frontiers | Research Topics

Integrated management of chronic kidney disease patients

Edited by
Li Zuo, Hong Liu, Jingyuan Xie and Jiannong Liu

Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-83252-049-9
DOI 10.3389/978-2-83252-049-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Integrated management of chronic kidney disease patients

Topic editors

Li Zuo — Peking University People's Hospital, China

Hong Liu — Central South University, China

Jingyuan Xie — Shanghai Jiao Tong University, China

Jiannong Liu — Hennepin Healthcare Research Institute, United States

Citation

Zuo, L., Liu, H., Xie, J., Liu, J., eds. (2023). *Integrated management of chronic kidney disease patients*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83252-049-9

Table of contents

frontiers in Medicine

SYSTEMATIC REVIEW
published: 17 March 2022
doi: 10.3389/fmed.2022.828189

Safety and Efficacy of Spironolactone in Dialysis-Dependent Patients: Meta-Analysis of Randomized Controlled Trials

Jing Liu[1], *WanYu Jia*[2] *and Chen Yu*[1]*

[1] Department of Nephrology, Tongji Hospital, Tongji University School of Medicine, Shanghai, China, [2] Department of Pediatrics, Clinical Center of Pediatric Nephrology of Henan Province, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Background: Patients with end-stage renal disease (ESRD) are characterized with high risk of heart failure. Although mineralocorticoid receptor antagonists have beneficial effect on relieving cardiac fibrosis and, thus, reduce the incidence of cardiovascular disease and cardiac death, the therapeutic benefits and adverse effects are still controversial. We conducted a meta-analysis to measure the safety and efficacy of spironolactone in patients undergoing dialysis.

Methods: A systematic search for randomized controlled trials (RCTs) was performed in PubMed, Embase, and Cochrane databases. Primary outcomes included changes in all-cause mortality (ACM), serum potassium concentration, incidence of hyperkalemia and gynecomastia (GYN). Secondary outcomes included changes in blood pressure (BP), left ventricular mass index (LVMI) and left ventricular ejection fraction (LVEF). Subgroup analysis and sensitivity analysis were further conducted. This research was registered with PROSPERO (International Prospective Register of Systematic Reviews; No. CRD42021287493).

Results: Fifteen RCTs with 1,258 patients were enrolled in this pooled-analysis. Spironolactone treatment significantly decreased ACM (RR = 0.42, $P < 0.0001$), CCV (RR = 0.54, $P = 0.008$) and LVMI (MD = −6.28, $P = 0.002$), also increased occurrence of GYN (RR = 4.36, $P = 0.0005$). However, LVEF (MD = 2.63, $P = 0.05$), systolic BP (MD = −4.61, $P = 0.14$) and diastolic BP (MD = −0.12, $P = 0.94$) did not change between two groups after treatment. Although serum potassium concentration was increased (MD = 0.22, $P < 0.0001$) after spironolactone supplement, the risk of hyperkalemia remained unchanged (RR = 1.21, $P = 0.31$). Further subgroup analysis found more obvious advantageous as well as disadvantageous effects in Asian subjects than European or American ones. Also, with more than 9 months of treatment duration, patients achieved more favorable influence than shorter duration.

Conclusions: These results highlight the therapeutic effects of spironolactone on cardiovascular indexes, including ACM, CCV, and LVMI. However, the unignorable increase of GYN incidence and serum potassium level indicate that close monitor in dialysis-dependent patients, especially Asian patients, is essential.

Keywords: dialysis, end-stage renal disease, mineralocorticoid receptor antagonists, spironolactone, meta-analysis

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Yu Pan,
Shanghai Jiao Tong University, China
Kyriakos Dimitriadis,
National and Kapodistrian University of Athens, Greece
Kwadwo Bonsu,
Memorial University of Newfoundland, Canada

***Correspondence:**
Chen Yu
yuchen@tongji.edu.cn

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *03 December 2021*
Accepted: *21 February 2022*
Published: *17 March 2022*

Citation:
Liu J, Jia W and Yu C (2022) Safety and Efficacy of Spironolactone in Dialysis-Dependent Patients: Meta-Analysis of Randomized Controlled Trials. Front. Med. 9:828189. doi: 10.3389/fmed.2022.828189

INTRODUCTION

Heart failure, which often occurs in patients with chronic kidney disease (CKD), may contribute to high cardiovascular morbidity and mortality (1, 2). Among all causes of death in patients undergoing dialysis, sudden cardiac death is the leading one, accounting for 25% of all-cause mortality (ACM) (3). Hypertension and left ventricular hypertrophy, which is directly associated with the risk for sudden cardiac death and ACM, occur in more than 70% patients with long-term dialysis (4, 5).

Aldosterone has been implicated as an important factor to keep cardiovascular homeostasis. As a pleiotropic hormone, aldosterone also can regulate various tissues, such as heart, kidney and liver, through activating the mineralocorticoid receptors (MRs) (6). In the presence of impaired renal function, the renin-angiotensin-aldosterone system (RAAS) are always activated abnormally, mediating high blood pressure and cardiac fibrosis (7). Former researches proved that utilization of mineralocorticoid receptor antagonists (MRA) can mitigate the deleterious effects on cardiovascular system and thus, improving the prognosis of patients with end-stage renal disease (ESRD) (8).

The role of MRAs therapy as a neurohormonal antagonist has been studied by prior studies, however, had various outcomes. Flevari et al. (9) found significant increased sodium potassium level and decreased blood pressure after MRAs treatment, while Lin et al. (10) and Gross et al. (11) suggested unchanged serum potassium and blood pressure, respectively. Previous meta-analyses have been limited by small number of clinical trials (12) or results for single system (13). Thus, spironolactone is not widely understood in subjects undergoing dialysis. These differences in adverse effect and efficacy prompted us to conduct a meta-analysis to determine the changes in dialysis-dependent patients after spironolactone supplement. Also, we will further explore the effect of some factors (e.g., country, dosage, intervention duration) on the results through subgroup analysis.

METHODS

This meta-analysis was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guideline, and was registered with PROSPERO (International Prospective Register of Systematic Reviews; No. CRD42021287493).

Search Strategy and Data Sources

Literature published up to October 2021 in PubMed, Embase, and Cochrane databases, without time or language restriction, were searched. The search strategies are provided in **Appendix A**. Also, the reference lists of review articles and original studies were manually searched for additional eligible reports.

Selection Criteria

Studies were considered to be eligible if they met the following criteria: (1) randomized-control study on humans; (2) dialysis patients (3) patients in the intervention group, were treated with spironolactone, while the control subjects received placebo or standard treatment. Exclusion criteria were: (1) compared different dosages of spironolactone; (2) failed to provide data on outcomes of interest: occurrence of adverse events (ACM, hyperkalemia and gynecomastia [GYN]) and cardiovascular benefits (including incidence of cardiocerebrovascular diseases [CCV], left ventricular mass index [LVMI], left ventricular ejection fraction [LVEF], systolic blood pressure [SBP] and diastolic blood pressure [DBP]).

Data Extraction and Quality Assessment

The data extraction from each studies includes study characteristics (year of publication, country, randomization method, type of study, sample size, duration and follow-up period) and patient characteristics (age, sex, and dialysis type).

Statistical Analyses

Review Manager (RevMan, version 5.4; the Nordic Cochrane Center, the Cochrane Collaboration, Copenhagen, Denmark) and Stata/SE (version 15.1; StataCorp LP, College Station, TX) were used for the analysis. Two authors extracted raw data from individual studies and then calculated pooled risk ratios (RRs) for dichotomous outcomes and mean differences (MDs) for continuous ones, and corresponding 95% confidence intervals (95% CIs) for each outcomes. The outcomes are presented as SMD if continuous indexes were measured in different methods. For research with more than one intervention groups, we split the shared control group into several groups with smaller sample size, and thus, included two or more comparisons (14). A fixed-effects model was used to perform meta-analysis, and a random-effects model was applied when severe heterogeneity was present. Heterogeneity of included studies was quantified by Q test and I^2 statistic. High heterogeneity was defined as $p < 0.1$ for Q statistic or $I^2 > 50\%$. To identify the source of heterogeneity, subgroup analysis was further conducted, according to country, dosage, and length of follow-up. Besides, two reviewers performed additional sensitivity analyses to explore the impact of a single article on the results. Cochrane Collaboration methodology (14) was used to assess included studies for bias.

RESULTS

Search Results

The process of study selection is schematically presented in the flowchart (**Figure 1**). Overall, the search terms identified 511 references. Of these, the reviewers excluded 259 articles from the initial screening. Subsequently, the majority of articles (217 articles) were excluded after reading the title and the abstract. After assessing the remaining 36 full text articles, we eliminated 23 additional articles since they failed to meet the inclusion criteria. Therefore, 15 randomized controlled trials (in 13 articles) were included in the meta-analysis.

Search Characteristics and Quality

The studies enrolled 1,258 patients, with the mean age spanned 52.92 ± 6.90 to 70.45 ± 9.70 years. Of these articles, three were conducted in China (10, 15, 16), two in the United States (11, 17), two in Grace (9, 18), two in Japan (19, 20), two in Iran (21, 22), one in Brazil (23) and one in Chile (24). In terms of

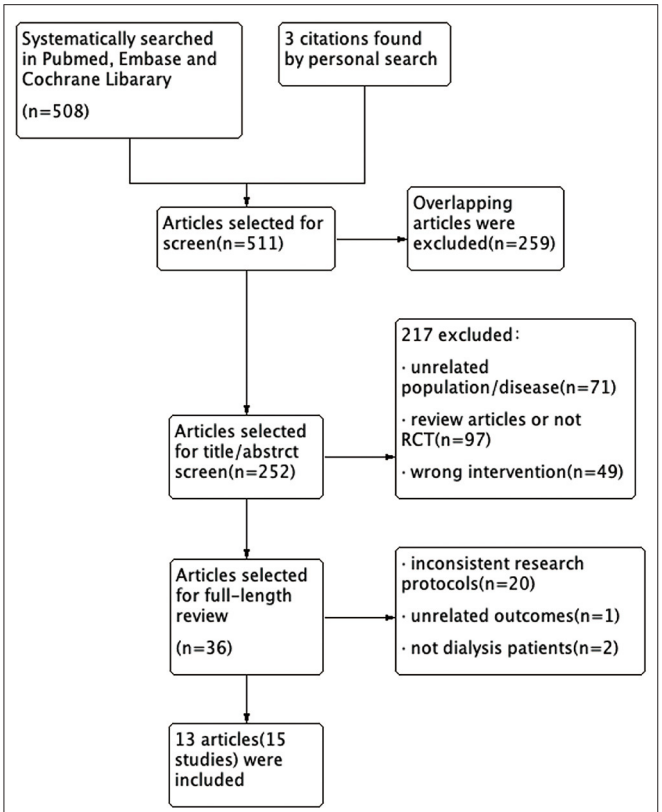


FIGURE 1 | Flow diagram of the literature search process.

ethnicity, seven RCTs were performed in Asians and 6 articles (8 RCTs) included non-Asians. Nine RCTs were performed in patients on maintenance HD, 3 articles involved PD patients, and other 3 researches reported data in both HD and PD population (**Table 1**).

Figure 2 summarizes the risk of bias of 13 RCTs. Eight studies and 3 studies provided information of random sequence or allocation concealment, respectively. Eleven studies were triple-blinded and 1 had a single-blind design.

Effects of Spironolactone on Primary Outcomes

ACM and CCV

Analysis of ACM in 1,074 patients in 11 studies found that spironolactone treatment significantly decreased ACM [RR = 0.42, 95%, CI = (0.28, 0.62), $P < 0.0001$]. Similarly, rate of CCV disease also decreased in spironolactone treatment group [RR = 0.54, 95%, CI = (0.35, 0.85), $P = 0.008$]. There were no heterogeneity (ACM: $Chi^2 = 6.19$, $P = 0.72$, $I^2 = 0\%$; CCV: $Chi^2 = 5.32$, $P = 0.38$, $I^2 = 6\%$) or publication bias ($P = 0.770$) (**Figure 3**).

Serum Potassium and Hyperkalemia

As illustrated in **Figure 4**, meta-analysis showed patients in spironolactone group had significantly higher serum potassium level compared with control ones [MD = 0.22, 95%, CI = (0.12, 0.31), $P < 0.0001$, $I^2 = 0\%$]. However, incidence of hyperkalemia in 1,072 patients in 10 studies showed no significant difference between treated and untreated groups [RR = 1.21, 95%, CI = [0.83, 1.77], $P = 0.31$, $I^2 = 0\%$]. There was no publication bias ($P = 0.119$).

GYN

Analysis of the effects of spironolactone on GYN occurrence in 674 subjects revealed an increase in experimental vs. control groups [RR = 4.36, 95%, CI = (1.90, 10.03), $P = 0.0005$], with no heterogeneity ($Chi^2 = 4.80$, $P = 0.57$, $I^2 = 0\%$) (**Figure 5**).

Effects of Spironolactone on the Secondary Outcomes

Blood Pressure

The pooled analysis of 10 researches showed that there was no significant difference in SBP [MD = −4.61, 95%, CI = (−10.78, 1.56), $P = 0.14$, $I^2 = 74\%$] or DBP [MD = −0.12, 95%CI = (−3.52, 3.27), $P = 0.94$, $I^2 = 59\%$]between two groups. The considerable heterogeneity was not linked to the country, treatment duration or type of renal replacement (**Figures 6A,B**). However, sensitivity analysis showed that the results of Flevari et al. (9) and Ni et al. (15) differed significantly from other studies, leading to unstable meta-analysis results for SBP and DBP (**Figures 6C–F**).

Heart Function

Available data of heart function included LVMI and LVEF. Data on LVMI and LVEF were respectively reported in several trials, and results indicated a significant decrease of LVMI [MD = −6.28, 95%, CI = (−10.29, −2.28), $P = 0.002$, $I^2 = 77\%$] between two groups. However there was no significant difference in LVEF (MD = 2.63, 95%, CI = (−0.03, 5.29), $P = 0.05$, $I^2 = 89\%$] in treated patients vs. untreated ones. Further subgroup analysis was conducted due to the non-ignorable heterogeneity. The data on LVMI showed that spironolactone was beneficial for Asian patients [MD = −9.66, 95%, CI= (−13.78, −5.53), $P < 0.00001$, $I^2 = 80\%$], while there was no significant difference between patients with and without spironolactone in Europe and the United States subgroup [MD = −0.59, 95%, CI = (−4.97, 3.78), $P = 0.79$, $I^2 = 0\%$]. Similarly, the benefit of spironolactone in increasing LVEF only existed in Asian patients (MD = 7.01, 95%, CI= (6.12, 7.91), $P < 0.00001$, $I^2 = 0\%$), not European or American subjects [MD = −0.90, 95%, CI = [−2.40, 0.61], $P = 0.24$, $I^2 = 0\%$] (**Figure 7**).

Subgroup Analysis

Further subgroup analysis based on country was performed. In Asian subgroup, the pooled analysis found significant difference in serum potassium level, SBP, ACM, LVMI, LVEF, and incidence of CCV and GYN between experimental and control patients. Besides, spironolactone supplementation did not cause a consistent change in DBP and hyperkalemia occurrence. However, in European and American subjects, meta-analysis showed that above mentioned indexes did not confer difference after spironolactone application (**Figure 8**).

TABLE 1 | Characteristics of enrolled researches.

Study, year, country	Population characteristics	Spironolactone dosage	Dialysis	Duration	Outcomes
Chaochao Wang (16); China	*N* = 96; Age (years) = 52.92 ± 6.91; Male (%) = 58.33 LVEF (%) = 39.78 ± 5.11; Dialysis vintage (months) = 32.32 ± 12.44	20 mg daily	PD	1 year	LVMI; LVEF
Charytan et al. (17); America	*N* = 102; Age (years) = 55.50 ± 12.00; Male (%) = 68.63 Hypertension (%) = 93.00; LVEF (%) = 68.11 ± 6.44 Dialysis vintage (years) = 3.4 (1.9–6.1)	25 mg; 50 mg daily	HD	9 months	Serum potassium; BP; LVMI; LVEF; ACM; CCV; side effects
Feniman (23); Brazil	*N* = 17; Age (years) = 54.12 ± 12.00; Male (%) = 52.94 LVEF (%) = 69.56 ± 4.06; Dialysis vintage (months) = 37.99 ± 58.26	25 mg daily	HD	6 months	Serum potassium; BP; LVMI; LVEF
Flevari et al. (9); Grace	*N* = 14; Age (years) = 59.5 ± 11.60; male (%) = 64.29 Hypertension (%) = 100% Dialysis vintage (years) = 2.4 ± 0.75	25 mg three times a week	HD	4 months	Serum potassium; BP; ACM; side effects
Gross et al. (11); America	*N* = 8; Age (years) = 53.00 ± 10.00; Male (%) = 37.50 Hypertension (%) = 37.50%	50 mg twice a week	HD	2 weeks	Serum potassium; BP
Hammer et al. (18); Grace	*N* = 97; Age (years) = 60.30 ± 13.20; Male (%) = 77.32 Hypertension (%) = 87.60; LVEF (%) = 59.76 ± 11.76 Dialysis vintage (months) = 42 (15.7–74.5)	50 mg daily	HD	10 months	BP; LVMI; LVEF; ACM; side effects
Ito et al. (19); Japan	*N* = 158; Age (years) = 56.49 ± 13.36; Male (%) = 71.52 LVEF (%) = 66.20 ± 10.31	25 mg Daily	PD	2 years	serum potassium; BP; LVMI; LVEF; ACM; CCV; side effects
Lin et al. (10); China	*N* = 253; Age (years) = 70.45 ± 9.70; Male (%) = 60.47 LVEF (%) = 57.75 ± 9.28; Dialysis vintage (months) = 42.70 ± 18.27	25 mg daily	HD/PD	2 years	Serum potassium; LVMI; LVEF; ACM; CCV; side effects
Matsumoto et al. (20); Japan	*N* = 309; Age (years) = 67.55 ± 11.75; Male (%) = 65.70 Hypertension (%) = 5.26; Dialysis vintage (months) = 113.58 ± 89.26	25 mg Daily	HD	3 years	ACM; CCV; side effects
Ni et al. (15); China	*N* = 76; Age (years) = 55.32 ± 13.15; Male (%) = 59.21 Hypertension (%) = 100; Dialysis vintage (months) = 55.74 ± 12.47	25 mg Daily	HD/PD	3 months	Serum potassium; BP; side effects
Taheri et al. (21); Iran	*N* = 16; Age (years) = 58.15 ± 7.88; Male (%) = 68.75 Hypertension (%) = 87.5; LVEF (%) = 32.5 ± 8.75	25 mg three times a week	HD	6 months	LVMI; LVEF; ACM; side effects
Taheri et al. (22); Iran	*N* = 18; Age (years) = 53.95 ± 15.31; Male (%) = 55.56 Hypertension (%) = 22.22; Dialysis vintage (months) = 44.05 ± 23.42	25 mg once every 2 days	PD	6 months	LVEF; ACM
Vukusich et al. (24); Chile	*N* = 53; Age (years) = 58.15 ± 5.06; Male (%) = 64.15 Hypertension (%) = 58.49; Dialysis vintage (years) = 8.29 ± 1.32	50 mg three times a week	HD	2 years	BP; ACM

ACM, all-cause mortality; BP, blood pressure; CCV, cardiocerebrovascular diseases; HD, hemodialysis; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; PD, peritoneal dialysis.

To eliminate the effect of duration, we also took subgroup analysis based on duration period. As shown in **Figure 9**, although longer intervention times (>9 months) had significant benefits on cardiac function (lower ACM and CCV occurrence, decreased LVMI, and increased LVEF), side effects (GYN, hyperkalemia, serum potassium) were significantly increased. However, in the subgroup with a treatment duration of less than or equal to 9 months, neither cardiac-related efficacy nor side effects were significant (**Figure 9**).

DISCUSSION

In this meta-analysis of 15 RCTs of 1,258 dialysis-dependent patients, we suggested that spironolactone treatment affected various parameters associated with cardiovascular system. Spironolactone decreased ACM, SBP, LVMI. Although the serum potassium level significantly increased, spironolactone did not elevate the occurrence of hyperkalemia. The further subgroup analysis implied the intergroup differences across countries. Spironolactone treatment had more obvious efficacy (decreased ACM, CCV, LVMI, and SBP, also increased LVEF) and more severe adverse effects (increased GYN occurrence and serum potassium) on Asian patients. However, spironolactone did not affect these markers in European and American population. Seven RCTs used spironolactone for more than 9 months, appearing more effective in reducing ACM and CCM as well as increasing incidence of GYN and hyperkalemia than those RCTs with shorter application duration.

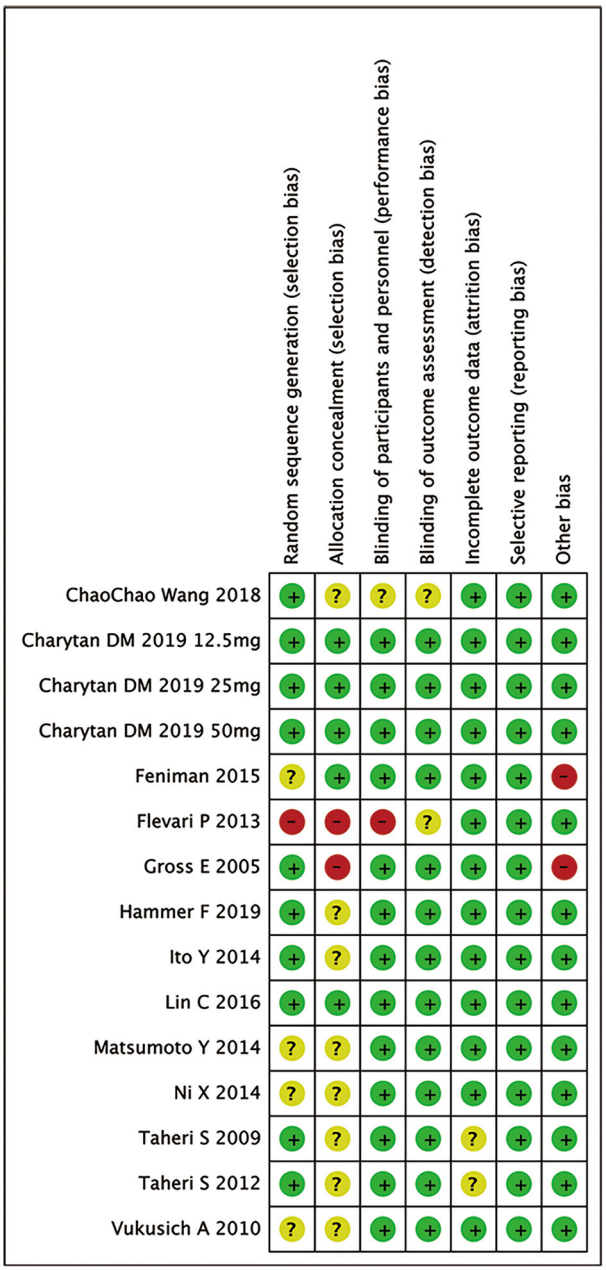


FIGURE 2 | Risk of bias in analyzed studies. Unclear risk of bias: "?", low risk of bias: "-", and high risk of bias: "+".

Hypertension often exists in patients with ESRD, accompanied by activated RASS and secondary hyperaldosteronemia (25). Aldosterone adversely affects blood pressure through both cellular and nervous mechanism. In blood vessels, mineralocorticoid not only decreases deformability of endothelial cells (26), but also suppresses bioactivity of nitric oxide in smooth muscle cells (27), therefore regulates blood pressure. Meanwhile, aldosterone receptors in the central nervous system may enhance sympathetic activity and thus contribute to hypertension (28). Although our meta-analysis didn't find antihypertensive effect of spironolactone, the high heterogeneity promoted us to perform sensitive analysis, which found data provided by Ni et al. (15) and Flevari et al. (9) were the source of heterogeneity. It may because all subjects included in these two studies were hypertensive, while other researches had a lower proportion of participants with hypertension (37.5 to 87.6%). Patients with hypertension, especially resistant hypertension, may be particularly prone to salt and water retention, therefore, more susceptible to spironolactone (29). Abnormal level of serum aldosterone promotes the production of profibrotic TGF-βsignaling and following cardiac fibrosis (30). Thus, using aldosterone antagonist can relief cardiac fibrosis in nephrectomized animals (31, 32). Similarly, we also observed rate of LVEF and LVMI significantly changed after MRAs treatment. Spironolactone may also benefit CCV system through following pathways: (1) blocking aldosterone effect on collagen formation, therefore, inhibiting left ventricle remodeling (33); (2) the antihypertensive effect of spironolactone improves vascular endothelial function (34); (3) preventing peritoneal inflammation and fibrosis thereby maintaining peritoneal function (35).

Our research also noted the safety of spironolactone in dialysis-dependent patients, with spironolactone treatment showing a tendency to increase serum potassium concentration, but an unchanged incidence of hyperkalemia. Hyperkalemia, which is well-recognized as adverse effect of MRAs, always prevent the physician from applying spironolactone. Notably, in our pooled-analysis, most of patients who dropped out of the study because of hyperkalemia were hemodialysis (HD) patients, not PD patients. These results are similar to previous findings showing the greater removal of potassium in PD than HD subjects (36, 37). Since dialysis patients are more dependent on dialysis rather than kidney to excrete potassium, using spironolactone maybe safer in dialysis patients than in non-dialysis ones. GYN also appeared with spironolactone application (38), and a significant occurrence of GYN was noted in treated group. The risk of GYN may be minimized if patients used low dose of spironolactone or switched to selective MRAs, such as eplerenone (19).

In subgroup analysis, the effects, including efficacy and side effects, of spironolactone were more significant in Asian patients. Actually, this kind of racial difference existed in several clinical studies on MRAs. Vardeny et al. reported that non-African Americans might have greater beneficial effect from spironolactone supplement than African Americans (39). Besides the unclear genetic mechanism of racial disparity, different selection of ACEI or ARB by ethnic groups may also cause inter-subgroup heterogeneity. The high incidence of cough among Asian patients makes them prone to choose ARB rather than ACEI (40, 41), which may contribute to different results on efficacy and side effects between Asian and non-Asian subgroups (42, 43). Besides, relatively longer intervention duration in Asian populations (3 ∼ 36 months) than in European and American ones (0.5 ∼ 24 months) should also be taken into account. Till now, the safe duration of spironolactone treatment is still controversial. Spironolactone exerts cardio-protective effects

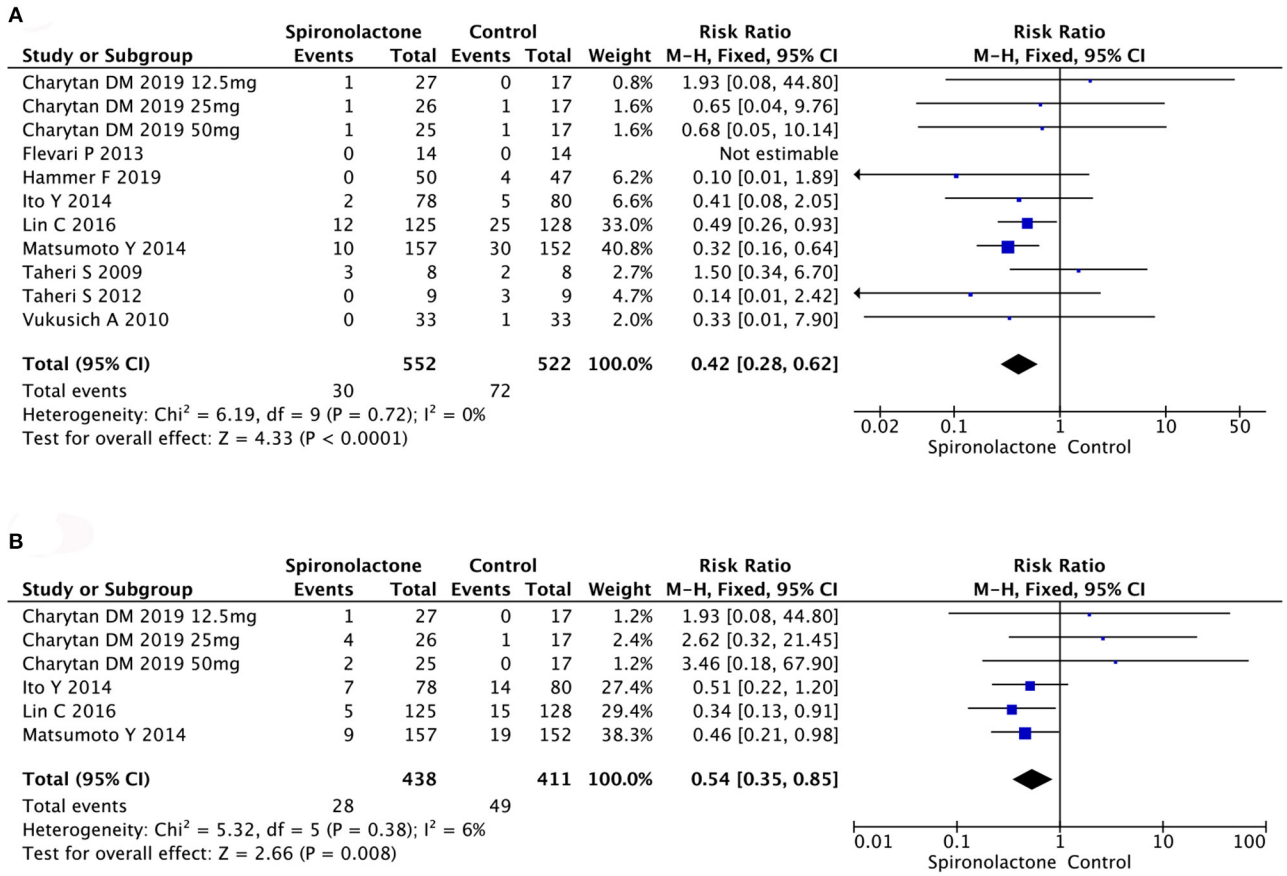


FIGURE 3 | (A) Effect of spironolactone on ACM; **(B)** Effect of spironolactone on CCV. ACM, all-cause mortality; CCV, cardiocerebrovascular diseases.

by inhibiting MRs on the one hand and reduces potassium excretion owing to Na^+/K^+ pump inhibition on the other hand. These double-edged sword effects do not occur simultaneously. Spironolactone elevates serum potassium at an early stage, while cardioprotective effects appear later (44), suggesting close laboratory surveillance for patients newly initiating therapy with MRAs. From this pooled-analysis, longer duration (more than 9 months) was related to an increased LVEF, decreased LVMI, ACM, and CCV, however, the incidence of GYN and ACM also raised. We also did further subgroup-analysis based on dosage, and surprisedly, the results indicated that the lower dosage (≤25 mg) had more obvious side effect and efficacy than higher dosage (50 mg) (**Supplementary Figure 2**). This may due to the possibility that Asians are more sensitive to drugs and therefore, based on former experience, all Asian groups have chosen smaller doses. Thus, optimal dosage in terms of safety and efficacy remains a critical question that needs to be addressed. Epidemiological evidence suggests that diabetes mellitus is one of the most common modifiable risk factors for CCV and ACM (45). However, all but one article [the study by Vukusich et al. (24)] included only non-diabetic patients, other 12 articles included diabetic and non-diabetic patients. Thus, we failed to perform a diabetes-based subgroup analysis. Besides, as an early sensitive indicator, carotid intima-media thickness has been used as a surrogate endpoint for CCV and ACM to access the efficacy of certain interventions in the past few years (46). Therefore, further studies limited to non-diabetic patients with reported data on carotid intima-media thickness are recommended.

The strength of this study is the strict selection range that only includes RCTs. Meanwhile, without the restrictions of follow-up duration and renal replacement type, we maximized the collected information while selection bias or other potential bias were minimized. Moreover, to the best of our knowledge, this is the first meta-analysis to provide evidence of racial differences in spironolactone use in dialysis-dependent patients. Our results should also be interpreted within the context of several limitations. These include the relatively small sample size and various duration of treatment period. Since spironolactone is an MRA, future studies should report serum as well as urine aldosterone level. Most RCTs included are single-center trials. However, the racial factor is one of the sources of intergroup heterogeneity, thus, multi-central research with patients from various continents is needed in following studies. Moreover, Ito et al. (19) proposed inconsistent changes in kidney-related indicators after spironolactone use in men and women,

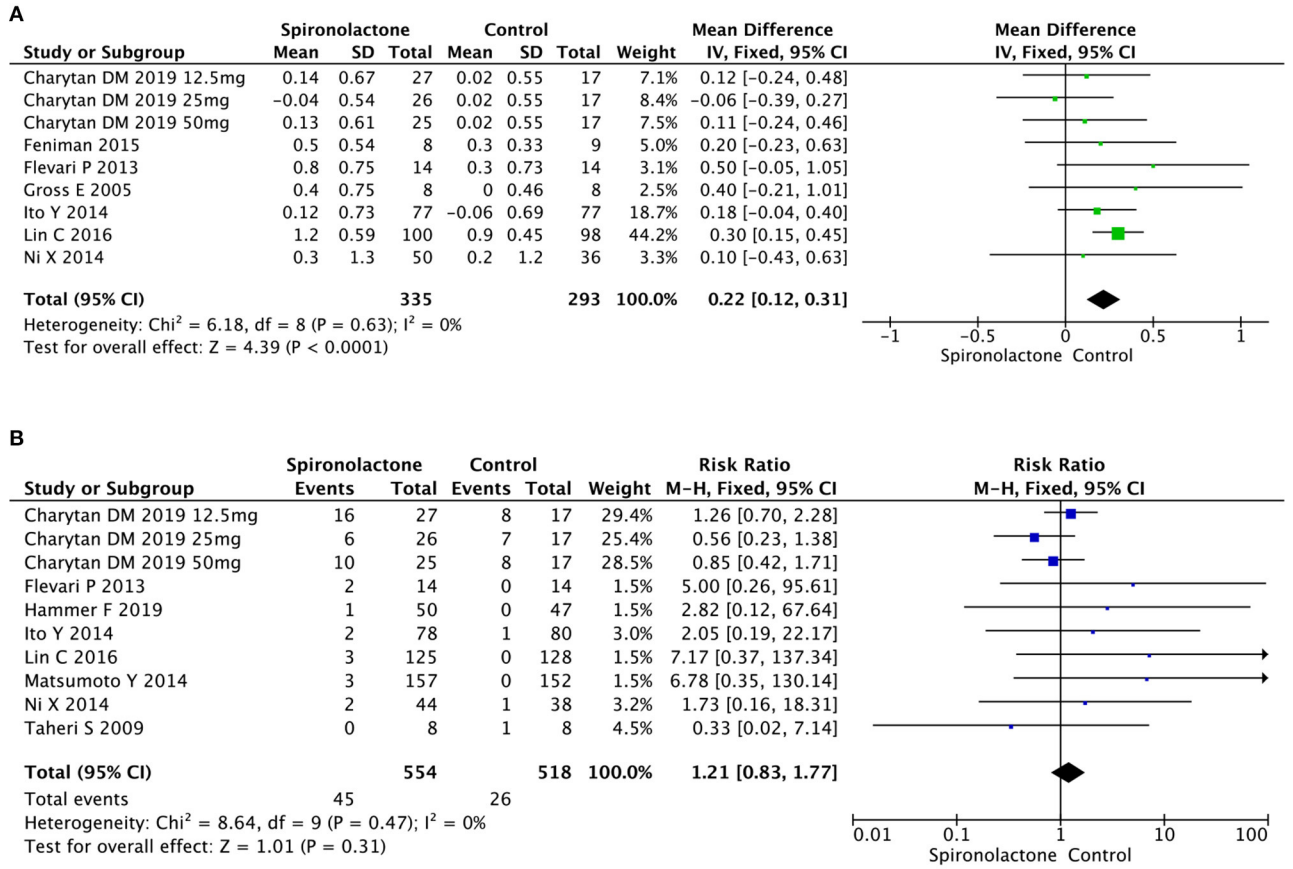


FIGURE 4 | (A) Effect of spironolactone on serum potassium; **(B)** Effect of spironolactone on hyperkalemia.

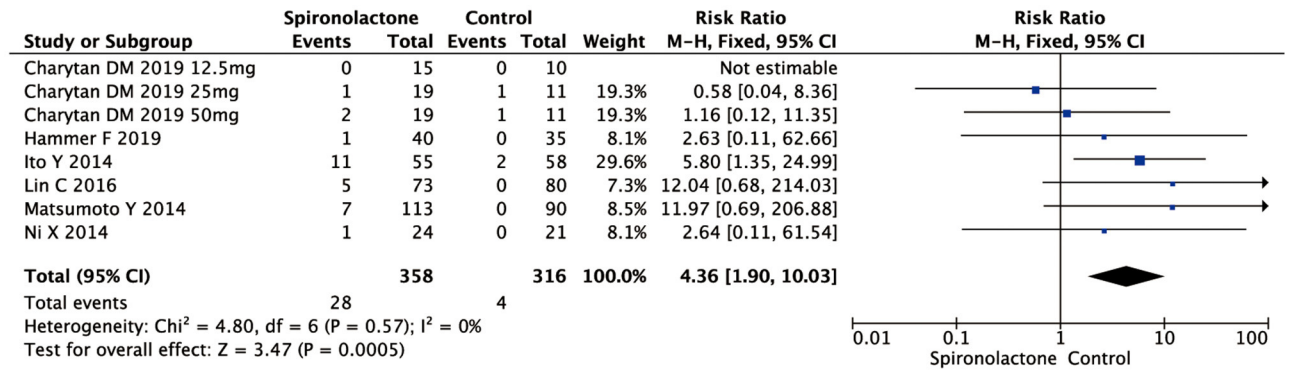


FIGURE 5 | Effect of spironolactone on GYN. GYN, gynecomastia.

suggesting further researches to report outcomes according to gender.

CONCLUSIONS

Patients undergoing dialysis can achieve cardiac benefit (LVEF, LVMI, CCV, and ACM) after spironolactone treatment, while the risk of GYN and serum potassium concentration also increased. However, spironolactone does not affect the incidence rate of hyperkalemia. Asian patients can achieve more obvious benefit, although more side effects, from spironolactone than European and American subjects. The use of spironolactone for more than 9 months can have a pharmacological effect compared to a shorter course of treatment. Studies with

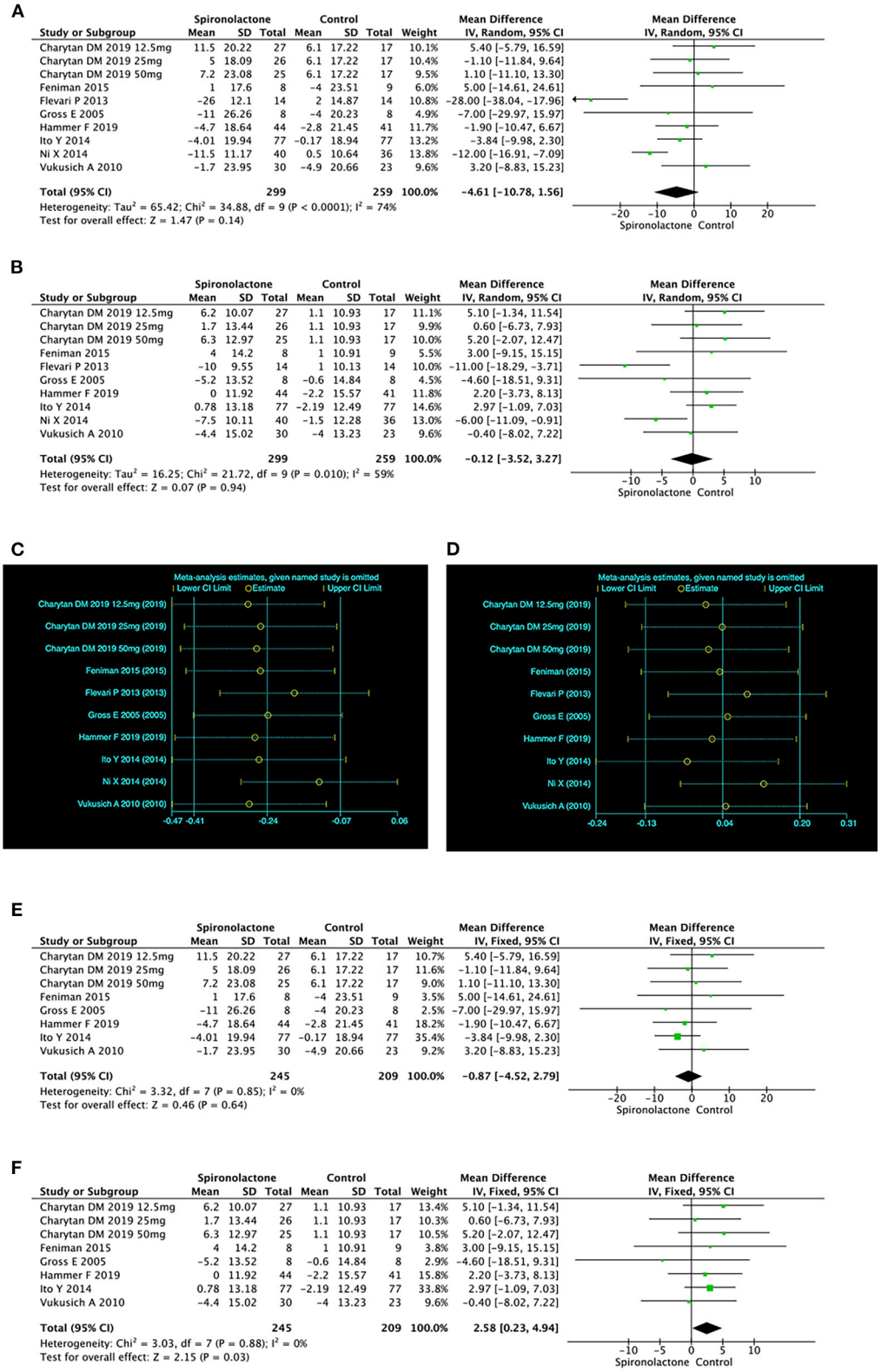


FIGURE 6 | (A) Effect of spironolactone on SBP; **(B)** Effect of spironolactone on DBP; **(C)** Sensitive analysis of SBP; **(D)** Sensitive analysis of DBP; **(E)** Effect of spironolactone on SBP after sensitive analysis; **(F)** Effect of spironolactone on DBP after sensitive analysis. DBP, diastolic blood pressure; SBP, systolic blood pressure.

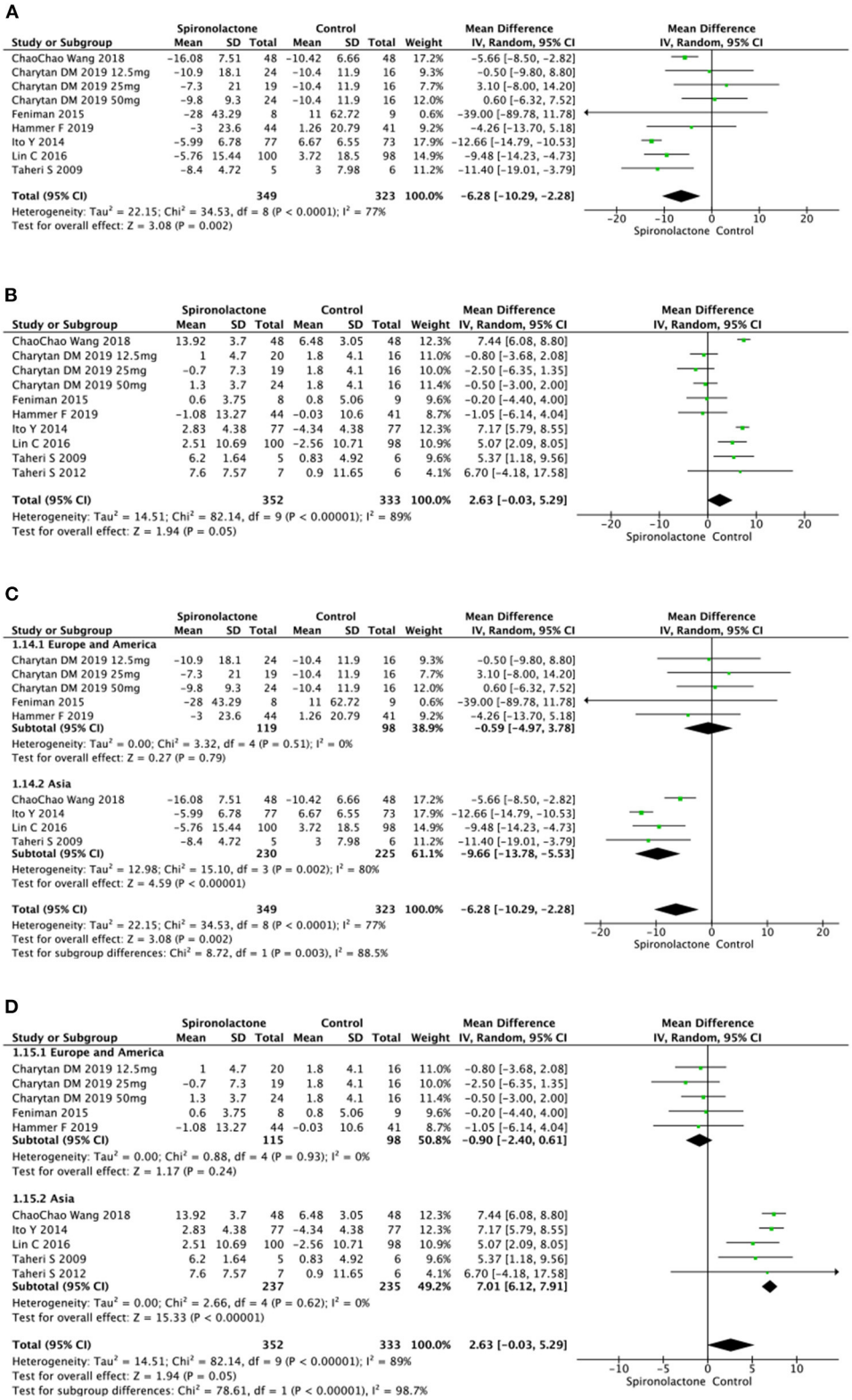


FIGURE 7 | (A) Effect of spironolactone on LVMI; **(B)** Effect of spironolactone on LVEF; **(C)** Effect of spironolactone on LVMI after subgroup analysis; **(D)** Effect of spironolactone on LVEF after subgroup analysis. LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index.

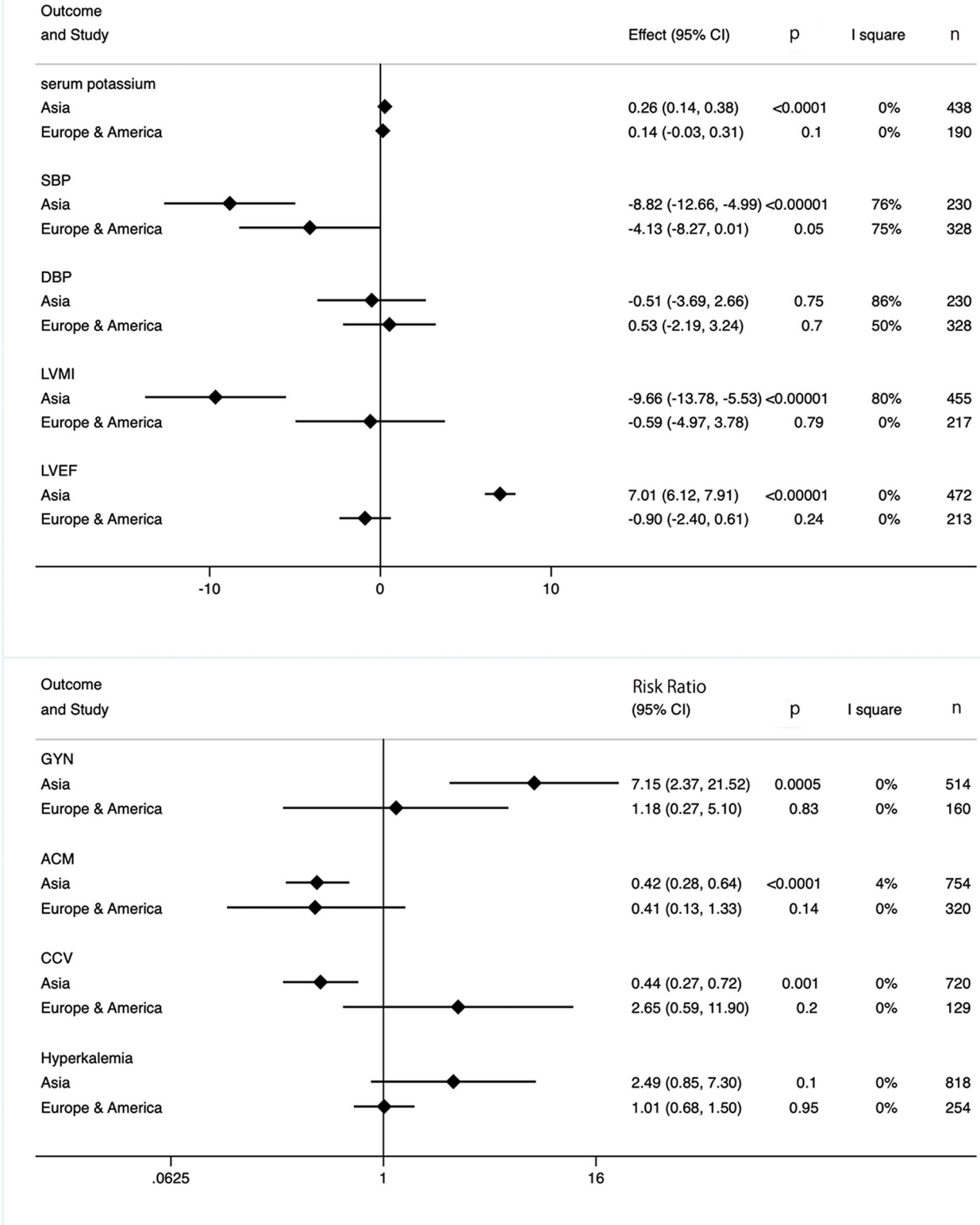
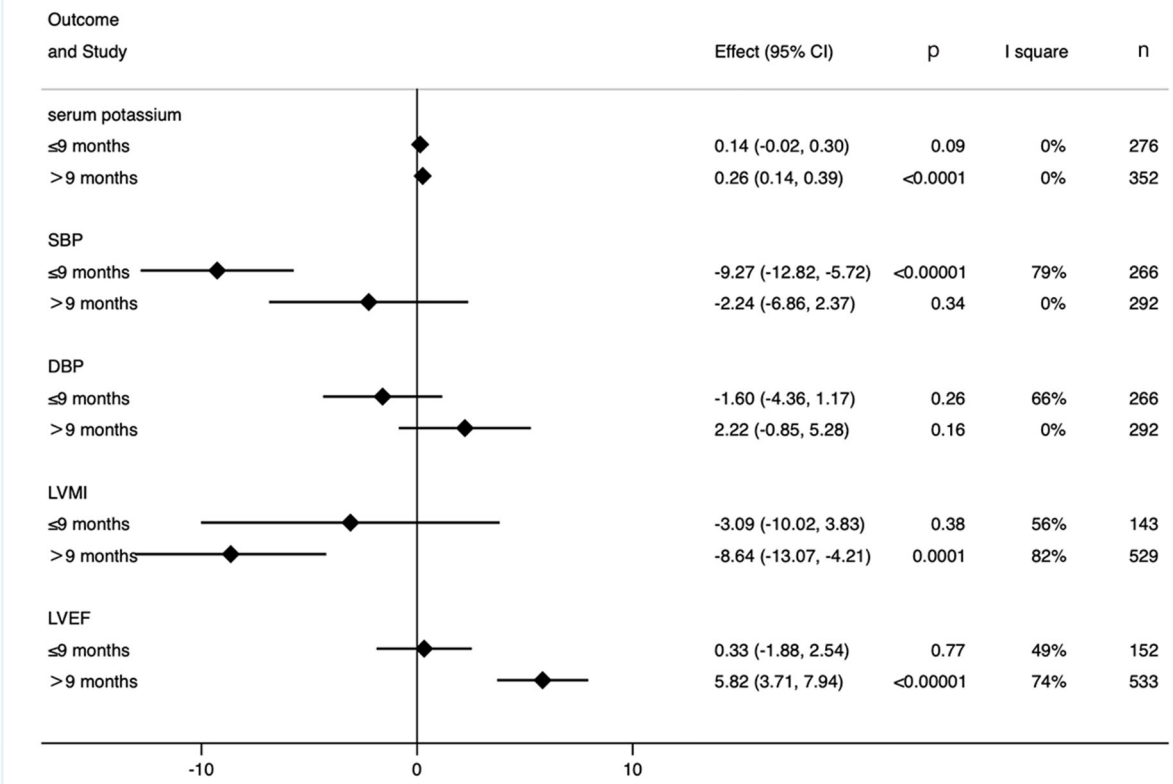


FIGURE 8 | Subgroup analysis based on countries.



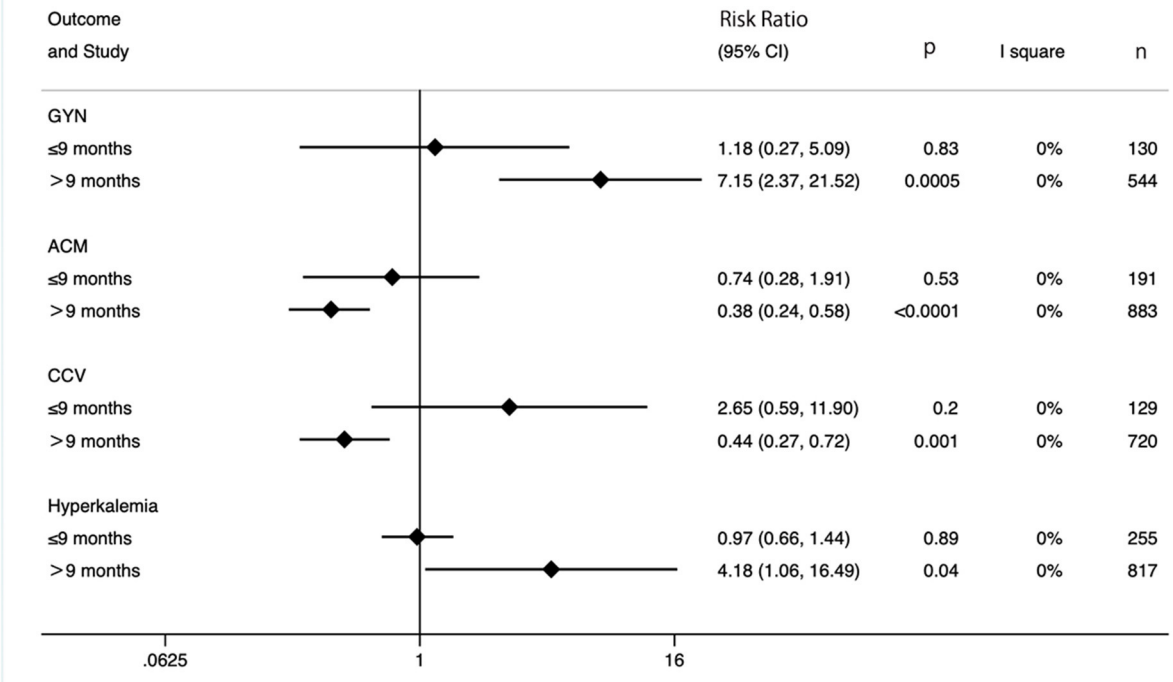


FIGURE 9 | Subgroup analysis based on duration.

larger scales and multi-countries are advocated to further evaluate the balance of efficacy and adverse effects in spironolactone use.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JL assisted in conceptualization, data curation, formal analysis, investigation, and writing—original draft preparation. WJ assisted in conceptualization, formal analysis, and writing—original draft preparation. CY assisted in resources, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was financially supported through grants from the National Natural Science Foundation of China (Nos. 81873609, 82170696, and 81800631), Shanghai Sailing Program (19YF1444200 and 19YF1444400), and Tongji Hospital Medical Records Program (TJ (DB)2103).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.828189/full#supplementary-material

Supplementary Figure 1 | Graphic abstract.

Supplementary Figure 2 | Subgroup analysis based on dosage.

REFERENCES

1. Ku E, Mitsnefes MM. Cardiovascular disease in young adults with incident ESRD. *Nat Rev Nephrol*. (2019) 15:390–1. doi: 10.1038/s41581-019-0154-3
2. Herzog CA, Ma JZ, Collins AJ. Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. *N Engl J Med*. (1998) 339:799–805. doi: 10.1056/NEJM199809173391203
3. Wheeler DC, London GM, Parfrey PS, Block GA, Correa-Rotter R, Dehmel B, et al. Effects of cinacalcet on atherosclerotic and nonatherosclerotic cardiovascular events in patients receiving hemodialysis: the evaluation of cinacalcet HCl therapy to lower cardiovascular events (EVOLVE) trial. *J Am Heart Assoc*. (2014) 3:e001363. doi: 10.1161/JAHA.114.001363
4. Salem MM. Hypertension in the hemodialysis population: a survey of 649 patients. *Am J Kidney Dis*. (1995) 2:461–8. doi: 10.1016/0272-6386(95)90492-1
5. Foley RN, Parfrey PS, Harnett JD, Kent GM, Martin CJ, Murray DC, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney Int*. (1995) 47:186–92. doi: 10.1038/ki.1995.22
6. Struthers AD, MacDonald TM. Review of aldosterone-and angiotensin II-induced target organ damage and prevention. *Cardiovasc Res*. (2004) 61:663–70. doi: 10.1016/j.cardiores.2003.11.037
7. Sato A, Funder JW, Saruta T. Involvement of aldosterone in left ventricular hypertrophy of patients with end-stage renal failure treated with hemodialysis. *Am J Hypertens*. (1999) 12:867–73. doi: 10.1016/S0895-7061(99)00066-7
8. Bolignano D, Palmer SC, Navaneethan SD, Strippoli GF. Aldosterone antagonists for preventing the progression of chronic kidney disease. *Cochrane Database Syst Rev*. (2014) 29:CD007004. doi: 10.1002/14651858.CD007004.pub3
9. Flevari P, Kalogeropoulou S, Drakou A, Leftheriotis D, Panou F, Lekakis J, et al. Spironolactone improves endothelial and cardiac autonomic function in non heart failure hemodialysis patients. *Am J Hypertens*. (2013) 31:1239–44. doi: 10.1097/HJH.0b013e32835f955c
10. Lin C, Zhang Q, Zhang H, Lin A. Long-term effects of low-dose spironolactone on chronic dialysis patients: a randomized placebo-controlled study. *J Clin Hypertens*. (2016) 18:121–8. doi: 10.1111/jch.12628
11. Gross E, Rothstein M, Dombek S, Juknis HI. Effect of spironolactone on blood pressure and the renin-angiotensin-aldosterone system in oligo-anuric hemodialysis patients. *Am J Kidney Dis*. (2005) 46:94–101. doi: 10.1053/j.ajkd.2005.03.005
12. Zeng Q, Zhou X, Xu G. Safety evaluation and cardiovascular effect of additional use of spironolactone in hemodialysis patients: a meta-analysis. *Drug Des Devel Ther*. (2019) 13:1487. doi: 10.2147/DDDT.S189454
13. Li Y, Xie N, Liang M. Aldosterone antagonists reduce the risk of cardiovascular mortality in dialysis patients: a meta-analysis. *Evid Based Complement Alternat Med*. (2019) 2019:1925243. doi: 10.1155/2019/1925243
14. Michaelis R, Tang V, Wagner JL, Modi AC, LaFrance WC Jr, Goldstein LH, et al. Cochrane systematic review and meta-analysis of the impact of psychological treatments for people with epilepsy on health-related quality of life. *Epilepsia*. (2018) 59:315–32. doi: 10.1111/epi.13989
15. Ni X, Zhang J, Zhang P, Wu F, Xia M, Ying G, et al. Effects of spironolactone on dialysis patients with refractory hypertension: a randomized controlled study. *J Clin Hypertens*. (2014) 16:658–63. doi: 10.1111/jch.12374
16. Wang C, Lin Y, Zhao R, Chen Q. Effects of spironolacone on cardiac and residual renal function in patients with peritoneal dialysis. *Chinese Gen. Pract*. (2018) 16:1303–7.
17. Charytan DM, Himmelfarb J, Alp Ikizler T, Raj DS, Hsu JY, Landis JR, et al. Safety and cardiovascular efficacy of spironolactone in dialysis-dependent ESRD (SPin-D): a randomized, placebo-controlled, multiple dosage trial. *Kidney Int*. (2019) 95:973–82. doi: 10.1016/j.kint.2018.08.034
18. Hammer F, Himmelfarb J, Ikizler TA, Raj DS, Hsu JY, Landis JR, et al. A randomized controlled trial of the effect of spironolactone on left ventricular mass in hemodialysis patients. *Kidney Int*. (2019) 95:983–91. doi: 10.1016/j.kint.2018.11.025
19. Ito Y, Mizuno M, Suzuki Y, Tamai H, Hiramatsu T, Ohashi H, et al. Long-term effects of spironolactone in peritoneal dialysis patients. *J Am Soc Nephrol*. (2014) 25:1094–102. doi: 10.1681/ASN.2013030273
20. Matsumoto Y, Mori Y, Kageyama S, Arihara K, Sugiyama T, Ohmura H, et al. Spironolactone reduces cardiovascular and cerebrovascular morbidity and mortality in hemodialysis patients. *J Am Coll Cardiol*. (2014) 63:528–36. doi: 10.1016/j.jacc.2013.09.056
21. Taheri S, Mortazavi M, Shahidi S, Pourmoghadas A, Garakyaraghi M, Seirafian S, et al. Spironolactone in chronic hemodialysis patients improves cardiac function. *Saudi J Kidney Dis Transpl*. (2009) 20:392–7. doi: 10.1002/clc.20838
22. Taheri S, Mortazavi M, Pourmoghadas A, Seyrafian S, Alipour Z, Karimi S. A prospective double-blind randomized placebo-controlled clinical trial to evaluate the safety and efficacy of spironolactone in patients with advanced congestive heart failure on continuous ambulatory peritoneal dialysis. *Saudi J Kidney Dis Transpl*. (2012) 23:507–12.
23. Feniman-De-Stefano GMM, Zanati-Basan SG, De Stefano LM, Xavier PS, Castro AD, Caramori JC, et al. Spironolactone is secure and reduces left ventricular hypertrophy in hemodialysis patients. *Ther Adv Cardiovasc Dis*. (2015) 9:158–67. doi: 10.1177/1753944715591448
24. Vukusich A, Kunstmann S, Varela C, Gainza D, Bravo S, Sepulveda D, et al. A randomized, double-blind, placebo-controlled trial of spironolactone on

carotid intima-media thickness in nondiabetic hemodialysis patients. *Clin J Am Soc Nephrol*. (2010) 5:1380–7. doi: 10.2215/CJN.09421209
25. Yamamoto N, Yasue H, Mizuno Y, Yoshimura M, Fujii H, Nakayama M, et al. Aldosterone is produced from ventricles in patients with essential hypertension. *Hypertension*. (2002) 39: 958–62. doi: 10.1161/01.HYP.0000015905.27598.E9
26. Ritz E, Koleganova N. Aldosterone in uremia–beyond blood pressure. *Blood Purif*. (2010) 29:111–3. doi: 10.1159/000245635
27. Ikeda U, Kanbe T, Nakayama I, Kawahara Y, Yokoyama M, Shimada K. Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells induced by interleukin-1 β. *Eur J Pharmacol*. (1995) 290:69–73. doi: 10.1016/0922-4106 (95)90018-7
28. Nakagaki T, Hirooka Y, Matsukawa R, Nishihara M, Nakano M, Ito K, et al. Activation of mineralocorticoid receptors in the rostral ventrolateral medulla is involved in hypertensive mechanisms in stroke-prone spontaneously hypertensive rats. *Hypertens Res*. (2012) 35:470–6. doi: 10.1038/hr.2011.220
29. Chapman N, Dobson J, Wilson S, Dahlöf B, Sever PS, Wedel H, et al. Effect of spironolactone on blood pressure in subjects with resistant hypertension. *Hypertension*. (2007) 49:839–45. doi: 10.1161/01.HYP.0000259805.18468.8c
30. Ritz E. Left ventricular hypertrophy in renal disease: beyond preload and afterload. *Kidney Int*. (2009) 75:771–73. doi: 10.1038/ki.2009.35
31. Michea L, Villagrán A, Urzúa A, Kuntsmann S, Venegas P, Carrasco L, et al. Mineralocorticoid receptor antagonism attenuates cardiac hypertrophy and prevents oxidative stress in uremic rats. *Hypertension*. (2008) 52:295–300. doi: 10.1161/HYPERTENSIONAHA.107.109645
32. Zhou H, Xi D, Liu J, Zhao J, Chen S, Guo Z. Spirolactone provides protection from renal fibrosis by inhibiting the endothelial-mesenchymal transition in isoprenaline-induced heart failure in rats. *Drug Des Devel Ther*. (2016) 10:1581–8. doi: 10.2147/DDDT.S100095
33. Kosmala W, Przewlocka-Kosmala M, Szczepanik-Osadnik H, Mysiak A, O'Moore-Sullivan T, Marwick TH. A randomized study of the beneficial effects of aldosterone antagonism on LV function, structure, and fibrosis markers in metabolic syndrome. *JACC Cardiovasc. Imaging*. (2011) 4:1239–49. doi: 10.1016/j.jcmg.2011.08.014
34. Farquharson CA, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation*. (2000) 101:594–7. doi: 10.1161/01.CIR.101.6.594
35. Zhang L, Hao JB, Ren LS, Ding JL, Hao LR. The aldosterone receptor antagonist spironolactone prevents peritoneal inflammation and fibrosis. *Lab Invest*. (2014) 94:839–50. doi: 10.1038/labinvest.2014.69
36. Spital A, Sterns RH, Potassium supplementation via the dialysate in continuous ambulatory peritoneal dialysis. *Am J Kidney Dis*. (1985) 6:173–6. doi: 10.1016/S0272-6386 (85)80022-6
37. Szeto CC, Chow KM, Kwan BC, Leung CB, Chung KY, Law MC, et al. Hypokalemia in Chinese peritoneal dialysis patients: prevalence and prognostic implication. *Am J Kidney Dis*. (2005) 46:128–35. doi: 10.1053/j.ajkd.2005.03.015
38. Bertocchio JP, Warnock DG, Jaisser F. Mineralocorticoid receptor activation and blockade: an emerging paradigm in chronic kidney disease. *Kidney Int*. (2011) 79:1051–60. doi: 10.1038/ki.2011.48
39. Vardeny O, Cavallari LH, Claggett B, Desai AS, Anand I, Rossignol P, et al. Race influences the safety and efficacy of spironolactone in severe heart failure. *Circ Heart Fail*. (2013) 6:970–6. doi: 10.1161/CIRCHEARTFAILURE.113.000530
40. Woo K.S, Norris RM, Nicholls G, Racial difference in incidence of cough with angiotensin-converting enzyme inhibitors (a tale of two cities). *Am J Cardiol*. (1995) 75:967–8. doi: 10.1016/S0002-9149 (99)80703-6
41. Luo JQ, He FZ, Wang ZM, Sun NL, Wang LY, Tang GF, et al. SLCO1B1 variants and angiotensin converting enzyme inhibitor (enalapril)-induced cough: a pharmacogenetic study. *Sci Rep*. (2015) 5:17253. doi: 10.1038/srep17253
42. Burnett H, Earley A, Voors AA, Senni M, McMurray JJ, Deschaseaux C, Cope S. Thirty years of evidence on the efficacy of drug treatments for chronic heart failure with reduced ejection fraction: a network meta-analysis. *Circ Heart Fail*. (2017) 10:e003529. doi: 10.1161/CIRCHEARTFAILURE.116.003529
43. Zhang L, Zeng X, Fu P, Wu HM. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers for preserving residual kidney function in peritoneal dialysis patients. *Cochrane Database Syst Rev*. (2014) 23:CD009120. doi: 10.1002/14651858.CD009120.pub2
44. Rossignol P, Frimat L, Zannad F. The safety of mineralocorticoid antagonists in maintenance hemodialysis patients: two steps forward. *Kidney Int*. (2019) 95:747–9. doi: 10.1016/j.kint.2018.12.006
45. Hedayatnia M, Asadi Z, Zare-Feyzabadi R, Yaghooti-Khorasani M, Ghazizadeh H, Ghaffarian-Zirak R, et al. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. *Lipids Health Dis*. (2020) 19:42. doi: 10.1186/s12944-020-01204-y
46. Lorenz MW, Markus HS, Bots ML, Rosvall M and Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation*. (2007) 115:459–67. doi: 10.1161/CIRCULATIONAHA.106.628875

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 18 March 2022
doi: 10.3389/fmed.2022.823835

Early Menopause May Associate With a Higher Risk of CKD and All-Cause Mortality in Postmenopausal Women: An Analysis of NHANES, 1999–2014

Duo Qian†, Zu-feng Wang†, Yi-chun Cheng, Ran Luo, Shu-Wang Ge and Gang Xu**

Division of Internal Medicine, Department of Nephrology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Michael Hutchens,
Oregon Health and Science University, United States
Jonathan P. Troost,
University of Michigan, United States

***Correspondence:**
Gang Xu
xugang@tjh.tjmu.edu.cn
Shu-Wang Ge
geshuwang@tjh.tjmu.edu.cn

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *28 November 2021*
Accepted: *11 February 2022*
Published: *18 March 2022*

Citation:
Qian D, Wang Z-f, Cheng Y-c, Luo R, Ge S-W and Xu G (2022) Early Menopause May Associate With a Higher Risk of CKD and All-Cause Mortality in Postmenopausal Women: An Analysis of NHANES, 1999–2014. Front. Med. 9:823835. doi: 10.3389/fmed.2022.823835

Background: Chronic kidney disease (CKD) in women is often accompanied by hormone disorders such as sex hormones, and most women with CKD are in the post-menopausal age group. Due to the close relationship between menopause and sex hormones, we aimed to explore the association between early menopause and CKD in post-menopausal women, and the influence of early menopause on longevity in the CKD population.

Methods: Information regarding 4,945 post-menopausal women was extracted from the database of the National Health and Nutrition Examination Survey (NHANES) 1999–2014, and then divided into 4 groups according to the type of menopause (natural or surgical) and early menopause (menopause at age <45) or not. The association between early menopause and CKD prevalence was examined using multivariable logistic regression, while we used multivariable Cox proportional hazards models to investigate the possible relationship between early menopause and all-cause mortality in CKD and non-CKD populations. The differences in the levels of sex hormones between women with and without CKD were also explored.

Results: Compared with women with natural menopause at age ≥45, women experiencing early natural menopause had a higher risk of CKD [OR = 1.26 (1.01–1.56)]. Similarly, as compared to women with surgical menopause at age ≥ 45, women in the early surgical menopause group were more likely to have CKD [OR = 1.38 (1.05–1.81)]. In addition, early surgical menopause was associated with higher mortality in the non-CKD group [HR = 1.62 (1.06–2.49)], but not in the CKD group. Women with CKD had a higher level of luteinizing hormone and follicle-stimulating hormone, combined with a lower level of testosterone and estradiol than the non-CKD women.

Conclusion: Both early natural and surgical menopause were associated with a higher risk of CKD. Early surgical menopause was a hazard factor for survival in the non-CKD group, but not in the CKD group. Further research is required to understand the mechanisms.

Keywords: menopause, women, chronic kidney disease, oophorectomy, mortality

INTRODUCTION

Chronic kidney disease (CKD), mainly manifested by decreased GFR and proteinuria (1), is a major public health problem. In the United States, 14.5% of the population meets the criteria for CKD (2). In women with CKD, hormone disorders such as sex hormones disorders are highly prevalent, which may lead to some gynecological problems, for example, menorrhagia and early menopause (3, 4). Moreover, compared with the mean age of 48.8 years at menopause in the general population (5), women with end-stage kidney disease (ESKD) have earlier menopause at a mean age of 45.9 years (6).

Previous studies have shown that early menopause, both natural and surgical, is associated with many diseases, including cardiovascular disease, diabetes, and osteoporosis (7–11). Considering the protective role of estrogens on the cardiovascular system, bone density, and insulin resistance, the shorter lifetime exposure to endogenous estrogens may be the main reason (7, 9). As a consequence, early menopause may influence lifespan (12, 13). Estrogen also appears to be renoprotective in women (14) and it is possible that early menopause is associated with a higher risk of CKD and may affect survival in CKD patients as a consequence of endocrine disturbances. However, the association of early menopause throughout the stages of CKD is inconclusive, and the information about early menopause and mortality in CKD patients is lacking.

We hypothesized that early menopause might be a risk factor of CKD and associated with higher mortality among women with CKD. We examined the National Health and Nutrition Examination Survey (NHANES) database from1999 to 2014 to evaluate the effect of early natural menopause and surgical menopause on CKD prevalence as well as all-cause mortality among women with CKD respectively. The differences in the levels of sex steroid hormone between women with and without CKD were also explored.

METHODS

Study Participants

All data in this study were from NHANES, a representative cross-section survey. It was designed to get information about civilian residents' health and nutritional status in the United States, using a multistage-stratified sample. The NHANES is a periodic survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The NCHS Research Ethics Review Board reviewed and approved the NHANES and all participants provided written informed consent. The NHANES Reproductive Health questionnaire surveys females to provide personal interview data on menstrual history, pregnancy history, lactation, oral contraceptive, and other related conditions. These questions were administered at the mobile examination center by trained interviewers, using the Computer-Assisted Personal Interviewing system as part of the Mobile Examination Center (MEC) interview.

We merged the data from the 1999 to 2014 NHANES strictly following the analytical guidelines. 28,688 women answered the reproduction health questionnaire during this period. 10,064 post-menopause women were selected according to the negative response to the question "Have you had at least one menstrual period in the past 12 months? (Please do not include bleedings caused by medical conditions, hormone therapy, or surgeries)." We then excluded women who reported their menopause status as attributable to pregnancy, breastfeeding, usually irregular period. For the surgical menopause group, 1,515 participants reported binary oophorectomy and the surgery time is the same as age at the last period were included. For the natural menopause group, according to the questions "What is the reason that you have not had a period in the past 12 months?" "Have you had a hysterectomy, including a partial hysterectomy, that is, surgery to remove your uterus or womb?" and "Have you had at least one of ovaries removed (either when had the uterus removed or at another time)?" we excluded 2,430 women with a history of hysterectomy, unilateral oophorectomy, or women who reported menopause are due to other medical treatments. Because no data were available on age at menopause, 404 women were excluded. Since this is a questionnaire survey, considering that participants may not be clear about the question, a lot of them reported the age of menopause was the same as age at screening, and most of the participants reported extreme values to have incomplete information. To ensure the accuracy of data, we ruled out 216 women with extreme age at menopause (<30 years old or >60) and 253 women younger than 50 years old at screening consistent with the past study (15–17). Both groups exclude women with missing data of age, race, age at the last period, serum creatinine, or urinary albumin-creatinine ratio. Finally, there were 3,574 participants in the natural menopause group and 1,377 in the surgical menopause group in the present study (**Figure 1**).

For the lack of sex hormone data of women in 1999–2012 NHANES, these laboratory data were from Sex Steroid Hormone—Serum data file in 2013–2016 NHANES. 1,754 women were included to explore the difference in the level of sex hormones between women with CKD and without CKD in the present study.

Definitions of Early Menopause

Natural menopause was defined as physiological amenorrhoea longer than 12 months and does not interfere with other causes, such as surgery or medical treatment (18). Surgical menopause was due to surgical removal of both ovaries before the natural age of menopause (19). Experiencing menopause before 45 was thought to be early menopause which is defined by European Menopause and Andropause Society (20, 21).

Assessment of CKD

Data related to eGFR calculation are accessed from NHANES. Serum Cr was calibrated for the 1999–2000 and 2005–2006 participants. The recalibration equations for 1999–2000 and 2005–2006 NHANES surveys were applied: standard serum Cr (mg/dl) $= 0.147 + 1.013 \times$ uncalibrated serum Cr (mg/dl) and standard serum Cr (mg/dl) $= -0.016 + 0.978 \times$ uncalibrated serum Cr (mg/dl). Estimating glomerular filtration rate (eGFR) was calculated by the CKD Epidemiology

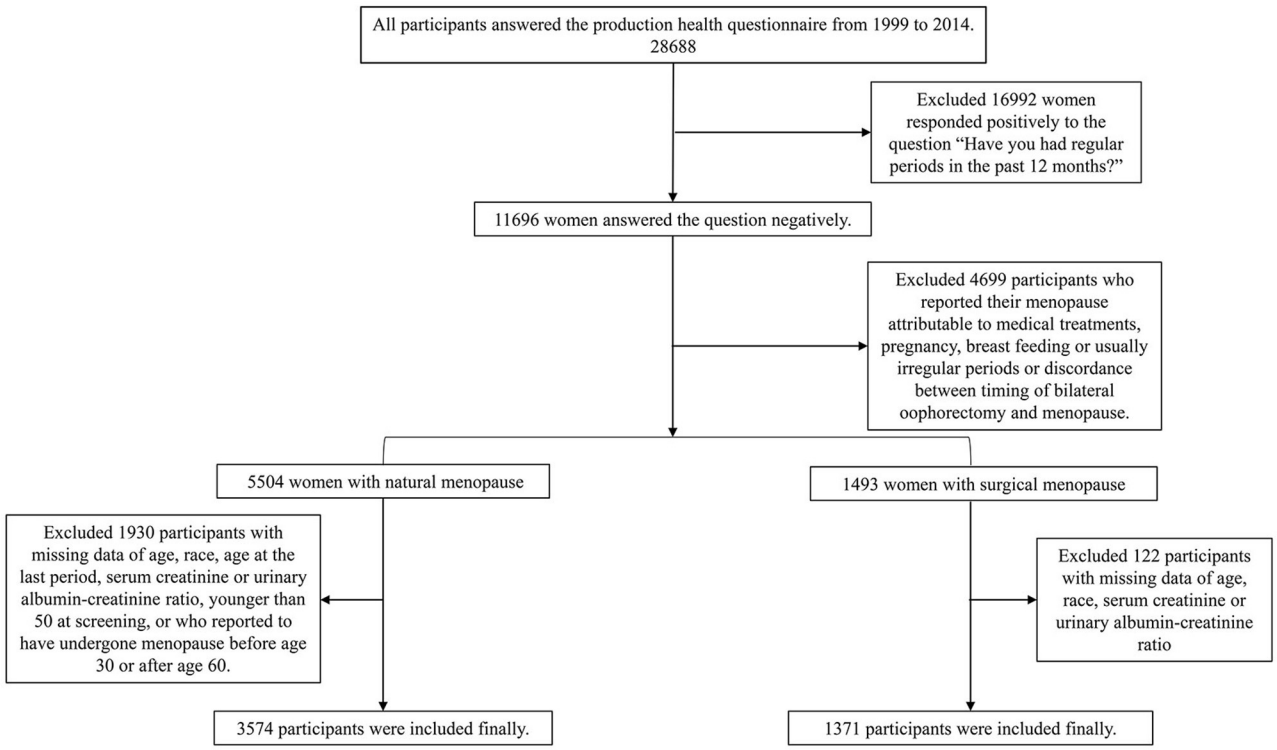


FIGURE 1 | The flowchart of participants' section progress.

Collaboration equation:

$$\text{eGFR} = 141 \times \min(\text{Scr}/\kappa, 1)\alpha \times \max(\text{Scr}/\kappa, 1) - 1.209 \times 0.993\text{Age} \times 1.018\ [\text{if female}]\ /1.159\ [\text{if black}]$$

(Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1) (22). Relevant data were obtained from laboratory data of NHANES. CKD was defined as participants with an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 or urinary albumin-to-creatinine ratio (UACR) ≥30 mg/g, and CKD patients were classified into stages according to GFR categories and albuminuria categories by the Kidney Disease: Improving Global Outcomes (KDIGO) organization (23).

Mortality Data

Public-use Linked Mortality Files are available for continuous NHANES 1999–2014. These files provide mortality data from the date of survey enrollment through December 31, 2015. The survival time data we use are the number of Person-Months of follow-up from NHANES Interview date, with a mean follow-up time of 89.5 months. Mortality source and cause of death were determined using death certificates similar to previous studies (24, 25).

Covariates

Clinical covariates were classified as demographics, medical conditions, and lifestyle factors. We used the information at screening from the survey in these analyses. Demographic factors included age, race/ethnicity, and marital status. Medical conditions included hypertension (defined as a history of physician-diagnosed hypertension, a measured average systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or current use of antihypertensive medication), diabetes (defined as a history of physician-diagnosed diabetes, hemoglobin A1c level ≥ 6.5% or current use of diabetes medication). Obesity (characterized by standard body index, under 25 kg/m^2, 25 to <30 kg/m^2, ≧30 kg/m^2), triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high-density lipoprotein cholesterol (HDL-c, mg/dl), coronary heart disease, congestive heart failure, stroke, cancer were also included in this analysis. Lifestyle factors included smoking status (never, ever, current) and alcohol consumption (never, ever, current).

Statistical Analysis

We reported the characteristics of post-menopause participants and compared those four groups, natural menopause before 45, natural menopause after 45, surgical menopause before 45, and surgical menopause after 45. For continuous variables, if data was of normality, we used ANOVA tests to compared different groups. Otherwise, non-parametric tests were used. And chi-square tests were used for classified variables. After that,

we performed logistic regression to evaluate the association of early menopause with CKD prevalence and CKD stages. Model I was a crude model, while model II had been adjusted for age and race/ethnicity, and model III were further adjusted for marital status, smoke, alcohol use, obesity, TG, TC, HDL-c, hypertension, and diabetes. To explore the association between early menopause and all-cause mortality, Cox proportional hazard regression was used in all participants. CKD stage was adjusted in model I,model II was further adjusted for age and race/ethnicity. The fully adjusted model further included smoke, alcohol use, obesity, TG, TC, HDL-c, hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, cancer. We also used Cox proportional hazard regression in CKD and non-CKD groups respectively. eGFR and UACR were adjusted in model I, and model II adjusted for age and race/ethnicity besides. The fully adjusted model included smoke, alcohol use, obesity, TG, TC, HDL-c, hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, cancer additionally. We also performed logistic regression and Cox regression using the same process with the age of menopause as a continuous variable. Finally, we compared the differences in the levels of sex steroid hormone between people with and without CKD, using an independent-samples t-test. A 2-sided $p < 0.05$ was considered statistically significant. All data management and analyses were performed in SPSS version 26.0.

RESULTS

Description of the Study Population

Table 1 described the characteristics of the women included in the study. Mean ± SD age was 64 ± 10.2 years of all participants, while the mean age at menopause was 47 ± 7.1 years. Women with early menopause, both natural and surgical, had a higher percentage of current smoking and having cardiovascular disease including stroke and congestive heart failure. In addition, the early menopause groups had a higher proportion of having CKD. Compared with people with natural menopause, women who had undergone surgical menopause tended to be obese, have cancer and hypertension, and have a higher level of TC. There were also significant differences in race, marital status, and alcohol use among the groups. The CKD stages of each group were also listed (**Supplementary Table 1**), few CKD women had UACR <3 mg/mmol, and most of them had a decrease in eGFR.

Early Menopause and CKD Prevalence

We used logistics regressions to compare the CKD risk between the early menopause group and the population experiencing menopause after 45 (**Table 2**). For the natural menopause group, in crude analysis, women with early natural menopause were more likely to have CKD [OR = 1.35 (1.11–1.64), $p = 0.003$], the model adjusted for demographics factors and the fully adjusted model both supported the result that women with early natural menopause tend to have CKD, and it was significant. For the surgical menopause group, although the unadjusted model showed there was no correlation between early surgical menopause and CKD, women with early surgical menopause had a significant risk of CKD after adjusted for age and race [OR = 1.41 (1.08–1.83), $p = 0.011$], the result remained the same as the further adjusted model. However, when we explored the relationship between early menopause and CKD-G1/2 and CKD-G3∼5 respectively (**Supplementary Tables 2**, **3**), except early natural menopause was associated with CKD-G3∼5 [OR = 1.37 (1.04–1.80), $p = 0.025$], there were no other significant findings. The regression which treated age at menopause as a continuous variable and included surgical vs. non-surgical as a covariate showed the same results (**Supplementary Table 4**). Older menopausal age was associated with a lower risk of CKD [OR = 0.98 (0.97–0.99), $p = 0.002$].

Early Menopause and All-Cause Mortality

When we treated the CKD stage as a covariate, both early natural menopause and surgical menopause were risk factors for survival (**Table 3**). Then we separated all participants into two groups according to conditions of CKD and non-CKD. For the CKD group, either early natural or surgical menopause had no significant correlation with all-cause mortality (**Table 4**). For the non-CKD group (**Table 5**), although the result of natural menopause is similar to the CKD group, surgical menopause differed from the CKD group. Higher mortality risk was observed in the non-CKD population with early surgical menopause [HR = 1.62 (1.06–2.49), $p = 0.027$]. However, when we treated age at menopause as a continuous variable (**Supplementary Table 5**), we found early natural menopause in the CKD group was associated with higher mortality risk [HR = 0.98 (0.96–1.00), $p = 0.024$]. The other results made no difference.

The Hormone Differences Between CKD and Non-CKD Group

To find out the connection between the CKD prevalence and early menopause in women, we compared the hormone level of CKD and the non-CKD group (**Table 6**). Several hormones showed a significant difference. CKD patients had a higher level of follicle-stimulating hormone (34.44 vs. 27.71, $p = 0.029$) and luteinizing hormone (22.66 vs. 19.63, $p = 0.029$), but a lower level of testosterone (153.37 vs. 176.29, $p = 0.033$) and estradiol (29.50 vs. 40.11, $p = 0.001$). For sex hormone-binding globulin (SHBG), CKD and non-CKD groups made no difference.

DISCUSSION

In the present study, both early natural menopause and early surgical menopause were associated with statistically significant increased risk of CKD, with persistent relation observed after adjusted for conventional factors. In our study, when we treated the CKD stage as a covariate, both early natural menopause and surgical menopause were risk factors for survival, but the results had some differences when we divided the participants into CKD and non-CKD. Early natural menopause was independent of longevity in the non-CKD group. However, in the CKD group, the regression results of the age of menopause as a continuous variable and early menopause as a categorical variable are different. As for surgical menopause, bilateral oophorectomy

TABLE 1 | Characteristics of the participants.

Characteristics	Natural menopause at age ≧45 y (*n* = 3,038)	Natural menopause at age <45 y (*n* = 536)	Surgical menopause at age ≧45 y (*n* = 516)	Surgical menopause at age <45 y (*n* = 815)	*P* value
Age, y	64.0 (58.0–72.0)	65.0 (58.0–74.0)	65.0 (59.0–73.0)	62.0 (52.0–71.0)	<0.001
Race/ethnicity, No. (%)					<0.001
Non-hispanic white	1,603 (52.8)	258 (48.1)	307 (59.5)	488 (59.9)	
Non-hispanic black	492 (16.2)	90 (16.8)	98 (19.0)	169 (20.7)	
Other	943 (31.0)	188 (35.1)	111 (21.5)	158 (19.4)	
Marital status, No. (%)					<0.001
Married/ cohabitating	1,544 (50.8)	226 (42.2)	296 (57.4)	434 (53.2)	
Widowed/seperated/divorced	1,311 (43.2)	269 (50.2)	199 (38.6)	339 (41.6)	
Never married	183 (6.0)	41 (7.6)	21 (4.1)	42 (5.2)	
Alcohol use, No. (%)					0.001
Never	751 (24.7)	158 (29.5)	98 (19.0)	182 (22.3)	
Ever	642 (21.1)	115 (21.5)	134 (26.0)	198 (24.3)	
Current	1,645 (54.2)	263 (49.1)	284 (55.0)	435 (53.4)	
Smoke, No. (%)					<0.001
Never	1,879 (61.9)	307 (57.3)	320 (62.0)	431 (52.8)	
Ever	820 (27.0)	135 (25.2)	151 (29.3)	223 (27.4)	
Current	339 (11.2)	94 (17.5)	45 (8.7)	161 (19.8)	
Hypertension, No. (%)	1,836 (60.4)	341 (63.6)	356 (69.0)	520 (63.8)	0.001
Diabetes, No. (%)	621 (20.4)	123 (23.0)	106 (20.5)	175 (21.5)	0.583
Body mass index (kg/m^2), No. (%)					0.023
<25	885 (29.1)	140 (26.1)	130 (25.2)	191 (23.4)	
25–<30	946 (31.1)	177 (33.0)	164 (31.8)	257 (31.5)	
≧30	1,207 (39.7)	219 (40.9)	222 (43.0)	367 (45.0)	
Triglycerides (mg/dl)	125.5 (88.0–184.0)	126.0 (86.3–179.3)	136.0 (93.0–194.0)	132.0 (92.0–198.0)	0.008
Total cholesterol (mg/dl)	209.0 (183.0–237.0)	207.0 (177.3–233.0)	210.5 (181.0–236.0)	207.0 (181.0–237.0)	0.215
HDL-cholesterol (mg/dl)	57.0 (47.0–68.0)	56.0 (46.0–68.0)	57.0 (48.0–69.8)	56.0 (46.0–68.0)	0.360
Congestive heart failure, No. (%)	109 (3.6)	36 (6.7)	23 (4.5)	45 (5.5)	0.003
Coronary heart disease, No. (%)	127 (4.2)	31 (5.8)	25 (4.8)	41 (5.0)	0.340
Stroke, No. (%)	133 (4.4)	32 (6.0)	24 (4.7)	68 (8.3)	<0.001
Cancer, No. (%)	368 (12.1)	65 (12.1)	104 (20.2)	160 (19.6)	<0.001
Ovary cancer, No. (%)	1 (0.0)	0 (0.0)	3 (0.0)	15 (0.0)	/
eGFR, ml/min/1.73 m^2	81.7 (66.8–94.7)	78.8 (62.4–93.2)	80.9 (66.4–95.9)	82.2 (66.4–100.5)	<0.001
UACR, mg/g	9.1 (5.8–18.4)	9.8 (6.3–23.5)	8.1 (5.4–15.8)	8.4 (5.4–18.3)	<0.001
CKD, No. (%)	829 (27.3)	180 (33.3)	140 (27.1)	237 (29.1)	0.023
Death, No. (%)	485 (16.0)	116 (21.6)	73 (14.1)	129 (15.8)	0.004

TABLE 2 | The association of early menopause and CKD prevalence by logistic regression.

Groups	Model I[a] OR	*P* value	Model II[b] OR	*P* value	Model III[c] OR	*P* value	*P* value of Hosmer/Lemeshow
Women with natural menopause							
Natural menopause at age ≧45	Ref (1.00)		Ref (1.00)		Ref (1.00)		
Early natural menopause	1.35 (1.11–1.64)	0.003	1.26 (1.02–1.55)	0.034	1.25 (1.00–1.55)	0.046	0.209
Women with surgical menopause							
Surgical menopause at age ≧45	Ref (1.00)		Ref (1.00)		Ref (1.00)		
Early surgical menopause	1.10 (0.86–1.41)	0.442	1.41 (1.08–1.83)	0.011	1.33 (1.01–1.76)	0.041	0.554

[a] *Unadjusted model.*

[b] *Adjusted for age and race/ethnicity.*

[c] *Adjusted for age, race/ethnicity, marital status, smoke, alcohol use, obesity, triglycerides (mg/dl), total cholesterol (mg/dl), HDL-cholesterol (mg/dl), hypertension, and diabetes.*

TABLE 3 | Association of early menopause and all-cause mortality by Cox proportional hazard regression models.

Groups	Model I[a] HR	*P* value	C-index	Model II[b] HR	*P* value	C-index	Model III[c] HR	*P* value	C-index
Women with natural menopause									
Natural menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early natural menopause	1.26 (1.03–1.55)	0.025	0.75 (0.72–0.77)	1.19 (0.97–1.45)	0.101	0.76 (0.74–0.78)	1.23 (1.00–1.51)	0.048	0.79 (0.78–0.80)
Women with surgical menopause									
Surgical menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early surgical menopause	1.14 (0.85–1.52)	0.379	0.73 (0.69–0.78)	1.51 (1.13–2.01)	0.005	0.78 (0.75–0.82)	1.46 (1.09–1.97)	0.012	0.81 (0.78–0.84)

[a]*Adjusted for CKD stage.*
[b]*Adjusted for age, race/ethnicity, and CKD stage.*
[c]*Adjusted for age, race/ethnicity, CKD stage, marital status, smoke, alcohol use, obesity, triglycerides (mg/dl), total cholesterol (mg/dl), HDL-cholesterol (mg/dl), hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, cancer.*

TABLE 4 | Association of early menopause and all-cause mortality by Cox proportional hazard regression models among CKD group.

Groups	Model I[a] HR	*P* value	C-index	Model II[b] HR	*P* value	C-index	Model III[c] HR	*P* value	C-index
Women with natural menopause									
Natural menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early natural menopause	1.25 (0.96–1.63)	0.095	0.64 (0.58–0.65)	1.20 (0.92–1.57)	0.177	0.69 (0.66–0.72)	1.22 (0.93–1.60)	0.158	0.73 (0.70–0.76)
Women with surgical menopause									
Surgical menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early surgical menopause	1.10 (0.74–1.63)	0.640	0.62 (0.56–0.68)	1.40 (0.94–2.08)	0.096	0.73 (0.68–0.78)	1.25 (0.81–1.92)	0.317	0.76 (0.71–0.81)

[a]*Adjusted for eGFR and UACR.*
[b]*Adjusted for age, race/ethnicity, eGFR, and UACR.*
[c]*Adjusted for age, race/ethnicity, eGFR, UACR, marital status, smoke, alcohol use, obesity, triglycerides (mg/dl), total cholesterol (mg/dl), HDL-cholesterol (mg/dl), hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, cancer.*

TABLE 5 | Association of early menopause and all-cause mortality by Cox proportional hazard regression models among the non-CKD group.

Groups	Model I[a] HR	*P* value	C-index	Model II[b] HR	*P* value	C-index	Model III[c] HR	*P* value	C-index
Women with natural menopause									
Natural menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early natural menopause	1.28 (0.93–1.76)	0.123	0.65 (0.61–0.69)	1.22 (0.89–1.68)	0.226	0.72 (0.69–0.76)	1.12 (0.801–1.55)	0.495	0.74 (0.71–0.78)
Women with surgical menopause									
Surgical menopause at age ≧45	Ref (1.00)			Ref (1.00)			Ref (1.00)		
Early surgical menopause	1.44 (0.95–2.17)	0.087	0.61 (0.54–0.69)	1.67 (1.10–2.53)	0.015	0.73 (0.67–0.79)	1.62 (1.06–2.49)	0.027	0.78 (0.73–0.83)

[a]*Adjusted for eGFR and UACR.*
[b]*Adjusted for age, race/ethnicity, eGFR, and UACR.*
[c]*Adjusted for age, race/ethnicity, eGFR, UACR, marital status, smoke, alcohol use, obesity, triglycerides (mg/dl), total cholesterol (mg/dl), HDL-cholesterol (mg/dl), hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, cancer.*

TABLE 6 | The difference in hormones between the population with and without CKD.

Variable	CKD group (*n* = 201)	Non-CKD group (*n* = 1,553)	*P* value
Follicle stimulating hormone (mIU/ml)	34.44	27.71	0.029
luteinizing hormone (mIU/ml)	22.66	19.63	0.029
Testosterone, total (ng/dl)	153.37	176.29	0.033
Estradiol (pg/ml)	29.50	40.11	0.001
SHBG (nmol/l)	61.43	57.48	0.782

before age 45 years increased the all-cause mortality in the non-CKD group, but such finding was not observed in the CKD group. Our study also revealed the apparent differences in sex hormone levels between CKD and non-CKD participants.

Our study revealed women with early menopause, both natural and surgical, had a higher percentage of current smoking and having cardiovascular disease including stroke and congestive heart failure. Studies have reported that smoking is associated with an earlier age of menopause, and a later age of menopause could reduce the risk of cardiovascular diseases (26). Our study confirmed this. In our study, compared with people with natural menopause, women who had undergone surgical menopause tended to be obese, have cancer and hypertension, and have a higher level of TC. This indicates that cancer may be an important cause of oophorectomy. Obesity, hyperlipidemia, and hypertension are associated with a higher risk of ovarian diseases (27), which make women more likely to undergo oophorectomy. There were also significant differences in race, marital status, and alcohol use among the groups. A meta-analysis has shown low and moderate alcohol consumption was associated with later menopause onset, compared to non-drinkers (28). In addition, alcohol may also cause lesions in the female reproductive organs leading to oophorectomy (29).

Our study suggested that early menopause, both natural and surgical, was associated with a higher risk of CKD and early natural menopause is mainly associated with CKD-G3∼5. A study based on the multiethnic Women's Health Initiative cohort had mentioned early natural menopause occurred more among women with CKD (3), which indicated a potential association between early natural menopause and CKD. Another cohort study found women who had undergone bilateral oophorectomy were more likely to develop CKD compared to age-matched referent women without surgery (30), but the relationship between the age at surgery and CKD prevalence is unclear. In this study, women who had bilateral oophorectomy before age 45 were at a higher risk of CKD than those who had surgery after age 45, suggesting the association between early surgical menopause and CKD prevalence.

The difference in sex hormone levels between the CKD group and the non-CKD group in this study proved that sex hormones may play crucial roles in the association between early menopause and CKD. As CKD develops, an inadequate cyclic release of gonadotropin-releasing hormone (GnRH) by the hypothalamus leads to loss of normal pulsatile gonadotropin secretion by the pituitary including FSH and LH (31–34). In this case, the pre-ovulatory rise of estrogen and progesterone is absent, causing ovulatory obstacles, then amenorrhea occurs, finally resulting in early menopause (35). On the other hand, after menopause, the level of estrogen declines, which may accelerate the progression of glomerulosclerosis (36). A recent study had mentioned that the risk for CKD was higher in women with shorter reproductive life span duration, indicating less endogenous estrogen exposure was closely associated with higher CKD risk in postmenopausal women (37). In addition, in patients with surgical menopause, surgery and subsequent drug use might increase the renal load and lead to injury of renal. Therefore, the relationship between early menopause and CKD is bi-directional. Nevertheless, the mechanism that confers the association of early menopause and CKD is uncertain.

In our study, early natural menopause did no effect on mortality in the non-CKD group. In the CKD group, there was a negative result when we take early menopause as a categories variable, however, when we treated age at menopause as a continuous variable, we found early natural menopause was associated with higher mortality. The relation between age at natural menopause and mortality is controversial. A study based on the Health, Well-Being and Aging study found women with early menopause had a 48% higher risk of all-cause mortality compared to the women with menopause at normal age (38), but a Taiwan study showed there was no significant difference in all-cause mortality between women with early menopause and the normal reference (39). Early menopause is associated with many other diseases and might result in an uncertain relationship between early menopause and mortality. Previous research has shown that early onset of menopause was connected with a higher risk of cardiovascular disease mortality (12, 40), whereas early menopause was known to decrease the risk of developing breast cancer, ovarian and endometrial cancer (41–43). These factors may also affect the longevity of postmenopausal women. More studies are needed to confirm the association of early natural menopause with mortality.

Bilateral oophorectomy undertaken before age 45 years is thought to increase mortality in the general population (44, 45), the similar conclusion was observed in the non-CKD group in our study. Oophorectomy-induced estrogen deficiency may partly explain the surgery's damage to survival. A low level of estrogen increases the risk of cardiovascular disease, osteoporosis, or neurological disease. Consequently, it influences the longevity of these patients (11). There was no data in the NHANES on why women had bilateral oophorectomy, but other studies have reported although some oophorectomies are performed to treat ovary-related pathology or to prevent ovarian cancer in women at increased risk of ovarian cancer, most oophorectomies are done in cases of grossly normal ovaries in women at average risk for ovarian cancer (46). Women who underwent hysterectomy and had mutations in the BCRA2 and BRCA1 genes were most likely to have prophylactic bilateral oophorectomy (47). Hysterectomy and gene mutations may also be the risk factors of mortality. Moreover, early menopause has been linked to psychological disorders such as depression and anxiety, which can accelerate the progression of diseases and increase mortality (48). However, in the CKD population we studied, early bilateral oophorectomy was irrelevant with survival. There were several possible explanations for this result. CKD may interfere with the pathogenesis of menopause. For example, Vasomotor symptoms occur in 30–80% of post-menopausal women in the form of peripheral vasodilation and sweating, while women with CKD have a lower risk to experience it (3). In addition, decreased estradiol catabolism in CKD patients might lead to a higher free estradiol level (49). Moreover, taking the same dose of estradiol, serum concentrations of estradiol and estrone in patients with CKD are 2–3 times higher than in healthy postmenopausal women (50) and improved the treatment efficacy of estradiol supplement

after the bilateral oophorectomy. Besides, for the regular medical examinations during the perioperative period of oophorectomy, CKD patients could be early diagnosed, which is also conducive to survival. The above factors may counteract the adverse effects of surgical menopause. However, the exact mechanisms remain inconclusive.

There are still many limitations in our study. First, the criterion of menopause was only 1 year without menstruation after excluding some special conditions, without considering the issue of hormone levels. Second, most of the data were from the questionnaire, including the time of menopause. Although some abnormal data had been screened out, the accuracy of the data was still lacking. As for women with surgical menopause, there are no specific reasons for women to undergo bilateral oophorectomy in the NHANES, and we can only speculate it according to other studies, that might interfere with the outcome. Additionally, there were inevitable censorings in the follow-up, which may have an influence on the results. Finally, because we can't get information about when the participants developed CKD, the sequence of CKD and menopause is unknown and the causal relationship between early menopause and CKD is not clear and requires further cohort studies. Despite these limitations, our study has the merit of using a representative national sample, and we have found evidence in support of the association between early menopause and CKD prevalence and all-cause mortality, and their difference between CKD and non-CKD patients.

CONCLUSION

Both early natural and surgical menopause were associated with a higher risk of CKD. In our study, early surgical menopause was a hazard factor for survival in the non-CKD group, but not in the CKD group. The mechanisms behind these associations need further research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The NHANES is a periodic survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The NCHS Research Ethics Review Board reviewed and approved the NHANES and all participants provided written informed consent. NCHS Ethics Review Board Protocol Number: Protocol #98-12, Protocol #2005-06 and Protocol #2011-17.

AUTHOR CONTRIBUTIONS

GX: conceptualization. DQ and Z-fW: data curation and writing—original draft. Z-fW: formal analysis. S-WG: methodology and writing—review and editing. DQ: resources and software. Y-cC and RL: validation. All authors contributed to the article and approved the submitted version.

FUNDING

This work was financially supported by the International (Regional) Cooperation and Exchange Projects (NSFC-DFG Grant No. 81761138041), National Natural Science Foundation of China (Grant No. 81570667), Major Research Plan of the National Natural Science Foundation of China (Grant No. 91742204), and the National Key R&D Program of China (Grant No. 2018YFC1314003-1).

ACKNOWLEDGMENTS

We acknowledge and thank members of the National Center for Health Statistics (NCHS) of the Centers for Disease Control (CDC) and Prevention and the participants who contributed time and data to the National Health and Nutrition Examination Survey.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.823835/full#supplementary-material

REFERENCES

1. Chen TK, Knicely DH, Grams ME. Chronic kidney disease diagnosis and management: a review. *JAMA*. (2019) 322:1294–304. doi: 10.1001/jama.2019.14745
2. Saran R, Robinson B, Abbott KC, Bragg-Gresham J, Chen X, Gipson D. US renal data system 2019 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* (2020) 75(1 Suppl 1): A6–7. doi: 10.1053/j.ajkd.2019.09.003
3. Cheung KL, Stefanick ML, Allison MA, LeBlanc ES, Vitolins MZ, Shara N, et al. (2015). Menopausal symptoms in women with chronic kidney disease. *Menopause.* 22:1006–1011. doi: 10.1097/GME.0000000000000416
4. Cochrane R, Regan L. Undetected gynaecological disorders in women with renal disease. *Hum Reprod.* (1997) 12:667–70. doi: 10.1093/humrep/12.4.667
5. Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M. Menopause. *Nat Rev Dis Primers.* (2015) 1:15004. doi: 10.1038/nrdp.2015.4
6. Kramer HM, Curhan GC, Singh A. Hemodialysis, Estrogen Levels in Postmenopausal Patients Study G. Permanent cessation of menses and postmenopausal hormone use in dialysis-dependent women: the HELP study. *Am J Kidney Dis*. (2003) 41:643–50. doi: 10.1053/ajkd.2003.50126
7. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL. Association of premature natural and surgical menopause with incident cardiovascular disease. *JAMA*. (2019) 322:2411–21. doi: 10.1001/jama.2019.19191
8. Gallagher JC. Effect of early menopause on bone mineral density and fractures. *Menopause.* (2007) 14:567–71. doi: 10.1097/gme.0b013e31804c793d
9. Anagnostis P, Christou K, Artzouchaltzi AM, Gkekas NK, Kosmidou N, Siolos P. Early menopause and premature ovarian insufficiency are associated with

increased risk of type 2 diabetes: a systematic review and meta-analysis. *Eur J Endocrinol.* (2019) 180:41–50. doi: 10.1530/EJE-18-0602
10. Zhu DS, Chung HF, Dobson A, Pandeya N, Giles GG, Bruinsma F. Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data. *Lancet Public Health.* (2019) 4:E553–64. doi: 10.1016/S2468-2667(19)30155-0
11. Lobo RA. Surgical menopause and cardiovascular risks. *Menopause.* (2007) 14:562–6. doi: 10.1097/gme.0b013e318038d333
12. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: a systematic review and meta-analysis. *JAMA Cardiol.* (2016) 1:767–76. doi: 10.1001/jamacardio.2016.2415
13. Ossewaarde ME, Bots ML, Verbeek AL, Peeters PH, van der Graaf Y, Grobbee DE. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology.* (2005) 16:556–62. doi: 10.1097/01.ede.0000165392.35273.d4
14. Ahmed SB, Ramesh S. Sex hormones in women with kidney disease. *Nephrol Dial Transplant.* (2016) 31:1787–95. doi: 10.1093/ndt/gfw084
15. Shenassa ED, Rossen LM. Telomere length and age-at-menopause in the US. *Maturitas.* (2015) 82:215–21. doi: 10.1016/j.maturitas.2015.07.009
16. van der Plaat D, Pereira M, Pesce G, Potts JF, Amaral AFS, Dharmage SC. Age at menopause and lung function: a Mendelian randomisation study. *Eur Respir J.* (2019) 54:1802421. doi: 10.1183/13993003.02421-2018
17. Fugiel J, Ignasiak Z, Skrzek A, Slawinska T. Evaluation of relationships between menopause onset age and bone mineral density and muscle strength in women from South-Western Poland. *Biomed Res Int.* (2020) 2020:5410253. doi: 10.1155/2020/5410253
18. Nelson HD. Menopause. *Lancet.* (2008) 371:760–70. doi: 10.1016/S0140-6736(08)60346-3
19. Rodriguez M, Shoupe D. Surgical menopause. *Endocrinol Metab Clin North Am.* (2015) 44:531–42. doi: 10.1016/j.ecl.2015.05.003
20. Kingsberg SA, Larkin LC, Liu JH. Clinical effects of early or surgical menopause. *Obstet Gynecol.* (2020) 135:853–68. doi: 10.1097/AOG.0000000000003729
21. Mishra GD, Chung HF, Cano A, Chedraui P, Goulis DG, Lopes P. EMAS position statement: predictors of premature and early natural menopause. *Maturitas.* (2019) 123:82–8. doi: 10.1016/j.maturitas.2019.03.008
22. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
23. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Internal Med.* (2013) 825–30. doi: 10.7326/0003-4819-158-11-201306040-00007
24. Chen, C., Ye, Y., Zhang, Y., Pan, X. F., and Pan, A. (2019). Weight change across adulthood in relation to all cause and cause specific mortality: prospective cohort study. *BMJ.* 367:l5584. doi: 10.1136/bmj.l5584
25. Ricci C, Schutte AE, Schutte R, Smuts CM, Pieters M. Trends in alcohol consumption in relation to cause-specific and all-cause mortality in the United States: a report from the NHANES linked to the US mortality registry. *Am J Clin Nutr.* (2020) 111:580–9. doi: 10.1093/ajcn/nqaa008
26. Gold EB. The timing of the age at which natural menopause occurs. *Obstet Gynecol Clin North Am.* (2011) 38:425–40. doi: 10.1016/j.ogc.2011.05.002
27. Azziz R. Polycystic ovary syndrome. *Obstetr Gynecol.* (2018) 132:321–36. doi: 10.1097/AOG.0000000000002698
28. Taneri PE, Kiefte-de Jong JC, Bramer WM, Daan NM, Franco OH, Muka T. Association of alcohol consumption with the onset of natural menopause: a systematic review and meta-analysis. *Hum Reprod Update.* (2013) 22:516–28. doi: 10.1093/humupd/dmw013
29. de Angelis C, Nardone A, Garifalos F, Pivonello C, Sansone A, Conforti A, Di Dato C, Sirico F, Alviggi C, Isidori A, Colao A. Smoke, alcohol and drug addiction and female fertility. *Reprod Biol Endocrinol.* 18:21. doi: 10.1186/s12958-020-0567-7
30. Kattah AG, Smith CY, Gazzuola Rocca L, Grossardt BR, Garovic VD, Rocca WA. CKD in patients with bilateral oophorectomy. *Clin J Am Soc Nephrol.* (2018) 13:1649–58. doi: 10.2215/CJN.03990318
31. Holley JL. The hypothalamic-pituitary axis in men and women with chronic kidney disease. *Adv Chronic Kidney Dis.* (2004) 11:337–41. doi: 10.1053/j.ackd.2004.07.004
32. Serret-Montaya J, Zurita-Cruz JN, Villasís-Keever MA, Aguilar-Kitsu A, del Carmen Zepeda-Martinez C, Cruz-Anleu I, et al. Hyperprolactinemia as a prognostic factor for menstrual disorders in female adolescents with advanced chronic kidney disease. *Pediatr Nephrol.* (2020) 35:1041. doi: 10.1007/s00467-020-04494-7
33. Huang W, Molitch ME. Prolactin and other pituitary disorders in kidney disease. *Semin Nephrol.* (2021) 41:156–67. doi: 10.1016/j.semnephrol.2021.03.010
34. Vellanki K, Hou S. Menopause in CKD. *Am J Kidney Dis.* (2018) 71:710–9. doi: 10.1053/j.ajkd.2017.12.019
35. Palmer BF, Clegg DJ. Gonadal dysfunction in chronic kidney disease. *Rev Endocr Metab Disord.* (2017) 18:117–30. doi: 10.1007/s11154-016-9385-9
36. Elliot SJ, Karl M, Berho M, Potier M, Zheng F, Leclercq B. Estrogen deficiency accelerates progression of glomerulosclerosis in susceptible mice. *Am J Pathol.* (2003) 162:1441–8. doi: 10.1016/S0002-9440(10)64277-0
37. Kang SC, Jhee JH, Joo YS, Lee SM, Nam KH, Yun HR. Association of reproductive lifespan duration and chronic kidney disease in postmenopausal women. *Mayo Clin Proc.* (2020) 95:2621–32. doi: 10.1016/j.mayocp.2020.02.034
38. Lay AA, do Nascimento CF, de Oliveira Duarte YA, Chiavegatto Filho AD. Age at natural menopause and mortality: a survival analysis of elderly residents of São Paulo. *Maturitas.* (2018) 117:29–33. doi: 10.1016/j.maturitas.2018.08.012
39. Shen TY, Strong C, Yu T. Age at menopause and mortality in Taiwan: a cohort analysis. *Maturitas.* (2020) 136:42–8. doi: 10.1016/j.maturitas.2020.04.008
40. van der Schouw YT, van der Graaf Y, Steyerberg EW, Eijkemans MJC, Banga JD. Age at menopause as a risk factor for cardiovascular mortality. *Lancet.* (1996) 347:714–8. doi: 10.1016/S0140-6736(96)90075-6
41. La Vecchia C. Ovarian cancer: epidemiology and risk factors. *Eur J Cancer Prev.* (2017) 26:55–62. doi: 10.1097/CEJ.0000000000000217
42. Collaborative Group on Hormonal Factors in Breast C. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol.* (2012) 13:1141–51. doi: 10.1016/S1470-2045(12)70425-4
43. Wu Y, Sun W, Liu H, Zhang D. Age at menopause and risk of developing endometrial cancer: a meta-analysis. *Biomed Res Int.* (2019) 2019:8584130. doi: 10.1155/2019/8584130
44. Tuesley KM, Protani MM, Webb PM, Dixon-Suen SC, Wilson LF, Stewart LM, Jordan SJ. Hysterectomy with and without oophorectomy and all-cause and cause-specific mortality. *Am J Obstetr Gynecol.* (2020) 223:723-e1. doi: 10.1016/j.ajog.2020.04.037
45. Rocca WA, Grossardt BR, de Andrade M, Malkasian GD, Melton III LJ. Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. *Lancet Oncology.* (2006) 7:821–8. doi: 10.1016/S1470-2045(06)70869-5
46. Evans EC, Matteson KA, Orejuela FJ, Alperin M, Balk EM, El-Nashar S. Salpingo-oophorectomy at the time of benign hysterectomy: a systematic review. *Obstet Gynecol.* (2016) 128:476–85. doi: 10.1097/AOG.0000000000001592
47. Eleje GU, Eke AC, Ezebialu IU, Ikechebelu JI, Ugwu EO, Okonkwo OO. Risk-reducing bilateral salpingo-oophorectomy in women with BRCA1 or BRCA2 mutations. *Cochrane Database Syst Rev.* (2018) 8:CD012464. doi: 10.1002/14651858.CD012464.pub2
48. Faubion SS, Kuhle CL, Shuster LT, Rocca WA. Long-term health consequences of premature or early menopause and considerations for management. *Climacteric.* (2015) 18:483–91. doi: 10.3109/13697137.2015.1020484
49. Ginsburg ES, Owen WF Jr, Greenberg LM, Shea BF, Lazarus JM, Walsh BW. Estrogen absorption and metabolism in postmenopausal women with end-stage renal disease. *J Clin Endocrinol Metab.* (1996) 81:4414–7. doi: 10.1210/jcem.81.12.8954051
50. Anderson GD, Odegard PS. Pharmacokinetics of estrogen and progesterone in chronic kidney disease. *Adv Chronic*

Kidney Dis. (2004) 11:357–60. doi: 10.1053/j.ackd.2004.07.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 18 March 2022
doi: 10.3389/fmed.2022.813329

Machine Learning Reveals Ets2 as a Novel Target for Membranous Nephropathy Treatment and Its Role in Immune Infiltration

Peng-Zhi Wan, Tian-Hua Xu, Bin-Yao Tian, Guang-Ying Guo, Xiao-Li Li and Li Yao**

Department of Nephrology, The First Affiliated Hospital of China Medical University, Shenyang, China

Background: Membranous nephropathy (MN) is a common pathological phenotype for adult nephrotic syndrome (NS). The occurrence of MN is increasing across China, but diagnostic methods for MN still rely on kidney biopsy and PLA2R and THSD7A detection in plasma and kidney tissue, and there has been no new biomarker for MN discovered since 2014. Immune infiltration status in MN patients suffers from the dearth of associated studies. In the present study, we aimed to find new bio-markers for MN and evaluate the role of immune cells infiltration in MN pathology.

Methods: We downloaded MN expression profile from the Gene Expression Omnibus database and used R-project to screen differentially expressed genes (DEGs) and performed functional correlation analysis. Least absolute shrinkage and selection operator (LASSO) logistic regression and Radom Forest algorithms were used to screen and verify the bio-markers of MN. Finally, CIBERSORT was used to evaluate the infiltration of immune cells in MN tissues.

Results: A total of 463 DEGs were screened from the MN tissue in this study. ETS2 was identified as bio-marker for MN. The CIBERSORT results showed that there were statistical differences in monocytes, plasma cells, regulatory T cells, and memory B cells. In addition, ETS2 was positively related to monocytes, M1 phase macrophages, and neutrophils and negatively correlated to plasma cells, CD4+ T memory cells, M2 macrophages, CD8+ T cells, memory B cells, and resting mast cells.

Conclusion: (1) Machine learning algorithms reveals Ets2 as a novel target for membranous nephropathy patients. (2) Immune infiltration plays an important part in membranous nephropathy. (3) Ets2 expression is related to immune cells infiltration.

Keywords: membranous nephropathy, machine learning, immune infiltration, Ets2, chronic kidney disease

OPEN ACCESS

Edited by:
Jingyuan Xie,
Shanghai Jiao Tong University, China

Reviewed by:
Huang Biao,
Zhejiang Sci-Tech University, China
Marc Hilhorst,
Academic Medical Center, Netherlands

****Correspondence:***
Xiao-Li Li
foxfinder99@sohu.com
Li Yao
liyao_cmu@163.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *11 November 2021*
Accepted: *08 February 2022*
Published: *18 March 2022*

Citation:
Wan P-Z, Xu T-H, Tian B-Y, Guo G-Y, Li X-L and Yao L (2022) Machine Learning Reveals Ets2 as a Novel Target for Membranous Nephropathy Treatment and Its Role in Immune Infiltration. Front. Med. 9:813329. doi: 10.3389/fmed.2022.813329

INTRODUCTION

Membranous nephropathy (MN) is one of the common causes of nephrotic syndrome (NS) in the adult population (1). Recent studies confirmed the occurrence of MN in primary glomerulopathy (2). The typical pathological changes for MN include diffuse thickening of the glomerular capillary basement membrane and diffuse granular deposits of IgG with or without C3 (3). Currently, the diagnosis of MN relies on kidney biopsy and detection of PLA2R (4) and THSD7A (5) in plasma and kidney biopsy tissue: there has been no new biomarker for MN discovered since 2014 (6). Finding new biomarkers for MN diagnosis and treatment target is important.

Based on previous studies on human MN patient kidney biopsy and animal models, MN pathophysiological changes involved B cell activation (7) and immune infiltration caused by B cell activation including macrophages (8) and T cells (9). Recently, Rituximab, a monoclonal antibody against CD20, which can cause B cell degeneration, had been approved as a first-line treatment for MN patients (10), indicating that immune infiltration, especially B cell action, plays an important role in MN activation. However, there has been no large-scale immune infiltration study on human MN patients based on RNA sequencing and the CIBERSORT algorithm.

Machine learning algorithms such as least absolute shrinkage and selection operator logistic regression (LASSO) and random forest algorithms were widely used in clinical practice. These machine learning algorithms provided new insight in COVID-19 mortality (11), progressive of idiopathic pulmonary fibrosis (12) and cancer outcomes (13). Based on these studies, we could use machine learning algorithms to find novel targets for MN patients.

In the present study, a microarray dataset of MN downloaded from the Gene Expression Omnibus (GEO) database was used. We performed differential expression gene analysis and used machine learning algorithms to screen and identify suitable targets for MN treatment and diagnosis. CIBERSORT (14) was then used to ascertain the difference in immune infiltration between normal tissue and MN tissue. In addition, MN treatment target and its relationship with immune infiltration in 22 immune cells were determined.

MATERIALS AND METHODS

Data Download

The "GEOquery" package in R (Version 4.10) was used to download the MN expression profile datasets GSE99340 and GSE108113 from the GEO database.

Data Processing and Deg Screening

The expression matrices of GSE99340 and GSE108113 were downloaded and combined. The control group and MN group samples were selected according to patient data (**Table 1**). Inter-batch differences were removed using the "sva" package. Two PCA cluster plots were used to demonstrate the effect of inter-sample correction. DEGs were scanned using the "limma" package and "ggplot2" software was used to show differential expression of DEGs.

Functional Analysis

The "clusterProfiler" package was used to perform Gene Ontology (GO) and Disease Ontology (DO) enrichment analyses on DEGs, respectively. KEGG pathway enrichment analyses were also conducted on the gene expression matrix through the "clusterProfiler" package. A false discovery rate (FDR) < 0.25 and $p < 0.05$ were considered to represent significant enrichment.

TABLE 1 | Sample number and group information.

Group	GEO accession	Sample
Control group	GSE99340	GSM2641153 GSM2641154 GSM2641155 GSM2641156
Control group	GSE108113	GSM2889865 GSM2889866 GSM2889867 GSM2889868 GSM2889869 GSM2889870 GSM2890047 GSM2890048 GSM2890049 GSM2890050 GSM2890051
MN group	GSE99340	GSM2641237 GSM2641238 GSM2641239 GSM2641240 GSM2641241 GSM2641242 GSM2641243 GSM2641244 GSM2641245 GSM2641246 GSM2641247 GSM2641248 GSM2641249 GSM2641250 GSM2641251 GSM2641252 GSM2641253 GSM2641254 GSM2642375 GSM2642376 GSM2642377 GSM2642378 GSM2642379 GSM2642380 GSM2642381 GSM2642382 GSM2642383 GSM2642384 GSM2642385 GSM2642386 GSM2642387 GSM2642388 GSM2642389 GSM2642390 GSM2642391 GSM2642392 GSM2642393
MN group	GSE108113	GSM2889876 GSM2889877 GSM2889878 GSM2889879 GSM2889880 GSM2889881 GSM2889882 GSM2889883 GSM2889884 GSM2889885 GSM2889886 GSM2889887 GSM2889888 GSM2889889 GSM2889890 GSM2889891 GSM2889892 GSM2889893 GSM2889894 GSM2889895 GSM2889896 GSM2889897 GSM2889923 GSM2889924 GSM2889925 GSM2889926 GSM2889927 GSM2889928 GSM2889929 GSM2889930 GSM2889931 GSM2889932 GSM2889933 GSM2889934 GSM2889935 GSM2889936 GSM2889937 GSM2889938 GSM2889939 GSM2889940 GSM2889941 GSM2889942 GSM2889943 GSM2889944 GSM2890058 GSM2890059 GSM2890060 GSM2890061 GSM2890062 GSM2890063 GSM2890064 GSM2890065 GSM2890066 GSM2890067 GSM2890068 GSM2890069 GSM2890070 GSM2890071 GSM2890072 GSM2890073 GSM2890074 GSM2890075 GSM2890076 GSM2890077 GSM2890078 GSM2890079 GSM2890080 GSM2890081 GSM2890082 GSM2890083 GSM2890084 GSM2890115 GSM2890116 GSM2890117 GSM2890118 GSM2890119 GSM2890120 GSM2890121 GSM2890122 GSM2890123 GSM2890124 GSM2890125 GSM2890126 GSM2890127 GSM2890128 GSM2890129 GSM2890130

Screening and Verification of Treatment Target

We used least absolute shrinkage and selection operator logistic regression (LASSO) and random forest algorithms to perform feature selection to screen treatment target for MN. The LASSO algorithm was applied using the "glmnet" package, and the random forest algorithm was established using the "randomForest" package the further to evaluate treatment targets. The GSE108113 matrix was used as a test matrix to examine random forest algorithm results. Then, we combined

genes from the results from running the LASSO and random forest algorithms.

Evaluation of Immune Cell Infiltration

CIBERSORT R-packages and the LM22 document were used to run the algorithm locally (14); we filtered out those samples with $p < 0.05$ and obtained the immune cell infiltration matrix. The "corrplot" package was used to draw a correlation heatmap to visualize the correlation between infiltrated immune cells; the "ggplot2" package was employed to plot diagrams allowing visualization of the correlations in immune cell infiltration.

Correlation Analysis of Treatment Target and Immune Cell Infiltration

Spearman correlation analysis was applied to the markers and infiltrating immune cells and the "ggplur" package was used to visualize the results.

RESULTS

Data Processing and DEGs Screening

First, we merged expression profile datasets GSE99340 and GSE108113. The "sva" package was then used to remove inter-batch differences between different expression matrices. The merged expression matrix was normalized and processed: two PCA cluster diagrams (before and after normalization, **Figures 1A,B**) were used, indicating that the sample source was reliable. After data normalization, R-project was used to extract a total of 463 DEGs from the gene expression matrix, as shown in volcano map (**Figure 1C**). The heatmap of the top-50 DEGs demonstrated that DEGs were expressed differently in normal and MN samples (**Figure 2A**).

Functional Correlation Analysis

GO analysis showed that DEGs were mainly related to biological process (BP) including inflammatory response, lymphocyte activation, response to bacterium, response to lipopolysaccharide, leukocyte proliferation, leukocyte migration, response to molecules of bacterial origin, and cellular response to biotic stimulus (**Figure 2B**). The cellular component (CC) aspect of the results of GO analysis of DEGs included external encapsulating, extracellular matrix, collagen containing, external side of plasma membrane, basolateral plasma membrane, basal part of cells, and apical plasma membrane (**Figure 2C**). The molecular function (MF) aspect of the GO analysis results included active transmembrane transporter activity, symporter activity, secondary active transmembrane transporter activity, active ion transmembrane transporter activity, solute cation symporter activity, solute sodium symporter activity, sodium ion transmembrane transporter activity, and cytokine binding (**Figure 2D**).

GO analysis showed that DEGs were mainly related to urinary system disease, kidney disease, mouth disease, lung disease, tooth disease, myeloma, bone marrow cancer, and multiple myeloma (**Figure 2E**). KEGG pathway enrichment showed that human papilloma virus infection pathway, chemokine signaling pathway, pathways in cancer, Epstein-Barr virus infection pathway, proteoglycans in cancer pathway, human cytomegalovirus infection pathway, human T-cell leukemia virus 1 infection pathway, Rap 1 signaling pathway, Wnt signaling pathway, and axon guidance pathway as the top-10 most up-regulated pathways (**Figure 2F**). These results indicating MN pathophysiological changes involved immune responses.

Screening of Biomarkers for MN

The LASSO logistic regression algorithm was employed to identify 23 genes from DEGs as biomarkers for MN combined results from two λ values (**Figure 3A**). The protein-protein interaction (PPI) was analyzed (**Figure 3B**); 30 genes were obtained through use of the random forest algorithm as MN biomarkers (**Figure 3C**) and PPI analysis (**Figure 3D**); the learning status of the random forest algorithm showed that the predictive results were useful when scanning MN patients (**Figure 3E**). The gene markers from two algorithms were overlapped and one related gene (ETS2) was obtained (**Figure 3F**).

Immune Cell Infiltration Results and Correlation Analysis Between Ets2 and Immune Cells

The CIBERSORT tool was used to identify the estimated proportion of immune cells in MN and normal tissues. The estimated proportion of immune cells for each sample is shown in **Figure 4A**. The immune cell composition in both MN and normal groups was calculated; results showed that there were statistical differences in monocytes, plasma cells, regulatory T cells, and memory B cells (**Figure 4B**). The correlation between infiltrated immune cells is shown in **Figure 4C**. The correlation between ETS2 and infiltered immune cells was also determined. ETS2 was positively related to monocytes (cor = 0.63795852, $p = 3.007799 \times 10^{-17}$), M1 phase macrophages (cor = 0.66141468, $p = 7.823412 \times 10^{-19}$), and neutrophils (cor = 0.66695409, $p = 3.149015 \times 10^{-19}$) and negatively correlated to plasma cells (cor = −0.69775865, $p = 1.369595 \times 10^{-21}$), CD4+ T memory cells (cor = −0.50377651, $p = 1.119549 \times 10^{-7}$), M2 macrophages (cor = −0.37665222, $p = 5.751199 \times 10^{-6}$), CD8+ T cells (cor = −0.37258185, $p = 6.280381 \times 10^{-6}$), memory B cells (cor = −0.35864338, $p = 1.455759 \times 10^{-5}$), and resting mast cells (cor = −0.28208991, $p = 7.672348 \times 10^{-4}$) (**Figure 4D**).

DISCUSSION

MN is one of the most common pathological phenotypes for adult NS (3). Treatment for MN includes Rituximab, immunosuppressants, and glucocorticoids (10). Although, some patients can self-heal, many suffer from a protracted course of MN and some would eventually progress to end-stage-kidney-disease and require a kidney transplant.

Immune infiltration plays an important role in MN development (15). B cell activation leads to deposition of immune complex causing classic pathological changes of MN. Immune complex and B cell activation also leads to immune cell infiltration by macrophages. Macrophage phenotypes also

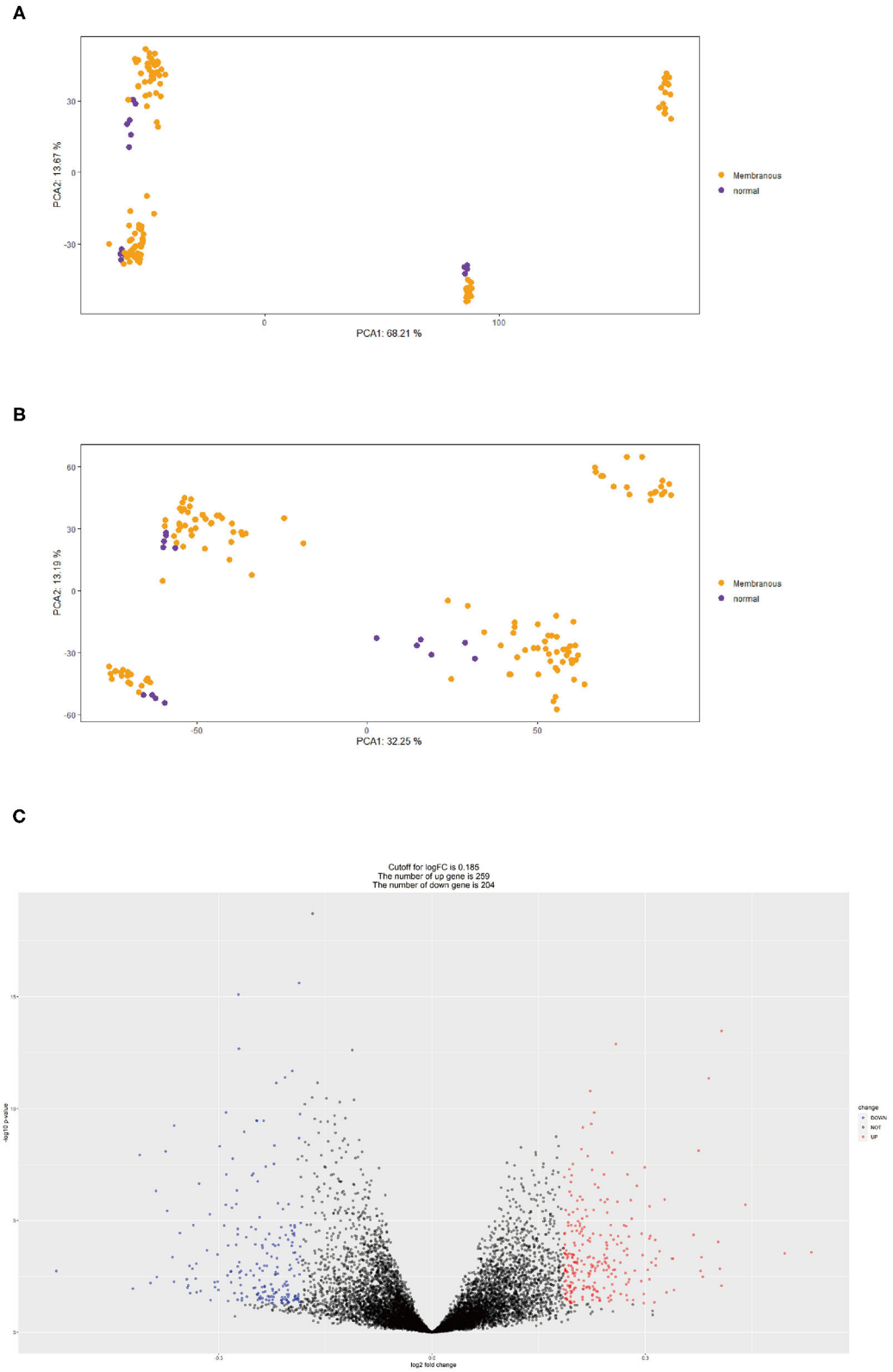


FIGURE 1 | (A) PCA clustering results of expression dataset before removing inter-batch difference: yellow dots represent MN samples and purple dots represent normal samples. **(B)** PCA clustering results of expression dataset after removing inter-batch difference, yellow dots represent MN samples and purple dots denote normal samples. **(C)** Volcano map for 463 DEGs, red represents up-regulated DEGs, blue represents down-regulated DEGs.

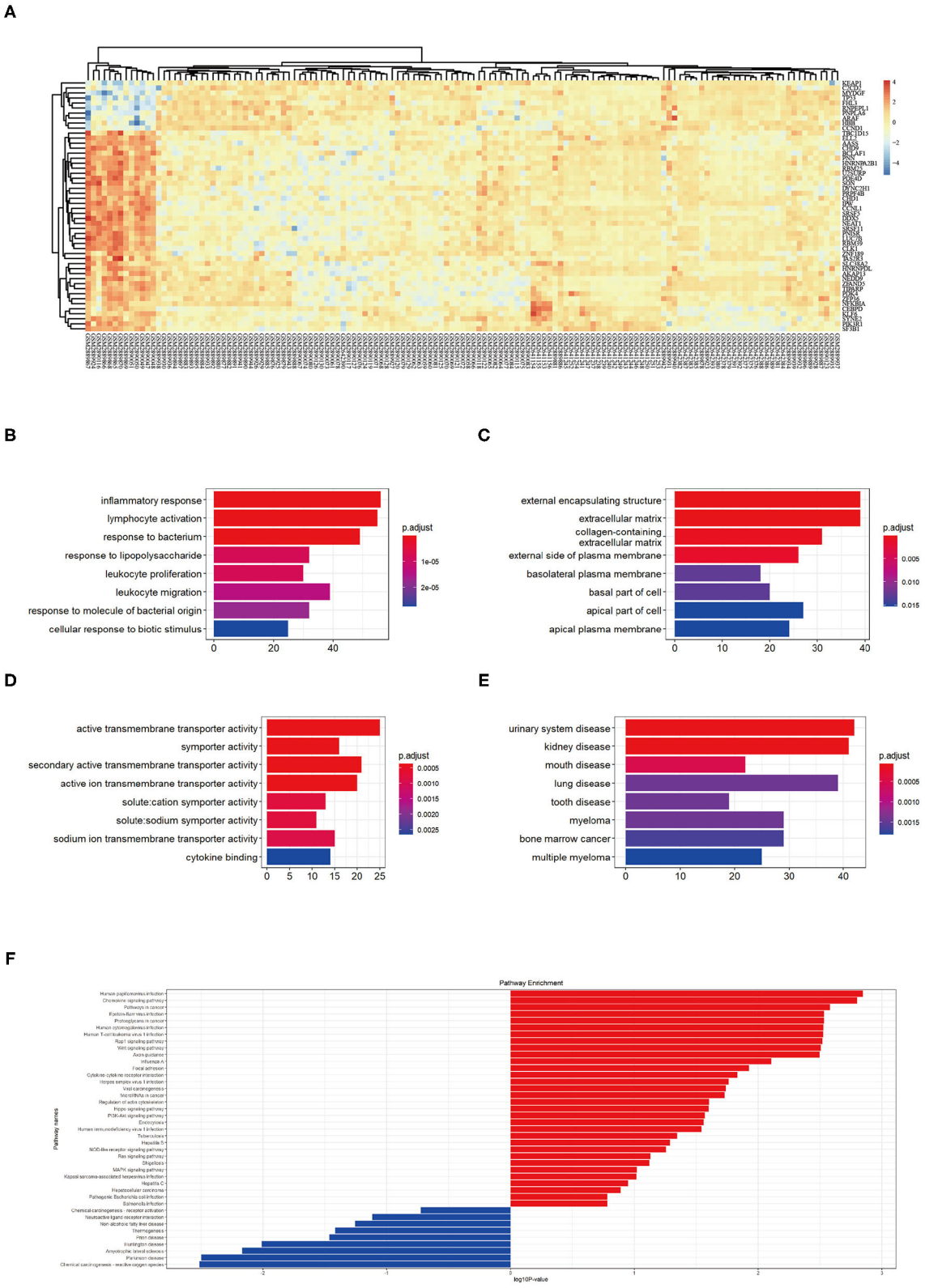


FIGURE 2 | (A) Top 50 DEG expressions in each sample, red represents a high level of expression and blue represents a low level of expression. **(B–D)** GO enrichment in biological process (BP), cellular component (CC), and molecular function (MF): color represents the *P*-value, red represents a low *P*-value and blue represents a high *P*-value. **(E)** DO enrichment: color represents the *P*-value, red represents a low *P*-value and blue represents a high *P*-value. **(F)** KEGG pathway enrichment results, red represents up-regulated pathways and blue represents down-regulated pathways.

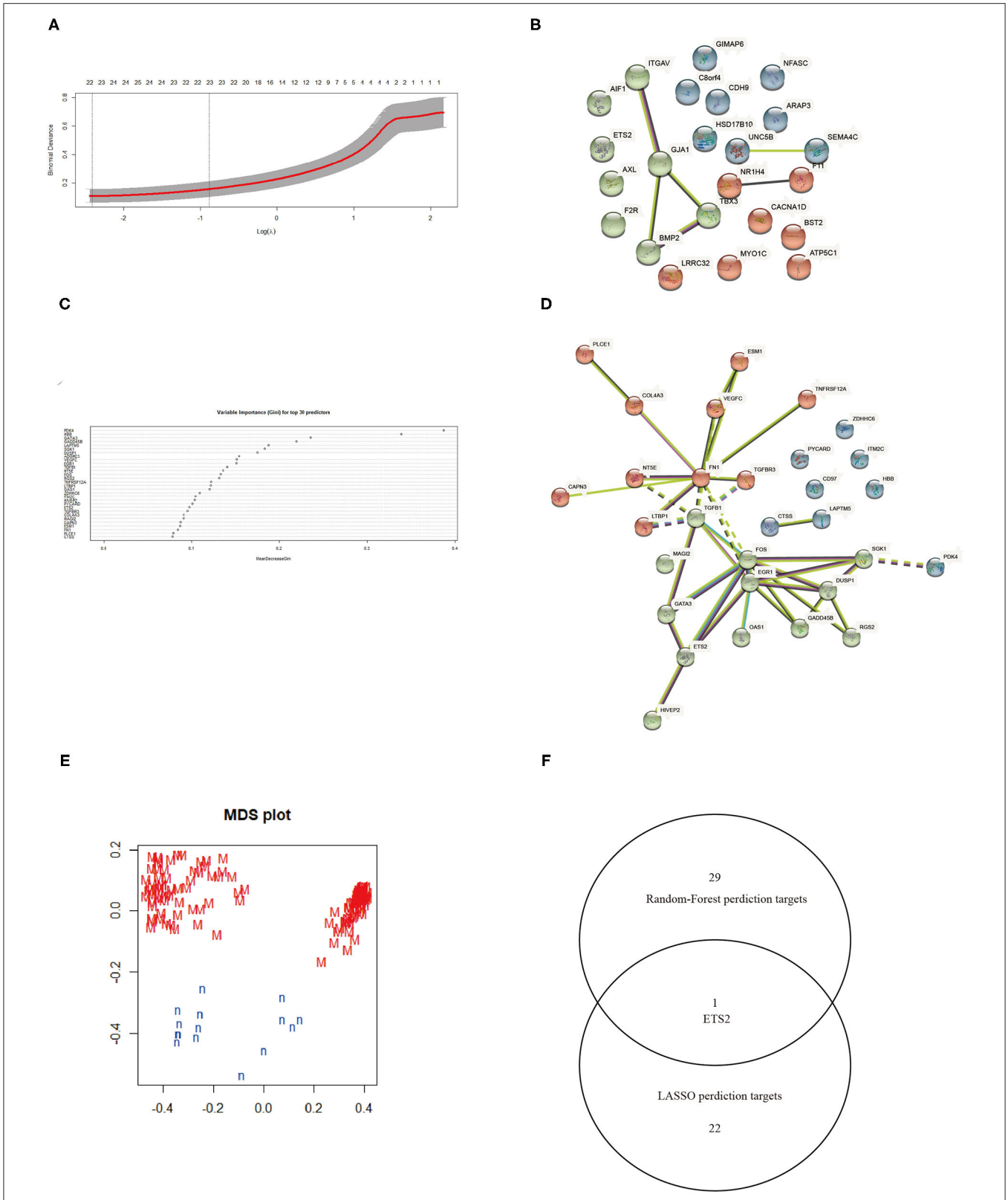


FIGURE 3 | (A) Selection of λ in the LASSO algorithm. **(B)** PPI network between LASSO algorithm results. **(C)** Top 30 results from the random forest algorithm. **(D)** PPI network between random forest algorithm results. **(E)** Random Forest algorithm learning status. **(F)** Venn map combining results from the LASSO and random forest algorithms.

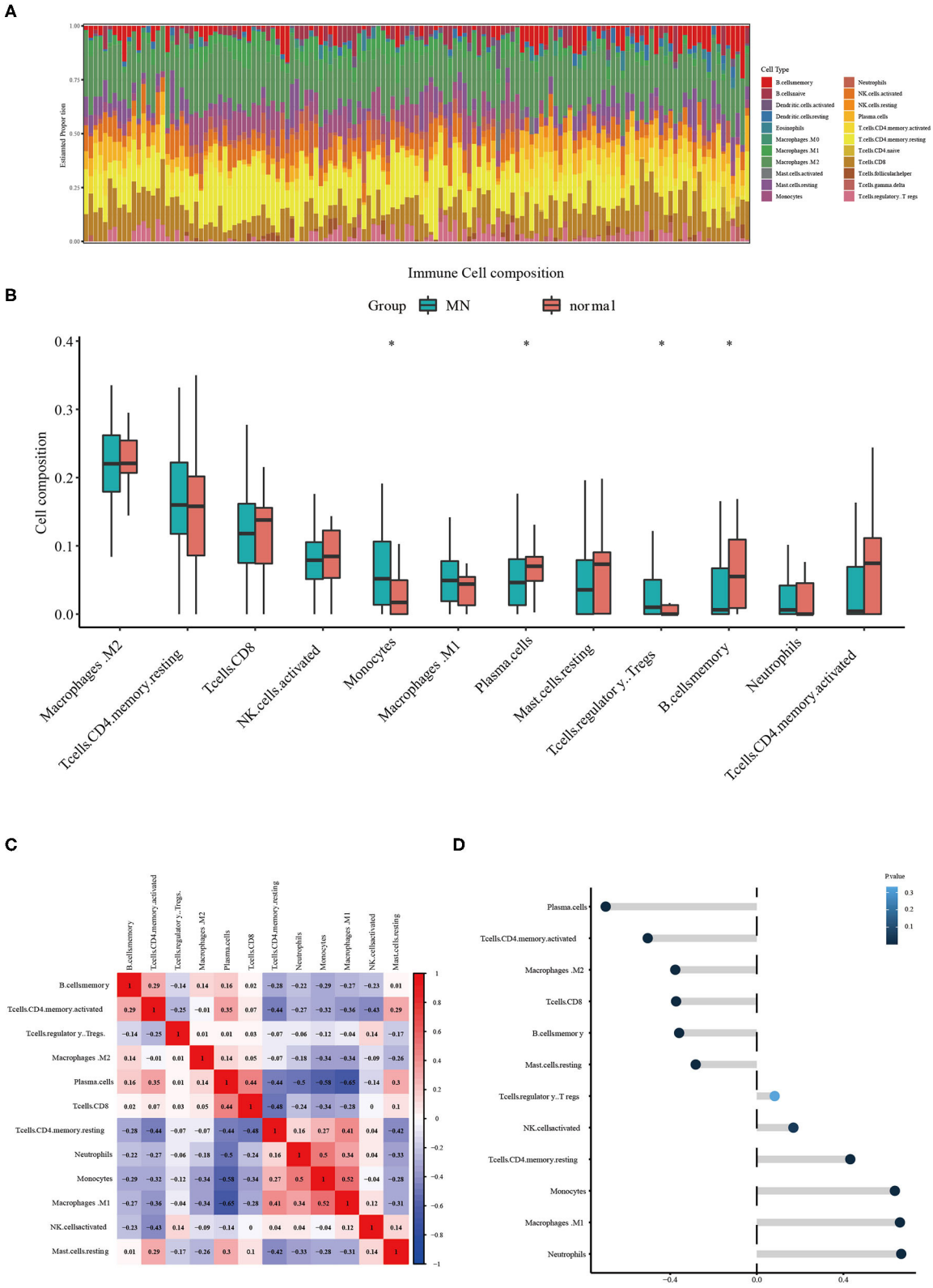


FIGURE 4 | (A) Estimated proportion of immune cells in each sample. **(B)** Different immune cell infiltration between MN and normal samples. The symbol * represents a *P*-value < 0.05. **(C)** Correlation between different infiltrated immune cells. **(D)** ETS2 Correlation upon different immune cell infiltrations.

play a critical role in the progress of MN; MN patients had a higher ratio of M1 macrophages to M2 phenotype, which led to increased inflammation and caused more damage (8).

We downloaded the expression profile dataset from the GEO database and identified a total of 463 DEGs. GO enrichment analysis showed that DEGs were mainly related to inflammatory response, lymphocyte activation, response to bacterium, response to lipopolysaccharide, leukocyte proliferation, leukocyte migration, response to molecules of bacterial origin, and cellular response to biotic stimulus. These results show that immune activity plays an important role in MN processes. KEGG enrichment analysis showed that up-regulated DEGs mainly belonged to the human papilloma virus infection pathway, chemokine signaling pathway, pathways in cancer, and Epstein-Barr virus infection pathway. These results showed that immune infiltration and immunomodulatory genes were changed during MN pathology changes. Also, targeting immune infiltration and virus-related innate immune could be next generation treatments (15) and diagnostic methods for MN (16).

CIBERSORT analysis result of 22 immune cell infiltration showed the statistical differences in monocytes, plasma cells, regulatory T cells, and memory B cells and indicated that pathways involved in immune cell activation and infiltration could be critical in MN progression. Moreover, these results demonstrated that these immune cells could be viable targets for MN immune suppression treatment.

The expression data were analyzed by using machine learning algorithms to screen possible targets for MN treatment. LASSO and random forest algorithms were used during the scan for treatment targets. We then combined the results from the LASSO algorithm and the random forest algorithm, showing that ETS2 was a suitable target for MN treatment. ETS2 was found to be positively related to monocytes, M1 phase macrophages, and neutrophils and negatively correlated to plasma cells, CD4+ T memory cells, M2 macrophages, CD8+ T cells, memory B cells, and resting mast cells. These results indicated that ETS2 participated in MN pathophysiological immune infiltration. According to previous studies, ETS2 participated in various activities: EST2 can control cytokine production and innate immune activation, which leads to IL-6 suppression and decreased amounts of macrophages (17). These functions of ETS2 could be related to its suppression of MAPK/NF-KB pathways (18). Also, deletion of ETS2 in pancreas leads to fibroblast continuous activation (17), however, studies in kidney showed that ETS2 could promote epithelial-to-mesenchymal transition and cause the progression of renal fibrosis. The study also indicated that these results could be caused by direct levels of regulation between ETS2 and JUNB transcription (19). Moreover, some studies showed that ETS2 interacts with the TGF-β/Smad pathway (20)—a pathway related to renal fibrosis—but studies of ETS2 function in MN are needed.

These results demonstrated that ETS2 was a crucial component for MN activation and immune infiltration, but direct intervention with ETS2 transcription function is not a wise way in which to treat MN. Further studies should be aimed at understanding of ETS2 function in MN and interaction with ETS2 promoting JUNB transaction. Inhibiting ETS2 and JUNB promotor binding could be the next-generation treatment used to cure MN. However, there are some limitations of this study, such as lack of the validations of Ets2 in MN patients at the protein level and Ets2 expression level's relation with patients' clinical outcomes. Further studies in this area are needed.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: GEO Database; GSE99340; GSE108113.

AUTHOR CONTRIBUTIONS

P-ZW: carried out the basic analysis of the study. T-HX and B-YT: provided clinical study of this work. G-YG: took part in statistical analysis. X-LL and LY: took part in study design. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Natural Science Foundation of China (Project number: 82070763), Key R&D Program of Liaoning Province (Project number: 2020JH 2/10300045 and 2019JH810300016), Population and Health Special Project of Shenyang Science and Technology Plan (Project number: 19-112-4-031), and Servier Kidney Disease Research and Development Project (Project number: 20010040796).

ACKNOWLEDGMENTS

The authors thank Dr. Jianming Zeng (University of Macau), and all the members of his bioinformatics team, biotrainee, for generously sharing their experience and codes.

REFERENCES

1. Couser WG. Primary membranous nephropathy. *Clin J Am Soc Nephrol.* (2017) 12:983–97. doi: 10.2215/CJN.11761116
2. Alsharhan L, Beck LH Jr. Membranous nephropathy: core curriculum 2021. *Am J Kidney Dis.* (2021) 77:440–53. doi: 10.1053/j.ajkd.2020.10.009
3. Ronco P, Debiec H. Molecular pathogenesis of membranous nephropathy. *Annu Rev Pathol.* (2020) 15:287–313. doi: 10.1146/annurev-pathol-020117-043811
4. Burbelo PD, Joshi M, Chaturvedi A, Little DJ, Thurlow JS, Waldman M, et al. Detection of PLA2R autoantibodies before the diagnosis of membranous nephropathy. *J Am Soc Nephrol.* (2020) 31:208–17. doi: 10.1681/ASN.2019050538
5. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med.* (2014) 371:2277–87. doi: 10.1056/NEJMoa1409354

6. Sethi S. New 'Antigens' in membranous nephropathy. *J Am Soc Nephrol.* (2021) 32:268–78. doi: 10.1681/ASN.2020071082
7. Su Z, Jin Y, Zhang Y, Guan Z, Li H, Chen X, et al. The Diagnostic and prognostic potential of the B-cell repertoire in membranous nephropathy. *Front Immunol.* (2021) 12:635326. doi: 10.3389/fimmu.2021.635326
8. Hu W, Li G, Lin J, Dong W, Yu F, Liu W, et al. M2 macrophage subpopulations in glomeruli are associated with the deposition of IgG subclasses and complements in primary membranous nephropathy. *Front Med.* (2021) 8:657232. doi: 10.3389/fmed.2021.657232
9. Rosenzwajg M, Languille E, Debiec H, Hygino J, Dahan K, Simon T, et al. B- and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab. *Kidney Int.* (2017) 92:227–37. doi: 10.1016/j.kint.2017.01.012
10. Fervenza FC, Appel GB, Barbour SJ, Rovin BH, Lafayette RA, Aslam N, et al. Rituximab or cyclosporine in the treatment of membranous nephropathy. *N Engl J Med.* (2019) 381:36–46. doi: 10.1056/NEJMoa1814427
11. Snider JM, You JK, Wang X, Snider AJ, Hallmark B, Zec MM, et al. Group IIA secreted phospholipase A2 is associated with the pathobiology leading to COVID-19 mortality. *J Clin Invest.* (2021) 131:e149236. doi: 10.1172/JCI149236
12. Clynick B, Corte TJ, Jo HE, Stewart I, Glaspole IN, Grainge C, et al. Biomarker signatures for progressive idiopathic pulmonary fibrosis. *Eur Respir J.* (2021). doi: 10.1183/13993003.01181-2021. [Epub ahead of print].
13. Barnes BM, Nelson L, Tighe A, Burghel GJ, Lin IH, Desai S, et al. Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes. *Genome Med.* (2021) 13:140. doi: 10.1186/s13073-021-00952-5
14. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nat Biotechnol.* (2019) 37:773–82. doi: 10.1038/s41587-019-0114-2
15. Motavalli R, Etemadi J, Kahroba H, Mehdizadeh A, Yousefi M. Immune system-mediated cellular and molecular mechanisms in idiopathic membranous nephropathy pathogenesis and possible therapeutic targets. *Life Sci.* (2019) 238:116923. doi: 10.1016/j.lfs.2019.116923
16. Mertowski S, Lipa P, Morawska I, Niedzwiedzka-Rystwej P, Bebnowska D, Hrynkiewicz R, et al. Toll-like receptor as a potential biomarker in renal diseases. *Int J Mol Sci.* (2020) 21:6712. doi: 10.3390/ijms21186712
17. Pitarresi JR, Liu X, Sharma SM, Cuitiño MC, Kladney RD, Mace TA, et al. Stromal ETS2 regulates chemokine production and immune cell recruitment during acinar-to-ductal metaplasia. *Neoplasia (New York, NY).* (2016) 18:541–52. doi: 10.1016/j.neo.2016.07.006
18. Ma X, Jiang Z, Li N, Jiang W, Gao P, Yang M, et al. Ets2 suppresses inflammatory cytokines through MAPK/NF-κB signaling and directly binds to the IL-6 promoter in macrophages. *Aging.* (2019) 11:10610–25. doi: 10.18632/aging.102480
19. Yao F, Wang X, Cui ZK, Lan H, Ai X, Song Q, et al. ETS2 promotes epithelial-to-mesenchymal transition in renal fibrosis by targeting JUNB transcription. *Lab Invest.* (2020) 100:438–53. doi: 10.1038/s41374-019-0331-9
20. Liu X, Zhang C, Zhang Z, Zhang Z, Ji W, Cao S, et al. E26 transformation-specific transcription factor ETS2 as an oncogene promotes the progression of hypopharyngeal cancer. *Cancer Biother Radiopharm.* (2017) 32:327–34. doi: 10.1089/cbr.2017.2296

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 31 March 2022
doi: 10.3389/fmed.2022.854300

Association Between Urinary Protein-to-Creatinine Ratio and Chronic Kidney Disease Progression: A Secondary Analysis of a Prospective Cohort Study

Xun Qin[1†], *Haofei Hu*[2,3,4†], *Ji Cen*[1†], *Xiaoyu Wang*[1†], *Qijun Wan*[2,3,4*] *and Zhe Wei*[1*]

[1] Department of Nephrology, Hechi People's Hospital, Hechi, China, [2] Department of Nephrology, Shenzhen Second People's Hospital, Shenzhen, China, [3] Department of Nephrology, The First Affiliated Hospital of Shenzhen University, Shenzhen, China, [4] Shenzhen University Health Science Center, Shenzhen, China

OPEN ACCESS

Edited by:
Jiannong Liu,
Hennepin Healthcare Research Institute, United States

Reviewed by:
Jonathan P. Troost,
University of Michigan, United States
Emanuele De Simone,
Azienda Sanitaria Locale "Città di Torino", Italy

***Correspondence:**
Qijun Wan
wanqijun12345@126.com
Zhe Wei
412429064@qq.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 13 January 2022
Accepted: 14 March 2022
Published: 31 March 2022

Citation:
Qin X, Hu H, Cen J, Wang X, Wan Q and Wei Z (2022) Association Between Urinary Protein-to-Creatinine Ratio and Chronic Kidney Disease Progression: A Secondary Analysis of a Prospective Cohort Study. Front. Med. 9:854300. doi: 10.3389/fmed.2022.854300

Objective: Studies on the association between urinary protein-to-creatinine ratio (UPCR) and chronic kidney disease (CKD) progression are limited. This study aimed to investigate the relationship between UPCR and CKD progression in a Japanese population.

Methods: The present research was a secondary analysis of a prospective cohort study. Eight hundred and ninety-six subjects from the research of CKD-ROUTE in Japan were included. All the patients were new visitors or first referred to the participating centers of nephrology between October 2010 and December 2011. The target-independent variable was UPCR measured at baseline. The dependent variable was CKD progression and the estimated glomerular filtration rate (eGFR) changes during follow-up. We used Cox proportional hazards regression to investigate the association between UPCR and CKD progression risk. To address UPCR and CKD progression's non-linearity, a multivariate Cox proportional hazards regression analysis with cubic spline functions model and smooth curve fitting (penalized spline method) were conducted. We further used a generalized linear mixed model to explore the relationship between UPCR and the changes of eGFR.

Result: The mean age of the included patients was 67.2 ± 13.4 years old. Two hundred and thirty-four people occurred CKD progression during follow-up. The present study showed that UPCR was independently associated with CKD progression in the multivariate analysis [HR = 1.164, 95% CI (1.116, 1.215)]. The non-linear relationship between UPCR and CKD progression was explored in a dose-dependent manner, with an obvious inflection point of 1.699. Furthermore, our findings indicated that the tendency of the effect sizes on both the left and right sides of the inflection point was not consistent [left HR: 4.377, 95% CI (2.956, 6.483); right HR: 1.100, 95% CI (1.049–1.153)]. Using the linear mixed-effects regression model, we found that UPCR was an independent predictor of the longitudinal changes in eGFR ($p < 0.001$ for the interaction term with time).

Conclusion: This study demonstrates a nonlinear positive relationship between UPCR and CKD progression in the Japanese population. UPCR is also an independent predictor of the longitudinal changes in eGFR.

Keywords: urinary protein-to-creatinine ratio, non-linearity, chronic kidney disease progression, Cox proportional hazards regression, linear mixed-effects regression model

BACKGROUND

Chronic kidney disease (CKD), resulting in end-stage renal disease (ESRD), has become a significant health problem worldwide. In recent years, billions of dollars have been charged to the National Health Insurance system, and the cost has continued to rise (1). CKD affects about 14% of the United States population, and the prevalence of ESRD is around 2,043 per 1 million people, which is ranked third in the world (2). There are ∼13.3 million people, accounting for 13% of the Japanese adult population, are estimated to have CKD (3). In addition, patients with CKD also have poorer cardiovascular outcomes and higher mortality (4). Therefore, studying the risk factors that may lead to the damage and deterioration of renal function has become the top priority of preventing and treating kidney diseases.

Diabetes mellitus (DM), age, gender, dyslipidemia, anemia, high-protein diet, smoking, obesity, hyperuricemia, proteinuria, family history for CKD and hypertension are traditional risk factors for the development of CKD (5, 6).

Proteinuria not only indicates the severity of CKD but is also strongly related to CKD progression (7). It is therefore essential to evaluate accurately each patient's proteinuria levels. In clinical practice, there are three indicators used to assess proteinuria: 24-h urine protein excretion (UPE), protein-to-creatinine ratio (UPCR), and urinary albumin-creatinine ratio (ACR). Although 24-h UPE is the most commonly used measure of proteinuria in randomized controlled clinical trials, it has several limitations and is unreliable if not validated by measuring 24-h urinary creatinine concomitantly. It is inconvenient and often inaccurate due to the 24-h urine collection required, and the quality of the urine is easily affected by the environment (8). Some studies support recommendations of using spot UPCR in screening and monitoring proteinuria in CKD patients (8), such as nephritis (9), diabetic nephropathy (10), and IgA nephropathy (11). Currently, a few studies have explored the relationship between UPCR and CKD progression (11–13). Nevertheless, most of these studies only used the logistic regression model or Cox proportional hazards regression to explore the relationship between UPCR and CKD progression. Therefore, evidence on the quantitative relationship between UPCR and CKD progression is still limited. Besides, few studies have investigated the relationship between baseline UPCR and the changes in estimated glomerular filtration rate (eGFR) and the role of other variables in modifying the relationship between UPCR and CKD progression. Moreover, fewer studies evaluated the possible non-linear relationship between UPCR and CKD progression.

Therefore, this study would use a multivariate Cox proportional hazards regression analysis, a multivariate Cox proportional hazards regression analysis with cubic spline functions model and smooth curve fitting (penalized spline method), and a generalized linear mixed model to investigate whether the baseline UPCR was independently related to renal function progression and the changes of eGFR in patients with CKD. A comprehensive understanding of the relationship between UCPR and the risk of CKD progression and changes in eGFR can provide a more scientific reference for clinically delaying renal function progression in CKD patients by controlling proteinuria.

Abbreviations: UPCR, urinary protein-to-creatinine ratio; CKD, chronic kidney disease; CKD-ROUTE, chronic kidney disease research of outcomes in treatment and epidemiology; KDIGO, Kidney Disease Improving Global Outcomes; ESRD, end-stage renal disease; ACR, albumin-creatinine ratio; UPE, urine protein excretion; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; ALB, serum albumin; Hb, hemoglobin; CVD, cardiovascular disease; ARB, angiotensin receptor blockers; ACEI, angiotensin-converting enzyme inhibitors; AP, angina pectoris; MI, myocardial infarction; CHF, congestive heart failure; PAD, peripheral arterial disease.

METHODS

Data Source and Participants

Data could be downloaded from the "DATADRYAD" database (www.datadryad.org), a website that allowed users to download raw data freely. All occurrence data for specimens included in this study were available as part of a Dryad (http://datadryad.org/) data package (doi: 10.5061/dryad.kq23s) (14). The variables used in the study were as follows: gender, age, systolic blood pressure (SBP), body mass index (BMI), serum creatinine (Scr), UPCR, urinary occult blood, eGFR, hemoglobin (Hb), serum albumin (ALB), causes of CKD, history of cardiovascular disease (CVD), diabetes, hypertension and anti-hypertensive therapy including calcium channel blocker, angiotensin receptor blockers (ARB), angiotensin-converting enzyme inhibitors (ACEI), and diuretics, years of follow up and CKD progression at follow up (14). According to the Dryad Terms of Service, researchers might apply these data in secondary analysis without infringing on the authors' rights. As written informed consent and research ethics approved were obtained in the previous research, no longer needed for this secondary study (14).

Data were obtained from the research on chronic kidney disease outcomes in treatment and epidemiology (CKD-ROUTE), a prospective, observational cohort study of a representative Japanese population with stage G2-G5 CKD. Stage of CKD was defined based on Kidney disease: improving global outcomes (KDIGO) classification (15). Details of the design in the study have been reported previously (14, 16, 17). More than 1,000

participants participated in Tokyo Medical and Dental University Hospital, and its 15 affiliated hospitals were enrolled (14).

New patients who were older than 20 years of age and who visited or were referred for the treatment of CKD stage 2–5 between October 2010 and December 2011, but were not on dialysis therapy, were recruited in this study (14). Patients with malignancy, transplant recipients, and/or active gastrointestinal bleeding; those who did not provide informed consent were excluded (14). Finally, 1,138 patients were assessed for eligibility in the original study (14). We excluded patients with missing values of UPCR ($n = 88$), and follow-up time was <3 months ($n = 154$). The final analysis included 896 subjects (629 male and 267 female) in the present study (see flowchart for details in **Figure 1**).

Study Design and Measurement of Variables

This was a prospective cohort study, and the study's design had been documented elsewhere (14, 17). All patients' medical history and current medications were recorded at enrollment. BMI was calculated from the body height and weight, obtained by anthropometric measurements. A standard sphygmomanometer was used to measure BP. Urine and blood samples were collected to measure creatinine, hemoglobin, albumin, urinary protein, urinary occult blood, and urinary creatinine (14). The eGFR was calculated by the following diet modification in renal disease equation modified for Japanese subjects (18): eGFR = 194 × serum creatinine $^{-1.094}$ × age $^{-0.287}$ (if female, × 0.739). Proteinuria was identified by urine dipstick test and the UPCR. Anemia was defined as hemoglobin level <10 g/dL because treatment for anemia was provided with a target Hb level of 10–12 g/dL. Low BMI (<23.5 kg/m^2) and low serum albumin level (<4 g/dL) were defined as cutoff values (19). All patients received standard treatment protocols according to the Japanese CKD guidelines (20). All patients were visited every 6 months for assessment of their clinical status. UPCR at baseline was the main independent variable. The dependent variable was CKD progression and eGFR changes during the follow-up period.

Definition of Diabetes, Hypertension, Cardiovascular Disease, and Etiology of Kidney Disease

Hypertension was defined as SBP at least 140 mmHg or DBP at least 90 mmHg or clinician-diagnosed hypertension, or currently on anti-hypertensive medication (14). Diabetes mellitus was defined as HbA1c ≥ 6.5% or antidiabetic therapy history (14). The etiology of CKD in each patient was determined by the physician who treated the patient at the time of enrollment, according to the patient's clinical characteristics, past medical history, and histological findings of renal biopsy specimens (14).

CVD was defined as having a history of coronary heart disease (including myocardial infarction, angina pectoris, coronary

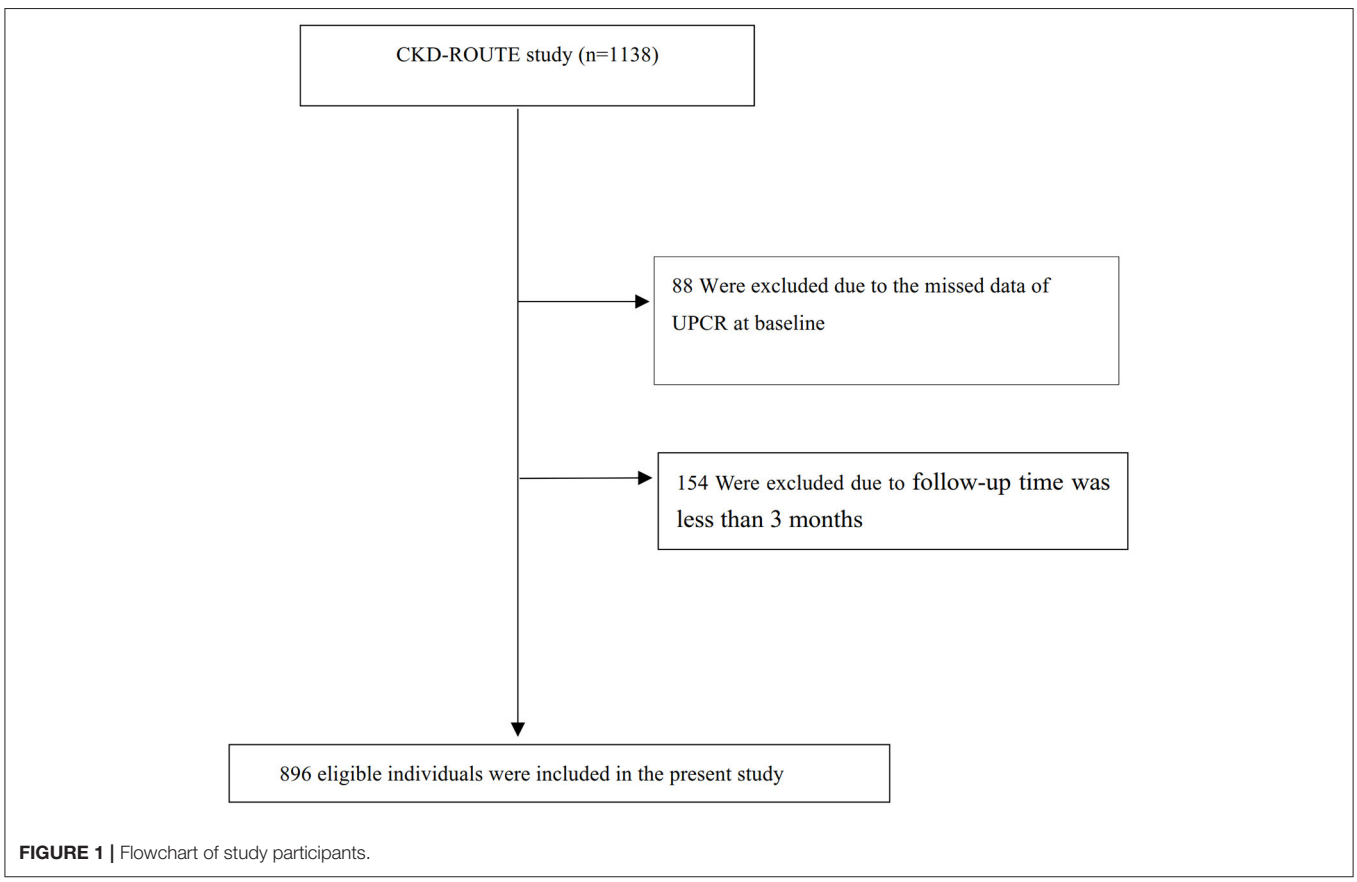


FIGURE 1 | Flowchart of study participants.

revascularization), congestive heart failure, peripheral arterial disease, or stroke (transient ischemic attack, cerebral infarction, subarachnoid hemorrhage, or cerebral hemorrhage) (14).

Study Endpoint

The primary outcome was CKD progression, defined as either initiation of dialysis during or 50% decline in eGFR from baseline (14). And the secondary endpoint was the changes of eGFR.

Statistical Analysis

First, we dealt with the missing values of covariates. The number of participants with missing SBP, BMI, and ALB data was 13, 89 and 3, respectively. Since only some continuous variables had missing data and were not much, it was unlikely to make a big impact. They all satisfied the normal distribution, so we used means to supplement the missing data (21).

Next, UPCR tertiles stratified baseline characteristics of all patients. Continuous variables with normal and skewed distribution were expressed as means with standard deviations or medians with interquartile ranges. Categorical variables were expressed as numbers and percentages. To compare differences among different UPCR groups (tertiles based on UPCR data), one-way analysis of variance (parametric distribution) or Kruskal–Wallis (non-parametric distribution) test was applied. Chi-square was used to compare categorical variables. Kaplan-Meier survival analysis with the log-rank test was used to compare CKD progression-free survival of different UPCR groups.

The process of data analysis was based on two criteria: (1) what was the real relationship between UPCR and CKD progression (linear or non-linear); (2) adjusted the confounding variables or after the stratified analysis, what was the true relationship between UPCR and CKD progression? Therefore, all of the results presented in this study were based on a three-step data analysis approach. Step 1: Univariate and multivariate Cox proportional hazard regression models were employed. According to the recommendation of the STROBE statement (22), we constructed three models: model 1, no covariates were adjusted; model 2, only adjusted for sociodemographic data (age, BMI, gender, SBP, hypertension, diabetes, history of CVD, and etiology of CKD); model 3, model 2+other covariates (HB, eGFR, ALB, urinary occult blood, use of calcium channel blocker, use of RAAS inhibitor, and use of diuretics). When added to the model, those covariates that changed the coefficient by more than 10% were considered confounders and adjusted for the multivariate analysis (23). Step 2: To address the non-linearity of UPCR and CKD progression, a Cox proportional hazards regression with cubic spline functions and smooth curve fitting (the cubic spline smoothing) were conducted. We calculated the inflection point using a recursive algorithm to detect a non-linearity. Then a two-piecewise Cox proportional hazard model was performed to calculate the threshold effect of the UPCR on CKD progression in terms of the smoothing plot. In the end, which model was more suitable for fitting the association between UPCR and CKD progression was mainly determined by the log-likelihood ratio test.

A sensitivity analysis was conducted to ensure the robustness of the data analysis. UPCR was converted into a categorical variable, and the *P*-value was calculated for the trend. The test's purpose was to verify the results of treating UPCR as a continuous variable and determine the possibility of non-linearity. Achieving complete remission predicts an excellent long-term renal prognosis (24). Therefore, when exploring the association between UPCR and CKD progression in other sensitivity analyses, we excluded participants with UPCR < 0.3.

Furthermore, eGFR levels were followed every 6 months until 36 months. The longitudinal changes in eGFR were analyzed with linear mixed-effects regression models (25), which easily accommodate unbalanced, unequally spaced observations (26). The dependent variable (i.e., eGFR) was assessed on the baseline visit and during all follow-up visits in these models. In contrast, the independent variable (i.e., UPCR) was only measured on the baseline visit. The following variables, measured or calculated on the baseline visit, were entered into all of the models as fixed effects: age, BMI, gender, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of calcium channel blocker, use of RAAS inhibitor, and use of diuretics. In mixed-effects regression models, the interaction term between a fixed effect variable and time assessed whether the variable was a predictor of longitudinal changes in the eGFR variable. Therefore, the interaction terms between time and UPCR were evaluated. All models also included intercept as random term. Random effects allowed each participant's beginning value to vary from the population average (intercept). Patient level (each patient has one intercept) was the level of random intercept. Since patients were assessed for eGFR every 6 months for a total of 6 times, time to repeated measures was treated as a categorical variable (0, 6, 12, 18, 24, 30, 36 months). No structure was imposed on the covariance matrix of these random effects, and the errors were assumed to be independent with constant variance.

All analyses were performed using R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc., Boston, MA, USA). *P*-values <0.05 were considered statistically significant.

RESULTS

Thus, a total of 895 participants (70.2% men and 29.8% women) were eventually included in this analysis. The mean age was 67.2 years old (SD = 13.4). The mean follow-up time was 26.53 $\pm$ 11.83 months, and 234 people developed CKD progression during follow-up. The baseline mean UPCR and eGFR were 2.09 $\pm$ 3.22 and 33.19 $\pm$ 17.97 ml/min per 1.73 m^2, respectively.

Baseline Characteristics of the Study Participants

Baseline characteristics of the study population were presented by tertiles of UPCR (**Table 1**). We divided participants into subgroup using UPCR tertiles (<0.224, 0.22–1.648, ≥ 1.648). In the highest UPCR group, we found that patients generally had higher Scr, SBP, BMI levels, higher rates of urinary occult

TABLE 1 | Baseline characteristics of all the patients at enrollment ($n = 896$).

UPCR	T1 (<0.224)	T2 (0.224–1.648)	T3 (≥1.648)	*P*-value
Participants	299	298	299	
Age (years)	68.44 ± 13.10	67.67 ± 13.64	65.46 ± 13.35	0.019
HB (g/dL)	12.91 ± 1.97	12.05 ± 2.28	11.31 ± 2.06	<0.001
Scr (mg/dL)	1.29 (1.08–1.72)	1.70 (1.20–2.50)	2.40 (1.68–3.45)	<0.001
eGFR (ml/min per 1.73 m^2)	41.56 ± 15.50	33.31 ± 18.23	24.69 ± 15.98	<0.001
UPCR (g/gCr)	0.07 (0.03–0.13)	0.70 (0.40–1.04)	4.22 (2.63–6.90)	<0.001
SBP(mmHg)	132.63 ± 19.30	138.19 ± 21.15	148.83 ± 22.62	<0.001
BMI(kg/m^2)	23.59 ± 3.39	23.70 ± 3.68	24.32 ± 4.26	0.039
ALB(g/dL)	4.20 ± 0.43	3.99 ± 0.47	3.44 ± 0.63	<0.001
Gender				0.439
Male	216 (72.24%)	211 (70.81%)	202 (67.56%)	
Female	83 (27.76%)	87 (29.19%)	97 (32.44%)	
Etiology of CKD				<0.001
Diabetic nephropathy, *n* (%)	24 (8.03%)	48 (16.11%)	160 (53.51%)	
Nephrosclerosis, *n* (%)	177 (59.20%)	121 (40.60%)	59 (19.73%)	
Glomerulonephritis, *n* (%)	23 (7.69%)	80 (26.85%)	60 (20.07%)	
Other, *n* (%)	75 (25.08%)	49 (16.44%)	20 (6.69%)	
Urinary occult blood, *n* (%)	50 (16.72%)	98 (33.11%)	142 (47.49%)	<0.001
Hypertension, *n* (%)	242 (80.94%)	272 (91.28%)	292 (97.66%)	<0.001
History of CVD, *n* (%)	65 (21.74%)	75 (25.17%)	101 (33.78%)	0.003
Diabetes, *n* (%)	78 (26.09%)	89 (29.87%)	178 (59.53%)	<0.001
Use of RAAS inhibitor, *n* (%)	173 (57.86%)	187 (62.75%)	226 (75.59%)	<0.001
Use of calcium channel blocker, *n* (%)	100 (33.44%)	150 (50.34%)	181 (60.54%)	<0.001
Use of diuretics, *n* (%)	69 (23.08%)	84 (28.19%)	138 (46.15%)	<0.001

Continuous variables are presented as mean ± standard deviation and median with interquartile ranges. Categorical data are presented as numbers and percentages. BMI, body mass index; SBP, Systolic blood pressure; Scr, Serum creatinine; ALB, Serum albumin; HB, Hemoglobin; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; UPCR, urinary protein/creatinine ratio; g/gCr, gram per gram creatinine; RAAS, renin-angiotensin aldosterone system.

blood, diabetes, hypertension, history of CVD, calcium channel blocker use, RAAS inhibitor use, and use of diuretics. Besides, patients in the highest UPCR group had a higher proportion of diabetic nephropathy as the primary disease. In contrast, patients generally had lower age, HB, ALB, and eGFR levels in the highest UPCR group.

Figure 2 showed the distribution of UPCR levels. It presented a skewed distribution while being in the range from 0.006 to 20.183. This result also indicated that most of the FLI were <6.329. Participants were divided into two groups according to whether they developed CKD progression during the follow-up. The UPCR values in the two groups were shown in **Figure 3**. The results indicated that the distribution level of UPCR in the CKD-progression group was higher, while the UPCR level in the CKD-progression-free group was relatively lower.

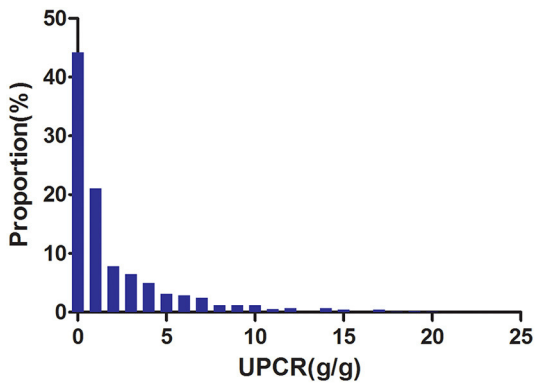


FIGURE 2 | Distribution of UPCR. It presented a skewed distribution while being in the range from 0.006 to 20.183.

The Incident Rate of CKD Progression

Table 2 revealed that 234 patients developed CKD progression in total. The total incident rate of all participants was 11.814 per 100 person-years. Specifically, the incident rates of the three UPCR groups were 0.957, 5.776, and 35.424 per 100 person-years, respectively.

Univariate Analysis

The results of the univariate Cox regression analysis were shown in **Table 3**. The results showed that UPCR, urinary occult blood, SBP, diabetes, hypertension, use of calcium channel blocker, use of RAAS inhibitor, and use of diuretics were positively associated with the risk of CKD progression. In contrast, HB, eGFR and ALB

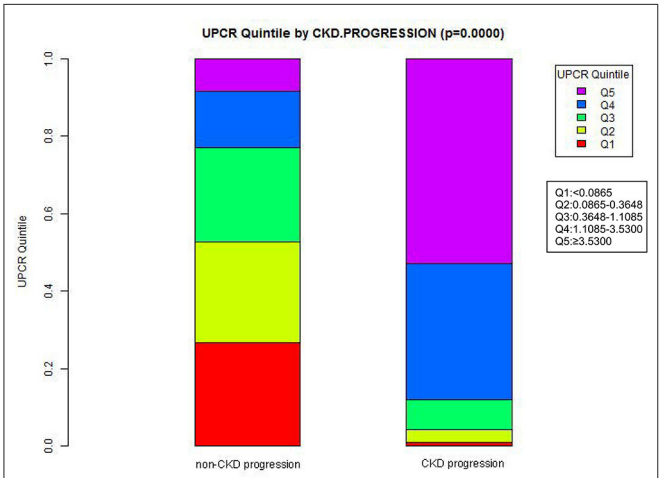


FIGURE 3 | Data visualization of UPCR of all participants from CKD progression and non-CKD progression groups. The results indicated that the distribution level of UPCR in the CKD progression group was higher, while the level of UPCR in the CKD progression-free group was relatively low.

TABLE 2 | Incident rate of incident CKD pregression.

UPCR	Participants (*n*)	CKD progression event (*n*)	Incident rate (Per 100 person-year)
Total	896	234	11.814
T1	299	7	0.957
T2	298	42	5.776
T3	299	185	35.423

were negatively related to the risk of CKD progression. We also found that patients with primary onset diabetic nephropathy had a high risk of CKD progression.

Figure 4 showed the Kaplan-Meier curves of the probability of CKD progression-free survival stratified by UPCR categories. The probability of CKD progression-free survival between the three UPCR groups was significantly different (log-rank test, $p < 0.0001$). The probability of CKD progression-free survival gradually decreased with the increasing UPCR group, indicating that the higher the UPCR group, the higher the risk of CKD progression.

The Results of the Relationship Between UPCR and CKD Progression

In this study, we constructed three models to analyze the independent effects of UPCR on CKD progression (univariate and multivariate Cox proportional hazard model). The effect sizes hazard ratio (HR) and 95% confidence intervals were listed in **Table 4**. In crude model, UPCR showed a positive association with CKD progression [HR = 1.210, 95% confidence interval (CI):1.760–1.903, $P < 0.00001$]. In the minimally adjusted model (adjusted gender, age, SBP, BMI, hypertension, diabetes, history of CVD, and etiology of CKD), the result did not have obvious change (HR: 1.180, 95% CI: 1.146–1.251). In the fully adjusted model (model II) (adjusted gender, age, SBP, BMI, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of calcium channel blocker, use of RAAS inhibitor, and use of diuretics), we could also detect the connection (HR = 1.164, 95% CI: 1.116–1.215, $P < 0.00001$). Namely, for each additional 1 unit of UPCR, the risk of CKD progression increased by 16.4%.

The Analyses of the Non-linear Relationship

We used a Cox proportional hazards regression model with cubic spline functions to evaluate the relationship between UPCR (as continuous and tertile variables) and incident CKD progression (**Figures 5A,B**). The result showed that the relationship between UPCR and CKD progression was non-linear after adjusting for related confounding factors. When UPCR was a continuous variable, we used both the Cox proportional hazard model and the two-piecewise Cox proportional hazard model to fit the association and select the best fit model based on P for log-likelihood ratio test.

Because the *P* for the log-likelihood ratio test was <0.05, we chose the two-piecewise Cox proportional hazard model for fitting the association between UPCR and CKD progression because it could accurately represent the relationship. By the two-piecewise Cox proportional hazard model and recursive algorithm, we calculated the inflection point was 1.699. We observed a stronger positive association between UPCR and CKD progression on the left side of the inflection point, the HR and 95% CI were 4.377, 2.956–6.483, respectively. The results showed that a 1-unit increase in UPCR levels was associated with a 4.377-fold greater risk of CKD progression when the UCR was less than the 1.699. On the right side of the inflection point, we only observed a relatively weaker positive relationship between UPCR and CKD progression, the HR and 95% CI were 1.100, 1.049–1.153, respectively. Which indicated that a 1 unit increase in the UPCR level was only associated with a 1.1 times greater in the risk of CKD progression when UPCR > 1.699 (**Table 5**).

When UCPR was used as a tertile variable, we found that as its grade increased, there was a corresponding increase in the risk of CKD progression, with a more pronounced increase in T2–T3 than T1–T2 (**Figure 5**).

Sensitivity Analysis

A series of sensitivity analyses were performed to confirm our findings' robustness. We first converted UPCR from a continuous variable to a categorical variable (according to tertile) and then put the categorical-transformed UPCR back into the model. The *P* for trend of UPCR as a categorical variable in the fully adjusted model was consistent with the result when UPCR was a continuous variable (*P* for trend <0.00001). Besides, we also found the trend of the effect size in different UPCR groups was non-equidistant.

In addition, we excluded patients with UPCR < 0.3 to explore the non-linear relationship between UPCR and CKD progression in other sensitivity analyses. The result showed that there was still a non-linear association between UPCR and CKD progression after adjusting for related confounding factors. By

TABLE 3 | The results of univariate analysis.

	Statistics	Effect size HR (95% CI)	*P*-value
Age	67.19 ± 13.41	0.99 (0.98, 1.00)	0.1350
Gender			
Male	629 (70.20%)	Ref.	
Female	267 (29.80%)	1.05 (0.79, 1.38)	0.7405
Etiology of CKD			
Diabetic nephropathy,	232 (25.89%)	Ref.	
Nephrosclerosis,	357 (39.84%)	0.17 (0.13, 0.24)	<0.0001
Glomerulonephritis	163 (18.19%)	0.27 (0.19, 0.39)	<0.0001
Other	144 (16.07%)	0.16 (0.10, 0.26)	<0.0001
HB	12.09 ± 2.20	0.70 (0.66, 0.74)	<0.0001
eGFR	33.18 ± 17.97	0.92 (0.91, 0.93)	<0.0001
Urinary occult blood			
No	604 (67.56%)	Ref.	
Yes	290 (32.44%)	1.72 (1.32, 2.22)	<0.0001
UPCR	2.09 ± 3.22	1.21 (1.18, 1.24)	<0.0001
Hypertension			
No	90 (10.04%)	Ref.	
Yes	806 (89.96%)	5.44 (2.24, 13.19)	0.0002
History of CVD			
No	655 (73.10%)	Ref.	
Yes	241 (26.90%)	1.25 (0.94, 1.66)	0.1211
Diabetes			
No	551 (61.50%)	Ref.	
Yes	345 (38.50%)	2.87 (2.21, 3.72)	<0.0001
Use of RAAS inhibitor			
No	310 (34.60%)	Ref.	
Yes	586 (65.40%)	1.75 (1.30, 2.37)	0.0003
Use of calcium channel blocker			
No	465 (51.90%)	Ref.	
Yes	431 (48.10%)	1.77 (1.36, 2.30)	<0.0001
Use of diuretics			
No	605 (67.52%)	Ref.	
Yes	291 (32.48%)	2.29 (1.77, 2.96)	<0.0001
SBP	139.88 ± 22.09	1.02 (1.01, 1.02)	<0.0001
BMI	23.87 ± 3.80	1.03 (0.99, 1.07)	0.0973
ALB	3.88 ± 0.61	0.35 (0.30, 0.41)	<0.0001

the two-piecewise Cox proportional hazard model and recursive algorithm, we calculated the inflection point was 1.607. We could still observe a stronger positive association between UPCR and CKD progression on the left side of the inflection point, and a relatively weaker positive relationship on the right side of the inflection point (**Table 6**, **Supplementary Figure S1**).

UPCR and Longitudinal eGFR

We took advantage of the repeated measurements of eGFR at each follow-up visit to characterize the longitudinal changes in eGFR over time in the cohort. Using a linear mixed-effects regression model, we evaluated baseline UPCR's profound influence on the longitudinal changes in eGFR. In this model, a statistically significant interaction term between time and UPCR indicated that the longitudinal changes in eGFR were influenced by UPCR. As shown in **Table 7** and **Figure 6**, after adjusting for BMI, gender, age, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of calcium channel blocker, use of RAAS inhibitor, and use of diuretics, baseline UPCR was independently associated with the longitudinal changes in eGFR ($p < 0.001$ for the interaction term with time). Besides, the negative association between baseline UPCR and eGFR was also gradually increased with the increase of follow-up time. Each 1 unit of UPCR increased, eGFR decreased by 1.023 ml/min per 1.73 m^2 at baseline, while half a year of follow-up, eGFR decreased by 0.489

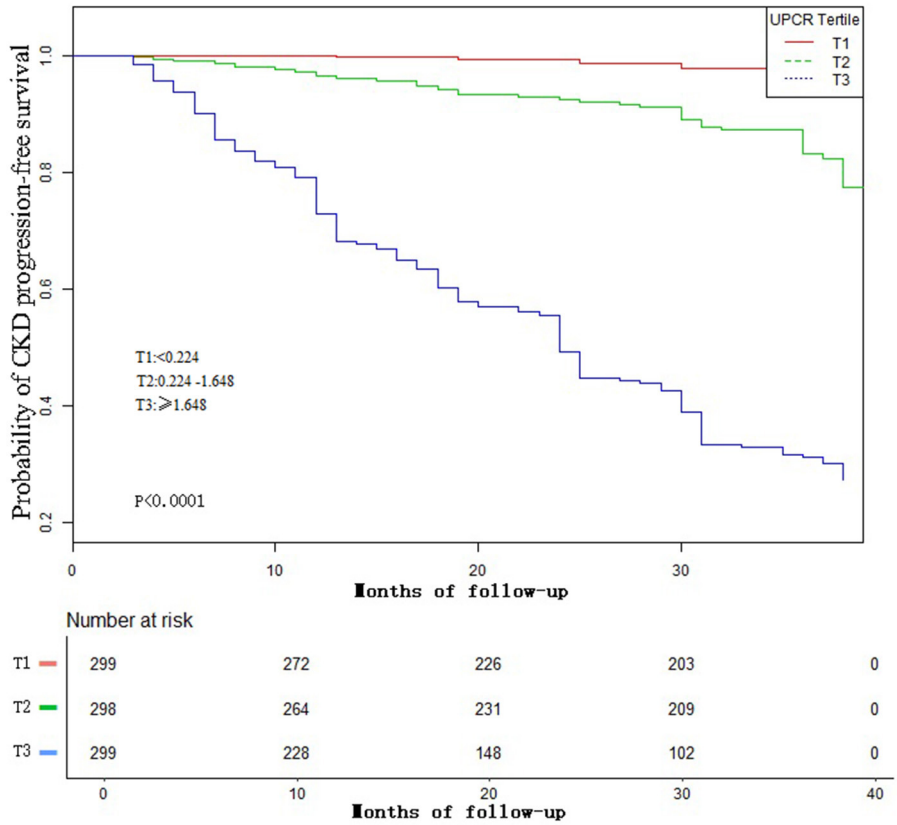


FIGURE 4 | Kaplan–Meier event-free survival curve. Kaplan–Meier analysis of incident CKD progression-free survival based on UPCR groups (log-rank, *P* <0.0001).

TABLE 4 | Relationship between UPCR and the chronic kidney disease progression in different models.

Variable	Crude model (HR, 95% CI, *P*)	Model I (HR, 95% CI, *P*)	Model II (HR, 95% CI, *P*)
UPCR	1.210 (1.184, 1.236) <0.00001	1.180 (1.146, 1.215) <0.00001	1.164 (1.116, 1.215) <0.00001
UPCR(Tertile)			
T1	Ref.	Ref.	Ref.
T2	6.007 (2.699, 13.372) 0.00001	5.544 (2.474, 12.423) 0.00003	2.673 (1.180, 6.057) 0.01846
T3	39.760 (18.678, 84.639) <0.00001	27.965 (12.772, 61.228) <0.00001	9.618 (4.272, 21.652) <0.00001
P for trend	<0.00001	<0.00001	<0.00001

Crude model: we did not adjust other covariants.
Model I: we adjust age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD.
Model II: we adjust age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of RAAS inhibitor, use of calcium channel blocker, use of diuretics.
CI, confidence; Ref, reference.

ml/min per 1.73 m^2 more than the level of decrease at baseline. After a year of follow-up, eGFR decreased by 0.936 ml/min per 1.73 m^2 more than the baseline. The baseline UPCR had the greatest effect on the decline of eGFR after 30 months of follow-up. Specifically, eGFR decreased by 2.556 ml/min per 1.73 m^2 after 30 months of follow-up with an increase of 1 unit of UPCR at baseline.

Figure 6 illustrated the effect of the baseline UPCR group on the longitudinal changes of eGFR. While stratified based on the tertiles of UPCR, the association between UPCR and eGFR changes was correspondingly divided into three parts. As UPCR was negatively correlated with eGFR, the patients with the lowest tertile of UPCR at baseline had the highest levels of eGFR at baseline and during follow-up. However, eGFR decreased rapidly

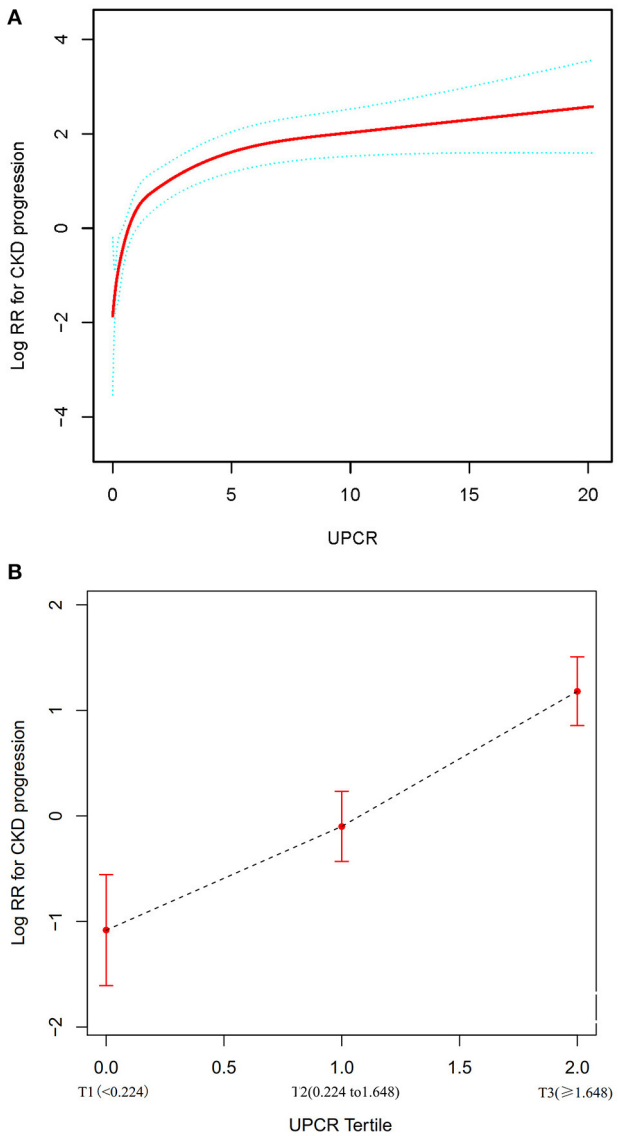


FIGURE 5 | The non-linear relationship between UPCR and CKD progression. **(A)** The non-linear relationship between UPCR (continuous variable) and CKD progression. **(B)** The non-linear relationship between UPCR (tertile variable) and CKD progression. We used a Cox proportional hazards regression model with cubic spline functions and smooth curve fitting (penalized spline method) to evaluate the relationship between UPCR and incident CKD progression. The result showed that the relationship between UPCR and CKD progression was non-linear after adjusting for age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of RAAS inhibitor, use of calcium channel blocker and use of diuretics.

in patients with the lowest UPCR group at baseline compared with the other two groups.

DISCUSSION

Our findings indicated UPCR was positively associated with CKD progression after adjusting other covariates. Besides, we calculated the inflection point of UPCR was 1.699, and we found the trend of the effect sizes on the left and right sides of the inflection point was not consistent [left (HR = 4.377, 95% CI: 2.956–6.483, $P < 0.0001$); right (HR = 1.100, 95% CI: 1.049–1.153, $P < 0.0001$)]. The result suggested a turning point effect on the independent association between UPCR and CKD progression. Using the linear mixed-effects regression model, we found that the impact of baseline UCPR on changes in eGFR was different at different follow-up times.

Proteinuria has been proposed as a surrogate endpoint in clinical trials on CKD progression (27). Evaluation of proteinuria is inexpensive and straightforward. Some previous studies have probed the association between proteinuria and renal progression in CKD patients. Mass sample of 106,177 participants from the general Japanese population identified proteinuria as the most powerful predictor of ESKD risk over 10 years (28). A similar study in France showed the most potent independent risk factors of poor renal outcome were proteinuria ≥1 g/day [proportional hazard risk (HR) =23.7, $P = 0.0001$] (29). Another study suggested that baseline UPCR and systolic BP levels were independently associated with CKD progression in children with non-glomerular CKD (12). In a retrospective study of 438 adults with IgA nephropathy, Hong Zhang et al.

TABLE 5 | The result of the two-piecewise linear regression model.

	CKD progression (HR, 95% CI, *P*)
Fitting model by standard linear regression	1.164 (1.116, 1.215) <0.0001
Fitting model by two-piecewise linear regression	
Inflection point of UPCR	1.699
≤1.699	4.377 (2.956, 6.483) <0.0001
>1.699	1.100 (1.049, 1.153) <0.0001
P for log-likelihood ratio test	<0.001

CI, Confidence interval.
We adjusted age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of RAAS inhibitor, use of calcium channel blocker and use of diuretics.

TABLE 6 | The result of the two-piecewise linear regression model in patients with UCPR > 0.3 for sensitivity analyses.

	CKD progression (HR, 95%CI, *P*)
Fitting model by standard linear regression	1.148 (1.099, 1.199) <0.0001
Fitting model by two-piecewise linear regression	
Inflection point of UPCR	1.607
≤1.699	5.466 (3.031, 9.856) <0.0001
>1.699	1.101 (1.050, 1.155) <0.0001
P for log-likelihood ratio test	<0.001

CI, Confidence interval.
We adjusted age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of RAAS inhibitor, use of calcium channel blocker and use of diuretics.

TABLE 7 | Relationship between baseline UPCR and longitudinal eGFR derived from a linear mixed-effects regression model.

Variable	Crude model (β, 95%CI, *P*)	Model I (β, 95% CI, *P*)
UPCR(baseline)	−1.653 (−2.010, −1.296) <0.001	−1.023 (−1.405, −0.641) <0.001
UPCR ×6th month	−0.487 (−0.641, −0.333) <0.001	−0.489 (−0.643, −0.335) <0.001
UPCR × 12th month	−0.932 (−1.106, −0.757) <0.001	−0.936 (−1.110, −0.762) <0.001
UPCR ×18th month	−1.200 (−1.386, −1.014) <0.001	−1.208 (−1.393, −1.023) <0.001
UPCR ×24th month	−1.189 (−1.382, −0.996) <0.001	−1.199 (−1.391, −1.006) <0.001
UPCR ×30th month	−1.521 (−1.735, −1.307) <0.001	−1.533 (−1.747, −1.319) <0.001
UPCR ×36th month	−1.441 (−1.667, −1.214) <0.001	−1.452 (−1.678, −1.226) <0.001

CI, Confidence interval.
We adjusted age, gender, BMI, SBP, hypertension, diabetes, history of CVD, etiology of CKD, HB, eGFR, ALB, urinary occult blood, use of RAAS inhibitor, use of calcium channel blocker and use of diuretics.

found that urine ACR, UPCR, and 24-h UPE had a comparable association with severe clinical and histologic findings. All of them showed good performance in predicting IgAN progression (11). On the contrary, another study that assessed the relationship between UPCR, ACR, and 24-h UPE in 6,842 patients with CKD focusing on performance at thresholds of 0.5 and 1 g/day of proteinuria, found that UPCR was a more sensitive screening test than ACR to predict clinically relevant proteinuria. The study also found that the relationship between ACR and UPCR was non-linear. UPCR was highly correlated with 24-h urine protein (Spearman's rho = 0.91), though ACR also performed well (rho = 0.84) (13). However, most of these studies explored the relationship between proteinuria and CKD progression only using the logistic regression model or Cox proportional hazards regression model. They neither further analyzed the possible non-linear relationship between UPCR and CKD progression nor explored the effect of UCPR on eGFR changes. In our research, having a similar sample size, the Cox proportional hazard regression model showed a positive association between UPCR and CKD progression, consistent with those studies. Besides, we found a non-linear relationship between UPCR and CKD progression through multivariate Cox proportional hazards regression analysis with cubic spline functions model and smooth curve fitting (penalized spline method). We also found the inflection point was 1.699. When UPCR was <1.699, there was a stronger positive relationship between UPCR and CKD progression. A 1 unit increase in the UPCR level was associated with 4.377 times greater in the risk of CKD progression (HR = 4.377, 95% CI: 2.956–6.483). However, when UPCR > 1.699, a 1 unit increase in the UPCR level was only associated with a 1.1 times greater in the risk of CKD progression (HR = 1.100, 95% CI: 1.049–1.153). The reason might be that other variables in the participant's baseline also influenced the risk of CKD progression. It could be seen from **Supplementary Table S1** that compared with the UPCR < 1.699 group, people with UPCR ≥ 1.699 generally had higher SBP, BMI levels, higher rates of urinary occult blood, hypertension, diabetes, history of CVD, RAAS inhibitor use, and use of diuretics. Besides, patients with UPCR ≥ 1.699 had a higher proportion of diabetic nephropathy as the primary disease. In contrast, patients generally had lower HB, ALB, and eGFR levels in the UPCR ≥ 1.699 group. However, the abnormality of the above indicators was closely related to the progress of CKD. When UPCR was >1.699, due to the presence of these CKD progression risk factors, UPCR had a relatively weak effect on the development of CKD progression. On the contrary, when UPCR was <1.699, the risk factors for CKD progressions, such as SBP, BMI, the proportion of hypertension, diabetes, and use of diuretics, were low. The impact on the occurrence of CKD progression was weakened; at this time, the effect of UPCR was relatively enhanced. It provided a further reference for the prevention of CKD progression in patients with different proteinuria states. The findings of this study should be helpful for future research on the establishment of diagnostic or predictive models of CKD progression. Combining the results in **Tables 4**, **5**, it should be pointed out that since the distribution of UPCR was skewed, the impact of a 50% reduction in proteinuria in the high range when UPCR > 1.699 is bigger than a 50% reduction in the lower range when UPCR < 1.699. In addition, using a linear mixed-effects regression model, we assessed the profound effect of baseline UPCR on longitudinal changes in eGFR. We found that the impact of baseline UCPR on changes in eGFR was different at different follow-up times. With a 1 unit increase in the UPCR level, its effect on the decline in eGFR increased continuously with follow-up time. This result may help clinicians understand the impact of baseline proteinuria on short- and long-term renal outcomes in patients with CKD.

One of the currently widely accepted proteinuria-mediated progressive renal injury mechanisms involves a tubulointerstitial injury caused by the direct toxicity of filtered urine protein. Recent studies support that excessive protein accumulation in podocytes is a factor in the progressive damage of glomerular cells through the release of transforming growth factor-beta, which ultimately leads to myofibroblastic differentiation of mesangial cells (30). The underlying pathophysiological mechanism that has been proposed to link albuminuria to cardiovascular disease is peripheral vascular dysfunction, particularly renal endothelial dysfunction, accelerating the atherothrombotic process and thereby increasing cardiovascular risk (31). Early studies have concluded that clustered plasma proteins in the glomerular mesangial region can cause mesangial cell damage and proliferation, increasing mesangial matrix production and eventually aggravating glomerulosclerosis (32).

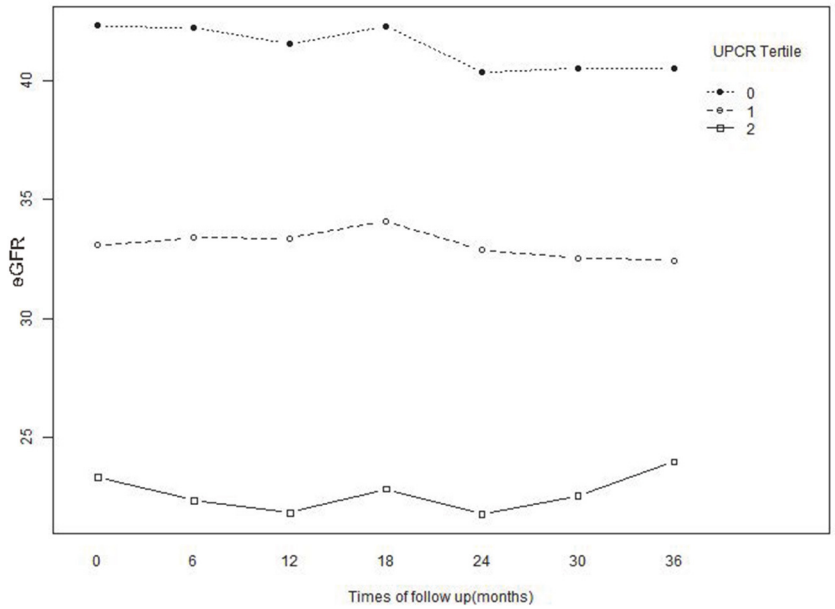


FIGURE 6 | Baseline and predicted 3-year longitudinal changes in eGFR for the patients with UPCR at baseline were divided into three groups according to tertile. The patients whose baseline UPCR was in the lowest tertile showed an accelerated decrease in eGFR compared with the other two tertiles.

Our study has some strengths. (1) Compared with the previous research, the research on the non-linearity addressing was a significant improvement. (2) This observational study was susceptible to residual bias due to unmeasured confounding factors. Therefore, strict statistical adjustment was used to minimize residual confounders. (3) In this study, we tested the robustness of the results through a series of sensitivity analyses (target independent variable transformation, log-likelihood ratio test, and reanalyzing the association between UPCR and CKD progression after excluding patients with UPCR < 0.3, etc.) to ensure the reliability of the results. (4) Using a linear mixed-effects regression model, we assessed the profound effect of baseline UPCR on longitudinal changes in eGFR.

Our research has the following shortcomings and needs attention. First, the data was obtained from the study of the CKD-ROUTE in Japan, and the data has been screened by Iimori et al. (14). Therefore, we could not conclude whether our findings are suitable for people in other areas of a different race. Because this was secondary data analysis, factors not measured in the original study could not be adjusted, such as blood phosphorus level. Second, the attending doctor's diagnosis determined the etiology of CKD. Many patients did not undergo renal biopsy. Third, the effect of diet therapy was not evaluated by a specialized nephrologist. Fourth, the participants enrolled in the present study were patients with CKD stages 2–5. The eGFR of all patients was <90. For patients with CKD stage 1, the relationship between UPCR and CKD progression needs to be further explored. Fifth, the present study only measured UPCR, and other parameters at baseline did not consider changes of UPCR over time. In the future, we can consider designing our studies or collaborating with other researchers to collect as many variables as possible, including patients with CKD stages 1–5 and information on the evolution of UPCR during patients follow-up, to facilitate our better analysis of the impact of change in proteinuria on outcomes as well.

CONCLUSION

This study demonstrates a positive and non-linear relationship between UPCR and incident CKD progression in the Japanese population. There is a threshold effect between the UPCR level and CKD progression. This result is expected to provide a reference for the clinicians to control UPCR. Reducing the UPCR level can significantly reduce the risk of CKD progression and slow down the decline in eGFR levels.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committees of Tokyo Medical and Dental University, School of Medicine (No. 883). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XQ, HH, JC, and XW contributed to the study concept and design, researched and interpreted the data, and drafted the

manuscript. QW and ZW are the guarantors of this work and, as such, had full access to all the data in the study and took responsibility for the data's integrity and the accuracy of the data analysis. All authors read and approved the final manuscript.

FUNDING

This study was supported in part by the Discipline Construction Ability Enhancement Project of the Shenzhen Municipal Health Commission (SZXJ2017031) and Shenzhen Key Medical Discipline Construction Fund (SZXK009).

ACKNOWLEDGMENTS

As this is a secondary analysis, the data and method description are mainly derived from the following research (14). We are grateful to all the authors of the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.854300/full#supplementary-material

REFERENCES

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med.* (2004) 351:1296–305. doi: 10.1056/NEJMoa041031
2. Saran R, Li Y, Robinson B, Abbott KC, Agodoa LY, Ayanian J, et al. US renal data system 2015 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* (2016) 67(3 Suppl. 1):S1–305. doi: 10.1053/j.ajkd.2015.12.014
3. Imai E, Horio M, Watanabe T, Iseki K, Yamagata K, Hara S, et al. Prevalence of chronic kidney disease in the Japanese general population. *Clin Exp Nephrol.* (2009) 13:621–30. doi: 10.1007/s10157-009-0199-x
4. Manns B, Hemmelgarn B, Tonelli M, Au F, Chiasson TC, Dong J, et al. Population based screening for chronic kidney disease: cost effectiveness study. *BMJ.* (2010) 341:c5869. doi: 10.1136/bmj.c5869
5. Chang HL, Wu CC, Lee SP, Chen YK, Su W, Su SL. A predictive model for progression of CKD. *Medicine (Baltimore).* (2019) 98:e16186. doi: 10.1097/MD.0000000000016186
6. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis.* (2000) 35(4 Suppl. 1):S117–31. doi: 10.1016/S0272-6386(00)70239-3
7. Tourojman M, Kirmiz S, Boelkins B, Noyes SL, Davis AT, O'Donnell K, et al. Impact of reduced glomerular filtration rate and proteinuria on overall survival of patients with renal cancer. *J Urol.* (2016) 195:588–93. doi: 10.1016/j.juro.2015.09.083
8. Price CP, Newall RG, Boyd JC. Use of protein:creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systematic review. *Clin Chem.* (2005) 51:1577–86. doi: 10.1373/clinchem.2005.049742
9. Leung YY, Szeto CC, Tam LS, Lam CWK, Li EK, Wong KC, et al. Urine protein-to-creatinine ratio in an untimed urine collection is a reliable measure of proteinuria in lupus nephritis. *Rheumatology.* (2006) 46:649–52. doi: 10.1093/rheumatology/kel360
10. Kulasooriya PN, Bandara SN, Priyadarshani C, Arachchige NS, Dayarathna RK, Karunarathna C, et al. Prediction of microalbuminuria by analysing total urine protein-to-creatinine ratio in diabetic nephropathy patients in rural Sri Lanka. *Ceylon Med J.* (2018) 63:72–7. doi: 10.4038/cmj.v63i2.8687
11. Zhao Y, Zhu L, Liu L, Shi S, Lv J, Zhang H. Measures of urinary protein and albumin in the prediction of progression of IgA nephropathy. *Clin J Am Soc Nephrol.* (2016) 11:947–55. doi: 10.2215/CJN.10150915
12. Fathallah-Shaykh SA, Flynn JT, Pierce CB, Abraham AG, Blydt-Hansen TD, Massengill SF, et al. Progression of pediatric CKD of nonglomerular origin in the CKiD cohort. *Clin J Am Soc Nephrol.* (2015) 10:571–7. doi: 10.2215/CJN.07480714
13. Methven S, MacGregor MS, Traynor JP, O'Reilly DS, Deighan CJ. Assessing proteinuria in chronic kidney disease: protein-creatinine ratio versus albumin-creatinine ratio. *Nephrol Dial Transplant.* (2010) 25:2991–6. doi: 10.1093/ndt/gfq140
14. Iimori S, Naito S, Noda Y, Sato H, Nomura N, Sohara E, et al. Prognosis of chronic kidney disease with normal-range proteinuria: The CKD-ROUTE study. *PLoS ONE.* (2018) 13:e190493. doi: 10.1371/journal.pone.0190493
15. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* (2013) 158:825–30. doi: 10.7326/0003-4819-158-11-201306040-00007
16. Iimori S, Naito S, Noda Y, Nishida H, Kihira H, Yui N, et al. Anaemia management and mortality risk in newly visiting patients with chronic kidney disease in Japan: The CKD-ROUTE study. *Nephrology.* (2015) 20:601–8. doi: 10.1111/nep.12493
17. Iimori S, Noda Y, Okado T, Naito S, Toda T, Chida Y, et al. Baseline characteristics and prevalence of cardiovascular disease in newly visiting or referred chronic kidney disease patients to nephrology centers in Japan: a prospective cohort study. *BMC Nephrol.* (2013) 14:152. doi: 10.1186/1471-2369-14-152
18. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis.* (2009) 53:982–92. doi: 10.1053/j.ajkd.2008.12.034
19. Kikuchi H, Kanda E, Mandai S, Akazawa M, Iimori S, Oi K, et al. Combination of low body mass index and serum albumin level is associated with chronic kidney disease progression: the chronic kidney disease-research of outcomes in treatment and epidemiology (CKD-ROUTE) study. *Clin Exp Nephrol.* (2017) 21:55–62. doi: 10.1007/s10157-016-1251-2
20. Japanese Society of Nephrology. Evidence-based practice guideline for the treatment of CKD. *Clin Exp Nephrol.* (2009) 13:537–66. doi: 10.1007/s10157-009-0237-8
21. Erviti J, Alonso Á, Oliva B, Gorricho J, López A, Timoner J, et al. Oral bisphosphonates are associated with increased risk of subtrochanteric and diaphyseal fractures in elderly women: a nested case–control study. *BMJ Open.* (2013) 3:e2091. doi: 10.1136/bmjopen-2012-002091
22. Yokoyama M, Watanabe T, Otaki Y, Takahashi H, Arimoto T, Shishido T, et al. Association of the aspartate aminotransferase to alanine aminotransferase ratio with BNP level and cardiovascular mortality in the general population: the yamagata study 10-year follow-up. *Dis Mark.* (2016) 2016:1–9. doi: 10.1155/2016/4857917
23. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. *Int J Surg.* (2014) 12:1500–24. doi: 10.1016/j.ijsu.2014.07.014
24. Sinico RA, Mezzina N, Trezzi B, Ghiggeri GM, Radice A. Immunology of membranous nephropathy: from animal models to humans. *Clin Exp Immunol.* (2016) 183:157–65. doi: 10.1111/cei.12729
25. Taavoni M, Arashi M. Estimation in multivariate t linear mixed models for longitudinal data with multiple outputs: application to PBCseq data analysis. *Biom J.* (2022) 64:539–56. doi: 10.1002/bimj.202000015
26. Gueorguieva R, Krystal JH. Move over ANOVA: progress in analyzing repeated-measures data and its reflection in papers published in the archives of general psychiatry. *Arch Gen Psychiatry.* (2004) 61:310–7. doi: 10.1001/archpsyc.61.3.310
27. Cravedi P, Ruggenenti P, Remuzzi G. Proteinuria should be used as a surrogate in CKD. *Nat Rev Nephrol.* (2012) 8:301–6. doi: 10.1038/nrneph.2012.42

28. Iseki K, Ikemiya Y, Iseki C, Takishita S. Proteinuria and the risk of developing end-stage renal disease. *Kidney Int.* (2003) 63:1468–74. doi: 10.1046/j.1523-1755.2003.00868.x
29. Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T, Nguyen AT, Gausson V, Mothu N, et al. Early prediction of IgA nephropathy progression: proteinuria and AOPP are strong prognostic markers. *Kidney Int.* (2004) 66:1606–12. doi: 10.1111/j.1523-1755.2004.00926.x
30. Barnes JL, Gorin Y. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. *Kidney Int.* (2011) 79:944–56. doi: 10.1038/ki.2010.516
31. Stehouwer CD, Smulders YM. Microalbuminuria and risk for cardiovascular disease: analysis of potential mechanisms. *J Am Soc Nephrol.* (2006) 17:2106–11. doi: 10.1681/ASN.2005121288
32. Burton C, Harris KP. The role of proteinuria in the progression of chronic renal failure. *Am J Kidney Dis.* (1996) 27:765–75. doi: 10.1016/S0272-6386(96)90512-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 25 April 2022
doi: 10.3389/fmed.2022.838256

Efficacy of Huangqi Injection in the Treatment of Hypertensive Nephropathy: A Systematic Review and Meta-Analysis

ZhongChi Xu[1†], LiChao Qian[2†], RuGe Niu[1], Ying Yang[1], ChunLing Liu[1] and Xin Lin[1]*

[1] Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [2] Nanjing Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China

Background: Huangqi injection (HQI) is the extract of Astragalus membranaceus (Fisch.) Bunge, which is widely used in the treatment of a variety of diseases in China. It is supposed to be an important adjuvant therapy for hypertensive nephropathy.

Objective: To evaluate the efficacy of HQI combined with antihypertensive drugs in the treatment of hypertensive nephropathy.

Materials and Methods: We systematically searched China National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), Wanfang Knowledge Service Platform (WanfangData), Chinese Biomedical Database (CBM), EMBASE, PubMed and Cochrane Library from their inception to April 23st, 2021. All studies were independently screened by two auditors according to the inclusion and exclusion criteria. Randomized controlled trials comparing HQI in combination with antihypertensive drugs vs. antihypertensive drugs alone were extracted.

Results: The meta-analysis included 15 studies involving 1,483 participants.The effect of HQI combined with antihypertensive drugs is better than that of antihypertensive drugs alone in regulating hypertensive nephropathy for reducing 24-h urinary total protein (24 h UTP) [WMD=-0.29, 95% CI (−0.40, −0.18), $P = 0.000$], microalbuminuria (mALB) [WMD = −17.04, 95% CI (−23.14, −10.94), $P = 0.000$], serum creatinine (SCr) [WMD = −40.39, 95% CI (−70.39, −10.39), $P = 0.008$], systolic blood pressure (SBP) [WMD = −9.50, 95% CI (−14.64, −4.37), $P = 0.000$], diastolic blood pressure (DBP) [WMD = −4.588, 95% CI (−6.036, −3.140), $P = 0.000$], cystatin-C (Cys-c) [WMD = −0.854, 95% CI (−0.99, −0.72), $P = 0.000$], blood urea nitrogen (BUN) [WMD = −4.155, 95% CI (−6.152, −2.157), $P = 0.000$].

Conclusion: The combination of HQI and antihypertensive drugs is more efficient in improving the related indexes of patients with hypertensive nephropathy than using antihypertensive drugs alone, and a moderate dose of HQI (no more than 30 mL) may benefit more. However, the quality of the methodology is low and the number of samples is small, the results need to be confirmed by more stringent randomized controlled trials.

Keywords: hypertensive nephropathy, Astragalus membranaceus (Fisch.) Bunge, Huangqi injection, systematic review, meta-analysis

OPEN ACCESS

Edited by:
Jiannong Liu,
Hennepin Healthcare Research Institute, United States

Reviewed by:
Yubin Lu,
American Academy of Acupuncture and Oriental Medicine, United States
Wen Qian Xu,
Jiangnan University, China

****Correspondence:***
Xin Lin
linxin@njucm.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *17 December 2021*
Accepted: *28 March 2022*
Published: *25 April 2022*

Citation:
Xu Z, Qian L, Niu R, Yang Y, Liu C and Lin X (2022) Efficacy of Huangqi Injection in the Treatment of Hypertensive Nephropathy: A Systematic Review and Meta-Analysis. Front. Med. 9:838256. doi: 10.3389/fmed.2022.838256

INTRODUCTION

Hypertension is one of the important risk factors for cardiovascular events and kidney disease, which is considered to be a potential cause of death and a major health problem in all regions of the world. The prevalence of hypertension in different countries ranges from 22 to 55%, which is expected to increase to 60% by 2025. It has become a global high-burden disease (1). Chronic kidney disease (CKD) is a common complication in patients with hypertension, which plays a major role in the progression of most forms of CKD, including diabetic nephropathy. Hypertension accelerates the progression of kidney disease, and the deterioration of renal function makes it more difficult to control blood pressure, resulting in a vicious circle of progressive renal failure (2, 3). When the glomerular filtration rate (GFR) falls below the critical level, CKD will continue to develop to end-stage renal disease (ESRD). Hypertensive nephropathy is the main cause of ESRD after diabetic nephropathy (4). In the United States, hypertension is the second major cause of ESRD patients. More than 30,000 people are diagnosed with hypertension-related ESRD every year, and the number of patients diagnosed with RSRD continues to grow steadily, which has become a major challenge in the field of public health care (5–7).

Studies found that hypertension is positively related to the occurrence and development of cardiovascular disease and kidney disease. Considering that reducing blood pressure can significantly reduce the risk of chronic kidney disease, active intervention and management of blood pressure should be carried out in patients with hypertension. Moreover, the development of hypertensive nephropathy is related to many factors, such as sympathetic nervous activity (SNA) change, renin-angiotensin-aldosterone system (RAAS) activation, arteriosclerosis, water and sodium retention, and genetic susceptibility (3). Current guidelines recommend that adults with hypertension and chronic kidney disease should control the blood pressure below 130/80 mmHg, with an emphasis on the management of blood pressure and urinary microalbumin, and prefer to RAAS inhibitor drugs, usually in combination with diuretics or calcium antagonists to slow the progression of kidney disease (8). Microalbumin (mALB) which was defined as the excretion rate of urinary albumin between 20–200 mg/min or 30–299 mg/d is an important indicator of cardiovascular events and renal function. The degree of mALB is closely related to the progression of ESRD (9, 10). Studies have shown that after active treatment, mALB can be reduced by more than 30%, while the risk of dialysis in 3–5 years is reduced by 39–72%. Progressive kidney disease can be minimized when albuminuria and blood pressure decrease simultaneously (11, 12). As a part of alternative medical adjuvant therapy, traditional chinese medicine is widely used in the treatment of hypertension and chronic kidney disease. More and more evidence support the point of view (13–15).

Huangqi injection (HQI) is a Chinese herbal medicine which is a water extraction and sterilization solution of dried roots of Astragalus membranaceus (Fisch.) Bunge. HQI has a wide range of pharmacological effects. Ultra-high performance liquid phase tandem quadrupole time-of-flight mass spectrometry has identified 46 active components of HQI, such as saponins, flavonoids and amino acids (16, 17). Studies have shown that HQI can dilate blood vessels, increase coronary and renal blood perfusion, reduce myocardial oxygen consumption, improve renal microcirculation, eliminate lipid peroxides and scavenge ROS (18–20). HQI can inhibit phosphodiesterase activity, reduce cAMP decomposition, increase extracellular calcium (CA^{2+}) inflow and sarcoplasmic reticulum CA^{2+} outflow, increase cardiomyocyte excitability, thus enhance myocardial contractility. HQI can inhibit thromboxane synthesis, reduce blood viscosity, alleviate water retention and increase eGFR by improving arginine vasopressin (AVP) system and AVP-dependent aquaporin2 levels (21). With the study of the pharmacological effects of Astragalus membranaceus (Fisch.) Bunge, HQI is widely used in clinical practice in China, such as in the treatment of coronary heart disease, cardiomyopathy, acute and chronic glomerulonephritis, diabetic nephropathy, as well as hypertensive nephropathy (20, 22).

In recent years, there have been many clinical practices comparing the efficacy of HQI combined with antihypertensive drugs with that of antihypertensive drugs alone in the treatment of hypertensive nephropathy, and the results show that the combined use of HQI and antihypertensive drugs benefits patients, but the efficacy of HQI in the treatment of hypertensive nephropathy has not been laborated. Therefore, we conducted a systematic review and meta-analysis to determine the efficacy of HQI in adjuvant treatment of hypertensive nephropathy.

MATERIALS AND METHODS

Systematic reviews and meta-analyses were designed in accordance with the guidelines of the 2009 Preferred Reporting Project for Systematic Analysis and Meta-analysis (PRISMA) statement (23).

Search Strategy

Literature retrieval is carried out from the electronic network databases from inception to April 23st, 2021, and the retrieval language is not limited. The databases include China National Knowledge Infrastructure (CNKI), China Scientific Journal Database (VIP), WanfangData Knowledge Service Platform (WanfangData), Chinese Biomedical Database (CBM), EMBASE, Cochrane Library and PubMed. The retrieval scheme was based on the combination of subject words and free words, and the search terms included "hypertension," "Hypertensive nephropathy," "Hypertension nephropathy," "Hypertensive renal injury," "Hypertensive renal damage," "Hypertensive kidney injury," "Hypertensive kidney damage," "Astragalus," "Astragalus injection," "Huangqi" and "Huangqi Injection."

Inclusion Criteries

Inclusion studies should meet the following criteria: (1) Randomized Controlled Trials (RCTs) regardless of blinding, publication status, type of publication, or language; (2) Patients meeting the diagnostic criteria of hypertensive nephropathy, ①meeting the diagnostic criteria of hypertension. Hypertension was defined as SBP $\geq$ 140 mmHg or DBP$\geq$90 mmHg, ②appearing clinical patterns of abnormal renal function, such

as increased urinary protein and serum creatinine, ③excluding secondary hypertension and primary renal disease caused by other reasons, other serious diseases or complications; (3) Comparing the intervention with HQI combined with antihypertensive drugs with the treatment in the control group. The intervention measures in the control group included antihypertensive drugs and conventional therapy, and there were no restrictions on the dosage, type, frequency or course of treatment.

Exclusion Criteria

Research that meets the following criteria will be excluded : (1) duplicate publications; (2) basic research, non-clinical studies, systematic review, case report and case discussion; (3) use of any other drugs and/or herbal medicines during the study; (4) clinical trials from which relevant data cannot be extracted; (5) clinical trials that did not meet the expected inclusion criteria.

Study Selection

The levels of serum creatinine (Scr), microalbuminuria (mALB) and 24-h urinary total protein (24 h UTP) were selected as the main outcome indicators. The secondary outcome included blood urea nitrogen (BUN), cystatin C (Cys-c), systolic blood pressure (SBP), diastolic blood pressure (DBP).

Data Extraction

Two researchers independently conducted searches according to the search strategy. Preliminary screening was based on topics and abstracts, excluding studies that were obviously unqualified. For studies that might be eligible, full text screening was performed according to inclusion and exclusion criteria, and data was extracted. Two researchers then cross-checked the studies.Any differences are resolved through discussion or final arbitration verified by a third researcher.

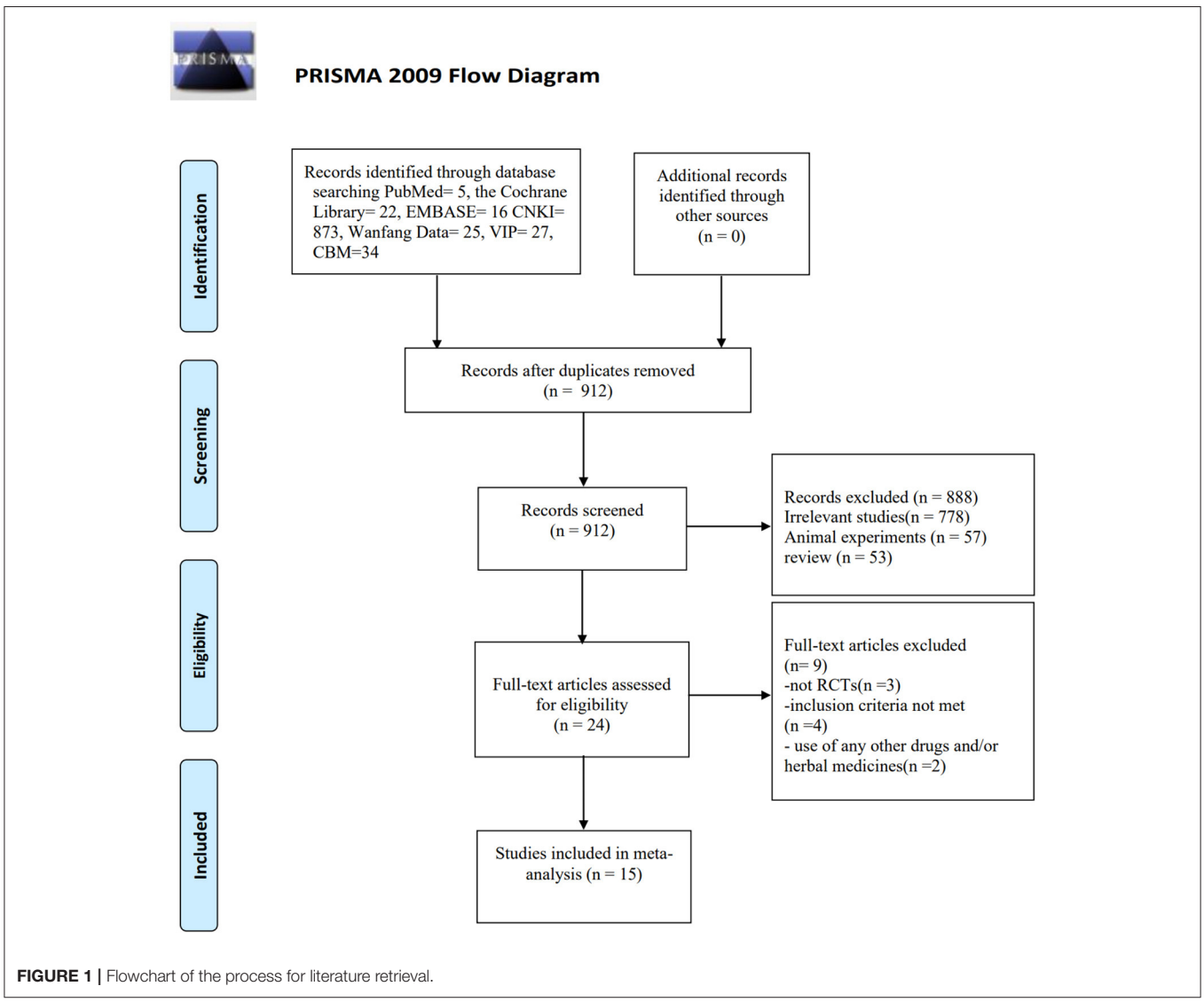


FIGURE 1 | Flowchart of the process for literature retrieval.

TABLE 1 | Characteristics of the included studies.

References	Sample size (T/C)	Sex M/F		Age (years)		Diagnosis standards	Treatment	Control	Duration of treatment	Outcomes	Adverse events
		T	C	T	C						
Chen, (38)	90/90	54/36	51/39	43.25 ± 9.61	42.67 ± 9.72	CGMH (2005)	HQI (30 mL ivgtt qd) +control	Antihypertension therapy (no details) +Low protein, low salt and low fat diet (no details)	30 days	SBP, DBP, Scr, 24 h UTP, MDA, SOD, NO, NOS, Ep, PWVβ, AC	no mentioned
Dong, (29)	26/23	19/7	17/6	65 ± 3.2	64.3 ± 4.5	Diagnosis and Treatment of Nephropathy (Ye Rengao)	HQI (20 mL ivgtt qd) +control	Fosinopril Sodium (10–20 mg/d)	30 days	BUN, Scr	no mentioned
Guo, (32)	47/47	33/14	34/13	57.6 ± 9.3	57.8 ± 9.1	Essential hypertension with proteinuria	HQI (30 mL ivgtt qd) +control	Low protein, low salt and low fat diet (no details) +Irbesartan (150 mg/d)	4 weeks	SBP, DBP, BUN, Scr, Cys-c, 24 h UTP, NAG, α1-MG	no mentioned
Han, (24)	40/38	26/14	22/16	57.2 ± 3.5	57.8 ± 3.3	CGMH (2009)	HQI (40 mL ivgtt qd) +control	Irbesartan (150 mg/d)	30 days	SBP, DBP, hs-CRP, UAER, β2-MG, BUN, Ccr, TC, TG, HDL-C, LDL-C	no mentioned
He, (35)	50/46	40/10	38/8	74.2 (mean)		Essential hypertension with proteinuria	HQI (40 mL ivgtt qd) +control (without PGE1 injection)	Low protein, low salt and low fat diet (no details) +PGE1 injection 200 mg ivgtt qd	45 days	BUN, Scr, Ccr	no mentioned
Huang, (30)	45/45	54/36 (no details)		62.25 ± 8.52 (no details)		CGMH (2005)	HQI (30 mL ivgtt qd) +control	Irbesartan (150 mg/d)	4 weeks	Cys-c, mALB, β2-MG, NAG	no mentioned
Huang, (27)	63/63	41/22	40/23	58.36 ± 6.87	57.93 ± 6.97	Diagnosis and Treatment of Nephropathy (Ye Rengao)	HQI (30 mL ivgtt qd) +control	Irbesartan (150mg/d)	4 weeks	BUN, Scr, 24 h UTP, mALB, Cys-c, LP	Treatment group 2, Control group 4
Ji, (28)	54/40	36/18	23/17	56.3 (mean)	54.6 (mean)	CGMH (2000)	HQI (25 mL ivgtt qd) +control	Antihypertension therapy (no details)	30 days	mALB, β2-MG	no mentioned
Song, (31)	39/39	26/13	25/14	56.87 ± 11.55	55.26 ± 10.47	Nephrology (Wang Haiyan)	HQI (30 mL ivgtt qd) +control	Irbesartan (150 mg/d)	28 days	SBP, DBP, 24 h UTP, Scr, BUN	no mentioned
Tang, (25)	30/30	18/12	16/14	67.10 ± 9.94	62.96 ± 9.54	Nephrology (Wang Haiyan)	HQI (60 mL ivgtt qd) +control	Antihypertension therapy (no details) +Low protein, low salt and low fat diet (no details)	4 weeks	NAG, 24 h UTP, ET-1	no mentioned

(Continued)

TABLE 1 | Continued

References	Sample size (T/C)	Sex M/F		Age (years)		Diagnosis standards	Treatment	Control	Duration of treatment	Outcomes	Adverse events
		T	C	T	C						
Wu, (37)	23/23	15/8	16/7	54 ± 8	55 ± 9	CGMH (1999)	HQI (50 mL ivgtt qd) +control	Antihypertension therapy (no details) +Low protein, low salt and low fat diet (no details)	4 weeks	24 h UTP	no mentioned
Yang, (34)	80/78	51/29	50/28	no mentioned		CGMH (2014)	HQI (40 mL ivgtt qd) +control	Losartan Potassium (50 mg/d) +Hydrochlorothiazide (12.5 mg/d)	4 weeks	SBP, DBP, 24 h UTP, UAER	No adverse events
Zhao, (26)	56/56	31/25	32/24	62.1 ± 7.9	61.6 ± 8.2	Essential hypertension with renal damage	HQI (30 mL ivgtt qd) +control	Captopril (25 mg/d) + Low protein, low salt and low fat diet (no details)	30 days	Scr, BUN, Cys-c, 24 h UTP, NAG, α1-MG	no mentioned
Zhao, (33)	66/66	76/56 (no details)		57.33 ± 10.29 (no details)		Internal Medicine (Lu Zaiying)	HQI (20 mL ivgtt qd) +control	Irbesartan (150 mg/d) + Low protein, low salt and low fat diet (no details)	4 weeks	SBP, DBP, Scr, BUN, 24 h UTP, mAlB	no mentioned
Zhaoyj, (36)	45/45	23/22	22/23	67.6 ± 8.2	68.4 ± 8.7	CGMH (2005)	HQI (40 mL ivgtt qd) +control	Antihypertension therapy (no details) +Low protein, low salt and low fat diet (no details) +Quitting cigarettes and alcohol	4 weeks	SBP, DBP, UAER, β2-MG, TC, TG, HDL-C, LDL-C	No adverse events

T, treatment group; C, control group; CGMH, Chinese guidelines for the management of hypertension; 24 h UTP, 24-h urinary total protein; AC, arterial compliance; BUN, blood urea nitrogen; Ccr, creatinine clearance rate; Cys-c, cystatin C; DBP, diastolic blood pressure; Ep, pressure-strain elasticty modulus; ET-1, endothelin-1; HDL-C, high density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; LP, lipoprotein; mAlB, microalbumin; MDA, malondialdehyde; NAG, N-acetyl-beta-D-glucosaminidase; NO, nitric oxide; NOS, nitric Oxide Synthase; PWVβ, pulse wave velocity β; SBP, systolic blood pressure; Scr, serum creatinine; SOD, superoxide dismutase; TC, total cholesterol; TG, triglycerides; UAER, urinary protein excretion rate; α1-MG, alpha-1- macroglobulin; β2-MG, beta-2-microglobulin.

Quality Assessment

According to the bias risk assessment tool in the Cochrane Handbook for Systematic Reviews, the methodological quality of the included study was evaluated by two researchers. The risk assessment tool consists of seven items: (1) generation of random sequences; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome data; (5) incomplete outcome data; (6) selective reporting; (7) other biases, These items were assessed as having "high bias risk," "low bias risk" or "unclear bias risk" according to the evaluation criteria.

Data Analysis

Stata14.0 software was used to analyze the meta-analysis. For continuous variables, weighted mean difference (WMD) or standardized mean difference (SMD) and 95% CI were used. Heterogeneity is evaluated using I^2 statistics and χ^2 statistics. The effect model was selected according to the results of heterogeneity test, and the fixed effect model was used when $P \geq 0.05$ and $I^2 < 50$. $P < 0.05$ and $I^2 \geq 50$ indicated that the heterogeneity of the results could not be ignored, and we used the random effect model. $P < 0.05$ was considered to be statistically significant. Potential publication bias was tested by egger. The possible abnormal studies were evaluated by sensitivity analysis, and the results were compared with the meta-analysis before exclusion to determine how the excluded study would affect the size of the merger effect and the stability of the meta-analysis. Then we analyzed or eliminated the possible sources of heterogeneity. The indicators with high heterogeneity could not be excluded by subgroup analysis in order to explore the potential causes of heterogeneity according to different interventions or other factors.

RESULTS

Search Results

A total of 1002 articles [Cochrane Library ($n = 22$), PubMed ($n = 5$), EMBASE ($n = 16$), CBM ($n = 34$), CNKI ($n = 873$), WanfangData ($n = 25$) and VIP ($n = 27$)] met the criteria through the search strategy, and 90 of them were excluded due to repeated publication. Eight hundred and eighty-eight articles were excluded after reviewing titles and abstracts. The remaining 24 articles were reviewed in full, a further 9 articles were excluded. Among them, 3 were not RCTs, 4 were not in accordance with the inclusion criteria, and 2 used any other drugs and/or herbal medicines during the study. In the end, the remaining 15 articles were included in the meta-analysis. The filtering flow chart is as follows (**Figure 1**).

Research Characteristics

All 15 RCTs (24–38) enrolled 1,483 patients, including the experimental group (n = 754) and the control group ($n = 729$). In all studies, the control therapy was followed by conventional antihypertensive regimen, while in the observation group, the control therapy was combined with HQI intervention with a dose range of 20–60 mg/d. Detailed information about the included studies is provided in **Table 1**.

Quality Assessment

All of the included studies mentioned randomization, and only five of the trials described the randomization method used in their studies, while the others did not describe specific allocation techniques. None of the studies had procedures for hidden assignment and blinding. Quality assessment is shown in **Figure 2**.

Results of Meta-Analysis

24-H Urinary Total Protein (24 h UTP)

There are 8 studies (25–27, 32–34, 37, 38) included a total of 908 participants reporting 24 h UTP. After heterogeneity was tested ($I^2 = 79.2\%$, $P = 0.000$, **Supplementary Figure 1**), a random effect model was used. A funnel chart analysis of 8 studies showed significant asymmetry, which may be related to publication bias or inclusion of low-quality studies (**Supplementary Figure 2**). Egger test ($P = 0.372$) (**Supplementary Figure 3**) was used to

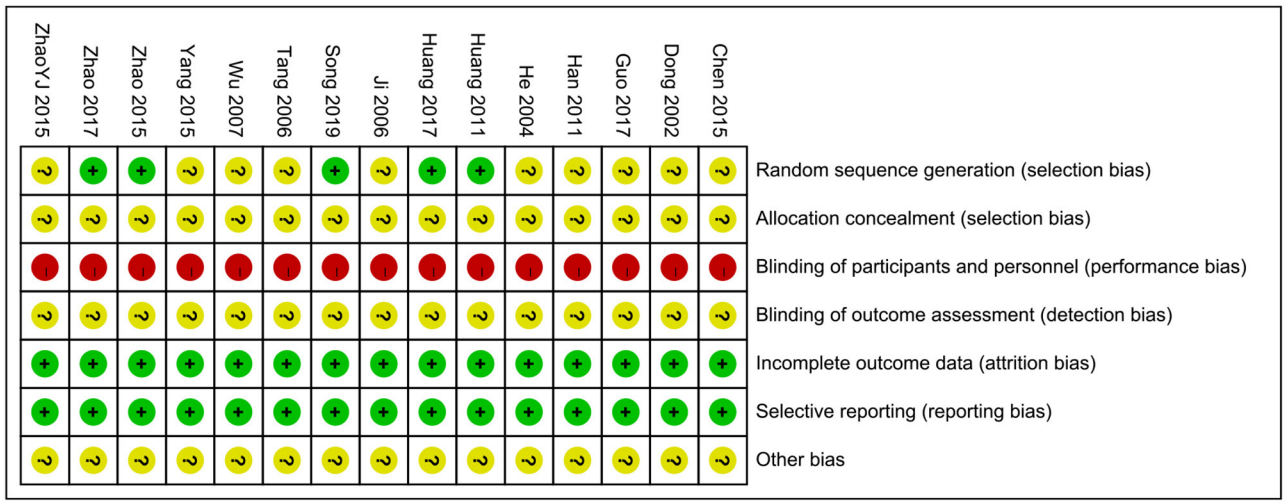


FIGURE 2 | Risk of bias summary.

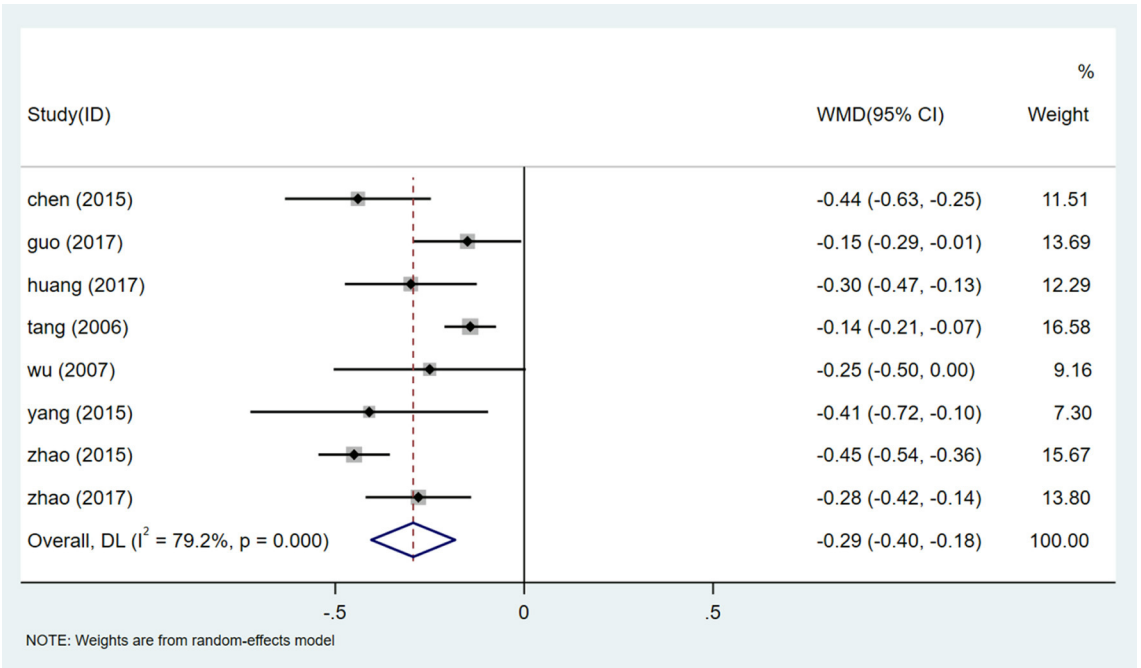


FIGURE 3 | Forest plot of 24 h UTP.

evaluate publication bias, and the results showed that there was no publication bias. The results of meta-analysis showed that the experimental group was superior to the control group in reducing 24h UTP [WMD = −0.29, 95% CI (−0.40, −0.18), $P = 0.000$] (**Figure 3**, **Supplementary Figure 1**). The difference was statistically significant, and patients with HQI combined with routine antihypertensive intervention had more significant efficacy in reducing 24 h UTP.

Sensitivity Analysis of 24 h UTP

We conducted a sensitivity analysis of 24 h UTP (**Supplementary Figure 4**). By excluding one inclusion study one by one, a meta-analysis of the remaining experiments was conducted to determine whether the results had changed significantly. Sensitivity analysis shows that the results of 24 h UTP are very similar and have relatively good stability.

Subgroup Analysis of 24 h UTP

There is a high degree of heterogeneity in the evaluation of 24 h UTP. In order to determine the source of heterogeneity, we included the dose of HQI, antihypertensive regimen, course of treatment, and the year in which the study was published. Univariate Meta regression analysis was performed on the parameters of 8 studies (**Supplementary Figure 5**). The results show that the source of heterogeneity of HQI intervention in 24 h UTP may be related to the course of treatment ($P = 0.001$). The subgroup analysis was carried out based on the course of treatment. The results of meta-analysis showed that the heterogeneity was lower in the subgroup with a treatment cycle of 4 weeks [WMD = −0.212, 95% CI (−0.287, −0.138), $P = 0.000$, $I^2 = 0.0\%$], The results were statistically significant (**Figure 4**). Meta-analysis showed that the results were still statistically significant for subgroups with more than 4 weeks of treatment [WMD = −0.448, 95% CI (−0.287, −0.138), $P = 0.000$, $I^2 = 0.0\%$] (**Supplementary Figure 6**). Results of the subgroup analysis showed a statistically significant reduction in 24h UTP levels in studies that treated for more than 4 weeks compared with studies that treated for 4 weeks ($P = 0.000$). It is suggested that prolonging the treatment period of HQI may benefit patients with a reduction of 24 h UTP.

Microalbumin (mALB)

A total of 442 participants from 4 studies reported mALB levels (27, 28, 30, 33). After heterogeneity test ($I^2 = 74.4\%$, $P = 0.008$, **Supplementary Figure 7**), random effects model was used to summarize the data. Publication bias was assessed by Egger test ($P = 0.629$) (**Supplementary Figure 8**), and sensitivity analysis of mALB was performed (**Supplementary Figure 9**). The results showed that there was no significant publication bias, and meta-analysis was conducted to exclude the study at a time. The results of mALB were similar and relatively stable. The results of meta-analysis show that HQI combined with conventional antihypertensive regimen is more effective in reducing mALB [WMD = −17.04, 95% CI (−23.14, −10.94), $P = 0.000$] (**Figure 5**, **Supplementary Figure 7**). Due to the heterogeneity, different doses of HQI were used for subgroup analysis in our study. The effect of subgroup of not <30 mL/d HQI was used [WMD = −13.375, 95% CI (−16.497, −10.252), $P = 0.000$, I^2

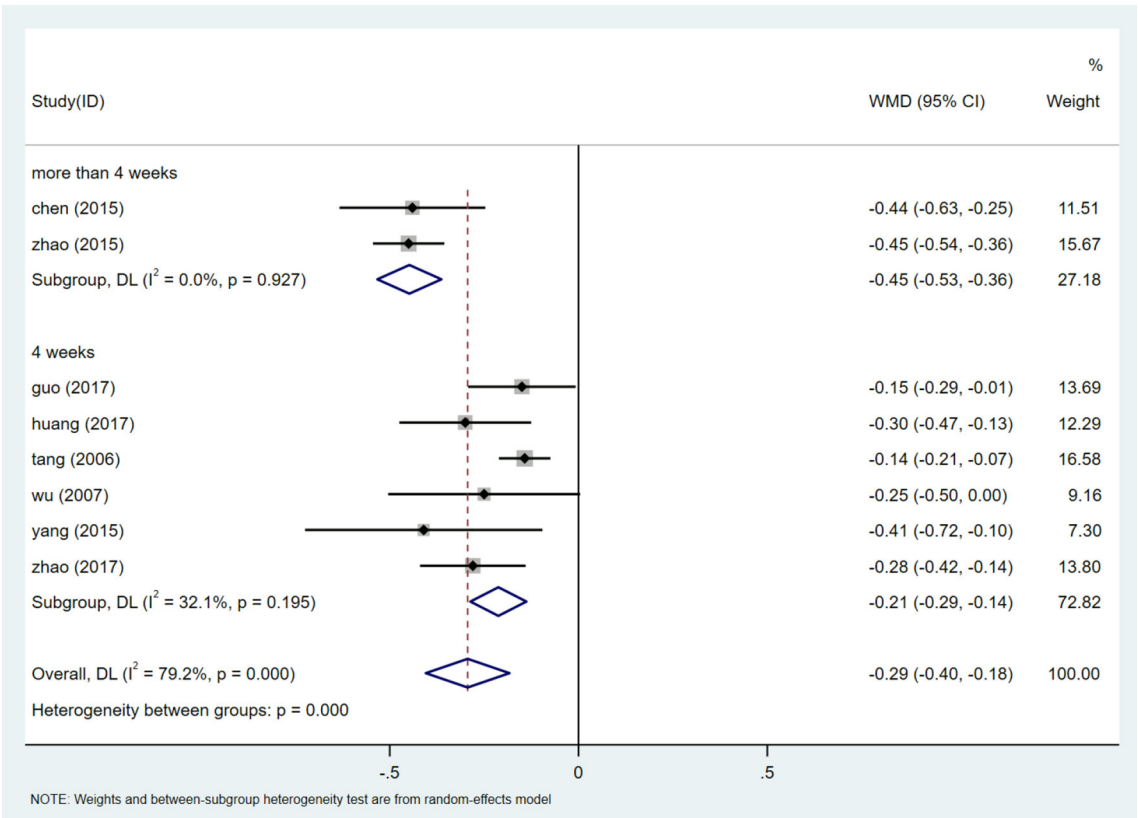


FIGURE 4 | Subgroups analysis of 24 h UTP.

= 0.0%] is more significant than subgroup using HQI below 30 mL/d [WMD = −23.71, 95% CI (−28.85, −18.56), P = 0.000, I^2 = 0.0%], the results were statistically significant (P = 0.001) (**Figure 6**, **Supplementary Figure 10**).

Serum Creatinine (Scr)

Five studies (26, 29, 32, 35, 38) reported Scr analysis, involving a total of 531 participants. After the heterogeneity test (I^2 = 97.6%, P = 0.000, **Supplementary Figure 11**), the random effect model was used to evaluate the data. Egger test (P = 0.659) (**Supplementary Figure 12**) showed that there was no significant publication bias. Sensitivity analysis shows the stability of the results (**Supplementary Figure 13**). Meta-analysis showed that the Scr of patients receiving HQI combined with routine antihypertensive regimen was significantly lower than that of the control group [WMD = −40.39, 95% CI (−70.39, −10.39), P = 0.008] (**Figure 7**, **Supplementary Figure 11**). We also carried out a subgroup analysis based on the selection of conventional antihypertensive schemes. The results showed that HQI combined with irbesartan was effective in reducing Scr [WMD = −61.346, 95% CI (−67.075, −55.617), P = 0.000, I^2 = 0.0%], In the subgroup of HQI combined with other antihypertensive drugs, the decreasing trend of Scr between the experimental group and the control group was not significant [WMD = −17.073, 95% CI (−34.315, 0.169), P = 0.052, I^2 = 41.6%] (**Figure 8**, **Supplementary Figure 14**).

Systolic Blood Pressure (SBP)

There are 7 studies (24, 31–34, 36, 38) involving 810 participants that reported SBP levels. The random effect model was used after heterogeneity test (I^2 = 96.5%, P = 0.000, **Supplementary Figure 15**). We used sensitivity analysis to test the stability of the results (**Supplementary Figure 16**), and Egger test (P = 0.188) (**Supplementary Figure 17**) to evaluate the publication bias of SBP. Meta-analysis showed that patients treated with HQI combined with conventional antihypertensive regimen had better SBP management [WMD = −9.50, 95% CI (−14.64, −4.37), P = 0.000] (**Figure 9**, **Supplementary Figure 15**). The subgroup analysis based on the dose of HQI showed that there was a correlation between the reduction of SBP and the dose of HQI. The results showed that the heterogeneity decreased in the subgroup using more than 30mL HQI [WMD = −3.451, 95% CI (−14.642, −4.366), P = 0.011, I^2 = 0.0%]. the subgroups using less than the dose of 30 mL HQI performed better [WMD = −13.570, 95% CI (−19.506, −7.633), P = 0.000, I^2 = 97.2%] (**Figure 10**, **Supplementary Figure 18**), the results are statistically significant (P = 0.002). It is suggested that patients receiving dose intervention of no more than 30 mL HQI will benefit more

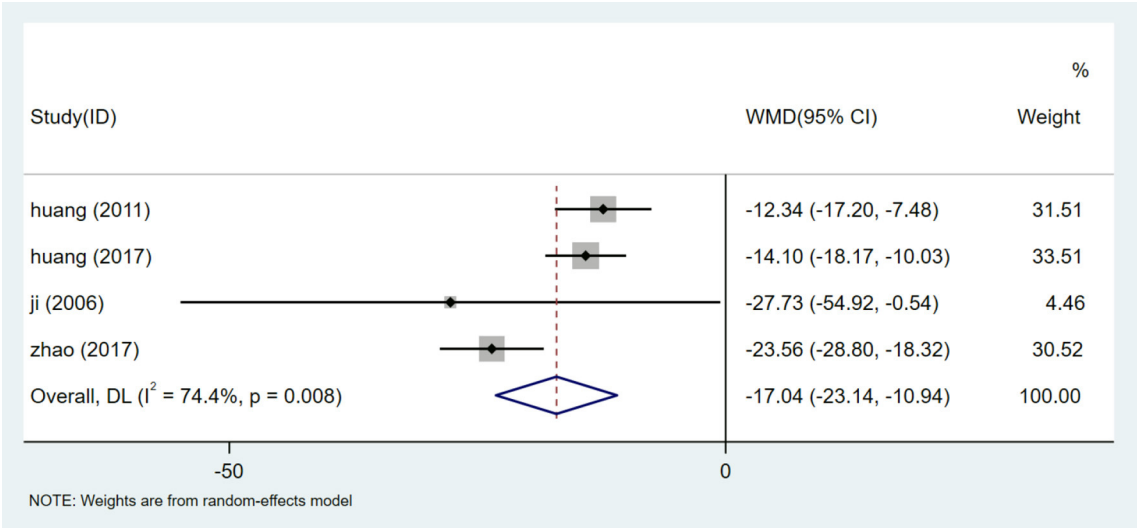


FIGURE 5 | Forest plot of mALB.

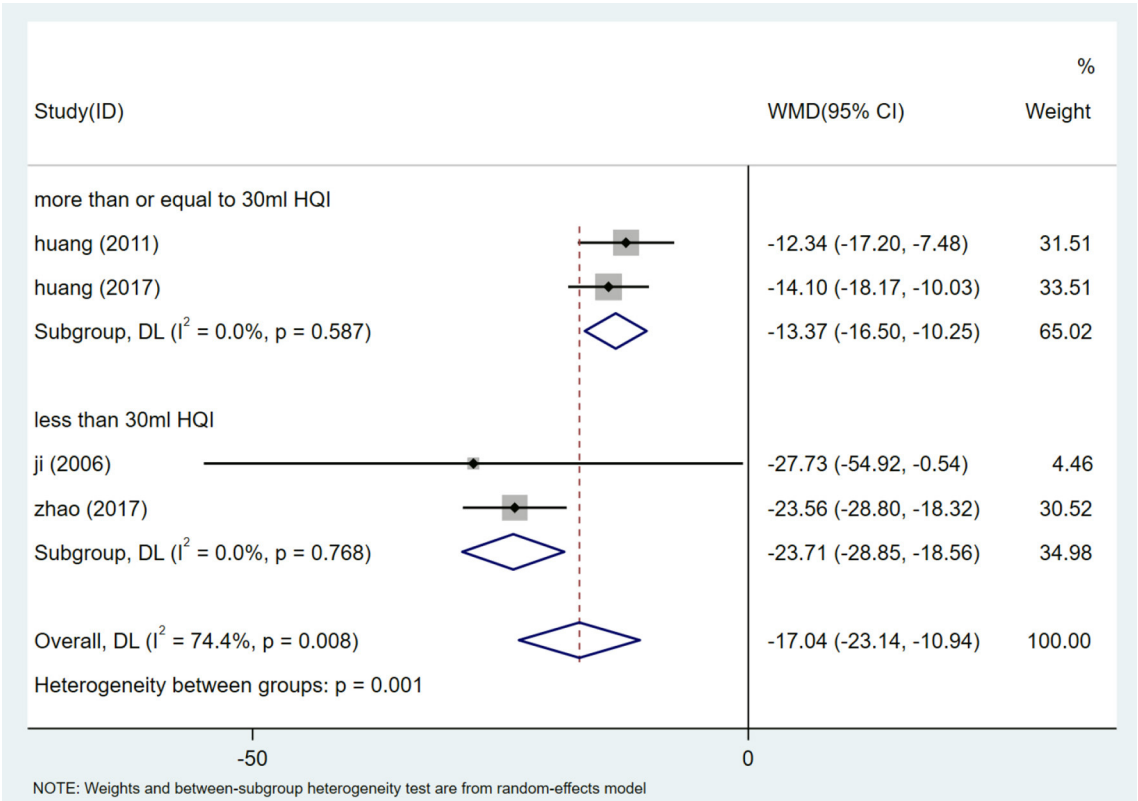


FIGURE 6 | Subgroups analysis of mALB.

than those who receive dose more than 30 mL HQI. However, the subgroup using <30 mL HQI still has high heterogeneity, suggesting that there are other sources of heterogeneity, which may be related to the low quality of the included study.

Diastolic Blood Pressure (DBP)

There are 7 studies (24, 31–34, 36, 38) that involved 810 participants assessed the level of DBP. After heterogeneity test (I^2 = 47.7%, P = 0.075, **Supplementary Figure 19**),

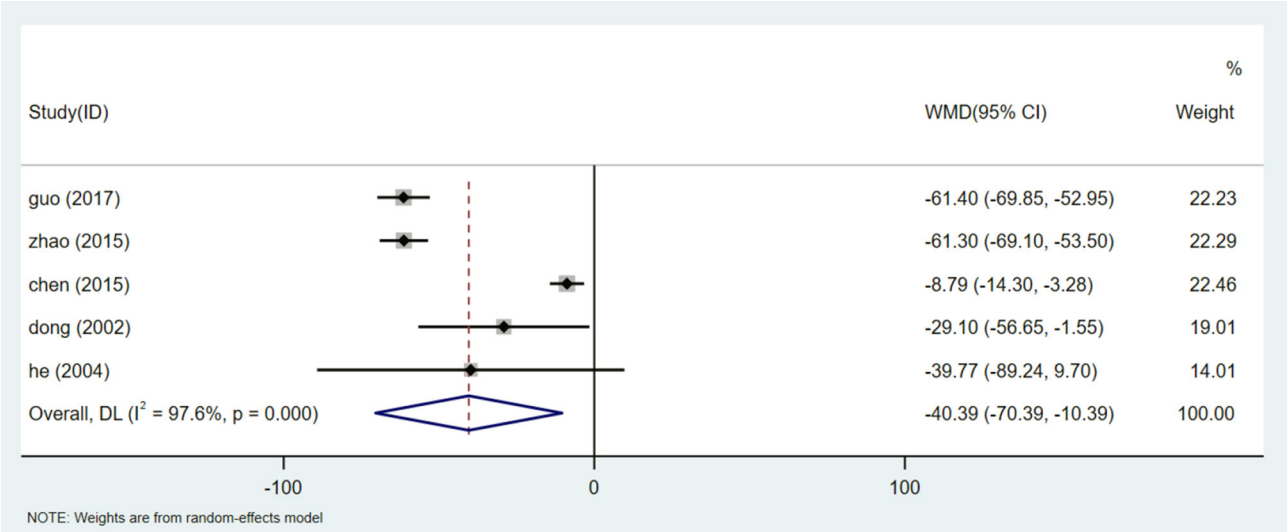


FIGURE 7 | Forest plot of Scr.

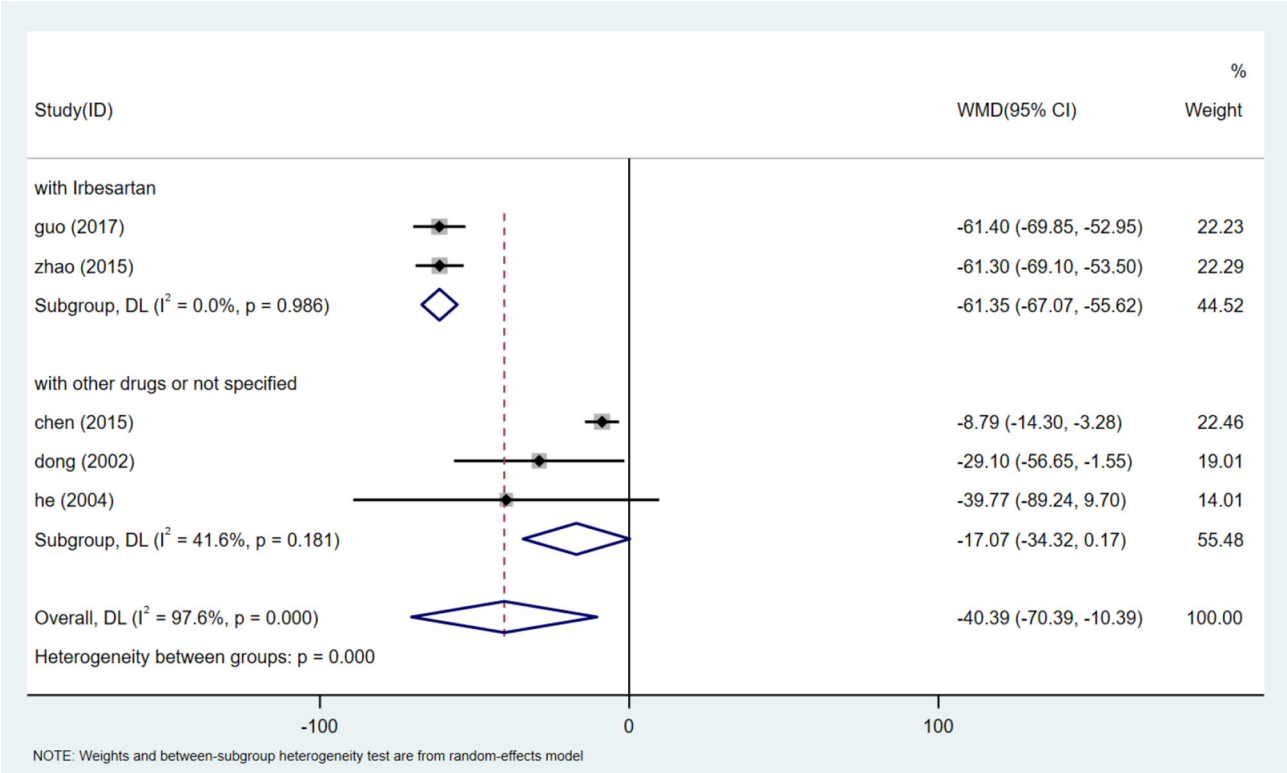


FIGURE 8 | Subgroups analysis of Scr.

a fixed-effect model was used.Egger test ($P = 0.900$) (**Supplementary Figure 20**) was used to evaluate DBP publication bias. Sensitivity analysis showed that the results of DBP were relatively analogical, while the results of successive exclusion of one trial and reanalysis of the meta-analysis were relatively stable (**Supplementary Figure 21**). The results showed that HQI combined with conventional antihypertensive regimen was superior to the control group in reducing DBP level [WMD = −4.588, 95% CI (−6.036, −3.140), $P = 0.000$] (**Figure 11**, **Supplementary Figure 19**). Subgroup analysis

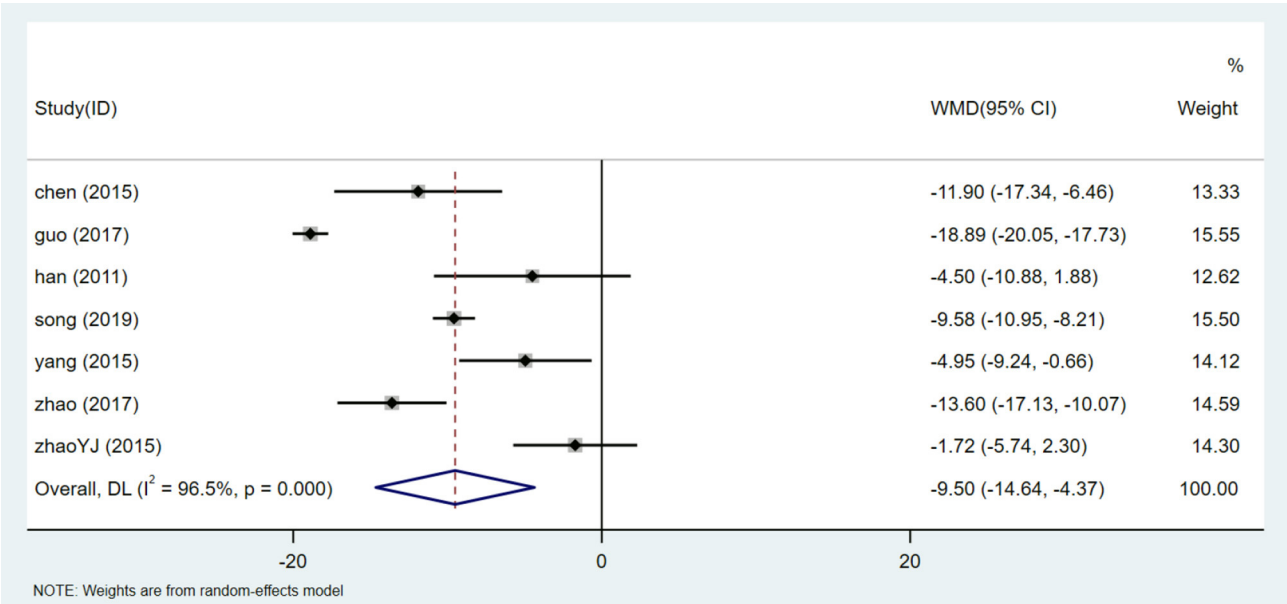


FIGURE 9 | Forest plot of SBP.

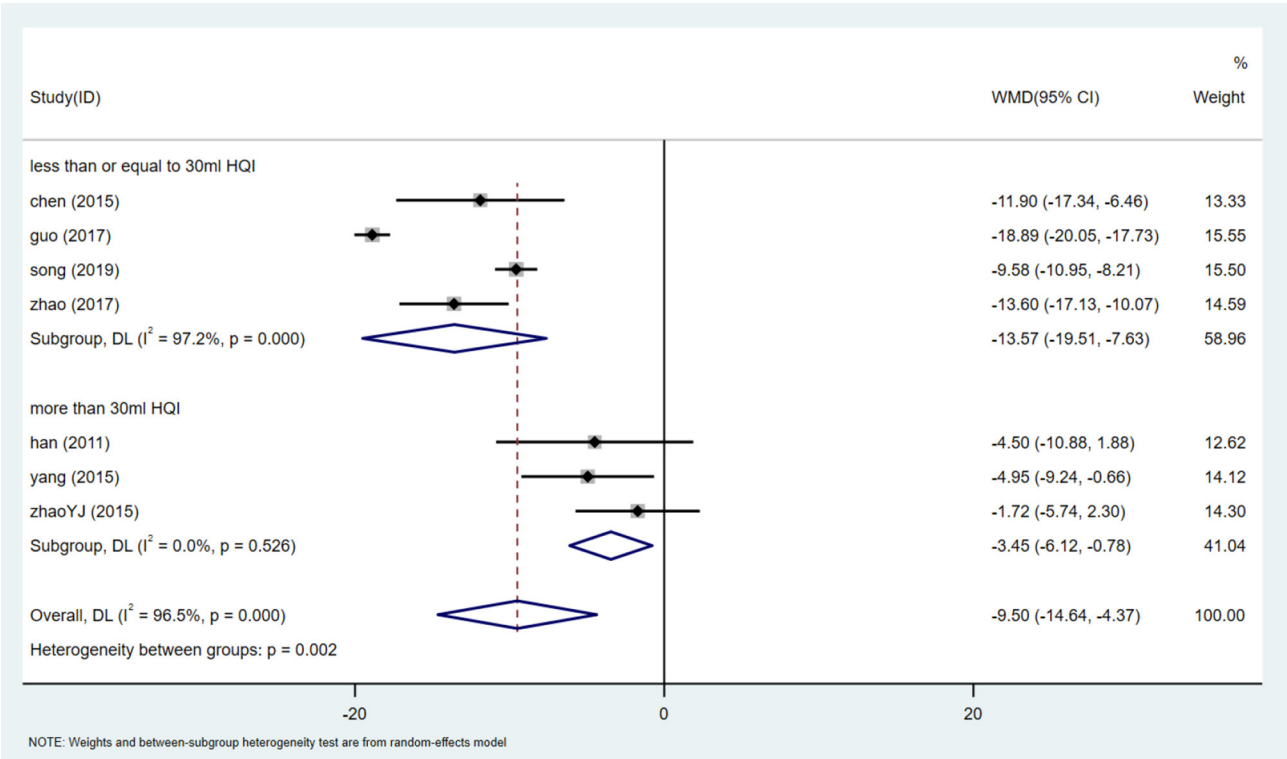


FIGURE 10 | Subgroups analysis of SBP.

based on HQI usage dose showed that subgroup using more than 30 ml HQI [WMD = −4.588, 95% CI (−6.036, −3.140), $P = 0.000$, $I^2 = 0.0\%$] and <30 mL HQI [WMD = −5.623, 95% CI (−7.033, −4.213), $P = 0.000$, $I^2 = 18.2\%$] (**Figure 12**, **Supplementary Figure 22**) both reduce heterogeneity, and the results were statistically significant ($P = 0.010$). There was a

correlation between the decrease of DBP and the dose of HQI, and the results were consistent with the intervention of SBP with HQI combined with conventional antihypertensive regimen.

Cystatin C (Cys-c)

Only four studies (26, 27, 30, 32) included Cys-c levels. After testing the heterogeneity ($I^2 = 76.9\%$, $P = 0.005$, **Supplementary Figure 23**), the random effect model was used. We performed an Egger test ($P = 0.336$) (**Supplementary Figure 24**) to assess publication bias. Considering the high degree of heterogeneity, sensitivity analysis was conducted on Cys-C data. By excluding one study one by one and re-meta-analysis of the other studies (**Supplementary Figure 25**), we found that the study from an article led to an increase in sensitivity. After elimination, meta-analysis was carried out. After heterogeneity test ($I^2 = 0.0\%$, $P = 0.933$, **Supplementary Figure 26**), a fixed effect model was used. Meta-analysis showed lower Cys-C in patients treated with HQI combined with conventional antihypertensive regimens [WMD $= -0.854$, 95% CI (-0.987, -0.721), $P = 0.000$] (**Figure 13**, **Supplementary Figure 26**).

Blood Urea Nitrogen (BUN)

There are 5 studies (26, 29, 32, 33, 35) that showed BUN results. After the heterogeneity test ($I^2 = 88.5\%$, $P = 0.000$, **Supplementary Figure 27**), the random effect model was used. The publication bias was assessed by Egger test ($P = 0.191$) (**Supplementary Figure 28**). High sensitivity was detected, and sensitivity analysis was used, and the results showed that the stability was high (**Supplementary Figure 29**). Meta-analysis showed that the therapeutic effect of HQI combined with conventional antihypertensive regimen was better than that of the control group [WMD $= -4.155$, 95% CI (-6.152, -2.157), $P = 0.000$] (**Figure 14**, **Supplementary Figure 27**).

DISCUSSION

The increase of blood pressure is closely related to the progress of CKD. The prevalence of CKD and ESRD caused by hypertension continues to rise worldwide. It is a challenge to identify biomarkers of early renal insufficiency due to the lack of unified criteria for the diagnosis of hypertensive nephropathy. Hypertension-related renal dysfunction can lead to an increase in serum creatinine (Scr) which eventually lead to irreversible kidney damage. Urinary protein can be used to evaluate hypertension-related kidney damage caused by glomerular disease, and urinary mALB is also considered as a potential marker of early renal dysfunction (9). Scr, urinary protein and urinary microprotein have become important indicators to evaluate the protective effect of antihypertensive drugs on kidney in clinical trials, and that is also the reason why we take them as the main evaluation indexes (39–41). Cys-c is another biomarker that reflects the level of renal function. Studies have shown that Cys-c-based eGFR is superior to Scr-based eGFR in predicting the risk of cardiovascular disease and chronic kidney disease (42). It has important reference value in evaluating the prognosis of hypertensive nephropathy. BUN is usually considered as an important serum marker to evaluate renal function. BUN plays an important role in the diagnosis of renal disease, prediction of cardiovascular events caused by acute heart failure and prognosis in patients with acute myocardial infarction (43, 44).

The incidence of hypertension and hypertension-related chronic kidney disease is increasing year by year, which is an independent risk factor for the morbidity and mortality of cardiovascular disease. Hypertension is not only the cause of kidney disease, but also the result of kidney disease, so hypertension nephropathy is bound to become a heavy burden in the field of public health and medical insurance. Strict antihypertensive treatment and measures to minimize proteinuria can significantly improve the prognosis of patients with hypertensive nephropathy (45–47). Although the pathogenesis of hypertensive nephropathy is unclear, current evidence suggest that RAAS is associated with hypertension and kidney disease (7, 48). The guidelines recommend that RAAS-inhibitor durgs are the first-line choice for hypertensive patients complicated with CKD (8). Unfortunately, the management of blood pressure in patients with hypertensive nephropathy is very difficult. A data from a trial of IDNT showed that only 30% of patients met the goal of systolic blood pressure management (49). Predictably, the proportion of patients whose blood pressure are effectively controlled in clinical practice is much lower than the reported data. Therefore, it is critical to explore other effective treatments for patients with hypertensive nephropathy.

HQI is the extract of Astragalus membranaceus (Fisch.) Bunge. More and more evidence show that HQI can reduce hypertension and protect kidney. Modern pharmacological studies have proved that HQI has a variety of active components. HQI has obvious advantages in the treatment of hypertensive nephropathy. Some pharmacological experiments on the efficacy and mechanism of HQI in treating hypertensive nephropathy have suggested that it plays an important role in improving renal perfusion, managing blood pressure and delaying renal function progression. (1) Improving hemodynamics. Studies have shown that HQI can affect the expression of renal vasoactive substances, improve renal hemodynamics and reduce tissue ischemia and hypoxia (50). (2) Anti-renal fibrosis. Astragalus polysaccharides can reduce the expression of Transforming Growth Factor β (TGF- β) and connective Tissue Growth Factor (CTGF) in renal tissue, reduce the excessive deposition of extracellular matrix and improve interrenal fibrosis (51, 52). (3) Regulation of vascular cell migration and proliferation. Astragaloside can regulate the effect of Protein Kinase D1 (PKD1)-Histone Deacetylase 5 (HDAC5)-Vascular Endothelial Growth Factor (VEGF) on vascular growth, migration and differentiation in rats (53). (4) Blood pressure management. Astragaloside IV can improve the diastolic and systolic function of the heart and has a two-way regulation, thus playing a role in the regulation of blood pressure (54). (5) Protective effect on myocardial ischemia injury. By increasing the content of cAMP, HQI can fully play the role of positive myodynamia, improve the stability of cardiomyocytes, reduce myocardial oxygen consumption, avoid ischemia-reperfusion injury, and improve the ability of myocardial antioxidation at the same time (55). These studies support the positive effects

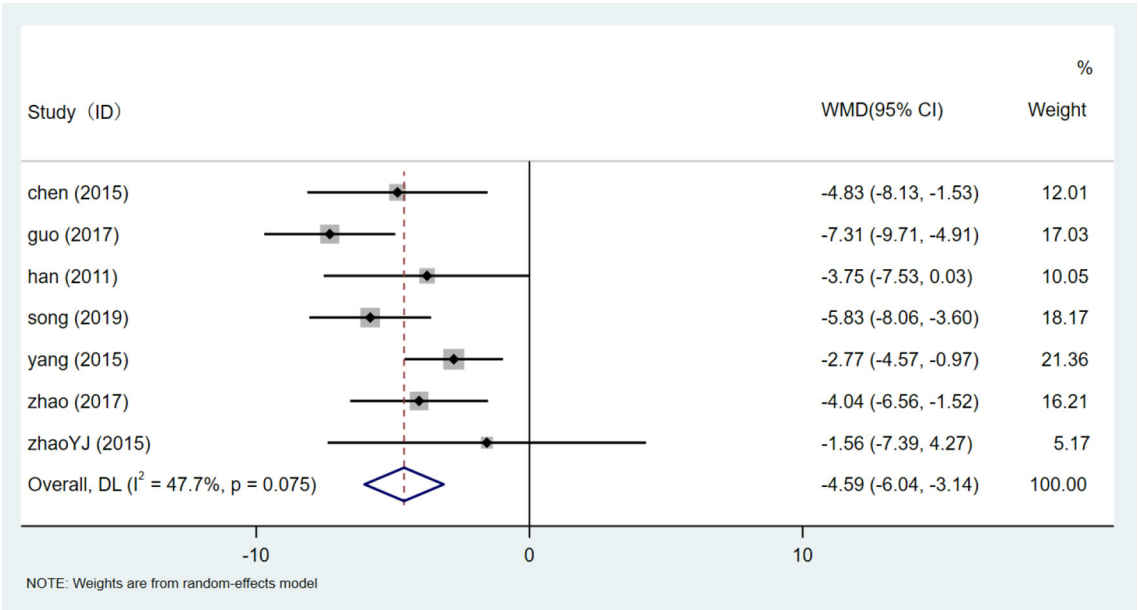


FIGURE 11 | Forest plot of DBP.

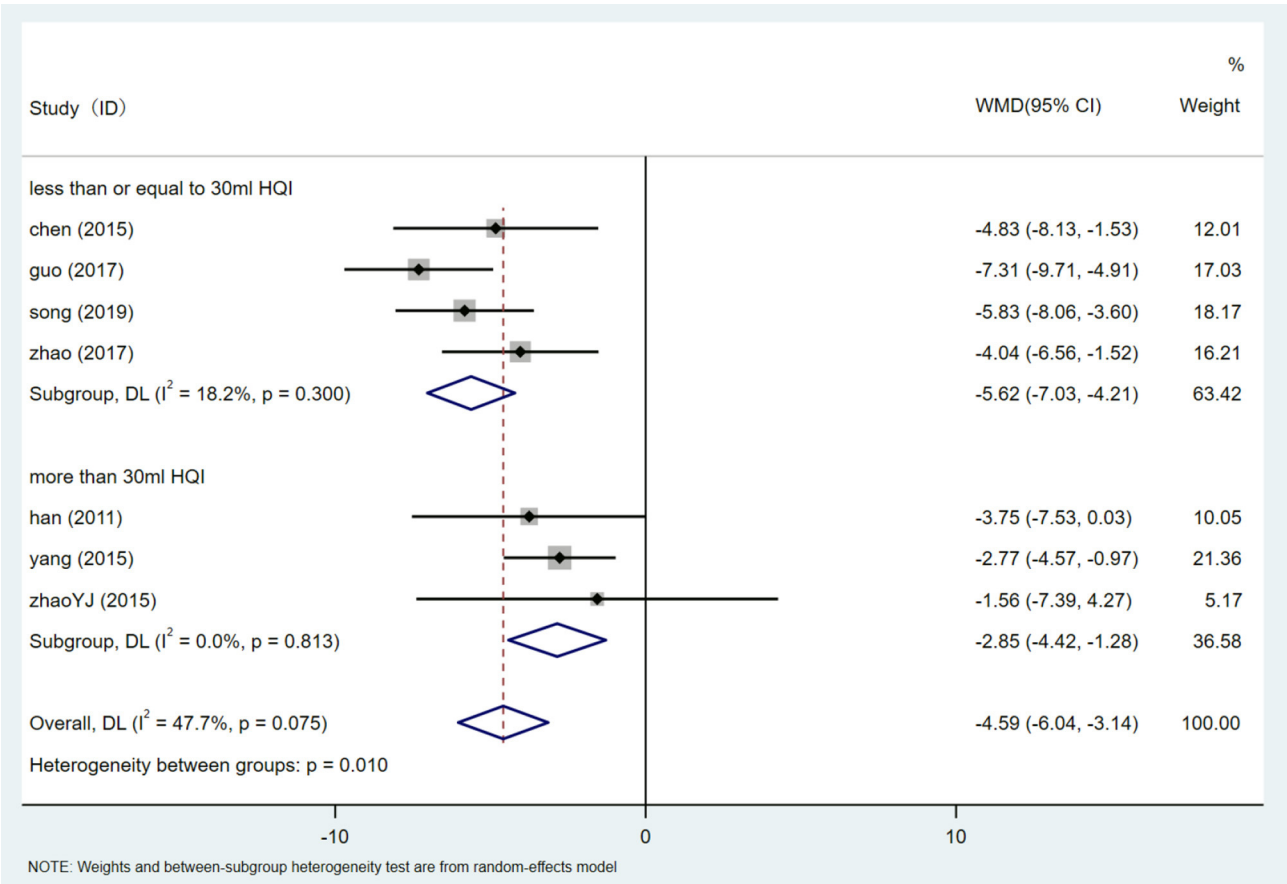


FIGURE 12 | Subgroups analysis of DBP.

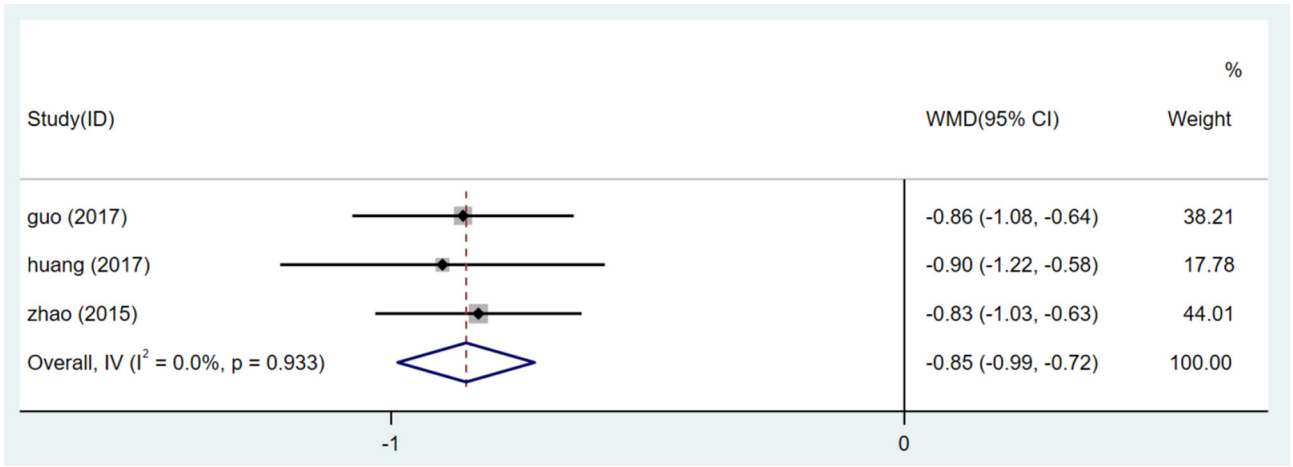


FIGURE 13 | Forest plot of Cys-c.

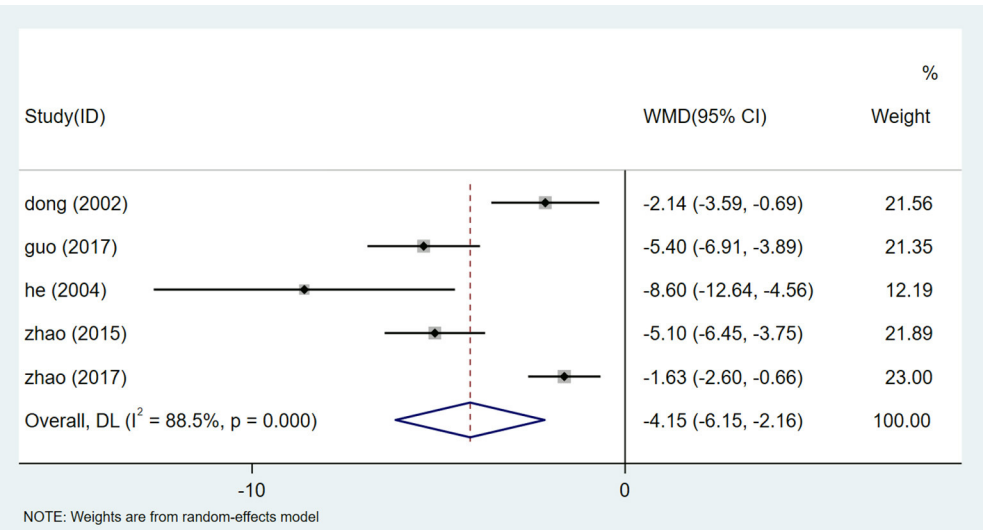


FIGURE 14 | Forest plot of BUN.

of HQI in relieving hypertensive nephropathy, but the efficacy of HQI combined with antihypertensive drugs in hypertensive nephropathy remains to be further reviewed and analyzed.

This meta-analysis included 15 RCTs and involved 1,483 patients to evaluating the relationship between HQI combined with antihypertensive agents and the use of antihypertensive agents alone in the treatment of hypertensive nephropathy. Based on the analysis of available data, we found that the efficacy of HQI combined with antihypertensive drugs is better than antihypertensive drugs used alone in the treatment of hypertensive nephropathy. The results showed that all of the involved patients improved from baseline, but the patients who received HQI combined with conventional antihypertensive therapies were more effective in improving 24 h UTP, mALB, Scr, SBP, DBP, Cys-c and BUN than those who only received conventional antihypertensive therapies. Compared with using antihypertensive drugs alone, patients with hypertensive nephropathy treated with HQI have significant advantages in reducing 24 h UTP. Although the included studies showed a high degree of heterogeneity, we conducted a subgroup analysis based on different courses of treatment. We observed the benefit of prolonging the course of HQI combination therapy in all subgroups with low heterogeneity. HQI combination treatment showed statistically significant advantages in the intervention of mALB, SBP and DBP. Interestingly, in the subgroup analysis based on the dosage of HQI, we observed that the intervention of antihypertensive drugs combined with not exceeding 30 mL HQI in hypertensive nephropathy was

more effective in reducing mALB, SBP and DBP than that of antihypertensive drugs combined with more than 30 mL HQI, and the difference was statistically significant. We believe that the combined treatment dose of 30 mL HQI is a key point, which can achieve the goal of controlling mALB, SBP and DBP, and the use of large doses of HQI will not increase the benefits of patients. Therefore, excessive HQI combination therapy may be unreasonable. Considering that the methodological quality of these randomized controlled trials is low and the number of cases included is relatively small, the reliability of this conclusion needs to be further tested by prospective studies. In terms of Scr level, HQI combined with antihypertensive drugs was more effective than antihypertensive drugs alone. Subgroup analysis showed that HQI combined with irbesartan was superior to HQI combined with other antihypertensive drugs in the treatment of Scr. However, in view of the ambiguity of the description of other types of antihypertensive drugs in the included study, some studies lack specific and detailed drug regimen, and their reliability needs to be further confirmed, and this finding should be carefully interpreted.

Limitations

Several limiting factors should be taken into account in this study. First of all, all the included studies were published in Chinese journals, which may lead to potential ethnic and geographical bias. Secondly, the methodological quality of the included randomized controlled trials is generally low, and all studies claim to be random, but only 5 studies mention the specific information generated by the sequence, so the so-called randomization method is questionable. Third, blinding is an important way to prevent research results from being affected by placebo effects or observer biases. All studies have no information about hidden allocation and participant blindness, which may lead to potential selection biases. Fourth, safety is the basic principle for the provision of herbal products for adjuvant therapy. Twelve studies have not reported adverse drug events and adverse reactions, and there is not enough evidence to conclude safety because some of the active components of the herbal may be unstable. Finally, there are no clear criteria for the diagnosis of hypertensive nephropathy, and the different diagnostic criteria used in the study may affect the reliability of the results.

CONCLUSION

The results of this meta-analysis show that the combination of HQI and antihypertensive drugs is more significant in improving the related indexes of patients with hypertensive nephropathy than using antihypertensive drugs alone, and a evidence dose of HQI (no more than 30 mL) may benefit more. HQI combined with antihypertensive drugs has significant advantages in blood pressure management and renal function improvement in patients with hypertensive nephropathy. However, the quality of the methodology is low and the number of samples is small, the results need to be confirmed by more stringent randomized controlled trials.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZX and CL conceived the study. ZX and LQ evaluated the included studies, conducted data extraction, and drafted manuscripts. RN checked the data with LQ. YY analyze the data. CL and XL supervised all aspects of the study. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (No. 81973762) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX20_1477 and KYCX20_1455).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.838256/full#supplementary-material

Supplementary File 1 | Details of each study.

Supplementary File 2 | Search Strategy.

REFERENCES

1. Lawes CM, Vander HS, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet.* (2008) 371:1513–8. doi: 10.1016/S0140-6736(08)60655-8
2. Rigo D, Orias M. Hypertension and kidney disease progression. *Clin Nephrol.* (2020) 93:103–7. doi: 10.5414/CNP92S118
3. Hart PD, Bakris GL. Hypertensive nephropathy: prevention and treatment recommendations. *Expert Opin Pharmaco.* (2010) 11:2675–86. doi: 10.1517/14656566.2010.485612
4. Bidani AK, Polichnowski AJ, Loutzenhiser R, Griffin KA. Renal microvascular dysfunction, hypertension and CKD progression. *Curr Opin Nephrol Hypertens.* (2013) 22:1–9. doi: 10.1097/MNH.0b013e32835b36c1
5. Udani S, Lazich I, Bakris GL. Epidemiology of hypertensive kidney disease. *Nat Rev Nephrol.* (2011) 7:11–21. doi: 10.1038/nrneph.2010.154
6. Bicescu G. Epidemiology of hypertensive kidney disease: diagnosis. *Maedica (Bucur).* (2010) 5:309–10.
7. Xu J, Zhang C, Shi X, Li J, Liu M, Jiang W, et al. Efficacy and Safety of Sodium Tanshinone IIA Sulfonate Injection on Hypertensive Nephropathy: A Systematic Review and Meta-Analysis. *Front Pharmacol.* (2019) 10:1542. doi: 10.3389/fphar.2019.01542
8. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the

American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. (2018) 138:e426–83.
9. Bakris GL. Implications of Albuminuria on Kidney Disease Progression. The journal of clinical hypertension (Greenwich, Conn.). (2004) 6(11 Suppl 3):18–22. doi: 10.1111/j.1524-6175.2004.04065.x
10. Khosla N, Sarafidis PA, Bakris GL. Microalbuminuria. *Clin Lab Med.* (2006) 26:635–53. doi: 10.1016/j.cll.2006.06.005
11. Atkins RC, Briganti EM, Lewis JB, Hunsicker LG, Braden G, Champion DCP, et al. Proteinuria reduction and progression to renal failure in patients with type 2 diabetes mellitus and overt nephropathy. *Am J Kidney Dis.* (2005) 45:281–7. doi: 10.1053/j.ajkd.2004.10.019
12. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, et al. Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int.* (2004) 65:2309–20. doi: 10.1111/j.1523-1755.2004.00653.x
13. Huang KC, Su YC, Sun MF, Huang ST. Chinese Herbal Medicine Improves the Long-Term Survival Rate of Patients With Chronic Kidney Disease in Taiwan: A Nationwide Retrospective Population-Based Cohort Study. *Front Pharmacol.* (2018) 9:1117. doi: 10.3389/fphar.2018.01117
14. Li Y, Yan S, Qian L, Wu L, Zheng Y, Fang Z. Danhong Injection for the Treatment of Hypertensive Nephropathy: A Systematic Review and Meta-Analysis. *Front Pharmacol.* (2020) 11:909. doi: 10.3389/fphar.2020.00909
15. Wu L, Liu M, Fang Z. Combined Therapy of Hypertensive Nephropathy with Breviscapine Injection and Antihypertensive Drugs: A Systematic Review and a Meta-Analysis. *Evid Based Complement Alternat Med.* (2018) 2018:2958717. doi: 10.1155/2018/2958717
16. Yu H. Analysis of Chemical Components in Huangqi Injection Based on Ultra Performance Liquid Chromatography-Quadrupole-Time-of-Flight Mass Spectrometry. *World Trad Chin Med.* (2019) 14:809–17.
17. Jing L, Zhong-Zhen Z, Hu-Biao C. Review of Astragali Radix. *Chin Herb Med.* (2011) 3:90–105.
18. Peng Z. Analysis of therapeutic effect of Huangqi injection on 80 cases of acute and chronic nephritis. *Chinese Foreign Med.* (2011) 30:28.
19. Ni Z. Effects of astragaloside on the secretion of stroma and expression of β1 integrin in human mesangial cells. *Chin J Nephrol.* (2000) 5:303–7.
20. Gao W. Research progress in clinical application of Huangqi injection. *J Chengde Med Coll.* (2014) 31:129–31.
21. Ma J. Study on arginine vasopressin, V_2 receptor and aquaporin-2 in rats with nephrotic syndrome and the therapeutic effect of astragalus. Journal of Nephrology and Dialysis and Kidney Transplantation. (1999) 4:315–8.
22. Guo X. Research progress on pharmacological effects and clinical application of Huangqi injection. *Chin Pharm.* (2015) 26:3018–21.
23. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
24. Han X. The effect of irbesartan combined with Huangqi injection on renal damage in patients with essential hypertension. *Hebei Trad Chin Med.* (2011) 33:1505–6.
25. Tang G. The Protective Effect of Astragalus on the Early Renal Damage of Hypertension. *Mod J Integr Trad Chin West Med.* (2006) 1:26–27.
26. Zhao Y. Effects of astragalus combined with irbesartan on renal function and urine protein in patients with hypertensive renal impairment. *Hainan Med.* (2015) 26:1028–30.
27. Huang L. Study on the Efficacy and Mechanism of Astragalus Combined with Irbesartan in Treating Hypertensive Nephropathy. *Mod Med Health.* (2017) 33:3456–8.
28. Ji Y. Effect of Huangqi Injection on Kidney Injury of Hypertension. *Chin Med Emerg.* (2006) 11:1237–8.
29. Dong G. Huangqi Injection and Fosinopril Sodium in the Treatment of Hypertensive Renal Damage. *J Hubei Coll Trad Chin Med.* (2002) 2:46.
30. Huang X. Preventive and therapeutic effects of Huangqi injection combined with irbesartan on early renal damage of hypertension. *Shaanxi Med J.* (2011) 40:1663–4.
31. Song P. The effect of huangqi injection combined with irbesartan in the treatment of hypertensive nephropathy. *J Univers Health.* (2019) 21:278.
32. Guo J. Analysis of the effect and mechanism of Huangqi injection combined with irbesartan in the treatment of hypertensive nephropathy. *China Contemp Med.* (2017) 24:76–8.
33. Zhao F. Study on the curative effect of Huangqi injection combined with irbesartan in the treatment of hypertensive nephropathy. *Shaanxi Trad Chin Med.* (2017) 38:51–2.
34. Yang J. Clinical Observation of Huangqi Injection Combined with Hyzaar in the Treatment of Essential Hypertension Complicated with Proteinuria. *TCM Clin Res.* (2015) 7:29–30.
35. He X. Effect of Huangqi Injection on Kidney Injury of Elderly Patients with Hypertension. *J Pract Med.* (2004) 8:712–3.
36. Zhaoyj Y. Clinical Observation of Huangqi Injection in Treating Renal Damage of Primary Hypertension. *Chin J Evid Based Cardiovasc Med.* (2015) 7:248–50.
37. Wu X. Observation on Curative Effect of Huangqi Injection in Treating Primary Hypertension with Renal Damage. *Prim Med Forum.* (2007) 16:717–8.
38. Chen X. Effect of Huangqi Injection on Antioxidant Capacity and Vascular Elasticity in Patients with Renal Hypertension. *J Hainan Med Coll.* (2015) 21:375–7.
39. Yan L, Ma J, Guo X, Tang J, Zhang J, Lu Z, et al. Urinary albumin excretion and prevalence of microalbuminuria in a general Chinese population: a cross-sectional study. *BMC Nephrol.* (2014) 15:165. doi: 10.1186/1471-2369-15-165
40. Kalaitzidis RG, Bakris GL. Serum creatinine vs. albuminuria as biomarkers for the estimation of cardiovascular risk. *Curr Vasc Pharmacol.* (2010) 8:604–11. doi: 10.2174/157016110792006914
41. Bakris GL. Clinical importance of microalbuminuria in diabetes and hypertension. *Curr Hypertens Rep.* (2004) 6:352–6. doi: 10.1007/s11906-004-0053-1
42. Shlipak MG, Matsushita K, Arnlov J, Inker LA, Katz R, Polkinghorne KR, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med.* (2013) 369:932–43. doi: 10.1056/NEJMoa1214234
43. Wei D, Ge M. The spatial distribution of BUN reference values of Chinese healthy adults: a cross-section study. *Int J Biometeorol.* (2018) 62:2099–107. doi: 10.1007/s00484-018-1585-4
44. Ke Z. The value of serum creatinine, cystatin-C, urea nitrogen and urine β_2-microglobulin in early renal injury of type 2 diabetes. *China Medical Herald.* (2013) 10:94–6.
45. Hohenstein K, Watschinger B. Hypertension and the kidney. *Wien Med Wochenschr.* (2008) 158:359–64. doi: 10.1007/s10354-008-0558-3
46. Ravera M, Re M, Deferrari L, Vettoretti S, Deferrari G. Importance of blood pressure control in chronic kidney disease. *J Am Soc Nephrol.* (2006) 17 (4 Suppl 2): S98–103. doi: 10.1681/ASN.2005121319
47. Palmer BF. Proteinuria as a therapeutic target in patients with chronic kidney disease. *Am J Nephrol.* (2007) 27:287–93. doi: 10.1159/000101958
48. Neumann J, Ligtenberg G, Klein II, Koomans HA, Blankestijn PJ. Sympathetic hyperactivity in chronic kidney disease: pathogenesis, clinical relevance, and treatment. *Kidney Int.* (2004) 65:1568–76. doi: 10.1111/j.1523-1755.2004.00552.x
49. Pohl MA, Blumenthal S, Cordonnier DJ, De Alvaro F, Deferrari G, Eisner G, et al. Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. *J Am Soc Nephrol.* (2005) 16:3027–37. doi: 10.1681/ASN.2004110919
50. Chen X, Wang H, Chen L. Effects of astragalus injection on antioxidant capacity and vascular elasticity in patients with renal hypertension. *J Hainan Med Coll.* (2015) 21:375–7.
51. Zhu Y. Antagonistic effect of astragaloside IV on renal interstitial fibrosis. *Liaoning J Trad Chin Med.* (2014) 41:2700–2.
52. Zhang X. Study on the intervention effect and Mechanism of warming Yang, purging turbidity and dredging collaterals on Renal Interstitial Fibrosis in Rats with chronic Renal failure. *Chin Herb Med.* (2019) 50:2133–8.
53. Fu W. Astragaloside IV promotes angiogenesis in rats with myocardial infarction by regulating the PKD1-HDAC5-VEGF pathway. *Chin J Pathophysiol.* (2018) 34:643–9.

54. Wang J. Effects of astragaloside IV on myocardial fibrosis and inflammation in hypertensive rats. *North Pharm.* (2020) 17:187–8.
55. Wei H. Research overview of Huangqi injection in the treatment of chronic cardiac insufficiency. *Chin Pract Med.* (2012) 7:171.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

BRIEF RESEARCH REPORT
published: 12 May 2022
doi: 10.3389/fmed.2022.914250

Fecal Capsule as a Therapeutic Strategy in IgA Nephropathy: A Brief Report

*Wenqiang Zhi[1], Wenzhu Song[2], Yasin Abdi Saed[1], Yi Wang[3] and Yafeng Li[1,4,5]**

[1] Department of Nephrology, The Fifth Hospital (Shanxi Provincial People's Hospital) of Shanxi Medical University, Taiyuan, China, [2] School of Public Health, Shanxi Medical University, Taiyuan, China, [3] The Third Clincial College, Shanxi University of Chinese Medicine, Taiyuan, China, [4] Shanxi Provincial People's Hospital, Shanxi Provincial Key Laboratory of Kidney Disease, Taiyuan, China, [5] Academy of Microbial Ecology, Shanxi Medical University, Taiyuan, China

In this brief report, we reported an IgA nephropathy (IgAN) patient who presented in November 2020 with an acute exacerbation with massive proteinuria and diarrhea. He had the earliest onset in 2018 when his IgAN was diagnosed by renal biopsy. He has been treated with active ACEI/ARB drugs for more than 90 days, intermittent steroid therapy, combined with anti-infective therapy. Although his acute symptoms resolved with each episode, he became increasingly severe as the interval between episodes shortened. Accordingly, the immunosuppressive drugs were administered under the KDIGO guidelines and related guidelines. However, the patient and his family refused this treatment. We pondered over the possible pathogenesis of IgAN, and after a full discussion with the patient and his family, FMT was administered to him after obtaining his informed consent. During the FMT procedure, one healthy volunteer (the doctor himself) also took the FMT capsules. In the end, the patient's urine protein dropped significantly and even turned negative after treatment. Neither the patient nor the healthy volunteer experienced any serious adverse effects during the use of the capsules and the subsequent 6-month follow-up period. We also used metagenomic sequencing to analyze the intestinal flora of patients before and after treatment, and a gradual increase stood out in the abundance of the patient's intestinal flora after drug administration.

Keywords: IgA nephropathy, fecal microbiota transplantation, gut microbiota, FMT capsule, 24-h urine protein quantification

OPEN ACCESS

Edited by:
Jingyuan Xie,
Shanghai Jiao Tong University, China

Reviewed by:
Guochun Chen,
Central South University, China
Xu-jie Zhou,
Peking University, China
Wei Qin,
Sichuan University, China

****Correspondence:***
Yafeng Li
Dr.yafengli@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *06 April 2022*
Accepted: *25 April 2022*
Published: *12 May 2022*

Citation:
Zhi W, Song W, Abdi Saed Y, Wang Y and Li Y (2022) Fecal Capsule as a Therapeutic Strategy in IgA Nephropathy: A Brief Report. Front. Med. 9:914250. doi: 10.3389/fmed.2022.914250

INTRODUCTION

IgA nephropathy (IgAN) is the most common primary glomerular disease worldwide and the leading cause of chronic renal insufficiency and renal failure (1). Approximately 25% of patients with IgAN would be subject to end-stage renal disease (ESRD), which requires renal replacement therapy. It has emerged as a major public health issue globally (2). Its pathogenesis remains to be elucidated, and there's no specific and effective treatments are available, except for optimized supportive care.

DIAGNOSIS AND TREATMENT PROCESS

Case Presentation

Herein, we report a case of a 21-year-old man who repeatedly visited the Department of Nephrology of Shanxi Provincial People's Hospital for hematuria with diarrhea.

The patient initially presented to the emergency department in 2018 with no obvious cause of tea-colored, foamy urine, occasional burning sensation, intermittent lumbar pain. He was examined for a 24-h urine protein, which stood at 3.19 g/24 h, and his blood pressure is 128/76 mmHg. An ultrasound-guided renal biopsy was performed on the second day of admission to clarify the cause of hematuria and proteinuria and the type of renal pathology.

Pathological reports revealed: Fourteen glomeruli, mesangial cells, and mesangial matrix were mildly segmentally hyperplastic with metanephrin deposition, and 1 small cellular crescent. Vacuolar degeneration of renal tubular epithelium, renal interstitium and arterioles showed no definite lesions. Immunofluorescence sections showed no glomeruli, and immunofluorescence with supplemental paraffin: IgG (–) IgA (+) IgM (–) C3 (–) FRA (–) C1q (–) was deposited in mesangial areas in a granular pattern. Immunohistochemical results: HBsAg (–), HBcAg (–), kappa (+), lambda (+). Consider mild mesangial proliferative IgA nephropathy, M0E0S0T0C1. Information on pathology pictures is provided in **Supplementary Material**.

Urine routine showed urine protein 1+, erythrocytes 2+, microscopy erythrocytes 45–50/HP, erythrocyte variability 85%. Prednisone 20 mg/day and Irbesartan 150 mg/day were given to the patient. Prednisone was reduced by 10 mg every 2 months, and further reduced to 5 mg daily for maintenance. 6 months later, the urine protein quantification was rechecked at 0.3 g/24 h with hematuria ±, so the patient stopped hormone intake.

Two years later, the patient had a fever with no apparent cause, with a maximum temperature of 37.6°C. He was readmitted to the Department of Nephrology for hematuria. The examination showed that urine red blood cells were 3+, urine protein was 3+, 24-h urine protein quantification was 3.83 g/24 h. As such, the patient received 5 g of piperacillin sulbactam and 0.1 g/day of anti-infective hydroxychloroquine. With improved symptoms, he was then discharged with regular oral prednisone acetate tablets, which were discontinued after 2 months.

In November 2020, the patient was again admitted to the hospital with meatus hematuria and with dilute stools 4–5 times/day. Clinical examination showed urine protein quantification 2.83 g/24 h, and urine routine examination showed red blood cells 1+, urine protein 3+. Therefore, 5 g of piperacillin sulbactam and 0.1 g/day of anti-infective hydroxychloroquine were given to the patient. Irbesartan 150 mg/day was used for protein reduction. Four days later, the 24-h urine protein quantification reached 4.98 g/24 h.

Fecal Microbiota Transplantation Procedure

Donor screening and FMT capsule preparation were conducted by Dongyuan Yikang, Beijing, China. Donors were first clinically evaluated by questionnaire, including medical history, behavioral risk, and current health status. Potential candidates are then screened fecally and serologically to rule out infectious diseases and potential intestinal dysregulation-related diseases. After screening, 2–3% were eligible enough to be donors. The fresh feces collected from donors were subsequently diluted, filtered, and centrifuged by professional technicians in Dongyuan Yikang's clean laboratory to produce FMT capsules. The patient started the FMT after antibiotic consumption for 1 month. Afterward, FMT capsules were given on days 1, 8, and 15 for one course of treatment. The patient continued to take irbesartan during the FMT treatment.

RESULTS

This patient was followed up for 6 months after treatment and showed a significant downward trend in 24-h urine protein quantification, which is a validated prognostic biomarker. It first decreased to 0.29 g/24 h 1 month after FMT. Surprisingly, his 24-h urine protein quantification eventually witnessed a negative conversion 3 months after FMT treatment (**Figure 1**).

We also used metagenomic sequencing to analyze the intestinal flora of patients before and after treatment. Alpha diversity is to describe the structure of an ecological community with regard to its richness (number of taxonomic groups), evenness (distribution of abundances of the groups), or both (3). Sobs index and Richness index are to show its richness, whereas Shannon index is to reflect its evenness. **Figures 2A–C** showed us the gradual increase of alpha-diversity, which was close to the healthy control, indicating the recovery of intestinal composition. The healthy control exhibited no obvious change but slight fluctuation. Meanwhile, no discomfort or side effects were observed, which, in a sense, demonstrated the efficacy and safety of the FMT capsule.

In general, a gradual increase stood out in the abundance of the patient's intestinal flora after drug administration. However, some bacterial abundance were on the increase, such as Bacteroides_fragilis, Bacteroides_ovatus and Bacteroides_stercoris, and some were on the wane, including Eggerthella_lenta, Bilophila_wadsworthia, and Escherichia_coli (**Figure 2D**).

DISCUSSION

New discoveries suggest that the pathogenesis of IgAN involves excessive circulation of gd-IgA which is target of anti-glycan antibodies. Immune complexes formed by gd-IgA and anti-glycan antibodies ultimately deposit in the mesangium, causing mesangial cell proliferation, secretion of pro-inflammatory cytokines and complement activation (4). More questions are being asked about the origin of focal aberrant IgA, mainly in the mucosal immune response. Previous studies have focused on the hyperresponsiveness of the upper respiratory tract and tonsil mucosa, but current studies exhibit that altered gut microbial composition and immune and metabolic dysregulation

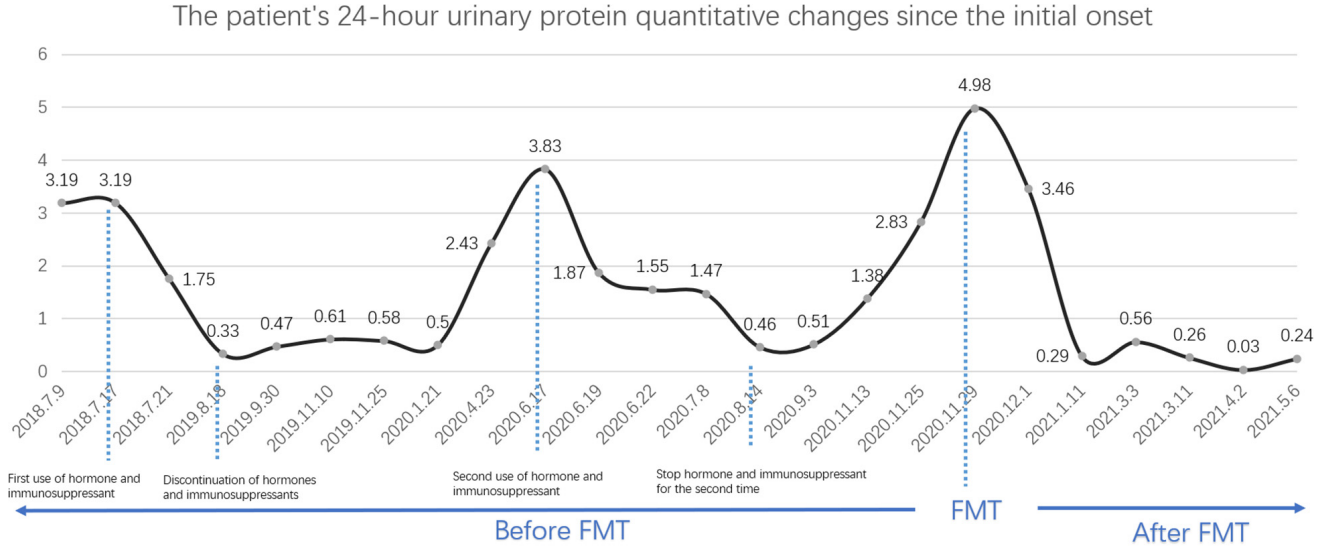


FIGURE 1 | Patient's 24-h urine protein quantitative changes since the initial onset.

of the gut may have a combined role in the development of IgAN through the intestinal-renal axis mechanism, a hypothesis that also provides a theoretical support for the treatment of IgAN by regulation of gut microbial composition. Some studies demonstrated that risk genotypes concerning gut microbial composition are associated with higher serum galactose-deficient IgA1 (GdIgA1) levels (5). Related studies have revealed that mucosal infections can cause acute episodes of IgAN (6), that changes in the microbiota are also associated with the severity of IgAN (7, 8).

Based on the current study, we believe that the future treatment of IgAN will be primarily targeted at the mucosal microbiota, together with some novel agents (such as hydroxychloroquine). Fecal microbiota transplantation (FMT), which involves functional flora transplantation into a diseased gut microbiota, helps to re-establish a normal intestinal flora composition and has been used in various intestinal diseases such as CDI with good efficacy and safety (9). After we performed FMT in this patient, his clinical symptoms were dramatically relieved, the intestinal microbial diversity was significantly increased, which undoubtedly provides a new idea for the treatment of IgAN.

Since the first episode in 2008, the patient has been treated with irbesartan at a starting dose of 150 mg/day and has maintained this dose for a long period, including during each acute episode and FMT implementation. On first admission, the patient's blood pressure fluctuated at 113/68 mmHg–135/78 mmHg with a maximum of 135/78 mmhg only once. Considering the patient's good pressure control, the physician did not treat this patient at the maximum dose. The patient discontinued hormonal therapy and anti-infective therapy in the middle of the disease course. Initially, significant results were obtained with hormonal and anti-infective therapy, but in the subsequent course, patients had shorter intervals between each acute episode, more severe infectious symptoms and increased proteinuria. After the first hormonal and anti-infective treatment, the patient was in remission and maintained so for 23 months. However, the most recent acute episode was only 3 months after previous episode, and the infection symptoms were significantly aggravated, accompanied by sore throat, expectoration, diarrhea 4–5 times/day in the form of loose stools. Before admission, he was self-administered amoxicillin 1 g/dose, 3 times/day, orally. There was an improvement in diarrhea symptoms but no significant improvement in hematuria. Antibiotic therapy was empirically used by the physician after admission, and irbesartan was maintained. Five days after admission, the patient's symptoms of hematuria and sore throat and diarrhea improved, but proteinuria was not effectively controlled and appeared progressively elevated. Seventeen days after discontinuation of antibiotics, the patient was treated with FMT.

In terms of gut microbiota, the patient presented with an increased intestinal flora diversity after FMT, accompanied by an increase of Clostridium_symbiosum, a bacterium whose abundance is positively correlated with renal ACE2 expression (10). Yet, ACE2 deficiency is associated with renal impairment, renal fibrosis, and other kidney-related diseases. Of note, Bacteroides_ovatus has also increased after FMT; It was found that different bacterial strains also vary in the induction of IgA (11). It remains unclear whether the increased Bacteroides_ovatus would induce more IgA production in humans. More follow-up studies are needed, and our ongoing recruitment of IgAN patients would shed light on the alteration of this bacterial strain.

FMT has now been applied to a variety of diseases associated with intestinal dysbioses, such as CDI, IBD, IBS, obesity, and diabetes; it shows efficacy and safety in the above different diseases (12), but a paucity of research on its use in IgAN has been carried out. The patient in our study was treated with FMT

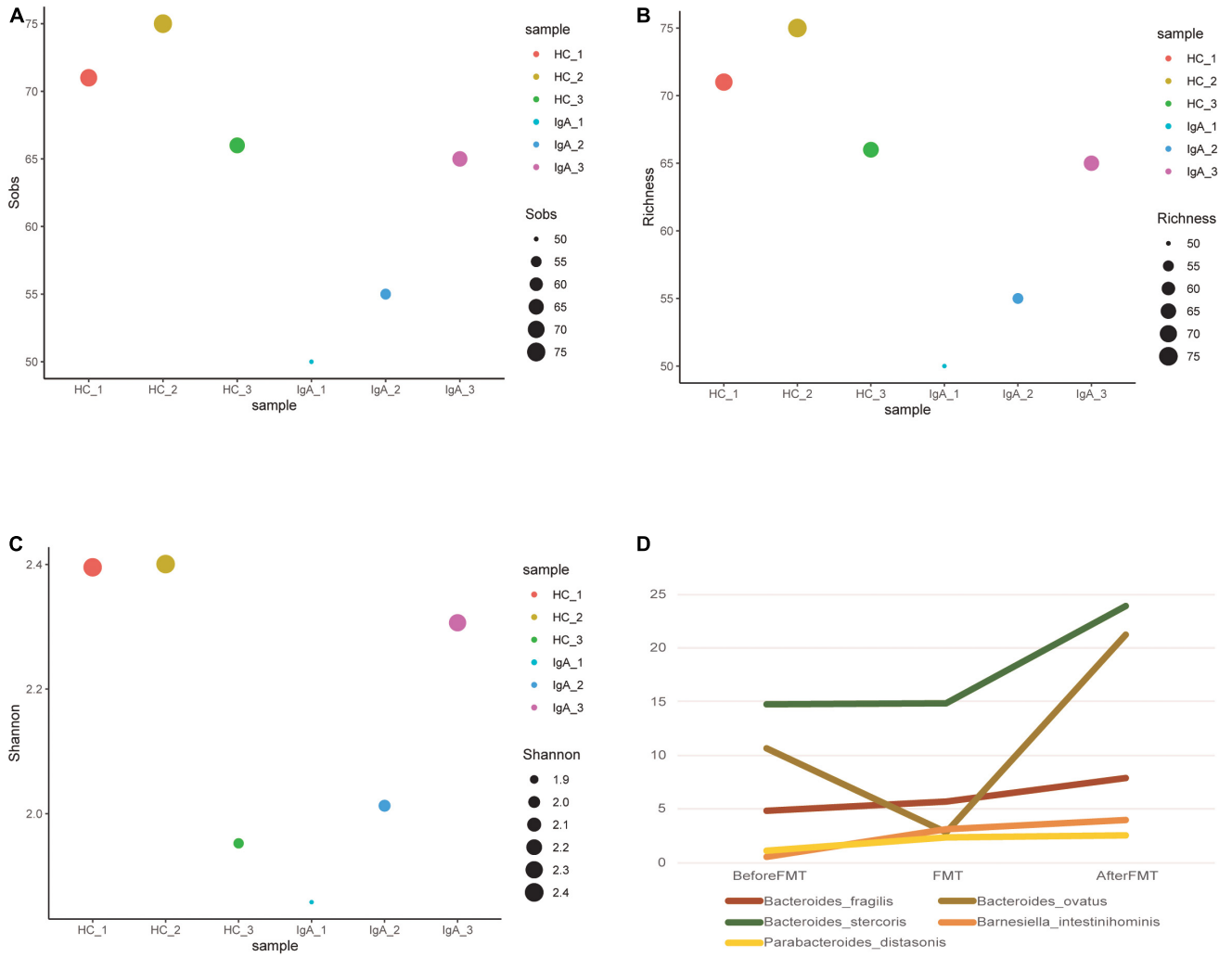


FIGURE 2 | Changes in alpha diversity and main bacterial species before and after FMT. **(A–C)** Were to show the diversity of IgAN patient and healthy control in this study. **(A,B)** Were to reflect the Richness, and **(C)** was to show the Evenness. The larger the point, the larger the value is. Different color means different samples before or after FMT. **(D)** Was to reflect the changes of main bacterial strains.

capsules, a simpler procedure with lower incidence of side effects, lower risk of bleeding or perforation by endoscopic operation and thus higher acceptance, compared with traditional enema or endoscopy. After FMT, the patient's proteinuria turned negative, with no significant side effects during treatment and follow-up, showing good efficacy and safety.

In this report, limitation also emerges that more clinical evidence is required to prove the efficacy and safety of FMT in IgAN. Regarding other indicators reflecting prognosis, we didn't conduct a regular monthly test for them, such as serum creatinine, as there were no significant abnormalities in the patient's renal function or serum albumin. As such, the effect of FMT on these indicators still needs to be further verified. Meanwhile, with regard to the timing of FMT capsule use as well as usage, we previously referenced the standard usage provided by the capsule preparer, and the optimal usage for patients with IgA still needs further validation. Since, we are preparing for a single-center clinical trial, whose protocol was approved by the ethics committee of Shanxi Provincial People's Hospital. Besides, we have managed to register the protocol in Chinese Clinical Trial Registry Center (ChiCTR2100053206), and in ClinicalTrials.gov (NCT05182775).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was gained from the patient and the treatment was approved by the ethics

committee for clinical trials of Shanxi Provincial People's Hospital.

AUTHOR CONTRIBUTIONS

WZ drafted the original manuscript. WS helped polish the manuscript and responsible for the Figure. YA and YW revised the manuscript. YL gave the overall design of this manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Natural Science Foundation of China, Gut microbiota promotes IgAN through CASP-1/IL-1β-driven increased intestinal permeability (grant no 82170716).

ACKNOWLEDGMENTS

We thank the patient and his family for participating in this study, and Shanxi Provincial Key Laboratory of Kidney Disease for bioinformatics analysis. We are also grateful to the physician who served as a volunteer.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.914250/full#supplementary-material

REFERENCES

1. Suzuki H, Novak J. IgA glycosylation and immune complex formation in IgAN. *Semin Immunopathol.* (2021) 43:669–78. doi: 10.1007/s00281-021-00883-8
2. Jarrick S, Lundberg S, Welander A, Carrero JJ, Höijer J, Bottai M, et al. Mortality in IgA nephropathy: a nationwide population-based cohort study. *J Am Soc Nephrol.* (2019) 30:866–76. doi: 10.1681/ASN.2018101017
3. Willis AD. Rarefaction, alpha diversity, and statistics. *Front Microbiol.* (2019) 10:2407. doi: 10.3389/fmicb.2019.02407
4. Lai KN, Tang SC, Schena FP, Novak J, Tomino Y, Fogo AB, et al. IgA nephropathy. *Nat Rev Dis Primers.* (2016) 2:16001.
5. He JW, Zhou XJ, Li YF, Wang YN, Liu LJ, Shi SF, et al. Associations of genetic variants contributing to gut microbiota composition in immunoglobin a nephropathy. *mSystems.* (2021) 6:e819–20. doi: 10.1128/mSystems.00819-20
6. Selvaskandan H, Barratt J, Cheung CK. Immunological drivers of IgA nephropathy: exploring the mucosa-kidney link. *Int J Immunogenet.* (2022) 49:8–21. doi: 10.1111/iji.12561
7. Watanabe H, Goto S, Mori H, Higashi K, Hosomichi K, Aizawa N, et al. Comprehensive microbiome analysis of tonsillar crypts in IgA nephropathy. *Nephrol Dial Transplant.* (2017) 32:2072–9. doi: 10.1093/ndt/gfw343
8. Cao Y, Qiao M, Tian Z, Yu Y, Xu B, Lao W, et al. Comparative analyses of subgingival microbiome in chronic periodontitis patients with and without IgA nephropathy by high throughput 16S rRNA sequencing. *Cell Physiol Biochem.* (2018) 47:774–83. doi: 10.1159/000490029
9. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, et al. Fecal microbiota transplantation: review and update. *J Formos Med Assoc.* (2019) 118(Suppl 1):S23–31. doi: 10.1016/j.jfma.2018.08.011
10. Snelson M, Muralitharan RR, Dinakis E, Nakai M, Jama HA, Shihata WA, et al. Renal ACE2 (Angiotensin-Converting Enzyme 2) expression is modulated by dietary fiber intake, gut microbiota, and their metabolites. *Hypertension.* (2021) 77:e53–5. doi: 10.1161/HYPERTENSIONAHA.121.17039
11. Yang C, Mogno I, Contijoch EJ, Borgerding JN, Aggarwala V, Li Z, et al. Fecal IgA levels are determined by strain-level differences in *Bacteroides ovatus* and are modifiable by gut microbiota manipulation. *Cell Host Microbe.* (2020) 27:467–75.e466. doi: 10.1016/j.chom.2020.01.016
12. Aroniadis OC, Brandt LJ. Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol.* (2013) 29:79–84. doi: 10.1097/mog.0b013e32835a4b3e

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 27 May 2022
doi: 10.3389/fmed.2022.874916

Induction of Pyroptosis in Renal Tubular Epithelial Cells Using High Glucose

*Yinghong Liu, Mingyue He, Hao Xiong and Fang Yuan**

Hunan Key Laboratory of Kidney Disease and Blood Purification, Department of Nephrology, The Second Xiangya Hospital of Central South University, Changsha, China

Background: The micro-inflammatory state is important for the occurrence of diabetic kidney disease (DKD). Here, we aimed to explore the expression of pyroptosis related indicators and ultrastructural characteristics in DKD, and investigate pyroptosis in renal tubular epithelial cells induced by high glucose.

Methods: Immunohistochemistry was used to detect expression of the inflammation-related protein NOD-like receptor protein 3 (NLRP3) and pyroptosis key protein gasdermin D (GSDMD) in kidney tissues of DKD patients. HK-2 cells were cultured *in vitro* and stimulated with different concentrations of glucose. The changes in HK-2 cell ultrastructure were observed using electronmicroscopy, and western blot was used to detect NLRP3, caspase-1 p20, GSDMD-N, interleukin (IL)-1β, and IL-18 expression.

Results: NLRP3 and GSDMD expression in kidney tissues of DKD patients was higher than that in control subjects. Further, GSDMD expression was positively correlated with that of NLRP3 ($r = 0.847$, $P = 0.02$). After stimulating HK-2 cells for 24 h with different glucose concentrations, compared with the control group, the 15 and 30 mmol/L glucose groups showed typical ultrastructural changes of pyroptosis. The protein expression of NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18 expression in high glucose group increased significantly compared with the control group, and was glucose-concentration-dependent.

Conclusion: High glucose can activate inflammasome, cause inflammatory cytokines release, and induce pyroptosis in HK-2 cells. NLRP3-caspase-1 may be involved in GSDMD-mediated pyroptosis. This study shows a novel relationship between glucose concentration and pyroptosis, which can be studied further to design better therapies for patients with DKD.

Keywords: diabetic kidney disease, GSDMD, NLRP3 inflammasome, pyroptosis, renal tubular epithelial cells

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Cheng Cheng Xiao,
Wuhan University, China
Fan Yang,
Jiangxi Agricultural University, China

****Correspondence:***
Fang Yuan
1278018214@qq.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *13 February 2022*
Accepted: *28 April 2022*
Published: *27 May 2022*

Citation:
Liu Y, He M, Xiong H and Yuan F (2022) Induction of Pyroptosis in Renal Tubular Epithelial Cells Using High Glucose. Front. Med. 9:874916. doi: 10.3389/fmed.2022.874916

INTRODUCTION

Diabetic kidney disease (DKD) is the primary cause of end-stage renal disease (1). In recent years, it has been found that renal tubular injury and renal interstitial fibrosis are important features of DKD. The micro-inflammatory state is an important basis for the occurrence of DKD (2). In this regard, pyroptosis is an inflammatory programmed cell death mediated by gasdermin D (GSDMD) (3). Inflammasomes are the key to triggering cell pyroptosis. NOD-like receptor protein 3 (NLRP3)

is one of inflammasomes, it can sense different pathogen-associated or danger-associated molecular patterns (PAMPs or DAMPs) and oligomerize these components into a very gigantic and efficient protein complexes, leading to Caspase-1 activation and IL-1β and IL-18 production and participate in the regulation of cell functions (4).

In recent years, pyroptosis has become a research hotspot in the field of inflammation globally (5–7). However, there are few studies on the relationship between pyroptosis and DKD. Recently, evidence indicates that inhibition of the NLRP3 inflammasome pathway may reduce the occurrence of pyroptosis in diabetes-related complications (8, 9). However, the specific relationship between NLRP3 inflammasome activation and pyroptosis remains to be clarified. In addition, there have been few experimental studies on whether pyroptosis is involved in the process of renal tubular epithelial cell damage in DKD.

In this study, we compared the expression of pyroptosis-related proteins in human renal tubular epithelial cells and in the kidney tissues of healthy people and patients with DKD. We also used transmission electron microscope (TEM) to observe the occurrence of pyroptosis in human renal tubular epithelial cells treated with different concentrations of glucose, and analyzed the possible mechanism of pyroptosis caused by high glucose concentration. This study may obtain information about the progression of DKD and provide theoretical basis for further study of the molecular mechanism of the pyroptosis-related signal transduction pathway.

MATERIALS AND METHODS

Materials

GSDMD (20770-1-AP) was purchased from Proteintech, China. Dulbecco's modified Eagle's medium/F12 medium, fetal bovine serum, and trypsin were purchased from Gibco, USA. Antibodies to NLRP3 (ab214185), Caspase-1 p20 (ab1872), GSDMD-N (ab215203), IL-1β (ab200478), IL-18 (ab207324), and GAPDH (ab9485) were purchased from Abcam, UK. Goat anti-rabbit (G1213-100UL) secondary antibody and diaminobenzidine chromogenic reagent (G1212-200T) were purchased from Servicebio, China.

Abbreviations: DKD, diabetic kidney disease; NLRP3, NOD-like receptor protein 3; GSDMD, Gasmedermin D; IL, Interleukin; TEM, Transmission electron microscope.

Tissue Specimen Collection

We collected renal tissues from four patients diagnosed with DKD after renal biopsy in the Second Xiangya Hospital of Central South University from December 2019 to May 2020 as the experimental group, and normal renal tissues from three patients undergoing surgical resection after renal trauma as the control group. None of the patients received any immune-related treatment, and all DKD patients were diagnosed by pathology. Informed consent was obtained from all patients prior to tissue sample collection.

Cell Culture and Experimental Groups

The human renal tubular epithelial cell line HK-2 was from the Nephrology Laboratory of the Second Xiangya Hospital of Central South University; it was cultured with Dulbecco's modified Eagle's medium/F12 medium, 10% fetal bovine serum and 1% double antibiotics (penicillin, streptomycin), and was placed in a cell incubator at 37°C and 5% CO_2. At the logarithmic growth phase, HK-2 cells were divided into four groups, with the complete medium being replaced with different concentrations of glucose (5.6, 15, 30, and 45 mmol/L), and were cultured for 24 h.

Biochemical Analyses and ELISA

Routine blood samples for measurement of serum creatinine, eGFR and microalbuminuria were assayed at the central laboratory of Second Xiangya Hospital. Serum NLRP3 was detected by ELISA method.

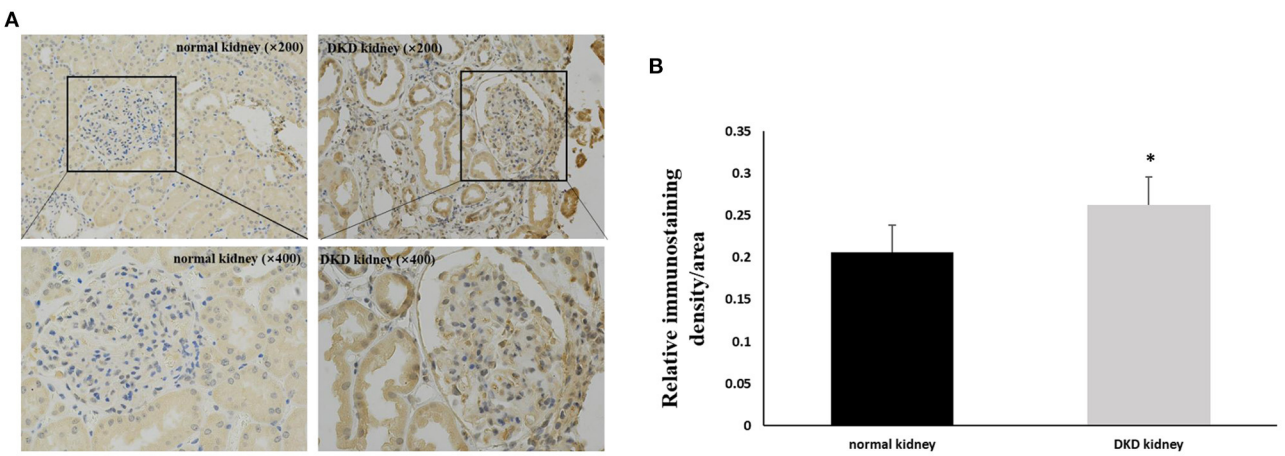


FIGURE 1 | Increased expression of NLRP3 in renal tissues of DKD patients. **(A)** IHC studies revealed increased expression of NLRP3 in DKD patients (×200 and ×400) **(B)** Averaged relative intensity for the staining of NLRP3 in kidney biopsies of normal vs. DKD patients (*$P < 0.05$).

Immunohistochemistry

Following gradient ethanol deparaffinization of kidney tissue slices, antigen retrieval was performed, followed by NLRP3 and GSDMD immunohistochemical staining. The slices were incubated overnight at 4°C with primary antibody. Diaminobenzidine staining was performed according to the method specified in the immunohistochemistry kit. In each slice of each group, at least three 200-fold fields of view were randomly selected for imaging; Image J software was used for analysis, and mean density was calculated.

Transmission Electron Microscope

TEM was used to observe the ultrastructure of cells. The electron microscope fixative solution glutaraldehyde was quickly added after discarding the culture medium for each group. Then, the cells were gently scraped off using a cell scraper and centrifuged. The cells were collected after it was visually observed that they precipitated to the size of sesame seeds or mung beans. New fixative solution was added for fixation, and the cells were dehydrated, embedded in paraffin, and then sliced. The slices were stained with lead citrate to observe the changes in cell ultrastructure.

Western Blot

The cells were lysed using RIPA lysate buffer containing protease inhibitors. 20 ug protein samples were resolved using 8% SDS-PAGE gel electrophoresis, transferred to polyvinylidene fluoride membrane, blocked using skim milk for 1 h, and incubated overnight at 4°C after adding primary antibodies to detect NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18. After washing three times with phosphate-buffered saline-Tween 20 (PBST), the membrane was incubated with the secondary antibody [HRP-conjugated goat anti-rabbit IgG (1:5000)] at 37°C for 1 h, then washed and exposured to Kodak film. Using GAPDH protein as an internal reference, the gray-scale ratio between the target protein band and the GAPDH band was semi-quantitatively analyzed using Image Pro Plus software.

Statistical Methods

The SPSS 23 software was used to perform statistical analysis, and GraphPad Prism 7 software was used for graph plotting. Measurement data are expressed as mean ± standard deviation, and inter-group differences were compared using *t*-test or analysis of variance. Spearman's correlation was used for the analysis, and $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Expression of NLRP3 and GSDMD in Kidney Tissues of Healthy People and Patients With DKD

Immunohistochemistry results showed that NLRP3 and GSDMD were primarily expressed in renal tubular epithelial cells. NLRP3

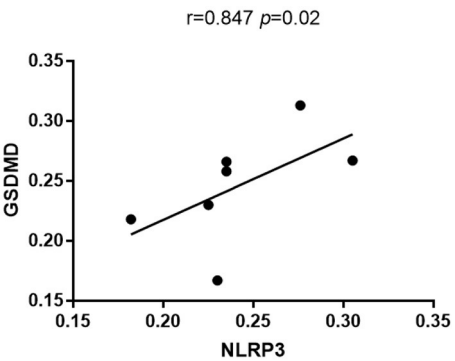


FIGURE 3 | GSDMD expression was positively correlated with that of NLRP3.

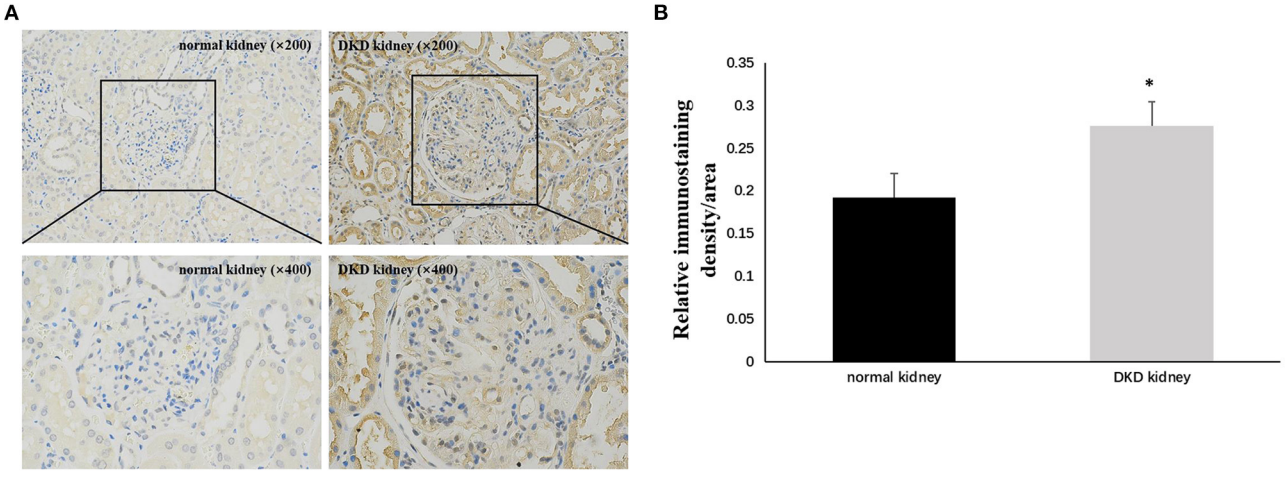


FIGURE 2 | Increased expression of GSDMD in renal tissues of DKD patients. **(A)** IHC studies revealed increased expression of GSDMD in DKD patients (×200 and ×400) **(B)** Averaged relative intensity for the staining of GSDMD in kidney biopsies of normal vs. DKD patients (*$P < 0.05$).

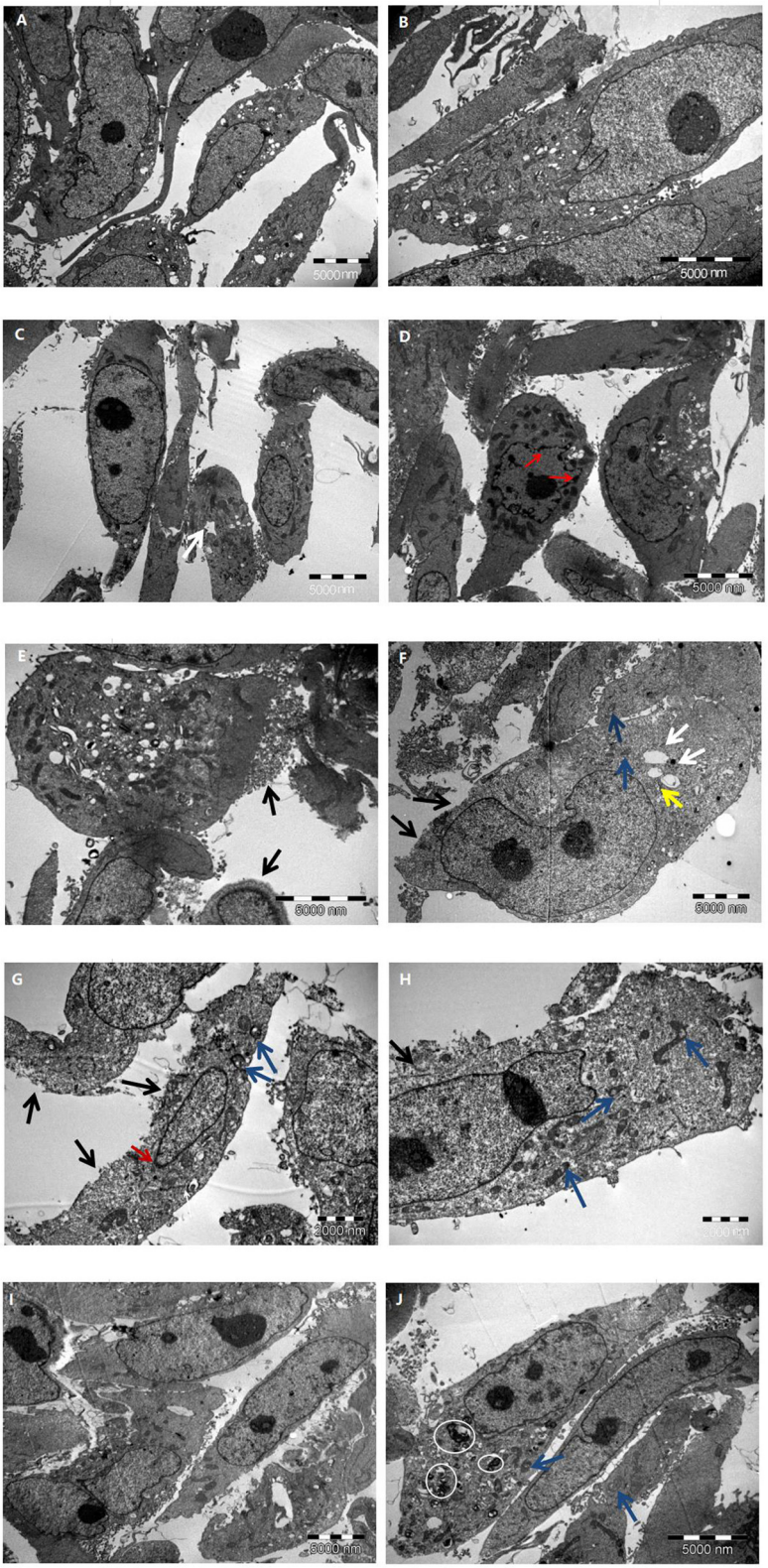


FIGURE 4 | The characteristic of HK-2 cells at different glucose concentrations. **(A,B)** Control group; **(C–E)** 15 mmol/L glucose group; **(F–H)** 30 mmol/L glucose group; **(I,J)** 45 mmol/L glucose group. Black arrow: cell membrane injure; White arrow: endoplasmic reticulum vesiculated expansion; Blue arrow: mitochondrial vacuolation; Yellow arrow: Golgi swelling; Red arrow: chromatin marginated; White circle: myeloid structure.

TABLE 1 | Comparison of HK-2 cell ultrastructure in different experimental groups.

	Control group	15 mmol/L group	30 mmol/L group	45 mmol/L group
Cell morphology	Normal	Slight swelling	Significant swelling	Significant swelling
Cell membrane	Intact and continuous	Partial membranous perforation and cytoplasmic content overflow	Membranous perforation and massive cytoplasm content overflow	Intact and continuous
Cytoplasm	The organelle structure was intact without damage	Vesiculation and expansion of endoplasmic reticulum	Vesiculation and expansion of endoplasmic reticulum, mitochondrial vacuolation, and Golgi swelling	Mitochondrial vacuolation and myeloid structure
Nucleus	No chromatin pyknosis or adherence to nuclear membrane	Partial nuclear chromatin pyknosis, margination and adhesion to the nuclear membrane	Disappearance of part of nucleoli, chromatin margination and adhesion to the nuclear membrane	No chromatin margination or adhesion to the nuclear membrane

was expressed in normal kidney tissue (0.206 ± 0.028); however, its expression in the kidney tissue of patients with DKD was significantly increased (0.263 ± 0.033) ($P < 0.05$; **Figure 1**). Similarly, GSDMD was also expressed in normal kidney tissue (0.192 ± 0.029), but its expression in the kidney tissue of patients with DKD was significantly increased (0.276 ± 0.028) ($P < 0.05$; **Figure 2**). Further, GSDMD expression was positively correlated with that of NLRP3 ($r = 0.847$, $P = 0.02$; **Figure 3**).

Ultrastructural Changes in HK-2 Cells at Different Glucose Concentrations

Different concentrations of glucose (control group 5.6 mmol/L, high glucose groups 15, 30, and 45 mmol/L) were used to stimulate HK-2 cells for 24 h. Using TEM, it was observed that the cells in the control group had an intact structure without swelling; the cell membrane structure was complete and continuous and did not have membranous perforation or cytoplasm content overflow; the cell organelle structure was intact, without swelling, vacuolation, or other damages; the structure of the cell nuclei was complete; and chromatin pyknosis and adhesion to the nuclear membrane was not observed. These observations indicated that the cell structure was normal and no pyroptosis had occurred (**Figures 4A,B**).

Compared with that in the control group, a higher proportion of cells in the 15 mmol/L group showed slight swelling; some cell membrane edges were fuzzy brush-like, with membranous perforations and cytoplasmic content overflow; some cells showed vesiculation and expansion of endoplasmic reticulum; and the nuclear chromatin showed pyknosis, margination, and adhesion to the nuclear membrane. These observations indicated that a small number of cells in this group had undergone pyroptosis (**Figures 4C–E**).

Compared with control group, the cell swelling in the 30 mmol/L group was significantly increased; a large number of cell membrane was perforated or had disappeared; a large amount of cytoplasmic content had overflowed; vesiculation and expansion of endoplasmic reticulum, mitochondrial vacuolation, and Golgi swelling were widely present; part of the nucleoli had disappeared; chromatin were marginated and adhered to the nuclear membrane; and the overall electron density of the cells had decreased (cell viability had decreased). These observations indicated that a large number of cells in this group had experienced pyroptosis (**Figures 4F–H**).

Further, compared with the control group, the 45 mmol/L group showed significant cell swelling; the cell membrane structure was intact and continuous, and did not show membranous perforation or cytoplasmic content overflow; a large number of myeloid structures was observed in the cytoplasm of some cells, showing mitochondrial vacuolation and swelling; there was no significant damage to the cell nuclei, and no phenomena such as chromatin margination were seen. It was revealed that the cells in this group had serious pathological changes, but no significant ultrastructural changes associated with pyroptosis (**Figures 4I,J**). The comparison of the ultrastructure of HK-2 cells in different group is summarized in **Table 1**.

Expression of NLRP3 and Pyroptosis-Related Proteins in HK-2 Cells Stimulated by Different Glucose Concentrations

Following stimulation of HK-2 cells with different glucose concentrations (control group 5.6 mmol/L and high glucose groups 15, 30, and 45 mmol/L) for 24 h, we observed that compared with control group, the expression of NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18 increased significantly in a glucose-concentration-dependent manner ($P < 0.05$; **Figure 5**).

DISCUSSION

DKD is the primary cause of chronic kidney disease. Effective treatment of DKD and exploration of new therapeutic targets has always been a major topic of research. In addition to abnormal blood glucose and lipid metabolism, abnormal activation of inflammasomes also plays a key role in the development of DKD (10, 11). Pyroptosis is a form of inflammatory programmed cell death, and NLRP3 inflammasome activation plays a central role in this process. In our study, we found that NLRP3 and GSDMD (key protein of pyroptosis) are primarily expressed in human renal tubular epithelial cells. Their expression in kidney

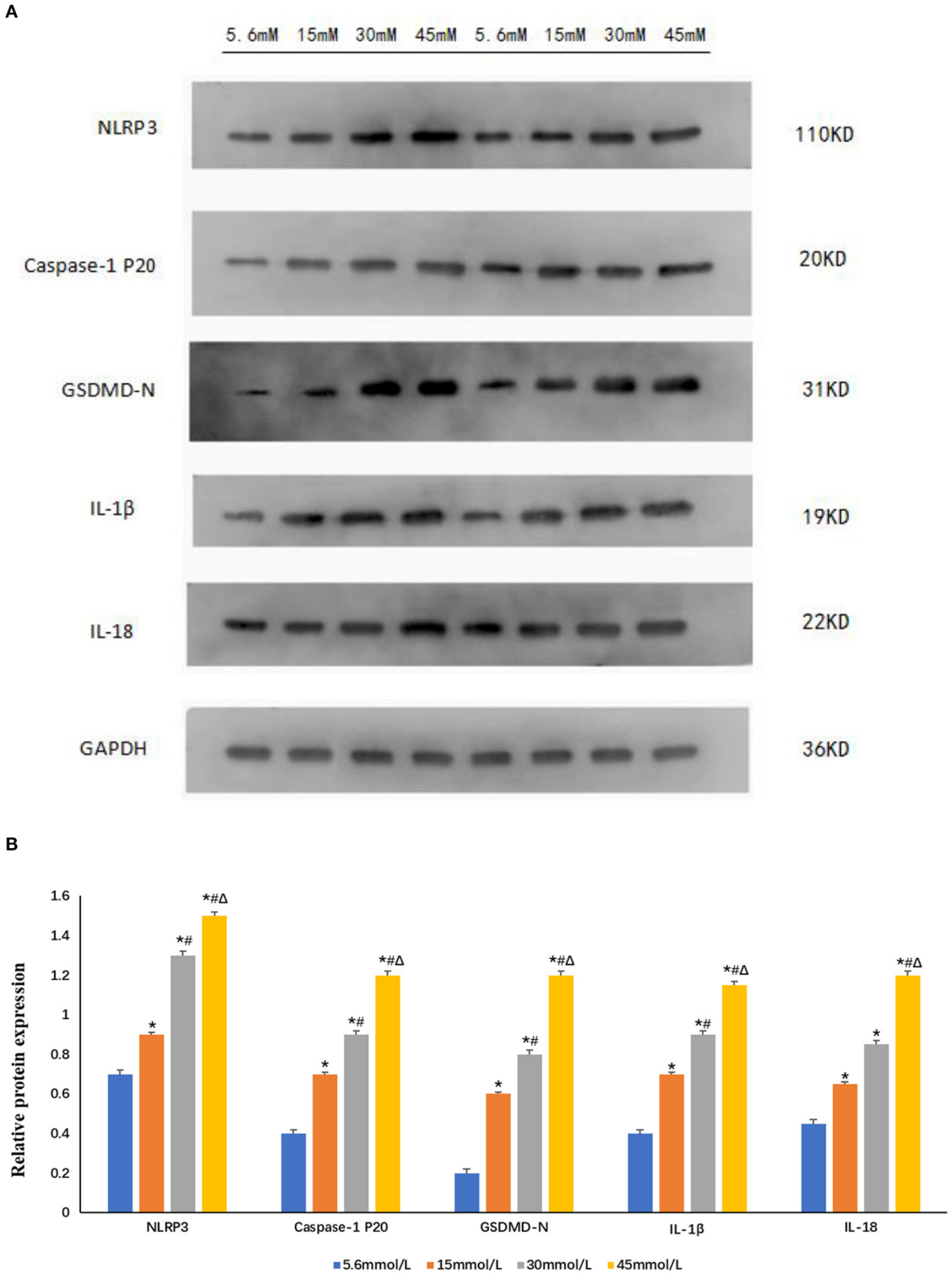


FIGURE 5 | Expression of NLRP3 and pyroptosis-related proteins increased after stimulation with different glucose concentrations in HK-2 cells. **(A)** Western blot showing the expression of NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18 increased in a glucose-concentration-dependent manner. **(B)** The protein fold change of NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18, using Gapdh as a reference gene. Data is presented as the average fold change compared to controls ± s.e.m. $n = 3$. $^{*}P < 0.05$ vs. 5.6 mmol/L glucose group; $^{\#}P < 0.05$ vs. 15 mmol/L glucose group; $^{\Delta}P < 0.05$ vs. 30 mmol/L glucose group. $n = 3$.

tissue of patients with DKD is significantly higher than that in normal human kidney tissues, and the expression of GSDMD is positively correlated with that of NLRP3. It is therefore speculated that NLRP3 cooperates with GSDMD to participate in the development and progression of DKD.

Inflammasomes are multi-protein complexes assembled by pattern recognition receptors in the cytoplasm. They can recognize a variety of stimuli such as pathogen-associated molecular patterns or damage-associated molecular patterns. By recruiting and activating caspase-1, they induce mature inflammatory factors such as IL-1β and IL-18. NLRP3 is the most widely studied inflammasome, which can be activated through classical or non-classical pathways and participates in the pathogenesis of acute kidney injury and chronic kidney disease (12). The activation of NLRP3 inflammasome in DKD has attracted widespread attention. The activated NLRP3 inflammasome promotes secretion of IL-1β and IL-18, which in turn promotes the progression of DKD (13). Researchers have observed upregulation of NLRP3 and caspase-1 expression in endothelial cells and podocytes in the kidneys of mice and patients with DKD (14). *In vitro* cell culture and animal models have confirmed the role of NLRP3 in DKD (15–17). In this study, we stimulated HK-2 cells with high concentrations of glucose and observed increased expression of NLRP3, caspase-1 p20, IL-1β, and IL-18, suggesting that high glucose concentration activates HK-2 cell inflammasome and causes the release of inflammatory factors. That consistent with previous studies.

Although pyroptosis was initially considered a unique feature of immune cells, recent studies have shown that it also plays a role in non-immune cells (6, 18). In contrast-induced acute kidney injury and renal ischemia-reperfusion injury, renal tubular epithelial cell pyroptosis is an indispensable process (19, 20). The activated NLRP3 activates caspase-1, cleaves GSDMD, breaks self-inhibition, and produces an N-terminal fragment. GSDMD-N targets the cell membrane to form pores and causes water influx, so that the ion gradients across the cell membrane disappear, and the cells undergo swelling and osmotic lysis, eventually leading to cell pyroptosis (21, 22). In our study, we also observed that renal tubular epithelial cells showed obvious pyroptosis with the increase expression of NLRP3 after stimulated by high glucose. In addition, for the first time, we observed through TEM that the HK-2 cells in the high-glucose groups (15 and 30 mmol/L) showed ultrastructural changes that are typical of pyroptosis, such as cell membrane damage, discontinuity, cytoplasmic content overflow, and chromatin margination and adhesion to the nuclear membrane. However, the cells in the control group did not undergo pyroptosis. Further, we found through western blot that compared with that in the normal control group, the expression of NLRP3, caspase-1 p20, GSDMD-N, IL-1β, and IL-18 in the high-glucose groups (15, 30, and 45 mmol/L) increased in a concentration-dependent manner, which also indicated that high glucose concentration promotes the occurrence of cell pyroptosis, and that NLRP3-caspase-1 may be related to GSDMD-mediated pyroptosis. Although the expression of pyroptosis-related proteins in the 45 mmol/L group was higher than that in the other groups, and TEM showed that the cells in this group were significantly swollen with part of cells appearing large number of myeloid structures and vacuolated mitochondria. This phenomenon indicated that the cells were seriously damaged, but there were no ultrastructural characteristics typical of pyroptosis. We speculated that the cells in the 45 mmol/L group have other complex cell damage patterns, such as autophagy.

In conclusion, our study confirmed that high glucose concentration can induce pyroptosis of human renal tubular epithelial cells. We stimulated human renal tubular epithelial cells with different concentrations of glucose, which showed a series of changes in NLRP3 inflammasomes, pyroptosis-related proteins, and inflammatory factors, suggesting that high glucose concentration affects the activation of NLRP3 inflammasome in the autoimmune system, leading to the occurrence of pyroptosis and release of inflammatory factors. This provides a basis for further animal and clinical experiments. With the elucidation of the relevant mechanisms, the targets in the pyroptosis-related signaling pathways are expected to become a new hotspot in the treatment of DKD, and targeting pyroptosis through inflammasome assembly, caspase activation, GSDMD-mediated nuclear pore formation, and other unknown upstream or downstream pathways may be a new way to treat DKD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FY and YL designed the research. MH and HX did the cell experiment. FY and MH analyzed the data and drafted the manuscript. FY revised the manuscript. All authors have read and approved the manuscript.

FUNDING

This work was supported by Grants from the National Natural Science Foundation of China (No. 81770730) and the Natural Science Fund of Changsha (No. kq2014235).

ACKNOWLEDGMENTS

The authors wish to Liuxia Xiang for her help in pathological assessment.

REFERENCES

1. Zhu B, Cheng X, Jiang Y, Cheng M, Chen L, Bao J, et al. Silencing of KCNQ1OT1 decreases oxidative stress and pyroptosis of renal tubular epithelial cells. *Diabetes Metab Syndr Obes.* (2020) 13:365–75. doi: 10.2147/DMSO.S225791
2. Qiu YY, Tang LQ. Roles of the NLRP3 inflammasome in the pathogenesis of diabetic nephropathy. *Pharmacol Res.* (2016) 114:251–64. doi: 10.1016/j.phrs.2016.11.004
3. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature.* (2015) 526:660–5. doi: 10.1038/nature15514
4. Schroder K, Tschopp J. The inflammasomes. *Cell.* (2010) 140:821–32. doi: 10.1016/j.cell.2010.01.040
5. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev.* (2017) 277:61–75. doi: 10.1111/imr.12534
6. Xu YJ, Zheng L, Hu YW, Wang Q. Pyroptosis and its relationship to atherosclerosis. *Clin Chim Acta.* (2018) 476:28–37. doi: 10.1016/j.cca.2017.11.005
7. Mamik MK, Power C. Inflammasomes in neurological diseases: emerging pathogenic and therapeutic concepts. *Brain.* (2017) 140:2273–85. doi: 10.1093/brain/awx133
8. Wu D, Yan ZB, Cheng YG, Zhong MW, Liu SZ, Zhang GY, et al. Deactivation of the NLRP3 inflammasome in infiltrating macrophages by duodenal-jejunal bypass surgery mediates improvement of beta cell function in type 2 diabetes. *Metabolism.* (2018) 81:1–12. doi: 10.1016/j.metabol.2017.10.015
9. Song Y, Yang L, Guo R, Lu N, Shi Y, Wang X. Long noncoding RNA MALAT1 promotes high glucose-induced human endothelial cells pyroptosis by affecting NLRP3 expression through competitively binding miR-22. *Biochem Biophys Res Commun.* (2019) 509:359–66. doi: 10.1016/j.bbrc.2018.12.139
10. Yaribeygi H, Atkin SL, Simental-Mendía LE, Barreto GE, Sahebkar A. Anti-inflammatory effects of resolvins in diabetic nephropathy: mechanistic pathways. *J Cell Physiol.* (2019) 234:14873–82. doi: 10.1002/jcp.28315
11. Tönnies T, Stahl-Pehe A, Baechle C, Castillo K, Yossa R, Holl RW, et al. Diabetic nephropathy and quality of life among youths with long-duration type 1 diabetes: a population-based cross-sectional study. *Pediatr Diabetes.* (2019) 20:613–21. doi: 10.1111/pedi.12837
12. Ding W, Guo H, Xu C, Wang B, Zhang M, Ding F. Mitochondrial reactive oxygen species-mediated NLRP3 inflammasome activation contributes to aldosterone-induced renal tubular cells injury. *Oncotarget.* (2016) 7:17479–91. doi: 10.18632/oncotarget.8243
13. Li LH, Lin JS, Chiu HW, Lin WY, Ju TC, Chen FH, et al. Mechanistic insight into the activation of the nlrp3 inflammasome by neisseria gonorrhoeae in macrophages. *Front Immunol.* (2019) 10:1815. doi: 10.3389/fimmu.2019.01815
14. Mulay SR. Multifactorial functions of the inflammasome component NLRP3 in pathogenesis of chronic kidney diseases. *Kidney Int.* (2019) 96:58–66. doi: 10.1016/j.kint.2019.01.014
15. Feng H, Gu J, Gou F, Huang W, Gao C, Chen G, et al. High glucose and lipopolysaccharide prime NLRP3 inflammasome via ROS/TXNIP pathway in mesangial cells. *J Diabetes Res.* (2016) 2016:6973175. doi: 10.1155/2016/6973175
16. Gao P, He FF, Tang H, Lei CT, Chen S, Meng XF, et al. NADPH oxidase-induced NALP3 inflammasome activation is driven by thioredoxin-interacting protein which contributes to podocyte injury in hyperglycemia. *J Diabetes Res.* (2015) 2015:504761. doi: 10.1155/2015/504761
17. Shahzad K, Bock F, Dong W, Wang H, Kopf S, Kohli S, et al. Nlrp3-inflammasome activation in non-myeloid-derived cells aggravates diabetic nephropathy. *Kidney Int.* (2015) 87:74–84. doi: 10.1038/ki.2014.271
18. Tan CC, Zhang JG, Tan MS, Chen H, Meng DW, Jiang T, et al. NLRP1 inflammasome is activated in patients with medial temporal lobe epilepsy and contributes to neuronal pyroptosis in amygdala kindling-induced rat model. *J Neuroinflammation.* (2015) 12:18. doi: 10.1186/s12974-014-0233-0
19. Zhang Z, Shao X, Jiang N, Mou S, Gu L, Li S, et al. Caspase-11-mediated tubular epithelial pyroptosis underlies contrast-induced acute kidney injury. *Cell Death Dis.* (2018) 9:983. doi: 10.1038/s41419-018-1023-x
20. Yang JR, Yao FH, Zhang JG, Ji ZY, Li KL, Zhan J, et al. Ischemia-reperfusion induces renal tubule pyroptosis via the CHOP-caspase-11 pathway. *Am J Physiol Renal Physiol.* (2014) 306:F75–84. doi: 10.1152/ajprenal.00117.2013
21. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, et al. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature.* (2016) 535:111–6. doi: 10.1038/nature18590
22. Sborgi L, Rühl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H, et al. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* (2016) 35:1766–78. doi: 10.15252/embj.201694696

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 30 May 2022
doi: 10.3389/fmed.2022.882692

Long-Term Kidney Prognosis and Pathological Characteristics of Late-Onset Lupus Nephritis

*Na Tian[1,2], Qian Zhou[1,3], PeiRan Yin[1,4], WenFang Chen[5], LingYao Hong[1], QiMei Luo[1], MengHua Chen[2], XueQing Yu[6] and Wei Chen[1]**

[1] Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Key Laboratory of National Health Commission, Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China, [2] Department of Nephrology, General Hospital of Ningxia Medical University, Ningxia, China, [3] Department of Medical Statistics, Clinical Trials Unit, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [4] Department of Nephrology, Second Affiliated Hospital of Soochow University, Suzhou, China, [5] Department of Pathology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [6] Guangdong Provincial People's Hospital, Guangzhou, China

Background: Arguments still exist on prognosis of late-onset SLE, especially their kidney function. The purpose of this study was to investigate long-term kidney outcomes in patients with late-onset lupus nephritis (LN).

Methods: A retrospective long-term cohort study was conducted in adult Chinese patients with LN. The patients were divided into late- (>50 years) and early-onset (<50 years) LN groups. The baseline characteristics, especially the kidney pathological characteristics, were compared. The cohort was followed-up for kidney outcome defined as doubling of serum creatinine or ESRD. Cox regression analysis was used to examine the association between late onset LN and its outcomes.

Results: A total of 1,264 patients were recruited, who were assigned to late-onset LN with 102 patients and early-onset LN with 1,162 patients. The late-onset LN group showed a worse baseline kidney function and more chronic pathological lesions than the early-onset LN group. During a follow-up time of 55 (3, 207) months, 114 (13.1%) deaths occurred, 107 (12.2%) had doubling of creatinine, and 80 (9.1%) developed end-stage kidney disease. The 5- and 10-year survival rates of the late-onset LN group were 67.6 and 50.5%, respectively, which were much worse than those of the early-onset LN group (89.8 and 84.6%, respectively). However, no significant difference was found on kidney survival (log-rank chi-square = 3.55, $p = 0.06$). Cox regression analysis showed that late-onset LN was an independent risk factor for patient survival (hazard ratio = 3.03, 95% CI (1.39, 6.58), $p = 0.005$). Increased baseline serum creatinine was an independent risk factor for kidney survival of patients with late-onset LN.

Conclusions: Patients with late-onset LN had milder active lesions but severer chronic lesions in kidney pathology. They have poorer overall outcome but relatively favorable kidney outcome.

Trial Registration: ClinicalTrials.gov Identifier: NCT03001973, 22 December 2016 retrospectively registered.

Keywords: lupus nephritis, late-onset, kidney outcome, renal pathology, Chinese population

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Marc Hilhorst,
Academic Medical Center, Netherlands
Giorgia Comai,
Alma Mater Studiorum University of Bologna, Italy

***Correspondence:**
Wei Chen
vvchen66@qq.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *24 February 2022*
Accepted: *09 May 2022*
Published: *30 May 2022*

Citation:
Tian N, Zhou Q, Yin P, Chen W, Hong L, Luo Q, Chen M, Yu X and Chen W (2022) Long-Term Kidney Prognosis and Pathological Characteristics of Late-Onset Lupus Nephritis. Front. Med. 9:882692. doi: 10.3389/fmed.2022.882692

INTRODUCTION

Systemic lupus erythematosus (SLE) is the most common cause (54.3%) of secondary glomerular nephropathy in China (1). SLE mainly occurs in young women of child-bearing age and is not commonly found in elderly population. Previous studies suggested that the onset age of lupus is an important risk factor associated with clinical manifestations and outcomes (2–6). Two nested case-control study from Brazil and US, respectively, Appenzeller et al. (7), Bertoli et al. (8) found that patients with late-onset SLE have milder symptoms, but present higher rate of organ damage and mortality than patients with early-onset SLE. While another found that late-onset SLE has a benign prognosis (9). In a Chinese study, kidney pathology analysis showed that activity lesions are milder and chronic lesions are more severe in patients with late-onset SLE compared with those in patients with early-onset SLE, but no difference in kidney outcomes was found between the two groups (3).

Although certain differences between late- and early-onset SLE have been reported, few distinct patterns of clinical manifestation, therapeutic response, or prognosis have been confirmed. In addition, to the best of our knowledge, only a few studies have investigated kidney pathological features and kidney outcome in population with late-onset lupus nephritis (LN). Therefore, we aimed to assess the kidney pathological characteristics and outcomes of patients with late-onset LN based on a large sample size and long-term follow-up data.

MATERIALS AND METHODS

Subjects

A single center, retrospective cohort study was designed. Clinical and kidney histopathological data were extracted from the LN database (ln.medidata.cn) of the Department of Nephrology, the First Affiliated Hospital of Sun Yat-sen University. Patients diagnosed with LN at the age of 16 years or older from January 1, 1996 to December 31, 2011 were enrolled. All patients were diagnosed using the 1997 revised SLE criteria of the American College of Rheumatology (10). Patients who had end-stage renal disease (ESRD), cancer, or drug-induced LN at the time of diagnosis were excluded. Each biopsy specimen with at least 10 glomeruli was included for histopathological analysis. The protocol was approved by the human ethics committee of the First Affiliated Hospital, Sun Yat-sen University. Written informed consent was obtained from each participant.

The cut-off age of 50 was employed referring to the previous studies to define the late-onset LN (11–15). Patients with LN aged 50 years or older at kidney biopsy were assigned to the late-onset LN group, whereas those with LN aged <50 years at kidney biopsy were assigned to the early-onset LN group (control).

Data Collection and Clinical Definitions

Age at onset was obtained in a retrospective manner as the first sign of kidney involvement was detected, including proteinuria ≥0.5 g/24 h, glomerular hematuria, and cellular cast. Cardiovascular complications included coronary heart disease, chronic heart failure, arrhythmia, and cardiomyopathy. Organ damage was assessed using the Systemic Lupus Erythematosus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) (16). To be scored, each manifestation in the SDI is required to be present for at least 6 months unless it is noted in the SDI instructions. Disease activity was evaluated using the SLE disease activity index (SLEDAI) (17). Acute kidney injury (AKI) was diagnosed based on 2012 KDIGO criteria (18). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (19).

Therapeutic variables included exposure to glucocorticoid at induction treatment, dose of prednisone at induction, high-dose glucocorticoid at first diagnosed (pulse therapy or oral prednisone ≥1 mg/kg day), immunosuppressive agents treatment (taken methotrexate, mycophenolate mofetil, cyclophosphamide, azathioprine, or combination), and use of angiotensin converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB).

Kidney histopathological data were reviewed by an experienced pathologist according to the 2003 International Society of Nephrology/Kidney Pathology Society classification of LN (20).

Study Outcomes

All the participants were followed up for at least 1 year until December 31, 2013. Patients were required to return to our hospital at least once a year for an overall medical evaluation and/or were interviewed annually through phone call by experienced doctors to assess the general conditions. The primary endpoint was a composite of kidney outcomes, including doubling of serum creatinine (serum creatinine doubled compared with the baseline) and ESRD (eGFR ≦15 mL/min; persistent dialysis; kidney transplant). The secondary endpoint was all cause mortality.

Statistical Analysis

Patient characteristics were presented as mean ± SD for normally distributed continuous variables, median (interquartile range) for skewed continuous variables, and frequencies and percentages for categorical variables. Comparisons between the late- and control groups were performed using the Student's t-test for normally distributed continuous variables, the Mann–Whitney U-test for non-normally distributed continuous variables, and the Chi-square test for categorical variables. Patients' cumulative survival and kidney survival rates (at a combined kidney endpoint of creatinine doubling and ESRD) were calculated using Kaplan–Meier curves. Unadjusted and adjusted Cox proportional hazards regression models were used to evaluate the risk factors for mortality in all participants and kidney mortality in patients with late-onset LN. Potential confounders were included in univariate Cox regression analysis. Significant variates with a p-value <0.10 in the univariate analysis were forced into the multivariate models. Other variates were selected into the multivariable models using the forward method (entry: 0.1, removal: 0.2). To adjust the impact of age on patient survival, we collected the data of life expectancy at LN onset for each participant based on the World Bank latest report

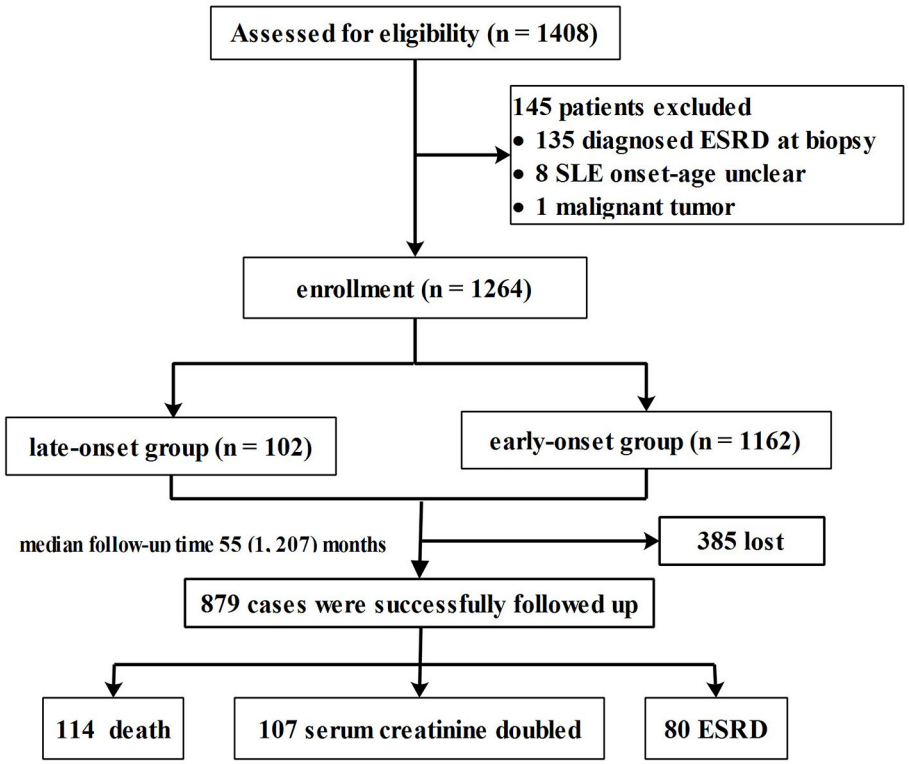


FIGURE 1 | Enrollment and follow-up of study population.

(The World Bank. Data of life expectancy at birth, total (years), China (2019). https://data.worldbank.org/indicator/SP.DYN.LE00.IN?end=2016&locations=CN&start=1960&view=chart&year_low_desc=true). Since only data from 1960 to 2016 could be obtained, we estimated life expectancy with the recent 5-year life growth rate of 0.21% for those who were born between 1920 and 1959. In the multivariable Cox regression, life expectancy was considered as a confounder into model to adjust the impact of age to patient survival.

A two-sided p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software, version 20.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinical Characteristics of Late-Onset LN

A total of 1,264 patients who met the inclusion criteria were enrolled from the 1,408 patients diagnosed with LN during the study period (**Figure 1**). Among the eligible patients, 102 (8.1%) were assigned to the late-onset LN group and 1,162 (91.9%) to the early-onset LN group.

Tables 1, **2** showed the baseline of the two groups. The male-to-female ratio in the late-onset LN group was 2:5, which was twice of that in the early-onset LN group ($p = 0.001$). More patients with late-onset LN had hypertension and cardiovascular disease (CVD) complications compared with the patients in the early-onset LN group ($p < 0.001$). Diabetes percentage was similar in both groups. Higher percentage of patients with late-onset LN had no skin or mucous lesions. The serositis frequency was lower in the late-onset LN group. The SDI and SLEDAI scores indicated no difference between the two groups. The baseline kidney function of the late-onset LN group was significantly worse, presenting more urine protein ($p = 0.03$), smaller kidney size, and lower eGFR ($p < 0.001$). AKI occurred more frequently in the late-onset LN group ($p = 0.005$).

As for the treatment, patients with late-onset LN were administered lower dose of prednisone at the induction phase. Immunosuppressant drugs and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker drugs were used in less proportion of patients with late-onset LN (**Table 3**).

Kidney Histopathological Evaluation

Kidney pathological analysis was conducted in 784 eligible patients. The proportion of biopsied patients showed no statistical difference between the late- and early-onset LN groups ($p = 0.07$). The distribution of LN pathological type (ISN/RPS, 2003) was similarly between the two groups. It generally showed that chronic lesions (glomerular sclerosis, interstitial fibrosis, tubular atrophy, and arterial wall thickening) were more severe in the late-onset LN group (all p-value < 0.05). Active lesions (including crescents, karyorrhexis, capillary tuft necrosis, glomerular capsule adhesion, subendothelial hyaline deposits and tubular necrosis) were not statistically different between the two groups (all p-value > 0.05) (**Table 4**).

TABLE 1 | Comparisons of baseline clinical manifestations between late- and early-onset LN groups.

Items	All	Early-onset LN group	Late-onset LN group	*p*
N	1,264	1,162	102	
Male/female	1:5	1:5	2:5	0.001
Age at diagnosis (years)	31.12 ± 12.21	28.81 ± 9.99	58.13 ± 6.80	<0.001
Duration before diagnosis (months)	4 (1, 24)	3 (1, 20)	3 (1, 12)	0.37
Hypertension, *n* (%)	35 (2.7)	21 (1.8)	14 (13.7)	<0.001
CVD history, *n* (%)	82 (6.5)	61 (5.2)	21 (20.6)	<0.001
Diabetes history, *n* (%)	18 (1.4)	13 (1.1)	5 (4.9)	0.12
BMI (kg/m^2)	21.09 ± 3.07	21.03 ± 3.12	21.85 ± 2.31	0.15
Fever, *n* (%)	415 (32.8)	389 (33.4)	26 (25.4)	0.11
Edema, *n* (%)	834 (65.9)	771 (66.3)	63 (61.7)	0.45
Skin and mucous, *n* (%)				
None	161 (13.2)	123 (10.6)	38 (37.2)	0.01
Rash	568 (44.7)	527 (45.3)	41 (40.2)	0.34
Alopecia	249 (19.6)	238 (20.5)	11 (10.8)	0.01
Mucous ulcer	121 (9.5)	115 (9.9)	6 (5.9)	0.19
Raynaud's phenomenon	18 (1.4)	18 (1.6)	0 (0)	0.20
Photosensitivity	147 (11.6)	141 (12.1)	6 (5.9)	0.06
Musculoskeletal, *n* (%)				
None	769 (60.8)	717 (61.7)	52 (51.0)	0.07
Arthritis	431 (34.1)	391 (33.6)	40 (39.2)	0.23
Myalgia	40 (3.2)	34 (2.9)	6 (5.9)	0.09
Muscle weakness	24 (1.9)	20 (1.7)	4 (3.9)	0.11
Serositis, *n* (%)	755 (59.8)	751 (64.7)	53 (51.2)	0.04
Systolic BP (mmHg)	127.7 ± 21.80	126.53 ± 21.40	138.48 ± 20.50	<0.001
Diastolic BP (mmHg)	81.45 ± 14.70	81.45 ± 14.76	79.81 ± 13.50	0.28
Hepatomegaly, *n* (%)	11 (0.8)	10 (0.9)	1 (0.9)	0.90
Splenomegaly, *n* (%)	7 (0.5)	7 (0.6)	0	0.43
SDI score	2.1 ± 1.9	2.0 ± 1.8	2.3 ± 1.9	0.12
SLEDAI score	14.74 ± 5.35	14.77 ± 5.33	14.35 ± 5.69	0.59

CVD, cardiovascular disease; BP, blood pressure; SDI, systemic damage index; SLEDAI, systemic lupus erythematosus disease activity index.

Patient Overall Survival and Kidney Survival

Flow chart of the enrollment, follow-up process, and study outcome is shown in **Figure 1**. At the end of the study period, 114 patients died; 27 belonged to the late-onset LN group and 87 in the early-onset LN group. The main causes of death were CVD (late vs. early: 22.2% vs. 19.8%, $p = 0.12$) and infection (late vs. early: 22.2% vs. 25.6%, $p = 0.28$). More patients with late-onset LN died of kidney failure (after the onset of ESRD) (late vs. early: 14.8% vs. 4.7%, $p = 0.02$).

Kaplan–Meier analysis showed that the patient survival of the late-onset LN group at 5 and 10 years (67.6 and 50.5%, respectively) was much poorer than that of the early-onset LN group (89.8 and 84.6%, respectively) (log-rank chi-square = 35.9, $p < 0.001$; **Supplementary Figure 2A**). However, no significant statistical differences were observed on kidney survival at the composite endpoint of serum creatinine doubling or ESRD (log-rank chi-square = 3.55, $p = 0.06$; **Figure 2**).

Association of the Onset Age of LN With Kidney Prognosis

The onset age of LN ≧50 years was an independent risk factor of mortality after adjusted for confounders, such as gender, life expectancy, comorbidity diseases, pathological characteristics, and treatment. No statistically significant association was found between the onset age of LN and kidney failure by using regression models 1 to 4 (**Table 5** and **Figure 2B**).

Risk Factors for Kidney Survival in Patients With Late-Onset LN

Further analysis was performed focusing on the risk factors for kidney survival in patients with late-onset LN. The results showed that the increased serum creatinine at baseline was an independent risk factor (hazard ratio = 1.45 (1.20–1.73), $p < 0.001$; **Table 6**).

TABLE 2 | Comparisons of baseline examination profiles between late- and early-onset LN groups.

Items	All	Early-onset LN group	Late-onset LN group	*p*
Hemoglobin (g/L)	97.81 ± 24.01	105.79 ± 24.87	96.98 ± 25.79	0.002
Serum creatinine (μmol/L)	87.0 (63.0,147.0)	85.0 (62.0,138.0)	112.0 (79.0,242.2)	<0.001
Serum albumin (g/L)	27.53 ± 7.44	27.48 ± 7.49	28.28 ± 6.98	0.29
hs-CRP (g/L)	2.52 (0.84, 7.15)	2.44 (0.84, 2.44)	3.05 (1.21, 10.90)	0.63
ESR (mm/h)	38.0 (19.0,63.0)	38.0 (20.0,62.0)	40.5 (16.0,73.0)	0.49
Anti-ds DNA positive, *n* (%)	941 (76.6)	863 (76.5)	78 (77.2)	0.87
Anti-SSA positive, *n* (%)	536 (44.7)	481 (43.7)	55 (56.7)	0.02
Anti-SSB positive, *n* (%)	241 (20.2)	217 (19.9)	24 (24.2)	0.29
Low C3, *n* (%)	994 (81.5)	915 (81.6)	79 (79.8)	0.65
Urine protein (g/24 h)	1.63 (0.72, 3.34)	1.70 (0.74, 3.41)	1.15 (0.67, 2.46)	0.02
Left kidney size (mm)				
Length	108.48 ± 11.82	108.85 ± 11.67	101.38 ± 22.22	<0.001
Width	50.38 ± 7.06	50.52 ± 7.21	47.47 ± 10.66	<0.001
Right kidney size (mm)				
Length	105.44 ± 16.14	105.67 ± 16.33	101.67 ± 13.87	0.02
Width	47.12 ± 7.43	49.32 ± 8.01	46.81 ± 7.34	0.01
AKI, *n* (%)	197 (16.3)	173 (15.7)	24 (23.5)	0.04
eGFR (ml/min/1.73 m^2)	100.8 (54.37, 128.03)	104.9 (58.28, 129.24)	59.59 (25.17, 93.81)	<0.001

ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein; ANA, anti-nuclear antibody; AKI, acute kidney injury; eGFR, estimated glomerular filtration rate.

TABLE 3 | Comparisons of treatment regimen between late- and early-onset LN groups.

Presentations	All	Early-onset LN group	Late-onset LN group	*p*
N	1,264	1,162	102	
Glucocorticoid at induction treatment, *n* (%)	1,220 (98.3)	1,126 (98.5)	94 (95.9)	0.06
Dose of prednisone at induction (mg/d)	50.4 ± 5.6	54.1 ± 5.8	40.1 ± 4.7	<0.001
High-dose glucocorticoid at first diagnosed, *n* (%)	835(66.1)	784(67.5)	51(49.6)	<0.001
Immunosuppression drug at induction treatment, *n* (%)	671 (53.2)	629 (54.3)	42 (41.2)	0.01
ACEI/ARB, *n* (%)	669 (52.9)	607 (52.2)	62 (60.7)	0.04

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

DISCUSSION

Late-onset SLE is a relatively minority in the SLE patients. Studies have shown differences on clinical manifestation, seroantibodies and damage accrual between late-onset and the early-onset, but the findings were variable (2–9). Our large retrospective cohort study indicated that the patient survival of late-onset LN population was poorer while the kidney outcome was comparable than that of patients with early-onset LN. The advanced age-onset of LN was closely related to the poor clinical outcome independent of potential confounders.

Late-onset SLE has been reported to occur in 3%−18% of patients with SLE. As early as 1989, a meta-analysis conducted by Ward and Polisson concluded that the clinical manifestation between early- and late-onset SLE is different (2). However, there remains a debate on the clinical characteristics and the outcomes of patients with late-onset SLE (21–25). The discrepancy in findings in previous studies may partially be due to the different cutoff ages used in different studies and the inter-ethnic differences (13, 25). In our cohort, we selected a cut-off age of 50 years because it was arbitrarily designated as late onset when the clinical diagnosis of the disease occurred after the age of 50 years (14, 26, 27). Based on the cut-off age, the incidence of late-onset LN was 8.1% in our cohort. The percentage of male in the late-onset SLE group doubled that in the early-onset SLE group, which may be explained by the less apparent female predominance among older patients with SLE, mainly owing to the decreased estrogen levels (26). Estrogen status is important in determining disease activity and prognosis in SLE (21, 26, 28), and sex and age factors may inevitably interact to influence SLE prognosis. However, when we specifically adjusted for sex as a confounder in the multivariate Cox regression model, the hazard ratio of death in patients with late-onset LN was three-fold higher than that in patients with early-onset LN. This may suggest the age plays an important role on patient survival independent of sex difference.

Previous studies reported that late-onset SLE has greater comorbidities (8, 14), lower SLE activity (14), and less major organ involvement (8, 12). As shown in our study, patients with late-onset LN had higher blood pressure, more CVD complications, and worse kidney function at baseline, while most systematic organ involvement associated with SLE are not different from the early-onset group. Moreover, the SLEDAI scores and the SDI scores were also similar in

TABLE 4 | Kidney pathological characteristics of late-onset LN.

Items	All	Early-onset group	Late-onset group	*p*
Number of biopsy, *n* (%)	784 (62.0)	726 (62.4)	58 (56.8)	0.07
LN classification, *n* (%)				
Class II	71 (9.6)	68 (10)	3 (5.5)	0.274
Class III	81 (11.0)	73 (10.7)	8 (14.5)	0.383
Class IV	308 (41.8)	287 (42.1)	21 (38.2)	0.567
Class V	105 (14.3)	96 (14.1)	9 (16.4)	0.644
Class V+III	58 (7.9)	52 (7.6)	6 (10.9)	0.386
Class V+IV	98 (13.3)	93 (13.7)	5 (9.1)	0.338
Class VI	9 (1.3)	8 (1.2)	1 (1.8)	0.676
Glomerular sclerosis (%)	0 (0,9)	0 (0,7.4)	10.7 (0,23.7)	<0.001
Crescents (%)	3 (0,16)	3 (0,16)	0 (0,23)	0.72
Endocapillary hypercellularity, *n* (%)				0.058
None	207 (26.4)	194 (26.7)	13 (22.4)	
(25–50%)	360 (45.9)	325 (45.9)	35 (60.3)	
(>50%)	217 (27.7)	207 (28.5)	10 (17.2)	
Glomerular leukocyte infiltration, *n* (%)				0.037
None	286 (36.5)	263 (36.2)	23 (39.7)	
(<25%)	314 (40.1)	284 (39.1)	30 (51.7)	
(25–50%)	161 (20.5)	156 (21.5)	5 (8.6)	
(>50%)	28 (3.6)	28 (3.8)	0 (0)	
Capillary tuft necrosis, *n* (%)				0.137
None	704 (89.8)	653 (89.9)	51 (87.9)	
(<25%)	71 (9.1)	66 (9.1)	5 (8.6)	
(25–50%)	8 (1.0)	7 (1.0)	1 (1.7)	
(>50%)	1 (0.1)	0 (0)	1 (1.7)	
Subendothelial hyaline deposits, *n* (%)				
None	333 (44.7)	307 (44.5)	26 (47.3)	0.559
Segmental	194 (26.0)	183 (26.5)	11 (20.0)	
Diffuse	218 (29.3)	200 (29.0)	18 (32.7)	
Interstitial leukocyte infiltration, *n* (%)				0.052
None	196 (25.0)	189 (26.0)	7 (12.3)	
<25%	461 (58.9)	427 (58.8)	34 (59.6)	
25–50%	87 (11.1)	76 (10.5)	11 (19.3)	
50–75%	30 (3.8)	26 (3.6)	4 (7.0)	
>75%	9 (1.1)	8 (1.1)	1 (1.8)	
Interstitial fibrosis, *n* (%)				0.023
None	330 (42.1)	315 (43.4)	15 (26.3)	
<25%	367 (46.9)	336 (46.3)	31 (54.4)	
25–50%	63 (8.0)	56 (7.7)	7 (12.3)	
50–75%	17 (2.2)	13 (1.8)	4 (7.0)	
>75%	6 (0.8)	6 (0.8)	0 (0)	
Tubular necrosis, *n* (%)	43 (5.5)	38 (5.2)	5 (8.8)	0.260
Tubular atrophy, *n* (%)				0.035
None	326 (41.6)	313 (43.1)	13 (22.8)	
<25%	360 (46.0)	326 (44.9)	34 (59.6)	
25–50%	73 (9.3)	65 (9.0)	8 (14.0)	
50–75%	19 (2.6)	19 (2.6)	2 (3.5)	
>75%	3 (0.4)	3 (0.4)	0 (0)	
Artery wall thickening, *n* (%)	231 (29.5)	203 (28.0)	28 (48.3)	0.001

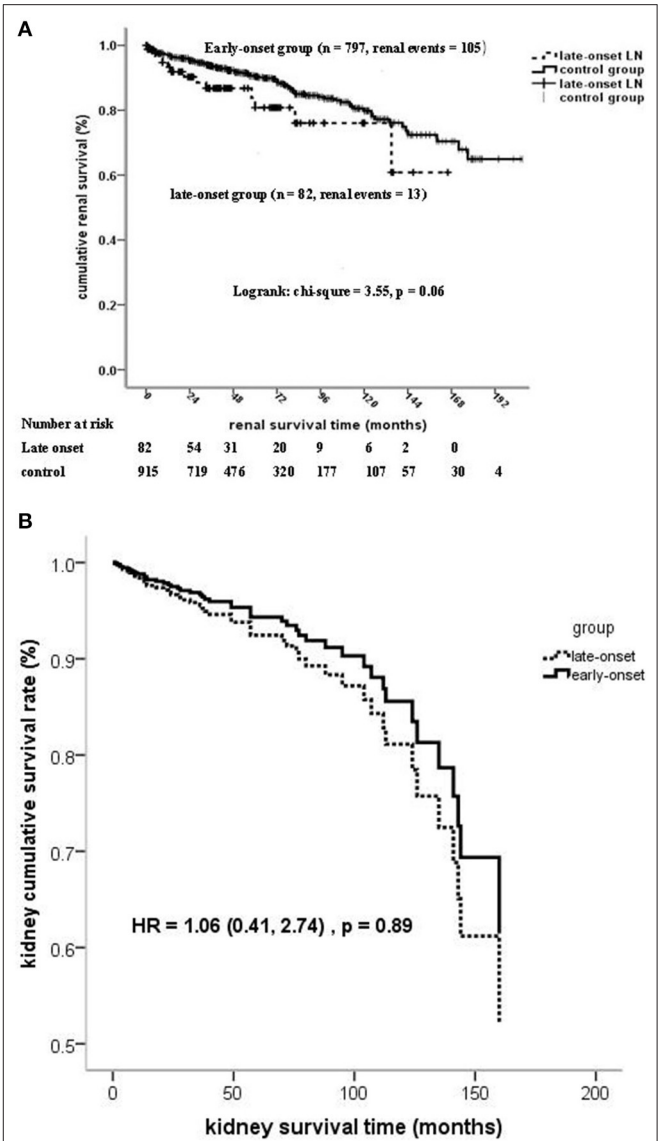


FIGURE 2 | (A) Kidney survival (at the composite endpoint of serum creatinine doubling and ESRD) stratified on the age of LN onset. **(B)** Kidney survival (at the composite endpoint of serum creatinine doubling and ESRD) stratified on the age of LN onset by cox regression.

the two groups, which is different from previous report on a lower SLEDAI score in patients with late-onset SLE (8, 14). Further analysis of kidney histopathology implied that the chronic lesions (glomerular sclerosis, interstitial fibrosis, tubular atrophy, and cortex wall thickening) were more severe in patients with late-onset LN. However, active lesions such as crescents, karyorrhexis, capillary tuft necrosis, glomerular capsule adhesion, tubular necrosis, were not significantly different between the two groups. The glomerular leukocyte infiltration and endocapillary hypercellularity showed more severe in the early-onset group. Therefore, it indicated that the relatively poorer conditions of patients with late-onset SLE at baseline are mainly associated with their advanced age, while

TABLE 5 | Association of late-onset LN with all-cause mortality and kidney mortality.

Model	All-cause mortality		Kidney outcome	
	HR [CI (95%)]	*p*	HR [CI (95%)]	*p*
Univariable Cox model	1.72 (1.32, 2.24)	<0.001	1.66 (0.93, 2.97)	0.08
Model 1	2.27 (2.10, 5.08)	<0.001	1.25 (0.69, 2.25)	0.45
Model 2	2.16 (1.18, 3.94)	0.012	1.17 (0.53, 2.57)	0.68
Model 3	2.63 (1.35, 5.13)	<0.001	1.11 (0.50, 2.46)	0.78
Model 4	3.03 (1.39, 6.58)	0.005	1.06 (0.41, 2.74)	0.89

Reference, LN onset age < 50; HR, hazard ratio; CI, confidence interval.
Model 1: adjusted for sex, life expectancy.
Model 2: adjusted for Model 1 and creatinine, uric acid, urine protein, C3, SLEDAI score.
Model 3: adjusted for Model 2 steroid dose and immunosuppression drugs.
Model 4: adjusted for Model 3 and crescent, glomerular sclerosis, interstitial fibrosis, tubular atrophy.

TABLE 6 | Predictors of kidney survival in patients with late-onset LN.

Variants	Univariate	*p*	Multivariate	*p*
	HR [CI (95%)]		HR [CI (95%)]	
Gender (F: refer)	2.26 (0.28, 18.09)	0.442		
CVD history	2.79 (0.57, 13.55)	0.202		
Diagnose duration (↑12 months)	0.97 (0.93, 1.02)	0.341		
Urine protein (↑1 g/L)	1.14 (0.94, 1.37)	0.168		
SBP (↑10 mmHg)	1.01 (0.98, 1.04)	0.472		
Oliguria/anuria (no: refer)	3.42 (0.96, 12.18)	0.057	-	NS
Hemoglobin (↑10 g/L)	0.68 (0.51, 0.91)	0.011	-	NS
Microscopic hematuria (no: refer)	1.53 (0.43, 5.47)	0.506		
Baseline creatinine (↑100 μmol/L)	1.38 (1.13, 1.69)	0.001	1.45 (1.20, 1.73)	<0.001
Baseline uric acid (↑100 mmol/L)	1.00 (1.00, 1.01)	0.059	–	NS
Serum albumin (↓10 g/L)	0.93 (0.84, 1.04)	0.099	–	NS
AKI (no: refer)	2.13 (0.62, 7.32)	0.229		
C3 (↓0.1 g/L)	0.66 (0.05, 8.76)	0.759		
hs-CRP (↑1 g/L)	1.00 (1.00, 1.01)	0.018	-	NS
SLEDA score (↑1)	1.06 (0.96, 1.18)	0.211	-	NS
Glomerular sclerosis (↑1%)	15.1 (0.55, 412.3)	0.108	-	NS
Crescents (↑1%)	0.62 (0.007, 55.8)	0.837		
Interstitial fibrosis>75% (no: refer)	1.88 (0.21, 16.8)	0.573		
Tubular necrosis (no: refer)	24.1 (0.00, 165.3)	0.575		
Tubular atrophy (no: refer)	1.31 (0.54, 3.14)	0.541		
Artery wall thickening (no: refer)	2.16 (0.42, 11.18)	0.355		
Glucocorticoid at initial treatment	0.30 (0.04, 2.41)	0.259		
Immune suppressor at initial treatment	1.06 (0.29, 3.78)	0.926		

CVD, Cardiovascular disease; SBP, systolic blood pressure; AKI, acute kidney injury; hs-CRP, high-sensitivity C-reactive protein; SLEDAI, systemic lupus erythematosus disease activity index.

not with SLE activity *per se*. To our knowledge, there is only few detailed information available on kidney histopathological features of late-onset LN in published papers (3, 23, 24). Both chronic lesion and active lesion associated influenced the outcome of LN. Therefore, the results of kidney survival analysis which showed no statistical difference even by Cox regression (adjusted for multivariate) was the combined effect of the two factors. In consideration of the more comorbid conditions and chronic impairment of histopathology, practitioners tended to give late-onset LN with lower corticosteroid dose and less frequent cyclophosphamide lest the side-effect. However, the present study failed to prove whether the limited therapeutic options for SLE due to the presence of comorbidities and concomitant therapies in elderly patients may contribute to the poorer outcome.

There were conflicting reports with regard to patient outcome of late-onset SLE. An epidemiological study from Canada (29) and long-term cohort studies from Brazil (7) and China (28) found that late-onset SLE is not a benign subgroup. Another study reported that older patients have less kidney involvement and better prognosis than their younger counterparts (9). In our cohort, the 5- and 10-year survival rates of patients with late-onset LN were 68.5 and 49.1%, respectively, which are much lower than those of patients with early-onset LN (95.5 and 92.1%). In patients with late-onset LN, kidney failure was a more common cause of death (14.8%) than in patients with early-onset LN (4.7%). Results of multivariate regression equation showed that onset at age >50 years was an independent risk factor of patient survival. Considering that age is naturally associated with survival, life expectancy was taken as a confounder into the multivariable Cox regression model. The independent association between late-onset LN and patient survival still existed, which made the conclusion more robust.

Only a small number of studies have reported on kidney outcome (3, 30, 31). In our study, although patients with late-onset LN had a poorer kidney baseline, no significant difference on the kidney outcome was found between late- and early-onset LN. The contradiction of worse kidney baseline and benign kidney prognosis may have partially been related to relatively milder SLE course in older patients (12). These might also be explained by the results of comparisons on histopathological characteristics. In general, no significant difference was found in the pathological type distribution between the two groups. With respect to the activity indices and chronicity indices, there were significantly lower scores in activity indices but significantly higher scores in chronic indices such as tubular atrophy and interstitial fibrosis in the late onset group. This is consistent with another report based on Chinese population (3). Nevertheless, in older patients, AKI occurred, to some extent, more frequently on conditions of aggravating factors, such as hypovolemia, infection, and

nephrotoxic medication. More attention should be paid on overall condition balancing and complication correcting in elder patients.

The strengths of our study are the relatively large sample size and long follow-up duration focusing on the kidney survival and taking a closer sight on the detailed pathological changing analysis to investigate the effect of onset-age of LN on the prognosis of late-onset LN.

An important potential limitation of our study is its retrospective design, where information bias, selection bias, and uncontrolled confounding effects could potentially influence the results. A critical and unresolved question is whether or not the poorer outcomes observed in patients with late-onset LN are solely age related. This issue warrants further investigation in a prospective controlled cohort study.

CONCLUSIONS

Age-related impairments are the most significant determinant of both clinical and pathological manifestations in patients with late-onset LN. Patients with late-onset LN have milder active lesions related to SLE but severer chronic lesions in kidney pathology, and they have an unfavorable patient outcome but rather acceptable kidney prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Human Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. Each participant provided written informed consent.

AUTHOR CONTRIBUTIONS

WC and XY designed the study. NT drafted the manuscript. NT, PRY, and QML participated in the data collection. WFC reviewed the pathology slices. NT, QZ, and LYH performed the statistical analysis. All authors read and approved the final manuscript.All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (Nos. 81970599, 82170737, and 81960144); Key Laboratory of National Health Commission, and Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China (Nos. 2002B60118 and 2020B1212060028).

ACKNOWLEDGMENTS

We appreciate the help and support from all the participants in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.882692/full#supplementary-material

REFERENCES

1. Li LS, Liu ZH. Epidemiologic data of kidney diseases from a single unit in China: analysis based on 13,519 kidney biopsies. *Kidney Int.* (2004) 66:920–3. doi: 10.1111/j.1523-1755.2004.00837.x
2. Ward MM, Polisson RPA. meta-analysis of the clinical manifestations of older-onset systemic lupus erythematosus. *Arthritis Rheum.* (1989) 32:1226–32. doi: 10.1002/anr.1780321007
3. Xu Y-X, Tan Y, Yu F, Zhao M- H. Late onset lupus nephritis in Chinese patients: classified by the 2003 International Society of Nephrology and Kidney Pathology Society system. *Lupus.* (2011) 20:801–8. doi: 10.1177/0961203310397563
4. Voulgari PV, Katsimbri P, Alamanos Y, Drosos AA. Gender and age differences in systemic lupus erythematosus. A study of 489 Greek patients with a review of the literature. *Lupus.* (2002) 11:722–9. doi: 10.1191/0961203302lu253oa
5. Tomic-Lucic A, Petrovic R, Radak-Perovic M, Milovanovic D, Milovanovic J, Zivanovic S, et al. Late-onset systemic lupus erythematosus: clinical features, course, and prognosis. *Clin Rheumatol.* (2013) 32:1053–8. doi: 10.1007/s10067-013-2238-y
6. Groot N, Shaikhani D, Teng YKO, de Leeuw K, Bijl M, Dolhain RJEM., et al. Long-term clinical outcomes in a cohort of adults with childhood-onset systemic lupus erythematosus. *Arthritis Rheumatol.* (2019) 71:290–301. doi: 10.1002/art.40697
7. Appenzeller S, Pereira DA, Costallat LT. Greater accrual damage in late-onset systemic lupus erythematosus: a long-term follow-up study. *Lupus.* (2008) 17:1023–8. doi: 10.1177/0961203308089695
8. Bertoli AM, Alarcón GS. Calvo-Alén J, Fernández M, Vilá LM, Reveille JD, LUMINA Study Group. Systemic lupus erythematosus in a multiethnic US cohort XXXIII Clinical [corrected] features, course, and outcome in patients with late-onset disease. *Arthritis Rheum.* (2006) 54:1580–7. doi: 10.1002/art.21765
9. Sohn IW, Joo YB, Won S, Bae SC. Late-onset systemic lupus erythematosus: Is it "mild lupus"? *Lupus.* (2018) 27:235–42. doi: 10.1177/0961203317716789
10. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* (1997) 40:1725. doi: 10.1002/art.1780400928
11. Medlin JL, Hansen KE, Fitz SR, Bartels CMA. Systematic review and meta-analysis of cutaneous manifestations in late- versus early-onset systemic lupus erythematosus. *Semin Arthritis Rheum.* (2016) 45:691–7. doi: 10.1016/j.semarthrit.2016.01.004
12. Alonso MD, Martinez-Vazquez F, de Teran, Miranda-Filloy TD, Dierssen JA, Blanco T, et al. Late-onset versus early-onset systemic lupus: characteristics and outcome in a national multicentre register (RELESSER). *Rheumatology (Oxford).* (2021) 60:1793-1803. doi: 10.1093/rheumatology/keaa477
13. Boddaert J, Huong DL, Amoura Z, Wechsler B, Godeau P, Piette JC. Late-onset systemic lupus erythematosus: a personal series of 47 patients and pooled analysis of 714 cases in the literature. *Medicine (Baltimore).* (2004) 83:348–59. doi: 10.1097/01.md.0000147737.57861.7c

14. Medhat BM, Behiry ME, Sobhy N, Farag Y, Marzouk H, Mostafa N, et al. Late-onset systemic lupus erythematosus: characteristics and outcome in comparison to juvenile- and adult-onset patients-a multicenter retrospective cohort. *Clin Rheumatol.* (2020) 39:435–42. doi: 10.1007/s10067-019-04776-y
15. Lopez P, Mozo L, Gutierrez C, Suarez A. Epidemiology of systemic lupus erythematosus in a northern Spanish population: gender and age influence on immunological features. *Lupus.* (2003) 12:860–5. doi: 10.1191/0961203303lu469xx
16. Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Fortin P, Ginzler E, et al. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus International Comparison. *J Rheumatol.* (2000) 27:373–6.
17. Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Bombardier C, Isenberg D, et al. Sensitivity to change of 3 systemic lupus erythematosus disease activity indices: international validation. *J Rheumatol.* (1994) 21:1468–71.
18. Stevens PE, Levin A. Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am J Kidney Dis 61.* (2013) 649–72. doi: 10.1053/j.ajkd.2013.02.349
19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
20. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. International Society of Nephrology Working Group on the Classification of Lupus Nephritis; Kidney Pathology Society Working Group on the Classification of Lupus Nephritis. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int.* (2004) 65:521–30. doi: 10.1111/j.1523-1755.2004.00443.x
21. Sayarlioglu M, Cefle A, Kamali S, Gul A, Inanc M, Ocal L, et al. Characteristics of patients with late onset systemic lupus erythematosus in Turkey. *Int J Clin Pract.* (2005) 59:183–7. doi: 10.1111/j.1742-1241.2004.00283.x
22. Delfino J, Dos Santos TAFG, Skare TL. Comparison of lupus patients with early and late onset nephritis: a study in 71 patients from a single referral center. *Adv Rheumatol.* (2020) 60:5. doi: 10.1186/s42358-019-0105-5
23. Tang Z, Chen D, Yang S, Zhang H, Hu W, Liu Z, et al. Late onset lupus nephritis: analysis of clinical manifestations and kidney pathological features in Chinese patients. *Rheumatol Int.* 31:1625–9. doi: 10.1007/s00296-010-1536-9
24. Martínez-Barrio J, Ovalles-Bonilla JG, López-Longo FJ, González CM, Montoro M, Valor L, et al. Juvenile, adult and late-onset systemic lupus erythematosus: a long term follow-up study from a geographic and ethnically homogeneous population. *Clin Exp Rheumatol.* (2015) 33:788–94.
25. Alonso MD, Martinez-Vazquez F, Diaz T, de Teran, Miranda-Filloy JA, Dierssen T, et al. Late-onset systemic lupus erythematosus in Northwestern Spain: differences with early-onset systemic lupus erythematosus and literature review. *Lupus.* (2012) 21:1135–48. doi: 10.1177/0961203312450087
26. Jorge IRS, Karin B, Johannes M, Dag L, Elisabet S, Andreas J, et al. Sex differences in clinical presentation of systemic lupus erythematosus. *Biol Sex Differ.* (2019) 10:60. doi: 10.1186/s13293-019-0274-2
27. Arnaud, L., Mathian, A., Boddaert, J., and Amoura, Z. Late-onset systemic lupus erythematosus: epidemiology, diagnosis and treatment. *Drugs Aging.* (2012) 29:181–9. doi: 10.2165/11598550-000000000-00000
28. Lin H, Wei JC, Tan CY, Liu YY, Li LiYH, Deng FX, et al. analysis of late-onset systemic lupus erythematosus: a cohort study in China. *Clin Rheumatol.* (2012) 31:1683–9. doi: 10.1007/s10067-012-2073-6
29. Lalani S, Pope J, de Leon, Peschken F, Clinical C. Features and prognosis of late-onset systemic lupus erythematosus: results from the 1000 faces of lupus study. J Rheumatol. (2010) 37:38–44. doi: 10.3899/jrheum.080957
30. Austin RHA, Muenz LR, Joyce KM, Antonovych TT, Balow JE. Diffuse proliferative lupus nephritis: Identification of specific pathologic features affecting kidney outcome. *Kidney Int.* (1984) 25:689–95. doi: 10.1038/ki.1984.75
31. Chen YM, Lin CH, Chen HH, Chang SN, Hsieh TY, Hung WT, et al. Onset age affects mortality and kidney outcome of female systemic lupus erythematosus patients: a nationwide population-based study in Taiwan. *Rheumatology (Oxford).* (2014) 53:180–5. doi: 10.1093/rheumatology/ket330

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

TYPE Correction
PUBLISHED 05 September 2022
DOI 10.3389/fmed.2022.1018311

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Wei Chen
vvchen66@qq.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 13 August 2022
ACCEPTED 15 August 2022
PUBLISHED 05 September 2022

CITATION
Tian N, Zhou Q, Yin P, Chen W, Hong L, Luo Q, Chen M, Yu X and Chen W (2022) Corrigendum: Long-term kidney prognosis and pathological characteristics of late-onset lupus nephritis. *Front. Med.* 9:1018311. doi: 10.3389/fmed.2022.1018311

Corrigendum: Long-term kidney prognosis and pathological characteristics of late-onset lupus nephritis

Na Tian[1,2], Qian Zhou[1,3], PeiRan Yin[1,4], WenFang Chen[5], LingYao Hong[1], QiMei Luo[1], MengHua Chen[2], XueQing Yu[6] and Wei Chen[1]*

[1]Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Key Laboratory of National Health Commission, Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China, [2]Department of Nephrology, General Hospital of Ningxia Medical University, Ningxia, China, [3]Department of Medical Statistics, Clinical Trials Unit, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [4]Department of Nephrology, Second Affiliated Hospital of Soochow University, Suzhou, China, [5]Department of Pathology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [6]Guangdong Provincial People's Hospital, Guangzhou, China

KEYWORDS

lupus nephritis, late-onset, kidney outcome, renal pathology, Chinese population

A corrigendum on
Long-term kidney prognosis and pathological characteristics of late-onset lupus nephritis

by Tian, N., Zhou, Q., Yin, P., Chen, W., Hong, L., Luo, Q., Chen, M., Yu, X., and Chen, W. (2022). *Front. Med.* 9:882692. doi: 10.3389/fmed.2022.882692

In the published article, there was an error in affiliation 1. Instead of "Key Laboratory of National Health Commission, Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China," it should be "Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Key Laboratory of National Health Commission, Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China."

Affiliation 7 has been deleted.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 02 June 2022
doi: 10.3389/fmed.2022.876229

Outcome Analysis of Transition From Peritoneal Dialysis to Hemodialysis: A Population-Based Study

*Ming-Hsien Tsai[1,2], Yun-Yi Chen[3,4], Tsrang-Neng Jang[2,5], Jing-Tong Wang[1] and Yu-Wei Fang[1,2]**

[1] Division of Nephrology, Department of Internal Medicine, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, [2] Department of Medicine, Fu Jen Catholic University School of Medicine, Taipei, Taiwan, [3] Department of Research, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, [4] Institute of Hospital and Health Care Administration, National Yang Ming Chiao Tung University, Taipei, Taiwan, [5] Department of Internal Medicine, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan

If a technical failure occurs during peritoneal dialysis (PD), the patients undergoing PD may be transitioned to hemodialysis (HD). However, the clinical outcomes of patients who have undergone such a transition are under studied. This study assessed whether patients undergoing HD who have transitioned from PD have the same clinical outcomes as HD-only patients. This research was a retrospective cohort study by searching a National Health Insurance research database for data on patients in Taiwan who had undergone HD between January 2006 and December 2013. The patients were divided into two groups, namely a case group in which the patients were transitioned from PD to HD and a HD-only control group, through propensity score matching at a ratio of 1:4 (n = 1,100 vs. 4,400, respectively). We used the Cox regression model to estimate the hazard ratios (HRs) for all-cause death, all-cause hospitalization, infection-related admission, and major adverse cardiac events (MACE). Those selected patients will be followed until death or the end of the study period (December, 2017), whichever occurs first. Over a mean follow-up of 3.2 years, 1,695 patients (30.8%) died, 3,825 (69.5%) required hospitalization, and 1,142 (20.8%) experienced MACE. Patients transitioning from PD had a higher risk of all-cause death (HR: 1.36; 95% CI: 1.21–1.53) than HD-only patients. However, no significant difference was noted in terms of MACE (HR: 0.91; 95% CI: 0.73–1.12), all-cause hospitalization (HR: 1.07; 95% CI: 0.96–1.18), or infection-related admission (HR: 0.97, 95% CI: 0.80–1.18) between groups. Because of the violation of the proportional hazard assumption, the piecewise-HRs showed that the risk of mortality in the case group was significant within 5 months of the transition (HR: 2.61; 95% CI: 2.04–3.35) not in other partitions of the time axis. In conclusion, patients undergoing HD who transitioned from PD had a higher risk of death than the HD-only patients, especially in the first 5 months after transition (a 161% higher risk). Therefore, more caution and monitoring may be required for patients undergoing HD who transitioned from PD.

Keywords: hemodialysis, peritoneal dialysis, peritoneal dialysis technique failure, mortality, hospitalization, major adverse cardiac outcomes

OPEN ACCESS

Edited by:
Jiannong Liu,
Hennepin Healthcare Research Institute, United States

Reviewed by:
Mark Dominik Alscher,
Robert Bosch Hospital, Germany
Maria-Eleni Roumelioti,
University of New Mexico, United States

***Correspondence:**
Yu-Wei Fang
m005916@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *15 February 2022*
Accepted: *09 May 2022*
Published: *02 June 2022*

Citation:
Tsai M-H, Chen Y-Y, Jang T-N, Wang J-T and Fang Y-W (2022) Outcome Analysis of Transition From Peritoneal Dialysis to Hemodialysis: A Population-Based Study. Front. Med. 9:876229. doi: 10.3389/fmed.2022.876229

INTRODUCTION

Taiwan has a high burden of chronic kidney disease (CKD) (1). In a comparison of international data from the United States Renal Data System in 2016, Taiwan was reported to have the highest incidence [0.455/1,000 person-years (PY)] and prevalence (0.32%) of end-stage renal disease (ESRD) requiring renal replacement therapy among surveyed countries, and it maintained high levels of incidence and prevalence in subsequent years (2, 3). The trend of choosing peritoneal dialysis (PD) as treatment for ESRD has gradually decreased in Taiwan, accounting for only 6–8% of dialysis treatments from 2000 to 2018. This may be due to a decrease in new-onset PD from 14.2% in 2007 to 9.4% in 2018. Because of this decrease, hemodialysis (HD) has gained prevalence (4).

Compared with HD, PD offers several clinical benefits. First, patients undergoing PD have been reported to have a better 3-year survival rate, although the age-adjusted life span for both treatments was similar (2). Second, the degree of residual kidney function preservation and quality of life has been reported to be more favorable in PD than in HD (5, 6). Third, PD is more cost-effective than HD (7, 8). Accordingly, PD has been widely recognized and employed as the first therapy of choice (9).

However, several limitations and risks have been reported for PD that lead to patients requiring a transition to HD. The most prevalent risk is PD-related peritonitis, which is the reason for 28–35% of PD dropouts (10). In addition, gradual loss of residual kidney function and progressive reduction of peritoneal function resulting from PD may cause insufficient clearance and ultrafiltration (11). These limitations often negate the initial benefits of PD, and approximately 35% of patients undergoing PD drop out of the therapy and require transition to HD (12).

Because transition from PD to HD is inevitable for patients with ESRD, no study has yet analyzed outcomes by using a real-world dataset. In this study, we compared the outcomes of patients who transitioned from PD to HD with those of patients undergoing HD-only using a nationwide population-based dataset.

MATERIALS AND METHODS

Data Sources and Research Samples

We used a CKD sub-database from the National Health Insurance Research Database (NHIRD) maintained by the Health and Welfare Data Science Center of the Ministry of Health and Welfare of Taiwan. The diagnosis of CKD includes 124 ICD-9-CM codes that were verified officially in the Chronic Kidney Disease Prevention Technology Research Project conducted by the Health Promotion Administration, Ministry of Health and Welfare (13). Since its launch in 1995, the NHIRD has compiled records of 99% of Taiwan's 23 million citizens who have enrolled in the Taiwan National Health Insurance program (14). Before they are released from the NHIRD, data are deidentified and identifying information is encrypted. The deidentified, personal information retained in the data are date of birth, sex, area of residence, diagnostic codes, medical procedures, and drug prescriptions. Before 2015, diseases listed in the NHIRD were defined according to International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes. After 2015, diseases were defined according to International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) codes. This study was exempted from full ethical review and was approved by the institutional review board of Shin-Kong Wu Ho-Su Memorial Hospital (IRB approval number: No. 20200806R). Informed consent was waived by the Ethics Committee of Shin-Kong Wu Ho-Su Memorial Hospital because the personal information of all beneficiaries listed in the NHIRD was deidentified.

Study Design and Population

This study was designed as a population-based longitudinal cohort study. The target population was patients who newly entering chronic dialysis (receiving dialysis more than 3 months) from January 1, 2006, to December 31, 2013 (n = 48,294). Patients who were under 20 years of age, were undergoing PD, and were given a diagnosis of malignancy or who underwent coronary artery bypass graft (CABG) 1 year prior to beginning dialysis were excluded. Moreover, patients who transitioned from PD to HD with a PD history of less than 3 months or who died within a month of dialysis transition were also excluded. A total of 40,108 patients undergoing dialysis were included in the analysis. To reduce baseline differences between study groups, we used 1:4 propensity score matching for variables age, sex, and Charlson comorbidity index score (15). Accordingly, our analysis included patients undergoing HD who had transitioned from PD (n = 1,100) and patients in a matched comparison group (n = 4,400; **Figure 1**). The index day for the first group was 1 month after the transition from PD to HD. The index day for the HD-only group was the day when they started to undergo chronic dialysis, plus the dialysis vintage of the matching case. Those selected patients will be followed until death or the end of the study period (December 31, 2017), whichever occurs first.

Baseline comorbidities, including hypertension, diabetes mellitus, coronary artery disease (CAD), chronic heart failure (CHF), atrial fibrillation, peripheral vascular disease (PVD), stroke, chronic obstructive pulmonary disease (COPD), hyperlipidemia, and polycystic kidney disease, were defined as present if the patient had at least three outpatient diagnoses or one inpatient diagnosis within the year preceding the index date (**Supplementary Table 1**). Use of drugs, including renin–angiotensin system blockers (RASB), beta-blockers, calcium channel blockers (CCB), anticoagulants, dipeptidyl peptidase-4, and lipid-lowering agents, was defined as present if the patient had used the drug for least 3 months within 1 year preceding the index date (**Supplementary Table 2**).

Definition of Outcomes

The primary outcomes of this study were all-cause mortality, all-cause hospitalization, infection-related admission, and major adverse cardiac events (MACE), which comprises myocardial infarction, cerebrovascular disease, heart failure, and arrhythmia (**Supplementary Table 1**). Data were analyzed from the index

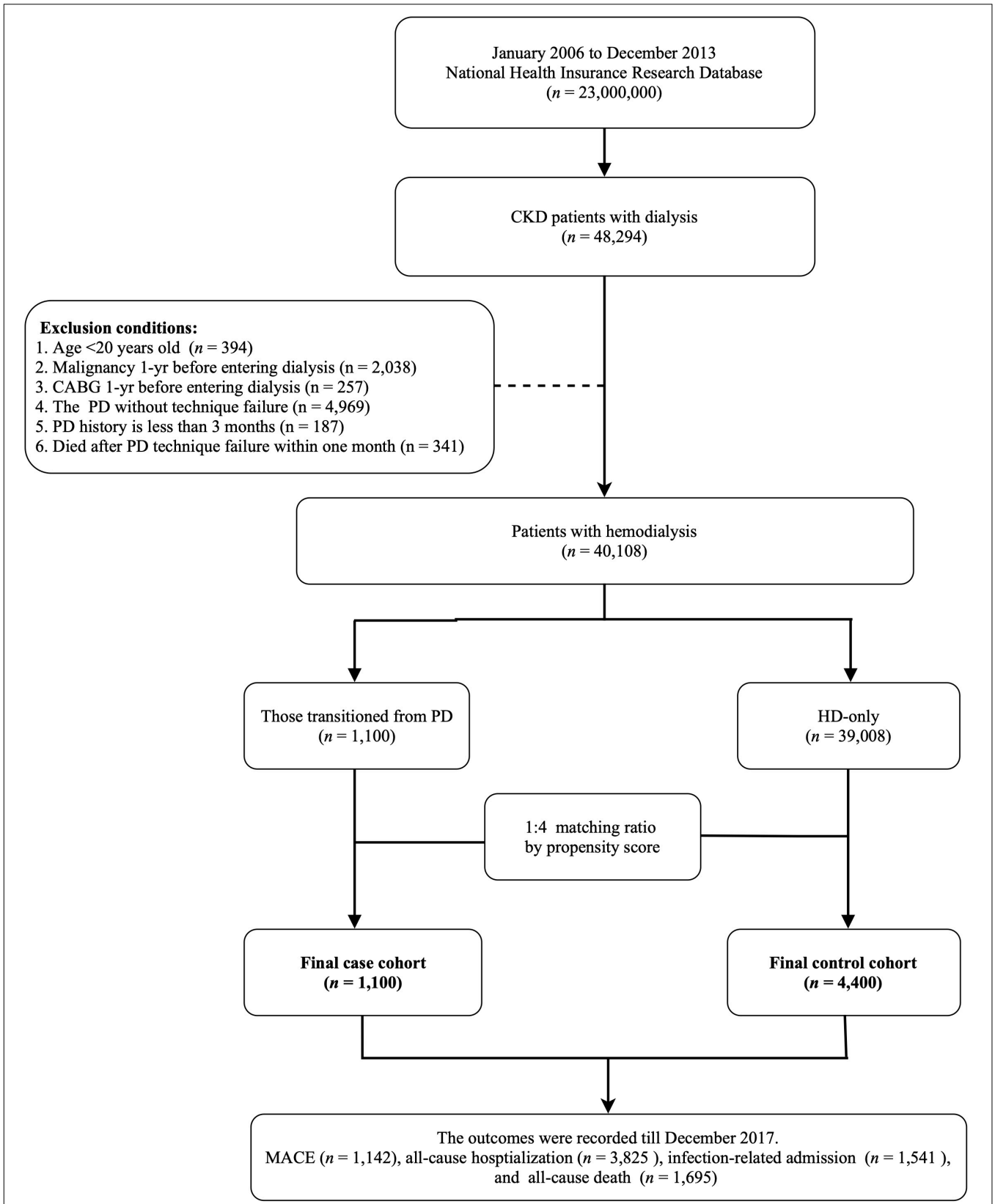


FIGURE 1 | Schematic of patient enrollment. CKD, chronic kidney disease; PD, peritoneal dialysis; CABG coronary artery bypass graft; HD, hemodialysis; MACE, major adverse cardiovascular events.

date to the first instance of the desired outcome (as previously defined) or to the end of the study period (December 31, 2017).

Statistical Analysis

Continuous data of the baseline characteristics are expressed as mean ± standard deviation, and categorical data are expressed as counts with proportions. The differences between the groups were determined using Chi-squared tests and *t* tests for proportions and continuous variables, respectively. The Kaplan–Meier method was employed for estimating and plotting event-free curves, which were then tested using a log-rank test. A Cox proportional-hazard model for clustered data was used to estimate the hazard ratios (HRs), and a 95% confidence interval (CI) was calculated for the risk of clinical outcomes. Both crude-adjusted and multivariable-adjusted analyses were performed. The assumption of proportional hazard was tested for the interaction between time and the variables. If the assumption was violated, the time axis was partitioned for the further analysis. Moreover, death was considered a competing risk for the development of desired outcomes in our analysis.

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, United States). For all tests, two-sided p values < 0.05 indicated statistical significance.

RESULTS

Patient Characteristics

In the present study, we enrolled 5,500 patients undergoing HD (**Figure 1**). Among these patients, 1,100 had transitioned from PD to HD and 4,400 were HD-only. The two groups were matched using propensity scores. The differences in the baseline characteristics between groups are presented in **Table 1**. Several significant differences in comorbidities, such as for hypertension, diabetes mellitus, atrial fibrillation, stroke, COPD, and hyperlipidemia (all $p < 0.05$), were identified between the groups. Moreover, the patients who had previously undergone PD had higher instances of the use of RASB, beta-blockers, CCB, anticoagulants, dipeptidyl peptidase-4, and lipid-lowering agents (all $p < 0.05$).

All-Cause Deaths Between Groups

Within the mean follow-up time of 3.2 years, all-cause death differed between the transition from PD to HD and HD-only groups, with 385 patients [incidence, 0.126/person-year (PY)] and 1310 patients (incidence, 0.089/PY) dying, respectively (**Table 2**). **Table 3** shows no significant difference of the causes of death between groups ($p = 0.748$). The deaths resulted from cancer, sepsis, cardiovascular disease and dialysis between the transition from PD to HD and HD-only groups were 22 (5.7%) versus 76 (5.8%); 43 (11.2%) versus 152 (11.6%); 97 (25.2%) versus 315 (24 %); and 85 (22.1%) versus 326 (24.9%), respectively. The Kaplan–Meier event-free curves for all-cause deaths are presented in **Figure 2A**. The log-rank test indicated that the difference between the two groups was significant ($\chi^2 = 33.02$, $p < 0.001$). However, the two survival curves came close as time went on, indicating it may violate the proportional hazard assumption.

As presented in **Table 2**, the patients who had transitioned from PD had a higher risk of all-cause death (HR: 1.39; 95% CI: 1.24–1.56) in crude analysis, and this significant association remained even after adjustment for comorbidities and drug use (HR: 1.36; 95% CI: 1.21–1.53). The parameter estimates for the covariates in the multivariable model of all-cause death are shown in **Supplementary Table 3**. Because of the assumption of proportional hazard was violated (time interaction, $p < 0.001$), the times axis were divided into four periods (≤5 months, 5–24 months, 2–5 years, and >5 years), a significant risk difference was observed only in the ≤5 months period (HR: 2.61; 95% CI: 2.04–3.35) (**Table 4**).

MACE and Hospitalization Between the Groups

As presented in **Table 2**, the incidence of all-cause hospitalization, infection-related admission, and MACE between the transition

TABLE 1 | Baseline characteristics of study population on hemodialysis after matching.

	Transitioned from PD	HD-only	*P* value
	(*n* = 1,100)	(*n* = 4,400)	
Age (years)			0.949
<65	836 (76.0)	3,340 (75.9)	
≤65	264 (24.0)	1,060 (24.1)	
Gender			0.859
Male	640 (58.2)	2,547 (57.9)	
Female	460 (41.9)	1,853 (42.1)	
Dialysis vintage	2.83 ± 0.57	2.83 ± 0.57	1
CCI score	1.35 ± 1.10	1.33 ± 1.08	0.57
Baseline comorbidities			
Hypertension	568 (51.6)	1,853 (42.1)	< 0.001
Diabetes mellitus	422 (38.4)	1,507 (34.2)	0.01
CAD	191 (17.4)	725 (16.9)	0.48
CHF	115 (10.5)	388 (8.8)	0.092
Atrial fibrillation	28 (2.6)	54 (1.2)	0.001
PVD	65 (5.9)	253 (5.8)	0.839
Stroke	109 (9.9)	331 (7.1)	0.001
COPD	81 (7.4)	236 (5.4)	0.01
Hyperlipidemia	180 (16.4)	546 (12.4)	< 0.001
Polycystic kidney	1 (< 0.1)	16 (< 0.1)	0.223
Prescriptions			
RASB	448 (40.7)	1,251 (28.4)	< 0.001
Beta-blocker	449 (40.8)	1,314 (29.9)	< 0.001
CCB	520 (42.3)	1,636 (37.2)	< 0.001
Anti-cogulants	369 (33.6)	951 (21.6)	< 0.001
DPP4	121 (11)	330 (7.5)	< 0.001
Lipid-lowering agents	294 (26.7)	809 (18.4)	< 0.001

HD, hemodialysis; PD, peritoneal dialysis; CCI, charlson comorbidity index; CAD, coronary artery disease; CHF, congestive heart failure; PVD, peripheral vascular disease; COPD, chronic obstructive pulmonary disease; RASB, renin-angiotensin system blockades; CCB, calcium channel blockers; DPP4, dipeptidyl peptidase-4.

TABLE 2 | Outcomes between hemodialysis patients with/without previous peritoneal dialysis.

Outcomes	Transitioned from PD		HD-only		Transitioned from PD vs. HD-only			
	Events	IR	Events	IR	Crude		Multivariable	
					HR (95%CI)	*P*	aHR* (95%CI)	*P*
All-cause Death	385	12.6	1,310	8.93	1.39 (1.24–1.56)	< 0.001	1.36 (1.21–1.53)	< 0.001
All-cause hospitalization	680	52.2	3,145	43.5	1.01 (0.91–1.12)	0.824	1.07 (0.96–1.18)	0.219
Infection-related admission	269	11.7	1,272	11	1.07 (0.89–1.30)	0.435	0.97 (0.80–1.18)	0.781
MACE	200	7.6	942	7.4	0.93 (0.75–1.14)	0.49	0.91 (0.73–1.12)	0.372

**Further adjusted comorbidities (hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, atrial fibrillation, peripheral vascular disease, stroke, chronic obstructive pulmonary disease, hyperlipidemia, polycystic kidney) and medications (renin-angiotensin system blockades, beta-blocker, calcium channel blockers, anti-coagulants, dipeptidyl peptidase-4, and Lipid-lowering agents).*

PD, peritoneal dialysis; HD, hemodialysis; IR, incidence rate (every 100 person-years); HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval; MACE, major adverse cardiovascular events.

TABLE 3 | The causes of death in hemodialysis patients.

Groups	The cause of death							
	Cancer	Sepsis	CVD	DM	Liver	ESRD	Others	*P value*
Transitioned from PD [*n* (%)]	22 (5.7)	43 (11.2)	97 (25.2)	74 (19.2)	17 (4.4)	85 (22.1)	47 (12.2)	0.748
HD-only [*n* (%)]	76 (5.8)	152 (11.6)	315 (24)	264 (20.2)	51 (3.9)	326 (24.9)	126 (9.6)	

HD, hemodialysis; PD, peritoneal dialysis; CVD, cardiovascular disease; DM, diabetes mellitus; ESRD, end stage of renal disease.

from PD to HD and HD-only groups were 0.522/PY versus 0.435/PY, 0.117/PY versus 0.11/PY, and 0.076/PY versus 0.074/PY, respectively. The Kaplan–Meier event-free curves for all-cause hospitalization ($\chi^2 = 33.02$, $p < 0.001$), infection-related admission ($\chi^2 = 0.22$, $p = 0.635$) and MACE ($\chi^2 = 0.07$, $p = 0.79$) are displayed in **Figures 2B–D**.

As indicated by the crude analysis presented in **Table 2**, no significant differences in risk of all-cause hospitalization (HR: 1.01; 95% CI: 0.91–1.12), infection-related admission (HR: 1.07; 95% CI: 0.89–1.30), and MACE (HR: 0.93; 95% CI: 0.75–1.14) were identified between the groups. These associations remained non-significant even after further adjustment for comorbidities and drug use, with HRs of 1.07 (95% CI: 0.96–1.18), 0.97 (95% CI: 0.80–1.18), and 0.91 (95% CI: 0.73–1.12) for all-cause hospitalization, infection-related admission, and MACE, respectively.

A sensitivity analysis was done by removing the exclusion criteria of CABG. There was still no significant difference in risk of MACE between the transition from PD to HD and HD-only groups (HR: 0.95; 95% CI: 0.81–1.12). The assumption of proportional hazard for all-cause hospitalization was violated ($p = 0.044$). Therefore, the times axis were partitioned into four groups (≤5 months, 5–24 months, 2–5 years, and >5 years) and no significant differences in risk of all-cause hospitalization was noted in the different time periods (**Table 4**).

Subgroup Analysis

The association of all-cause deaths in the transition from PD to HD group stratified by covariates is presented in **Figure 3**. The hazardous outcomes in this group were consistent and significant in all subgroups, with the exception of the subgroup with patients aged < 65 years (HR: 1.12; 95% CI: 0.96–1.31) and with a history of CAD (HR: 1.10; 95% CI: 0.88–1.38), CHF (HR: 1.27; 95% CI: 0.94–.1.71), PVD (HR: 1.25; 95% CI: 0.85–1.83), and hyperlipidemia (HR: 0.96; 95% CI: 0.73–1.27).

In the patients with a DM history, the association of all-cause deaths in the transition from PD kept significant (HR: 1.35; 95% CI: 1.16–1.57), but the association of all-cause hospitalization, infection-related admission, and MACE didn't (**Supplementary Table 4**).

DISCUSSION

Through analysis of data from a nationwide database, we demonstrated the outcomes of patients with ESRD who transitioned from PD to HD due to PD failure. The results indicate no significant differences in hospitalization and MACE in the transition from PD to HD group compared with the HD-only group. However, a higher rate of all-cause mortality was observed in the transition from PD to HD group, with the significance of this association remaining

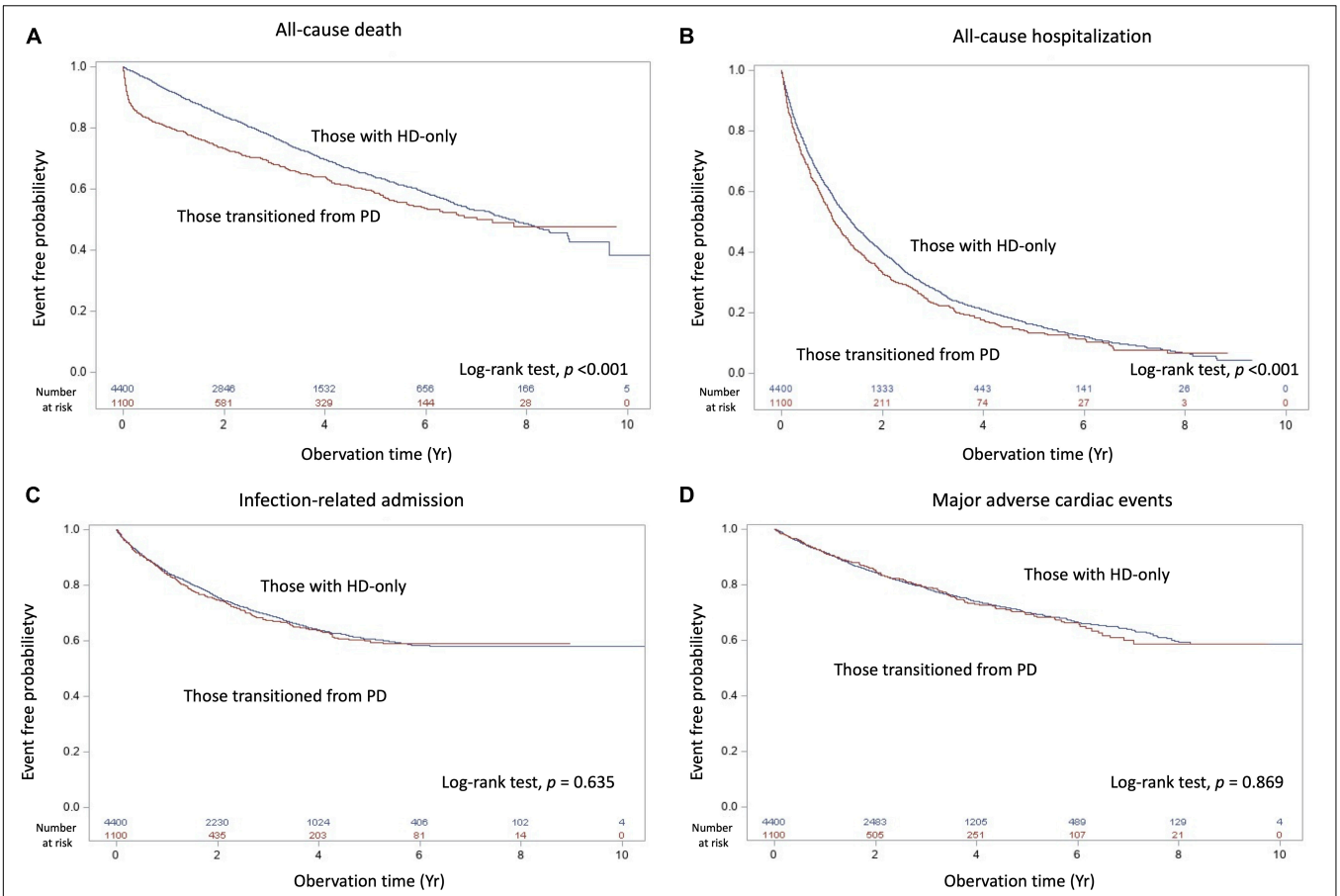


FIGURE 2 | Kaplan–Meier cumulative event-free plots of **(A)** all-cause death, **(B)** all-cause hospitalization, **(C)** infection-related hospitalization, and **(D)** major adverse cardiac events in the study population based on having experienced peritoneal dialysis; the results of log-rank tests were $\chi^2 = 33.01$ ($p < 0.001$), $\chi^2 = 12.89$ ($p < 0.001$), $\chi^2 = 0.22$ ($p = 0.635$) and $\chi^2 = 0.03$ ($p = 0.869$), respectively. Abbreviation: HD, hemodialysis; PD, peritoneal dialysis.

TABLE 4 | Outcome analysis of mortality and hospitalization by times axis in hemodialysis patients.

	Transitioned from PD vs. HD-only					
			All-cause death		All-cause hospitalization	
Partition of time axis	**Events of Death (%)[#]**	**Events of Hospitalization (%)[#]**	**aHR* (95%CI)**	***P***	**aHR* (95%CI)**	***P***
≤5 _months	177/150	266/964	2.61	< 0.001	0.91	0.184
	(46 vs. 11)	(39 vs. 31)	(2.04–3.35)		(0.80–1.05)	
5–24 _months	93/495	299/1446	0.87	0.218	1.05	0.585
	(24 vs. 38)	(44 vs. 46)	(0.69–1.09)		(0.91–1.17)	
2–5 _years	88/507	102/634	0.85	0.164	0.91	0.37
	(23 vs. 39)	(15 vs. 20)	(0.67–1.07)		(0.73–1.12)	
> 5 _years	27/158	13/101	0.73	0.145	0.71	0.269
	(7 vs. 12)	(2 vs. 3)	(0.48–1.11)		(0.39–1.30)	

Multivariable adjusting model was the same as that in **Table 2.*
[#]The portion of deaths in the different time axis by total deaths.
HD, hemodialysis; PD, peritoneal dialysis; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval.

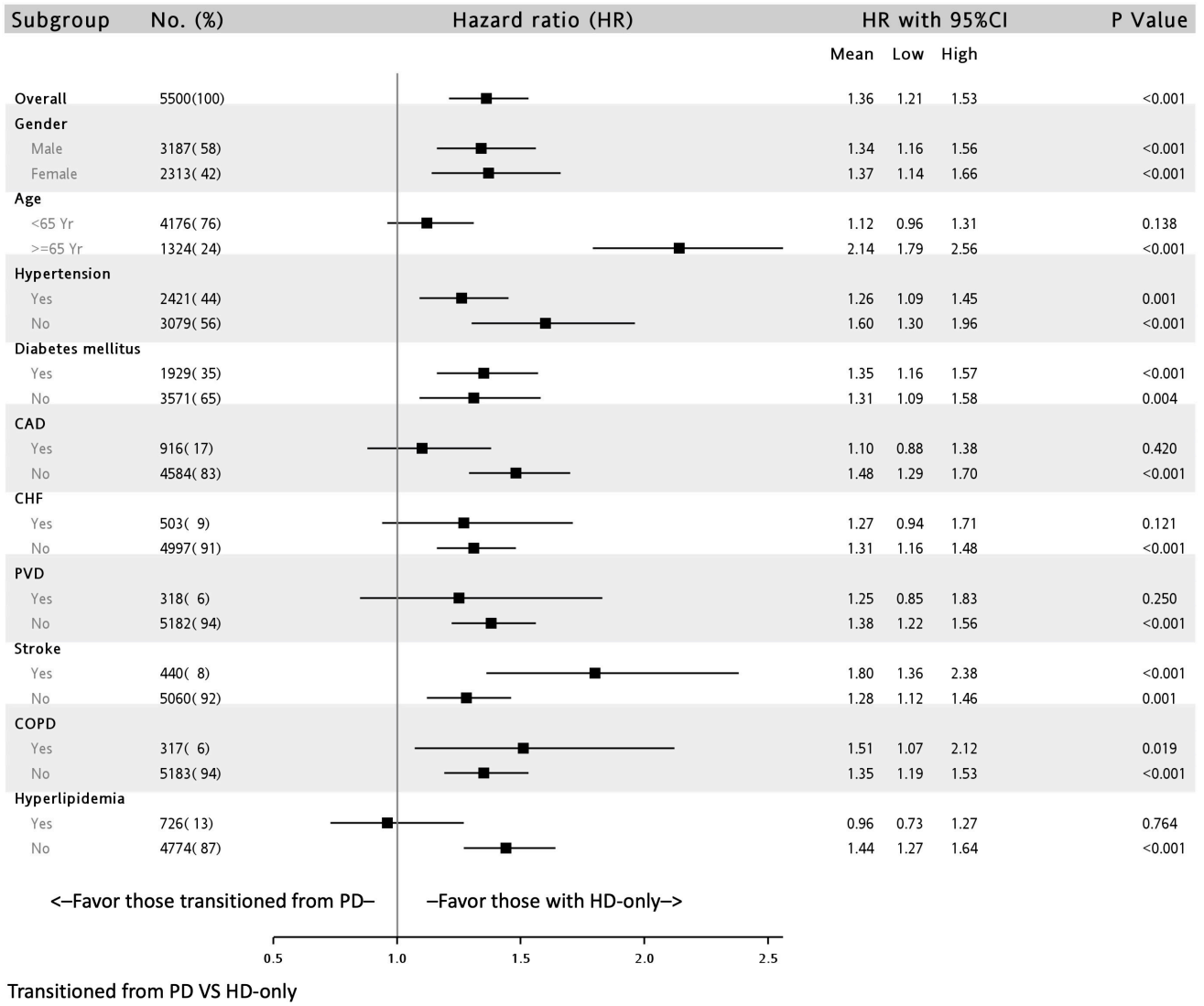


FIGURE 3 | Subgroup analysis of all-cause mortality of patients undergoing dialysis with and without previous peritoneal dialysis in a multivariable adjusting model*. *Full adjusted model is the same as that in **Table 2**. CAD, coronary artery disease; CHF, congestive heart failure; PVD, peripheral vascular disease; COPD, chronic obstructive pulmonary disease.

consistent in most subgroups. Moreover, the risk of mortality was noted to be the highest in the 5 months following dialysis transition. The results of our study not only expand on current understanding of dialysis but also indicate that greater care should be given to patients undergoing HD who have transitioned from PD.

Whether PD or HD is a more favorable treatment option for patients with ESRD has long been a topic of debate (16). Generally, PD offers more favorable preservation of residual renal function (17), quality of life (18), and stability of hemodynamic changes than HD (19). In addition, the survival outcomes did not seem to differ between PD and HD (20, 21). Accordingly, PD has been identified as a favorable first treatment choice. However, such favorability was not indicated in a small randomized controlled trial conducted by Korevaar JC et al. (22). Therefore, clinicians should view both PD and HD as viable options for treating ESRD.

Transitioning from PD to HD is not uncommon during the disease course among patients with dialysis-dependent ESRD. The average mortality-censored medial technique survival for this group is only 3.7 years (11). In a report that used data from the NECOSAD Study Group, the 2-year PD technique survival was 64%, indicating that one-third of the patients had dropped out of the treatment (23). Transition from PD to HD is a common and gradual process in patients with ESRD who undergo PD as their first dialysis modality (11). Jaar et al. (24) conducted a prospective study of 262 patients and reported no difference in survival between a PD group that transitioned to HD and a PD group that did not (HR, 0.89; 95% CI: 0.41–1.93). Chen et al. by using Australian and New Zealand Dialysis and Transplant

(ANZDATA) registry data, reported infection and social-related technique failure to be associated with deaths within 2 years of technique failure in patients undergoing PD (25). To our knowledge, no study has compared the outcomes of transitioning from PD to HD with those of HD-only.

In our study, transitioning from PD to HD yielded the same clinical outcomes as (all-cause hospitalization, infection-hospitalization, and MACE) but poorer survival outcomes than HD-only. Furthermore, the piecewise HR showed that such worse outcome occurred especially in the first 5 months after transitioning to HD. This is possibly because the early period after transitioning to HD are key with respect to premature mortality in such a group, which is consistent with the results of Chen et al. (25). Therefore, a personalized care program for patients undergoing HD who have transitioned from PD may be warranted to prevent mortality.

In the subgroup analysis, we found that older adults had the poorest mortality outcomes (HR: 2.14; 95% CI, 1.79–2.56). Dialysis in older patients presents several challenges; the choice of dialysis modality for older patients is mostly dependent on caregiver (such as family) preferences. In the literature, whether mortality is higher in PD than in HD is highly debated. Several reports have indicated no difference in mortality between PD and HD in older patients (26, 27). However, some studies have reported that the mortality rate of patients undergoing PD was higher than that of those undergoing HD (28, 29). In a study conducted by Sakai K et al. older adults undergoing PD had similar rates of peritonitis or catheter-related complications but lower rates of transition to HD compared with adult patients undergoing PD (30). This may explain our findings. The conditions and complications of transitioning to HD may have been severe for the older adults included in our study, which may have led to poorer outcomes in the older adults in this group than in those in the HD control group. Moreover, in our study, the association was non-significant in patients with severe comorbidities, such as CAD, CHF, PVD, and hyperlipidemia. These comorbidities may, therefore, be hypothesized to play a major role in mortality in older adult populations transitioning from PD to HD. Atherosclerosis and stenosis of the large vessels could result in reduced blood flow to the smaller arteries and arterioles of the peritoneum leading to reduced ultrafiltration and clearance, which resultantly to PD technique failure (31). In addition to the above hypothesis, the other possible explanation for these insignificant results is the small sample size of these subgroups in our study.

Notably, we also found that patients with prior stroke had higher mortality rates when they had transitioned from PD to HD (HR: 1.80; 95% CI: 1.36–2.38). According to a retrospective study by Wu et al. patients who underwent continual ambulatory PD with prior stroke had a higher rate of all-cause mortality and death-censored technique failure compared with those without prior stroke. The study further reported older age and nutritional status to be independent risk factors (32). In this cohort study, patients with prior stroke undergoing PD had higher rates of peritonitis, leading to a need for transition to HD. Malnutrition has been well documented as a risk factor for mortality in PD (33, 34). Patients who have experienced previous stroke are prone to malnutrition (35). This may be due to underlying chronic inflammation, infection, and an inability to achieve optimal intake. In our cohort, patients with stroke transitioning from PD to HD had higher mortality rates, suggesting that these two factors have additive effects, which may be caused by malnutrition.

Our study had several strengths. First, this was a nationwide population-based cohort study (real-world evidence), which ensured a large sample size and the generalizable results. Second, our follow-up duration was sufficiently long to obtain primary outcomes. Finally, we used propensity scores to remove baseline imbalances and adjusted for potential confounders, which improved the reliability of our inferences. However, our study also has some limitations. First, data on potential confounding factors, such as body mass index, blood pressure, laboratory data, lifestyle, and dialysis quality, were not available in the NHI database. However, the consistency in the majority of the subgroup analyses indicates that our study results are robust. Second, the reasons for PD failure were also unavailable in the NHI database, which prevented us from conducting further risk analyses for deaths in patients who had undergone PD. Chen et al. reported that infection and social-related PD technique failure are associated with premature death within 2 years in patients who transition from PD to HD (25). Therefore, further study of risk assessment in such a population is required. Third, the medication use between groups was not balanced. The more prescription of RASB, beta-blockers, CCB and anticoagulants in the group transitioned from PD to HD might indicate volume overload, which lead to higher mortality. Nevertheless, further multiple variables adjusted in the data analysis can reduce this bias. Finally, the results derived from this Taiwanese population of patients undergoing HD may require further verification to be applied to other ethnic populations.

CONCLUSION

Patients transitioning from PD to HD may experience poorer mortality outcomes compared with patients who are HD-only, especially within 5 months of the transition. Although this finding does not evidence the superiority of either PD or HD, it indicates that clinicians transitioning patients from PD to HD should be well trained and well prepared.

DATA AVAILABILITY STATEMENT

The dataset used in this study is held by the Taiwan Ministry of Health and Welfare (MOHW). Due to the General Data Protection Regulation, the dataset is not available on request from the corresponding author. Any researcher interested in accessing this dataset can apply for access. The MOHW will evaluate the application for accessing this dataset. Please visit the website of the National Health Informatics Project of the MOHW (https://dep.mohw.gov.tw/dos/np-2497-113.html).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional review board of Shin-Kong Wu Ho-Su Memorial Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

M-HT, T-NJ, and Y-WF: conceived and designed the experiments. Y-YC and J-TW: performed the experiments. Y-YC: analyzed the data. M-HT and Y-WF: wrote the manuscript. All authors contributed reagents, materials, and analysis tools and approved the manuscript.

FUNDING

This study was sponsored by Shin Kong Wu Ho-Su Memorial Hospital (Grant No. 2019SKHADR008) and a cooperative project between Shin Kong Wu Ho-Su Memorial Hospital and National Yang Ming Chiao Tung University (Grant No. 109GB006-2).

ACKNOWLEDGMENTS

This manuscript was edited by Wallace Academic Editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.876229/full#supplementary-material

REFERENCES

1. Tsai MH, Hsu CY, Lin MY, Yen MF, Chen HH, Chiu YH, et al. Incidence, prevalence, and duration of chronic kidney disease in Taiwan: results from a community-based screening program of 106,094 individuals. *Nephron.* (2018) 140:175–84. doi: 10.1159/000491708
2. Saran R, Robinson B, Abbott KC, Agodoa LY, Albertus P, Ayanian J, et al. US renal data system 2016 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* (2017) 69:A7–8. doi: 10.1053/j.ajkd.2016.12.004
3. Johansen KL, Chertow GM, Foley RN, Gilbertson DT, Herzog CA, Ishani A, et al. US renal data system 2020 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* (2021) 77:A7–8. doi: 10.1053/j.ajkd.2021.01.002
4. Lai TS, Hsu CC, Lin MH, Wu VC, Chen YM. Trends in the incidence and prevalence of end-stage kidney disease requiring dialysis in Taiwan: 2010-2018. *J Formos Med Assoc.* (2022) 121:S5–11. doi: 10.1016/j.jfma.2021.12.013
5. Jansen MA, Hart AA, Korevaar JC, Dekker FW, Boeschoten EW, Krediet RT. Predictors of the rate of decline of residual renal function in incident dialysis patients. *Kidney Int.* (2002) 62:1046–53. doi: 10.1046/j.1523-1755.2002.00505.x
6. Dąbrowska-Bender M, Dykowska G, Żuk W, Milewska M, Staniszewska A. The impact on quality of life of dialysis patients with renal insufficiency. *Patient Prefer Adherence.* (2018) 12:577–83. doi: 10.2147/ppa.s156356
7. Tang CH, Chen HH, Wu MJ, Hsu BG, Tsai JC, Kuo CC, et al. Out-of-pocket costs and productivity losses in haemodialysis and peritoneal dialysis from a patient interview survey in Taiwan. *BMJ Open.* (2019) 9:e023062. doi: 10.1136/bmjopen-2018-023062
8. Pike E, Hamidi V, Ringerike T, Wisloff T, Klemp M. More use of peritoneal dialysis gives significant savings: a systematic review and health economic decision model. *J Clin Med Res.* (2017) 9:104–16. doi: 10.14740/jocmr2817w
9. Ghaffari A, Kalantar-Zadeh K, Lee J, Maddux F, Moran J, Nissenson A. PD first: peritoneal dialysis as the default transition to dialysis therapy. *Semin Dial.* (2013) 26:706–13. doi: 10.1111/sdi.12125
10. Ambruso SL, Teitelbaum I. Prevention of peritoneal dialysis drop-out. *Adv Perit Dial.* (2018) 34:19–23.
11. Boissinot L, Landru I, Cardineau E, Zagdoun E, Ryckelycnk JP, Lobbedez T. Is transition between peritoneal dialysis and hemodialysis really a gradual process? *Perit Dial Int.* (2013) 33:391–7. doi: 10.3747/pdi.2011.00134
12. Chaudhary K. Peritoneal dialysis drop-out: causes and prevention strategies. *Int J Nephrol.* (2011) 2011:434608. doi: 10.4061/2011/434608
13. Chen YH, Chen YY, Fang YW, Tsai MH. Protective effects of angiotensin receptor blockers on the incidence of dementia in patients with chronic kidney disease: a population-based nationwide study. *J Clin Med.* (2021) 10:5175. doi: 10.3390/jcm10215175
14. Hsieh CY, Su CC, Shao SC, Sung SF, Lin SJ, Kao Yang YH, et al. Taiwan's national health insurance research database: past and future. *Clin Epidemiol.* (2019) 11:349–58. doi: 10.2147/CLEP.S196293
15. Yang H, Chen YH, Hsieh TF, Chuang SY, Wu MJ. Prediction of mortality in incident hemodialysis patients: a validation and comparison of CHADS2, CHA2DS2, and CCI scores. *PLoS One.* (2016) 11:e0154627. doi: 10.1371/journal.pone.0154627
16. Sinnakirouchenan R, Holley JL. Peritoneal dialysis versus hemodialysis: risks, benefits, and access issues. *Adv Chronic Kidney Dis.* (2011) 18:428–32. doi: 10.1053/j.ackd.2011.09.001
17. Marron B, Remon C, Perez-Fontan M, Quiros P, Ortiz A. Benefits of preserving residual renal function in peritoneal dialysis. *Kidney Int Suppl.* (2008) 108:S42–51. doi: 10.1038/sj.ki.5002600
18. Zazzeroni L, Pasquinelli G, Nanni E, Cremonini V, Rubbi I. Comparison of quality of life in patients undergoing hemodialysis and peritoneal dialysis: a systematic review and meta-analysis. *Kidney Blood Press Res.* (2017) 42:717–27. doi: 10.1159/000484115
19. Ryuzaki M. Blood pressure control in peritoneal dialysis patients. *Contrib Nephrol.* (2018) 196:148–54. doi: 10.1159/000485715
20. Wong B, Ravani P, Oliver MJ, Holroyd-Leduc J, Venturato L, Garg AX, et al. Comparison of patient survival between hemodialysis and peritoneal dialysis among patients eligible for both modalities. *Am J Kidney Dis.* (2018) 71:344–51. doi: 10.1053/j.ajkd.2017.08.028
21. Choi SJ, Obi Y, Ko GJ, You AS, Eriguchi R, Wang M, et al. Comparing patient survival of home hemodialysis and peritoneal dialysis patients. *Am J Nephrol.* (2020) 51:192–200. doi: 10.1159/000504691
22. Korevaar JC, Feith GW, Dekker FW, van Manen JG, Boeschoten EW, Bossuyt PM, et al. Effect of starting with hemodialysis compared with peritoneal dialysis in patients new on dialysis treatment: a randomized controlled trial. *Kidney Int.* (2003) 64:2222–8. doi: 10.1046/j.1523-1755.2003.00321.x
23. Jager KJ, Merkus MP, Dekker FW, Boeschoten EW, Tijssen JG, Stevens P, et al. Mortality and technique failure in patients starting chronic peritoneal dialysis: results of the Netherlands cooperative study on the adequacy of dialysis. NECOSAD study group. *Kidney Int.* (1999) 55:1476–85. doi: 10.1046/j.1523-1755.1999.00353.x
24. Jaar BG, Plantinga LC, Crews DC, Fink NE, Hebah N, Coresh J, et al. Timing, causes, predictors and prognosis of switching from peritoneal dialysis to hemodialysis: a prospective study. *BMC Nephrol.* (2009) 10:3. doi: 10.1186/1471-2369-10-3
25. Chen JHC, Johnson DW, Hawley C, Boudville N, Lim WH. Association between causes of peritoneal dialysis technique failure and all-cause mortality. *Sci Rep.* (2018) 8:3980. doi: 10.1038/s41598-018-22335-4

26. Lamping DL, Constantinovici N, Roderick P, Normand C, Henderson L, Harris S, et al. Clinical outcomes, quality of life, and costs in the North Thames dialysis study of elderly people on dialysis: a prospective cohort study. *Lancet.* (2000) 356:1543–50. doi: 10.1016/s0140-6736(00)03123-8
27. Brown EA, Johansson L. Dialysis options for end-stage renal disease in older people. *Nephron Clin Pract.* (2011) 119:c10–3. doi: 10.1159/000328019
28. Kim H, Kim KH, Park K, Kang SW, Yoo TH, Ahn SV, et al. A population-based approach indicates an overall higher patient mortality with peritoneal dialysis compared to hemodialysis in Korea. *Kidney Int.* (2014) 86:991–1000. doi: 10.1038/ki.2014.163
29. Jassal SV, Watson D. Offering peritoneal dialysis to the older patient: medical progress or waste of time? *Semin Nephrol.* (2011) 31:225–34. doi: 10.1016/j.semnephrol.2011.01.010
30. Sakai K, Nihei H. Peritoneal dialysis in elderly patients. *Contrib Nephrol.* (2018) 196:141–7. doi: 10.1159/000485714
31. Stepanova N, Burdeyna O. Association between dyslipidemia and peritoneal dialysis technique survival. *Open Access Maced J Med Sci.* (2019) 7:2467–73. doi: 10.3889/oamjms.2019.664
32. Wu X, Yang X, Liu X, Yi C, Guo Q, Feng X, et al. Patient survival and technique failure in continuous ambulatory peritoneal dialysis patients with prior stroke. *Perit Dial Int.* (2016) 36:308–14. doi: 10.3747/pdi.2014.00030
33. Avram MM, Sreedhara R, Fein P, Oo KK, Chattopadhyay J, Mittman N. Survival on hemodialysis and peritoneal dialysis over 12 years with emphasis on nutritional parameters. *Am J Kidney Dis.* (2001) 37:S77–80. doi: 10.1053/ajkd.2001.20754
34. Fung F, Sherrard DJ, Gillen DL, Wong C, Kestenbaum B, Seliger S, et al. Increased risk for cardiovascular mortality among malnourished end-stage renal disease patients. *Am J Kidney Dis.* (2002) 40:307–14. doi: 10.1053/ajkd.2002.34509
35. Kim EJ, Yoon YH, Kim WH, Lee KL, Park JM. The clinical significance of the mini-nutritional assessment and the scored patient-generated subjective global assessment in elderly patients with stroke. *Ann Rehabil Med.* (2013) 37:66–71. doi: 10.5535/arm.2013.37.1.66

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 09 June 2022
doi: 10.3389/fmed.2022.877220

Red Blood Cell Distribution Width Is Associated With Adverse Kidney Outcomes in Patients With Chronic Kidney Disease

Xinwei Deng[1,2,3], *Bixia Gao*[1,2,3,4,5], *Fang Wang*[1,2,3,4,5], *Ming-hui Zhao*[1,2,3,4,5,6], *Jinwei Wang*[1,2,3,4,5*†] and *Luxia Zhang*[1,2,3,4,5,7*†]

[1] Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China, [2] Institute of Nephrology, Peking University, Beijing, China, [3] Research Units of Diagnosis and Treatment of Immune-Mediated Kidney Diseases, Chinese Academy of Medical Sciences, Beijing, China, [4] Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China, [5] Key Laboratory of Chronic Kidney Disease Prevention and Treatment, Ministry of Education of China, Beijing, China, [6] Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China, [7] National Institute of Health Data Science, Peking University, Beijing, China

OPEN ACCESS

Edited by:
Jiannong Liu,
Hennepin Healthcare Research Institute, United States

Reviewed by:
James Wetmore,
Hennepin Healthcare, United States
Dorey Glenn,
University of North Carolina at Chapel Hill, United States

***Correspondence:**
Luxia Zhang
zhanglx@bjmu.edu.cn
Jinwei Wang
wangjinwei@bjmu.edu.cn

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 16 February 2022
Accepted: 09 May 2022
Published: 09 June 2022

Citation:
Deng XW, Gao BX, Wang F, Zhao M-H, Wang JW and Zhang LX (2022) Red Blood Cell Distribution Width Is Associated With Adverse Kidney Outcomes in Patients With Chronic Kidney Disease. Front. Med. 9:877220. doi: 10.3389/fmed.2022.877220

Background: Chronic kidney disease (CKD) is a global public health issue. Red blood cell distribution width (RDW) is a recently recognized potential inflammatory marker, which mirrors the variability in erythrocyte volume. Studies indicate that elevated RDW is associated with increased risk of mortality in CKD patients, while evidence regarding the impact of RDW on kidney outcome is limited.

Methods: Altogether 523 patients with CKD stage 1–4 from a single center were enrolled. We identified the cutoff point for RDW level using maximally selected log-rank statistics. The time-averaged estimated glomerular filtration rate (eGFR) slope was determined using linear mixed effects models. Rapid CKD progression was defined by an eGFR decline >5 ml/min/1.73 m^2/year. The composite endpoints were defined as doubling of serum creatinine, a 30% decline in initial eGFR or incidence of eGFR < 15 ml/min/1.73 m^2, whichever occurred first. Multivariable logistic regression or Cox proportional hazards regression was performed, as appropriate.

Results: During a median follow-up of 26 [interquartile range (IQR): 12, 36] months, 65 (12.43%) patients suffered a rapid CKD progression and 172 (32.89%) composite kidney events occurred at a rate of 32.3/100 patient-years in the high RDW group, compared with 14.7/100 patient-years of the remainder. The annual eGFR change was clearly steeper in high RDW group {−3.48 [95% confidence interval (CI): −4.84, −2.12] ml/min/1.73 m^2/year vs. −1.86 [95% CI: −2.27, −1.45] ml/min/1.73 m^2/year among those with RDW of >14.5% and ≤14.5%, respectively, *P* for between-group difference <0.05}. So was the risk of rapid renal function loss (odds ratio = 6.79, 95% CI: 3.08–14.97) and composite kidney outcomes (hazards ratio = 1.51, 95% CI: 1.02–2.23). The significant association remained consistent in the sensitivity analysis.

Conclusion: Increased RDW value is independently associated with accelerated CKD deterioration. Findings of this study suggest RDW be a potential indicator for risk of CKD progression.

Keywords: chronic kidney disease (CKD), rapid CKD progression, kidney outcomes, red blood cell distribution width, estimated glomerular filtration rate (eGFR) slope

INTRODUCTION

Chronic kidney disease (CKD) is a growing public health issue, affecting approximately 8–16% of global population, posing a significant public burden both socially and economically (1, 2). The co-occurrence of chronic malnutrition and inflammation is considered as a common pathophysiologic status in CKD progression, triggering deterioration of renal function and poor prognosis (3, 4). Measurable parameters in blood which could provide early detection of these specific conditions are of utmost importance.

Red blood cell distribution width (RDW) is a common coefficient of heterogeneity in red blood cell (RBC) size, which is routinely reported in complete blood count. RDW is calculated by dividing the standard deviation of the mean cell size by the mean cell volume (MCV) of RBC (5, 6). Therefore, RDW elevation is mathematically caused by decrease in MCV or increase in RBC size variance (7). Previous literatures revealed that the correlation between RDW elevation and impaired erythropoiesis might be attributed to bone marrow dysfunction and poor nutritional status (8). Recently, the RDW level was reported to be influenced by the rate of RBC turnover, which allows persistence of older and smaller cells in circulation. Thus, delayed RBC clearance would have an impact on RBC size variance (9, 10). All the above-mentioned research suggests that increased RDW be an emerging biomarker of abnormal erythrocyte metabolism and survival, potentially representing oxidative stress, inflammation, and a variety of disorders.

It seems hence rational that this simple parameter may reflect the adverse prognosis in many clinical conditions. There are several studies indicating the prognostic performance of advanced RDW level in coronary artery disease (11, 12), heart failure (13, 14), atrial fibrillation (15, 16), and new-onset stroke (17, 18). Existing reports on the relationship between RDW and CKD are comparatively sparse. While some studies showed that RDW was significantly correlated with markers of kidney damage and adverse outcomes in CKD patients (19, 20), others failed to reveal this relationship (21). Despite accumulating evidence, the majority of studies were retrospective, conducted among patients with advanced CKD and/or converged on clinical hard endpoints or ambiguous kidney outcomes.

Furthermore, according to our review of published literature, there are no studies focused on the association between RDW and rate of estimated glomerular filtration rate (eGFR) decline. From this perspective, we attempted to evaluate whether the RDW level was independently associated with eGFR decline and could serve as a novel biomarker for CKD progression among a prospective cohort of patients with stage 1–4 CKD.

MATERIALS AND METHODS

Cohort Participants

This study was conducted based on the prospective CKD cohort at Peking University First Hospital, which is the first renal division and a renal reference center in China. The patients were from all over China, particularly from northern China. The criteria for the registration consist of aged >18 years, diagnosis of CKD but not on dialysis. Patients were excluded for being with acute kidney injury, having had kidney transplant, active malignancy, or being under pregnancy. The diagnosis of CKD was the presence of persistent anatomical kidney lesions, continuous kidney damage markers or decreased eGFR below 60 ml/min/1.73 m^2 existing for at least 3 months. All data were collected prospectively. Between January 2003 and December 2020, there were 974 patients with a total of 16,457 records in the database. Among the cohort, 902 patients had at least one measurement of RDW. We identified the date for the first measurement of RDW as baseline date. We excluded patients without eGFR within 3 months prior to the first measurement of RDW (n = 69), with a baseline eGFR of < 15 ml/min/1.72 m^2 (n = 126), with number of eGFR measurements <3 over the first-year (n = 159), and with follow-up time less than 3 months (n = 25), leading to the final study cohort involving 523 participants for the analysis (**Figure 1**).

Written informed consent was obtained from all participants for use of the clinical data in future studies before they were registered in the cohort. The conduct of the study has been approved by the Ethics Committee of Peking University First Hospital.

Data Collection

Assessment of Red Blood Cell Distribution Width

In this study, the optimal cut-off point for RDW as a dichotomous classification of the study participants was chosen based on the maximally selected log-rank statistic for the study outcome, as well as by taking into account the upper limit of normal value of the parameter (14.5%) defined by the local laboratory (the clinical laboratory center of Peking University First Hospital). Coincidentally, the statistic-maximization driven method found out the same threshold of 14.5% (**Figure 2**).

Definition of Hypertension, Anemia, and Diabetes

Hypertension was defined by systolic blood pressure (BP) persistently ≥140 mmHg and/or diastolic BP ≥90 mmHg on two different visits, or self-reported history of hypertension, or use of antihypertensive medications (22). Diabetes was defined by either a fasting blood glucose (FBG) ≥7.0 mmol/L (126 mg/dL) or use of antidiabetic medications (23). We used a criterion for anemia

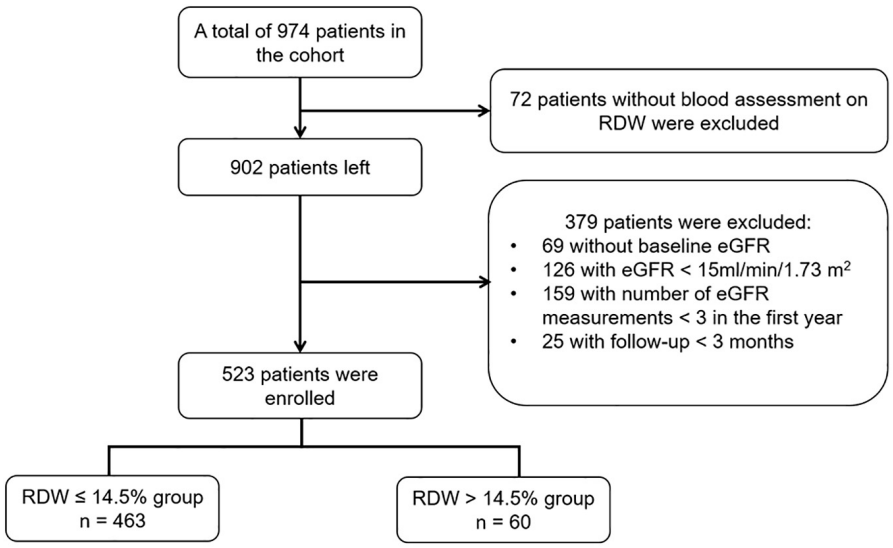


FIGURE 1 | Flow chart of study population. eGFR, estimated glomerular filtration rate; RDW, red blood cell distribution width.

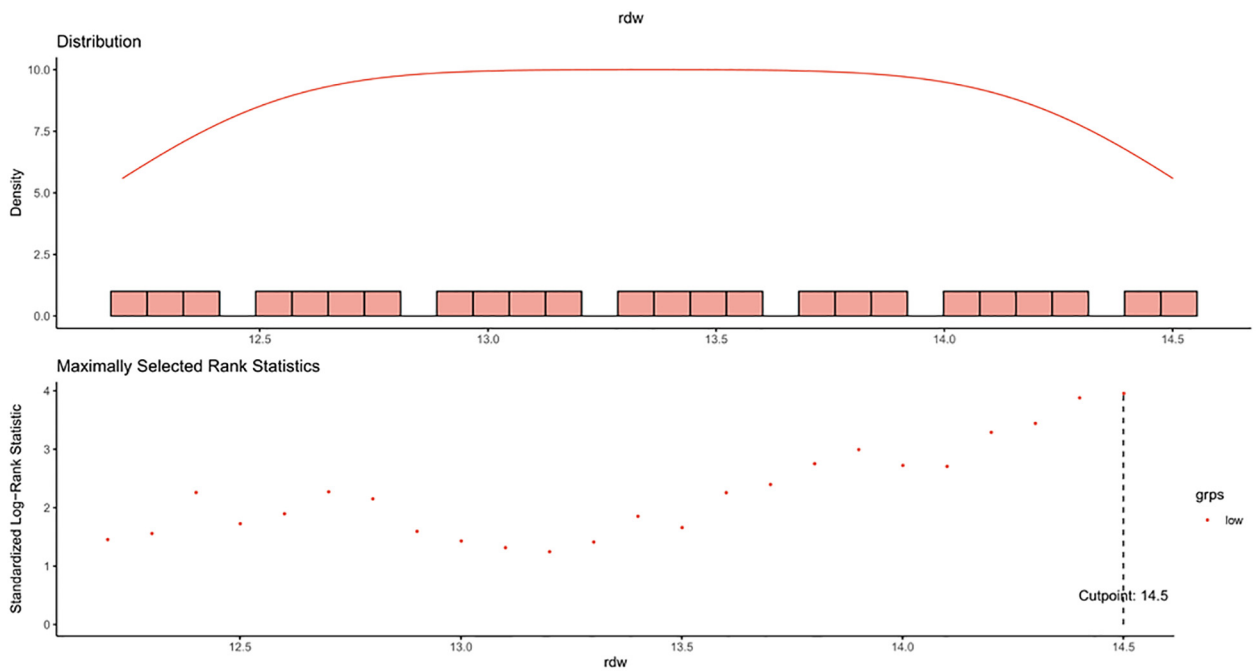


FIGURE 2 | Maximally selected log-rank statistics for cut-off point of RDW. RDW, red blood cell distribution width.

as hemoglobin concentration <13 g/dL in men and <12 g/dL in women, respectively (24).

Assessment of Covariates

Patients were followed up regularly every 3–6 months, depending on the patients' disease condition. We set a time window of 3 months prior to baseline to obtain information of the covariates. Baseline characteristics consisting of demographics (age, sex, and original cause of renal disease), medical history (hypertension and diabetes), medication use and laboratory variables were considered as the most proximate results prior to baseline date. The medication use included iron supplements, erythropoietin (EPO) stimulating agents, angiotensin converting–enzyme inhibitors (ACEI), angiotensin II–receptor blockers (ARB), alpha-blockers, beta-blockers, calcium-channel blockers, and loop diuretics. The laboratory variables included white blood cell (WBC), RBC, hemoglobin, percentage of lymphocyte, MCV, blood glucose, serum albumin, uric acid, serum bicarbonate,

calcium, phosphorus, serum iron, urinary albumin-creatinine ratio (UACR), 24-h urine protein, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TCHO), and triglyceride (TG). eGFR was computed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The baseline eGFR for each individual established the staging of CKD according to the standards proposed by Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (25). Follow-up data were obtained from the clinical records at the outpatient visits. All of the measurements were performed according to the standard procedure in the clinical laboratory center of Peking University First Hospital.

Outcomes

To reflect CKD progression, the primary outcome of the study was the slope of eGFR for each individual estimated by linear mixed effect model. We identified a rapid renal function decline as $>$ 5 ml/min/1.73 m^2/year decline in eGFR, consistent with the 2012 KDIGO guideline (25).

The composite kidney outcome was defined as a confirmed doubling of serum creatinine concentration, a 30% decline in baseline eGFR or incident eGFR $<$15 ml/min/1.73 m^2, whichever occurred first. The follow-up was censored at the earliest date among the study end date (31 December 2020), the date of mortality, and the last follow-up date of serum creatinine.

Statistical Analysis

Descriptive data were presented as number (percentage), mean $\pm$ standard deviation (SD) and median with interquartile range, as appropriate. Variables with skewed distributions were log transformed or square root transformed before analysis. Comparisons of between-group variance in baseline characteristics were analyzed using two-sample *t*-test or Mann–Whitney U test for continuous data, as appropriate, and Chi-squared test for categorical data.

Spearman's correlation analysis was performed to evaluate correlation between RDW value and other laboratory parameters, and correlation coefficients were calculated to determine the strength of these associations. Multivariable analysis was performed among those significant parameters in univariate analysis.

After adjusting for baseline eGFR, we calculated eGFR slope over the entire follow-up period for each patient by using a linear mixed effects model with a random patient-specific intercept and time effect. Category of RDW, follow-up time, category of RDW $\times$ time and eGFR recorded at baseline was treated as fixed effect items. An unstructured variance-covariance matrix was employed for each individual. Individual-level eGFR slope was calculated using the fixed-portion linear predictor plus the corresponding predictor in random effects. The distribution was depicted by RDW groups and summarized into categories. The between-group difference in eGFR slope was evaluated by calculating the interaction of RDW groups with time for eGFR decline. Subsequently, univariate and multivariable logistic regression analysis was performed with rapid eGFR decline treated as the outcome. The stepwise elimination with a threshold of $P < 0.05$ was used to select covariates in the multivariable analysis to determine the impact of elevated RDW value on the event.

The incidence rates of composite kidney outcomes were calculated as number of events per 100 person-years. Survival analysis was performed by Kaplan–Meier curves to compare the event rates between RDW categories, with the differences tested by log-rank test. Furthermore, we performed univariate and multivariable Cox proportional hazards regression to estimate the effect of increased RDW value on the composite outcome. Similarly, a stepwise variable selection was employed to select covariates. Hazard ratios were calculated to indicate the strength of association. We checked the proportional hazards assumption by involving an interaction term between the category of RDW and follow-up time to see if there is a statistical significance.

We performed the following sensitivity analyses to test the robustness of our findings. First, we reimplemented our primary analyses using the data over the first-year after enrollment in the cohort. Second, as proposed by several studies, we used an alternative cutoff of 3 ml/min/1.73 m^2/year to define a rapid renal function decline. Third, given the arbitrary, although not unreasonable, definition of rapid renal function decline, we redefined it as the lowest quartile of eGFR slope among participants included in the cohort. Fourth, to assess whether differences in the frequency of eGFR measurements influence study results, we repeated the primary analyses among patients with at least 2 eGFR measurements during the first-year. Regarding the potential relationship between anemia and RDW value, we tested their cross-product for each of the study outcomes. All analyses were conducted using SAS 9.4 (version 9.4, SAS institute, CA, United States). The threshold for significance was set at $P < 0.05$ (two-side).

RESULTS

Baseline Characteristics

In total, 523 patients with a median age 57 (IQR: 44, 69) years were enrolled. Of these patients, 52.77% were men and 38.24% exhibited anemia. The median eGFR at baseline was 37.30 (IQR: 26.86, 49.01) ml/min/1.73 m^2, and the majority of patients (89.29%) had CKD stage 3 or 4. The cutoff point of RDW level in this study was defined as 14.5%. Thus, enrolled patients were divided into RDW $\leq$14.5% group and RDW $>$14.5% group. Baseline characteristics according to dichotomy of RDW are displayed in **Table 1**. Patients with elevated RDW presented a higher prevalence of anemia and were more likely of receiving iron supplements, EPO stimulating agents, beta-blockers and calcium-channel blockers. In addition, patients with RDW $>$14.5% had lower values of eGFR, hemoglobin, RBC, MCV, percentage of lymphocyte, serum albumin, calcium, bicarbonate, TCHO, LDL-C and serum iron, but higher levels of UACR, and 24-h urine protein.

Laboratory Variables in Relation to Red Blood Cell Distribution Width

As illustrated in **Table 2**, the spearman correlation coefficients show that seven parameters correlated inversely with RDW,

TABLE 1 | Baseline characteristics of the study populations according to the RDW level.

Characteristics	Total (*n* = 523)	RDW group		*P*-value
		RDW ≤ 14.5% (*n* = 463)	RDW > 14.5% (*n* = 60)	
Demographics				
Age (years)	57 (44, 69)	56 (43, 69)	60 (48, 73)	0.12
Sex (*n*%)				0.12
Male	276 (52.77)	250 (54.00)	26 (43.33)	
Female	247 (47.23)	213 (46.00)	34 (56.67)	
Cause of renal disease (*n*%)				0.59
Glomerulonephritis	125 (23.90)	111 (23.97)	14 (23.33)	
Diabetic nephropathy	88 (16.83)	80 (17.28)	8 (13.33)	
Hypertensive renal disease	130 (24.86)	112(24.19)	18 (30.00)	
Tubulointerstitial disease	33 (6.31)	32 (6.91)	1 (1.67)	
Polycystic kidney disease	10 (1.91)	8 (1.73)	2 (3.33)	
Pyelonephritis	3 (0.57)	3 (0.65)	0 (0.00)	
Other	36 (6.88)	30 (6.48)	6 (10.00)	
Unknown	98 (18.74)	87 (18.79)	11 (18.33)	
Clinical				
Hypertension (*n*%)	151 (28.87)	132 (28.51)	19 (31.67)	0.61
Diabetes (*n*%)	238 (45.51)	213 (46.00)	25 (41.67)	0.52
Anemia (*n*%)				<0.001
Mild	192 (36.71)	153 (33.05)	39 (65.00)	
Moderate	8 (1.53)	4 (0.86)	4 (6.67)	
CKD stages (*n*%)				<0.001
CKD stage 1	9 (1.72)	9 (1.94)	0 (0.00)	
CKD stage 2	47 (8.99)	44 (9.50)	3 (5.00)	
CKD stage 3	293 (56.02)	270 (58.32)	23 (38.33)	
CKD stage 4	174 (33.27)	140 (30.24)	34 (56.67)	
Laboratory				
WBC ($\times 10^{12}$/L)	6.40 (5.40, 7.60)	6.33 (5.40, 7.60)	6.41 (5.33, 7.55)	0.96
RBC ($\times 10^{12}$/L)	4.19 ± 0.64	4.22 ± 0.63	3.92 ± 0.67	<0.001
Hemoglobin (g/L)	129.6 ± 19.7	131.3 ± 19.4	116.1 ± 17.0	<0.001
MCV (fl)	89.96 ± 6.00	90.23 ± 5.80	87.83 ± 7.06	0.01
RDW (%)	13.2 (12.6, 13.8)	13.1 (12.6, 13.6)	15.1 (14.8, 15.7)	<0.001
Lymphocyte (%)	27.39 ± 8.06	27.77 ± 7.88	24.44 ± 8.79	0.002
Albumin (g/dL)	43.0 (40.8, 45.1)	43.1 (41.0, 45.2)	41.9 (38.1, 43.6)	<0.001
Blood glucose (mmol/L)	5.83 ± 1.36	5.80 ± 1.33	6.06 ± 1.54	0.16
Bicarbonate (mmol/L)	24.60 ± 2.93	24.75 ± 2.91	23.43 ± 2.78	0.001
Calcium (mmol/L)	2.31 (2.24, 2.38)	2.32 (2.24, 2.38)	2.26 (2.18, 2.34)	<0.001
Phosphorus (mmol/L)	1.18 (1.04, 1.31)	1.17 (1.03, 1.31)	1.23 (1.10, 1.32)	0.17
Serum iron (μmol/L)	14.99 (13.05, 16.80)	15.18 (13.20, 16.90)	13.41 (11.15, 15.61)	0.002
eGFR (ml/min/1.73 m^2)	37.30 (26.86, 49.01)	38.27 (27.85, 49.57)	28.40 (22.36, 38.93)	<0.001
UACR (mg/g)	324.71 (128.67, 679.79)	311.43 (117.54, 621.77)	525.70 (279.68, 975.51)	0.004
24-h urine protein (g/24 h)	0.74 (0.21, 1.45)	0.72 (0.19, 1.39)	1.04 (0.59, 1.77)	0.02
Uric acid (μmol/L)	415.3 ± 93.9	415.9 ± 94.8	410.5 ± 87.4	0.68
TG (mmol/L)	1.55 (1.11, 2.24)	1.58 (1.11, 2.27)	1.40 (1.04, 1.89)	0.08
TCHO (mmol/L)	4.55 (3.86, 5.31)	4.57 (3.92, 5.38)	4.08 (3.41, 4.84)	0.003
LDL-C (mmol/L)	2.55 (2.01, 3.09)	2.60 (2.07, 3.14)	2.08 (1.71, 2.77)	<0.001
Treatment				
Iron supplements (*n*%)	219 (41.87)	176 (38.01)	43 (71.67)	<0.001
EPO-stimulating agents (*n*%)	143 (27.34)	114 (24.62)	29 (48.33)	<0.001
ACEI or ARB (*n*%)	402 (77.91)	355 (77.85)	47 (78.33)	0.93
Alpha-blockers (*n*%)	145 (28.10)	122 (26.75)	23 (38.33)	0.06

(Continued)

TABLE 1 | (Continued)

Characteristics	Total (n = 523)	RDW group		P-value
		RDW ≤ 14.5% (n = 463)	RDW > 14.5% (n = 60)	
Beta-blockers (n%)	124 (24.03)	102 (22.37)	22 (36.67)	0.01
Calcium-channel blockers (n%)	379 (73.45)	326 (71.49)	53 (88.33)	0.006
Loop diuretics (n%)	188 (36.43)	160 (35.09)	28 (46.67)	0.08

Data are expressed as mean ± SD, median value (interquartile range) or n (%).
ACEI, angiotensin converting–enzyme inhibitors; ARB, angiotensin II–receptor blockers; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; LDL-C, low-density lipoprotein cholesterol; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red blood cell distribution width; SD, standard deviation; TCHO, total cholesterol; TG, triglyceride; UACR, urinary albumin-creatinine ratio; WBC, white blood cell.

TABLE 2 | Correlations between baseline RDW and laboratory parameters.

Variables	Univariate analysis		Multivariable analysis	
	r	P-value	r	P-value
WBC (× 10^{12}/L)	−0.027	0.54		
RBC (× 10^{12}/L)	−0.174	<0.001	−0.125	0.004
Hemoglobin (g/L)	−0.258	<0.001	−0.154	<0.001
MCV (fl)	−0.073	0.10		
Lymphocyte (%)	−0.132	0.003	−0.041	0.35
Albumin (g/dL)	−0.205	<0.001	−0.102	0.02
Blood glucose (mmol/L)	0.076	0.08		
Bicarbonate (mmol/L)	−0.124	0.005	−0.119	0.006
Calcium (mmol/L)	−0.141	0.001	−0.119	0.007
Phosphorus (mmol/L)	0.018	0.69		
Serum iron (μmol/L)	−0.192	<0.001	−0.093	0.03
eGFR (ml/min/1.73 m^2)	−0.111	0.01	−0.110	0.01
UACR (mg/g)	0.097	0.03	0.101	0.02
24-h urine protein (g/24 h)	0.060	0.17		
Uric acid (μmol/L)	−0.051	0.25		
TG (mmol/L)	−0.096	0.03	−0.023	0.60
TCHO (mmol/L)	−0.104	0.02	−0.033	0.45
LDL-C (mmol/L)	−0.101	0.02	−0.061	0.17

eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein cholesterol; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red blood cell distribution width; TCHO, total cholesterol; TG, triglyceride; UACR, urinary albumin-creatinine ratio; WBC, white blood cell.

including RBC, hemoglobin, serum albumin, bicarbonate, calcium, serum iron, and eGFR values in the multivariable adjustment analysis. Conversely, we observed a significant positive association between the RDW level and UACR. Hemoglobin appeared to be the strongest factor correlating with RDW (Spearman's rank test correlation coefficient, −0.154, $P < 0.001$).

Associations of Red Blood Cell Distribution Width With Estimated Glomerular Filtration Rate Decline

The observed change rate in eGFR after a median follow-up of 26 (IQR: 12, 36) months was −1.86 (95% CI: −2.27, −1.45) ml/min/1.73 m^2/year in the RDW ≤14.5% group compared with −3.48 (95% CI: −4.84, −2.12) ml/min/1.73 m^2/year in the RDW >14.5% group, with a significant between-group difference of 1.62 (95% CI: 0.20, 3.04) ml/min/1.73 m^2/year ($P = 0.03$).

The distribution of eGFR slope was graphically depicted across RDW groups in **Figure 3**. In total, 29.64 and 7.84% of enrolled patients suffered an eGFR decline rate of 3–6 ml/min/1.73 m^2/year and over 6 ml/min/1.73 m^2/year, with higher proportions in those with RDW >14.5% (**Figure 3**).

Consequently, a total of 65 patients (12.43%) underwent rapid renal function decline (eGFR loss >5 ml/min/1.73 m^2/year). 44 (9.50%) of these were in the RDW ≤14.5% group and 21 (35.00%) in the RDW >14.5% group (**Table 3**).

Multivariable logistic regression reveals that elevated RDW values at baseline was associated with rapid eGFR deterioration. Adjusted odds ratio exceeded 5.79 for RDW >14.5% with 95% confidence interval of 3.08–14.97 ($P < 0.001$) (**Table 3**). Covariate selection is displayed in **Supplementary Table 1**.

Associations of Red Blood Cell Distribution Width With Composite Kidney Outcomes

There were 172 events (32.89%) of composite kidney outcomes including 33 (55.00%) in the RDW >14.5% group and 139 (30.02%) in the RDW ≤14.5% group, following crude incidence rates of 32.3 and 14.7 per 100 patient-years, respectively. The overall kidney survival rate was 64.20% in the RDW ≤14.5% group and 43.20% in the RDW >14.5% group (P for log-rank test < 0.001) (**Figure 4**).

Table 4 shows the multivariable adjusted hazard ratios of RDW categories for progression to composite kidney outcomes. Presence of elevated RDW was significantly associated with kidney events (fully adjusted hazard ratio: 1.51, 95% CI: 1.02–2.23, $P = 0.03$). Nonetheless, interactions between the concentration of hemoglobin and baseline eGFR were not significant through all models (data not shown).

Sensitivity Analyses

Similar associations were confirmed in several sensitivity analyses. Individual 1-year eGFR slope consistently diverged significantly between RDW >14.5% group (−5.29 [95% CI: −7.97, −2.62] ml/min/1.73 m^2/year) and RDW ≤14.5% group (−1.60 [95% CI: −2.42, −0.79] ml/min/1.73 m^2/year) with between-group difference of 3.69 (95% CI: 0.89, 6.49) ml/min/1.73 m^2/year ($P = 0.009$).

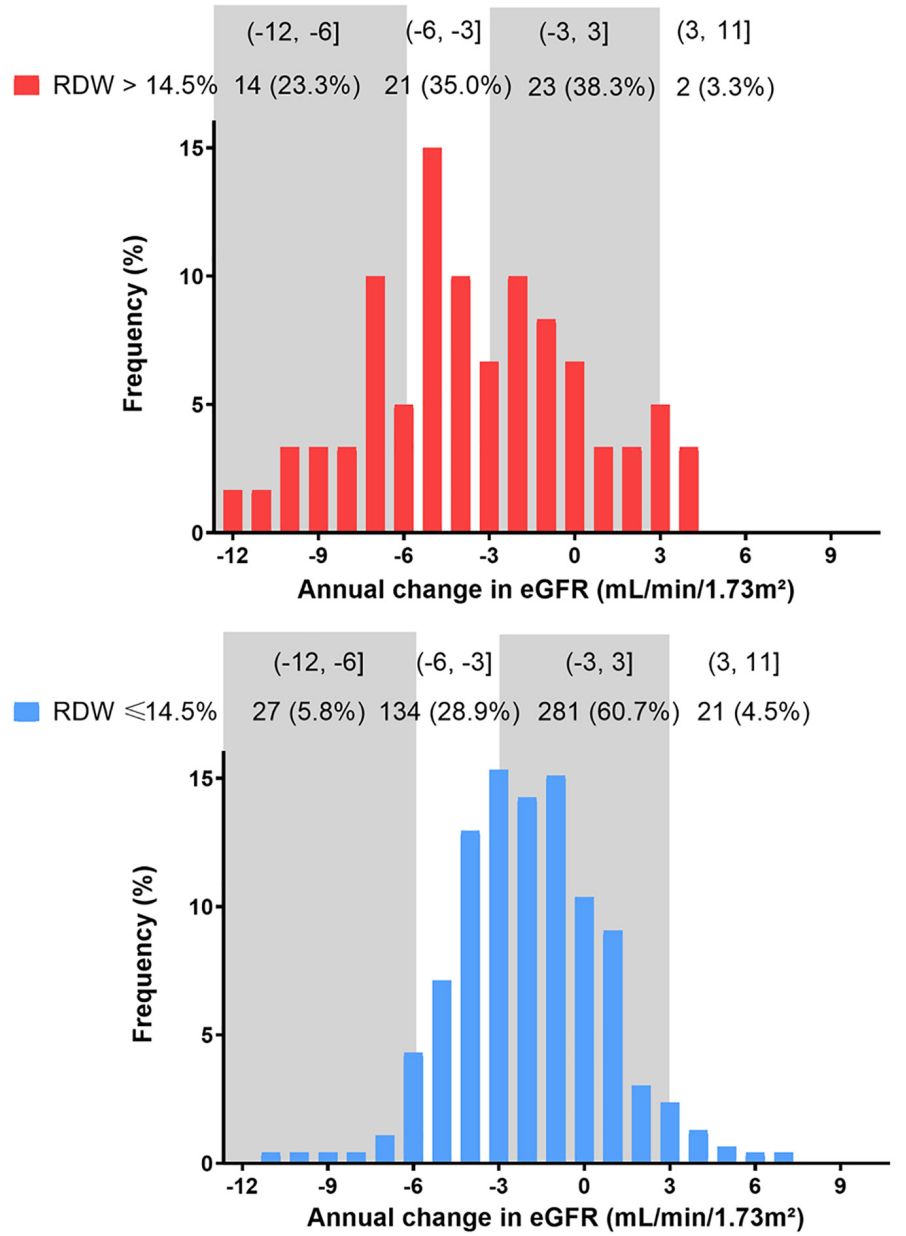


FIGURE 3 | Distribution of eGFR slope during the entire study period by RDW group. eGFR, estimated glomerular filtration rate.

(**Supplementary Table 2** and **Supplementary Figure 1**). The association of RDW with rapid function decline and composite kidney outcomes remained consistently significant over the first-year after enrollment (**Supplementary Tables 2**, **3** and **Supplementary Figure 2**). Consistent findings were observed when rapid function decline was redefined by the lowest quartile of eGFR slope or an alternative cutoff of 3 ml/min/1.73 m^2/year (**Supplementary Tables 4**, **5**). Among patients with at least 2 eGFR measurements available over the first-year, we found that RDW >14.5% remained a statistically significant association with fast eGFR decline (**Supplementary Table 6**).

DISCUSSION

In this prospective study of 523 patients with non-dialysis-dependent CKD (NDD-CKD) stage 1–4, we demonstrated that RDW >14.5% was independently associated with faster eGFR loss rate and the presence of rapid kidney disfunction. Survival analysis provided further validation of observed associations between RDW and adverse CKD prognostic outcomes.

Despite multiple epidemiological studies, the impact of RDW on the prognosis of kidney disease remains controversial and data observed in populations with earlier stages of CKD are limited. To date, higher RDW level has been proved to associate with

TABLE 3 | Univariate and multivariable adjusted odds ratios for rapid eGFR decline.

Variables	Events of RFD (*n*, %)	Univariate analysis		Multivariable analysis[a]	
		OR (95% CI) for RFD	*P*-value	OR (95% CI) for RFD	*P*-value
RDW ≤ 14.5%	44 (9.50)	Ref		Ref	
RDW > 14.5%	21 (35.00)	5.13 (2.77, 9.48)	<0.001	6.79 (3.08, 14.97)	<0.001

Rapid function decline was defined as eGFR loss >5 ml/min/1.73 m^2/year.
[a]The model was further adjusted for tubulointerstitial disease as the primary cause of renal failure, usage of iron supplements (yes vs. no), usage of EPO-stimulating agents (yes vs. no), usage of loop diuretics (yes vs. no), usage of alpha-blockers (yes vs. no), usage of calcium-channel blockers (yes vs. no), log (10)-transformed age, percentage of lymphocyte, natural log-transformed baseline eGFR, log (10)-transformed albumin, log (10)-transformed calcium, natural log-transformed UACR, log (10)-transformed 24-h urine protein, and log (10)-transformed LDL-C.
ACEI, angiotensin converting–enzyme inhibitors; ARB, angiotensin II–receptor blockers; CI, confidence interval; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; LDL-C, low-density lipoprotein cholesterol; RDW, red blood cell distribution width; RFD, rapid function decline; OR, odds ratio; UACR, urinary albumin-creatinine ratio.

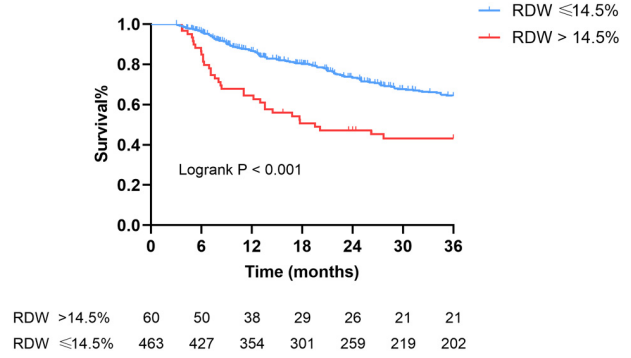


FIGURE 4 | Kaplan–Meier survival curves of composite kidney outcomes according to RDW levels. (Log-rank test, $P < 0.001$). The numbers below the *x*-axis indicate the number of event-free patients observed at 6, 12, 18, 24, 30, and 36 months.

TABLE 4 | Hazard ratios (95% CI) for composite kidney outcomes over the entire study period.

Variables	RDW ≤ 14.5% (*n* = 463)	RDW > 14.5% (*n* = 60)	*P*-value
Entire study period analysis			
Events (*n*, %)			<0.001
Doubling of SCR	5 (1.08)	0	
30% decline in eGFR	116 (25.05)	28 (46.67)	
eGFR <15 ml/min/1.73 m^2	56 (12.10)	14 (23.33)	
HR (95% CI) for composite kidney outcomes			
Model 1	Ref	2.24 (1.53, 3.28)	<0.001
Model 2	Ref	1.55 (1.05, 2.83)	0.02
Model 3	Ref	1.51 (1.02, 2.23)	0.03

Model 1: non-adjusted.
Model 2: adjusted for sex, log (10)-transformed age, history of hypertension (yes vs. no), usage of iron supplements (yes vs. no), usage of EPO-stimulating agents (yes vs. no), usage of ACEI or ARB (yes vs. no), usage of beta-blockers (yes vs. no), usage of alpha-blockers (yes vs. no), usage of calcium-channel blockers (yes vs. no).
Model 3: adjusted for Model 2 + RBC, hemoglobin, percentage of lymphocyte, MCV, log (10)-transformed serum iron, natural log-transformed baseline eGFR, natural log-transformed UACR, log (10)-transformed 24-h urine protein, log (10)-transformed albumin, bicarbonate, log (10)-transformed calcium, log (10)-transformed phosphorus, uric acid, blood glucose, and log (10)-transformed LDL-C.
ACEI, angiotensin converting–enzyme inhibitors; ARB, angiotensin II–receptor blockers; CI, confidence interval; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MCV, mean corpuscular volume; RDW, red blood cell distribution width; RBC, red blood cell; UACR, urinary albumin-creatinine ratio.

all-cause mortality risk in both hemodialysis (26) and peritoneal dialysis patients (27). A retrospective study on 282 NDD-CKD patients found that RDW ≥14.5% was associated with an enhanced risk of major composite cardiovascular outcomes (28). Additionally, a significantly higher risk of requiring dialysis or doubling of serum creatinine was observed in NDD-CKD patients with sustained, higher RDW (29). On the contrary, a large registry-based cohort study did not confirm that RDW was a prognostic factor of persistent requirement for dialysis therapy (21). Given the unclear relationship between RDW and CKD prognosis, our study extended the previous results by demonstrating that RDW was correlated with rapid renal function loss, concerning both long-term effects and short-term effects over 1-year follow-up.

Since some inherent explanations of RDW variation have been previously studied, including aging, inflammation, metabolic disorders and nutritional deficiencies (30), we investigated the associations between RDW and possibly related variables. Notably significant correlations between RDW value and a series of laboratory variables including erythrocytes count, hemoglobin, initial eGFR, UACR, serum albumin, and serum iron were observed in this study, indicating the potential role of RDW as manifestation of malnutrition and impaired erythropoiesis, thus poorer overall health, which was consistent with previous articles (5). Although it is hypothesized that factors like age, sex, lipid profiles, co-existing diabetes, or hypertension might also associate with RDW, we found no constant connection between RDW level and these variables after adjustments for covariates.

Previous studies reported that RDW is either normal or elevated. The circumstance of a RDW value below the normal reference range is infrequent and considered to be clinically meaningless (31, 32). Taken together, we noted that elevated RDW was associated with higher risk of CKD progression, after adjusting for baseline eGFR, albuminuria, serum iron, blood level

of hemoglobin, and albumin. Although the hidden mechanisms were not fully understood, we have several speculations. Very importantly, the presence of anemia was associated with adverse clinical outcomes in CKD patients (33). It was demonstrated in experimental researches that RDW first became abnormal than other measures including hemoglobin, MCV and transferrin saturation as iron deficiency anemia (IDA) developed (34), suggesting an early biochemical clue. Because advanced CKD often results in deficiency of EPO production and impaired iron absorption, RDW may be an essential reflection of progressive renal disease. Another reasonable explanation is probably related to systemic inflammation. A strong, graded association between RDW and C-reactive protein (CRP), along with erythrocyte sedimentation rate (ESR) independently of multiple confounders was revealed in a large-size cohort (35). Chronic inflammation and subsequent oxidative stress were suspected to serve as the key intermediates linking increased RDW value to kidney injury (36). However, the pathophysiologic effect of inflammatory biomarkers could not be assessed in our study. In addition, whether RDW is simply an epiphenomenon of underlying inflammation as the result of kidney disease or an essential risk factor of progressive renal damage, remained to be confirmed in future exploration. On top of that, atherosclerosis is commonplace in the context of CKD, and may help explain the correlates of RDW and cardiovascular risk (37). Previous studies concluded that the cholesterol content of erythrocytes membranes was positively associated with RDW values independently of inflammatory, nutritional and hematological confounders (38). Partially owing to the fact that anisocytosis might result in turbulence of blood flow, RDW was associated with thrombotic occlusions and vascular injuries in glomeruli (39). In summary, impaired body metabolism might contribute to elevated RDW level and thereby facilitate inflammatory process and impaired antioxidant status, thus triggering adverse kidney outcomes.

The strengths of this study included the sequential measurements of serum creatinine and calculation of eGFR slope. As former studies primarily concentrated on clinical hard endpoints, this is the first research that evaluated the impact of RDW on eGFR decline rate, a dynamic parameter with strong prognostic utility (40), in NDD-CKD patients. Previous studies have emphasized that the accuracy in estimating eGFR slope lies in the number of repeated eGFR measurements (41). Our study cohort was conducted in a busy outpatient nephrology clinic, providing frequent measurements of eGFR, which helped mitigate bias from acute effect of intervention or random fluctuations of eGFR. Also, we used linear mixed effects models to calculate eGFR slope, which are more robust than ordinary linear regression models when the baseline eGFR values significantly vary among study participants. As there is no consensus definition of a rapid eGFR decline, our primary analysis was performed using the original criteria recommended by the KDIGO guideline (eGFR decline >5 ml/min/1.73 m^2/year) (25). Furthermore, as proposed by other studies (42), we conducted the sensitivity analysis under alternative thresholds of eGFR decline >3 ml/min/1.73 m^2/year and that based on a natural distribution of eGFR change in our own study sample. Since the length of observation may influence the effect of the exposure, another sensitivity analysis was carried out using only the first-year data. Additionally, to mitigate the possible selection bias, we added participants with only 2 eGFR available during the first-year observation period into analysis. Overall, the above sensitivity analyses suggested consistent results.

There are several limitations that must be acknowledged. Firstly, this cohort with an observational design was conducted at a single center on a moderate sample size, which might restrict the cause-and-effect relationship and the extrapolation of findings to other populations. Secondly, the 3-year follow-up was relatively short and thus confined implication for long-term effects on CVD events or death. Thirdly, our database is lacking inflammatory biomarkers, such as CRP or interleukin-6 (IL-6), because only a small proportion of patients maintained routine tests of these indices. Fourthly, our data source was also short of information on nutrition intake or anthropometric parameters. Hence, serum albumin, TG, TC, and LDL-C were regarded as substitute nutritional data. Finally, some unknown confounders might affect the observed associations even after extensive adjustments.

CONCLUSION

In conclusion, our results revealed that RDW $>14.5\%$ was independently associated with rapid CKD progression and adverse kidney outcomes, after adjusting for initial kidney function, nutritional state, and presence of anemia. Since chronic inflammation or ineffective erythropoiesis might be prevalent silently until a later stage in the course of CKD progression, RDW might serve as an indicator for the disease progression. With its wide availability and low cost for measurement, RDW might be useful in monitoring NDD-CKD patients.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University First Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XD searched the literature, designed the study, analyzed the data, interpreted the results, and drafted the manuscript. LZ and JW supervised the study and revised the manuscript. M-HZ, LZ, FW, BG, and JW were involved in data collection and data cleaning. All authors have read and approved the final manuscript.

FUNDING

This study was supported by grants from the National Key R&D Program of the Ministry of Science and Technology of China (2020YFC2005003 and 2016YFC1305405), the National Natural Science Foundation of China (91846101, 81771938, 81301296, and 81900665), Beijing Nova Program Interdisciplinary Cooperation Project (Z191100001119008), Chinese Scientific and Technical Innovation Project 2030 (2018AAA0102100), the University of Michigan Health System-Peking University Health Science Center Joint Institute for Translational and Clinical Research (BMU20160466, BMU2018JI012, and BMU2019JI005), CAMS Innovation Fund for Medical Sciences (2019-I2M-5-046), PKU-Baidu Fund (2019BD017) and from Peking University (BMU2018MX020 and PKU2017LCX05). The funders had no role in study design; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication.

ACKNOWLEDGMENTS

We would like to thank all the nurses and doctors involved in the process of data collection and the patients for their support and cooperation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.877220/full#supplementary-material

REFERENCES

1. Chen TK, Knicely DH, Grams ME. Chronic kidney disease diagnosis and management: a review. *JAMA.* (2019) 322:1294–304.
2. Kalantar-Zadeh K, Jafar TH, Nitsch D, Neuen BL, Perkovic V. Chronic kidney disease. *Lancet.* (2021) 398:786–802.
3. Ruiz-Andres O, Sanchez-Nino MD, Moreno JA, Ruiz-Ortega M, Ramos AM, Sanz AB, et al. Downregulation of kidney protective factors by inflammation: role of transcription factors and epigenetic mechanisms. *Am J Physiol Renal Physiol.* (2016) 311:1329–40. doi: 10.1152/ajprenal.00487.2016
4. Zoccali C, Vanholder R, Massy ZA, Ortiz A, Sarafidis P, Dekker FW, et al. The systemic nature of CKD. *Nat Rev Nephrol.* (2017) 13:344–58. doi: 10.1038/nrneph.2017.52
5. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: a simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci.* (2015) 52:86–105. doi: 10.3109/10408363.2014.992064
6. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol.* (2016) 38:123–32. doi: 10.1111/ijlh.12500
7. Lushbaugh CC, Maddy JA, Basmann NJ. Electronic measurement of cellular volumes. I. Calibration of the apparatus. *Blood.* (1962) 20:233–40.
8. Patel HH, Patel HR, Higgins JM. Modulation of red blood cell population dynamics is a fundamental homeostatic response to disease. *Am J Hematol.* (2015) 90:422–8. doi: 10.1002/ajh.23982
9. Malka R, Delgado FF, Manalis SR, Higgins JM. In vivo volume and hemoglobin dynamics of human red blood cells. *PLoS Comput Biol.* (2014) 10:e1003839. doi: 10.1371/journal.pcbi.1003839
10. Higgins JM, Mahadevan L. Physiological and pathological population dynamics of circulating human red blood cells. *Proc Natl Acad Sci U S A.* (2010) 107:20587–92. doi: 10.1073/pnas.1012747107
11. Hou HF, Sun T, Li C, Li YM, Guo Z, Wang W, et al. An overall and dose-response meta-analysis of red blood cell distribution width and CVD outcomes. *Sci Rep.* (2017) 7:10. doi: 10.1038/srep43420
12. Isik T, Kurt M, Tanboga IH, Ayhan E, Gunaydin ZY, Kaya A, et al. The impact of admission red cell distribution width on long-term cardiovascular events after primary percutaneous intervention: a four-year prospective study. *Cardiol J.* (2016) 23:281–8. doi: 10.5603/CJ.a2015.0080
13. Melchio R, Rinaldi G, Testa E, Giraudo A, Serraino C, Bracco C, et al. Red cell distribution width predicts mid-term prognosis in patients hospitalized with acute heart failure: the RDW in acute heart failure (RE-AHF) study. *Intern Emerg Med.* (2019) 14:239–47. doi: 10.1007/s11739-018-1958-z
14. Yamada S, Yoshihisa A, Kaneshiro T, Amami K, Hijioka N, Oikawa M, et al. The relationship between red cell distribution width and cardiac autonomic function in heart failure. *J Arrhythm.* (2020) 36:1076–82. doi: 10.1002/joa3.12442
15. Wang Z, Korantzopoulos P, Roever L, Liu T. Red blood cell distribution width and atrial fibrillation. *Biomark Med.* (2020) 14:1289–98.
16. Korantzopoulos P, Sontis N, Liu T, Chlapoutakis S, Sismanidis S, Siminelakis S, et al. Association between red blood cell distribution width and postoperative atrial fibrillation after cardiac surgery: a pilot observational study. *Int J Cardiol.* (2015) 185:19–21. doi: 10.1016/j.ijcard.2015.03.080
17. Lappegård J, Ellingsen TS, Skjelbakken T, Mathiesen EB, Njølstad I, Wilsgaard T, et al. Red cell distribution width is associated with future risk of incident stroke. The Tromsø study. *Thromb Haemost.* (2016) 115:126–34. doi: 10.1160/TH15-03-0234
18. Song SY, Hua C, Dornbors D III, Kang RJ, Zhao XX, Du X, et al. Baseline red blood cell distribution width as a predictor of stroke occurrence and outcome: a comprehensive meta-analysis of 31 studies. *Front Neurol.* (2019) 10:1237. doi: 10.3389/fneur.2019.01237
19. Gromadziński L, Januszko-Giergielewicz B, Pruszczyk P. Red cell distribution width is an independent factor for left ventricular diastolic dysfunction in patients with chronic kidney disease. *Clin Exp Nephrol.* (2015) 19:616–25. doi: 10.1007/s10157-014-1033-7
20. Ujszaszi A, Molnar MZ, Czira ME, Novak M, Mucsi I. Renal function is independently associated with red cell distribution width in kidney transplant recipients: a potential new auxiliary parameter for the clinical evaluation of patients with chronic kidney disease. *Br J Haematol.* (2013) 161:715–25. doi: 10.1111/bjh.12315
21. Yeh HC, Lin YT, Ting IW, Huang HC, Chiang HY, Chung CW, et al. Variability of red blood cell size predicts all-cause mortality, but not progression to dialysis, in patients with chronic kidney disease: a 13-year pre-ESRD registry-based cohort. *Clin Chim Acta.* (2019) 497:163–71. doi: 10.1016/j.cca.2019.07.035
22. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 Practice guidelines for the management of arterial hypertension of the European society of hypertension and the European society of cardiology: ESH/ESC task force for the management of arterial hypertension. *J Hypertens.* (2018) 36:2284–309.
23. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. *Lancet.* (2017) 389:2239–51.
24. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis.* (2014) 63:713–35. doi: 10.1053/j.ajkd.2014.01.416
25. Levin A, Stevens PE. Summary of KDIGO 2012 CKD guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int.* (2014) 85:49–61. doi: 10.1038/ki.2013.444
26. Vashistha T, Streja E, Molnar MZ, Rhee CM, Moradi H, Soohoo M, et al. Red cell distribution width and mortality in hemodialysis patients. *Am J Kidney Dis.* (2016) 68:110–21.
27. Hsieh YP, Tsai SM, Chang CC, Kor CT, Lin CC. Association between red cell distribution width and mortality in patients undergoing continuous ambulatory peritoneal dialysis. *Sci Rep.* (2017) 7:45632. doi: 10.1038/srep45632

28. Lu YA, Fan PC, Lee CC, Wu VC, Tian YC, Yang CW, et al. Red cell distribution width associated with adverse cardiovascular outcomes in patients with chronic kidney disease. *BMC Nephrol.* (2017) 18:361. doi: 10.1186/s12882-017-0766-4
29. Yonemoto S, Hamano T, Fujii N, Shimada K, Yamaguchi S, Matsumoto A, et al. Red cell distribution width and renal outcome in patients with non-dialysis-dependent chronic kidney disease. *PLoS One.* (2018) 13:e0198825. doi: 10.1371/journal.pone.0198825
30. May JE, Marques MB, Reddy VVB, Gangaraju R. Three neglected numbers in the CBC: the RDW, MPV, and NRBC count. *Cleve Clin J Med.* (2019) 86:167–72. doi: 10.3949/ccjm.86a.18072
31. Arbel Y, Weitzman D, Raz R, Steinvil A, Zeltser D, Berliner S, et al. Red blood cell distribution width and the risk of cardiovascular morbidity and all-cause mortality. A population-based study. *Thromb Haemost.* (2014) 111:300–7. doi: 10.1160/TH13-07-0567
32. Foy BH, Carlson JCT, Reinertsen E, Padros I Valls R, Pallares Lopez R, Palanques-Tost E, et al. Association of red blood cell distribution width with mortality risk in hospitalized adults with SARS-CoV-2 infection. *JAMA Netw Open.* (2020) 3:e2022058. doi: 10.1001/jamanetworkopen.2020.22058
33. Fishbane S, Spinowitz B. Update on anemia in ESRD and earlier stages of CKD: core curriculum 2018. *Am J Kidney Dis.* (2018) 71:423–35. doi: 10.1053/j.ajkd.2017.09.026
34. McClure S, Custer E, Bessman JD. Improved detection of early iron deficiency in nonanemic subjects. *JAMA.* (1985) 253:1021–3.
35. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med.* (2009) 133:628–32. doi: 10.5858/133.4.628
36. Semba RD, Patel KV, Ferrucci L, Sun K, Roy CN, Guralnik JM, et al. Serum antioxidants and inflammation predict red cell distribution width in older women: the women's health and aging study I. *Clin Nutr.* (2010) 29:600–4. doi: 10.1016/j.clnu.2010.03.001
37. Parizadeh SM, Jafarzadeh-Esfehani R, Bahreyni A, Ghandehari M, Shafiee M, Rahmani F, et al. The diagnostic and prognostic value of red cell distribution width in cardiovascular disease; current status and prospective. *Biofactors.* (2019) 45:507–16. doi: 10.1002/biof.1518
38. Tziakas D, Chalikias G, Grapsa A, Gioka T, Tentes I, Konstantinides S. Red blood cell distribution width: a strong prognostic marker in cardiovascular disease: is associated with cholesterol content of erythrocyte membrane. *Clin Hemorheol Microcirc.* (2012) 51:243–54. doi: 10.3233/CH-2012-1530
39. Valdivielso JM, Rodríguez-Puyol D, Pascual J, Barrios C, Bermúdez-López M, Sánchez-Niño MD, et al. Atherosclerosis in chronic kidney disease. *Arterioscler Thromb Vasc Biol.* (2019) 39:1938–66.
40. Levey AS, Gansevoort RT, Coresh J, Inker LA, Heerspink HL, Grams ME, et al. Change in Albuminuria and GFR as end points for clinical trials in early stages of CKD: a scientific workshop sponsored by the national kidney foundation in collaboration with the US food and drug administration and European medicines agency. *Am J Kidney Dis.* (2020) 75:84–104. doi: 10.1053/j.ajkd.2019.06.009
41. Rifkin DE, Shlipak MG, Katz R, Fried LF, Siscovick D, Chonchol M, et al. Rapid kidney function decline and mortality risk in older adults. *Arch Intern Med.* (2008) 168:2212–8. doi: 10.1001/archinte.168.20.2212
42. Kovesdy CP, Coresh J, Ballew SH, Woodward M, Levin A, Naimark DM, et al. Past decline versus current eGFR and subsequent ESRD risk. *J Am Soc Nephrol.* (2016) 27:2447–55. doi: 10.1681/ASN.2015060687

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 15 June 2022
doi: 10.3389/fmed.2022.910840

Hepatitis C Prevalence, Incidence, and Treatment in Chinese Hemodialysis Patients: Results From the Dialysis Outcomes and Practice Patterns Study-China (2019–21)

*Liangying Gan[1], Dongyu Wang[2], Brian Bieber[2], Keith McCullough[2], Michel Jadoul[3], Ronald L. Pisoni[2], Fanfan Hou[4], Xinling Liang[5], Zhaohui Ni[6], Xiaonong Chen[7], Yuqing Chen[8] and Li Zuo[1]**

[1] Department of Nephrology, Peking University People's Hospital, Beijing, China, [2] Arbor Research Collaborative for Health, Ann Arbor, MI, United States, [3] Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium, [4] State Key Laboratory of Organ Failure Research, Division of Nephrology, National Clinical Research Center of Kidney Disease, Nanfang Hospital, Southern Medical University, Guangzhou, China, [5] Division of Nephrology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangdong, China, [6] Renal Division, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [7] Division of Nephrology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [8] Renal Division, Peking University First Hospital, Beijing, China

OPEN ACCESS

Edited by:
Terry Cheuk-Fung Yip, The Chinese University of Hong Kong, Hong Kong SAR, China

Reviewed by:
Jimmy Che to Lai, The Chinese University of Hong Kong, China
Jonathan Soldera, University of Caxias do Sul, Brazil

***Correspondence:**
Li Zuo
zuoli@bjmu.edu.cn

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *01 April 2022*
Accepted: *24 May 2022*
Published: *15 June 2022*

Citation:
Gan L, Wang D, Bieber B, McCullough K, Jadoul M, Pisoni RL, Hou F, Liang X, Ni Z, Chen X, Chen Y and Zuo L (2022) Hepatitis C Prevalence, Incidence, and Treatment in Chinese Hemodialysis Patients: Results From the Dialysis Outcomes and Practice Patterns Study-China (2019–21). Front. Med. 9:910840. doi: 10.3389/fmed.2022.910840

Background: Prior work from the Dialysis Outcomes and Practice Patterns Study (DOPPS) showed HCV prevalence in China in 2012–2015 being in the upper third and HCV incidence the 2nd highest among 15 different countries/regions investigated. The goal of the present investigation was to: (1) determine if HCV prevalence and incidence has changed, and (2) collect detailed data to understand how HCV is treated, monitored, and managed in Chinese HD facilities and non-dialysis chronic kidney disease (CKD) clinics.

Data and Methods: Detailed data for 1,700 randomly selected HD patients were reported by 39 randomly selected HD facilities from Beijing, Shanghai, and Guangzhou participating in the DOPPS 7-China study from 2019 to 2021. The study site medical directors completed a survey regarding numerous aspects of HCV treatment and management in HD and ND-CKD patients.

Results: In this 2019 to 2021 cohort, HCV prevalence was 7.4%, which was lower than the 14.8 and 11.5% HCV prevalence for the 2009–2011 and 2012–2015 cohorts, respectively. HCV incidence of 1.2 cases per 100 pt-yrs also was lower compared to the incidence of 2.1 for the 2012–2015 cohort. Although the great majority of study site medical directors indicated that all or nearly HCV+ patients should be treated for their HCV, very few HCV+ patients have been treated presumably due to substantial cost barriers for affording the new direct acting antivirals (DAAs). The randomly selected facilities in our DOPPS 7-China study appear to have excellent programs in place for frequent monitoring of patients and staff for HCV, education of staff, and referral of HCV cases to external infectious disease, gastroenterology, and liver disease specialists.

Liver biopsies were not commonly performed in HCV+ HD patients. HCV genotyping also was rarely performed in participating units.

Conclusions: Our study indicates a 50% decline in HCV prevalence and a >40% decline in HCV incidence in Chinese HD patients over the past 10–12 yrs. Chinese HD facilities and associated specialists appear to be well-equipped and organized for successfully treating and managing their HCV+ HD and CKD patients in order to achieve the WHO goal of eliminating HCV by 2030.

Keywords: chronic kidney disease, hemodialysis, hepatitis C virus, prevalence, incidence, Dialysis Outcomes and Practice Patterns Study

INTRODUCTION

During the past 3 decades, patients receiving chronic dialysis as kidney replacement therapy for end stage kidney failure have been indicated to be at high risk for hepatitis C virus (HCV) infection. We have previously reported in the international, prospective Dialysis Outcomes and Practice Patterns Study (DOPPS) that HCV infection is common among in-center hemodialysis (HD) patients in >20 countries (1) and is associated with elevated all-cause mortality and morbidity, highly elevated rates of hepatic-related mortality and morbidity, and worse quality of life (2).

HCV has been reported to be the fourth-most commonly reported infectious disease in China, with ∼10 million people infected (3). In addition, a disease sentinel surveillance program in China between 2010 and 2012, identified that the highest HCV seropositive rates in China were among persons using drugs and patients receiving hemodialysis (3). Previously, Jadoul et al. (1) reported an HCV prevalence of 11.5% among Chinese in-center chronic HD patients and an HCV incidence (95% CI) of 2.1(1.0, 4.2) new HCV cases per 100 patient-yrs based on a cohort of patients participating in DOPPS phase 5 data (2012–2015). The above HCV prevalence in China was in the upper third and HCV incidence the 2nd highest among the 15 different countries/regions investigated by Jadoul et al. (1).

However, the above HCV prevalence and incidence reported for China in DOPPS phase 5 was before direct acting antivirals (DAAs) were approved for treating HCV in China in 2017, and the management of blood-borne infectious diseases in hemodialysis facilities has been strengthened after establishing Hemodialysis Quality Control and Improvement Centers (HDQCIC) in each province. The current study was designed to describe the practice of HCV treatment among Chinese HD patients in phase 7 of the China DOPPS (2019–2021) and assess the impact of the availability of DAAs upon the trend in HCV prevalence and incidence.

METHODS AND MATERIALS

Patients and Data Collection

Analyses were based on data collected in DOPPS 7-China as part of the 7th phase of the broader international Dialysis Outcomes and Practice Patterns Study (DOPPS). DOPPS is a prospective cohort study of HD patients ≥18 years of age. The present study included 1,700 Chinese HD patients, who participated in DOPPS 7 anytime between April 2019 and October 2021. Study participants were randomly selected for study participation from a representative sample of HD dialysis facilities in Beijing ($N = 14$ facilities), Shanghai ($N = 13$ facilities), and Guangzhou ($N = 15$ facilities) as described previously (4). All study sites were hospital-based with 56% being academic vs. 44% non-academic, and 55% designated as grade 3 hospitals vs. 45% as grade 2 hospitals. All Chinese study sites which participated in DOPPS 7 also had previously participated in DOPPS 5 (2012–2015), although there were 3 military hospital dialysis units that participated in China-DOPPS 5 which did not participate in DOPPS 7. The current investigation was based on patient- and facility-level data from 39 randomly selected Chinese HD facilities participating in DOPPS 7-China from April 2019 to October 2021 and which had completed a Medical Director's Survey on HCV Management and Monitoring. Study approval was obtained by a central institutional review board. Additional study approval and patient consent were obtained as required by national and local ethics committee regulations.

Patients from the initial prevalent cross-section of DOPPS 7 participants were included in analyses of overall HCV prevalence and comparison of patient characteristics by HCV status. From a total population of 1,700 study patients, the following exclusions were implemented (a) two facilities did not accept HCV+ patients ($n = 69$), (b) an additional 350 patients were not in the initial prevalent cross section, and (c) 2 patients were missing HCV status at enrollment for an overall sample of 1,279 patients used for prevalence analyses. When calculating HCV incidence among all patients the following exclusion criteria were applied: (a) two facilities did not accept HCV+ patients ($n = 69$), (b) 92 patients had an indication of HCV infection history at study enrollment, and (c) 342 patients had fewer than two HCV antibody tests during follow-up for an overall sample of 1,197 patients when calculating HCV incidence.

Demographic data, comorbid conditions, and laboratory values were abstracted from patient records. Baseline HCV status was determined based on an established diagnosis of HCV infection or positive HCV serology at DOPPS enrollment. HCV serology (quantitative and qualitative) were collected monthly. Information regarding each study site's treatment, management, screening and monitoring practices for HCV in their HD patients and non-dialysis chronic kidney disease (CKD) stages 3–5 patients was obtained by administering a specific HCV survey

for each facility's Medical Director. These HCV surveys were completed by 39 of the 42 DOPPS 7-China study site Medical Directors, with responses used for analyses described in this investigation. Completion dates for these surveys were from December 2020 to September 2021, with 90% of the surveys completed between December 2020 and June 2021.

Data Analysis

Seroconversion models and rates were restricted to patients with an initial negative HCV antibody measurement and at least one follow-up HCV antibody value. Time at risk started at the lab date for the first negative HCV antibody measurement (at study baseline or during follow-up) and ended at the seroconversion lab date or last negative HCV antibody lab date. Patients departing the study before a follow-up HCV antibody was measured were not included in the incident HCV seroconversion analyses. The median follow-up time for HCV seroconversion was 1.2 years.

Standard descriptive statistics were used to characterize the DOPPS patients included in the study as well as HCV prevalence, incidence, and facility HCV control practices. All analyses used SAS software, version 9.4 (SAS institute, Cary, NC).

RESULTS

Study Sample Characteristics

Of the 39 facilities, 12, 12, and 15 of the facilities were from Beijing, Shanghai, and Guangzhou, respectively. **Table 1** provides a comparison of the characteristics of HCV+ vs. HCV- patients from the initial cross-section of 1,279 Chinese HD patients in facilities participating in DOPPS 7. In this cross-section, 81 of the 1,279 patients were HCV+. This corresponds to 6.3% of patients but 7.4% when weighted by the facility sampling fraction. HCV+ patients had a lower mean age of 58.9 yrs (vs. 60.9 yrs among HCV- patients), and a much longer mean time on dialysis of 10.8 yrs (vs. 6.1 yrs among HCV- patients). HCV+ patients were almost evenly split between males and females whereas HCV- patients were more likely to be male. The prevalence of hepatitis B was 1.6 fold higher in HCV+ patients (8%) compared to HCV- patients (5%). However, the prevalence of coronary artery disease, hypertension, other cardiovascular disease, congestive heart failure, and cerebrovascular disease was lower among HCV+ vs. HCV- patients, whereas the prevalence of diabetes and peripheral vascular disease was very similar in HCV+ vs. HCV- patients. The prevalence of other displayed characteristics was quite low overall (<5%) with absolute differences consequently being relatively small in magnitude.

TABLE 1 | Baseline patient characteristics of Chinese HD study patients, by HCV+ (China DOPPS, 2019–2021; initial cross-section of patients).

Characteristics	HCV+	HCV-	Overall
Number of patients	81	1,198	1,279
Demographics			
Age	58.9 (12.7)	60.9 (13.5)	60.7 (13.5)
Male Sex	51%	58%	58%
ESRD vintage, years	10.8 (8.1)	6.1 (4.9)	6.4 (5.3)
Comorbidities			
Hypertension	81%	87%	86%
Diabetes	33%	33%	33%
Coronary artery disease	20%	31%	30%
Cerebrovascular disease	10%	15%	15%
Peripheral vascular disease	11%	11%	11%
Congestive heart failure	22%	23%	23%
Cardiovascular disease, other	15%	19%	19%
Cancer	1%	4%	4%
Lung disease	5%	4%	4%
GI bleed in the last year	3%	2%	2%
Recurrent cellulitis	1%	2%	2%
Neurologic disorder	0%	6%	5%
Psychiatric disorder	3%	1%	1%
HIV/AIDS	0%	0%	0%
Hepatitis B	8%	5%	5%
Substance abuse in last 12 mo	0%	0%	0%
Cirrhosis	1%	1%	1%

TABLE 2 | HCV prevalence among Chinese HD Patients in DOPPS, at baseline by study phase.

DOPPS Study Phase	Time frame	Prevalence	*N* Pts
4*	2009–2011	14.8%	1,223
5*	2012–2015	11.5%	967
7	2019–2021	7.4%	1,279

*Prevalence weighted by facility sampling fraction, excluding facilities that did not accept HCV+ patients. *Published previously by Jadoul et al. (1).*

Prevalence and Incidence of HCV in Chinese HD Patients in DOPPS 7-China (2019–2021)

Although 6.3% of the initial cross-section of 1,229 study patients were HCV+, once analyses were weighted by the fraction of patients sampled within each HD facility, the prevalence of HCV+ was calculated to be 7.4% (**Table 2**). For comparison, we have shown that this HCV+ prevalence seen among Chinese HD patients in DOPPS 7 is lower than that which we reported previously in DOPPS for China. These new results from DOPPS 7 show a continual decline in HCV+ prevalence in Chinese HD patients from 14.8% in DOPPS 4 (2009–2011) to 11.5% in DOPPS 5 (2012–2015), and now to 7.4% in DOPPS 7 (2019–2021) despite 48.7% of HD facilities used the more sensitive PCR method in routine HCV screening (**Table 3**). DOPPS did not collect these data in China during the period from 2016 to 2018.

Further analyses revealed an incidence rate of 1.2 new cases of HCV per 100 patient-years based on 16 new cases arising during follow-up of 1,197 patients who had data that met the analysis criteria introduced earlier (**Table 4**). This incidence rate is approximately half the rate of 2.1 new cases of HCV per 100 patient-years that we had determined previously for Chinese HD patients in DOPPS 5 (2012–2015). HCV antibody tests were reported as the method used for routine HCV screening by 51% of medical directors while 44% reported using both HCV antibody and HCV RNA (PCR) tests (**Table 4**). Although the number of incident HCV cases was too small for formal

TABLE 3 | Method typically used for routine HCV screening in facility HD patients (China Medical Director HCV survey, 2021).

HCV screening test	% of respondents ($n = 39$)
HCV antibody test	51.3
HCV RNA test (PCR)	5.1
Both	43.6

TABLE 4 | Incidence of becoming HCV+ during study follow-up, among Chinese HD Patients in DOPPS, by study phase.

DOPPS study phase	Time frame	HCV+ Incidence (per 100 patient-years)	*N* Cases	N patients
4*	2009–2011	–		
5¶	2012–2015	2.1	8	450
7	2019–2021	1.2	16	1,197

**Not determined in phase 4 data because HCV antibodies were not collected longitudinally in China DOPPS Study Phase 4. ¶Published previously by Jadoul et al. (1). Restricted to patients with at least two HCV antibody measurements and in whom the initial HCV antibody measurement was negative, excluding facilities that did not accept HCV+ patients.*

TABLE 5 | Treatment of HCV+ patients based on patient-level prescription data (China DOPPS, 2019–2021).

Medication	*N* Patients
Epclusa (sofosbuvir + velpatasvir)	4
Daklinza (daclatasvir)	1
Interferon beta	1
Sovaldi (sofosbuvir)	1
Other	2

statistical analyses, several risk factors or HCV seroconversion were explored descriptively. The median (IQR) facility percent of patients with HCV at enrollment for units without any seroconverters ($n = 28$) vs. those with a seroconverter ($n = 10$) was 2.5% (0.0–4.8%) vs. 5.3% (2.5–7.9%). One seroconverter received a transfusion among 70 unique patients with a transfusion in China DOPPS 7. All incident HCV+ patients were HIV negative and had no substance abuse history in the last 12 months. The percentage of hepatitis B positivity in incident HCV+ patients vs. HCV- patients was 6.3 vs. 4.8%.

HCV Treatment in DOPPS 7-China HD Patients During Study Follow-Up

Among the 97 patients noted to be HCV+ at study baseline or who became HCV+ during study follow-up, anti-HCV medications were prescribed for 9 of these patients (**Table 5**): 4 of 9 patients (44%) were prescribed Epclusa (sofosbuvir + velpatasvir), 1 each prescribed Daklinza (daclatasvir), Interferon beta, and Sovaldi (sofosbuvir), and with no drug name reported for 2 treated patients.

The type of HCV medication used to treat HCV+ dialysis patients was also indicated by 13 of the 39 China-DOPPS 7 medical directors (**Table 6**), showing greater diversity in the range of medications used for treating HCV compared to the HCV medications prescribed for the relatively small number of 9 patients treated during follow-up as described above. At 4 of these 13 HD units, HCV was typically treated with interferon alone or with ribavirin; 3 of the 13 HD units used only Zepatier, and 1 site used only Epclusa, 1 used Epclusa or Zepatier, 1 used Epclusa or Viekira Pak, 1 used Epclusa, interferon or ribavirin, 1 used Epclusa, Harvoni, Ribavirin or Interferon, and 1 used either Epclusa, Harvoni, Sovaldi, Vosevi, Viekira Pak, or interferon + ribavirin; 2 of the 39 facilities indicated they did not accept HCV+ patients, 3 facilities indicated that they didn't currently have any HCV+ patients (and didn't answer the medication question), and 21 facilities (53%) indicated that HCV treatment was administered outside of their facility.

Medical Director Survey Responses on HCV Management and Monitoring

Perspectives on Treating HCV

Surveys regarding the treatment, management and monitoring of HCV were completed by the medical directors from 39 of the 42 DOPPS 7-China study sites. Nearly all medical directors (92 and 95%) indicated that all or nearly all HCV+ dialysis patients on a kidney transplant waiting list or HCV+ CKD stage 3 patients should be treated with antiviral medication for HCV (**Table 7**), and with a similar, although slightly lower percentage for the treatment of HCV+ dialysis patients NOT on a kidney transplant waiting list or having CKD stage 4–5 patients. In contrast to the above perspectives, when asked what % of current HCV+ patients had ever been treated with anti-HCV medications at any time, by any physicians managing HCV care (**Table 8**), ∼50% of medical directors indicated that none of their current HCV+ dialysis patients had ever been treated with an anti-HCV medication, whereas ∼20% of medical directors indicated that >50% of current HCV+ dialysis patients had ever been treated with an anti-HCV medication. Similar results were noted in HCV treatment of CKD stage 3–5 HCV+ patients.

Type of Doctor Who Typically Treats HCV in Dialysis or ND-CKD Patients

When dialysis unit medical directors were asked to what degree they felt comfortable or adequately trained in treating HCV in their HCV+ patients, 15% of medical directors indicated being somewhat or very comfortable, 44% were neutral, and 41% felt somewhat or very uncomfortable in treating HCV in their HCV+ patients (**Table 9**). Consequently, at the majority of DOPPS 7-China HD units, the type of doctor who usually prescribes antiviral medications to treat HCV in their dialysis patients typically is not a nephrologist with: 40% of study sites indicating this to be an infectious disease specialist, 30% indicating a liver or gastroenterology specialist, only 11% indicating a nephrologist, 8% indicating a type of doctor different than those above, and 11% of sites indicating that their facility's practice is to NOT have hepatitis C treated in their HCV+ patients (**Table 10A**). Furthermore, 85% of medical directors indicated that their

TABLE 6 | Medical director responses regarding the types of medications used by DOPPS 7-China HD units for treating HCV+ in their HD patients (China Medical Director HCV survey, 2021).

Medication (s)							Frequency ($n = 39$)
Interferon and/or ribavirin							4
Interferon and/or ribavirin	Epclusa						1
Interferon and/or ribavirin		Harvoni					1
Interferon and/or ribavirin	Epclusa	Harvoni	Sovalid	Viekira Pack	Vosevi		1
	Epclusa						1
						Zepatier	3
	Epclusa					Zepatier	1
	Epclusa			Viekira Pack			1
HCV+ patients not accepted at facility							2
Not currently any HCV+ patients at facility							3
HCV treatment not administered at facility							21

TABLE 7 | Medical director perspectives of whether HCV+ dialysis or CKD patients should be treated with an anti-viral medication (China Medical Director HCV survey, 2021).

	HD-tx waitlisted (% of $n = 38$)	HD-not tx waitlisted (% of $n = 39$)	CKD stage 3 (% of $n = 39$)	CKD stage 4 (% of $n = 38$)
Agree	92.1	84.6	94.9	86.8
Neutral	7.9	15.4	5.1	13.2
Disagree	0.0	0.0	0.0	0.0

TABLE 8 | Estimated % of clinic HCV+ patients that have been treated with anti-HCV medications, by ESKD stage (China Medical Director HCV survey, 2021).

% of patients treated	HD-tx waitlisted (% of $n = 39$)	HD-not tx waitlisted (% of $n = 39$)	CKD stage 3 (% of $n = 38$)	CKD stage 4 (% of $n = 39$)
0%	56.4	46.2	44.7	48.7
1–5%	10.3	20.5	18.4	15.4
6–10%	0.0	5.1	7.9	7.7
11–25%	0.0	2.6	2.6	2.6
26–50%	10.3	7.7	2.6	5.1
>50%	23.1	17.9	23.7	20.5

HCV+ dialysis patients typically were referred to a liver or gastroenterology specialist as part of their care (**Table 10B**). Medical director responses were nearly identical for HCV treatment of non-dialysis CKD stage 3–5 patients vs. dialysis patients with regard to the above two questions.

Testing for HCV Genotypes and Frequency of Liver Biopsies

In asking about the percent of a HD facility's HCV+ patients for whom HCV genotyping was performed either for new HCV cases or when the HCV genotype is unknown, 85% of unit medical directors indicated that HCV genotyping is performed only for 1–20% of such HCV+ patients in their dialysis units, with 6 and 9% of medical directors, respectively, indicating that

TABLE 9 | Do you feel comfortable/adequately trained in treating HCV in your facility's HCV+ patients? (China Medical Director HCV survey, 2021).

Comfort level treating HCV	% of respondents ($n = 39$)
Very comfortable	5.1
Somewhat comfortable	10.3
Neutral	43.6
Somewhat uncomfortable	23.1
Very uncomfortable	17.9

TABLE 10A | Physician type who usually prescribed antiviral medications to treat HCV+ patients, by ESKD stage (China Medical Director HCV survey, 2021).

HCV medication prescriber	HD (% of $n = 38$)	CKD stage 3 (% of $n = 38$)	CKD stage 4 (% of $n = 37$)
Nephrologist	10.5	10.5	10.8
Liver or gastroenterology specialist	28.9	28.9	29.7
Primary care physician	0.0	0.0	0.0
Infectious disease specialist	42.1	42.1	43.2
Other	7.9	7.9	8.1
HCV+ patients not treated	10.5	10.5	8.1

TABLE 10B | HCV+ patients are usually referred to a liver/gastroenterology specialist, by ESKD stage (China Medical Director HCV survey, 2021).

Referred to specialist	HD (% of $n = 39$)	CKD stage 3 (% of $n = 39$)	CKD stage 4 (% of $n = 39$)
Yes	84.6	87.2	87.2
No	15.4	12.8	12.8

TABLE 11 | HCV genotyping practices (China Medical Director HCV survey, 2021).

Genotyping practice	% of responses ($n = 39$)
% HCV+ patients genotyped[a]	
1–20%	84.6
21–40%	2.6
41–60%	2.6
61–80%	2.6
81–100%	7.7
Genotyping awareness	
NS5A resistance associated polymorphisms[b]	12.8
NS3 Q80K mutation[c]	5.1

[a]*Either for new HCV cases or when the HCV genotype is unknown.* [b]*Are you typically aware whether your HD facility's HCV genotype 1 dialysis patients are tested for NS5A resistance associated polymorphisms (e.g., amino acid substitutions at positions 28, 30, 31, 58, 93)?* [c]*Are you typically aware whether your HD facility's HCV genotype 1a dialysis patients are tested for the NS3 Q80K mutation?*

HCV genotyping was performed for 41–80% and 81–100% of their HCV+ HD patients (**Table 11**). Only a small fraction of unit medical directors–13 and 5%, respectively, —were aware of whether their HD unit's HCV genotype 1 patients were tested for NS5A resistance polymorphisms or whether their HCV genotype 1a dialysis patients were tested for the NS3 Q80K mutation (**Table 11**). Medical directors were also questioned about what % of HCV+ patients had ever had a liver biopsy (**Table 12**). Medical director responses indicated that no liver biopsies had been performed for any of their current HCV+ patients in ∼70% of HD units and 72% of CKD clinics treating CKD stage 3 and 4 patients. Medical directors indicate that only 3–5% of HD facilities and 3–5% of ND-CKD clinics had performed a liver biopsy in >50% of their HCV+ patients.

Screening for HCV in HD Facility or in ND-CKD Patients

Medical directors were asked the frequency at which HD patients in their facility were typically screened for HCV (**Table 13A**), with 92% indicating twice per year or more often, 6% indicating once per year or less often, and 3% only when a patient joins their facility. However, compared to HCV screening in dialysis patients, the frequency of HCV screening of non-dialysis CKD stage 3 or 4 patients at many facilities is considerably less frequent, with ∼50% indicating twice per year or more often, 28% indicating only once per year or less often than once per year, and ∼22% screening for HCV only when a patient joins their CKD clinic. A difference was seen as well in practices for monitoring HCV trends in dialysis vs. non-dialysis CKD stage 3–5 patients (**Table 13B**): 31, 54, and 5% of medical directors indicated that their facilities monitor HCV trends in their dialysis patients on a monthly, twice per year, or once per year frequency, respectively; by contrast, 62, 12, and 20% of medical directors indicate that their facilities monitor HCV trends in their ND-CKD patients on a monthly, twice per year, or once per year frequency, respectively. Finally, nearly all HD unit medical directors (95–97%) indicated that their HD patients are typically screened for HCV after returning from travel or a long-term hospitalization (**Table 13C**).

TABLE 12 | Estimated percent of facility HCV+ patients who have ever had a liver biopsy, by ESKD stage (China Medical Director HCV survey, 2021).

% of patients with biopsy	HD-tx waitlisted (% of $n = 39$)	HD-not tx waitlisted (% of $n = 38$)	CKD stage 3 (% of $n = 39$)	CKD stage 4 (% of $n = 39$)
0%	79.5	73.7	69.2	71.8
1–5%	2.6	10.5	12.8	10.3
6–10%	2.6	2.6	5.1	5.1
11–25%	0.0	2.6	2.6	2.6
26–50%	12.8	5.3	5.1	7.7
>50%	2.6	5.3	5.1	2.6

TABLE 13A | Frequency of screening for HCV, by ESKD stage (China Medical Director HCV survey, 2021).

HCV screening frequency	HD (% of $n = 39$)	CKD stage 3 (% of $n = 39$)	CKD stage 4 (% of $n = 39$)
At least every 3 months	25.6	33.3	33.3
Twice per year	66.7	17.9	15.4
Once per year	2.6	20.5	20.5
Less than once per year	2.6	7.7	7.7
Only upon entry to our facility	2.6	20.5	23.1

Dialysis Unit Staff HCV Education and HCV Screening

Medical directors indicated that screening of dialysis unit staff members for HCV was very common across nearly all China-DOPPS HD study sites with most sites (92%) screening staff members once per year, 5% screening twice per year, and 3% screening less often than once per year (**Table 14**). In addition, 95% of dialysis units provide staff members with educational courses on prevention of transmission of blood borne viruses (including HCV) once per year, 3% once every 2 years, and 3% less often than every 2 years (**Table 14**). In addition, 97% of dialysis units instruct new staff members about HCV precautions as part of their orientation.

DISCUSSION

In China, the main high-risk populations for HCV infection have been reported to be intravenous drug users, patients receiving hemodialysis, and patients co-infected with HIV or hepatitis B (5). In view of the large population of individuals receiving hemodialysis for end stage kidney failure in China, great efforts have been underway for many years to reduce HCV infections in this patient population. The present study represents the most detailed investigation of the prevalence, incidence, treatment, and monitoring of HCV in chronic HD patients in China since direct acting antiviral medications were approved for HCV treatment in China in 2017.

TABLE 13B | Frequency of monitoring facility HCV trends, by ESKD stage (China Medical Director HCV survey, 2021).

Facility HCV trend monitoring frequency	HD (% of *n* = 39)	CKD stage 3 (% of *n* = 39)	CKD stage 4 (% of *n* = 39)
At least monthly	30.8	61.5	59
∼3 times per year	10.3	2.6	5.1
∼2 times per year	53.8	12.8	10.3
∼1 time per year	5.1	17.9	20.5
Less than 1 time per year	0.0	5.1	5.1

TABLE 13C | Situations after which HCV testing is typically performed (China Medical Director HCV survey, 2021).

Situation	Yes (%)	No (%)	Not applicable (%)	Responses (n)
Travel to a country known to have high HCV prevalence	97.4	0.0	2.6	39
Return to facility after long-term hospitalization	94.9	5.1	–	39

TABLE 14 | HCV education and screening of dialysis staff members (China Medical Director HCV survey, 2021).

Practice	Prevalence (%)
Staff HCV screening frequency[a]	
At least 2 times per year	5.1
∼1 time per year	92.3
Less often than 1 time per year	2.6
Staff HCV training frequency[b]	
1 time per year	94.9
One time every 2 years	2.6
Less often than every 2 years	2.6
Staff orientation includes HCV precaution instructions[c]	
Yes	97.4
No	2.6

[a]*N = 39 responses.* [b]*n = 39 responses; How often does your dialysis unit provide staff members with educational courses on prevention of transmission of blood borne viruses (including HCV)?* [c]*n = 38 responses; Does your dialysis unit instruct new staff members about HCV precautions as part of their orientation?*

Our study results from the DOPPS 7-China indicate a 50% decline in HCV+ prevalence in Chinese HD patients over the past 10–12 yrs, with a prevalence of 7.4% seen in 2019–2021 compared to 11.5% in DOPPS 5 (2012–2015) and 14.8% in DOPPS 4 (2009–2011) (1). The above possible substantial decline in HCV prevalence within the DOPPS-China study sites over time has been seen despite the possibility that detection of HCV infected HD patients may have increased due to PCR more commonly being used as part of HCV monitoring activities in more recent years. Similar to what we had seen previously in an analysis of HCV across 15 countries/international regions in DOPPS (1), HCV+ HD patients in China were more likely to be younger, had a considerably longer mean number of years on dialysis, and were much more likely to also be hepatitis B+. However, the percentage of HD patients who were female was higher among HCV+ vs. HCV- patients in China in contrast to our prior international findings in which the proportion of female patients was lower among HCV+ individuals. The lower prevalence of cardiovascular disease comorbidities seen among HCV+ patients in our Chinese study sample may be a reflection in part of the younger mean age of HCV+ patients.

Consistent with the above trend in declining HCV prevalence among Chinese HD patients, our analyses also indicate an HCV incidence of 1.2 new cases of HCV per 100 patient-yrs seen among study patients who became HCV antibody positive during study follow-up in DOPPS 7-China (2019–2021). This incidence rate is >40% lower than the rate of 2.1 new cases of HCV per 100 patient-yrs that Jadoul et al. (1) had reported previously for Chinese HD patients in DOPPS 5 (2012–2015).

The large improvement seen in the incidence and prevalence of HCV in HD patients in China-DOPPS could be a reflection of the extensive HCV management program in place at many of the DOPPS 7-China study sites under the direction of local HDQCIC which was first established in Shanghai in 1999, followed by Beijing in 2002 (6). Strict adherence to infection control procedures and routine serologic screening plays a pivotal role in preventing transmission of HCV within hemodialysis units (7, 8). HDQCIC pushes hemodialysis facilities to formulate infection control procedures including routine training, HCV screening for new HD patients or patients transferring from another hemodialysis facility followed by retesting every 6–12 months for all HCV- hemodialysis patients, and implementation of optimal hygienic precautions. Also HDQCIC does routine observational audits of various infection control practices combined with feedback of results to clinical staff in accordance with KDIGO hepatitis C guidelines (8).

This well-developed infection control monitoring program is reflected by nearly all medical directors in DOPPS China facilities reporting frequent monitoring for HCV, with 26 and 67% of HD units screening their patients for HCV every 3 or 6 months, respectively. Additionally, 31% of HD units monitor their trends in HCV positivity every month, while 64% monitor their HCV+ trends 2–3 times per yr. Moreover, in ∼95% of facilities, patients are typically tested for HCV when returning to the facility after a long-term hospitalization or having traveled to a country known to have high HCV prevalence. Regular HCV testing is also applied to staff, with 97% of DOPPS 7-China HD units screening dialysis unit staff at least once per year for HCV. Furthermore, 95% of HD units have a HCV training course for staff each year, and orientation of new staff includes HCV precaution instructions at 97% of HD units.

DAAs were approved for treating HCV in China in 2017 which is later than some other developed countries and the safety and efficacy of DAA therapy in CKD have been demonstrated by a number of studies (9–11). From the medical directors survey we get the answer about whether application of DAAs in HD patients is a reason for the declining HCV prevalence and incidence in Chinese HD patients. Although nearly all China DOPPS medical directors (92+%) indicated that their HCV+ should be treated with DAAs, ∼50% of medical directors indicated that none of their current HCV+ dialysis patients had ever been treated with

an anti-HCV medication (consistent with patient-level data). Reasons are likely multifactorial for the low level of antiviral treatment of HCV+ patients in practice among the DOPPS 7-China HD units, but we suspect the main factor is the high cost of DAAs accompanied by lack of reimbursement by the national insurance system during this study time period (12). A similar situation occurred in Taiwan, where DAAs were approved for use in 2015, but the number of patients receiving DAAs treatment was very limited until 2018 due to medical insurance reimbursement policy (13). In our study, HCV+ patients were referred to a liver/gastroenterology specialist in 85% of HD units. Furthermore, in most facilities, prescription of antiviral medications to treat HCV+ patients was typically managed by an infectious disease or liver/gastroenterology specialist, with nephrologists prescribing antiviral medications for their HCV+ patients in only 11% of HD units. So adequate access to DAAs in dialysis patients in China also depends on good collaboration between nephrologists and other specialists (14).

When DAAs were used, Medical Director responses and the few patient-level prescriptions demonstrate that a broad array of different DAAs are being used across these study sites—and in different combinations—indicating substantial diversity in how DAAs are being applied in different HD units. This is partly due to the fact that these drugs were not available at the same time in China, and the drug choices available in different hospitals were not equal. Daclatasvir was one of the earliest DAAs available in China, while DAAs such as Pibrentasvir/Glecaprevir or Elbasvir/Grazoprevir, which are more suitable for patients with end-stage renal disease, entered China much later.

Although HCV was not treated in many HCV+ HD patients as discussed above, Medical Director responses provide a clear picture that management and monitoring of HCV appeared to be well-organized across the vast majority of study site HD units. After 2020, Elbasvir/Grazoprevir, Ledipasvir/Sofosbuvir, and Sofosbuvir/Velpatasvir were successfully reimbursed by the national insurance system, with an average price drop of more than 85% (15). These drugs are available gradually in different hospitals and the costs of these drugs are affordable after reimbursement now. Physicians, nephrologists, or other specialists will be free to choose DAAs suitable for dialysis patients. With proper quality improvement initiatives, HCV elimination from dialysis facilities is possible (16, 17).

Since 7.4% is still a relatively high HCV prevalence, the nosocomial transmission signs in China DOPPS7 should also be explored. One of the 16 incident HCV+ patients had history of a blood transfusion during DOPPS follow-up. Transfusion safety has been greatly improved in China with more sensitive nucleic acid testing (NAT) screening for HCV being widely used in blood donors (1). However, the HCV incidence in repeat blood donors in China was 15.2 per 100,000 person-years which indicates that there remains some infection risk because of the time window/period of donation and thus HCV infection is still an important concern for transfusion safety (18). As previously published in DOPPS (1), facility HCV prevalence is higher in facilities with seroconverters than in facilities without seroconverters (5.3 vs. 2.5%). Facility HCV prevalence is also a risk factor of seroconversion in China. As suggested by KDIGO hepatitis guideline, strategies to prevent HCV transmission within hemodialysis units should prioritize adherence to standard infection control practices (8).

Although a strength of the DOPPS 7–China study is the random selection of HD units for participation in the study, stratified to represent both tier 2 and 3 hospitals, a limitation of our study is that it does not represent all Chinese dialysis/CKD practice in being limited to hospital-based facilities located in the metropolitan areas of Beijing, Shanghai, and Guangzhou, and HCV prevalence is known to vary considerably across different regions of China (19, 20). However, the current study facility sample provides the ability to compare with earlier DOPPS-China results of HCV prevalence and incidence which included many of the same facilities as those participating in DOPPS 7-China. Additionally, the methodology and surveys used in the present study hopefully can serve as the basis for broader capture of these numerous aspects of HCV care in HD units in other Chinese jurisdictions to more thoroughly understand the variability in HD patient HCV incidence, prevalence and treatment across Chinese HD facilities. Another limitation of our study is that since HCV treatment for most HD patients is managed by non-nephrologist specialists, it is conceivable that HCV genotyping and anti-HCV medications prescribed for HCV+ dialysis patients were not always captured within a dialysis unit's medical record for such care provided outside of the dialysis unit. Additionally, even though PCR HCV viral load data were reported for >500 viral load measurements for study patients during follow-up, the diversity in how these values were reported for patients by different HD study sites, and in the common occurrence of duplicate values being reported over a 3 month period made interpretation challenging. This latter experience suggests the need for standardized measurement and reporting systems for PCR HCV viral load levels to be implemented across Chinese HD units if desiring to capture viral load data in a meaningful and interpretable fashion across HD facilities as one aspect of a broad-based HCV surveillance system for China.

In conclusion, this latest investigation of HCV in Chinese HD patients as viewed through the lens of the DOPPS-China study in Beijing, Shanghai, and Guangzhou documents a decline in HCV prevalence and incidence in Chinese HD patients when compared to earlier DOPPS-China results. The randomly selected HD units in the DOPPS 7-China study appear to have excellent programs in place for frequent monitoring of patients and staff for HCV, education of staff, and referral of HCV cases in most HD units to external infectious disease, gastroenterology, and liver disease specialists. Even though the great majority of study site medical directors feel that all or nearly HCV+ patients should be treated, very few if any HCV+ patients have been treated presumably due to substantial cost barriers for affording the new DAAs. DAAs are now covered by the Chinese National Medical Insurance reimbursement system which will likely lead to widespread use of DAAs and further reductions in the incidence and prevalence of HCV in this large patient population.

DATA AVAILABILITY STATEMENT

Data may be made available to qualified researchers for approved scientific uses. Some limitations and fees may apply. Arbor

Research Collaborative for Health encourages investigators, whether or not previously affiliated with the Dialysis Outcomes and Practice Patterns Study (DOPPS), to submit proposals for data use, collaboration, and ancillary studies.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University People's Hospital (ethical approval number: 2018PHB028-01). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This work has been supported in part by a research grant from Investigator-Initiated Studies Program of Merck Sharp & Dohme Corp. Global support for the ongoing DOPPS Programs is provided without restriction on publications by a variety of funders. For details, see https://www.dopps.org/AboutUs/Support.aspx. All grants were made to Arbor Research Collaborative for Health and not to coauthors directly.

ACKNOWLEDGMENTS

We wish to thank Dr. David Goodkin for providing input in the development of the HCV Medical Director Survey.

REFERENCES

1. Jadoul M, Bieber BA, Martin P, Akiba T, Nwankwo C, Arduino JM, et al. Prevalence, incidence, and risk factors for hepatitis C virus infection in hemodialysis patients. *Kidney Int.* (2019) 95:939–47. doi: 10.1016/j.kint.2018.11.038
2. Goodkin DA, Bieber B, Jadoul M, Martin P, Kanda E, Pisoni RL. Mortality, hospitalization, and quality of life among patients with hepatitis c infection on hemodialysis. *Clin J Am Soc Nephrol.* (2017) 12:287–97. doi: 10.2215/CJN.07940716
3. Qin Q, Smith MK, Wang L, Su Y, Wang L, Guo W, et al. Hepatitis C virus infection in China: an emerging public health issue. *J Viral Hepat.* (2015) 22:238–44. doi: 10.1111/jvh.12295
4. Bieber B, Qian J, Anand S, Yan Y, Chen N, Wang M, et al. Two-times weekly hemodialysis in China: frequency, associated patient and treatment characteristics, and quality of life in the China dialysis outcomes and practice patterns study. *Nephrol Dial Transplant.* (2014) 29:1770–77. doi: 10.1093/ndt/gft472
5. Li M, Zhuang H, Wei L. How would China achieve WHO's target of eliminating HCV by 2030? *Expert Rev Anti Infect Ther.* (2019) 17:763–73. doi: 10.1080/14787210.2019.1675509
6. Gan L, Zuo L. Current ESRD burden and its future trend in Beijing, China. *Clin Nephrol.* (2015) 83:17–20. doi: 10.5414/CNP83S017
7. Fabrizi F, Messa P. Transmission of hepatitis C virus in dialysis units: a systematic review of reports on outbreaks. *Int J Artif Organs.* (2015) 38:471–80. doi: 10.5301/ijao.5000437
8. Kidney Disease: Improving Global Outcomes (KDIGO) Hepatitis C Work Group. KDIGO 2018 clinical practice guideline for the prevention, diagnosis, evaluation, and treatment of hepatitis c in chronic kidney disease. *Kidney Int Suppl 2011.* (2018) 8:91–165. doi: 10.1016/j.kisu.2018.06.001
9. Borgia SM, Dearden J, Yoshida EM, et al. Sofosbuvir/velpatasvir for 12weeks in hepatitis C virus-infected patients with end-stage renal disease undergoing dialysis. *J Hepatol.* (2019) 71:660–66. doi: 10.1016/j.jhep.2019.05.028
10. Chuang WL, Hu TH, Buggisch P, Moreno C, Su WW, Biancone L, et al. Ledipasvir/Sofosbuvir for 8, 12, or 24 weeks in hepatitis c patients undergoing dialysis for end-stage renal disease. *Am J Gastroenterol.* (2021) 116:1924–28. doi: 10.14309/ajg.0000000000001281
11. Gane E, Lawitz E, Pugatch D, Papatheodoridis G, Bräu N, Brown A, et al. Glecaprevir and pibrentasvir in patients with HCV and severe renal impairment. *N Engl J Med.* (2017) 377:1448–55. doi: 10.1056/NEJMoa1704053
12. Xie Q, Xuan J-W, Tang H, Ye X-G, Xu P, Lee I-H, et al. Hepatitis C virus cure with direct acting antivirals: clinical, economic, societal and patient value for China. *World J Hepatol.* (2019) 11:421–41. doi: 10.4254/wjh.v11.i5.421
13. Lee JJ, Chang JM, Yang LJ, Hsu CC, Lin MH, Lin MY. Trends of treated hepatitis B, hepatitis C, and tuberculosis infection in long-term hemodialysis patients in Taiwan: a nationwide survey in 2010-2018. *J Formos Med Assoc.* (2022) 121(Suppl. 1):S73–81. doi: 10.1016/j.jfma.2021.12.019
14. Okubo T, Atsukawa M, Tsubota A, Koeda M, Yoshida Y, Arai T, et al. Epidemiological survey of patients with hemodialysis complicated by hepatitis C in Japan. *Ther Apher Dial.* (2019) 23:44–8. doi: 10.1111/1744-9987.12747
15. Available online at: http://www.nhsa.gov.cn/art/2019/11/28/art_14_2052.html (accessed May 31, 2022).
16. Hu TH, Su WW, Yang CC, Yang CC, Kuo WH, Chen YY, et al. Changhua hepatitis c elimination task force. elimination of hepatitis c virus in a dialysis population: a collaborative care model in Taiwan. *Am J Kidney Dis.* (2021) 78:511–9. doi: 10.1053/j.ajkd.2021.03.017
17. Jadoul M, Labriola L, Gordon CE. HCV can and should be eliminated from dialysis units. *Am J Kidney Dis.* (2021) 78:487–8. doi: 10.1053/j.ajkd.2021.06.001
18. Fu P, Lv Y, Zhang H, Liu C, Wen X, Ma H, et al. International component of the NHLBI recipient epidemiology and donor evaluation study-III (REDS-III). Hepatitis C virus prevalence and incidence estimates among Chinese blood donors. *Transfusion.* (2019) 59:2913–21. doi: 10.1111/trf.15432
19. Mei X, Lu H. Prevalence, diagnosis, and treatment of hepatitis C in Mainland China. *Glob Health Med.* (2021) 3:270–5. doi: 10.35772/ghm.2021.01080
20. Zhang M, Wu R, Xu H, Uhanova J, Gish R, Wen X, et al. Changing incidence of reported viral hepatitis in China from 2004 to 2016: an observational study. *BMJ Open.* (2019) 9. doi: 10.1136/bmjopen-2018-028248

Author Disclaimer: The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp.

Conflict of Interest: MJ has been a speaker or consultant for Abbvie and Merck and has received an unrestricted educational grant from Merck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 21 June 2022
doi: 10.3389/fmed.2022.912764

In-center Nocturnal Hemodialysis Reduced the Circulating FGF23, Left Ventricular Hypertrophy, and All-Cause Mortality: A Retrospective Cohort Study

Meizi Kang[1,2†], Jing Chen[2†], Lingling Liu[2], Cheng Xue[2], Xiaojing Tang[2], Jiayi Lv[2], Lili Fu[2], Changlin Mei[2], Zhiguo Mao[2], Yawei Liu[2,3‡] and Bing Dai[2*‡]*

[1] Division of Nephrology, The Second Affiliated Hospital of Nantong University, Nantong, China, [2] Division of Nephrology, Kidney Institute of People's Liberation Army of China, Shanghai Changzheng Hospital, Naval Medical University, Shanghai, China, [3] Department of Internal Medicine, Shanghai Changzheng Hospital, Naval Medical University, Shanghai, China

OPEN ACCESS

Edited by:
Jingyuan Xie,
Shanghai Jiao Tong University, China

Reviewed by:
Pablo Antonio Urena Torres,
AURA Paris, France
Valentin David,
Northwestern University, United States

***Correspondence:**
Bing Dai
czdaibing@smmu.edu.cn
Yawei Liu
lyw1025@126.com

†These authors have contributed equally to this work and share first authorship
‡These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 04 April 2022
Accepted: 20 May 2022
Published: 21 June 2022

Citation:
Kang M, Chen J, Liu L, Xue C, Tang X, Lv J, Fu L, Mei C, Mao Z, Liu Y and Dai B (2022) In-center Nocturnal Hemodialysis Reduced the Circulating FGF23, Left Ventricular Hypertrophy, and All-Cause Mortality: A Retrospective Cohort Study. Front. Med. 9:912764. doi: 10.3389/fmed.2022.912764

Fibroblast growth factor 23(FGF23) is the most important biomarker and pathogenic factor in Chronic Kidney Disease–Mineral and Bone Disorder (CKD–MBD). In the moderate and severe stages of chronic renal failure, abnormally elevated circulating FGF23 can lead to some complications, including myocardial hypertrophy, which is positively correlated with all-cause mortality. However, the circulating FGF23 level of different hemodialysis modalities, the underlying essential regulatory factors, and potential clinical benefits remain to be elucidated. In this retrospective cohort study, 90 in-center nocturnal hemodialysis (INHD) and 90 matched conventional hemodialysis (CHD) patients were enrolled. The complete blood count, intact FGF23(iFGF23), calcium, phosphorus, PTH, and other biochemical and echocardiographic parameters of INHD and CHD patients were collected and analyzed at 1-year follow-up. The all-cause mortality was recorded during the 7-year follow-up. Furthermore, the regulatory factors of iFGF23 and its association with echocardiographic parameters and mortality were investigated by multivariate regression. The levels of iFGF23 and serum phosphate in patients undergoing INHD were significantly lower than those in patients undergoing CHD. The left ventricular volume index (LVMI) in patients with INHD was significantly attenuated and positively correlated with the drop of serum iFGF23. The INHD group had reduced all-cause mortality compared to the CHD group. Multivariate analysis showed that iFGF23 was positively correlated with serum calcium, serum phosphorus, and calcium-phosphate product. The calcium-phosphate product is an independent determining factor of serum iFGF23. Compared with the CHD group, the INHD group presented with a significantly reduced circulating iFGF23 level, which was closely associated with attenuation of left ventricular hypertrophy, but INHD reduced all-cause mortality in an FGF23 independent manner.

Keywords: in-center nocturnal hemodialysis, fibroblast growth factor 23, Chronic Kidney Disease96Mineral and Bone Disorder (CKD-MBD), calcium-phosphate product, left ventricular hypertrophy

INTRODUCTION

Fibroblast growth factor 23 (FGF23) is the most important biomarker and pathogenic factor in CKD–MBD, which is one of the most common complications in dialysis patients. As a potent calcium and phosphorus regulator, FGF23 is produced by osteoblasts and osteocytes. It can reduce phosphorus by promoting phosphorus excretion and inhibiting the formation of $1,25(OH)_2D_3$ (1). In the early stage of chronic renal failure, FGF23 secretion is stimulated by an uncertain mechanism to maintain phosphorus balance, subsequently, leading to decreased $1,25(OH)_2D_3$ synthesis and triggering secondary hyperparathyroidism (2). With the deterioration of renal function, its capacity to promote phosphorus excretion gradually lessens due to reduced functional nephrons, abnormally elevated FGF23 instead becomes a uremic toxin and is highly associated with adverse clinical outcomes (3). So, FGF23 has been recognized as an important prognosis biomarker of chronic kidney disease (4–6).

Hemodialysis is the most widely used modality for renal replacement therapy, which has greatly improved the prognosis of patients with uremia (7). In contrast to conventional hemodialysis, which is performed three times a week for 4 h each, intensive dialysis includes daily dialysis, in-center nocturnal hemodialysis, and home nocturnal hemodialysis (8). The mortality of conventional hemodialysis (CHD) patients is still relatively high, it is ∼4 times more than healthy people under the age of 30, and six times more than healthy people over the age of 65 (9). Intensive dialysis has been reported to have many clinical benefits, including improved blood pressure, anemia, serum phosphorus, and reduced left ventricular hypertrophy (10). A large multicenter study showed that patients with INHD have a higher quality of life, prolonged survival, and a lower hospital admission rate (11). In our previous studies, regression of left ventricular mass index (LVMI) also had been detected when patients with ESRD convert from CHD to INHD (12, 13).

Since elevated FGF23 was found to be associated with increased cardiovascular mortality and all-cause mortality (4), we speculated that INHD might attenuate left ventricular hypertrophy and reduce mortality through lowering the serum FGF23 due to modification of CKD–MBD parameters. However, the effect of different hemodialysis modalities on the circulating FGF23 levels, the underlying essential regulatory mechanism, and potential clinical benefits remain to be elucidated. This study is aimed to further explore whether INHD can improve the prognosis by reducing the level of FGF23. We performed this retrospective cohort study to compare serum FGF23, CKD–MBD related parameters, hemoglobin, albumin, echocardiographic, and survival data in patients undergoing CHD and INHD. The regulatory factors of FGF23 and their association with echocardiographic parameters and mortality were investigated by multivariate regression.

PATIENTS AND METHODS

Study Design and Population

This retrospective study included prevalent long-term hemodialysis patients aged over 18 years old at Shanghai Changzheng Hospital in January 2015. Patients diagnosed with malignant tumors, serious infections or severe heart failure, and liver failure were excluded. A total of 90 patients were enrolled and converted from CHD to INHD. In total, 90 matched patients were enrolled and persistently received CHD (**Supplementary Figure S1**). They were followed up until February 2022. This study was approved by the Ethics Committee of Changzheng Hospital. All patients were dialyzed on a Rexeed 15uc dialyzer (polysulfone membrane, 1.5 m^2). INHD patients received thrice-weekly hemodialysis for 7.5 h each session. Blood flow rates were 200–300 ml/min. Dialysate flow rates were 300–500 ml/min. CHD patients received thrice-weekly hemodialysis (or twice hemodialysis plus single hemodiafiltration) for 4 h each session. Blood flow rates were 200–360 ml/min. Dialysate flow rates were 500 ml/min. Anticoagulation was achieved with heparin or low-molecular-weight heparin.

Data Collection

The demographic data, dialysis vintage, and primary diseases of ESRD were collected from the electronic database of our hemodialysis center. The baseline biochemical parameters, including calcium (Ca), phosphorus (P), hemoglobin (Hb), albumin (Alb), serum creatinine (Scr), ferritin, lipid profiles, 25-hydroxyvitamin D, intact parathyroid hormone (iPTH), and KT/V were collected. The blood samples for laboratory tests, including iFGF23, were obtained before the midweek hemodialysis session. Serum intact FGF23 concentrations with biological activity were measured using a human FGF23 ELISA assay kit (Kainos Lab, Tokyo, Japan). Medications during the enrollment period, including antihypertensive drugs, erythropoietin, phosphate binders, calcitriol, and intravenous iron were reviewed. One year later after enrollment, all the laboratory tests and medications were evaluated again. M-mode echocardiography was used to measure the echocardiographic parameters of 40 matched patients in each group, including interventricular septum thickness diastolic (IVSTd), left ventricular end-diastolic diameter (LVDd), and left ventricular posterior wall thickness (LVPWT), etc. The echocardiography data together with total ultrafiltration volume (ml), the ultrafiltration rate (ml/h/kg), and BP were collected from the electronic database at 1-year follow-up. LVMI was calculated according to the Devereux formula. Left ventricular mass (LVM) $= 1.04 \times [(\text{IVSTd} + \text{LVDd} + \text{LVPWT})^3 - \text{LVDd}^3] - 13.6$, body surface area (BSA) $= 0.0061 \times$ height (cm) $+ 0.0128 \times$ weight (kg) $- 0.1529$. LVMI = LVM/BSA. The all-cause mortality and survival data of the two groups were reviewed during 7 year follow-up.

Statistical Analysis

A propensity-score matching (PSM) analysis was performed to adjust for patient selection. All the quantitative data were presented as mean ± SD. The non-normally distributed variable iFGF-23 was described as medians (interquartile range) and ln-transformed to achieve a normal distribution. The laboratory test data of the two groups were analyzed by the independent sample *t*-test. The association of iFGF23 with patient demographics and laboratory variables was evaluated by Pearson correlation analysis. The correlations between iFGF23

and potential regulatory factors were analyzed by multivariate regression, which was typically shown by some scatter plots. The association of left ventricular volume index (LVMI) with iFGF23 in two groups and overall patients was detected by univariate correlation analysis. We adopted the Kaplan–Meier analysis (log-rank method) to evaluate the survival differences between the two groups. A multivariable Cox proportional hazards regression model was used to identify the risk factors for all-cause mortality. To better clarify the association between iFGF23 and mortality, adjusted HR (95% CI) for mortality was analyzed by both lnFGF23 and quartiles of FGF23 using the first quartile as the reference.

RESULTS

Characteristics of Patients

This retrospective cohort study included 90 INHD patients with an average age of 53.23 ± 10.26 and 90 CHD patients with an average age of 53.32 ± 9.53. The baseline characteristics of the study subjects are shown in **Table 1**. After PSM, there was no significant difference in age, gender, dialysis vintage, primary disease, medications, and biochemical parameters, including serum calcium, phosphorus PTH, and iFGF23 (**Supplementary Table S1**) between the two groups.

Biochemical Parameters and Medications at 1-Year Follow-Up

The serum iFGF23 level of patients in the INHD group was significantly lower than that in the CHD group, as presented by lnFGF23 (7.57 ± 1.62 vs. 8.56 ± 1.17) ($P < 0.05$). INHD patients had lower mean phosphorus levels (1.42 ± 0.41 vs. 2.03 ± 0.62 mmol/L) and calcium-phosphate product levels (3.47 ± 1.02 vs. 5.0 ± 1.52 $mmol^2/L^2$). The good control rate within the KDIGO recommendation target of serum calcium in the INHD group was higher than that in the CHD group (61.2 vs. 59%). The INHD group exhibited a higher good control rate of phosphorus (69.2 vs. 52.9%) and iPTH (73.3 vs. 63%). There was no significant difference in calcium, albumin, 25-(OH)D, and hemoglobin levels between the two groups. Patients undergoing INHD had a higher mean KT/V than those undergoing CHD (2.30 ± 0.71 vs. 1.53 ± 0.30), representing better dialysis adequacy. Compared with CHD, patients in the INHD group used fewer types of antihypertensive drugs on average, and the dosage of erythropoietin and phosphate binder was lower. There was no difference in the dosage and number of patients who used calcitriol and intravenous iron as shown in **Table 2**.

TABLE 1 | Baseline characteristics of patients undergoing hemodialysis after PSM.

Item	INHD ($n = 90$)	CHD ($n = 90$)
Sex		
Male	42	43
Female	48	47
Age (year)	53.23 ± 10.26	53.32 ± 9.53
Dialysis vintages (year)	8.32 ± 5.25	8.43 ± 5.12
Primary disease		
CGN	56	57
Hypertensive renal sclerosis	12	13
DN	15	14
Obstructive nephropathy	1	2
PKD	4	3
Others	2	1

CGN, chronic glomerulonephritis; DN, diabetic nephropathy; PKD, polycystic kidney disease I.

Regulatory Factors of FGF23

Univariate linear regression analysis showed that serum phosphate ($r = 0.480$, $p < 0.001$), calcium ($r = 0.413$, $P < 0.001$), calcium-phosphate product ($r = 0.593$, $P < 0.001$), KT/V ($r = -0.395$, $P < 0.001$), and iPTH ($r = 0.334$, $P < 0.001$) were significantly associated with iFGF23 levels (**Table 3**). Among all the parameters, multivariable regression analysis showed that serum phosphate ($\beta = 0.429$, $P < 0.001$), calcium ($\beta = 0.354$, $P = 0.001$), calcium-phosphate product ($\beta = 0.600$, $P < 0.001$), but not KT/V ($\beta = -0.218$, $P = 0.053$) and iPTH ($\beta = 0.235$, $P = 0.353$) independently correlated with iFGF23 levels (**Table 4**). The scatter plots showed that lnFGF23 was linear with serum calcium, phosphorus, and calcium-phosphate product (**Figure 1**).

Echocardiographic Parameters, BP, and Ultrafiltration

Patients in the INHD group displayed improvement in the cardiac structure and function manifested by lower LVMI (98.7 ± 32.1 vs. 113.9 ± 69.2 g/m^2) ($P < 0.01$), LAD, LVDD, and LVPW, slightly higher LVEF (63.75 ± 3.3 vs. 52.44 ± 5.29%) with a non-significantly different E/A ratio (0.78 ± 0.28 vs. 0.67 ± 0.13) ($P = 0.058$) compared with the CHD group (**Table 5**). Univariate linear regression showed lnFGF23 was significantly correlated with LV mass index (LVMI) in the INHD group ($r = 0.424$, $P = 0.01$), CHD group ($r = 0.619$, $P < 0.001$), and within overall patients ($r = 0.583$, $P < 0.001$) (**Figure 2**).

The INHD group had better BP control, with lower pre-dialysis SBP (132 ± 12 vs. 140 ± 11 mmHg) ($P = 0.028$) and post-dialysis SBP (126 ± 11 vs. 136 ± 13 mmHg) ($P = 0.032$). INHD group also had higher total ultrafiltration volume (4.2 ± 1.0 vs. 2.8 ± 1.2 L) ($P = 0.003$) and slower ultrafiltration rate (7.5 ± 1.8 vs. 9.3 ± 3.5 ml/h/kg) ($P = 0.029$; **Figure 3**).

Survival Analysis

The overall survival rate of INHD was better than CHD as depicted using the Kaplan–Meier method ($\chi^2 = 6.860$, $P = 0.009$) (**Figure 4**). Multivariable Cox proportional analyses were adjusted for age, gender, primary disease, ferritin, etc. Serum phosphate, dialysis vintage, and albumin were independent predictors of all-cause mortality in patients with ESRD. The hemodialysis modality of INHD was a protective factor for survival. However, iFGF23 and KT/V were not significantly

TABLE 2 | Comparison of parameters between CHD and INHD at 1-year follow-up.

Parameters	INHD ($n = 90$)	CHD ($n = 90$)
iFGF23 ($pg \cdot ml^{-1}$)	3,114.41 (1,023.23–9,582.05)	9,626.18 (4,258.53–10,123.23)
LnFGF23	7.57 ± 1.62*	8.56 ± 1.17
Pitch	306.02 ± 142.08	412.92 ± 212.10
Cholesterol ($mmol \cdot L^{-1}$)	3.82 ± 0.97	3.73 ± 0.92
Triglycerides ($mmol \cdot L^{-1}$)	2.25 ± 1.52	2.24 ± 1.45
LDL cholesterol ($mmol \cdot L^{-1}$)	2.08 ± 0.55	2.04 ± 0.60
HDL cholesterol ($mmol \cdot L^{-1}$)	0.89 ± 0.29	0.87 ± 0.27
Phosphate ($mmol \cdot L^{-1}$)	1.42 ± 0.41*	2.03 ± 0.62
Calcium ($mmol \cdot L^{-1}$)	2.44 ± 0.22	2.47 ± 0.33
Calcium-phosphate product ($mmol^2 \cdot L^{-2}$)	3.47 ± 1.02*	5.00 ± 1.52
Albumin ($g \cdot L^{-1}$)	42.32 ± 5.37	42.21 ± 4.38
KT/V	2.30 ± 0.71*	1.53 ± 0.30
25(OH)D ($ng \cdot ml^{-1}$)	27.6 ± 9.3	27.6 ± 9.3
Hemoglobin ($g \cdot L^{-1}$)	117.98 ± 13.76	111.69 ± 14.09
Ferritin ($ug \cdot L^{-1}$)	195.53 ± 159.28	237.30 ± 173.21
β2-MG ($mg \cdot L^{-1}$)	16.7 ± 3.3	17.4 ± 4.7
Medications		
Kinds of antihypertensive drugs/pts number	1.1 ± 0.3*/68	1.8 ± 0.5/74
EPO (U/week)/pts number	5,280.0 ± 3,120.0*/63	6,841.0 ± 3,210.0/70
$CaCO_3$(g/d)/pts number	1.6 ± 1.2*/51	2.5 ± 1.0/53
Calcitriol (ug/d)/pts number	0.23 ± 0.19/49	0.22 ± 0.14/52
Iron sucrose (mg)/pts number	950 ± 296/16	964 ± 285/18
Iron dextran (mg)/pts number	783 ± 182/5	800 ± 167/6

IFGF23, intact Fibroblast growth factor 23; IPTH, intact parathyroid hormone; LDL, low-density lipoprotein; HDL, high-density lipoprotein; KT/V, urea clear index; 25-(OH)D, 25-Hydroxy vitamin D; β2-MG, β2-Microglobulin.
*$P < 0.05$.

TABLE 3 | Univariate correlation analysis of FGF23 and other parameters.

Variables	*r*	*T*	*P*
Age	0.432	0.702	0.484
Dialysis vintage	−0.182	1.823	0.071
Triglycerides	0.211	2.090	0.093
Cholesterol	0.186	1.839	0.069
LDL cholesterol	0.196	1.864	0.066
HDL cholesterol	−0.091	0.856	0.394
Phosphate	0.480	5.388	< 0.001*
Calcium	0.413	4.465	< 0.001*
Calcium-phosphate product	0.593	7.253	< 0.001*
Albumin	0.073	0.702	0.484
KT/V	−0.395	3.542	< 0.001*
iPTH	0.334	3.396	< 0.001*
25(OH)D	0.232	2.435	0.342

LDL, low-density lipoprotein; HDL, high-density lipoprotein; KT/V, urea clear index; iPTH, intact parathyroid hormone; 25-(OH)D, 25-Hydroxy vitamin D.
*$p < 0.05$.

TABLE 4 | Multivariate regression analysis of regulatory factors of FGF23.

Variables	β	SE	*P*
Calcium	0.354	0.641	0.001*
Phosphate	0.429	0.285	< 0.001*
iPTH	0.235	0.310	0.353
KT/V	−0.218	0.232	0.053
Calcium-phosphate product	0.600	0.104	< 0.001*

Take the variables which satisfied $P < 0.3$ in the univariate correlation analysis as candidates, multivariate regression analysis has been done. Then we adopt stepwise regression, taking $P < 0.05$ as inclusion criteria and $P > 0.10$ as exclusion criteria. When calcium-phosphate product was taken into consideration, calcium and phosphate is excluded. The other results are derived from the global entry method.
*$p < 0.05$.

associated with mortality in this adjusted model (**Table 6**). Compared with patients in the first FGF23 quartile, there was no trend toward an association between elevated iFGF23 and mortality in the adjusted model shown in **Table 7**.

DISCUSSION

Nocturnal hemodialysis was first reported by Shaldon in 1963. Thrice-weekly in-center nocturnal hemodialysis has been adopted as the standard therapeutic strategy in Tassin since 1968, and more than 50 years of long-term experience has been gained until now (14). Our center initiated the INHD program in February 2009. Consistent with the previous studies, patients in the INHD group showed increased urea clearance

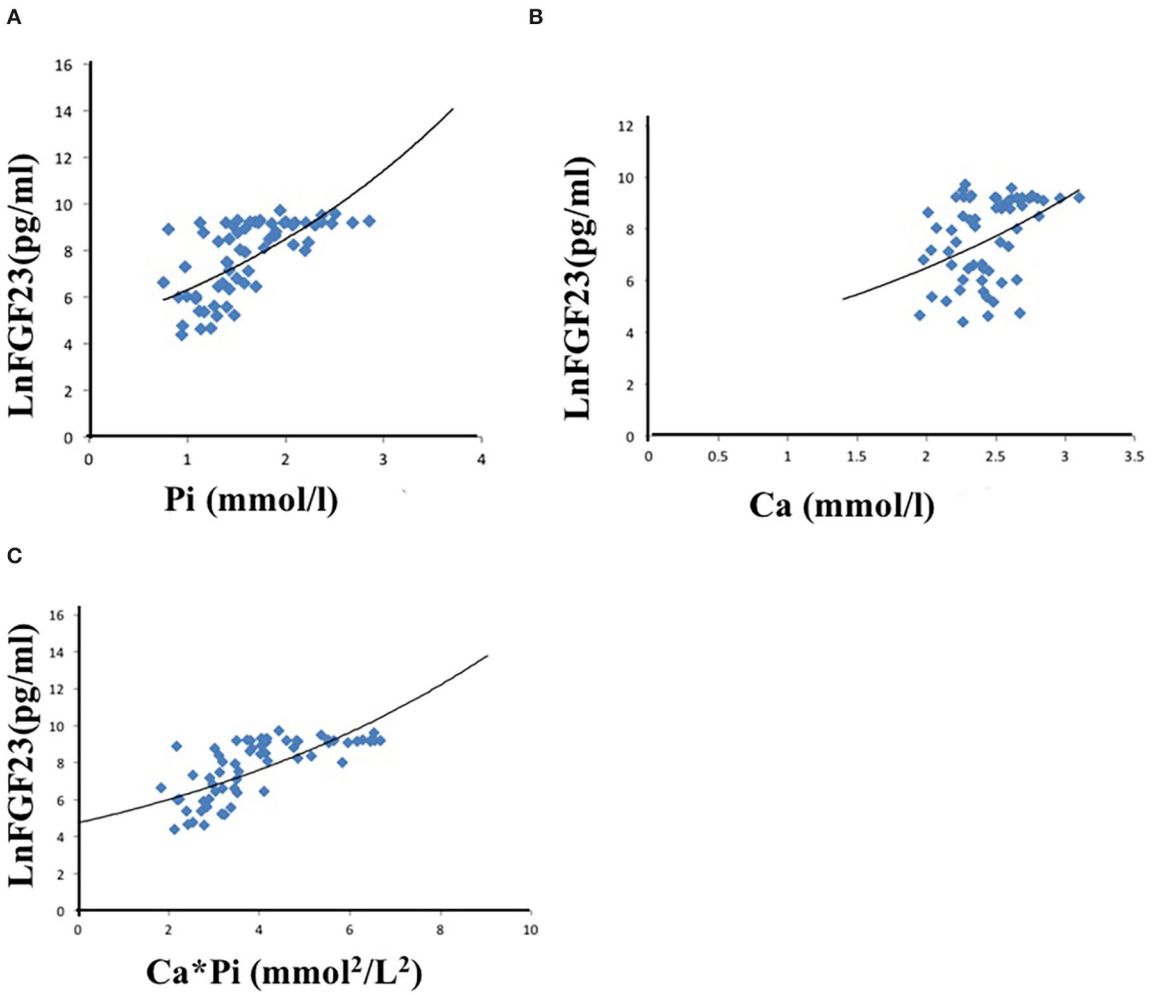


FIGURE 1 | Univariate linear correlation between phosphate **(A)**, calcium **(B)**, calcium-phosphate product **(C)**, and FGF23.

(KT/V), lower serum phosphorus, and significantly reduced requirements of EPO and phosphate binders, together with the improvement of echocardiographic parameters and mortality (11). In addition, for the first time, we found that circulating iFGF23 was significantly reduced in INHD compared to CHD, which might work as a bridge to connect the CKD–MBD with left ventricular hypertrophy and all-cause mortality.

First, the potential regulatory factors of iFGF23 in dialysis patients were evaluated. Although there were many studies focused on the regulation of elevated FGF23 in chronic renal failure, the conclusions were inconsistent. It is well known that phosphate load could enhance FGF23 secretion (15), but the molecular mechanism of how the osteocytes and osteoblasts sensed the extracellular phosphorus and stimulate the downstream FGF23 expression remains unclear. $1,25(OH)_2D_3$ was a positive regulator on FGF23. It can stimulate the transcription of FGF23 through binding to a vitamin D-responsive element on the FGF23 promoter (16). To avoid the interference of medication on circulating iFGF23 levels in the two groups of patients, we collected the medication data and found that there was no significant difference in the patient numbers taking active vitamin D and the dosage of these

TABLE 5 | Comparison of echocardiographic parameters between CHD and INHD.

Item	INHD ($n = 40$)	CHD ($n = 40$)
LVMI (g/m^2)	98.7 ± 32.1*	113.9 ± 69.2
LAD (mm)	33.25 ± 5.57*	41.44 ± 8.40
LVDD (mm)	45.63 ± 4.37*	53.33 ± 6.78
LVPW (mm)	9.84 ± 1.91*	11.53 ± 1.65
LVEF (%)	63.75 ± 3.3	52.44 ± 5.29
E/A	0.78 ± 0.28	0.67 ± 0.13

LVMI, left ventricular mass index; LAD, left atrial dimension; LVDD, left ventricular end diastolic dimension; LVPW, left ventricular posterior wall; LVEF, left ventricular ejection fraction.

*$p < 0.05$.

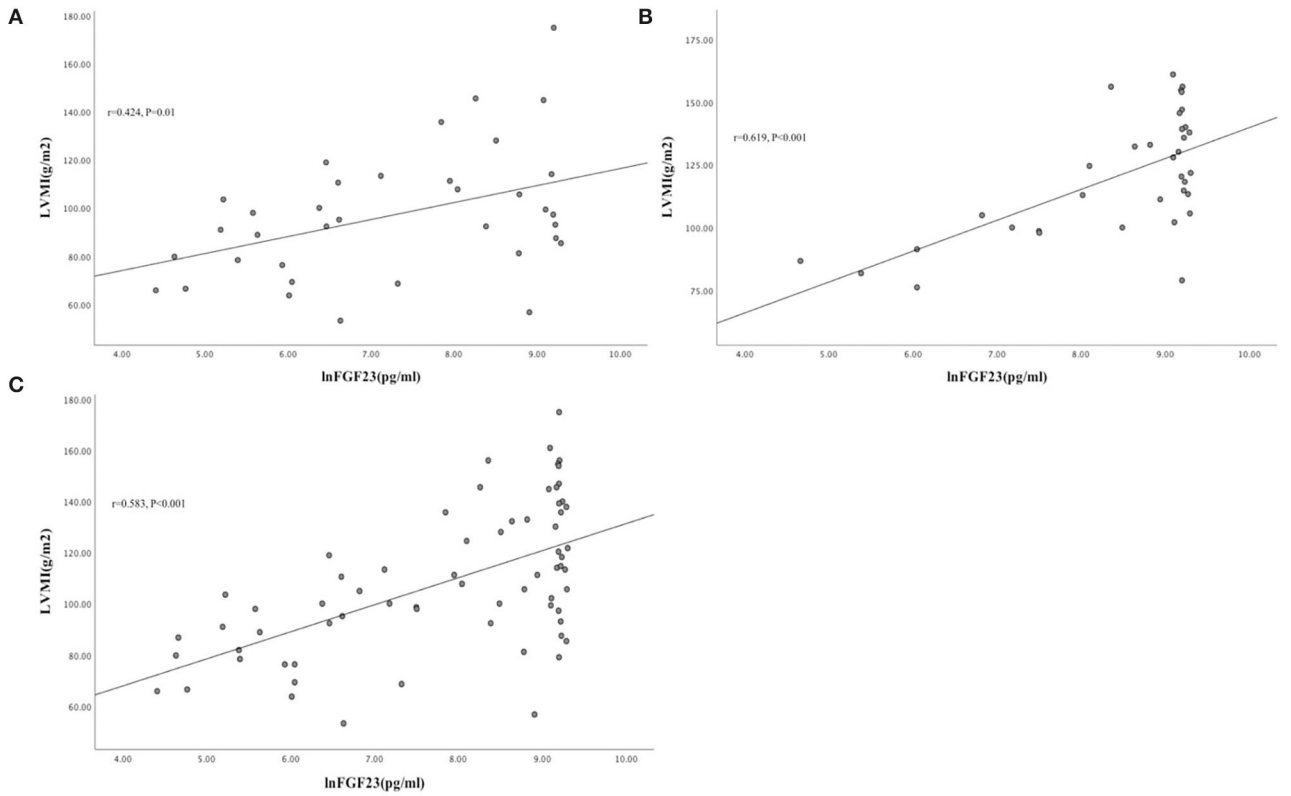


FIGURE 2 | Univariate linear correlation between lnFGF23 and LVMI in INHD group **(A)**, CHD group **(B)**, and within overall patients **(C)** (**Figure 3**).

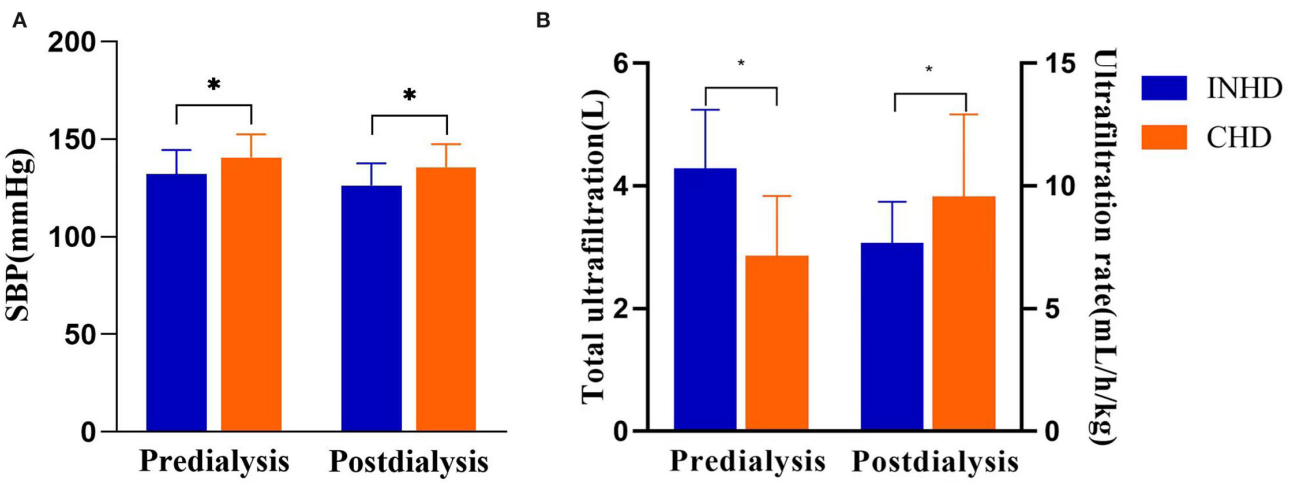


FIGURE 3 | (A) Comparison of systolic blood pressure between patients undergoing INHD and CHD. **(B)** Comparison of volume control between patients undergoing INHD and CHD. *$P < 0.05$.

categories of drugs between the two groups, but the dosage of calcium-containing phosphate binders was significantly reduced in the INHD group. Therefore, the decreased level of iFGF23 in INHD was not related to the difference in active vitamin D. PTH is another potential regulator of FGF23 in chronic renal failure since PTH and FGF23 were both elevated at the same time. Lavi-Moshayoff et al. suggested that PTH could stimulate the FGF23 expression *in vitro,* but other studies could not prove the direct regulation of FGF23 (17). Our previous study found that significantly elevated PTH could not stimulate

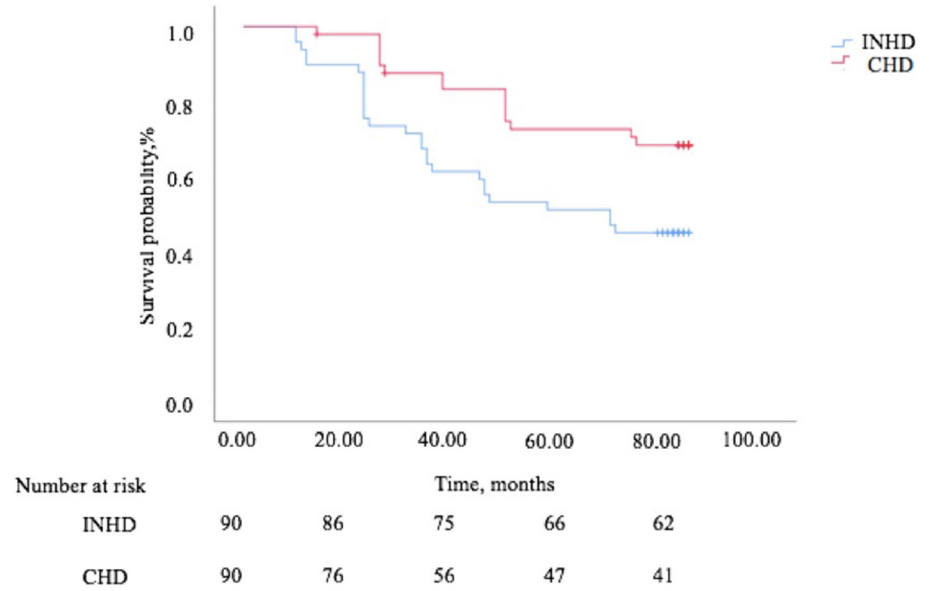


FIGURE 4 | The Kaplan–Meier survival curves comparing patients undergoing INHD and CHD in terms of mortality.

TABLE 6 | Cox regression analyses of factors predicting all-cause mortality.

Variables	β	SE	*P*	HR	95%CI
Age	0.092	0.463	0.842	1.097	0.442, 2.719
Dialysis vintage	0.079	0.040	0.047*	1.082	1.001, 1.170
Hemoglobin	−0.265	0.390	0.496	0.767	0.357, 1.647
Albumin	−1.068	0.495	0.031*	0.344	0.130, 0.906
Calcium	1.037	0.811	0.201	2.821	0.575, 13.838
Phosphate	0.545	0.366	0.001*	2.304	1.842, 3.532
iPTH	0.011	0.325	0.937	1.011	0.535, 1.911
KT/V	−1.552	0.452	0.230	0.932	0.087, 1.514
LnFGF23	0.418	0.562	0.457	1.519	0.505, 4.567
INHD	−0.814	0.323	0.012*	0.443	0.235, 0.834

Take the variables which satisfied P < 0.3 in the univariate cox regressive and some other factors as adjustment factors, a multivariable Cox proportional hazards regression has been done. Multivariable Cox regression analyses were adjusted for age, gender, dialysis vintage, hemoglobin, albumin, calcium, phosphate, KT/V, lnFGF23, ferritin. iPTH, intact parathyroid hormone; KT/V, urea clear index; FGF23, Fibroblast growth factor 23; INHD, In-center nocturnal hemodialysis.
**p < 0.05.*

TABLE 7 | Adjusted HR (95% CI) for mortality by quartile of FGF23.

	Quartile of FGF23			
Model	**<2,235 pg/ml**	**2,235–4,791 pg/ml**	**4,791–8,503 pg/ml**	**>8,503 pg/ml**
No adjustment	1 (ref)	1.967 (1.324, 2.500)	1.073 (0.519, 2.220)	1.168 (0.544, 2.508)
Model 1	1 (ref)	1.153 (0.972, 1.365)	0.841 (0.278, 2.546)	1.087 (0.362, 3.267)
Model 2	1 (ref)	1.139 (0.992, 1.327)	0.943 (0.852, 1.034)	0.913 (0.765, 1.042)

Model 1 was adjusted for age, gender, prior cardiovascular disease, dialysis vintage, hemoglobin, albumin, calcium, phosphate, and ferritin.
Model 2 was adjusted for Model 1 plus dialysis modality.

the expression of FGF23 in 1α-hydroxylase knockout mice with $1,25(OH)_2D_3$ deficiency and hypocalcemia (18). Similarly, the circulating iFGF23 in double knockout CKD mice with impaired production of PTH and calcitriol was significantly abated and could be restored by administration of a high calcium high phosphorus diet, suggesting that neither PTH nor active

vitamin D was indispensable for elevated iFGF23 in chronic renal failure (18, 19).

Multivariate analysis showed that only phosphorus, calcium, and calcium-phosphorus products were positively correlated with FGF23. Among them, it was an important finding that the serum calcium was positively correlated with FGF23 under the circumstances that the serum total calcium was not statistically different between the two groups, which indicated that calcium could positively regulate FGF23 by augmenting calcium load and calcium-phosphorus product before the serum calcium rose beyond the normal range. In our previous experimental study (18), we found that circulating iFGF23 significantly increased 6 h after calcium chloride administration in wild type and PTH-deficient mice. *In vitro*, we found that after adding different concentrations of calcium (1, 2, 4, 6, and 8 mmol/L) to the MC3T3-E1 osteoblast cell line, FGF23 transcription assayed by reporter gene reached maximum activation at 6 mmol/L calcium, and this effect could be inhibited by calcium channel blocker, which showed that extracellular calcium could directly regulate the transcription of FGF23. In a previous *in vivo* study, it was found that at least 50 mg/L of serum phosphorus was required for calcium to promote FGF23 secretion, and at least 80 mg/L of calcium was required for phosphorus to stimulate FGF23 expression (20). Therefore, in dialysis patients, the abnormally elevated calcium-phosphorus product rather than a single mineral ion might be the most essential regulatory factor to enhance the serum iFGF23 secretion. With regard to the underlying molecular mechanism, we speculate that the osteocytes could sense the calcium and phosphorus overload, and extracellular calcium and phosphorus deposited in the skeleton microenvironment might stimulate the secretion of circulating iFGF23 through a probably sodium/phosphate cotransporter (PiT1,2) or FGF receptor (FGFR1) dependent mechanism (21, 22).

It is not a novel idea to emphasize the calcium-phosphorus product in CKD–MBD. However, we suggest this finding has important clinical implications since there are many available strategies to modify the calcium-phosphorus product, which is an independent determining factor of serum iFGF23 levels in dialysis patients. At present, several clinical studies have supported the assumption that lowering calcium-phosphorus products led to an abated serum iFGF23 level in patients with CKD. For example, non-calcium-containing phosphate binder sevelamer was proved to significantly reduce serum iFGF23 compared with calcium-containing phosphate binder, through the mechanism of reducing elemental calcium intake and calcium-phosphorus product (23). Cinacalcet can also attenuate calcium-phosphate products and decrease serum iFGF23 levels in patients with CKD (24).

Two elegant experimental studies have proved that FGF23 can directly induce left ventricular hypertrophy *in vivo* and *in vitro*. The non-classic effect of FGF23 on the heart was independent of Klotho but through binding to FGFR4 on cardiomyocytes. FGF23 activated the PLCγ phosphorylation and the calcineurin/NFAT signaling pathway, finally upregulating the expression of cardiac hypertrophy-related genes (25, 26). There were also different views on whether FGF23 could directly cause myocardial damage (27, 28). Takashi et al. found that although serum iFGF23 was elevated in animal models and patients with X-linked hereditary hypophosphatemia, notable cardiac hypertrophy was not found (27). A recent meta-analysis compared the association between cardiovascular events and FGF23 in the general population, non-dialysis patients with CKD, and dialysis patients, although serum FGF23 went up with the progression of CKD, there was no powerful evidence that increased FGF23 contribution to heart failure, stroke, or myocardial infarction either in the overall or any of the three separate groups (29). To interpret the paradoxical phenomena, we surmised that there were many factors leading to cardiac hypertrophy in uremic patients, while elevated FGF23 was only one of the complex risk factors. Consistent with our previous study and the findings of Culleton et al., this cohort study showed that INHD could significantly improve echocardiographic parameters and reduce left ventricular hypertrophy (12, 13, 30). Based on the positive correlation between serum iFGF23 and LVMI in this study, we speculated that INHD might improve left ventricular hypertrophy partially by reducing serum iFGF23 levels. Better blood pressure control, slower ultrafiltration rate, and more optimized volume management might also be involved.

Many studies had shown that the increased FGF23 in patients with CKD was closely related to the worsened mortality (4, 31). The underlying mechanism comprises not only FGF23 induced myocardial toxicity but also aggravating inflammation, immunologic dysfunction, anemia, etc. (32, 33). Therefore, lowering circulating FGF23 holds the promise to bring survival benefits. Although in some clinical trials, the hypothesis seemed to be true, the causal relationship was hard to confirm. If we take overall hemodialysis patients into consideration, the conclusion becomes ambiguous. J-DOPPS found that as the dialysis vintage increased, the association between iFGF23 and mortality weakened and disappeared in the highest tertile (>9.4 years), indicating that the predictive value of iFGF23 as a prognosis biomarker was blunted with long-term dialysis (34). In a Dutch FGF23 cohort from the CONTRAST study, no association between a single value of cFGF23 and all-cause mortality was found. In addition, decreased cFGF23 in the hemodiafiltration group was not associated with improved survival compared to the hemodialysis group (35). In this study, the association between attenuated iFGF23 and reduced mortality also could not be established in patients with INHD. A plausible explanation was that most of the patients we enrolled had a longer dialysis vintage (average 8.3 years), and serum iFGF23 was already at a very high level, which probably masked the survival benefit.

Consistent with previous studies, this study showed that serum phosphorus, dialysis vintage, and albumin were independent predictors of mortality in long-term hemodialysis patients. In addition, no correlation between all-cause mortality and hemoglobin, KT/V was found, which suggested that the

improved survival of INHD was not due to the ameliorative dialysis adequacy of small-molecule uremic solutes. We believe that INHD persistently conveyed a protective effect on survival through modifying the CKD–MBD parameters, such as hyperphosphatemia, representing an FGF23 independent mechanism involved.

This study has the following limitations: First, this study is a single-center retrospective cohort study with a small number of patients. Some relevant parameters were missing or incomplete, such as c-terminal FGF23 (cFGF23), BNP, pro-BNP, echocardiographic data, and cardiovascular mortality. Second, the complex internal environment of hemodialysis patients and multiple regulatory mechanisms made the causal relationship between those variables could not be explicitly judged. Third, the majority of clinical data were collected and analyzed during the enrollment period as well as at the 1-year follow-up. Further studies on longitudinal changes in those parameters might greatly enhance the scientific value and make the conclusion robust. Fourth, most of the patients we enrolled had long dialysis vintage with no residual kidney function, although there was no statistically significant difference between the two groups, the outcomes still might be biased. Further studies on incident hemodialysis patients with shorter dialysis vintages might yield some different results.

In summary, the INHD group presented with modification of CKD–MBD parameters, including serum phosphorus, calcium-phosphorus product, and iFGF23 compared with the CHD in dialysis patients. The calcium-phosphorus product is the most important and independent regulatory factor of iFGF23 in hemodialysis patients. Decreased serum iFGF23 in patients with INHD might be partially involved in attenuating cardiac structural parameters. INHD significantly reduces all-cause mortality, which was not correlated with abated circulating iFGF23 suggesting other FGF23 independent mechanisms were involved and remained to be clarified.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Changzheng Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MK, JC, CX, and BD conceived and designed the protocol and study. MK, JC, and LL identified cases for eligibility. MK, XT, ZM, and JL extracted data of included cases. MK and LF performed the data analysis with assistance of CM, YL, and BD. MK and BD drafted the article for important content. BD and YL reviewed and revised this manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China under Grant No. 81970640.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.912764/full#supplementary-material

Supplementary Figure S1 | Study flow diagram.

Supplementary Table S1 | Baseline biochemical parameters of patients undergoing hemodialysis.

REFERENCES

1. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiol Rev.* (2012) 92:131–55. doi: 10.1152/physrev.00002.2011
2. Shimada T, Urakawa I, Yamazaki Y, Hasegawa H, Hino R, Yoneya T, et al. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun.* (2004) 314:409–14. doi: 10.1016/j.bbrc.2003.12.102
3. Galitzer H, Ben-Dov IZ, Silver J, Naveh-Many T. Parathyroid cell resistance to fibroblast growth factor 23 in secondary hyperparathyroidism of chronic kidney disease. *Kidney Int.* (2010) 77:211–8. doi: 10.1038/ki.2009.464
4. Chathoth S, Al-Mueilo S, Cyrus C, Vatte C, Al-Nafaie A, Al-Ali R, et al. Elevated fibroblast growth factor 23 concentration: prediction of mortality among chronic kidney disease patients. *Cardiorenal Med.* (2015) 6:73–82. doi: 10.1159/000440984
5. Munoz Mendoza J, Isakova T, Ricardo AC, Xie H, Navaneethan SD, Anderson AH, et al. Fibroblast growth factor 23 and Inflammation in CKD. *Clin J Am Soc Nephrol.* (2012) 7:1155–62. doi: 10.2215/CJN.13281211
6. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis.* (2009) 205:385–90. doi: 10.1016/j.atherosclerosis.2009.01.001
7. Goodkin DA, Bragg-Gresham JL, Koenig KG, Wolfe RA, Akiba T, Andreucci VE, et al. Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *J Am Soc Nephrol.* (2003) 14:3270–7. doi: 10.1097/01.ASN.0000100127.54107.57
8. Bugeja A, Dacouris N, Thomas A, Marticorena R, McFarlane P, Donnelly S, et al. In-center nocturnal hemodialysis: another option in the management of chronic kidney disease. *Clin J Am Soc Nephrol.* (2009) 4:778–83. doi: 10.2215/CJN.05221008
9. McFarlane PA. Nocturnal hemodialysis: effects on solute clearance, quality of life, and patient survival. *Curr Opin Nephrol Hypertens.* (2011) 20:182–8. doi: 10.1097/MNH.0b013e3283437046
10. Gubensek J, Buturovic-Ponikvar J, Knap B, Marn Pernat A, Benedik M, Ponikvar R. Effect of switching to nocturnal thrice-weekly hemodialysis on clinical and laboratory parameters: our experience. *Ther Apher Dial.* (2013) 17:412–5. doi: 10.1111/1744-9987.12088
11. Lacson E, Jr., Wang W, Lester K, Ofsthun N, Lazarus JM, Hakim RM. Outcomes associated with in-center nocturnal hemodialysis from a large multicenter program. *Clin J Am Soc Nephrol.* (2010) 5:220–6. doi: 10.2215/CJN.06070809
12. Jin X, Rong S, Mei C, Ye C, Chen J, Chen X. Effects of thrice-weekly in-center nocturnal vs. conventional hemodialysis on

integrated backscatter of myocardial tissue. *Hemodial Int.* (2011) 15:200–10. doi: 10.1111/j.1542-4758.2011.00537.x
13. Jin X, Rong S, Mei C, Ye C, Chen J, Chen X. Effects of in-center nocturnal versus conventional hemodialysis on endothelial dysfunction. *Ther Apher Dial.* (2012) 16:334–40. doi: 10.1111/j.1744-9987.2012.01070.x
14. Koh TJK. Nocturnal hemodialysis: improved quality of life and patient outcomes. *Int J Nephrol Renovasc Dis.* (2019) 12:59–68. doi: 10.2147/IJNRD.S165919
15. Ferrari SL, Bonjour JP, Rizzoli R. Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. *J Clin Endocrinol Metab.* (2005) 90:1519–24. doi: 10.1210/jc.2004-1039
16. Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, et al. Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest.* (2006) 116:3150–9. doi: 10.1172/JCI29463
17. Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T, PTH. increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol.* (2010) 299:F882–9. doi: 10.1152/ajprenal.00360.2010
18. David V, Dai B, Martin A, Huang J, Han X, Quarles LD. Calcium regulates FGF-23 expression in bone. *Endocrinology.* (2013) 154:4469–82. doi: 10.1210/en.2013-1627
19. Dai B, David V, Martin HM, Showkat A, Gyamlani G, Horst RL, et al. 1,25(OH)2D Is Essential for PTH Stimulation of FGF23. *J Am Soc Nephrol.* (2011) 22:6A. (ASN Oral Abstract).
20. Rodriguez-Ortiz ME, Lopez I, Munoz-Castaneda JR, Martinez-Moreno JM, Ramirez AP, Pineda C, et al. Calcium deficiency reduces circulating levels of FGF23. *J Am Soc Nephrol.* (2012) 23:1190–7. doi: 10.1681/ASN.2011101006
21. Bon N, Couasnay G, Bourgine A, Sourice S, Beck-Cormier S, Guicheux J, et al. Phosphate (Pi)-regulated heterodimerization of the high-affinity sodium-dependent Pi transporters PiT1/Slc20a1 and PiT2/Slc20a2 underlies extracellular Pi sensing independently of Pi uptake. *J Biol Chem.* (2018) 293:2102–14. doi: 10.1074/jbc.M117.807339
22. Takashi Y, Kosako H, Sawatsubashi S, Kinoshita Y, Ito N, Tsoumpra MK, et al. Activation of unliganded FGF receptor by extracellular phosphate potentiates proteolytic protection of FGF23 by its O-glycosylation. *Proc Natl Acad Sci USA.* (2019) 116:11418–27. doi: 10.1073/pnas.1815166116
23. Damment SJ, Pennick M. Clinical pharmacokinetics of the phosphate binder lanthanum carbonate. *Clin Pharmacokinet.* (2008) 47:553–63. doi: 10.2165/00003088-200847090-00001
24. Koizumi M, Komaba H, Nakanishi S, Fujimori A, Fukagawa M. Cinacalcet treatment and serum FGF23 levels in haemodialysis patients with secondary hyperparathyroidism. *Nephrol Dial Transplant.* (2012) 27:784–90. doi: 10.1093/ndt/gfr384
25. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest.* (2011) 121:4393–408. doi: 10.1172/JCI46122
26. Grabner A, Amaral AP, Schramm K, Singh S, Sloan A, Yanucil C, et al. Activation of cardiac fibroblast growth factor receptor 4 causes left ventricular hypertrophy. *Cell Metab.* (2015) 22:1020–32. doi: 10.1016/j.cmet.2015.09.002
27. Takashi Y, Kinoshita Y, Hori M, Ito N, Taguchi M, Fukumoto S. Patients with FGF23-related hypophosphatemic rickets/osteomalacia do not present with left ventricular hypertrophy. *Endocr Res.* (2017) 42:132–7. doi: 10.1080/07435800.2016.1242604
28. Pastor-Arroyo EM, Gehring N, Krudewig C, Costantino S, Bettoni C, Knopfel T, et al. The elevation of circulating fibroblast growth factor 23 without kidney disease does not increase cardiovascular disease risk. *Kidney Int.* (2018) 94:49–59. doi: 10.1016/j.kint.2018.02.017
29. Marthi A, Donovan K, Haynes R, Wheeler DC, Baigent C, Rooney CM, et al. Fibroblast growth factor-23 and risks of cardiovascular and noncardiovascular diseases: a meta-analysis. *J Am Soc Nephrol.* (2018) 29:2015–27. doi: 10.1681/ASN.2017121334
30. Culleton BF, Walsh M, Klarenbach SW, Mortis G, Scott-Douglas N, Quinn RR, et al. Effect of frequent nocturnal hemodialysis vs. conventional hemodialysis on left ventricular mass and quality of life: a randomized controlled trial. *JAMA.* (2007) 298:1291–9. doi: 10.1001/jama.298.11.1291
31. Chonchol M, Greene T, Zhang Y, Hoofnagle AN, Cheung AK. Low vitamin D and high fibroblast growth factor 23 serum levels associate with infectious and cardiac deaths in the HEMO study. *J Am Soc Nephrol.* (2016) 27:227–37. doi: 10.1681/ASN.2014101009
32. Rossaint J, Unruh M, Zarbock A. Fibroblast growth factor 23 actions in inflammation: a key factor in CKD outcomes. *Nephrol Dial Transplant.* (2017) 32:1448–53. doi: 10.1093/ndt/gfw331
33. Ratsma DMA, Zillikens MC, van der Eerden BCJ. Upstream regulators of fibroblast growth factor 23. *Front Endocrinol.* (2021) 12:588096. doi: 10.3389/fendo.2021.588096
34. Komaba H, Fuller DS, Taniguchi M, Yamamoto S, Nomura T, Zhao J, et al. Fibroblast growth factor 23 and mortality among prevalent hemodialysis patients in the Japan dialysis outcomes and practice patterns study. *Kidney Int Rep.* (2020) 5:1956–64. doi: 10.1016/j.ekir.2020.08.013
35. Bouma-de Krijger A, de Roij van Zuijdewijn CLM, Nube MJ, Grooteman MPC, Vervloet MG, Group CS. Change in FGF23 concentration over time and its association with all-cause mortality in patients treated with haemodialysis or haemodiafiltration. *Clin Kidney J.* (2021) 14:891–7. doi: 10.1093/ckj/sfaa028

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 29 June 2022
doi: 10.3389/fmed.2022.864667

The Significance of Crescents on the Clinical Features and Outcomes of Primary Immunoglobin A Nephropathy

Yongjing Du[1,2†], Shasha Chen[1†], Fengping Wang[2], Ping Zhang[1], Mijia Liu[1], Chi Liu[1], Xiang Zhong[1], Jianhua Qin[3], Guisen Li[1] and Wei Wang[1]**

[1] Department of Nephrology, Institute of Nephrology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China, [2] Department of Nephrology, Chengdu Second People's Hospital, Chengdu, China, [3] Department of Nephrology, Affiliated Hospital of Southwest Medical University, Luzhou, China

OPEN ACCESS

Edited by:
Jingyuan Xie,
Shanghai Jiao Tong University, China

Reviewed by:
Yan Ouyang,
Shanghai Jiao Tong University, China
Beom Jin Lim,
Yonsei University College of Medicine, South Korea

***Correspondence:**
Guisen Li
guisenli@163.com
Wei Wang
wangweisz@med.uestc.edu.cn

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 28 January 2022
Accepted: 09 June 2022
Published: 29 June 2022

Citation:
Du Y, Chen S, Wang F, Zhang P, Liu M, Liu C, Zhong X, Qin J, Li G and Wang W (2022) The Significance of Crescents on the Clinical Features and Outcomes of Primary Immunoglobin A Nephropathy. Front. Med. 9:864667. doi: 10.3389/fmed.2022.864667

Background: It is still controversial whether the proportion of crescents below 50% can be an independent predictive risk factor for poor prognosis in IgAN patients. We reported the significance of different proportions of crescents on the clinical features and the cut-off value of crescents in predicting the occurrence of end-stage kidney disease (ESKD) in patients with IgAN.

Methods: We retrospectively analyzed biopsy-proven primary IgAN patients in Sichuan Provincial People's Hospital from 2007 to 2019. The patients were divided into 5 groups on the basis of crescent proportion as follows: 0 (n = 647), < 10% (n = 221), 10 to 24% (n = 272), 25 to 49% (n = 80), and ≥50% (n = 22). The primary endpoint was defined as ESKD, and the secondary endpoint was the combined renal endpoint (≥50% reduction in eGFR or ESKD). A validation cohort of 346 patients were enrolled from Affiliated Hospital of Southwest Medical University. Cox regression model and Kaplan-Meier survival analysis were performed.

Results: A total of 1242 eligible patients with biopsy-proven IgAN were recorded in the database, compared with the non-crescent group, patients in the crescent group had lower levels of hemoglobin (Hb) and albumin (Alb), higher levels of blood urea nitrogen (BUN), 24h urinary protein and hematuria, a higher proportion of mesangial hypercellularity (M1), endocapillary hypercellularity (E1), segmental glomerulosclerosis (S1), and tubular atrophy/interstitial fibrosis (T1/T2) (p < 0.05). A higher crescent proportion was associated with lower levels of Hb, ALB, eGFR and serum IgG (p < 0.05), higher levels of SCr, BUN, increasing amounts of 24 h urinary protein, increasing proportion of M1 and E1, and increasing severity of interstitial inflammatory infiltration. During the median follow-up of 43 months (range 6-151), 63 individuals (7.0%) reached the primary outcome of ESKD and 99 patients (11.1%) reached the combined renal endpoint. 34(7.5%), 21 (13.3%), 24(12.2%), 14(21.5%) and 6(31.6%) patients reached the combined renal endpoint in the above five groups in crescents 0, <10%, 10~24%, 25~49% and ≥50%, respectively. A total of

274(62.6%) cases in the crescent group and 254 (55.7%) cases in the non-crescent group received immunosuppressive therapy. Multivariate Cox regression showed that crescents ≥50% was an independent risk factor for the progression of ESKD (p = 0.003) and crescents ≥25% was an independent risk factor for the combined renal endpoint(p < 0.001). The receiver operating characteristic curve showed that IgAN patients with crescents ≥43.7% had a higher risk of ESKD, even with immunosuppressants (Sensitivity = 75.7%,specificity = 89.6%,p < 0.001). This discovery cohort and the validation cohort further confirmed that patients with crescents <43.7% had better renal prognosis than those with crescents ≥43.7% in the whole group and those with immunosuppressants (p < 0.001).

Conclusion: IgAN patients with crescents had more severe clinicopathological features and poorer prognosis. Crescents ≥50% was an independent risk factor for the progression of ESKD and crescents ≥25% was an independent risk factor for ≥50% reduction in eGFR or ESKD in treated and untreated IgAN patients. Crescents ≥43.7% was an independent risk factor for ESKD in those with immunosuppressants.

Keywords: crescent, IgA nephropathy, clinical features, prognosis, ESKD

BACKGROUND

Immunoglobin A nephropathy (IgAN) is the most common primary glomerulonephritis in the world. There is considerable heterogeneity in clinicopathological features, rate of disease progression, and prognosis of different IgAN patients. The crescent formation is a common histopathological finding, occurring in approximately 20-60% of IgAN patients. In the original Oxford classification and validation studies for IgAN, the presence or absence of cellular/fibrocellular crescents was not included and crescents also were not considered as a significant predictive and prognostic factor (1). But there were some limitations to these studies, in which individuals with eGFR <30 ml/min/1.73 m^2 or the rapid progression to end-stage kidney disease (ESKD) were excluded. However, other studies with less restrictive inclusion criteria found a linear relationship between the proportion of crescents and the proportion of IgAN patients with composite end-point events and crescents crescentic were independently related to renal survival. Based on these findings, crescents have been added to Oxford classification, updating to the MEST-C scores (2). According to the revised Oxford Classification, the crescent-score was defined as C0 (no crescents), C1 (crescents in at least 1 but < 25% of glomeruli), or C2 (crescents in at least 25% of glomeruli). There were 61% of patients with crescents <10%, which a multicenter study reported among IgAN patients with crescents (3). Similarly, another study found pathological findings in IgAN patients with the initial eGFR <30 ml/min/1.73 m^2 had a median crescent of 10% (4). At present, it is widely recognized that crescentic IgAN (crescents involving more than 50% of glomeruli) is a risk factor for the prognosis of patients with IgAN and patients should receive intensive treatment, such as steroids or other immunosuppressive according to the origin Kidney Disease: Improving Global Outcomes (KDIGO) clinical guideline released in 2012 (5). Nevertheless, the prognosis and treatment of patients with crescents <50% is still uncertain (4, 6–14). The above contradictory conclusions suggest that the differences in race, research objects and the inclusion criteria may affect the predictive value of crescents in IgAN. More importantly, the cut-off value of crescents in predicting the occurrence of ESKD in patients with IgAN is also currently inconclusive.

Therefore, our study assessed the value of different proportions of crescents by comparing the clinicopathological features and prognosis of IgAN patients. Furthermore, we also explored the impact of immunosuppressive therapy on the prognosis of IgAN patients with crescents.

MATERIALS AND METHODS

Study Design

Patients

This is a single-center retrospective cohort study. Our research protocol was approved by the ethics committee of Sichuan Provincial People's Hospital. There were 1242 biopsy-proven primary IgAN patients in Sichuan Provincial People's Hospital from 2007 to 2019. The inclusion criteria were biopsy-proven primary IgAN and age ≥14 years old. Individuals with the number of glomeruli in renal biopsy less than 8, repeating renal biopsy, secondary IgAN caused by Henoch–Schönlein purpura, Sjogren's syndrome or systemic lupus erythematosus and so on, loss of complete medical records, less than 6 months of follow-up were excluded. The end-point follow-up time was October 1, 2020. Subjects were divided into the crescent group and the non-crescent group based on the presence or absence of crescents. The crescent group was further divided into 4 subgroups based on the proportion of crescents involving glomeruli: <10%, 10∼24%, 25∼49% and ≥50%. To determine the risk factors for ESRD and test the prediction model of IgAN, we further included a validation cohort of 346 patients from Affiliated Hospital of

Southwest Medical University (validation cohort; $n = 346$) during 2010 to 2019. The inclusion and exclusion criteria is consistent with our research.

Clinical and Laboratory Data at Biopsy

We collected data on individuals' clinicopathological characteristics at the time of renal biopsy and during follow-up, including blood pressure (BP), serum creatinine (Scr), albumin (Alb), estimated glomerular filtration rate (eGFR), 24 h urine protein, urine red blood cell counts, the medication regimen and so on.

Renal Pathological Data at Biopsy

Renal biopsy samples from all patients were reviewed and scored by three experienced renal pathologists. The updated Oxford Classification (MEST-C) was used in this study (mesangial hypercellularity (M0/M1, <or equal to >50% of glomeruli with >4 mesangial cells/area); endocapillary hypercellularity (E0/E1, present/absent); segmental glomerulosclerosis (S0/S1, present/absent); tubular atrophy/interstitial fibrosis (T0/T1/T2, <25%, 25–50%, >50%) (1). A crescent was defined as extra-capillary lesions involving 25% of the glomerular circumference. The proportion of crescents (regardless of the component) was calculated according to the number of crescent affected glomeruli divided by the total number of glomeruli; cellular/fibrocellular/fibrous crescents were considered a component ratio.

Treatment and Renal Prognosis

During follow-up, subjects were divided into subgroups based on whether receiving immunosuppressive therapy: the immunosuppressive treatment group and the non-immunosuppressive treatment group. The primary outcome was ESKD, and the combined event was defined as either ESKD or ≥50% decline in eGFR.

Definitions

Estimated glomerular filtration rate was calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). Mean arterial pressure was calculated as 1/3 systolic blood pressure + 2/3 diastolic blood pressure. Immunosuppressive therapy was defined as the use of any immunosuppressive agent, including glucocorticoid (GC), cyclophosphamide (CTX), mycophenolate mofetil (MMF), leflunomide (LEF), cyclosporin A (CsA), tacrolimus (FK506) or hydroxychloroquine (HCQ) and so on, regardless of drug dose and duration (15). Renin-angiotensin-aldosterone system inhibitor (RASi) was defined as any angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB). ESKD was defined as eGFR <15 ml/min/1.73m2 or the initiation of renal replacement therapy or renal transplantation.

Statistical Analyses

SPSS 18.0 software was used for data analysis. Continuous variables that followed a normal distribution were expressed as mean ± standard deviation and compared using the *t-test*. Non-parametric variables were expressed as medians (interquartile ranges) and compared using the Mann–Whitney *U* test. Categorical variables were expressed as frequency (percentage) and compared using the chi-square test.

The cumulative survival rate was calculated by the Kaplan-Meier method. Univariate and multivariate Cox regression models were performed to analyze the prognosis of patients with different proportions of crescents by *p*-value [hazard ratio (HR) and 95% confidence interval (95% CI)]. The clinical and pathological factors with a *p*-value <0.1 on univariate analysis were included in the multivariable model. A *p*-value of less than 0.05 was regarded as statistically significant. Receiver operating characteristic (ROC) was used to analyze the diagnostic efficacy of crescents to predict the prognosis of IgAN patients treated with immunosuppressive therapy. We used the area under the curve (AUC) to analyze the relationship between the proportion of crescents and the prognosis of IgAN patients treated with immunosuppressive and calculated the cut-off value of crescents in predicting the occurrence of ESKD in patients with IgAN. Cox regression model and Kaplan-Meier survival analysis were performed in the validation cohort.

RESULTS

Demographic Characteristics

A total of 1242 eligible patients with biopsy-proven IgA nephropathy from 2007 to 2019 in Sichuan Provincial People's Hospital were recorded in the database, of which 595 individuals (47.9%) had different proportions of crescents. The median crescent proportion was 12.5%. The percentage of patients with 0, <10%, 10∼24%, 24∼49% and ≥50% was 52.1% (647 cases), 17.8% (221 cases), 21.9% (272 cases), 6.4% (80 cases) and 1.8% (22 cases), respectively (**Supplementary Figure 1**). The flow chart of this study was shown in **Figure 1**.

Comparisons of Baseline Clinical and Laboratory Variables

Compared with the non-crescent group, the crescent group had lower levels of hemoglobin (Hb) ($p < 0.001$) and albumin (Alb) ($p < 0.001$), higher levels of blood urea nitrogen (BUN) ($p = 0.021$), 24 h urinary protein ($p < 0.001$) and hematuria ($p < 0.001$), a higher ratio of gross hematuria ($p = 0.03$). There was no significant difference in SCr between the two groups, but it showed an upward trend in patients in the crescent group [85.70 (61.91-21.40) μmol/L vs. 80.80 (59.95-115.63) μmol/L, $p = 0.098$] (**Supplementary Table 1**).

Pathological characteristics of IgAN patients with and without crescents were detailed in **Supplementary Table 2**. The immunofluorescence findings showed that C3 deposition was more significant in renal tissue of patients with crescents than that without crescents; however, there was no significant difference in IgA, IgG, and IgM deposition between the two groups. The pathological manifestations of patients in the crescent group were severer, including the increase of the proportion of M1, E1, S1, T1 and T2, and the aggravation of interstitial inflammatory infiltration ($p < 0.05$).

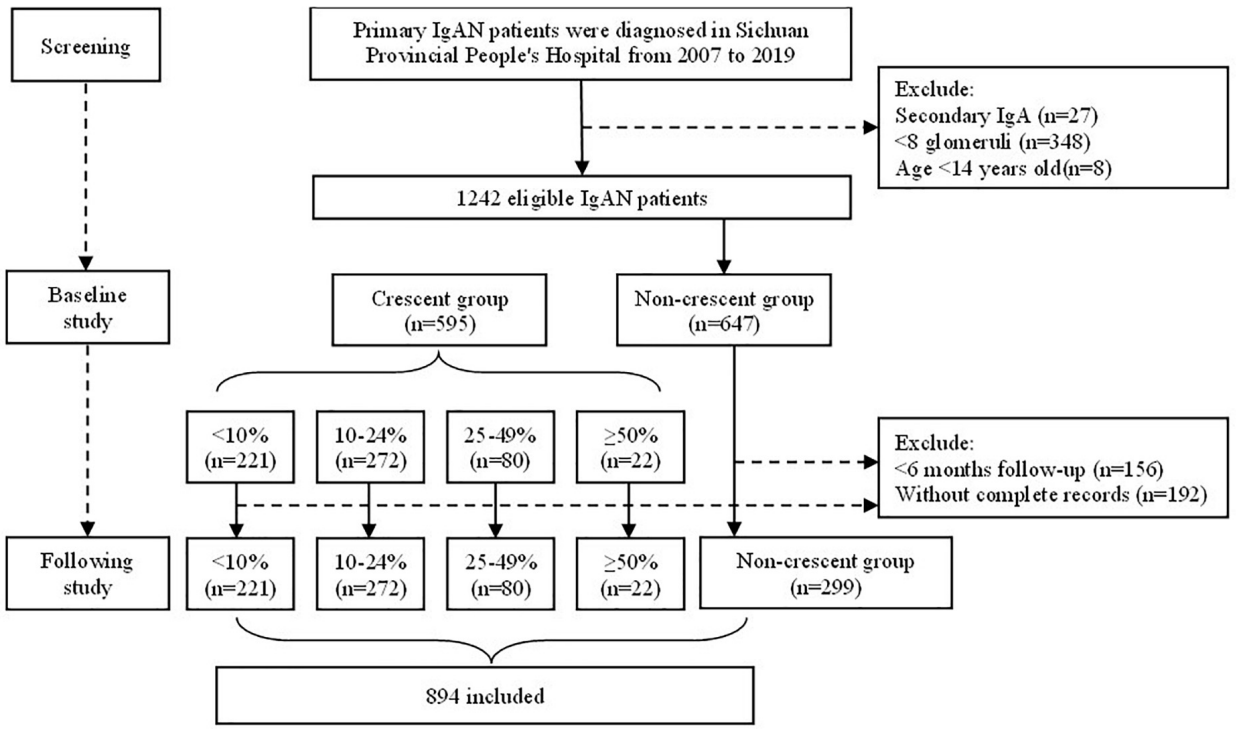


FIGURE 1 | The flow chart of the enrolment of IgAN patients.

We further compared baseline characteristics of four subgroups of IgAN with crescents, as shown in **Table 1**. A higher crescent proportion was associated with lower levels of Hb, ALB, eGFR and serum IgG ($p < 0.05$), higher levels of SCr, BUN and increasing amounts of 24 h urinary protein. With the increasing ratio of crescents, patients were more prone to acute kidney injury (AKI), especially in the group with crescents ≥50, which was significantly higher than that in other groups.

There was no significant difference in the expression of IgA, IgG, IgM, and C3 in renal tissue between different proportions of crescent subgroups (**Table 2**). Light microscopy findings showed that a higher crescent proportion was associated with increasing proportion of M1, E1, and increasing severity of interstitial inflammatory infiltration.

Immunosuppressive and/or Renin-Angiotensin-Aldosterone System Inhibitor Therapy

A total of 894 cases were included in the following study, among which, there were 254 of 456 cases (55.7%) treated with immunosuppressive therapy in the non-crescent group and 274 of 438 cases (62.6%) received immunosuppressive therapy in the crescent group. With the increasing ratio of crescents, the proportion of patients who received immunosuppressive therapy also increased ($p < 0.001$). In this cohort, we found no significant difference in the proportion of individuals treated with RASi among the groups (**Table 3**).

Prognosis

After an average follow-up of 43 months (range 6-151 months), 99 patients (11.1%) reached the combined renal endpoint, of which 63 patients (7.0%) experienced ESKD, 36 patients (4.0%) experienced a ≥50% reduction in eGFR. 21 (13.3%) cases with crescents <10%, 24 (12.2%) cases with crescents 10∼24%, 14 (21.5%) cases with crescents 25∼49% and 6 (31.6%) cases with crescents ≥50% reached combined end point, respectively. In the non-crescent group, 34 (7.5%) cases reached the combined renal endpoint.

In the discovery cohort, we used univariate Cox regression to analyze the factors affecting renal outcomes in IgAN patients (**Supplementary Table 3**). The results showed that age, SCr, UA, BUN, 24 h urinary protein, serum IgA, M1, S1, T1, T2, global glomerulosclerosis, crescents and interstitial inflammatory infiltration were related to the development of combined renal endpoint. The above factors were analyzed by multivariate analysis through COX proportional hazard regression model (**Table 4**). The results showed that MAP, SCr, 24 h urinary protein, serum IgA, T1, T2, crescents ≥50% and global glomerulosclerosis were independent risk factors for ESKD and composite renal outcomes in IgAN patients. We also compared combined events in treated versus untreated individuals using multivariate analysis through COX proportional hazard regression model (**Table 5**). The results showed that crescent ≥25% was an independent risk factor for combined renal outcomes in IgAN patients with immunosuppressive therapy and any proportion of crescents was an independent risk factor for poor prognosis in IgAN patients.

TABLE 1 | Baseline characteristics of IgAN patients with different proportions of crescents.

	<10% (*n* = 221)	10∼24% (*n* = 272)	25∼49% (*n* = 80)	≥ 50% (*n* = 22)	*P-value*
Gender, Male/Female[c]	95/126	118/154	34/46	12/10	0.770
Age, Year[a]	33.03 ± 11.73	34.97 ± 12.58	36.59 ± 14.83	33.86 ± 14.48	0.138
MAP, mmHg[a]	94.93 ± 11.91	94.88 ± 12.64	95.55 ± 12.18	98.86 ± 14.18	0.519
Hb, g/L[a]	132.20 ± 17.91	127.37 ± 19.44	124.55 ± 23.43	119.32 ± 19.65	0.001*
Cr, μmol/L	76.60 (60.30-104.75)	84.50 (61.00-122.98)	101.75 (73.30-134.80)	154.50 (91.03-261.00)	<0.001**
eGFR, ml/min/1.73m^2[b]	101.41 (72.75-131.16)	88.98 (58.98-124.05)	68.32 (42.51-107.98)	41.53 (23.34-91.28)	<0.001**
UA, μmol/L[a]	369.25 ± 111.25	381.57 ± 113.96	376.09 ± 106.40	376.11 ± 104.51	0.689
BUN, mmol/L[a]	5.71 ± 2.24	6.57 ± 2.99	7.36 ± 3.77	9.05 ± 5.68	<0.001**
Alb, g/L[b]	40.75 (36.53-43.90)	38.60 (34.05-42.75)	34.40 (27.78-40.15)	26.85 (24.38-33.88)	<0.001**
Gross hematuria, n (%)[c]	75(33.9)	79(29.0)	29(36.3)	8(36.4)	0.432
Urinary RBC, n (%)[c]					<0.001**
1 +	36(16.3)	41(15.1)	9(11.3)	1(4.5)	
2 +	63(28.5)	76(27.9)	15(18.8)	5(22.7)	
3 +	91(41.2)	147(54.0)	53(66.3)	11(50.0)	
24h urinary protein, g/24h[b]	1.28 (0.35-2.04)	1.59 (1.00-3.10)	2.55 (1.60-4.16)	3.59 (0.64-2.32)	<0.001**
Serum C3, g/L[a]	1.08 ± 0.25	1.11 ± 0.37	1.05 ± 0.26	1.14 ± 0.44	0.41
Serum C4, g/L[a]	0.27 ± 0.09	0.27 ± 0.09	0.28 ± 0.11	0.28 ± 0.10	0.795
Serum IgG, g/L[a]	10.47 ± 3.59	9.79 ± 3.55	9.41 ± 5.41	6.14 ± 2.55	<0.001**
Serum IgA, g/L[a]	3.03 ± 1.15	3.02 ± 1.22	2.95 ± 1.41	2.35 ± 1.02	0.166
AKI, n (%)[c]	6(2.7)	4(1.5)	6(7.5)	6(27.3)	<0.001**
CKD, n (%)[c]					<0.001**
CKD1	135(61.1)	142(52.2)	28(35.0)	5(22.7)	
CKD2	45(20.4)	54(19.9)	22(27.5)	3(13.6)	
CKD3	20(9.0)	47(17.3)	9(11.3)	4(18.2)	
CKD4	7(3.2)	17(6.3)	10(12.5)	1(4.5)	
CKD5	8(3.6)	8(2.9)	5(6.3)	3(13.6)	

*MAP, mean arterial pressure; Hb, hemoglobin; Cr, creatinine; eGFR, estimated glomerular filtration rate; UA, uric acid; BUN, blood urea nitrogen; Alb, albumin; AKI, acute kidney injury; CKD, chronic kidney disease. [a]Normal distribution was expressed as mean ± standard deviation and compared using the t-test. [b]Non-parametric variables were expressed as medians (interquartile ranges) and compared using the Mann–Whitney U test. [c]Categorical variables were expressed as frequency (percentage) and compared using the chi-square test *$p < 0.05$; **$p < 0.001$.*

Kaplan Meier survival curve was used to analyze the effect of immunosuppressive therapy on the renal survival rate of IgAN patients with or without crescents (**Figure 2**). The results showed that, for patients treated with immunosuppressive therapy, the 5-year renal survival rate was 86.2% in the crescent group. For patients without immunosuppressive therapy, there were 84.5 and 92.2% individuals in the crescent group and non-crescent group, respectively. There was no significant difference in renal survival among the three groups. However, for patients without immunosuppressive therapy, the 100-month renal survival rate in the non-crescent group was higher than that in the crescent group [59.2 *vs.* 75.8%, $p < 0.05$].

We drew a ROC curve to analyze the diagnostic efficacy of crescents to predict the prognosis of IgAN patients treated with immunosuppressants (**Figure 3**). AUC under ROC curve was 0.899 (95% CI = 0.845-0.959, $p < 0.001$). The cut-off value of the proportion of crescents in predicting the occurrence of ESKD in IgAN patients calculated by the Youden Index was 43.7% (Sensitivity = 75.7%,specificity = 89.6%, $p < 0.001$). The prognosis of IgAN patients with crescent ≥43.7% was still poor, even after receiving immunosuppressive therapy.

We further compared the risk of ESKD associated with crescents ≥43.7% in our whole cohort and within subgroups treated with immunosuppressants in **Figure 4**. Patients with crescents <43.7% had better renal prognosis than those with crescents ≥43.7% ($p < 0.001$), supporting the predictive value of 43.7% in IgA patients with or without immunosuppressive therapy.

Validation of Prediction Model

To determine the risk factors and test the prediction model of crescentic IgAN, we further confirmed the above results in a validation cohort of 346 patients from Affiliated Hospital of Southwest Medical University. Comparison of baseline characteristics of IgAN patients in this discovery cohort and validation cohort was shown in **Supplementary Table 4**. On multivariate cox regression analyses in the validation cohort, glomerular sclerosis ($p < 0.001$), crescents ($p < 0.001$), and

TABLE 2 | Pathological characteristics of IgAN patients with different proportions of crescents.

	<10% (n = 221)	10∼24% (n = 272)	25∼49% (n = 80)	≥50% (n = 22)	*P-value*
IgA, n (%)[c]					0.636
1∼2 +	53(24.0)	66(24.3)	26(32.5)	7(31.8)	
3 +	158(71.5)	196(72.1)	50(62.5)	14(63.6)	
4 +	10(4.5)	10(3.7)	4(5.0)	1(4.5)	
IgG, n (%)[c]					0.599
Negative	172(77.8)	224(82.4)	65(81.3)	16(72.7)	
1 +	41(18.6)	39(14.3)	13(16.3)	4(18.2)	
2∼3 +	8(3.6)	9(3.3)	2(2.5)	2(9.1)	
IgM, n (%)[c]					0.785
Negative	53(24.0)	59(21.7)	17(21.3)	6(27.3)	
1 +	125(56.6)	157(57.7)	41(51.3)	11(50.0)	
2∼3 +	43(19.5)	56(20.6)	22(27.5)	5(22.7)	
C3, n (%)[c]					0.888
Negative ∼1 +	49(22.2)	56(20.6)	20(25.0)	4(18.2)	
2 +	114(51.6)	138(50.7)	36(45.0)	10(45.5)	
3∼4 +	58(26.2)	78(28.7)	24(30.0)	8(36.4)	
M1, n (%)[c]	85(38.5)	134(49.3)	56(70.0)	16(72.7)	<0.001**
E1, n (%)[c]	50(22.6)	96(35.3)	35(43.8)	9(40.9)	0.001*
S1, n (%)[c]	123(55.7)	151(55.5)	42(52.5)	8(36.4)	0.358
T1, n (%)[c]	20(9.0)	24(8.8)	8(10.0)	5(22.7)	0.225
T2, n (%)[c]	6(2.7)	17(6.3)	5(6.3)	2(9.1)	0.137
Global glomerulosclerosis,%[b]	0(0-11.11)	0(0-13.13)	0(0-13.84)	0(0-8.04)	0.849
Interstitial inflammation, *n* (%)[c]					0.004*
Negative	59(26.7)	53(19.5)	24(30.0)	4(18.2)	
Mild	141(63.8)	190(69.9)	50(62.5)	10(45.5)	
Moderate	14(6.3)	12(4.4)	3(3.8)	4(18.2)	
Severe	7(3.2)	17(6.3)	3(3.8)	4(18.2)	
Cellular crescent, *n* (%)[c]	0(0-50.0)	0(0-50.0)	25.0(0-97.5)	26.8(0-84.7)	0.004*
Fibrous crescent,%[b]	0(0-100.0)	25.0(0-66.7)	25.0(0-50.0)	5.0(0-52.1)	0.604
Fibro-cellular crescent,%[b]	0(0-100.0)	25.0(0-66.7)	25.0(0-50.0)	21.1(0-53.6)	0.503

*The results of IgA, IgG, IgM, and C3 were manifestations of immunofluorescence. Cellular/fibrous/fibrocellular crescents were calculated according to the relative ratio. [b]Non-parametric variables were expressed as medians (interquartile ranges) and compared using the Mann–Whitney U test. [c]Categorical variables were expressed as frequency (percentage) and compared using the chi-square test *p < 0.05; **p < 0.001.*

TABLE 3 | During follow-up patients with or without immunosuppression and RASi.

	Crescent group (*n* = 438)				Non-crescent group (*n* = 456)	*P-value*
	<10% (*n* = 158)	10∼24% (*n* = 196)	25∼49% (*n* = 65)	≥ 50% (*n* = 19)		
Receiving Immunosuppression, n (%)[c]	80 (50.6)	122 (62.2)	53 (81.5)	19 (100)	254 (55.7)	<0.001**
Receiving RASi, n (%)[c]	137 (86.7)	166 (84.7)	56 (86.2)	13 (68.4)	391(85.7)	0.354

*RASi, renin-angiotensin-aldosterone system inhibitor. [c]Categorical variables were expressed as frequency (percentage) and compared using the chi-square test. **p < 0.001.*

serum Creatinine ($p < 0.001$) were risk factors for the development of ESRD. As shown in **Figure 5**, after adjusted for proteinuria, eGFR, blood pressure, Oxford-MEST score, patients with crescents <43.7% had better renal prognosis than those with crescents ≥43.7% in the whole group and those with immunosuppressants($p < 0.001$).

DISCUSSION

Although the origin Oxford classification for IgAN has been extended to the MEST-C scores, the impact of the crescents on the poor outcomes in IgAN patients is still uncertain, especially for the proportion of crescent below 50%, and

TABLE 4 | Multivariate analysis of clinical and pathological factors for the combined events.

Parameters	ESKD			Combined events		
	HR	95%CI	*p* Value	HR	95%CI	*P-value*
MAP	1.019	1.002-1.036	0.026*	1.020	1.003-1.037	0.021*
Cr, μmol/L	1.002	1.001-1.004	0.001*	1.001	1.000-1.003	0.045*
24 h urinary protein, g/24h	1.305	1.208-1.410	<0.001**	1.253	1.172-1.341	<0.001**
Serum IgA, g/L	1.424	1.067-1.901	0.016*	1.261	1.023-1.555	0.03*
M1(M0/M1)	1.315	0.620-2.791	0.475	0.775	0.470-1.277	0.317
E1(E0/E1)	1.303	0.638-2.662	0.467	1.255	0.763-2.061	0.371
S1(S0/S1)	0.723	0.321-1.629	0.434	0.945	0.562-1.589	0.832
T1(T0/T1/T2)	7.005	3.012-16.291	<0.001**	7.747	4.314-13.913	<0.001**
Proportions of crescents						
None		Reference			Reference	
<10%	2.567	0.956-6.890	0.061	1.695	0.952-3.018	0.073
10∼24%	1.999	0.792-5.046	0.143	1.623	0.919-2.866	0.095
25∼49%	1.675	0.474-5.913	0.423	4.109	2.046-8.254	<0.001**
≥50%	8.652	2.068-36.194	0.003*	5.259	1.971-14.025	0.001*
Global glomerulosclerosis,%	1.019	1.000-1.039	0.047*	1.016	1.001-1.032	0.039*
Immunosuppressive therapy						
No Immunosuppression		Reference			Reference	
Any Immunosuppression	0.378	0.189-0.753	0.006*	0.195	0.110-0.346	<0.001**

*The clinical and pathological factors with a p-value <0.1 on univariate analysis were included in the multivariable model. MAP, mean arterial pressure; Cr, creatinine *p < 0.05; **p < 0.001.*

TABLE 5 | Analysis of immunosuppressive therapy in subgroups of the crescents group.

Parameters	Untreated with immunosuppression			treated with immunosuppression		
	HR	95%CI	*p* Value	HR	95%CI	*P-value*
Proportions of crescents						
None		Reference			Reference	
<10%	3.978	1.437-11.011	0.008*	1.653	0.525-5.202	0.39
10∼24%	5.090	1.639-15.809	0.005*	1.633	0.568-4.694	0.362
25∼49%	6.746	1.524-29.873	0.012*	3.944	1.342-11.592	0.013*
≥50%	—	—	—	4.332	1.113-16.867	0.035*

*The clinical and pathological factors with a p-value <0.1 on univariate analysis were included in the multivariable model *p < 0.05.*

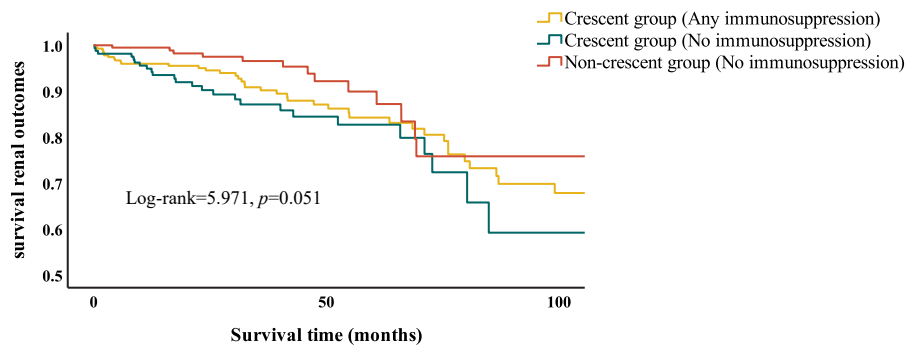


FIGURE 2 | Kaplan Meier survival curve of IgAN patients with or without crescents.

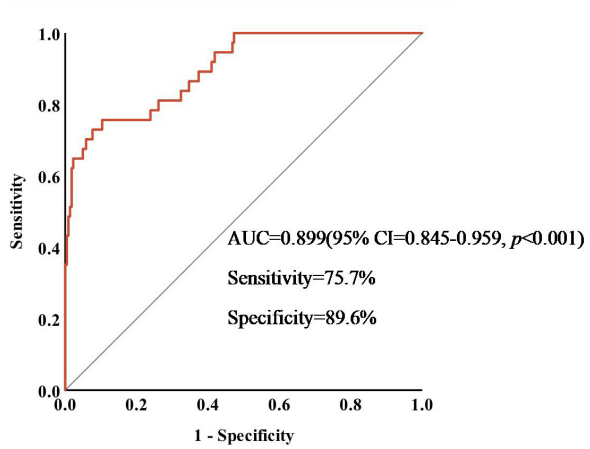


FIGURE 3 | Sensitivity and specificity of crescents in predicting the occurrence of ESKD in IgAN patients.

the threshold of crescent proportion for predicting poor prognosis of IgAN is also controversial. The crescent score wasn't included in the International IgAN Prediction Tool recommended for quantifying the risk of IgAN progression. Furthermore, a multicentre study from China found that the presence of crescents was not a predictor of poor prognosis in IgAN patients (9), which excluded individuals with eGFR<30 ml/min/1.73m^2 and 24 h urinary protein <0.5 g/24 h. Therefore, our study aimed to evaluate the value of different proportions of crescents (including crescent below 50%) by comparing the clinicopathological features and prognosis of IgAN patients including those with rapid progression renal function.

The glomerular crescent is a histomorphological indicator of a rupture of glomerular capillaries, which is related to various clinical manifestations, such as blood pressure, SCr, 24-h urinary protein, anemia and so on. Our study confirmed that the baseline clinical and pathological indicators were more severe with the increase of the proportion of crescent, which was similar to the results of other cohort studies (15–17). Crescents formation leads to fading of eGFR by increasing counter pressure and collapse of the glomerular tuft or by obstructing the tubular outflow (18). A multicenter study showed that the blood pressure of IgAN patients in group C2 was higher than that of C0 and C1 (15). However, there was no significant difference in MAP between the -crescent and the none-crescent group, in our study. The possible reason for this result is that we did not exclude patients who had received RASi before enrolment.

Our current report represents the largest cohort to date to investigate the effect of different proportions of crescents on the clinical outcomes of IgAN patients. In our study, after adjusting clinical and pathological factors, we showed crescents ≥50% was associated with the progression of IgAN patients to ESKD, and crescents ≥25% was an independent risk factor for combined renal outcomes, consistent with previous reports. Moreover, we

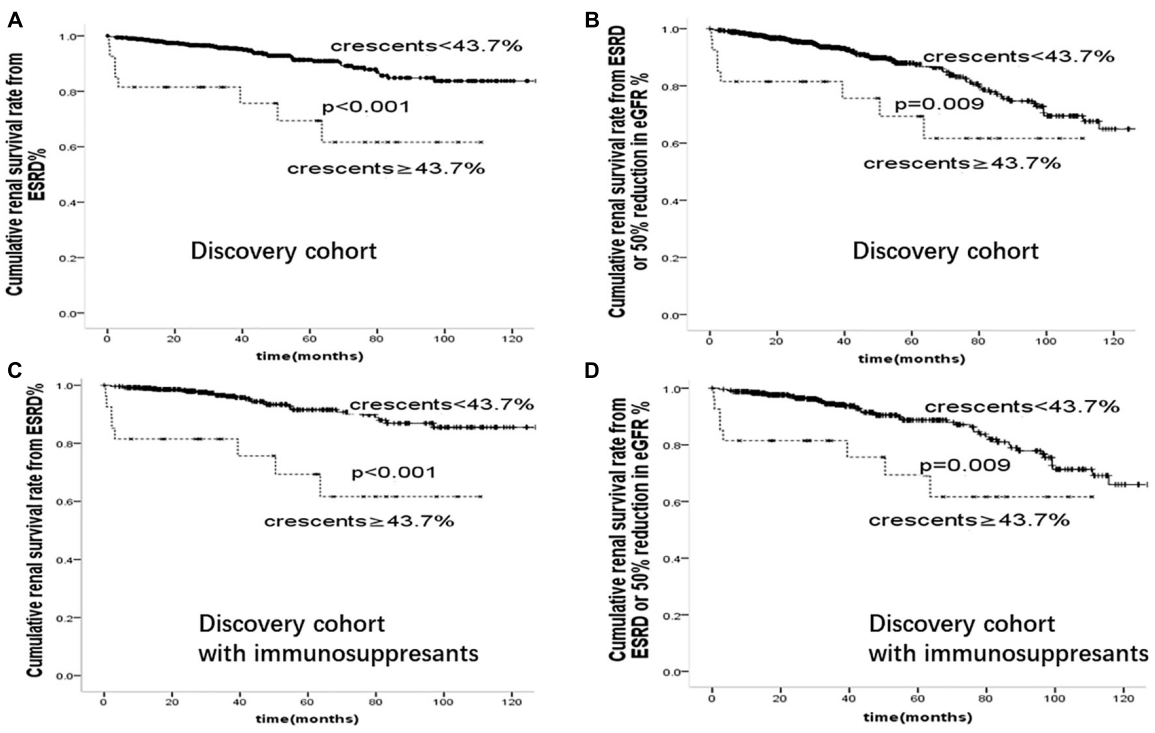


FIGURE 4 | Cumulative renal survival rate from **(A)**: ESRD **(B)**: ESRD or 50% reduction in eGFR in 894 cases of IgAN patients. Cumulative renal survival rate from **(C)**: ESRD **(D)**: ESRD or 50% reduction in eGFR in 529 cases of IgAN patients treated with immunosuppressants.

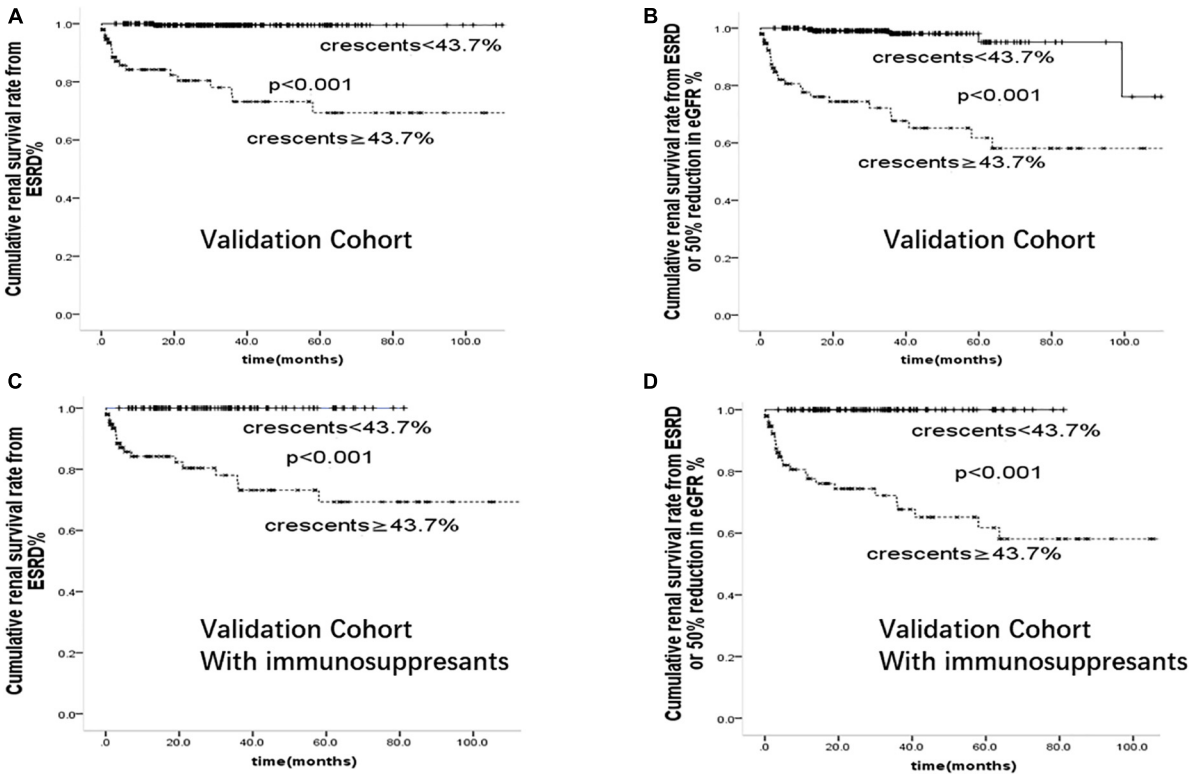


FIGURE 5 | Cumulative renal survival rate from **(A)**: ESRD **(B)**: ESRD or 50% reduction in eGFR in 346 cases of IgAN patients from validation Cohort. Cumulative renal survival rate from **(C)**: ESRD **(D)**: ESRD or 50% reduction in eGFR in 206 cases of IgAN patients treated with immunosuppressants from validation Cohort.

found that among patients without immunosuppressive therapy, there was no significant difference in baseline SCr between patients with crescents and without crescents, but the former's long-term renal survival rate was significantly lower. The results of another retrospective analysis also supported our conclusions (13). Similarly, this study showed that, after adjusting for age, gender, 24 h urinary protein and baseline SCr, the presence of crescents was independently associated with composite point events (doubling of baseline SCr or ESKD or death). Research at home and abroad shows that the value of crescents in predicting renal function progression is inconsistent in IgAN patients. On the one hand, it is considered that race, geographical distribution, inclusion criteria may affect the predictive value of crescents on the progression of IgAN. On the other hand, calculating the percentage of crescents is not a precise method, because it is affected by the size of the biopsy sample and the number of histologic sections examined (13). In addition, our baseline data analysis showed that the patients with crescents <10% were 37.1%. In fact, it's easy to ignore the crescent lesion in these patients, especially for those without severe renal function injury. Therefore, clinicians should pay more attention to the crescent lesion, even if the proportion of crescents is less than 10%, and early treatment can effectively delay the progression and improve the prognosis of IgAN.

According to the origin KDIGO clinical guideline released in 2012 (5), IgAN patients with crescentic IgAN were recommended to receive more intensive treatment, such as GC or other immunosuppressive. However, for IgAN patients with crescents < 50%, the specific treatment of these patients was still unclear. Additionally, with reference to the 2021 edition "KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases," it pointed out that there was insufficient evidence to make treatment decisions based on the presence and number of crescents in renal biopsy (19). Our study showed that IgAN patients with crescents received more intensive treatment. In these patients, crescents <25% was not an independent risk factor for poor prognosis. However, for IgAN patients without immunosuppressive therapy, any proportion of crescents was a predictor of composite renal outcome. Therefore, immunosuppressive therapy may delay the progression and improve the prognosis of IgAN, which was similar to the conclusion of Haas (3).

This discovery cohort and the validation cohort both confirmed that patients with crescents <43.7% had better renal prognosis than those with crescents 43.7% in the whole group and those with immunosuppressants ($p < 0.001$). Based on the above conclusions, we speculated that immunosuppressive therapy may delay the occurrence of composite renal outcomes, and patients with crescents ≥43.7% may still have a poor prognosis, even with intensive immunosuppressive therapy. Therefore, it's important to perform a renal biopsy as early as possible in patients with suspected IgA nephropathy.

There were some limitations to this study. Firstly, as a retrospective study, we didn't describe the kinds of immunosuppressive, drug doses and duration of treatment in detail. Secondly, this was a single-center retrospective observational analysis, it was difficult to control for all factors that may affect renal survivorship. We hope to perform multicenter prospective research with a large sample size to further verify this conclusion.

CONCLUSION

Immunoglobin A nephropathy patients with crescents had more severe clinicopathological features and poorer prognosis. Crescents ≥50% was an independent risk factor for the progression of ESKD and crescents ≥25% was an independent risk factor for ≥50% reduction in eGFR or ESKD in treated and untreated IgAN patients. Crescents ≥43.7% was an independent risk factor for ESKD in those with immunosuppressants.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital. Written informed consent from the participants/patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YD, SC, WW, PZ, ML, XZ, JQ, and WW: data collection. GL and WW: study design. YD, WW, FW, and ML: statistical analyses. YD, WW, SC, and CL: writing. All authors have read and approved the manuscript.

FUNDING

This work was supported by a grant from the University of Electronic Science and Technology of China Central University Research Fund (ZYGX2019J104), a grant from the Science and Technology Project of Sichuan Province (2020YJ0447), a grant from the Key R & D plan of Sichuan Province (2019YFS0538).

ACKNOWLEDGMENTS

We are grateful to all the subjects who participated in this work. This study was reviewed and approved by the effect of crescents on the clinical features and outcomes of primary IgAN.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.864667/full#supplementary-material

REFERENCES

1. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, et al. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int.* (2009) 76:534–45. doi: 10.1038/ki.2009.243
2. Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, et al. Oxford classification of IgA nephropathy 2016: an update from the IgA nephropathy classification working group. *Kidney Int.* (2017) 91:1014–21. doi: 10.1016/j.kint.2017.02.003
3. Haas M, Verhave JC, Liu ZH, Alpers CE, Barratt J, Becker JU, et al. A multicenter study of the predictive value of crescents in IgA nephropathy. *J Am Soc Nephrol.* (2017) 28:691–701. doi: 10.1681/ASN.2016040433
4. Katafuchi R, Ninomiya T, Nagata M, Mitsuiki K, Hirakata H. Validation study of oxford classification of IgA nephropathy: the significance of extracapillary proliferation. *Clin J Am Soc Nephrol.* (2011) 6:2806–13. doi: 10.2215/CJN.02890311
5. Radhakrishnan J, Cattran DC. The KDIGO practice guideline on glomerulonephritis: reading between the (guide)lines–application to the individual patient. *Kidney Int.* (2012) 82:840–56. doi: 10.1038/ki.2012.280
6. Edstrom Halling S, Soderberg MP, Berg UB. Predictors of outcome in paediatric IgA nephropathy with regard to clinical and histopathological variables (Oxford classification). *Nephrol Dial Transplant.* (2012) 27:715–22. doi: 10.1093/ndt/gfr339
7. Shi SF, Wang SX, Jiang L, Lv JC, Liu LJ, Chen YQ, et al. Pathologic predictors of renal outcome and therapeutic efficacy in IgA nephropathy: validation of the oxford classification. *Clin J Am Soc Nephrol.* (2011) 6:2175–84. doi: 10.2215/CJN.11521210
8. Walsh M, Sar A, Lee D, Yilmaz S, Benediktsson H, Manns B, et al. Histopathologic features aid in predicting risk for progression of IgA nephropathy. *Clin J Am Soc Nephrol.* (2010) 5:425–30. doi: 10.2215/CJN.06530909
9. Zeng CH, Le W, Ni Z, Zhang M, Miao L, Luo P, et al. A multicenter application and evaluation of the oxford classification of IgA nephropathy in adult chinese patients. *Am J Kidney Dis.* (2012) 60:812–20. doi: 10.1053/j.ajkd.2012.06.011
10. Coppo R, Troyanov S, Bellur S, Cattran D, Cook HT, Feehally J, et al. Validation of the Oxford classification of IgA nephropathy in cohorts with different presentations and treatments. *Kidney Int.* (2014) 86:828–36. doi: 10.1038/ki.2014.63
11. Rafieian-Kopaei M, Baradaran A, Nasri H. Significance of extracapillary proliferation in IgA-nephropathy patients with regard to clinical and histopathological variables. *Hippokratia.* (2013) 17:258–61.
12. Lee MJ, Kim SJ, Oh HJ, Ko KI, Koo HM, Kim CH, et al. Clinical implication of crescentic lesions in immunoglobulin A nephropathy. *Nephrol Dial Transplant.* (2014) 29:356–64. doi: 10.1093/ndt/gft398
13. Lv J, Yang Y, Zhang H, Chen W, Pan X, Guo Z, et al. Prediction of outcomes in crescentic IgA nephropathy in a multicenter cohort study. *J Am Soc Nephrol.* (2013) 24:2118–25. doi: 10.1681/ASN.2012101017

14. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Hashimura Y, et al. Validity of the Oxford classification of IgA nephropathy in children. *Pediatr Nephrol.* (2012) 27:783–92. doi: 10.1007/s00467-011-2061-0
15. Park S, Baek CH, Park SK, Kang HG, Hyun HS, Park E, et al. Clinical significance of crescent formation in IgA nephropathy - a multicenter validation study. *Kidney Blood Press Res.* (2019) 44:22–32. doi: 10.1159/000497808
16. Zhang W, Zhou Q, Hong L, Chen W, Yang S, Yang Q, et al. Clinical outcomes of IgA nephropathy patients with different proportions of crescents. *Medicine.* (2017) 96:e6190. doi: 10.1097/MD.0000000000006190
17. Zhang X, Shi S, Ouyang Y, Yang M, Shi M, Pan X, et al. A validation study of crescents in predicting ESRD in patients with IgA nephropathy. *J Transl Med.* (2018) 16:115. doi: 10.1186/s12967-018-1488-5
18. Anguiano L, Kain R, Anders HJ. The glomerular crescent: triggers, evolution, resolution, and implications for therapy. *Curr Opin Nephrol Hypertens.* (2020) 29:302–9. doi: 10.1097/MNH.0000000000000596
19. Kidney Disease: Improving Global Outcomes Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int.* (2021) 100:S1–276. doi: 10.1016/j.kint.2021.05.021

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 06 July 2022
doi: 10.3389/fmed.2022.904299

Association of Exercise With Vascular Function in Patients With CKD: A Meta-Analysis of Randomized Controlled Trials

Huan Wang[1†], *Dengpiao Xie*[1*†], *Lisheng Wu*[2] *and Liangbin Zhao*[1*]

[1] *Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China,* [2] *Southern Medical University, Guangzhou, China*

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Dorin Dragoş,
Carol Davila University of Medicine and Pharmacy, Romania
Tuanwei Sun,
Shenzhen University, China
Gaizun Hu,
University of Virginia, United States

***Correspondence:**
Dengpiao Xie
2876522253@qq.com
Liangbin Zhao
zlb31928856@163.com

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *25 March 2022*
Accepted: *07 June 2022*
Published: *06 July 2022*

Citation:
Wang H, Xie D, Wu L and Zhao L (2022) Association of Exercise With Vascular Function in Patients With CKD: A Meta-Analysis of Randomized Controlled Trials. Front. Med. 9:904299. doi: 10.3389/fmed.2022.904299

Background and Aim: Vascular function is associated with an increased risk of cardiovascular events in patients with chronic kidney disease (CKD). Whether exercise improves vascular function in such patients remains controversial. This study aimed to conduct a meta-analysis on the effect of exercise training on the vascular function of patients with CKD.

Methods: Embase, the Cochrane Central Register of Controlled Trials, and Medline were searched from inception until November 15, 2021. The terms exercise, CKD, dialysis, kidney transplant, and randomized controlled trial (RCT) were searched alone or in combination. RCTs were included when studies compared exercise with active control, usual care, or no intervention, and the studies reported vascular function on patients with CKD.

Results: This meta-analysis included 18 RCTs with 817 patients. Exercise training was significantly associated with decreased pulse wave velocity weighted mean difference (WMD), −0.56; 95% confidence interval (CI), −1.02 to −0.09, $P = 0.02$ and augmentation index (WMD, −3.26; 95% CI, −5.46 to −1.05, $P = 0.004$). It was also significantly associated with improved peak VO2 (WMD, 2.64; 95% CI, 1.94–3.35, $P < 0.00001$), general health (WMD, 7.03; 95% CI, 0.65–13.42, $P = 0.03$), and vitality (WMD, 9.1; 95% CI, 2.50–15.69, $P = 0.007$).

Conclusions: The meta-analysis suggested that exercise training improved vascular function in patients with CKD. An exercise program should be considered as one of the management strategies for vascular dysfunction in patients with CKD. Further studies are needed to demonstrate that exercise training improves cardiovascular diseases in patients with CKD.

Keywords: chronic kidney disease, dialysis, exercise, vascular function, meta-analysis

INTRODUCTION

The increasing number of patients with chronic kidney disease (CKD) poses a challenge to health care. More than 15% of American adults or 37 million people were estimated to have CKD in 2021 based on data from the Centers for Disease Control and Prevention. Patients with CKD are twice more likely to develop cardiovascular disease (CVD). CVD remains the leading cause of mortality in patients with CKD (1). The increased arterial stiffness is one of the major factors contributing to CVD in such patients. The mechanisms that lead to the arterial disease in CKD include endothelial dysfunction, disorders of nitric oxide metabolism, vascular calcification, and elevation of the levels of pro-inflammatory cytokines (2–4). The complicated mechanisms explain why the treatment focusing on a single risk factor cannot achieve satisfactory outcomes. Previous studies showed that aortic stiffness and carotid stiffness are strongly associated with CVD in patients with CKD (5, 6). Therefore, improving vascular function might bring benefits to these patients.

The safety of exercise training is questioned in patients with CKD because renal perfusion is reduced and proteinuria is more severe in some cases during exercise (7). However, studies also proved the benefits of exercise in patients with CKD. The studies demonstrated that voluntary exercise was an effective therapy to improve endothelial function in rats with CKD (8, 9). In addition, exercise training was shown to improve endothelial function, physical function, inflammatory status, hypertension, nitric oxide availability, and lipid metabolism disorders (10, 11).

Some studies were conducted to assess the effect of exercise on arterial stiffness in patients with CKD. However, most of the studies were non-randomized controlled trials (RCTs), the sample sizes were small, and the results were inconsistent. Therefore, the conclusion was not convincing. Given the lack of high-quality evidence on the effects of exercise on the vascular function of patients with CKD, we conducted a meta-analysis of randomized trials to assess the effect of exercise on the vascular function of such patients.

METHODS

Methods and Search Strategy

The meta-analysis was performed and reported following Preferred Reporting Items for Systematic Reviews and Meta-analysis (12). The study protocol was registered in the International Prospective Register of Systematic Reviews; registration number: CRD42021283470. Studies were searched in the following databases; Medline, Cochrane Trials, and Embase. The search deadline was November, 12, 2021. The details of the search strategy and terms are presented in **Supplementary Table 1**. In addition, clinical trial registries and references of similar clinical studies, as well as review articles or systemic reviews on a similar topic, were reviewed to search for potentially relevant studies.

Data Sources and Study Selection

Two independent reviewers (H.W, and D.P.X) evaluated the titles and abstracts and screened the full-text versions of the relevant trials. Disagreements were resolved by consensus between the reviewers, and if necessary, by consulting with other reviewers. The studies were considered for inclusion if they compared exercise with active control, usual care, or no intervention, or they were randomized trials and reported the vascular function in patients with CKD. The flow diagram of study selection is outlined in **Figure 1**.

Inclusion and Exclusion Criteria

Patients with CKD, including non-dialysis and dialysis, or patients with kidney transplants were included in the study. The outcome should include indices of vascular function, pulse wave velocity (PWV), or augmentation index. All RCTs that compared exercise with control in managing patients with CKD were also included. This review focused on exercise training. Non-exercise management, such as electrical stimulation and water-based therapy, were excluded. The exercise training included aerobic exercises (such as walking, and cycling on a treadmill, ergometer, or elliptical machine) or resistance training (such as lifting or suppressing the muscle groups). Trials were excluded if they were case reports, comments, letters, or reviews.

Data Extraction and Quality Assessment

The data on the characteristics of methods, participants, interventions, and outcomes were extracted by two independent reviewers. The Cochrane risk-of-bias tool was used to assess the included trials. It contained the following criteria: selection bias, performance and detection bias, attrition bias, reporting bias, and other sources of bias. Two independent reviewers performed the assessment. The third author resolved the discrepancies. The corresponding authors were responsible for obtaining missing information and unpublished data.

Data Synthesis and Analysis

The primary outcome was defined as the changes in vascular function, including PWV or augmentation index from baseline to the end of treatment. The changes in peak VO2, health-related quality of life (HRQoL), blood pressure, and C-reactive protein (CRP) level were regarded as the secondary outcomes. If clinical outcomes were measured more than once in a study, we selected the data reported the last time. Data reported as median, interquartile range, 95% confidence interval (CI), or standard error were converted into mean and standard deviation (SD) using the formula (13, 14). We assessed effect size by weighted mean differences (WMDs) for continuous outcomes. The CI was 95%. We assessed heterogeneity with I^2 statistics. An I^2 value $<25\%$, between 25 and 50%, and $>75\%$ indicated a low degree of heterogeneity, a moderate degree of heterogeneity, and significant heterogeneity, respectively (15). If the results were not significantly heterogeneous, a fixed-effects model was

Abbreviations: RCTs, randomized controlled trials; CKD, chronic kidney disease; PWV, pulse wave velocity; WMD, weighted mean difference; CI, confidence interval; CVD, cardiovascular disease; PRISMA, Systematic Reviews and Meta-analysis; HRQoL, health-related quality of life; CRP, C-reactive protein; SD, standard deviation; WMD, weighted mean difference.

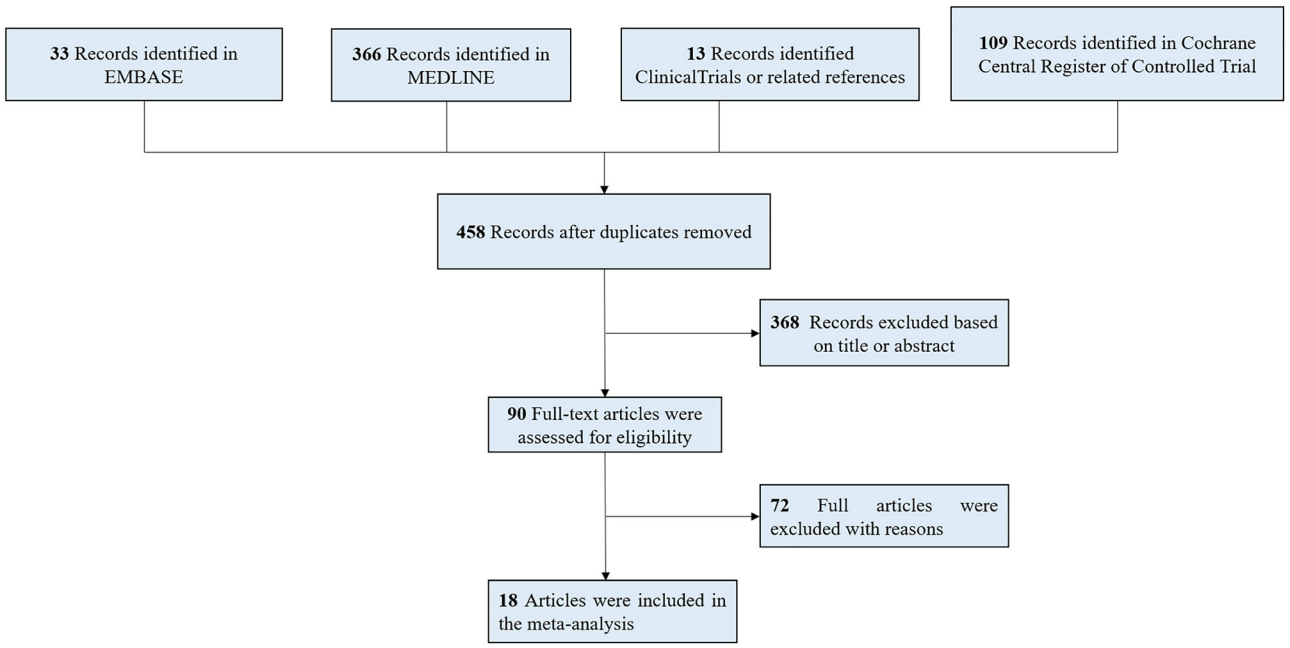


FIGURE 1 | Flow of the search strategy and included studies.

used. If the results were significantly heterogeneous, a random-effects model was used. The possibility of publication bias for the primary outcome was evaluated using the Egger test and the visual estimate of funnel plot. Sensitivity analyses were conducted by outlier identification and influence analysis using Stata 15. The subgroup analyses were performed based on the duration of the intervention. Exercise training lasting <6 months was defined as short term, while the training lasting ≥6 months was defined as long term. The data were assessed using Review Manager, version 5.3 (Oxford, UK).

RESULTS

Literature Selection and Study Characteristics

We identified 521 relevant studies or abstracts by the initial search. After removing 63 duplicates and 368 studies by screening the titles and abstracts, 90 full-text studies were further reviewed in detail. Two articles were considered as the same study for the analyses (16, 17). One study was removed for combining exercise with other lifestyle interventions (18). Finally, 18 studies were included in this meta-analysis.

The summary characteristics of studies included in the meta-analysis are shown in **Table 1**. All studies were RCTs, enrolling 817 patients. The sample size ranged from 12 to 156 patients, mean sample size of 45 (SD 37). A total of 7 trials (19–24) and NCT03197038 included pre-dialysis patients with CKD, 10 trials included dialysis patients (25–33), and 2 trials included kidney transplant patients (17, 34). Participants received aerobic training in these trials and NCT0319703, resistance training in these trials (17), and both aerobic and resistance trainings in these trials (22, 34). Most of the trials had an exercise frequency of three to four times per week; exercise was performed daily in only one trial (23). The exercise duration varied from 10 to 65 min as can be seen in references (23) and (29), respectively, in each session; only one trial did not report the exercise duration (27). The duration of exercise management was from 2.5 months to 12 months. Two trials contributed to two comparator categories (17, 26). One trial was a cross-over study (25).

Risk-of-Bias Assessment

All studies were randomized trials included in this meta-analysis. Among these studies, 12 trials (17, 19, 21–26, 28, 31, 32, 34) reported the concrete randomization methods. The performance bias was considered as high risk in all trials because it was impossible to blind the participants and researches for the exercise training. The intention-to-treat approach was employed in these trials (19, 29, 33). The risk-of-bias assessments are presented in **Figure 2** in the supplement.

Primary Outcome: PWV and Augmentation Index

A total of 17 trials (17, 20–34) and NCT03197038 were included in the meta-analysis for PWV between the two groups. The result showed that exercise training significantly decreased PWV in patients with CKD (WMD, −0.56; 95% CI, −1.02 to −0.09, $P = 0.02$, without significant heterogeneity; $P = 0.005$, $I^2 = 52\%$, **Figure 3**).

The augmentation index was measured in 11 trials (19–21, 23–27, 29, 30) and NCT03197038. The pooled result showed that exercise training significantly decreased the augmentation index

TABLE 1 | Basic characteristics of subjects and treatments of trials.

References	No. of patients (exercise/control)	Type of patient	Intervention		Duration (months)
			Exercise	Control	
Toussaint et al. (25)	19 (9/10)	Dialysis	Bicycling for a minimum of 30 min in each hemodialysis session	Usual care	3
Koh et al. (26)	46 (30/16)	Dialysis	Intradialytic-exercise: Cycling from 15 to 45 min during each dialysis three times per week on the Borg RPE of 12–13. Home-based-exercise: Walking from 15 to 45 min three times per week at Borg RPE of 12–13.	Usual care	6
Mustata et al. (19)	20 (10/10)	CKD3-4	Supervised training included the choice of treadmill, stationary, cycle and elliptical trainer twice per week throughout the study. Home training (walking) was initiated in the 2nd month and progressed over 3 months to a frequency of 3 days/week. Exercise was started at an intensity of 40–60% of peak VO_2 and duration was up to 60 min at Borg RPE of 12–15	Usual care	12
Kosmadakis et al. (20)	32 (18/14)	CKD4-5	Walking for at least 30 min, five times per week at an RPE of 12–14 and/or achieving the heart rate elicited by this effort level during the tread mill exercise test.	Usual physical activity	6
Riess et al. (34)	31 (16/15)	Kidney transplant	Endurance training was performed on a cycle ergometer and treadmill at 60–80% peak VO_2 for 30–60 min/session (3 days/week). Strength training was performed at 50% 1RM for 2 sets of 10–15 repetitions (2 days/week)	Usual	3
Headley et al. (21)	46 (25/21)	CKD3	Participants worked at 50–60% peak oxygen uptake using a variety of apparati three times per week.	Usual care	4
Greenwood et al. (22)	18 (8/10)	CKD3-4	Aerobic exercise was performed on recumbent stationary exercise cycles at about RPE of 11 for 40 min three times per week. Resistance training include life or press for upper- and lower- body, starting point of 1–2 sets × 10 repetitions with the aim to increase to 3 sets and 8–10 repetitions three times per week	Usual care	12
Greenwood et al. (17)	46 (26/20)	Kidney transplant	Aerobic exercise was performed on recumbent stationary exercise cycles, a treadmill, and elliptical trainer at PRE of 13–15 for 60 min three times per week. Resistance include lift or press for the upper and lower body muscle groups, starting with 1–2 sets of 10 repetitions with the aim of 3 sets of 8–10 repetitions.	Usual care	3
Van Craenenbroek et al. (23)	40(19/21)	CKD3-4	Four daily cycling sessions of 10 min at a target heat rate calculated as 90% of the heart rate achieved at the anaerobic threshold on baseline testing	Usual care	3
Cooke et al. (27)	20(10/10)	Dialysis	Pedaling exercise to reach 12–16 of RPE for Three times per week.	Usual care	4
Mcgregor et al. (28)	34(18/16)	Dialysis	Cycling was performed for up to 1 h per session to achieve 40–60% oxygen uptake reserve three times per week.	Usual care	2.5
Kirkman et al. (24)	31(16/15)	CKD3-5	Aerobic exercise (cycling, walking/jogging, elliptical) at 60–85% heart rate reserve for 45 min (three times per week)	Usual care	3

(Continued)

TABLE 1 | Continued

References	No. of patients (exercise/control)	Type of patient	Intervention		Duration (months)
			Exercise	Control	
Sliva et al. (29)	30 (15/15)	Dialysis	The aerobic training using a cycloergometer lasted 30 min at between 65 and 75% of the maximal heart rate with a Borg scale score around 13 (3 times a week).	Usual care	4
Jeong et al. (30)	67 (29/38)	Dialysis	Cycling 45 min during each dialysis session, and receiving protein supplement.	Usual care and protein supplement	12
Assawasaksakul et al. (31)	12 (6/6)	Dialysis	Cycling for 60 min during each dialysis session with Borg scale score of 13	Usual care	6
Graham-Brown et al. (33)	130(65/65)	Dialysis	Cycling for 30 min with 12–14 of RPE three times per week during dialysis.	Usual care	6
Greenwood et al. (32)	156 (78/78)	Dialysis	Cycling start from 21 min and progressing to 40 min per dialysis session.	Usual care	6
NCT03197038	39(22/17)	CKD	Participants exercised (a brisk walk) at home, for 30–60 min, 3 times per week	Usual care	6

CKD, Chronic kidney disease.

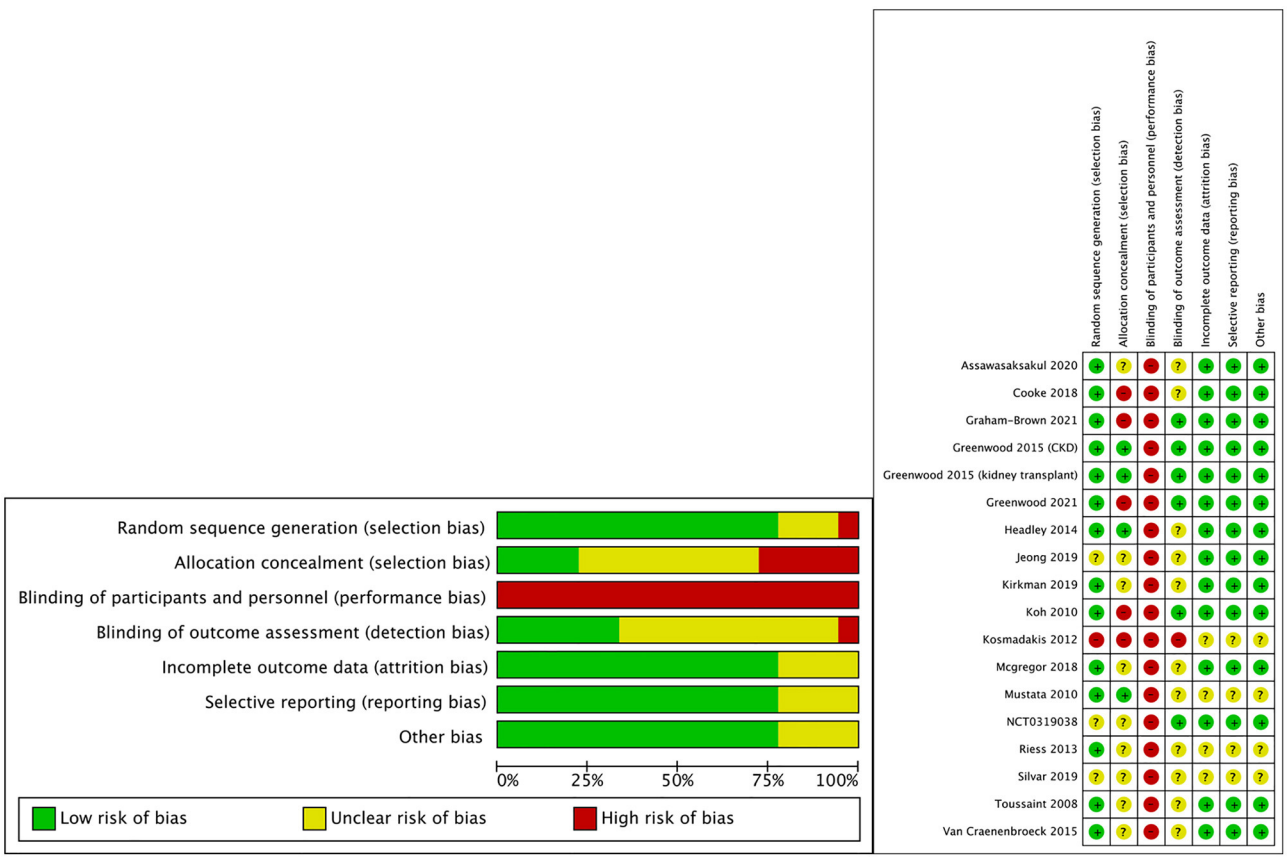


FIGURE 2 | Risk of bias graph of included studies.

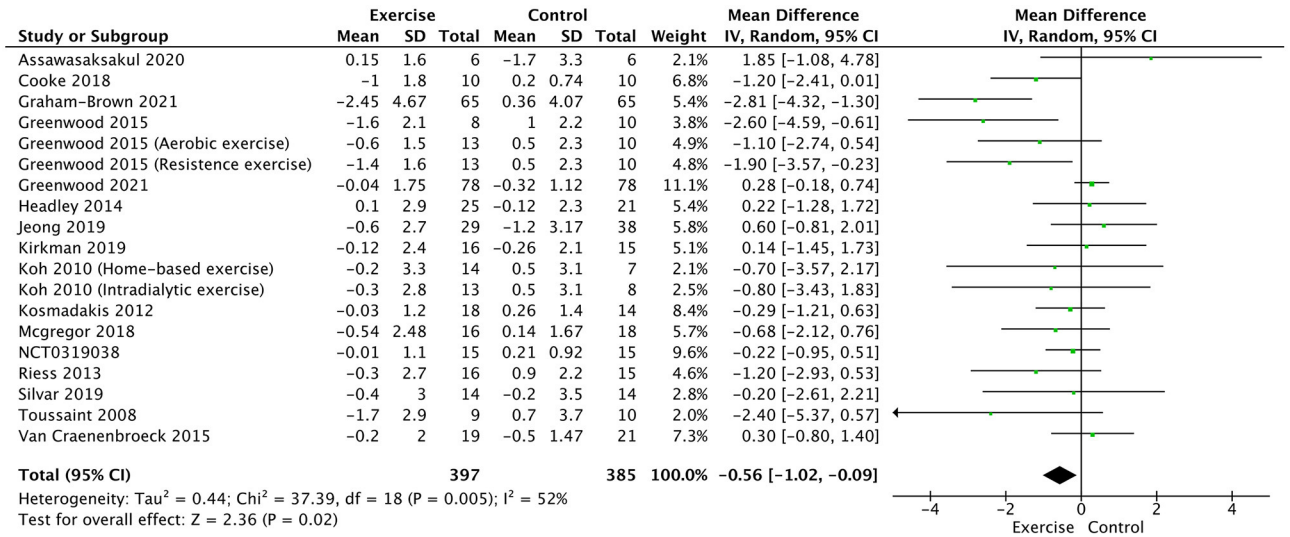


FIGURE 3 | Forest plot of exercise training on PWV.

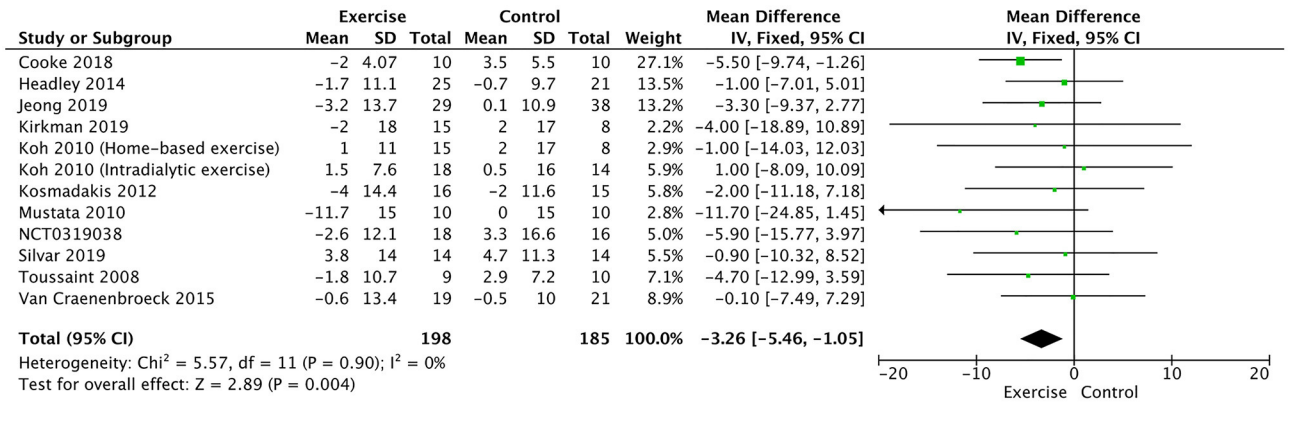


FIGURE 4 | Forest plot of exercise training on augmentation index.

(WMD, −3.26; 95% CI, −5.46 to −1.05, $P = 0.004$; without heterogeneity: $P = 0.90$, $I^2 = 0\%$, **Figure 4**).

Secondary Outcome: Peak VO2, CRP, Blood Pressure, and HRQoL

Peak VO2 was compared in 10 trials (17, 19, 21–24, 28, 31, 32, 34). It significantly increased in the exercise training group compared with the usual control group (WMD, 2.64; 95% CI, 1.94–3.35, $P < 0.00001$; without heterogeneity: $P = 0.24$, $I^2 = 22\%$, **Figure 5**).

The blood pressure was compared in 11 trials (17, 21–28, 30, 33). the exercise training had no effect on either systolic blood pressure (WMD, −0.70; 95% CI, −4.28 to 2.87, $P = 0.70$; without heterogeneity: $P = 0.87$, $I^2 = 0\%$, **Supplementary Figure 1**) or diastolic blood pressure (WMD, −0.55; 95% CI, −2.83 to 1.74, $P = 0.64$; without heterogeneity: $P = 0.84$, $I^2 = 0\%$, **Supplementary Figure 2**).

CRP was compared in seven trials (17, 21, 23, 25, 29–31). The exercise training had no effect on the levels of CRP (WMD, −0.09; 95% CI, −0.26 to 0.09, $P = 0.33$; without heterogeneity: $P = 0.57$, $I^2 = 0\%$, **Supplementary Figure 3**).

HRQoL was compared in four trials (19, 21, 23, 26), including vitality, general health, social function pain, and mental health. No significant difference in mental health was found between the training and control groups (WMD, 1.09; 95% CI, −4.21 to 6.4, $P = 0.69$; without heterogeneity: $P = 0.31$, $I^2 = 16\%$, **Supplementary Figure 4**), social function (WMD, 4.08; 95% CI, −2.52 to 10.69, $P = 0.23$; without heterogeneity: $P = 0.88$, $I^2 = 0\%$, **Supplementary Figure 5**). However, exercising training improved general health (WMD, 7.03; 95% CI, 0.65–13.42, $P = 0.03$; without heterogeneity: $P = 0.75$, $I^2 = 0\%$, **Supplementary Figure 6**) and vitality (WMD, 9.1; 95% CI, 2.50–15.69, $P = 0.007$; without heterogeneity: $P = 0.91$, $I^2 = 0\%$, **Supplementary Figure 7**).

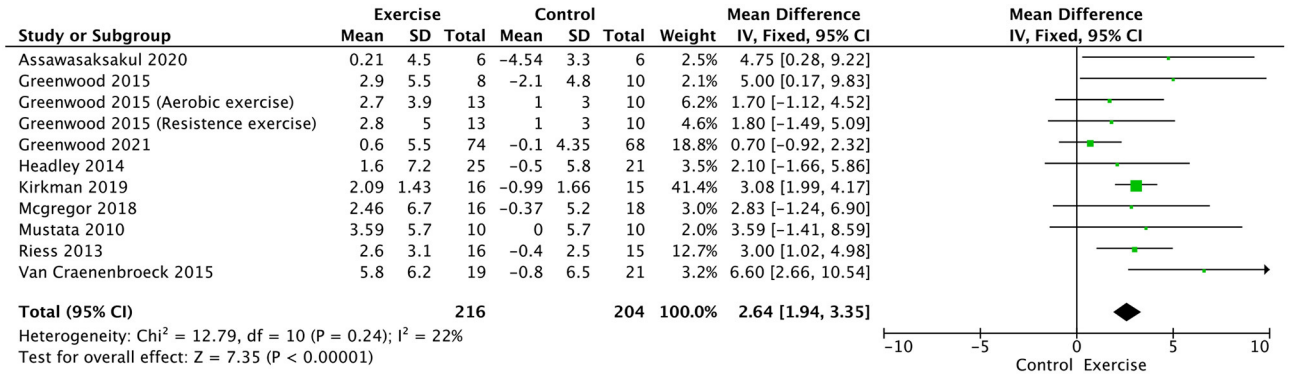


FIGURE 5 | Forest plot of exercise training on peak VO2.

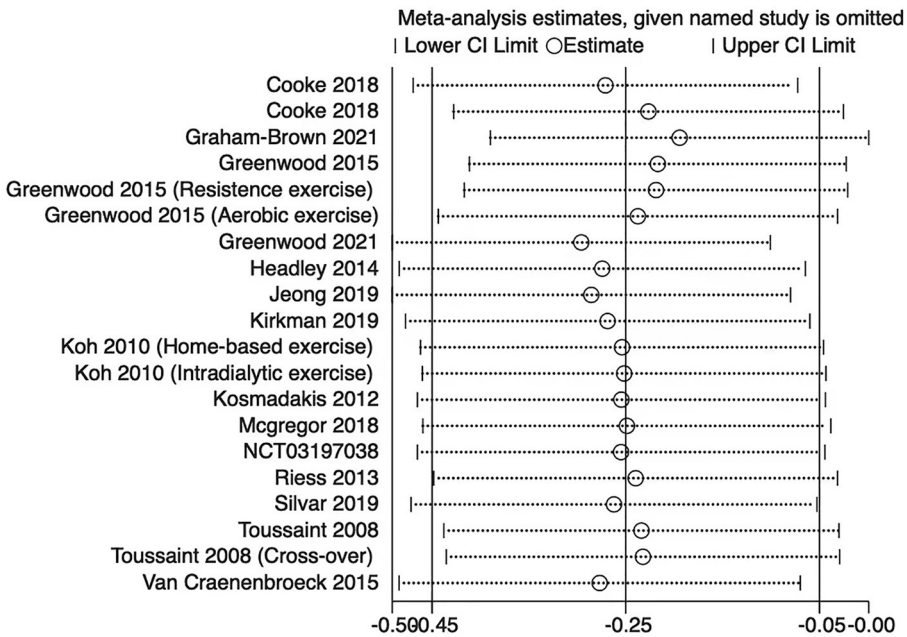


FIGURE 6 | Leave-one-out forest plot for the PWV.

Subgroup Analysis

The subgroup analysis revealed that PWV was significantly lower in patients with short-term exercise training and without heterogeneity (**Supplementary Figure 8**). However, no difference was observed between the long-term exercise training group and the control group in patients with significant heterogeneity (**Supplementary Figure 8**).

Adverse Events

Among the studies, nine trials (17, 19, 22, 24–28, 31, 34) reported no adverse events with exercise. Two trials (31, 32) reported no difference in adverse events between exercise training groups and control group. One trial (33) reported that the exercise training groups had more adverse events than the control group; the adverse events were judged to have no relationship with exercise. The study by Graham-Brown et al. (33) reported two deaths in each group, while Greenwood et al. (32) reported three deaths in the exercise training group and four deaths in the control group.

Sensitivity Analysis and Publication Bias

The sensitivity analysis was performed by leave-one-out analysis in the primary outcomes. The leave-one-out analysis showed that the pooled result and heterogeneity had no significant change in PWV (**Figure 6**) and augmentation index (**Figure 7**).

The funnel plot analysis showed the symmetry in **Figure 8**, and the Egger test ($P > 0.05$) did not detect the significant publication bias for the augmentation index. However, the funnel

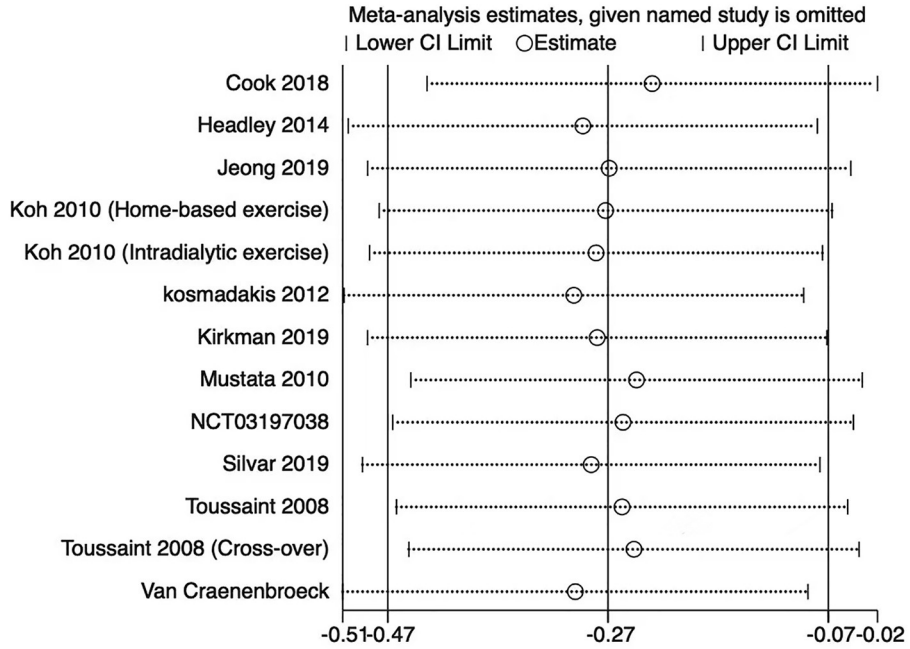


FIGURE 7 | Leave-one-out forest plot for the augmentation index.

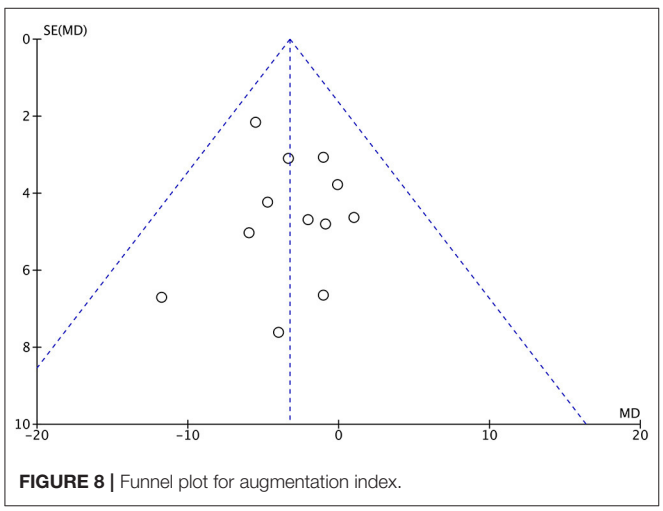


FIGURE 8 | Funnel plot for augmentation index.

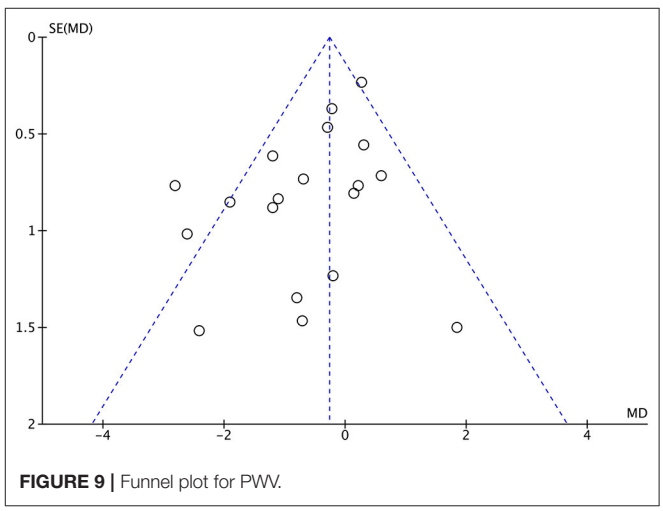


FIGURE 9 | Funnel plot for PWV.

plot analysis showed some asymmetry in **Figure 9**, and the Egger test ($P < 0.05$) detected the publication bias for PWV.

DISCUSSION

This meta-analysis involved 18 trials with 817 patients and showed that exercise training was significantly associated with reduced arterial stiffness evidenced by decreased PWV and augmentation index. In addition, exercise training was associated with improved peak VO2, general health, and vitality. However, no association of exercise training with improved CRP, mental health, and social function was found in this meta-analysis. Finally, this meta-analysis found no association between exercise training and adverse events.

Vascular stiffness is common in patients with CKD, and worsens as kidney function declines (35). The calcification of arteries is associated with vascular stiffness, which is an independent risk factor for CVD (36). PWV is the most widely used parameter for assessing arterial stiffness; it has become a useful method for diagnosis, risk stratification, and prognosis of cardiovascular diseases (37). It has been demonstrated to be associated with cardiovascular and all-cause mortality in

patients with end-stage renal disease (ESRD) (38). Blacher et al. demonstrated an increase of 1 m/s in PWV in patients with ESRD, while the all-cause mortality increased by 1.39 times (6). The augmentation index is another useful tool to reflect the arterial stiffness and predict cardiovascular outcomes (39, 40). London et al. provided direct evidence that an increased effect of augmentation index was a predictor of all-cause and cardiovascular mortality in patients with CKD (41). Therefore, PWV and augmentation index were considered as the primary outcomes in this meta-analysis. Although PWV and augmentation index are independent predictors of cardiovascular events, they are affected by different factors. PWV might be affected by blood pressure, distensibility of the arterial wall, and peripheral vascular resistance, while augmentation index might be affected by ventricular ejection and heart rate (42, 43). This meta-analysis had similar results, showing that exercise training reduced PWV and augmentation index, which made the conclusion of this meta-analysis more convincing. In these non-RCTs, the results showed that exercise improved vascular function, as evidenced by improved flow-mediated dilation (44, 45), which were consistent with our results.

Several meta-analyses assessed the effect of exercise on patients with CKD. However, these studies focused on aerobic capacity, muscular function, or health-related quality of life. No meta-analysis study focused on the effect of exercise on arterial stiffness in patients with CKD. In 2014, a meta-analysis included 928 patients with CKD. It found that exercise improved aerobic capacity, muscular function, and health-related quality of life (46). In 2019, similar meta-analyses found that aerobic exercise improved aerobic capacity, exercise duration, and health-related quality of life (47, 48) in patients with CKD and those undergoing hemodialysis. In 2019, a meta-analysis helped reinforce our findings. The review found that exercise improved PWV; however, only two trials were included (49).

Considering limited data on patients with CKD in terms of the benefits and risks of exercise interventions, the Kidney Disease Improving Global Outcomes (KDIGO) guideline followed the guideline of the American Heart Association (AHA), in which exercise was suggested for preventing cardiovascular diseases in patients with CKD. This meta-analysis provided a rationale for the KDIGO and AHA recommendation of exercise in the management strategy for cardiovascular diseases in patients with CKD. These findings indicated that three to four times of aerobic exercise was appropriate for such patients. However, the optimal duration of exercise each time and the beginning exercise of the CKD stage to achieve maximal benefits remain unknown. Further trials are needed to examine the suitable duration and type of exercise with a personalized condition for patients with CKD who are more likely to adhere and achieve benefit.

Limitations

This review had some limitations. First, we observed moderate levels of heterogeneity in PWV using I^2 statistics. We further conducted subgroups analysis to reduce heterogeneity based on the duration of exercise training. The heterogeneity of PWV decreased significantly in short-term exercise training; however, the heterogeneity of PWV was even higher in long-term exercise training, which might be the main reason for the reverse outcome. We were unable to use more meaningful subgroups to reduce heterogeneity for PWV. Second, although we conducted a comprehensive search of clinical trial registries and literature to reduce the risk of missing any study, an asymmetry funnel plot and Egger test detected publication bias. The potential sources of publication bias might include selective outcome reporting, English language bias, and differences in methodological quality among trials (50). Third, we did not evaluate the important covariates, such as the association of age with the primary outcomes, due to the low number of trials to conduct a convincing meta-regression. Fourth, this meta-analysis found that exercise training reduced arterial stiffness. However, we did not compare the effects of aerobic exercise training and resistance exercise training on these patients due to the low number of trials.

CONCLUSIONS

The meta-analysis suggested that exercise training improved vascular function in patients with CKD. An exercise program should be considered as one of the management strategies for vascular dysfunction in patients with CKD. Further studies are needed to demonstrate that exercise training improves CVD in patients with CKD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HW, DX, and LZ contributed to the collection of data, bias assessment, data analysis, and manuscript writing. LW and LZ contributed to bias assessment and data extraction. LZ and DX contributed to the design of the study. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Sichuan Administration of Traditional Chinese Medicine (Grant no. 2021MS123) and Special Project for the Development of Traditional Chinese Medicine (Grant no. CYW2022046).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.904299/full#supplementary-material

REFERENCES

1. Ali S, Dave N, Virani SS, Navaneethan SD. Primary and secondary prevention of cardiovascular disease in patients with chronic kidney disease. *Curr Atherosclerosis Rep.* (2019) 21:32. doi: 10.1007/s11883-019-0794-6
2. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulat Res.* (2000) 87:840–4. doi: 10.1161/01.RES.87.10.840
3. Zoccali C, Mallamaci F, Tripepi G. Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease. *Nephrol Dialysis Transplant.* (2004) 19:V67–72. doi: 10.1093/ndt/gfh1059
4. Vallianou NG, Mitesh S, Gkogkou A, Geladari E. Chronic kidney disease and cardiovascular disease: is there any relationship? *Curr Cardiol Rev.* (2019) 15:55–63. doi: 10.2174/1573403X14666180711124825
5. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension.* (1998) 32:570–4. doi: 10.1161/01.HYP.32.3.570
6. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation.* (1999) 99:2434–9. doi: 10.1161/01.CIR.99.18.2434
7. Headley S, Germain M, Milch C, Pescatello L, Coughlin MA, Nindl BC, et al. Exercise training improves HR responses and V?O2peak in predialysis kidney patients. *Med Sci Sports Exerc.* (2012) 44:2392–9. doi: 10.1249/MSS.0b013e318268c70c
8. Adams GR, Zhan CD, Haddad F, Vaziri ND. Voluntary exercise during chronic renal failure in rats. *Med Sci Sports Exerc.* (2005) 37:557–62. doi: 10.1249/01.MSS.0000159006.87769.67
9. Martens CR, Kuczmarski JM, Kim J, Guers JJ, Harris MB, Lennon-Edwards S, et al. Voluntary wheel running augments aortic l-arginine transport and endothelial function in rats with chronic kidney disease. *Am J Physiol Renal Physiol.* (2014) 307:F418–26. doi: 10.1152/ajprenal.00014.2014
10. Roberts CK, Vaziri ND, Barnard RJ. Effect of diet and exercise intervention on blood pressure, insulin, oxidative stress, and nitric oxide availability. *Circulation.* (2002) 106:2530–2. doi: 10.1161/01.CIR.0000040584.91836.0D
11. Khan AA, Mundra PA, Straznicky NE, Nestel PJ, Wong G, Tan R, et al. Weight loss and exercise alter the high-density lipoprotein lipidome and improve high-density lipoprotein functionality in metabolic syndrome. *Arteriosclerosis Thrombosis Vasc Biol.* (2018) 38:438–47. doi: 10.1161/ATVBAHA.117.310212
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6, e1000097. doi: 10.1371/journal.pmed.1000097
13. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res.* (2018) 27:1785–805. doi: 10.1177/0962280216669183
14. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135
15. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
16. O'Connor EM, Koufaki P, Mercer TH, Lindup H, Nugent E, Goldsmith D, et al. Long-term pulse wave velocity outcomes with aerobic and resistance training in kidney transplant recipients - A pilot randomised controlled trial. *PLoS ONE.* (2017) 12:e0171063. doi: 10.1371/journal.pone.0171063
17. Greenwood SA, Koufaki P, Mercer TH, Rush R, O'Connor E, Tuffnell R, et al. Aerobic or resistance training and pulse wave velocity in kidney transplant recipients: A 12-week pilot randomized controlled trial (the exercise in renal transplant [ExeRT] Trial). *Am J Kidney Dis.* (2015) 66:689–98. doi: 10.1053/j.ajkd.2015.06.016
18. Howden EJ, Leano R, Petchey W, Coombes JS, Isbel NM, Marwick TH. Effects of exercise and lifestyle intervention on cardiovascular function in CKD. *Clin J Am Soc Nephrol: CJASN.* (2013) 8:1494–501. doi: 10.2215/CJN.10141012
19. Mustata S, Groeneveld S, Davidson W, Ford G, Kiland K, Manns B. Effects of exercise training on physical impairment, arterial stiffness and health-related quality of life in patients with chronic kidney disease: a pilot study. *Int Urol Nephrol.* (2011) 43:1133–41. doi: 10.1007/s11255-010-9823-7
20. Kosmadakis GC, John SG, Clapp EL, Viana JL, Smith AC, Bishop NC, et al. Benefits of regular walking exercise in advanced pre-dialysis chronic kidney disease. *Nephrol Dialysis Transplant.* (2012) 27:997–1004. doi: 10.1093/ndt/gfr364
21. Headley S, Germain M, Wood R, Joubert J, Milch C, Evans E, et al. Short-term aerobic exercise and vascular function in CKD stage 3: a randomized controlled trial. *Am J Kidney Dis.* (2014) 64:222–9. doi: 10.1053/j.ajkd.2014.02.022
22. Greenwood SA, Koufaki P, Mercer TH, MacLaughlin HL, Rush R, Lindup H, et al. Effect of exercise training on estimated GFR, vascular health, and cardiorespiratory fitness in patients with CKD: a pilot randomized controlled trial. *Am J Kidney Dis.* (2015) 65:425–34. doi: 10.1053/j.ajkd.2014.07.015
23. Van Craenenbroeck AH, Van Craenenbroeck EM, Van Ackeren K, Vrints CJ, Conraads VM, Verpooten GA, et al. Effect of moderate aerobic exercise training on endothelial function and arterial stiffness in CKD stages 3-4: A randomized controlled trial. *Am J Kidney Dis.* (2015) 66:285–96. doi: 10.1053/j.ajkd.2015.03.015
24. Kirkman DL, Ramick MG, Muth BJ, Stock JM, Pohlig RT, Townsend RR, et al. Effects of aerobic exercise on vascular function in nondialysis chronic kidney disease: a randomized controlled trial. *Am J Physiol Renal Physiol.* (2019) 316:F898–905. doi: 10.1152/ajprenal.00539.2018
25. Toussaint ND, Polkinghorne KR, Kerr PG. Impact of intradialytic exercise on arterial compliance and B-type natriuretic peptide levels in hemodialysis patients. *Hemodial Int.* (2008) 12:254–63. doi: 10.1111/j.1542-4758.2008.00262.x
26. Koh KP, Fassett RG, Sharman JE, Coombes JS, Williams AD. Effect of intradialytic versus home-based aerobic exercise training on physical function and vascular parameters in hemodialysis patients: a randomized pilot study. *Am J Kidney Dis.* (2010) 55:88–99. doi: 10.1053/j.ajkd.2009.09.025
27. Cooke AB, Ta V, Iqbal S, Gomez YH, Mavrakanas T, Barré P, et al. The impact of intradialytic pedaling exercise on arterial stiffness: a pilot randomized controlled trial in a hemodialysis population. *Am J Hyperten.* (2018) 31:458–66. doi: 10.1093/ajh/hpx191
28. McGregor G, Ennis S, Powell R, Hamborg T, Raymond NT, Owen W, et al. Feasibility and effects of intra-dialytic low-frequency electrical muscle stimulation and cycle training: A pilot randomized controlled trial. *PLoS ONE.* (2018) 13:e0200354. doi: 10.1371/journal.pone.0200354
29. Oliveira ESVR, Stringuetta Belik F, Hueb JC, de Souza Gonçalves R, Costa Teixeira Caramori J, Perez Vogt B, et al. Aerobic exercise training and nontraditional cardiovascular risk factors in hemodialysis patients: results from a prospective randomized trial. *Cardiorenal Med.* (2019) 9:391–9. doi: 10.1159/000501589
30. Jeong JH, Biruete A, Tomayko EJ, Wu PT, Fitschen P, Chung HR, et al. Results from the randomized controlled IHOPE trial suggest no effects of oral protein supplementation and exercise training on physical function in hemodialysis patients. *Kidney Int.* (2019) 96:777–86. doi: 10.1016/j.kint.2019.03.018
31. Assawasaksakul N, Sirichana W, Joosri W, Kulaputana O, Eksakulkla S, Ketanun C, et al. Effects of intradialytic cycling exercise on daily physical activity, physical fitness, body composition, and clinical parameters in high-volume online hemodiafiltration patients: a pilot randomized-controlled trial. *Int Urol Nephrol.* (2021) 53:359–71. doi: 10.1007/s11255-020-02677-7
32. Greenwood SA, Koufaki P. Exercise programme to improve quality of life for patients with end-stage kidney disease receiving haemodialysis: the PEDAL RCT. *Health Technol Assess.* (2021) 25:1–52. doi: 10.3310/hta25400
33. Graham-Brown MPM, March DS, Young R, Highton PJ, Young HML, Churchward DR, et al. A randomized controlled trial to investigate the effects of intra-dialytic cycling on left ventricular mass. *Kidney Int.* (2021) 99:1478–86. doi: 10.1016/j.kint.2021.02.027
34. Riess KJ, Haykowsky M, Lawrance R, Tomczak CR, Welsh R, Lewanczuk R, et al. Exercise training improves aerobic capacity, muscle strength, and quality of life in renal transplant recipients. *Appl Physiol Nutrit Metab.* (2014) 39:566–71. doi: 10.1139/apnm-2013-0449
35. Temmar M, Liabeuf S, Renard C, Czernichow S, Esper NE, Shahapuni I, et al. Pulse wave velocity and vascular calcification at different stages of chronic kidney disease. *J Hyperten.* (2010) 28:163–9. doi: 10.1097/HJH.0b013e328331b81e
36. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective

observational data from 17,635 subjects. *J Am College Cardiol.* (2014) 63:636–46. doi: 10.1016/j.jacc.2013.09.063
37. Kim HL, Kim SH. Pulse wave velocity in atherosclerosis. *Front Cardiovasc Med.* (2019) 6:41. doi: 10.3389/fcvm.2019.00041
38. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int.* (2003) 63:1852–60. doi: 10.1046/j.1523-1755.2003.00932.x
39. Vyas M, Izzo JL, Jr., Lacourcière Y, Arnold JM, Dunlap ME, et al. Augmentation index and central aortic stiffness in middle-aged to elderly individuals. *Am J Hyperten.* (2007) 20:642–7. doi: 10.1016/j.amjhyper.2007.01.008
40. Nichols WW, Singh BM. Augmentation index as a measure of peripheral vascular disease state. *Curr Opin Cardiol.* (2002) 17:543–51. doi: 10.1097/00001573-200209000-00016
41. London GM, Blacher J, Pannier B, Guérin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. *Hypertension.* (2001) 38:434–8. doi: 10.1161/01.HYP.38.3.434
42. Obara S, Hayashi S, Hazama A, Murakawa M, Katsuda S. Correlation between augmentation index and pulse wave velocity in rabbits. *J Hyperten.* (2009) 27:332–40. doi: 10.1097/HJH.0b013e32831ac951
43. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol.* (2000) 525:263–70. doi: 10.1111/j.1469-7793.2000.t01-1-00263.x
44. Sprick JD, Mammino K, Jeong J. Aerobic exercise training improves endothelial function and attenuates blood pressure reactivity during maximal exercise in chronic kidney disease. *J Appl Physiol.* (2022) 132:785–93. doi: 10.1152/japplphysiol.00808.2021
45. Katulka EK, Hirt AE, Kirkman DL, Edwards DG, Witman MAH. Altered vascular function in chronic kidney disease: evidence from passive leg movement. *Physiol Rep.* (2019) 7:e14075. doi: 10.14814/phy2.14075
46. Heiwe S, Jacobson SH. Exercise training in adults with CKD: a systematic review and meta-analysis. *Am J Kidney Dis.* (2014) 64:383–93. doi: 10.1053/j.ajkd.2014.03.020
47. Pei G, Tang Y, Tan L, Tan J, Ge L, Qin W. Aerobic exercise in adults with chronic kidney disease (CKD): a meta-analysis. *Int Urol Nephrol.* (2019) 51:1787–95. doi: 10.1007/s11255-019-02234-x
48. Huang M, Lv A, Wang J, Xu N, Ma G, Zhai Z, et al. exercise training and outcomes in hemodialysis patients: systematic review and meta-analysis. *Am J Nephrol.* (2019) 50:240–54. doi: 10.1159/000502447
49. Chen G, Gao L. Effects of exercise training on cardiovascular risk factors in kidney transplant recipients: a systematic review and meta-analysis. *Ren Fail.* (2019) 41:408–18. doi: 10.1080/0886022X.2019.1611602
50. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 08 July 2022
doi: 10.3389/fmed.2022.904963

Low Klotho/Fibroblast Growth Factor 23 Ratio Is an Independent Risk Factor for Renal Progression in Chronic Kidney Disease: Finding From KNOW-CKD

*Hyo Jin Kim[1,2], Yunmi Kim[3], Minjung Kang[4], Seonmi Kim[4], Sue Kyung Park[5], Suah Sung[6], Young Youl Hyun[7], Ji Yong Jung[8], Curie Ahn[9] and Kook-Hwan Oh[4,10]**

[1] Department of Internal Medicine, Pusan National University School of Medicine, Busan, South Korea, [2] Biomedical Research Institute, Pusan National University Hospital, Busan, South Korea, [3] Department of Internal Medicine, Inje University Busan Paik Hospital, Busan, South Korea, [4] Department of Internal Medicine, Seoul National University Hospital, Seoul, South Korea, [5] Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, South Korea, [6] Department of Internal Medicine, Eulji Medical Center, Eulji University, Seoul, South Korea, [7] Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, South Korea, [8] Department of Internal Medicine, Gachon University Gil Medical Center, Gachon University College of Medicine, Incheon, South Korea, [9] Department of Internal Medicine, National Medical Center, Seoul, South Korea, [10] Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

OPEN ACCESS

Edited by:
Li Zuo,
Peking University People's Hospital, China

Reviewed by:
Javier Donate-Correa,
University Hospital of the Nuestra Señora de Candelaria, Spain
Yuri Battaglia,
University Hospital of Ferrara, Italy

***Correspondence:**
Kook-Hwan Oh
khoh@snu.ac.kr

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 26 March 2022
Accepted: 07 June 2022
Published: 08 July 2022

Citation:
Kim HJ, Kim Y, Kang M, Kim S, Park SK, Sung S, Hyun YY, Jung JY, Ahn C and Oh K-H (2022) Low Klotho/Fibroblast Growth Factor 23 Ratio Is an Independent Risk Factor for Renal Progression in Chronic Kidney Disease: Finding From KNOW-CKD. Front. Med. 9:904963. doi: 10.3389/fmed.2022.904963

Background: We aimed to evaluate soluble Klotho and circulating fibroblast growth factor 23 (FGF23) ratio as a risk factor for renal progression, cardiovascular (CV) events, and mortality in chronic kidney disease (CKD).

Methods: We analyzed 2,099 subjects from a CKD cohort whose soluble Klotho and C-terminal FGF23 levels were measured at enrollment. The Klotho to FGF23 ratio was calculated as Klotho values divided by FGF23 values + 1 (hereinafter called the Klotho/FGF23 ratio). Participants were categorized into quartiles according to Klotho/FGF23 ratio. The primary outcome was renal events, defined as the doubling of serum creatinine, 50% reduction of estimated glomerular filtration rate from the baseline values, or development of end-stage kidney disease. The secondary outcomes consisted of CV events and death. Changes in CV parameters at the time of enrollment and during follow-up according to the Klotho/FGF23 ratio were also examined.

Results: During the follow-up period of 64.0 ± 28.2 months, 735 (35.1%) and 273 (13.0%) subjects developed renal events and composite outcomes of CV events and death, respectively. After adjustment, the first (HR: 1.36; 95% CI: 1.08–1.72, $P = 0.010$) and second (HR: 1.45; 95% CI: 1.15–1.83, $P = 0.002$) quartiles with regard to the Klotho/FGF23 ratio showed elevated risk of renal events as compared to the fourth quartile group. There was no significant association between Klotho/FGF23 ratio and the composite outcome of CV events and death.

The prevalence of left ventricular hypertrophy and vascular calcification was higher in the low Klotho/FGF23 ratio quartiles at baseline and at the fourth-year follow-up.

Conclusions: Low Klotho/FGF23 ratio was significantly associated with increased renal events in the cohort of Korean predialysis CKD patients.

Keywords: Klotho, fibroblast growth factor 23, chronic kidney disease, renal progression, mortality

INTRODUCTION

Klotho and fibroblast growth factor 23 (FGF23) are early laboratory parameters of chronic kidney disease (CKD)-mineral bone disorder (MBD), and the Klotho/FGF23 axis plays an important role in this disorder (1, 2). Klotho, which is an anti-aging protein, is closely associated with CKD, since the kidney is the major organ for the production of Klotho, and CKD is known to be a Klotho-deficient state (3, 4). FGF23 is a phosphorus-regulating protein secreted by bone cells, and serum FGF23 level increases as kidney function declines (5, 6). In most previous studies, low soluble Klotho levels were associated with increased adverse kidney outcomes (7). Among CKD patients, the subjects with lower serum Klotho levels (lower than median: ≤396.3 pg/mL) exhibited poorer outcomes [doubling serum creatinine, end stage kidney disease (ESKD), or death] than those with higher levels (8). A community-based elderly cohort study showed that higher soluble Klotho level was independently associated with a lower risk of decline in kidney function, defined as eGFR decline ≥30% or eGFR decline >3 ml/min per year (9). However, there was also a study in which soluble Klotho was not related to kidney function and did not predict adverse outcomes in CKD patients (10). In addition, previous studies showed that both C-terminal and intact FGF23 independently predicted the progression of CKD after adjustment for multiple factors in patients with non-diabetic CKD (11). Higher FGF23 levels were likely associated with coronary calcification (12, 13) and all-cause mortality in CKD (12).

FGF23 binds to Klotho and FGF receptors to exert its physiological effects on traditional, on-target organs, such as the kidney and parathyroid glands, thereby regulating phosphate homeostasis and mineral metabolism. Recently, it has been shown that FGF23 could also target cell types that lack Klotho. In CKD patients, excess FGF23 also exerts Klotho-independent effects on non-traditional, off-target organs, such as the heart, cells of the immune system, and the liver (14). The off-target effect is activated at high FGF23 concentrations and may cause pathologic cellular changes, leading to poor outcomes in CKD patients (15). Therefore, it is meaningful to examine the effects of the relative ratio of Klotho and FGF23 on the outcome of CKD patients, rather than investigating Klotho and FGF23 individually. High FGF23 is associated with greater risks of severe inflammation (16, 17), and chronic inflammation is involved in renal progression (18). Therefore, FGF23 may affect renal progression in CKD patients. There is little data on the long-term clinical outcomes of Klotho/FGF23 together in CKD patients. Therefore, we aimed to investigate the association between the Klotho/FGF23 ratio and renal progression, all-cause mortality, and CV outcomes in CKD patients including all CKD stages, using data from a large-scale Korean CKD cohort. Changes in CV parameters at the time of enrollment and during follow-up according to the Klotho/FGF23 ratio were also examined.

METHODS

Study Design and Population

The KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease (KNOW-CKD) was a multicenter prospective cohort study in Korea that enrolled subjects with CKD stages 1 to 5 (predialysis) from nine university-affiliated hospitals. The detailed study methods and design of the KNOW-CKD have been described previously (19). Among the 2,238 participants enrolled in the KNOW-CKD between 2011 and 2016, 2,099 subjects whose serum Klotho and FGF23 levels were obtained at enrollment were included in the analysis. The study protocol was approved in 2011 by the ethical committee of each participating clinical center and by the institutional review boards of Seoul National University Hospital (1104-089-359), Yonsei University Severance Hospital (4-2011-0163), Seoul St. Mary's Hospital (KC11OIMI0441), Seoul National University Bundang Hospital (B-1106/129-008), Kangbuk Samsung Medical Center (2011-01-076), Gil Hospital (GIRBA2553), Eulji General Hospital (201105-01), Chonnam National University Hospital (CNUH-2011-092), and Pusan Paik Hospital (11-091). All study subjects provided written informed consent. The study protocol followed the principles of the Declaration of Helsinki.

Clinical Data Collection and Laboratory Measurements

Baseline demographic characteristics such as age, sex, body mass index (BMI), comorbidities, cause of CKD, and laboratory parameters at enrollment were extracted from an electronic data management system (http://www.phactax.org) with assistance from the Division of Data Management at the Seoul National University Medical Research Collaborating Center. Patients with a history of diabetes mellitus (DM), a fasting serum glucose ≥126 mg/dL, or those on anti-diabetic medication were considered to have DM. Patients with a history of hypertension (HTN), a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or those on antihypertensive drugs were considered to have HTN. Patients considered to have CV disease were those with a history of coronary artery disease, cerebrovascular disease, congestive heart failure, arrhythmia, or peripheral vascular disease. The following laboratory variables were measured using a ≥8-h fasting blood sample at each participating center: hemoglobin, uric acid, albumin, total

cholesterol, C-reactive protein, phosphorus, calcium, and intact parathyroid hormone (PTH). Serum creatinine was measured at a central laboratory (Lab Genomics, Korea) using an isotope dilution mass spectrometry-traceable method (20). The estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (21). CKD stages were defined according to the Kidney Disease: Improving Global Outcomes guidelines (22). Second voided or random urine samples were immediately sent to a central laboratory to measure urine creatinine and protein levels. The urinary protein excretion was quantified using the random urinary protein-to-creatinine ratio (UPCR, g/g). Alcohol consumption pattern was investigated: non-drinker, occasional drinker (<6 standard drinks/week), regular drinker (≥6 standard drinks/week), moderate drinker (<5 standard drinks/occasion and no alcohol-related problem within the past year), binge drinker [≥5 standard drinks/occasion (23) or the presence of an alcohol-related problem within the past year]. Physical activity was measured using the Korean form of the International Physical Activity Questionnaire (24, 25). Health-enhancing physical activity was defined as achieving at least 150 min/week of moderate-intensity physical activity, 75 min/week of vigorous-intensity physical activity, or an equivalent combination of moderate-vigorous physical activity (MVPA) (26). Frequency of MVPA per week was also investigated.

Klotho and FGF23 Measurement

The serum α-Klotho level was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories Co., Gunma, Japan) according to the manufacturer's protocol. The intra-assay and inter-assay coefficients of variation were 2.7–3.5% (Klotho levels: 186.64–2,968.78 pg/mL) and 2.9–11.4% (Klotho levels: 165.47–2,903.01 pg/mL), respectively. Serum C-terminal FGF23 was measured using a commercial ELISA kit (Immutopics, San Clemente, CA, USA) according to the manufacturer's protocol. The intra-assay and inter-assay coefficients of variation as reported by the manufacturer were 1.4–2.4% (FGF23 levels: 33.7–302 RU/mL) and 2.4–4.7% (FGF23 levels: 33.6–293 RU/mL), respectively.

Echocardiographic and Cardiovascular Parameters

Two-dimensional echocardiography was conducted, and left ventricular (LV) mass index was calculated by dividing the LV mass by the body surface area. Left ventricular hypertrophy (LVH) was defined as an LV mass index >115 g/m^3 in men and >95 g/m^3 in women, according to the American Society of Echocardiography guidelines (27). LV geometry was classified by LV mass index and relative wall thickness (RWT = [2 × PWTd]/LVIDd) into the following categories: normal geometry (normal LVMI with a RWT ≤ 0.42); concentric remodeling (normal LVMI with a RWT > 0.42); eccentric LVH (LVH with a RWT ≤ 0.42); concentric LVH (LVH with a RWT > 0.42). LV ejection fraction and the ratio (E/E′ ratio) of mitral peak velocity of early filling (E) to the early diastolic mitral annular velocity (E′) were evaluated to find systolic and diastolic dysfunction, respectively. Abdominal aorta calcification (AAC) score (28) and coronary artery calcification score (CACS) (29, 30) were measured to evaluate vascular calcification. The presence of abdominal aortal calcification was defined as a AAC score ≥1 in the present study. The presence of coronary artery calcification was defined as a CACS >100 in the present study (31). The ankle-brachial index was also measured (32).

Study Outcomes

The primary outcome was renal events, defined as a composite of a 50% decrease in eGFR from baseline, doubling of serum creatinine level, or development of ESKD. ESKD was defined as the initiation of renal replacement therapy, including dialysis or renal transplantation. The secondary composite outcome consisted of CV events and all-cause mortality. Patients were followed until March 2020. The eGFR decline during the follow-up period was also analyzed. In subgroup analyses, changes in echocardiography parameters, CACS, and AAC scores at 4 years of follow-up were investigated.

Statistical Analyses

Continuous variables were analyzed using analysis of variance or Kruskal–Wallis test. The Kolmogorov–Smirnov test was used to analyze the normality of the distributions of parameters. The results were presented as mean ± standard deviation for variables with normal distributions and as median (interquartile range) for variables with skewed distributions. Categorical variables were evaluated using the χ^2-test or Fisher's exact test and were presented as frequencies and percentages. The Klotho to FGF23 ratio was calculated as Klotho values divided by FGF23 values + 1 (hereinafter called the Klotho/FGF23 ratio). A log transformation was used to normalize the Klotho/FGF23 ratio. Participants were categorized into quartiles according to Klotho/FGF23 ratio. Cox proportional hazards models with adjustments, including variables that were significant in a univariable analysis or other clinically relevant variables, were used to analyze the association between the Klotho/FGF23 ratios and study outcomes. The results were expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). Subjects who were lost to follow-up were censored at the date of their last examination. The rates of renal function decline per year were determined using the slope of eGFR analyzed using a generalized linear mixed model. Only 1,851 (88.1%) patients whose eGFR values were measured three or more times during the follow-up period were included in the eGFR decline analysis. The rapid decline of eGFR was defined as eGFR <-3 ml/min/1.73 m^2/year. Binary logistic regression analysis was used to identify the risk factors for the rapid decline of kidney function. Multivariable linear regression model analysis was also used to investigate the association between eGFR slope and Klotho/FGF23 ratio. In addition, Harrell's C-index and receiver operating characteristic (ROC) curve analysis were conducted to evaluate the prognostic value of Klotho and FGF23 levels and the Klotho/FGF23 ratio for renal events. *P*-values < 0.05 were considered statistically significant. The SPSS statistical software (SPSS version 20.0, IBM Corporation, Armonk, NY, USA) was used for all analyses.

RESULTS

Baseline Clinical Characteristics of the Study Subjects

The clinical characteristic of the study subjects by Klotho/FGF23 ratio quartiles are shown in **Table 1**. The mean age was 53.6 ± 12.2 years, and 1,280 (61.0%) of the subjects were male. The mean eGFR was 53.0 ± 30.7 mL/min/1.73 m^2. The mean age was younger in the high Klotho/FGF23 ratio quartiles ($P = 0.020$). The prevalence of DM ($P < 0.001$), HTN ($P < 0.001$), and CV disease ($P = 0.008$) was lower in the high Klotho/FGF23 ratio quartiles. Estimated GFR ($P < 0.001$) and hemoglobin ($P < 0.001$) were higher in the high Klotho/FGF23 ratio quartiles. Serum phosphorus ($P < 0.001$) and PTH ($P < 0.001$) were lower in the high Klotho/FGF23 ratio quartiles. Binge drinkers were more in the 4th Klotho/FGF23 ratio group ($P = 0.033$). Health-enhancing physical activity was lower in the 1st and 4th Klotho/FGF23 ratio groups ($P = 0.022$).

Klotho/FGR23 Ratio and Renal Events

During the follow-up period of 64.0 ± 28.2 months, 735 (35.1%) subjects developed renal events. **Figure 1** presents the renal events according to the Klotho/FGF23 ratio groups. The first quartile of Klotho/FGF23 ratio group was at a greater risk of developing renal events compared to the other quartile groups ($P < 0.001$). The Kaplan–Meier curves showed that the low Klotho/FGF23 ratio group had a significantly higher cumulative incidence of renal events ($P < 0.001$; **Figure 2**). The multivariable Cox regression analysis presented that the first (HR: 1.36; 95% CI: 1.08–1.72, $P = 0.010$) and second (HR: 1.45; 95% CI: 1.15–1.83, $P = 0.002$) quartiles of the Klotho/FGF23 ratio group showed increased renal events as compared to the fourth quartile group (**Table 2**). As a continuous variable, as the log (Klotho/FGF23 ratio) increased, the development of renal events decreased (HR: 0.85; 95% CI: 0.75–0.96, $P = 0.008$). Similarly, when physical activity, smoking status, and alcohol consumption variables were added in model 4, the low Klotho/FGF23 ratio was significantly associated with developing renal events. To confirm whether the Klotho/FGF23 ratio is a valuable predictor of renal events, we compared Harrell's C-index between Klotho and FGF23 levels and the Klotho/FGF23 ratio described in the statistical analysis section. The Harrell's C-index for Klotho/FGF23 ratio was 0.644. The Harrell's C-indices for Klotho and FGF23 were 0.535 and 0.642, respectively. Furthermore, Harrell's C-index for Klotho/FGF23 ratio added to an adjusted model (model 4) was 0.841. When Klotho and FGF23 levels were added instead of Klotho/FGF23 ratio in the adjusted model (model 4), Harrell's C-index was 0.840. These findings suggest that Klotho/FGF23 ratio can have predictive value for renal events development.

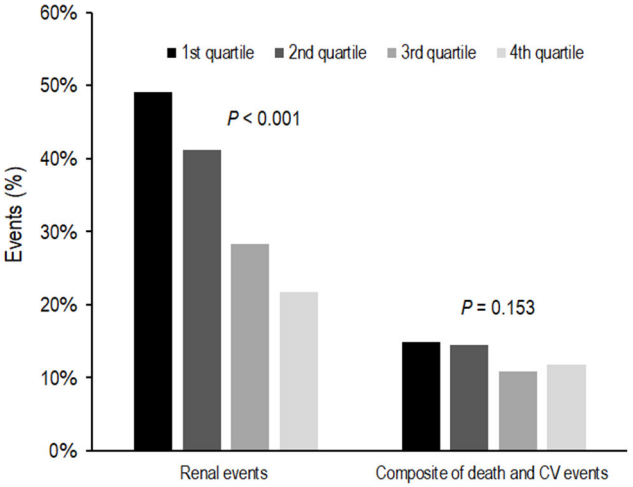


FIGURE 1 | Event rates for renal events and composites of death and CV events according to Klotho/FGF23 ratio. The first quartile of the Klotho/FGF23 ratio group was at a greater risk of developing renal events compared to the other quartile groups ($P < 0.001$). Composites of death and CV events were not significantly different according to Klotho/FGF23 ratio groups ($P = 0.153$). FGF23, fibroblast growth factor 23; CV, cardiovascular.

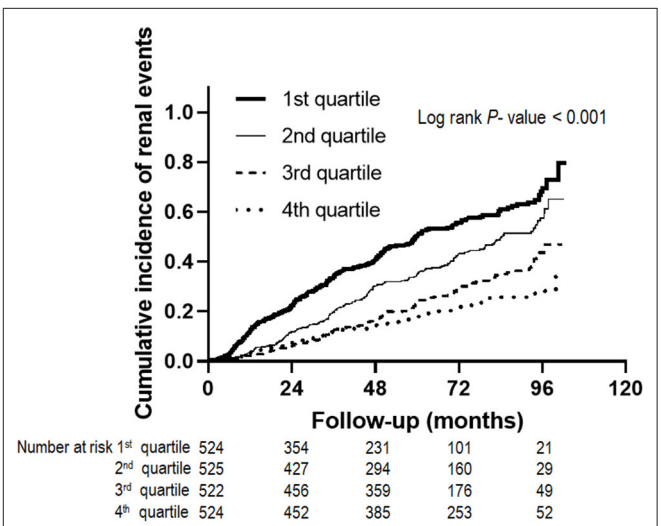


FIGURE 2 | Renal events according to Klotho/FGF23 ratio. The Kaplan-Meier curves show that the low Klotho/FGF23 ratio group had a significantly higher cumulative incidence of renal events ($P < 0.001$). FGF23, fibroblast growth factor 23.

Klotho/FGR23 Ratio and CV Events and All-Cause Mortality

During the follow-up period, 273 subjects developed a composite of CV events and death. Composites of death and CV events were not significantly different among the Klotho/FGF23 ratio groups ($P = 0.153$; **Figure 1**). In an unadjusted Cox proportional hazards model, the logarithm of the Klotho/FGF23 ratio was inversely associated with the composite of CV events and death (HR: 0.72; 95% CI: 0.60–0.87, $P < 0.001$; **Table 3**). After adjustment, there was no significant association between the Klotho/FGF23 ratio groups and the composite outcome of CV events and death (**Table 3**).

TABLE 1 | Clinical characteristics of the study subjects at enrollment, stratified by Klotho/FGF23 ratio quartiles.

Characteristics	Total (*N* = 2,099)	Klotho/FGF23 ratio				*P*-value
		1st quartile (*n* = 524)	2nd quartile (*n* = 525)	3rd quartile (*n* = 525)	4th quartile (*n* = 525)	
Age (mean ± SD)	53.6 ± 12.2	54.4 ± 12.0	54.4 ± 12.2	52.7 ± 12.0	52.8 ± 12.6	0.020
Sex, male, *n* (%)	1,280 (61.0)	304 (58.0)	342 (65.1)	315 (60.0)	319 (60.8)	0.113
BMI (kg/m^2)	24.6 ± 3.4	24.5 ± 3.5	24.8 ± 3.5	24.6 ± 3.3	24.4 ± 3.2	0.374
SBP (mmHg)	127.8 ± 16.2	131.4 ± 19.0	126.7 ± 15.0	126.6 ± 15.1	126.4 ± 14.7	<0.001
DM, *n* (%)	711 (33.9)	228 (43.5)	188 (35.9)	155 (29.5)	140 (26.8)	<0.001
HTN, *n* (%)	2,013 (95.9)	510 (97.3)	508 (96.8)	506 (96.4)	489 (93.1)	0.003
Preexisting CV disease, *n* (%)	334 (15.9)	104 (19.8)	90 (17.1)	70 (13.3)	70 (13.3)	0.008
CAD, *n* (%)	129 (6.1)	42 (8.0)	38 (7.2)	24 (4.6)	25 (4.8)	0.077
Cerebrovascular ds, *n* (%)	131 (6.2)	38 (7.8)	35 (6.7)	31 (5.9)	27 (5.1)	0.520
HF, *n* (%)	30 (1.4)	12 (2.3)	4 (0.8)	7 (1.3)	7 (1.3)	0.214
Arrhythmia, *n* (%)	54 (2.6)	22 (4.2)	13 (2.5)	11 (2.1)	8 (1.5)	0.068
PVD, *n* (%)	77 (3.7)	24 (4.6)	24 (4.6)	14 (2.7)	15 (2.9)	0.178
Cause of CKD						<0.001
DN, *n* (%)	490 (23.3)	171 (32.6)	131 (25.0)	109 (20.8)	79 (15.0)	
Hypertension, *n* (%)	388 (18.5)	84 (16.0)	101 (19.2)	92 (17.5)	111 (21.1)	
GN, *n* (%)	746 (35.5)	155 (29.6)	183 (34.9)	202 (38.5)	206 (39.2)	
PKD, *n* (%)	346 (16.5)	81 (15.5)	80 (15.2)	91 (17.3)	94 (17.9)	
Others, *n* (%)	129 (6.1)	33 (6.3)	30 (5.7)	31 (5.9)	35 (6.7)	
Smoking status, *n* (%)						0.022
Never	1,117 (53.3)	269 (51.3)	256 (49.0)	304 (57.9)	288 (54.9)	
Current or former	979 (46.7)	255 (48.7)	266 (51.0)	221 (42.1)	237 (45.1)	
Klotho (Q1, Q3) (pg/mL)	536 (419, 666)	444 (335, 565)	547 (451, 644)	591 (462, 739)	556 (429, 718)	<0.001
FGF23 (Q1, Q3) (RU/mL)	19.6 (1.7, 34.6)	51.6 (36.3, 76.7)	26.2 (21.2, 32.5)	9.7 (4.8, 17.8)	0.04 (0.0, 0.5)	<0.001
eGFR (mL/min/1.73 m^2)	53.0 ± 30.7	41.2 ± 29.4	48.5 ± 26.9	59.5 ± 30.2	62.8 ± 31.3	<0.001
Hemoglobin (g/dL)	12.8 ± 2.0	11.9 ± 2.0	12.9 ± 2.0	13.2 ± 1.9	13.4 ± 1.8	<0.001
Uric acid (mg/dL)	7.0 ± 1.9	7.4 ± 2.0	7.2 ± 1.8	6.9 ± 1.9	6.7 ± 1.9	<0.001
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.5	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	<0.001
Total cholesterol (mg/dL)	174.3 ± 39.4	172.8 ± 42.4	172.2 ± 38.2	175.6 ± 36.5	176.6 ± 38.2	0.199
CRP, median, (Q1, Q3) (mg/L)	0.6 (0.2, 1.7)	0.7 (0.3, 2.0)	0.7 (0.3, 1.6)	0.5 (0.2, 1.4)	0.7 (0.2, 1.5)	0.018
Phosphorus (mg/dL)	3.7 ± 0.7	4.0 ± 0.8	3.7 ± 0.6	3.6 ± 0.6	3.5 ± 0.6	<0.001
Corrected Ca (mg/dL)*	9.0 ± 0.4	8.9 ± 0.5	9.0 ± 0.5	9.0 ± 0.4	9.0 ± 0.4	<0.001
PTH, median (Q1, Q3) (pg/mL)	51.5 (33.3, 84.0)	70.9 (41.3, 122.5)	53.1 (36.0, 81.7)	45.5 (31.0, 74.0)	44.7 (29.1, 69.1)	<0.001
UPCR (Q1, Q3) (g/g)	0.49 (0.14, 1.51)	0.73 (0.24, 2.30)	0.57 (0.17, 1.66)	0.40 (0.12, 1.12)	0.33 (0.09, 0.97)	<0.001
Urine calcium (mg/day)	45 (21.6, 95.8)	33.0 (16.2, 69.4)	39.1 (19.5, 84.2)	50.0 (23.0, 110.5)	65.0 (29.6, 122.6)	<0.001
Urine phosphorus (mg/day)	571 (400, 737)	500 (400, 700)	588 (400, 770)	600 (400, 780)	600 (400, 793)	0.036
Medications						
ACEi or ARB, *n* (%)	1,797 (85.7)	450 (85.9)	449 (85.9)	464 (88.4)	434 (82.7)	0.071
Diuretics, *n* (%)	670 (32.0)	225 (42.9)	168 (32.1)	129 (24.6)	148 (28.2)	<0.001
Ca-based phosphorus binder, *n* (%)	183 (8.7)	69 (13.2)	39 (7.5)	40 (7.6)	35 (6.7)	0.001
Active vitamin D, *n* (%)	50 (2.4)	21 (4.0)	9 (1.7)	10 (1.9)	10 (1.9)	0.047

(Continued)

TABLE 1 | Continued

Characteristics	Total (N = 2,099)	Klotho/FGF23 ratio				P-value
		1st quartile (n = 524)	2nd quartile (n = 525)	3rd quartile (n = 525)	4th quartile (n = 525)	
LV mass index (g/m^3)	93.1 ± 24.5	99.9 ± 28.7	92.8 ± 22.8	90.7 ± 22.0	89.2 ± 22.4	<0.001
LVH, *n* (%)**	512 (25.0)	179 (35.1)	123 (24.0)	103 (20.2)	107 (20.7)	<0.001
LV ejection fraction (%)	64.0 ± 6.3	64.1 ± 7.1	63.9 ± 5.9	64.3 ± 6.1	63.8 ± 6.1	0.542
E/E′	9.9 ± 3.9	10.6 ± 4.9	10.0 ± 3.4	9.7 ± 3.4	9.4 ± 3.6	<0.001
AAC ≥ 1, *n* (%)	704 (35.1)	227 (45.4)	175 (35.3)	160 (32.1)	142 (27.9)	<0.001
CACS (Q1, Q3)	1 (0, 82)	5 (0, 140)	1 (0, 94)	0 (0, 62)	0 (0, 42)	<0.001
CACS >100, *n* (%)	454 (23.0)	133 (27.5)	123 (24.7)	107 (21.4)	91 (18.5)	0.005
Ankle-brachial index	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.080
Binge drinker†, *n* (%)	318 (16.7)	80 (16.8)	73 (15.4)	75 (15.89)	90 (18.9)	0.033
Health-enhancing physical activity††, *n* (%)	774 (42.4)	170 (37.6)	209 (45.4)	212 (46.2)	183 (40.1)	0.022
Frequency of MVPA per week (Q1, Q3)	1 (0, 4)	0 (0, 4)	1 (0, 5)	1 (0, 4)	0 (0, 4.7)	0.034

**Corrected Ca (mg/dL) = measured total Ca (mg/dL) + 0.8 × [4 – measured serum albumin (g/dL)].*

***LVH was defined as LV mass index > 115 g/m^3 in men and > 95 g/m^3 in women.*

†Binge drinker was defined as the consumption of ≥ 5 standard drinks per occasion or the presence of an alcohol-related problem within the past year.

††Health-enhancing physical activity was defined as achieving at least 150 min/week of moderate-intensity physical activity, 75 min/week of vigorous-intensity physical activity, or an equivalent combination of MVPA.

FGF23, fibroblast growth factor 23; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DM, diabetes mellitus; HTN, hypertension; CV, cardiovascular; CAD, coronary artery disease; HF, heart failure; PVD, peripheral vascular disease; CKD, chronic kidney disease; DN, diabetic nephropathy; GN, glomerulonephritis; PKD, polycystic kidney disease; eGFR, estimated glomerular filtration rate as determined by the CKD-EPI creatinine equation; CRP, C-reactive protein; Ca, calcium; PTH, parathyroid hormone; UPCR, urine protein creatinine ratio; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; LV, left ventricular; LVH, left ventricular hypertrophy; EF, ejection fraction; E/E′, ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E′); AAC, Abdominal aorta calcification; CACS, coronary artery calcium score; MVPA, Moderate-vigorous physical activity.

Association of Klotho/FGR23 Ratio With Renal Function Decline

We analyzed renal function decline as the slope of eGFR for 1,851 patients whose eGFR values were measured three times or more during the follow-up period. The eGFR slope was lower in the low Klotho/FGF23 ratio group ($P < 0.001$; **Figure 3A**). The proportion of patients showing a rapid decline of eGFR was higher in the first quartile of Klotho/FGF23 ratio group ($P < 0.001$; **Figure 3B**). The multivariable binary logistic regression analysis revealed that the first [odds ratio (OR): 1.68; 95% CI: 1.23–2.28, $P = 0.001$], second (OR: 1.73; 95% CI: 1.29–2.32, $P < 0.001$), and third (OR: 1.63; 95% CI: 1.21–2.19, $P = 0.001$) quartiles of Klotho/FGF23 ratio groups showed a significantly rapid decline in eGFR compared to the fourth quartile group. eGFR slope was significantly associated with log transformed Klotho/FGF23 ratio (β: 0.26; 95% CI: 0.12–0.41; P <0.001; **Supplementary Table S1**).

Echocardiography and Vascular Calcification Parameters

We further examined the relationship between the Klotho/FGF2 ratio and CV parameters. Baseline echocardiography and vascular calcification parameters are shown in **Table 1**. LV mass index ($P < 0.001$) and E/E′ ($P < 0.001$) were higher in the lower Klotho/FGF23 ratio groups. The LV geometry pattern according to Klotho/FGF23 ratio is shown in **Supplementary Figure S1**. Low Klotho/FGF23 ratio quartiles showed greater increases in the prevalence of LVH (**Supplementary Figure S1A**). AAC score ≥1 was 45.4% in the first Klotho/FGF23 ratio group and higher in the lower quartiles ($P < 0.001$). CACS > 100 was 27.5% in the first Klotho/FGF23 ratio group and was also higher in the lower quartiles ($P = 0.005$). Ankle-brachial index was similar according to Klotho/FGF23 ratio quartiles.

At 4 years of follow-up, echocardiography and vascular calcification parameters were evaluated in approximately 59% of subjects. Fourth-year echocardiography and vascular calcification parameters are presented in **Supplementary Table S2**. LV mass index ($P = 0.012$) and E/E′ ($P = 0.001$) at 4 years were higher in the low Klotho/FGF23 ratio quartiles. The prevalence of LVH was higher in the first Klotho/FGF23 ratio quartile at 4 years (**Supplementary Figure S1B**). Of the 987 patients who did not have LVH at baseline, 135 (13.7%) subjects developed *de novo* LVH. *De novo* LVH incidence was higher in the first Klotho/FGF23 ratio quartile (first, second, third, and fourth quartiles: 20.3, 14.7, 11.3, and 11.1%, respectively; $P = 0.020$). The presence of abdominal aortal calcification ($P = 0.001$) and coronary artery calcification ($P = 0.038$) were also higher in the low Klotho/FGF23 ratio quartiles.

TABLE 2 | Renal events according to Klotho/FGF23 ratio.

	Model 1		Model 2		Model 3		Model 4	
Klotho/FGF23 ratio	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Categorical variable								
1st quartile	3.52 (2.82, 4.39)	<0.001	2.92 (2.33, 3.65)	<0.001	1.37 (1.08, 1.73)	0.008	1.36 (1.08, 1.72)	0.010
2nd quartile	2.31 (1.84, 2.90)	<0.001	2.12 (1.69, 2.66)	<0.001	1.44 (1.14, 1.81)	0.002	1.45 (1.15, 1.83)	0.002
3rd quartile	1.43 (1.12, 1.83)	0.004	1.35 (1.05, 1.72)	0.017	1.25 (0.98, 1.60)	0.079	1.26 (0.98, 1.62)	0.068
4th quartile	Reference	-	Reference	-	Reference	-	Reference	-
Continuous variable								
Klotho/FGF23 ratio*	0.47 (0.42, 0.53)	<0.001	0.52 (0.46, 0.58)	<0.001	0.84 (0.75, 0.95)	0.007	0.85 (0.75, 0.96)	0.008

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, HTN, DM, preexisting CVD, systolic blood pressure, BMI.
Model 3: Model 2 + eGFR, phosphorous, corrected calcium, hemoglobin, log PTH.
Model 4: Model 3 + ACEi or ARB use, diuretics, Ca-baseline phosphorus binder, and active vitamin D use.
FGF23, fibroblast growth factor 23; HR, hazard ratio; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate by CKD-EPI creatinine equation; PTH, parathyroid hormone; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; Ca, calcium.
**Data for Klotho/FGF23 ratio was log transformed.*

TABLE 3 | Composite of mortality and cardiovascular events according to Klotho/FGF23 ratio.

	Model 1		Model 2		Model 3		Model 4	
Klotho/FGF23 ratio	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Categorical variable								
1st quartile	1.44 (1.03, 2.01)	0.034	1.07 (0.76, 1.51)	0.691	0.92 (0.64, 1.33)	0.668	0.93 (0.65, 1.34)	0.694
2nd quartile	1.34 (0.96, 1.88)	0.085	1.09 (0.78, 1.53)	0.613	1.01 (0.71, 1.42)	0.972	1.03 (0.73, 1.46)	0.879
3rd quartile	0.98 (0.69, 1.41)	0.932	0.91 (0.64, 1.31)	0.619	0.89 (0.62, 1.28)	0.525	0.91 (0.63, 1.32)	0.629
4th quartile	Reference	-	Reference	-	Reference	-	Reference	-
Continuous variable								
Klotho/FGF23 ratio*	0.72 (0.60, 0.87)	<0.001	0.86 (0.72, 1.04)	0.117	0.95 (0.78, 1.15)	0.614	0.95 (0.78, 1.15)	0.605

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, HTN, DM, preexisting CVD, systolic blood pressure, BMI.
Model 3: Model 2 + eGFR, phosphorous, corrected calcium, hemoglobin, log PTH.
Model 4: Model 3 + ACEi or ARB use, diuretics, Ca-baseline phosphorus binder, and active vitamin D use.
FGF23, fibroblast growth factor 23; HR, hazard ratio; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate by CKD-EPI creatinine equation; PTH, parathyroid hormone; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; Ca, calcium.
**Data for Klotho/FGF23 ratio was log transformed.*

DISCUSSION

In the present study, the incidence of renal events during the follow-up period was higher in low Klotho/FGF23 ratio quartile. Subjects in the first quartile Klotho/FGF23 ratio group showed a significantly rapid decline in eGFR. In our study, there was no significant association between the Klotho/FGF23 ratio and the composite outcome of CV events and death. The presence of LVH and vascular calcification were higher in the low Klotho/FGF23 ratio quartile group at enrollment. In subgroup analysis, during the follow-up period, LV mass index and development of *de novo* LVH were higher in the low Klotho/FGF23 ratio quartile group. The presence of vascular calcification was also higher in the low Klotho/FGF23 ratio quartiles.

A previous study reported that patients with higher soluble Klotho levels exhibited a reduced risk of adverse kidney outcomes, including ESKD, and of serum creatinine doubling in CKD stage 3–5 (33). In this study, FGF23 was not a significant risk factor for renal events after variable adjustment. Another study showed that low Klotho and high FGF23 were significant risk factors of composite renal outcomes including serum creatinine doubling, ESKD, and death (8). In this study, the areas under the ROC curve for soluble Klotho and FGF23 were comparable. Intact serum FGF23 was a predictor of doubling of creatinine, dialysis initiation, and death in diabetic nephropathy patients (34). In another study, FGF23 was a risk factor for dialysis initiation alone or dialysis initiation and death in advanced CKD (eGFR < 30 m/min/1.73 m^2) (35). Klotho and FGF23 are earlier markers of CKD-MBD that change before the alteration of such biochemical parameters as phosphorus and PTH, and the Klotho/FGF23 axis could be an early marker for the outcome of CKD patients. Furthermore, Klotho acts like a hormone that exerts anti-senescent, anti-oxidant, and anti-apoptotic effects (36, 37). In previous experimental studies, Klotho was reduced in kidney injury, and kidney function and tubulointerstitial injury improved when the Klotho gene was transferred to the damaged kidney (38–40).

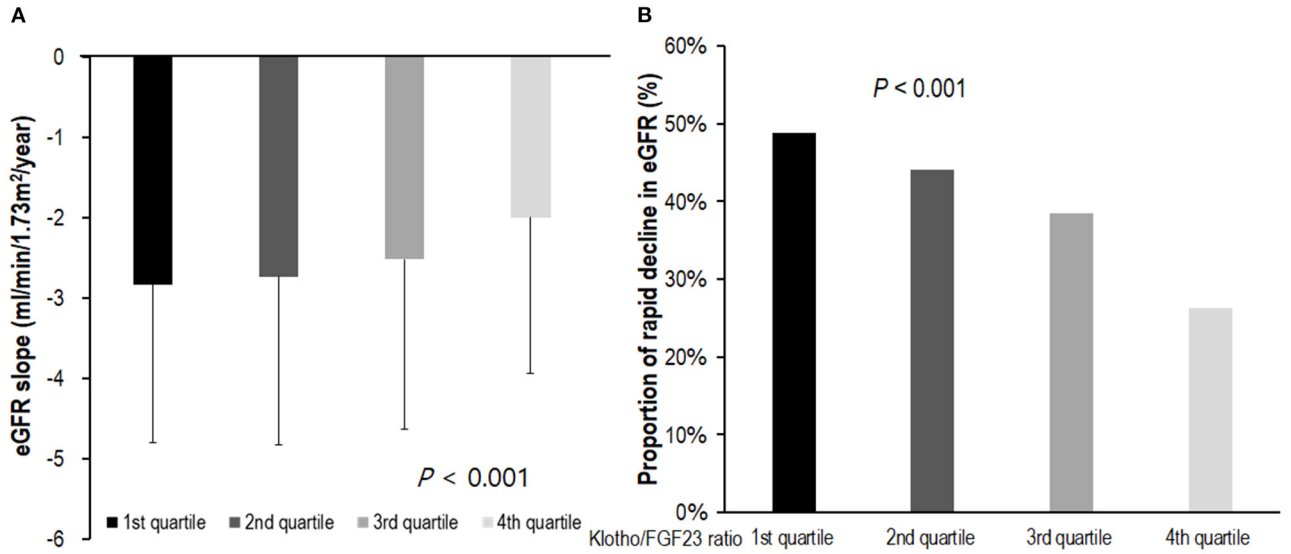


FIGURE 3 | The eGFR slope and rapid decline of eGFR according to Klotho/FGF23 ratio. **(A)** The eGFR slope according to Klotho/FGF23 ratio; **(B)** rapid decline of eGFR according to Klotho/FGF23 ratio. The eGFR slope was analyzed in 1,851 patients for whom eGFR was measured more than three times during the follow-up period. The eGFR slope was lower in the low Klotho/FGF23 ratio group ($P < 0.001$; **A**). The rapid decline of kidney function was defined as an estimated glomerular filtration rate (eGFR) <-3 ml/min/1.73 m^2/year. The proportion of patients exhibiting a rapid decline of eGFR was higher in the first quartile of Klotho/FGF23 ratio group ($P < 0.001$; **B**). eGFR, estimated glomerular filtration rate by CKD-EPI creatinine equation; FGF23, fibroblast growth factor 23.

Although FGF23 has Klotho-dependent traditional and on-target effects, a recent study showed that FGF23 also had Klotho-independent, non-traditional, off-target effects (14, 15). Pathologically increased FGF23 causes hypertrophy in heart cardiomyocytes (41–43) and inflammation in liver hepatocytes (44). Off-target effects of FGF23 also affect immune cells such as neutrophils and macrophages. A previous *in vitro* study showed that FGF23 was released by proinflammatory M1 macrophages and acted locally to increase tumor necrosis factor-α (TNF-α) production in M0 macrophages in the absence of Klotho (45). In animal experiments using a murine CKD model, FGF23 regulated genes involved in inflammation and renal fibrosis (transforming growth factor-β, TNF-α) (46). In addition, FGF23 inhibited neutrophil recruitment in a Klotho-independent manner in CKD (47). CKD is a state of acquired immune deficiency involving humoral and cellular immunity (48). As renal progression in CKD is associated with macrophage tissue infiltration and inflammation, these off-target effects of FGF23 related to the immune system provide a possible mechanistic link between elevated FGF23 and renal progression (14). Because FGF23 acts on target tissues with and without Klotho, it is crucial to evaluate their effects in CKD patients, not only for Klotho or FGF23 alone, but also the relative ratio of Klotho and FGF23. In the present study, a low Klotho/FGF23 ratio was associated with renal events after adjustment. In addition, the area under the ROC of the Klotho/FGF23 ratio was higher than those of either Klotho and FGF23 alone.

In the present study, a low Klotho/FGF23 ratio was associated with the presence of LVH and vascular calcification. At baseline, the lower Klotho/FGF23 ratio quartile group was associated with the presence of LVH and vascular calcification. In addition, after follow-up, the lower Klotho/FGF23 ratio quartile group exhibited a higher incidence of *de novo* development of LVH and vascular calcification. Of the 987 patients who did not have LVH at baseline, 135 (13.7%) subjects developed *de novo* LVH, and it developed more frequently in the low Klotho/FGF23 ratio group. In an experimental study, Klotho-deficient CKD mice had accelerated cardiac hypertrophy and cardiac fibrosis compared to wild-type CKD mice (49). Intravenous delivery of a transgene encoding soluble Klotho mitigated cardiac hypertrophy in the Klotho-deficient CKD mice. Serum Klotho levels are related with the development of LVH in CKD patients (50). Faul et al. (41) showed that FGF23 can cause LVH independently of Klotho. This indicates that the off-target effects of FGF23, independent of Klotho, affect cardiac myocytes. In patients with elevated FGF23, there have been studies in which aortic or coronary artery calcification scores were higher than in those with lower FGF23 (13), but there have been conflicting results (51). Moreover, up-regulation of Klotho expression protects against vascular calcification in CKD (52). The disruption of the balance between FGF23 and Klotho may be important in vascular calcification, rather than FGF23 or Klotho alone. In our study, the Klotho/FGF23 ratio was not associated with mortality or CV outcome. In the present study, we included all stages of CKD, and patients with early CKD (11.9% with CKD stage 1 and 18.4% with CKD stage 2) were also included. The incidence rates of death and CV outcomes were lower than in another study (35); hence, the results may differ. In addition, statistical significance may be decreased due to the low incidence of death and CV outcomes. However, *de novo* LVH

and vascular calcification, which were surrogate parameters of CV outcome, were significantly higher in the low Klotho/FGF23 ratio quartiles.

The advantage of our study is that long-term follow-up results were obtained in a large-scale CKD cohort. Instead of analyzing Klotho or FGF23 alone, we considered the relative ratio of Klotho and FGF23, which might have an interconnection. However, this study also has several limitations. First, Klotho and FGF23 have circadian variations (53), but blood sampling time could not be fixed. Second, we measured C-terminal FGF23 in the present study. Lack of agreement between intact FGF23 and C-terminal FGF23 measurements and also differences in their associations with other biochemical parameters have been reported (54). However, the C-terminal ELISA kit theoretically detects both intact FGF23 and its C-terminal fragments; since virtually all circulating FGF23 is intact, the C-terminal assay measures biologically active FGF23 (55). Third, we did not investigate the phosphorus intake of subjects.

CONCLUSION

Low Klotho/FGF23 ratio was significantly associated with increased risk of renal events in this cohort of Korean predialysis CKD patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of each participating clinical center and the Institutional Review Boards of Seoul National University Hospital (1104-089-359), Seoul National University Bundang Hospital (B-1106/129-008), Yonsei University Severance Hospital (4-2011-0163), Kangbuk Samsung Medical Center (2011-01-076), Seoul St. Mary's Hospital (KC11OIMI0441), Gil Hospital (GIRBA2553), Eulji General Hospital (201105-01), Chonnam National University Hospital (CNUH-2011-092), and Pusan Paik Hospital (11-091). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HJK and K-HO were involved with the conception and design of the study. HJK, YK, MK, SK, SKP, SS, YYH, JYJ, CA, and K-HO were involved with patient data collection and acquisition. HJK, MK, SKP, and K-HO performed the analysis and interpretation of data. Article draft and revision were carried out by HJK and K-HO. All authors approved the final manuscript.

FUNDING

This study was supported by the Research Program funded by the Korea Center for Disease Control and Prevention (2011E3300300, 2012E3301100, 2013E3301600, 2013E3301601, 2013E3301602, 2016E3300200, 2016E3300201, 2016E3300202, 2019E320100, 2019E320101, and 2022-11-007), the Bio and Medical Technology Development Program of the National Research Foundation (NRF), and funded by the Korean Government (MSIT) (2017M3A9E4044649). The funders had no role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

The authors gratefully thank the clinical research staff and nurses that participated in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.904963/full#supplementary-material

REFERENCES

1. Lu X, Hu MC. Klotho/FGF23 axis in chronic kidney disease and cardiovascular disease. *Kidney Dis.* (2017) 3:15–23. doi: 10.1159/000452880
2. Hu MC, Shiizaki K. Kuro-o M, Moe OW. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol.* (2013) 75:503–33. doi: 10.1146/annurev-physiol-030212-183727
3. Hu MC, Shi M, Zhang J, Quinones H, Griffith C, Kuro-o M, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* (2011) 22:124–36. doi: 10.1681/ASN.2009121311
4. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. *Am J Kidney Dis.* (2013) 62:339–51. doi: 10.1053/j.ajkd.2012.11.051
5. Seiler S, Heine GH, Fliser D. Clinical relevance of FGF-23 in chronic kidney disease. *Kidney Int Suppl.* (2009) 114:S34–42. doi: 10.1038/ki.2009.405
6. Isakova T, Wahl P, Vargas GS, Gutierrez OM, Scialla J, Xie H, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int.* (2011) 79:1370–8. doi: 10.1038/ki.2011.47
7. Liu QF Yu LX, Feng JH, Sun Q, Li SS, Ye JM. The prognostic role of klotho in patients with chronic kidney disease: a systematic review and meta-analysis. *Dis Markers.* (2019) 2019:6468729. doi: 10.1155/2019/6468729
8. Kim HR, Nam BY, Kim DW, Kang MW, Han JH, Lee MJ, et al. Circulating alpha-klotho levels in CKD and relationship to progression. *Am J Kidney Dis.* (2013) 61:899–909. doi: 10.1053/j.ajkd.2013.01.024
9. Drew DA, Katz R, Kritchevsky S, Ix J, Shlipak M, Gutierrez OM, et al. Association between soluble klotho and change in kidney function: the health aging and body composition study. *J Am Soc Nephrol.* (2017) 28:1859–66. doi: 10.1681/ASN.2016080828
10. Seiler S, Wen M, Roth HJ, Fehrenz M, Flugge F, Herath E, et al. Plasma Klotho is not related to kidney function and does not predict adverse

outcome in patients with chronic kidney disease. *Kidney Int.* (2013) 83:121–8. doi: 10.1038/ki.2012.288
11. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, et al. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. *J Am Soc Nephrol.* (2007) 18:2600–8. doi: 10.1681/ASN.2006080936
12. Zhang M, Yan J, Zhu M, Ni Z. Fibroblast growth factor 23 predicts coronary calcification and poor prognosis in patients with chronic kidney disease stages 3-5D. *Ann Clin Lab Sci.* (2015) 45:17–22.
13. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, et al. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int.* (2012) 23:2017–25. doi: 10.1007/s00198-011-1838-0
14. Marco GD, Brand M. Off-target effects and adverse outcomes of fibroblast growth factor 23 in chronic kidney disease. *Port J Nephrol Hypert.* (2018) 32:57–63.
15. Richter B, Faul C. FGF23 actions on target tissues-with and without klotho. *Front Endocrinol.* (2018) 9:189. doi: 10.3389/fendo.2018.00189
16. Hanudel M, Juppner H, Salusky IB. Fibroblast growth factor 23: fueling the fire. *Kidney Int.* (2016) 90:928–30. doi: 10.1016/j.kint.2016.08.013
17. Manghat P, Fraser WD, Wierzbicki AS, Fogelman I, Goldsmith DJ, Hampson G. Fibroblast growth factor-23 is associated with C-reactive protein, serum phosphate and bone mineral density in chronic kidney disease. *Osteoporos Int.* (2010) 21:1853–61. doi: 10.1007/s00198-009-1142-4
18. Silverstein DM. Inflammation in chronic kidney disease: role in the progression of renal and cardiovascular disease. *Pediatr Nephrol.* (2009) 24:1445–52. doi: 10.1007/s00467-008-1046-0
19. Oh KH, Park SK, Park HC, Chin HJ, Chae DW, Choi KH, et al. KNOW-CKD (KoreaN cohort study for Outcome in patients With Chronic Kidney Disease): design and methods. *BMC Nephrol.* (2014) 15:80. doi: 10.1186/1471-2369-15-80
20. Siekmann L. Determination of creatinine in human serum by isotope dilution-mass spectrometry. Definitive methods in clinical chemistry, IV. *J Clin Chem Clin Biochem.* (1985) 23:137–44. doi: 10.1515/cclm.1985.23.3.137
21. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, III, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
22. Chapter 1: Definition and classification of CKD. *Kidney Int Suppl 2011.* (2013) 3:19–62. doi: 10.1038/kisup.2012.64
23. National Institute on Alcohol Abuse and Alcoholism. NIAAA council approves definition of binge drinking. *NIAAA Newsletter.* (2004) 17:2
24. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* (2003) 35:1381–95. doi: 10.1249/01.MSS.0000078924.61453.FB
25. Chun MY. Validity and reliability of korean version of international physical activity questionnaire short form in the elderly. *Korean J Fam Med.* (2012) 33:144–51. doi: 10.4082/kjfm.2012.33.3.144
26. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955
27. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* (2015) 28:1–39.e14. doi: 10.1016/j.echo.2014.10.003
28. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis.* (1997) 132:245–50. doi: 10.1016/S0021-9150(97)00106-8
29. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* (1990) 15:827–32. doi: 10.1016/0735-1097(90)90282-T
30. Budoff MJ, Nasir K, McClelland RL, Detrano R, Wong N, Blumenthal RS, et al. Coronary calcium predicts events better with absolute calcium scores than age-sex-race/ethnicity percentiles: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol.* (2009) 53:345–52. doi: 10.1016/j.jacc.2008.07.072
31. Russo D, Corrao S, Battaglia Y, Andreucci M, Caiazza A, Carlomagno A, et al. Progression of coronary artery calcification and cardiac events in patients with chronic renal disease not receiving dialysis. *Kidney Int.* (2011) 80:112–8. doi: 10.1038/ki.2011.69
32. Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, et al. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation.* (2012) 126:2890–909. doi: 10.1161/CIR.0b013e318276fbcb
33. Liu QF, Ye JM, Yu LX, He AL, Sun Q, He DW, et al. Plasma s-Klotho is related to kidney function and predicts adverse renal outcomes in patients with advanced chronic kidney disease. *J Investig Med.* (2018) 66:669–75. doi: 10.1136/jim-2017-000560
34. Titan SM, Zatz R, Graciolli FG, dos Reis LM, Barros RT, Jorgetti V, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin J Am Soc Nephrol.* (2011) 6:241–7. doi: 10.2215/CJN.04250510
35. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. *J Am Soc Nephrol.* (2011) 22:1913–22. doi: 10.1681/ASN.2010121224
36. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone Klotho. *Science.* (2005) 309:1829–33. doi: 10.1126/science.1112766
37. Kuro-o M. Klotho as a regulator of oxidative stress and senescence. *Biol Chem.* (2008) 389:233–41. doi: 10.1515/BC.2008.028
38. Mitani H, Ishizaka N, Aizawa T, Ohno M, Usui S, Suzuki T, et al. *In vivo* klotho gene transfer ameliorates angiotensin II-induced renal damage. *Hypertension.* (2002) 39:838–43. doi: 10.1161/01.HYP.0000013734.33441.EA
39. Zhao Y, Banerjee S, Dey N, LeJeune WS, Sarkar PS, Brobey R, et al. Klotho depletion contributes to increased inflammation in kidney of the db/db mouse model of diabetes *via* RelA (serine)536 phosphorylation. *Diabetes.* (2011) 60:1907–16. doi: 10.2337/db10-1262
40. Hu MC, Shi M, Zhang J, Quinones H. Kuro-o M, Moe OW. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int.* (2010) 78:1240–51. doi: 10.1038/ki.2010.328
41. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest.* (2011) 121:4393–408. doi: 10.1172/JCI46122
42. Di Marco GS, Reuter S, Kentrup D, Ting L, Ting L, Grabner A, et al. Cardioprotective effect of calcineurin inhibition in an animal model of renal disease. *Eur Heart J.* (2011) 32:1935–45. doi: 10.1093/eurheartj/ehq436
43. Grabner A, Amaral AP, Schramm K, Singh S, Sloan A, Yanucil C, et al. Activation of cardiac fibroblast growth factor receptor 4 causes left ventricular hypertrophy. *Cell Metab.* (2015) 22:1020–32. doi: 10.1016/j.cmet.2015.09.002
44. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, et al. Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. *Kidney Int.* (2016) 90:985–96. doi: 10.1016/j.kint.2016.05.019
45. Han X, Li L, Yang J, King G, Xiao Z, Quarles LD. Counter-regulatory paracrine actions of FGF-23 and 1,25(OH)2 D in macrophages. *FEBS Lett.* (2016) 590:53–67. doi: 10.1002/1873-3468.12040
46. Dai B, David V, Martin A, Huang J, Li H, Jiao Y, et al. A comparative transcriptome analysis identifying FGF23 regulated genes in the kidney of a mouse CKD model. *PLoS ONE.* (2012) 7:e44161. doi: 10.1371/journal.pone.0044161
47. Rossaint J, Oehmichen J, Van Aken H, Reuter S, Pavenstadt HJ, Meersch M, et al. FGF23 signaling impairs neutrophil recruitment and host defense during CKD. *J Clin Invest.* (2016) 126:962–74. doi: 10.1172/JCI83470
48. Cohen G, Haag-Weber M, Horl WH. Immune dysfunction in uremia. *Kidney Int Suppl.* (1997) 62:S79–82.
49. Xie J, Yoon J, An SW, Kuro-o M, Huang CL. Soluble klotho protects against uremic cardiomyopathy independently of fibroblast growth factor 23 and phosphate. *J Am Soc Nephrol.* (2015) 26:1150–60. doi: 10.1681/ASN.2014040325

50. Yang K, Wang C, Nie L, Zhao X, Gu J, Guan X, et al. Klotho protects against indoxyl sulphate-induced myocardial hypertrophy. *J Am Soc Nephrol.* (2015) 26:2434–46. doi: 10.1681/ASN.2014060543
51. Leifheit-Nestler M, Haffner D. How FGF23 shapes multiple organs in chronic kidney disease. *Mol Cell Pediatr.* (2021) 8:12. doi: 10.1186/s40348-021-00123-x
52. Zhao Y, Zhao MM, Cai Y, Zheng MF, Sun WL, Zhang SY, et al. Mammalian target of rapamycin signaling inhibition ameliorates vascular calcification *via* Klotho upregulation. *Kidney Int.* (2015) 88:711–21. doi: 10.1038/ki.2015.160
53. Carpenter TO, Insogna KL, Zhang JH, Ellis B, Nieman S, Simpson C, et al. Circulating levels of soluble klotho and FGF23 in X-linked hypophosphatemia: circadian variance, effects of treatment, and relationship to parathyroid status. *J Clin Endocrinol Metab.* (2010) 95:E352–7. doi: 10.1210/jc.2010-0589
54. Smith ER, Cai MM, McMahon LP, Holt SG. Biological variability of plasma intact and C-terminal FGF23 measurements. *J Clin Endocrinol Metab.* (2012) 97:3357–65. doi: 10.1210/jc.2012-1811
55. Shimada T, Urakawa I, Isakova T, Yamazaki Y, Epstein M, Wesseling-Perry K, et al. Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. *J Clin Endocrinol Metab.* (2010) 95:578–85. doi: 10.1210/jc.2009-1603

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

REVIEW
published: 19 July 2022
doi: 10.3389/fmed.2022.877237

Application of Angiotensin Receptor–Neprilysin Inhibitor in Chronic Kidney Disease Patients: Chinese Expert Consensus

*Liangying Gan[1], Xiaoxi Lyu[2], Xiangdong Yang[3], Zhanzheng Zhao[4], Ying Tang[5], Yuanhan Chen[6], Ying Yao[7], Fuyuan Hong[8], Zhonghao Xu[9], Jihong Chen[10], Leyi Gu[11], Huijuan Mao[12], Ying Liu[13], Jing Sun[14], Zhu Zhou[15], Xuanyi Du[16], Hong Jiang[17], Yong Li[18], Ningling Sun[19], Xinling Liang[6] and Li Zuo[1]**

[1] Department of Nephrology, Peking University People's Hospital, Beijing, China, [2] Institute of Materia Medica, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China, [3] Qilu Hospital of Shangdong University, Qingdao, China, [4] First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [5] The Third Affiliated Hospital of Southern Medical University, Guangzhou, China, [6] Guangdong Provincial People's Hospital, Guangzhou, China, [7] Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China, [8] Fujian Provincial Hospital, Fuzhou, China, [9] Bethune First Hospital of Jilin University, Changchun, China, [10] Shenzhen Bao'an People's Hospital, Shenzhen, China, [11] Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, [12] Jiangsu Province Hospital, Nanjing, China, [13] First Affiliated Hospital of Dalian Medical University, Dalian, China, [14] Shandong Provincial Hospital, Jinan, China, [15] First Affiliated Hospital of Kunming Medical University, Kunming, China, [16] The Second Affiliated Hospital of Harbin Medical University, Harbin, China, [17] People's Hospital of Xinjiang, Urumqi, China, [18] Huashan Hospital, Fudan University, Shanghai, China, [19] Peking University People's Hospital, Beijing, China

OPEN ACCESS

Edited by:
Hong Liu,
Central South University, China

Reviewed by:
Vladimir Tesar,
Charles University, Czechia
Aniruddha Adhikari,
University of California, Los Angeles, United States

***Correspondence:**
Li Zuo
zuolimd@hotmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *16 February 2022*
Accepted: *20 June 2022*
Published: *19 July 2022*

Citation:
Gan L, Lyu X, Yang X, Zhao Z, Tang Y, Chen Y, Yao Y, Hong F, Xu Z, Chen J, Gu L, Mao H, Liu Y, Sun J, Zhou Z, Du X, Jiang H, Li Y, Sun N, Liang X and Zuo L (2022) Application of Angiotensin Receptor–Neprilysin Inhibitor in Chronic Kidney Disease Patients: Chinese Expert Consensus. Front. Med. 9:877237. doi: 10.3389/fmed.2022.877237

Chronic kidney disease (CKD) is a global public health problem, and cardiovascular disease is the most common cause of death in patients with CKD. The incidence and prevalence of cardiovascular events during the early stages of CKD increases significantly with a decline in renal function. More than 50% of dialysis patients die from cardiovascular disease, including coronary heart disease, heart failure, arrhythmia, and sudden cardiac death. Therefore, developing effective methods to control risk factors and improve prognosis is the primary focus during the diagnosis and treatment of CKD. For example, the SPRINT study demonstrated that CKD drugs are effective in reducing cardiovascular and cerebrovascular events by controlling blood pressure. Uncontrolled blood pressure not only increases the risk of these events but also accelerates the progression of CKD. A co-crystal complex of sacubitril, which is a neprilysin inhibitor, and valsartan, which is an angiotensin receptor blockade, has the potential to be widely used against CKD. Sacubitril inhibits neprilysin, which further reduces the degradation of natriuretic peptides and enhances the beneficial effects of the natriuretic peptide system. In contrast, valsartan alone can block the angiotensin II-1 (AT1) receptor and therefore inhibit the renin–angiotensin–aldosterone system. These two components can act synergistically to relax blood vessels, prevent and reverse cardiovascular remodeling, and promote natriuresis. Recent studies have repeatedly confirmed that the first and so far the only angiotensin receptor–neprilysin inhibitor (ARNI) sacubitril/valsartan can reduce blood pressure more effectively than renin–angiotensin system inhibitors and

improve the prognosis of heart failure in patients with CKD. Here, we propose clinical recommendations based on an expert consensus to guide ARNI-based therapeutics and reduce the occurrence of cardiovascular events in patients with CKD.

Keywords: chronic kidney disease, consensus, angiotensin receptor-neprilysin inhibitor, hypertension, ACEI/ARB

INTRODUCTION

Chronic kidney disease (CKD) is a global public health issue (1). In China, the prevalence of CKD is 7.18% and approximately 132 million patients have CKD, accounting for one fifth of all CKD patients in the world (2). CKD is usually progressive, and therefore worsens over time. In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) proposed CKD staging criteria based on the estimated glomerular filtration rate (eGFR) and urinary albumin levels because eGFR levels and the urinary albumin/creatinine ratio are both correlated with poor prognosis (3).

The risk of cardiovascular events is significantly increased under CKD, with cardiovascular diseases representing the major cause of death in patients with CKD (4). Compared with the general population, the incidence and prevalence of cardiovascular events are significantly higher during the early stages of CKD (CKD stages 1–3), and the risk increases exponentially as renal function declines (5). In patients undergoing hemodialysis and peritoneal dialysis, the prevalence of cardiovascular diseases is as high as 76.5 and 65%, respectively (6). In China, over 50% of dialysis patients die of cardiovascular and cerebrovascular events (7).

Traditional risk factors such as hypertension, hyperglycemia, and dyslipidemia, as well as non-traditional risk factors such as abnormal calcium and phosphorus metabolism, in addition to inflammation, are all implicated in the high incidence of cardiovascular events in patients with CKD (4).

Hypertension is one of the most common complications in patients with CKD (8), where the control rate is poor. That is, if 130/80 mmHg is taken as the target for blood pressure control, the control rate is only 11.9% (9). Elevated blood pressure not only promotes the progression of CKD (10), but also causes myocardial remodeling in patients with CKD and increases the risk of cardiovascular events (11). Cardiovascular complications in patients with CKD include coronary heart disease, heart failure, arrhythmia, sudden death, and others. Myocardial changes observed in patients with CKD, such as early-onset left ventricular hypertrophy, significant myocardial fibrosis, and sparse capillaries, constitute the pathological basis leading to these cardiovascular events (4). The occurrence of heart failure is also related to hypertension and increased volume load, which become more difficult to control with the progression of CKD.

Generally, the blood pressure target for patients with CKD and hypertension is stricter than that for regular patients with hypertension. The SPRINT study confirmed that intensive blood pressure reduction benefited patients with CKD by alleviating cardiovascular and cerebrovascular events (12). Based on the results of the SPRINT study and a meta-analysis, the 2021 KDIGO CKD hypertension management guidelines recommended a systolic blood pressure of 120 mmHg as the optimal blood pressure reduction target (13).

Antihypertensive treatment for patients with CKD usually includes lifestyle intervention in conjunction with drug treatment (13). All five major types of antihypertensive drugs can be used for blood pressure control in patients with CKD (13). Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) not only lower blood pressure, but also exert a protective effect on target organs, as exemplified by their positive influence on renal hemodynamics and urinary albumin-reducing effects (14), which can delay the progression of CKD. As such, these drugs represent first-line treatment options for hypertension management in patients with CKD (13, 15). However, because of the complicated pathogenesis of hypertension in these patients, most require two or more antihypertension drugs at higher doses for adequate blood pressure control. Epidemiological surveys in China show that more than 60% of patients with CKD failed to achieve the blood pressure target (16, 17). In addition, ACEIs and ARBs are the first-line treatment for reducing all-cause and cardiovascular-related deaths in patients with CKD as well as heart failure with reduced ejection fraction (HFrEF) (18, 19). Renin–angiotensin–aldosterone system (RAAS) inhibitors should also be considered because of their effects on serum potassium and fluctuations in serum creatinine levels caused by hemodynamic changes, such as reduced eGFR (18). However, there is almost no evidence that RAAS inhibition improves the prognosis of patients with CKD exhibiting heart failure with preserved ejection fraction (HFpEF) (20).

Natriuretic peptides, including atrial, B-type, and C-type natriuretic peptides, are a group of polypeptides with a wide range of physiological effects, including the promotion of natriuresis and diuresis, vasodilation, RAAS inhibition, reduction of sympathetic nerve activity, and the suppression of proliferation and fibrosis (21). Sacubitril/valsartan, a compound with a co-crystal structure composed of the neprilysin inhibitor sacubitril and angiotensin receptor blocker valsartan, is the first dual angiotensin receptor and neprilysin inhibitor (22, 23). Sacubitril is metabolized into LBQ657, which is an active metabolite that effectively inhibits neprilysin, thereby suppressing the neprilysin-mediated degradation of natriuretic peptides and enhancing the beneficial effect of the natriuretic peptide system. Valsartan can block the angiotensin II-1 (AT1) receptor to inhibit RAAS. Thus, these two components can act synergistically to dilate blood vessels, prevent or reverse cardiovascular remodeling, and promote natriuresis (23).

In recent years, studies have successively confirmed that the first and so far the only angiotensin receptor–neprilysin inhibitor (ARNI) sacubitril/valsartan can reduce blood pressure

in patients with CKD more effectively than RAAS inhibitors and can improve the prognosis in patients with heart failure (24). Considering current approaches to the diagnosis and treatment of CKD accompanied by hypertension and heart failure, as well as the need to better guide the application of ARNI in patients with CKD, we formulate a consensus based on clinical evidence and experience. In this consensus, ARNI refers specifically to sacubitril/valsartan. The strength of recommendation in this consensus is indicated as Level 1 or Level 2, the quality of supporting evidence is shown as A, B, C, or D following KDIGO guidelines (13).

EFFECTS OF CKD ON THE *IN VIVO* METABOLISM OF ARNI, DOSE ADJUSTMENT, AND DRUG INTERACTIONS

Is It Necessary to Adjust the Dosage of ARNI for Patients With Hypoproteinemia?

Suggestion 2.1: When ARNI is used for patients with hypoproteinemia (serum albumin <25 g/L), titration should be started from a low dose. The dosage and frequency that is suitable for patients should then fall within a safe-dose range (see Suggestion 2.2 for hypoproteinemia caused by hepatic dysfunction). *(2D)*

Rationale: ARNI has a high protein binding rate (16, 25). The protein binding rates of sacubitril, valsartan, and sacubitril metabolite LBQ657 are 97, 96, and 97%, respectively (25). Therefore, under hypoproteinemia, the conventional dose may result in a higher free drug concentration and enhanced efficacy of ARNI, in turn leading to side effects such as hypotension, diuresis, electrolyte disturbance, and a rapid increase in serum creatinine levels. Further, the apparent distribution volume and drug clearance of LBQ657 and valsartan increase under hypoproteinemia. As drugs are excreted faster, the frequency of medication should be adjusted accordingly.

Adjusting the Dose for Patients With Hepatic Dysfunction

Suggestion 2.2: For patients with mild hepatic impairment (Child–Pugh A), there is no need to adjust the loading dose, whereas in cases of moderate hepatic impairment (Child–Pugh B), the loading dose should be halved and the dose should be gradually increased to the most suitable dose that the patient can tolerate. ARNI is not recommended for patients with severe hepatic impairment. *(1B)*

Rationale: A pharmacokinetic study in patients with mild to moderate hepatic impairment revealed that, compared with healthy subjects, exposure to sacubitril, LBQ657, and valsartan was slightly increased in patients with mild hepatic impairment (by 1.5, 1.5, and 1.2 times, respectively). In patients with moderate hepatic impairment, the exposure increased by 3.4, 1.9, and 2.1 times, respectively (16). In another clinical trial involving 32 patients with mild to moderate hepatic dysfunction, the AUC of sacubitril increased by 53–245%, that of LBQ657 increased by 48–90%, and that of valsartan increased by 19–109%. Further, the C_{max} of LBQ657 and valsartan were not significantly different between patients with mild and moderate hepatic dysfunction (26). Therefore, we suggest that there is no need to adjust the loading dose in patients with mild hepatic impairment; however, the loading dose should be halved in those with moderate hepatic impairment. The above liver function staging refers to Child-Pugh staging, see **Supplementary Table 1** for details. At present, there are no pharmacokinetic data for patients with severe hepatic impairment, biliary cirrhosis, or cholestasis; thus, the use of ARNI is not recommended in the case of severe hepatic impairment (16).

Use of ARNI in Patients With Heart Failure

ARNI in Patients With Chronic Heart Failure

Suggestion 2.3.1: In patients with chronic heart failure, we suggest beginning with a low dose then gradually increasing the dosage. In patients who have not previously taken ACEIs or ARBs, the recommended loading dose is 50 mg twice-daily (BID). Thereafter, depending on the patient's tolerance, the dose should be doubled every 2–4 weeks until the target dose of 200 mg BID is reached. For patients who have previously used ARBs, we suggest converting to the appropriate ARNI dose according to the previous ARB dose. For patients who have used ACEIs, it is necessary to discontinue the drug for more than 36 h before converting to an appropriate ARNI dose with reference to the original ACEI dose.

Rationale: For patients with heart failure, it is usually appropriate to start ARNI treatment at a relatively low dose to improve tolerance to the initial treatment. The target dose of 200 mg BID is based on previous studies, which showed that 200 mg ARNI can fully inhibit neprilysin and enhance the activity of natriuretic peptides (27, 28). In the PARADIGM-HF study, ARNI was administered to patients with HFrEF, starting with 100 mg BID and gradually increasing to 200 mg BID. By the end of the study, 76% of the patients could still maintain the target dose, which indicated that this dosage regimen was well tolerated (29). The same dosage regimen of ARNI was also adopted for HFpEF patients in the PARAGON-HF study. By the end of the study, although no significant benefit was seen for the primary endpoint in the overall HFpEF population, 82% of the patients could maintain the target dose, which indicated that this regimen was also well tolerated (30).

How Long After Acute Heart Failure Can ARNI Be Administered?

Suggestion 2.3.2: In patients with acute heart failure, ARNI treatment should be initiated from a low dose immediately after reaching hemodynamic stability. The criteria for hemodynamic stability are shown in **Supplementary Table 2**. *(1A)*

Rationale: The PIONEER-HF and TRANSITION studies evaluated the benefits of initial ARNI treatment after stabilization of acute heart failure (31, 32). The PIONEER-HF study showed that starting ARNI directly after reaching hemodynamic stability in patients with acute heart failure significantly reduced the risk of composite clinical endpoints (death, rehospitalization owing to heart failure, and left ventricular assist device implantation) by 31% ($P < 0.002$) (32). Analysis of a high-risk subgroup in the PIONEER-HF study (**Supplementary Tables 3, 4**) indicated

that the benefits of ARNI in reducing cardiovascular death or rehospitalization owing to heart failure were comparable to those of ACEI treatment in the high-risk subgroup. Further, the risks of adverse reactions, including the deterioration of renal function, symptomatic hypotension, and hyperkalemia, were similar between high-risk and low-risk patients (33). The TRANSITION study demonstrated that, in patients with HFrEF who required hospitalization because of acute decompensated heart failure, the early initiation of ARNI treatment after reaching hemodynamic stability during hospitalization reduced the rehospitalization rate after discharge, with nearly half of the patients reaching the target dose within 10 weeks. ARNI initiation before discharge was well tolerated, and relatively few patients permanently discontinued the drug because of adverse reactions (31). Therefore, for patients with hemodynamic stability, ARNI should be initiated as early as possible with dose titration.

Are There Any Drug Interactions or Other Matters Requiring Attention When Using ARNI in Combination With Other Agents?

Can ARNI Be Used With ACEI?

Suggestion 2.4.1: Concomitant use of ARNI and ACEI should be avoided (**Table 1**), and it is recommended to wait 36 h after ACEI is discontinued before using ARNI. *(1C)*

Rationale: The concomitant use of ACEI while inhibiting neprilysin may increase the risk of angioedema (16). The development of a compound preparation comprising an neprilysin inhibitor and ACEI was previously halted because of severe angioedema and a significantly increased mortality rate (34). Therefore, it is recommended to initiate ARNI 36 h after the last dose of ACEI (16).

Can ARNI Be Used With SGLT2i?

Suggestion 2.4.2: ARNI can be used with SGLT2i in patients with heart failure. When used with diuretics, the dose should be adjusted. *(2B)*

Rationale: The mechanisms of action for ARNI and SGLT2i are relatively clear. ARNI acts on the natriuretic peptide system and RAAS, which blocks the adverse effects of the latter, while also enhancing the beneficial effects of the former, thereby playing a role in reversing cardiac remodeling and improving heart failure (35). SGLT2i can increase urine glucose excretion, natriuresis, and osmotic diuresis, and can act on myocardial cells to improve cardiac function (36). As ARNI and SGLT2i act through distinct mechanisms without overlap, they can be used in combination. In a randomized controlled study of SGLT-2i dapagliflozin (DAPA-HF study), 11% of patients used the combination with ARNI, which was shown to be safe and effective (37). Therefore, the two drugs can be used together by patients with heart failure. In a multicenter observational study, adding ARNI to SGLT2i for T2DM patients with HFrEF afforded better protection against renal function (38). Moreover, a study evaluated the safety and efficacy of the ARNI+SGLT2i combination in patients with HFrEF and diabetes, indicated that ARNI+SGLT2i improved the clinical and renal prognosis more significantly than ACEI/ARB+other hypoglycemic drug regimens (39). Moreover, combining SGLT2i with ARNI further reduced the risk of hospitalization owing to heart failure as well as that of major composite endpoints (hospitalization owing to heart failure or all-cause death). The combination was well tolerated, and the risks of creatinine elevation and hyperkalemia were lower than those for ACEI/ARB (39). However, in clinical practice, attention should be paid to the increased risk of hypovolemia when combining ARNI with SGLT2i. In the DAPA-HF study, SGLT2i combined with ARNI resulted in hypovolemia in 10.8% of patients (37). When used in combination, diuretics may synergistically act on nephrons with ARNI, mineralocorticoid receptor antagonists (MRA), and SGLT-2i to enhance the effects on natriuresis and diuresis. Therefore, it is necessary to adjust the doses of diuretics in time, then adjust the ARNI and SGLT-2i dosage as necessary.

Incompatibilities and Precautions for Concomitant Use With Other Drugs

Suggestion 2.4.3.1: ARNI is contraindicated in patients with a previous history of angioedema (**Table 1**). *(1C)*

Rationale: Patients with a previous history of angioedema may have an increased risk of angioedema during ARNI treatment (16), which is related to the reduced inactivation of bradykinin and substance P (40). Therefore, ARNI should not be used in patients with a known history of angioedema associated with ACEI/ARB treatment or hereditary angioedema (37). In the PARADIGM-HF study, 0.5% of patients treated with ARNI developed angioedema (37). Angioedema limited to the face and lips can generally be relieved without treatment, and antihistamines can also be applied to help relieve symptoms. Angioedema with laryngeal edema may endanger the life of patients; therefore, timely and appropriate treatment is required to keep the airway unobstructed (16).

Suggestion 2.4.3.2: ARNI in combination with aliskiren is not recommended (**Table 1**). *(1C)*

Rationale: Aliskiren is a direct inhibitor of renin, and its combination with ACEI or ARB may cause dual inhibition of the renin–angiotensin system and increase the risk of poor prognosis (41). According to several randomized controlled trials using aliskiren conducted by the European Medicines Agency, aliskiren used in combination with ACEI/ARB, especially in patients with impaired renal function, increased the risk of adverse events (e.g., hypotension, syncope, hyperkalemia, and acute renal failure) (42). Therefore, we suggest avoiding the use of aliskiren

TABLE 1 | Conditions and reasons not treated with ARNI.

Conditions and reasons not treated with ARNI
Concomitant use with ACEI: may increase the risk of angioedema
In patients with a previous history of angioedema: may increase the risk of angioedema
Concomitant use with aliskiren: may increase the risk of adverse events
In pregnant women: may cause fetal injury

ARNI, angiotensin receptor–neprilysin inhibitor.

combined with ACEI or ARB. Although there is currently no evidence related to the combination of ARNI and aliskiren, from a mechanistic perspective, ARNI combined with aliskiren may also lead to dual inhibition of the renin–angiotensin system and increase the risk of adverse reactions; therefore, their combined use is also not recommended (16).

Suggestion 2.4.3.3: ARNI combined with spironolactone, amiloride, or potassium salt increases the risk of hyperkalemia (*1A*).

Rationale: ARNI may induce hyperkalemia. In the PARADIGM-HF study, 12% of patients receiving ARNI and 14% of those receiving enalapril developed hyperkalemia. Thus, the concomitant use of spironolactone, amiloride, or potassium salt with ARNI may increase potassium levels in the blood. In severe cases, hyperkalemia can lead to renal failure, muscular paralysis, arrhythmia, and cardiac arrest. Therefore, when using ARNI in combination with any of these three agents, close attention should be paid to serum potassium levels to avoid hyperkalemia.

Suggestion 2.4.3.4: ARNI is contraindicated in pregnant women to avoid the risk of teratogenicity at the early stage as well as the inconvenience and risk of subsequent drug adjustment (**Table 1**). *(2C)*

Rationale: The application of ARNI in pregnant women may cause fetal injury. Further, the application of drugs that inhibit the RAAS in the second and third trimesters may affect fetal renal function and increase the risk of illness and death of the fetus and neonate (16). In animal studies, the use of ARNI in rats and rabbits during organogenesis led to an increase in embryo-fetal mortality, and teratogenicity was observed in rabbits (16). Therefore, ARNI is not recommended for use in pregnant women in order to avoid the risk of early-stage teratogenicity as well as the inconvenience and risk of subsequent drug adjustment.

Will the Efficacy of ARNI Be Affected if Taken With a Meal?

Suggestion 2.5: ARNI intake with food has no effect on its clinical efficacy. *(2B)*

Rationale: When ARNI was taken with a meal, exposure to sacubitril and valsartan was significantly lower, whereas exposure to the active metabolite LBQ657 exhibited no significant change (43). Although both sacubitril and valsartan exposure decreased, this decrease did not result in a clinically significant decrease in efficacy (16, 43). Therefore, ARNI can be taken with or without a meal (16, 43).

USE OF ARNI IN NON-DIALYSIS PATIENTS WITH CKD

What Are the Indications of ARNI in Non-dialysis Patients With CKD?

Suggestion 3.1.1: ARNI is recommended for non-dialysis patients with CKD and heart failure. *(1A)*

Rationale: The efficacy of ARNI in non-dialysis patients with CKD and heart failure has been confirmed by several studies. Analysis of the CKD subgroup in the PARADIGM-HF study (eGFR: 30–60 mL/min/1.73 m^2) revealed that ARNI reduced the risk of cardiovascular events compared with the ACEI-treatment group, with the risk of cardiovascular death decreasing by 24%, the risk of hospitalization owing to heart failure and all-cause death decreasing by 21%, and the risk of preset composite kidney events was reduced by 36% (the first occurrence of eGFR decreasing by 50% compared to the patient's baseline, decreasing from >30 to <60 mL/min/1.73 m^2, or progressing to end-stage renal disease) (29, 44). At the same time, UACR was slightly increased in the ARNI treatment group accompanied by a delay in the decline of eGFR, suggesting that improved renal perfusion due to improved cardiac function may be the reason for the increase of UACR (44). A real-world study in Taiwan also showed that, in patients with CKD and HFrEF with different eGFR values (including 102 patients with GFR<30 mL/min/1.73 m^2), ARNI treatment significantly reduced the risk of cardiovascular death and hospitalization owing to heart failure (45). In addition, a real-world study in Italy also showed that ARNI treatment for 12 months significantly increased eGFR in patients with CKD from the baseline ($P = 0.011$) (46). In patients with CKD exhibiting HFpEF, analysis of the CKD subgroup (eGFR: 30–60 mL/min/1.73 m^2) in the PARAGON-HF study showed that ARNI significantly reduced the risk of the renal composite endpoint by 50% compared with ARB (30). A recent retrospective cohort study in Taiwan showed that, in patients with CKD exhibiting HFpEF (average eGFR: 70.9 mL/min/1.73 m^2), the eGFR under ARNI treatment was significantly higher than that under valsartan treatment ($P < 0.01$) (47). Further, a recent meta-analysis revealed that ARNI significantly improved eGFR and reduced the level of NT-proBNP compared with ACEI/ARBs (48).

Suggestion 3.1.2: ARNI is recommended for non-dialysis patients with CKD exhibiting hypertension. *(1B)*

Rationale: ARNI can simultaneously act on the RAAS and natriuretic peptide system, thereby reducing blood pressure via multiple mechanisms and protecting target organs (49). More than 10 randomized controlled studies have confirmed the antihypertensive efficacy of ARNI (50). A meta-analysis involving five randomized controlled studies compared the efficacy of ARNI and ARB in patients with hypertension. After 12 weeks of treatment, ARNI more significantly reduced the mean sitting systolic blood pressure (SBP), mean sitting diastolic blood pressure (DBP), mean ambulatory SBP, and mean ambulatory DBP by 5.41 mmHg, 1.22 mmHg, 4.58 mmHg, and 2.17 mmHg, respectively, than ARB (olmesartan or valsartan) (51). The efficacy of ARNI in patients with CKD exhibiting hypertension has also been confirmed. In a multicenter, open-label study in Japan, 32 patients with CKD exhibiting hypertension were included; after ARNI treatment for 8 weeks, their average SBP and DBP in the sitting position decreased by 20.5 ± 11.3 and 8.3 ± 6.3 mmHg, respectively (52). The PARAMETER study showed that ARNI could control nocturnal blood pressure better than olmesartan. Further, as nocturnal hypertension is common in patients with CKD, ARNI could be the preferred treatment for patients with CKD exhibiting hypertension (53).

When ARNI Is Used in Patients With Renal Impairment, How Should the Dose Titration Be Performed?

Suggestion 3.2.1: In patients with heart failure accompanied by mild renal impairment, there is no need to reduce the dose. In those with moderate or severe renal impairment, 25–50 mg BID is recommended as the loading dose (depending on the patient's blood pressure). If the patient can tolerate this dose, the dose should be doubled every 2–4 weeks until reaching the target maintenance dose of 200 mg BID (**Table 2**). *(2B)*

Rationale: In patients with renal impairment, exposure to sacubitril active metabolite LBQ657 was affected, whereas exposure to sacubitril and valsartan was not affected, and the difference in LBQ657 exposure was not significant under mild renal impairment. However, in patients with moderate, severe, and end-stage renal disease (non-dialysis), the exposure increased by 2.29, 2.90, and 3.27 times, respectively (54). Furthermore, the LBQ657 half-life was prolonged from 12 h to 21.1 h, 23.7 h, and 38.5 h, respectively, in patients with mild, moderate, and severe renal impairment (54). Therefore, it is not necessary to adjust the dose of ARNI for patients with mild renal impairment. For those with moderate and severe renal impairment, it is recommended to start with 25–50 mg BID and gradually increase the dose (16). The diagnosis criteria for mild, moderate, and severe renal impairment are shown in **Supplementary Table 5**.

Suggestion 3.2.2: When used in patients with essential hypertension, the conventional loading dose is 200 mg once-daily (QD). For patients whose blood pressure cannot be sufficiently controlled via a single administration daily, the dose can be increased to 400 mg QD *(1B)*. In patients with CKD, a twice-daily dosage regimen can also be adopted *(2C)*. For patients with mild renal impairment (eGFR 60–90 mL/min/1.73 m^2), there is no need to adjust the dose (1B), whereas for those with moderate and severe renal impairment (eGFR 15–60 mL/min/1.73 m^2), it is recommended to reduce the loading dose to 100mg QD (**Table 2**) *(2C)*. However, considering the minimal experience of patients with end-stage renal disease, these clinical findings should also be considered in this case *(2C)*.

Rationale: In terms of pharmacokinetics, the half-lives of LBQ657 and valsartan after a single dose of ARNI 200 mg are 11.5 h and 9.9 h (55). Thus, once-daily administration can be adopted. The choice of 200 mg or 400 mg QD for patients with hypertension is based on two Phase II dose-finding studies, which showed that these dosages could improve ambulatory blood pressure and office blood pressure more effectively than the control treatment, while being safe and tolerable (28, 56). In several randomized control trials on ARNI for the treatment of hypertension, once-daily administration was adopted, and the results indicated that ARNI was more effective at lowering blood pressure, including ambulatory blood pressure and nocturnal blood pressure, than the control conditions (53, 57–60). Among patients with renal impairment, mild impairment did not affect exposure to the main components and metabolites of ARNI, whereas in patients with moderate and severe renal impairment, exposure to LBQ657 was significantly increased, by 2–3 times. Therefore, for patients with moderate and severe renal impairment, we suggest adopting a low loading dose then gradually increasing to a suitable target dose (54). In a study from Japan, patients with CKD and hypertension (eGFR15–60 mL/min/1.73m^2) were treated with ARNI 100–400 mg QD for 8 weeks according to their blood pressure. At the end of the study, 26 patients received LCZ696 at 200 mg QD, whereas 18 patients received LCZ696 at 400 mg QD. During the eight-week treatment period, no serious adverse reactions were noted. These results indicated that the regimen was generally well tolerated, with no adverse events such as hypotension and angioedema (52). At present, there is no medical data on patients with end-stage renal disease and eGFR<15 mL/min/1.73 m^2. Thus, it is necessary to evaluate the benefits and risks in these patients based on clinical observations. Currently, there is no evidence as to whether BID administration of ARNI can further improve nocturnal blood pressure compared to once-daily administration. However, patients with CKD often suffer from nocturnal hypertension. Thus, a twice-daily regimen can also be used in these patients if necessary.

What Possible Side Effects Should Be Considered During Titration of the ARNI Dose?

Hypovolemia and Hypotension

Suggestion 3.3.1: For patients with symptomatic hypovolemia, the blood pressure should be closely monitored. For patients with SBP<100 mmHg or hypotension symptoms, we recommend first adjusting the doses of diuretics and combined antihypertensive medication, treating other causes of hypotension, and correcting hypovolemia via oral or intravenous rehydration. *(1C)*

Rationale: In the PARADIGM-HF study, 14% of patients treated with ARNI developed symptomatic hypotension (SBP<100 mmHg), which was slightly higher than the proportion in the enalapril group (9.2%) (29). In a meta-analysis of three studies comparing ARNI and ACEI/ARB therapies, although the incidence of hypotension in patients receiving ARNI was slightly higher than that in those receiving ACEI/ARB, there was no statistically significant difference (48). We suggest that hypovolemia should be corrected before initiating ARNI, or a low loading dose should be used to reduce the risk of hypotension. If hypotension occurs during treatment, we suggest first adjusting the doses of diuretics and combined antihypertensive drugs, then treating other causes of hypotension (16). If hypotension persists after taking the above measures, dose reduction or temporary discontinuation of ARNI may be considered (16). Usually, there is no need to permanently discontinue treatment.

Hyperkalemia and Other Electrolyte Disorders

Suggestion 3.3.2: For patients with hyperkalemia (≥5.0 mmol/L), we recommend stopping potassium supplementation and MRA, and lowering potassium. If necessary, dose reduction or discontinuation of ARNI may be considered. *(1B)*

Rationale: ARNI treatment may lead to an increase in serum potassium because of its effect on the RAAS (16).

TABLE 2 | Dosage recommendations for ARNI in CKD.

Conditions treated with ARNI			Initial doses	Target doses
Patients with non-dialysis CKD	Heart failure	eGFR≥60 mL/min/1.73 m^2	50 mg BID	200 mg BID
		eGFR<60 mL/min/1.73 m^2	25–50 mg BID	
	Hypertension	eGFR≥60 mL/min/1.73 m^2	200 mg QD or 100 mg BID	400 mg QD or 200 mg BID
		eGFR 15-60 mL/min/1.73 m^2	100 mg QD or 50 mg BID	
Patients with CKD undergoing maintenance dialysis: heart failure or hypertension			25–50 mg QD	100–150 mg QD to BID

ARNI, angiotensin receptor–neprilysin inhibitor; eGFR, estimated glomerular filtration rate; QD, once-daily; BID, twice-daily.

In the PARADIGM-HF study, 12.3% of patients treated with ARNI reported hyperkalemia (serum potassium >5 mmol/L); the incidence was slightly lower than that after treatment with enalapril (13.5%) (61). In the UK HARP-III study, the incidence of hyperkalemia in patients with CKD treated with ARNI or irbesartan (GFR: 20–60 mL/min/1.73 m^2) was similar (32 vs. 24%, $P = 0.10$) (62). For patients with hyperkalemia, priority should be given to controlling the risk factors that may lead to high potassium, potassium supplementation and MRA should be discontinued, potassium-lowering drugs should be used as necessary, and down-titration or discontinuation of ARNI should be considered according to the situation (16). The serum potassium levels of patients should be closely monitored, and ARNI treatment should be gradually resumed after the serum potassium level returns to normal (<5.0 mmol/L).

Rapid Decline of Renal Function

Suggestion 3.3.3: There are no available clinical trial data regarding the respective evaluation criteria or treatment methods for patients exhibiting a rapid decline in renal function resulting from ARNI treatment. Thus, treatment methods for renal function decline due to RAAS inhibitor treatment can be referred to instead. First, it is necessary to clarify the reasons for the decline of renal function. After excluding renal artery stenosis as the culprit, if the serum creatinine levels increase by <30% compared with the baseline value, ARNI can be used continuously. If serum creatinine levels exceed the baseline level by 30%, the dose should be reduced or ARNI should be discontinued in time, and the underlying reasons should be identified. If serum creatinine levels exceed the baseline level by 50%, it is recommended to discontinue ARNI. *(1C)*

Rationale: Because of its effect on the RAAS, ARNI treatment may lead to an increase in creatinine. In the PARADIGM-HF study, 5% of patients treated with ARNI reported renal failure (63). For patients exhibiting a rapid decline of renal function, we suggest correcting/excluding other potential culprits (e.g., drugs affecting renal function, hypovolemia, or urinary tract infection), then considering down-titration of ARNI according to the situation. If the patient's serum creatinine increases by <30% compared with the baseline value, ARNI can be used continuously. If the serum creatinine level exceeds the baseline level by 30%, the dose should be reduced or ARNI should be discontinued in time, and the reasons should be identified (64).

Dose Adjustment of Hypoglycemic Drugs

Suggestion 3.3.4.: In patients with Stage 3–5 CKD, doses of hypoglycemic drugs should be adjusted according to their renal function. When ARNI is combined with common oral hypoglycemic drugs, no clinically significant drug interaction is observed; however, ARNI can improve blood glucose control. Therefore, we suggest monitoring blood glucose levels and appropriately adjusting the dose of insulin secretagogue or insulin. *(2B)*

Rationale: When patients with diabetes exhibit renal insufficiency, renal gluconeogenesis is weakened and the clearance rates of some drugs are reduced, which may in turn increase the risk of hypoglycemia. In addition, some hypoglycemic drugs or their metabolites can accumulate in the kidneys, which further aggravates renal insufficiency. Therefore, it is necessary to select appropriate hypoglycemic drugs and dosage based on the renal function of patients with CKD (65). At present, it has only been observed that ARNI combined with metformin might cause a slight decrease in metformin exposure, with no effect on clinical efficacy. Thus, there is no need to adjust the dose of metformin. To date, no drug interaction between ANRI and other hypoglycemic drugs has been reported (16). Some studies have shown that ACEI-enkephalinase inhibitor treatment could improve insulin-mediated glucose processing, induce insulin sensitization, and improve blood glucose control (66, 67). Therefore, when ARNI is used in combination with hypoglycemic drugs, we suggest closely monitoring blood glucose and appropriately reducing the dose of insulin secretagogue or insulin in patients with a high risk of hypoglycemia to reduce this risk.

Who Should Switch to ARNI Among Patients Using ACEIs/ARBs?

For Patients With Heart Failure, We Recommend Switching to ARNI

Suggestion 3.4.1: For patients with CKD exhibiting heart failure, ACEI/ARB can be switched to ARNI, as long as there is no contraindication, to improve eGFR, reverse cardiac remodeling, and reduce the risk of end-stage renal disease and cardiovascular events. *(1A)*

Rationale: The PARADIGM-HF study showed that, compared with ACEI, ARNI significantly delayed the decline rate of eGFR in patients with HFrEF by 23.5%, and reduced the risk of cardiac and renal adverse events in patients with CKD and HFrEF (29). The PARAGON study confirmed that, compared with ARB, ARNI significantly reduced the risk of adverse renal outcomes in patients with HFpEF and eGFR<60 mL/min/1.73 m^2 (30). The EVALUATE-HF study showed that, compared with enalapril, ARNI rapidly reversed cardiac remodeling after 3 months of treatment. The UK-HARP-III study revealed that, compared with ARB, ARNI could significantly improve NT-proBNP and troponin levels, while effectively reducing blood pressure and proteinuria in patients with CKD. A meta-analysis including 3,640 patients with heart failure and CKD from three randomized controlled trials (HARP-III, PARADIGM-HF, and PARAMOUNT) indicated that, compared with ACEI/ARB, ARNI significantly improved eGFR and decreased blood pressure and NT-proBNP levels (48).

If Blood Pressure Is Not Well Controlled, We Recommend Switching to ARNI

Suggestion 3.4.2.1: For patients with CKD and poor blood pressure control, after a whole day of treatment with ACEI/ARB, we suggest switching ACEI/ARB to ARNI to further improve blood pressure control. *(1B)*

Rationale: A meta-analysis including five randomized controlled studies confirmed that, compared with ACEI/ARB, ARNI more effectively reduced the mean sitting arterial pressure (weighted mean difference: −5.41 mmHg, $P < 0.01$) and diastolic pressure (weighted mean difference: −1.22 mmHg, $P < 0.01$) (68). An eight-week multicenter, open-label study showed that ARNI treatment for 1 week reduced the office SBP, DBP, and pulse pressure of patients with severe hypertension by 18.7, 10.3, and 8.3 mmHg, respectively, with a more significant decrease observed in the 8 week (69). A meta-analysis including 6,064 patients with hypertension from 12 studies, of which four studies compared the antihypertensive effects of 400 mg ARNI and ARB for 8 weeks, revealed that ARNI caused a more significant reduction in 24 h ambulatory SBP (by 4.31 mmHg) and ambulatory DBP (by 1.69 mmHg) than ARB (50).

Suggestion 3.4.2.2: For patients with CKD and poor nocturnal blood pressure control treated with ACEI/ARB, we suggest switching ACEI/ARB to ARNI to further improve nocturnal blood pressure control. *(1B)*

Rationale: A randomized controlled study in patients with hypertension in Asia showed that ARNI treatment for 8 weeks effectively reduces nocturnal ambulatory SBP by up to 16.14 mmHg (56). Another randomized, double-blind, placebo-controlled, and active drug-controlled study including 1,328 patients with hypertension showed that, compared with ARB, the nocturnal mean sitting SBP of patients treated with ARNI decreased significantly by up to 9.01 mmHg (28). A different randomized controlled study revealed that, compared with olmesartan, ARNI further reduced the nocturnal mean ambulatory SBP of elderly patients with hypertension by 5.9 mmHg (53).

For Patients With Heart Failure Whose Serum Potassium Tends to Increase When Using ACEIs/ARBs, We Suggest Switching to ARNI After Serum Potassium Decreases to the Normal Range

Suggestion 3.4.3: For patients with CKD and heart failure, and whose serum potassium tends to increase when using ACEIs/ARBs, we recommend switching to ARNI after serum potassium levels decrease to the normal range so as to reduce the risk of hyperkalemia. *(2B)*

Rationale: The use of ACEI/ARB will decrease potassium excretion through the kidneys and increase serum potassium levels. In the PARADIGM-HF study (which excluded patients with eGFR<30 mL/min/1.73 m^2), the serum potassium level was lower in the ARNI treatment group than in the enalapril group, and the incidence of hyperkalemia and severe hyperkalemia was significantly reduced, with even patients treated in combination with MRA exhibiting a significantly lower risk of hyperkalemia (61, 70). Moreover, the incidence of hyperkalemia was significantly lower in the ARNI group than in the valsartan group (13.2 vs. 15.3%, $P = 0.048$) (30). A meta-analysis of 11 randomized controlled trials comparing ARNI with RAAS inhibitors showed that, compared with those receiving ACEI/ARB, the ARNI group had a slightly lower risk of hyperkalemia (RR = 0.95; 95% CI = 0.88–1.02) (71). A meta-analysis published in ASN in 2020 showed similar results (72); however, patients with heart failure were mainly included in these studies, whereas patients with eGFR<30 mL/min/1.73 m^2 or serum creatinine level > 221 μmol were excluded. To data, there are no data on patients without heart failure.

For Patients With Heart Failure Whose Serum Creatinine Levels Rise Sharply, We Suggest Discontinuing ACEI/ARB and Switching to ARNI

Suggestion 3.4.4: For patients with CKD exhibiting heart failure who are treated with ACEI/ARB and whose serum creatinine has increased substantially (by >50% or ≥266 μmol/L), we recommend discontinuing ACEI/ARB, monitoring renal function, and switching to ARNI after renal function stabilizes or improves, so as to reduce the risk of entering end-stage renal disease. *(1B)*

Rationale: A review published in 2021 noted that the sympathetic system was strongly activated in patients with heart failure, and using a RAAS inhibitor alone to dilate efferent arterioles could significantly reduce the hydrostatic pressure in capillaries, resulting in an increase in creatinine. When ARNI was used instead of RAAS inhibitors, efferent and afferent arterioles were dilated synchronously, the renal plasma flow increased, the effective filtration area increased, and the filtration permeability of mesangial cells increased, thus improving eGFR (73). In the PARADIGM-HF study, the risk of increased serum creatinine was significantly lower in the ARNI group than in the enalapril group. A meta-analysis published in ASN in 2020 reported the same benefits of ARNI, as well as a reduction in the risk of elevated serum creatinine by 14% compared with ACEI/ARB (ORR = 0.86; 95% CI = 0.78–0.95, $P = 0.002$) (72).

If CKD Is Accompanied by Overhydration That Persists, We Recommend Switching to ARNI

Suggestion 3.4.5: For patients with CKD who still exhibit overhydration after treatment with ACEI/ARB, we suggest switching ACEI/ARB to ARNI to improve volume control. *(2C)*

Rationale: ARNI not only inhibits the RAAS system, but also inhibits enkephalinase and enhances the natriuretic peptide system. The natriuretic peptide system is a potential target for renal disease treatment, which can promote natriuresis and diuresis, help reduce sodium as well as water retention, and improve volume control (73).

Can ARNI Directly Replace ACEI/ARB?

Suggestion 3.5: For patients with HFrEF not previously treated with ACEI/ARB, ARNI treatment should be the first choice to reduce the risk of cardiovascular death and hospitalization owing to heart failure. *(1B)*

Rationale: Subgroup analysis of the PIONEER-HF study confirmed that direct initiation of ARNI had more clinical benefits than switching from ACEI to ARNI. Further, the benefits were consistent among all patients in the high-risk subgroup (**Supplementary Table 3**). The risk of adverse reactions was similar in both high-risk and low-risk patients. This indicated that, in the former, direct initiation of ARNI during hospitalization had more benefits and was well tolerated (33). The TRANSITION study further confirmed that early initiation of ARNI during hospitalization was safe and effective (31). Therefore, in patients with heart failure, directly initiating ARNI can avoid the treatment delay caused by initial ACEI intake followed by a switch to ARNI. The 2021 US expert consensus on the management of patients with HFrEF states that ARNI should be preferred over ACEI/ARB for patients with HFrEF (74).

ARNI IN PATIENTS WITH CKD UNDERGOING MAINTENANCE DIALYSIS (HEMODIALYSIS AND PERITONEAL DIALYSIS)

What Are the Indications of ARNI in Patients Undergoing Maintenance Dialysis?

Suggestion 4.1.1: For patients with heart failure undergoing maintenance dialysis, ARNI is recommended for improving myocardial remodeling, controlling the symptoms of heart failure, protecting residual renal function, and reducing the risk of cardiovascular events. *(1B)*

Rationale: Data exist on the use of ARNI in patients with heart failure undergoing maintenance dialysis. A clinical study conducted in hemodialysis patients in China showed that, when ARNI 100 mg BID was used to treat hemodialysis patients with HFrEF or heart failure with midrange ejection fraction, the maximum blood concentration of LBQ657 was within the safe drug concentration range, and ARNI effectively improved their left ventricular ejection fraction ($P < 0.05$) (75). Another retrospective study including 23 patients also confirmed that ARNI could improve the left ventricular ejection fraction and myocardial marker levels in dialysis patients with HFrEF (76). A Chinese study including 21 peritoneal dialysis patients with HFpEF confirmed that ARNI could also significantly improve the symptoms and signs of heart failure and reduce the levels of heart failure markers, while also exhibiting a tendency to improve cardiac function (77).

Suggestion 4.1.2: ARNI can be used in patients with hypertension undergoing maintenance dialysis to lower blood pressure, protect cardiac function, and reduce the risk of cardiovascular events. *(2D)*

Rationale: Approximately 90% of dialysis patients in China have hypertension (78). The blood pressure of dialysis patients is mostly managed *via* combination therapy (79). However, the blood pressure control rate in patients after antihypertensive treatment is only 25.5% (78). Persistent hypertension in dialysis patients can be correlated not only with left ventricular hypertrophy, but also with ischemic heart disease, heart failure, and cardiovascular mortality (80). Therefore, ARNI can be used in patients with hypertension undergoing maintenance dialysis to lower blood pressure, protect cardiac function, and reduce the risk of cardiovascular events (80). At present, there are limited data on the use of ARNI in dialysis patients with hypertension; therefore, clinical experience should be considered.

TABLE 3 | Future research suggestions.

ARNI use in patients with hypoproteinemia Hypoalbuminemia is common in patients with CKD. The pharmacokinetic characteristics and ARNI administration approaches in patients with hypoalbuminemia require further investigation.
Safety and efficacy of ARNI combined with ARB There is no clinical research on the combination of ARNI and ARB. Whether the combination of ARNI and ARB can elicit stronger long-term cardiovascular protection and further enhance the urine protein-reducing effect requires further investigation.
ARNI combined with SGLT2i for non-heart failure patients SGLT2i can reduce the risk of heart failure and renal failure as well as cardiovascular and all-cause death. In non-heart failure patients, whether combining ARNI with SGLT2i can have greater clinical benefits requires further investigation.
Do hypertensive patients with moderate and severe renal impairment require a lower loading dose? In patients with moderate and severe renal impairment, the exposure to LBQ657 is significantly increased (by 2–3 times). Whether the loading dose of ARNI should be reduced for these patients requires further investigation.
What is the efficacy and safety of twice-daily ARNI administration in patients with CKD exhibiting hypertension? The incidence of nocturnal hypertension is higher in patients with CKD than in the general population. For patients with CKD and hypertension, the safety and efficacy of twice-daily ARNI administration should be further studied.
What are the application results of ARNI in patients undergoing maintenance dialysis with hypertension? Whether ARNI exhibits better antihypertensive and target organ protective effects than other antihypertension drugs in patients with hypertension undergoing maintenance dialysis requires further investigation.

What Precautions Should Be Taken When Using ARNI in Patients Undergoing Maintenance Dialysis?

Suggestion 4.2: In patients undergoing maintenance dialysis, we recommend starting ARNI from a low dose of 25–50 mg QD, paying attention to blood pressure during treatment, and adjusting the dose gradually to 100–150 mg QD to BID according to the patient's tolerance (**Table 2**). *(1C)*

Rationale: When used reasonably, ARNI is well tolerated in dialysis patients. In a retrospective study, 23 patients who were switched from ACEI/ARB to ARNI treatment exhibited an average dose of ARNI at the baseline of 90 ± 43 mg/d. After a follow-up period with a median length of 132 days, the dose at the last follow-up was 123 ± 62 mg/d. The most common adverse reaction during treatment was hypotension (SBP<100 mmHg), which usually occurred during or immediately after hemodialysis. During treatment, the dose of ARNI was reduced for approximately 21.7% of patients because of adverse reactions; however, these reactions did not lead to drug discontinuation (76). Therefore, for dialysis patients, we suggest starting with a low dose, monitoring blood pressure during treatment, and gradually adjusting the dose according to tolerance. Research has shown that ARNI at a dose of 100–150 mg BID is safe and tolerable for hemodialysis patients (75, 81).

CONCLUSION

When using ARNI in patients with CKD, its dosage and frequency should be determined according to renal function, cardiac function, and the occurrence of hypoproteinemia or abnormal liver function. Whether it is necessary to start with a low dose in patients with moderate and severe renal impairment should be further addressed (**Table 3**). Although ARNI can be used in combination with SGLT2i for patients with heart failure, its combination with ARB requires further investigation. When used with diuretics, close attention should be paid to adjusting the dosage. When used with spironolactone, amiloride, or potassium salt, close attention should be paid to serum potassium levels, while avoiding ACEI or aliskiren use. ARNI is recommended for non-dialysis patients with CKD exhibiting heart failure or hypertension. In the case of heart failure or poor blood pressure control, we suggest that patients using ACEI/ARB should switch to ARNI. ARNI is recommended to improve cardiac structure and function in maintenance dialysis patients with heart failure. In dialysis patients with hypertension, the efficacy of ARNI requires further investigation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

ACKNOWLEDGMENTS

We acknowledge Zhongguancun Nephrology & Blood Purification Innovation Alliance for organizing the process of writing the expert consensus. Thank to Jing Liu, Ying Shan, Ruizhen Chen, Zhaochu He, Shuo Zhang, Min Liu, Weihong Jiang, Yang Zheng, Xiangqing Kong, Tao Ye, Peili Bu, Xiaoping Chen, Zhuhua Yao, Zhaohui Wang, Jianjun Mu, Shenjiang Hu, Liqun Hu, etc. for their valuable comments and suggestions in the consensus writing and review process.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.877237/full#supplementary-material

REFERENCES

1. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl.* (2011) 12:7–11. doi: 10.1016/j.kisu.2021.11.003
2. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2020) 395:709–33. doi: 10.1016/S0140-6736(20)30045-3
3. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group M. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* (2013) 158:825–30. doi: 10.7326/0003-4819-158-11-201306040-00007
4. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular disease in chronic kidney disease: Pathophysiological insights and therapeutic options. *Circulation.* (2021) 143:1157–72. doi: 10.1161/CIRCULATIONAHA.120.050686
5. Ryu H, Kim J, Kang E, Hong Y, Chae DW, Choi KH, et al. Incidence of cardiovascular events and mortality in Korean patients with chronic kidney disease. *Sci Rep.* (2021) 11:1131. doi: 10.1038/s41598-020-80877-y
6. United States Renal Data System. *2020 USRDS Annual Data Report: Epidemiology of kidney disease in the United States.* Bethesda, MD: National Institutes of Health; National Institute of Diabetes and Digestive and Kidney Diseases (2020).
7. Zhao X, Niu Q, Gan L, Hou FF, Liang X, Ni Z, et al. Thrombocytopenia predicts mortality in Chinese hemodialysis patients-an analysis of the China DOPPS. *BMC Nephrol.* (2022) 23:11. doi: 10.1186/s12882-021-02579-5
8. Cai G, Zheng Y, Sun X, Chen X. Survey of prevalence, awareness, and treatment rates in chronic kidney disease patients with hypertension in china collaborative group. Survey of prevalence A, treatment rates in chronic kidney disease patients with hypertension in China collaborative. *J Am Geriatr Soc.* (2013) 61:2160–7. doi: 10.1111/jgs.12551
9. Zheng Y, Tang L, Zhang W, Zhao D, Zhang D, Zhang L, et al. Applying the new intensive blood pressure categories to a nondialysis chronic kidney disease population: the prevalence, awareness and treatment rates in chronic kidney disease patients with hypertension in China survey. *Nephrol Dial Transplant.* (2020) 35:155–61. doi: 10.1093/ndt/gfy301
10. Hsu CY, McCulloch CE, Darbinian J, Go AS, Iribarren C. Elevated blood pressure and risk of end-stage renal disease in subjects without baseline kidney disease. *Arch Intern Med.* (2005) 165:923–8. doi: 10.1001/archinte.165.8.923
11. Paoletti E, De Nicola L, Gabbai FB, Chiodini P, Ravera M, Pieracci L, et al. Associations of left ventricular hypertrophy and geometry with adverse outcomes in patients with CKD and hypertension. *Clin J Am Soc Nephrol.* (2016) 11:271–9. doi: 10.2215/CJN.06980615
12. SPRINT Research Group, Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM, et al. A randomized trial of intensive vs. standard blood-pressure control. *N Engl J Med.* (2015) 373:2103–16. doi: 10.1056/NEJMoa1511939
13. Kidney Disease: Improving Global Outcomes (KDIGO) Blood Pressure Work Group. KDIGO 2021 clinical practice guideline for the management of blood pressure in chronic

kidney disease. *Kidney Int.* (2021) 99:S1–S87. doi: 10.1016/j.kint.2020.11.003
14. Mishima E, Haruna Y, Arima H. Renin-angiotensin system inhibitors in hypertensive adults with non-diabetic CKD with or without proteinuria: a systematic review and meta-analysis of randomized trials. *Hypertens Res.* (2019) 42:469–82. doi: 10.1038/s41440-018-0116-3
15. Nagata D, Hishida E, Masuda T. Practical strategy for treating chronic kidney disease (CKD)-associated with hypertension. *Int J Nephrol Renovasc Dis.* (2020) 13:171–8. doi: 10.2147/IJNRD.S259931
16. https://reference.medscape.com/drug/entresto-sacubitril-valsartan-1000010
17. Zheng Y, Cai GY, Chen XM, Fu P, Chen JH, Ding XQ, et al. Prevalence, awareness, treatment, and control of hypertension in the non-dialysis chronic kidney disease patients. *Chin Med J (Engl).* (2013) 126:2276–80.
18. Damman K, Tang WH, Felker GM, Lassus J, Zannad F, Krum H, et al. Current evidence on treatment of patients with chronic systolic heart failure and renal insufficiency: Practical considerations from published data. *J Am Coll Cardiol.* (2014) 63:853–71. doi: 10.1016/j.jacc.2013.11.031
19. House AA, Wanner C, Sarnak MJ, Piña IL, McIntyre CW, Komenda P, et al. Heart failure in chronic kidney disease: conclusions from a kidney disease: Improving Global Outcomes (KDIGO) controversies conference. *Kidney Int.* (2019) 95:1304–17. doi: 10.1016/j.kint.2019.02.022
20. Zhang R, Sun X, Li Y, He W, Zhu H, Liu B, et al. The efficacy and safety of sacubitril/valsartan in heart failure patients: A review. *J Cardiovasc Pharmacol Ther.* (2022) 27:10742484211058681. doi: 10.1177/10742484211058681
21. Gondek A, Jagodzińska A, Pietrzak B, Mamcarz A, Cudnoch-Jedrzejewska A. Relevance of the assessment of natriuretic peptide plasma concentrations in hypertensive pregnant women. *Biomarkers.* (2020) 25:449–57. doi: 10.1080/1354750X.2020.1795264
22. Zhang Y, Du X, Wang H, He Z, Liu H. Sacubitril-valsartan cocrystal revisited: Role of polymer excipients in the formulation. *Expert Opin Drug Deliv.* (2021) 18:515–26. doi: 10.1080/17425247.2021.1860940
23. McCormack PL. Sacubitril/Valsartan: a review in chronic heart failure with reduced ejection fraction. *Drugs.* (2016) 76:387–96. doi: 10.1007/s40265-016-0544-9
24. Kuang H, Huang X, Zhou Z, Cheng X, Xu G. Sacubitril/valsartan in chronic kidney disease: From pharmacological mechanism to clinical application. *Eur J Pharmacol.* (2021) 907:174288. doi: 10.1016/j.ejphar.2021.174288
25. Ayalasomayajula S, Langenickel T, Pal P, Boggarapu S, Sunkara G. Erratum to: Clinical pharmacokinetics of Sacubitril/Valsartan (LCZ696): A novel angiotensin receptor-neprilysin inhibitor. *Clin Pharmacokinet.* (2018) 57:105–23. doi: 10.1007/s40262-017-0558-9
26. Kulmatycki KM, Langenickel T, Ng WH, Pal P, Zhou W, Lin TH, et al. Pharmacokinetics and safety of sacubitril/valsartan (LCZ696) in patients with mild and moderate hepatic impairment. *Int J Clin Pharmacol Ther.* (2017) 55:728–39. doi: 10.5414/CP202988
27. Gu J, Noe A, Chandra P, Al-Fayoumi S, Ligueros-Saylan M, Sarangapani R, et al. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting angiotensin receptor-neprilysin inhibitor (ARNi). *J Clin Pharmacol.* (2010) 50:401–14:(Ar.Ni). doi: 10.1177/0091270009343932
28. Ruilope LM, Dukat A, Böhm M, Lacourcière Y, Gong J, Lefkowitz MP. Blood-pressure reduction with LCZ696, a novel dual-acting inhibitor of the angiotensin II receptor and neprilysin: A randomised, double-blind, placebo-controlled, active comparator study. *Lancet.* (2010) 375:1255–66. doi: 10.1016/S0140-6736(09)61966-8
29. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med.* (2014) 371:993–1004. doi: 10.1056/NEJMoa1409077
30. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, et al. Angiotensin-neprilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med.* (2019) 381:1609–20. doi: 10.1056/NEJMoa1908655
31. Wachter R, Senni M, Belohlavek J, Straburzynska-Migaj E, Witte KK, Kobalava Z, et al. Initiation of sacubitril/valsartan in haemodynamically stabilised heart failure patients in hospital or early after discharge: Primary results of the randomised TRANSITION study. *Eur J Heart Fail.* (2019) 21:998–1007. doi: 10.1002/ejhf.1498
32. DeVore AD, Braunwald E, Morrow DA, Duffy CI, Ambrosy AP, Chakraborty H, et al. Initiation of angiotensin-neprilysin inhibition after acute decompensated heart failure: Secondary analysis of the open-label extension of the Pioneer-HF trial. *JAMA Cardiol.* (2020) 5:202–7. doi: 10.1001/jamacardio.2019.4665
33. Berg DD, Samsky MD, Velazquez EJ, Duffy CI, Gurmu Y, Braunwald E, et al. Efficacy and safety of Sacubitril/Valsartan in high-risk patients in the Pioneer-HF trial. *Circ Heart Fail.* (2021) 14:e007034. doi: 10.1161/CIRCHEARTFAILURE.120.007034
34. Zanchi A, Maillard M, Burnier M. Recent clinical trials with omapatrilat: new developments. *Curr Hypertens Rep.* (2003) 5:346–52. doi: 10.1007/s11906-003-0045-6
35. Docherty KF, Vaduganathan M, Solomon SD, McMurray JJV. Sacubitril/Valsartan: Neprilysin inhibition 5 years after PARADIGM-HF. *JACC Heart Fail.* (2020) 8:800–10. doi: 10.1016/j.jchf.2020.06.020
36. Pradhan A, Vohra S, Vishwakarma P, Sethi R. Review on sodium-glucose cotransporter 2 inhibitor (SGLT2i) in diabetes mellitus and heart failure. *J Fam Med Prim Care.* (2019) 8:1855–62. doi: 10.4103/jfmpc.jfmpc_232_19
37. Solomon SD, Jhund PS, Claggett BL, Dewan P, Køber L, Kosiborod MN, et al. Effect of dapagliflozin in patients with HFrEF treated with Sacubitril/Valsartan: The DAPA-HF trial. *JACC Heart Fail.* (2020) 8:811–8. doi: 10.1016/j.jchf.2020.04.008
38. de la Espriella R, Bayés-Genís A, Morillas H, Bravo R, Vidal V, Núñez E, et al. Renal function dynamics following co-administration of sacubitril/valsartan and empagliflozin in patients with heart failure and type 2 diabetes. *ESC Heart Fail.* (2020) 7:3792–800. doi: 10.1002/ehf2.12965
39. Hsiao FC, Lin CP, Tung YC, Chang PC, McMurray JJV, Chu PH. Combining sodium-glucose cotransporter 2 inhibitors and angiotensin receptor-neprilysin inhibitors in heart failure patients with reduced ejection fraction and diabetes mellitus: a multi-institutional study. *Int J Cardiol.* (2021) 330:91–7. doi: 10.1016/j.ijcard.2021.02.035
40. Hubers SA, Brown NJ. Combined angiotensin receptor antagonism and neprilysin inhibition. *Circulation.* (2016) 133:1115–24. doi: 10.1161/CIRCULATIONAHA.115.018622
41. Ryu H, Kim J, Kang E, Hong Y, Chae DW, Choi KH, et al. Author Correction: Incidence of cardiovascular events and mortality in Korean patients with chronic kidney disease. *Sci Rep.* (2021) 11:9488. doi: 10.1038/s41598-021-88996-w
42. https://www.ema.europa.eu/en/documents/product-information/entresto-epar-product-information_en.pdf
43. Ayalasomayajula S, Langenickel TH, Chandra P, Wolfson ED, Albrecht D, Zhou W, et al. Effect of food on the oral bioavailability of the angiotensin receptor - Neprilysin inhibitor sacubitril/valsartan (LCZ696) in healthy subjects. *Int J Clin Pharmacol Ther.* (2016) 54:1012–8. doi: 10.5414/CP202604
44. Damman K, Gori M, Claggett B, Jhund PS, Senni M, Lefkowitz MP, et al. Renal Effects and Associated Outcomes During Angiotensin-Neprilysin Inhibition in Heart Failure. *JACC Heart Fail.* (2018) 6:489–98. doi: 10.1016/j.jchf.2018.02.004
45. Chang HY, Feng AN, Fong MC, Hsueh CW, Lai WT, Huang KC, et al. Sacubitril/valsartan in heart failure with reduced ejection fraction patients: Real world experience on advanced chronic kidney disease, hypotension, and dose escalation. *J Cardiol.* (2019) 74:372–80. doi: 10.1016/j.jjcc.2019.03.010
46. Spannella F, Marini M, Giulietti F, Rosettani G, Francioni M, Perna GP, et al. Renal effects of Sacubitril/Valsartan in heart failure with reduced ejection fraction: a real life 1-year follow-up study. *Intern Emerg Med.* (2019) 14:1287–97. doi: 10.1007/s11739-019-02111-6
47. Hsieh HL, Chen CY, Chen CH, Hsu SC, Huang WC, Sue YM, et al. Renal protective effect of sacubitril/valsartan in patients with heart failure. *Sci Rep.* (2021) 11:4593. doi: 10.1038/s41598-021-84118-8
48. Kang H, Zhang J, Zhang X, Qin G, Wang K, Deng Z, et al. Effects of sacubitril/valsartan in patients with heart failure and chronic kidney disease: a meta-analysis. *Eur J Pharmacol.* (2020) 884:173444. doi: 10.1016/j.ejphar.2020.173444
49. Kario K. The Sacubitril/Valsartan, a first-in-class, angiotensin receptor neprilysin inhibitor (ARNI): Potential uses in hypertension, heart failure, and beyond. *Curr Cardiol Rep.* (2018) 20:5. doi: 10.1007/s11886-018-0944-4
50. Geng Q, Yan R, Wang Z, Hou F. Effects of LCZ696 (Sacubitril/Valsartan) on blood pressure in patients with hypertension: a meta-analysis of randomized controlled trials. *Cardiology.* (2020) 145:589–98. doi: 10.1159/000507327
51. De Vecchis R, Ariano C, Soreca S. Antihypertensive effect of sacubitril/valsartan: a meta-analysis. *Minerva Cardioangiol.* (2019) 67:214–22. doi: 10.23736/S0026-4725.19.04869-2
52. Ito S, Satoh M, Tamaki Y, Gotou H, Charney A, Okino N, et al. Safety and efficacy of LCZ696, a first-in-class angiotensin receptor neprilysin inhibitor,

in Japanese patients with hypertension and renal dysfunction. *Hypertens Res.* (2015) 38:269–75. doi: 10.1038/hr.2015.1
53. Williams B, Cockcroft JR, Kario K, Zappe DH, Brunel PC, Wang Q, et al. Effects of Sacubitril/Valsartan versus olmesartan on central hemodynamics in the elderly with systolic hypertension: The PARAMETER study. *Hypertension.* (2017) 69:411–20. doi: 10.1161/HYPERTENSIONAHA.116.08556
54. Ayalasomayajula SP, Langenickel TH, Jordaan P, Zhou W, Chandra P, Albrecht D, et al. Effect of renal function on the pharmacokinetics of LCZ696 (sacubitril/valsartan), an angiotensin receptor neprilysin inhibitor. *Eur J Clin Pharmacol.* (2016) 72:1065–73. doi: 10.1007/s00228-016-2072-7
55. Ayalasomayajula S, Langenickel T, Pal P, Boggarapu S, Sunkara G. Clinical pharmacokinetics of Sacubitril/Valsartan (LCZ696): a novel angiotensin receptor-neprilysin inhibitor. *Clin Pharmacokinet.* (2017) 56:1461–78. doi: 10.1007/s40262-017-0543-3
56. Kario K, Sun N, Chiang FT, Supasyndh O, Baek SH, Inubushi-Molessa A, et al. Efficacy and safety of LCZ696, a first-in-class angiotensin receptor neprilysin inhibitor, in Asian patients with hypertension: a randomized, double-blind, placebo-controlled study. *Hypertension.* (2014) 63:698–705. doi: 10.1161/HYPERTENSIONAHA.113.02002
57. Cheung DG, Aizenberg D, Gorbunov V, Hafeez K, Chen CW, Zhang J. Efficacy and safety of sacubitril/valsartan in patients with essential hypertension uncontrolled by olmesartan: a randomized, double-blind, 8-week study. *J Clin Hypertens (Greenwich).* (2018) 20:150–8. doi: 10.1111/jch.13153
58. Huo Y, Li W, Webb R, Zhao L, Wang Q, Guo W. Efficacy and safety of sacubitril/valsartan compared with olmesartan in Asian patients with essential hypertension: a randomized, double-blind, 8-week study. *J Clin Hypertens (Greenwich).* (2019) 21:67–76. doi: 10.1111/jch.13437
59. Schmieder RE, Wagner F, Mayr M, Delles C, Ott C, Keicher C, et al. The effect of sacubitril/valsartan compared to olmesartan on cardiovascular remodelling in subjects with essential hypertension: The results of a randomized, double-blind, active-controlled study. *Eur Heart J.* (2017) 38:3308–17. doi: 10.1093/eurheartj/ehx525
60. Supasyndh O, Wang J, Hafeez K, Zhang Y, Zhang J, Rakugi H. Efficacy and safety of Sacubitril/Valsartan (LCZ696) compared with olmesartan in elderly Asian patients (≥65 Years) with systolic hypertension. *Am J Hypertens.* (2017) 30:1163–9. doi: 10.1093/ajh/hpx111
61. Ferreira JP, Mogensen UM, Jhund PS, Desai AS, Rouleau JL, Zile MR, et al. Serum potassium in the PARADIGM-HF trial. *Eur J Heart Fail.* (2020) 22:2056–64. doi: 10.1002/ejhf.1987
62. Haynes R, Judge PK, Staplin N, Herrington WG, Storey BC, Bethel A, et al. Effects of Sacubitril/Valsartan versus irbesartan in patients with chronic kidney disease. *Circulation.* (2018) 138:1505–14. doi: 10.1161/CIRCULATIONAHA.118.034818
63. Fala L. Entresto (Sacubitril/Valsartan): First-in-class angiotensin receptor neprilysin inhibitor FDA approved for patients with heart failure. *Am Health Drug Benefits.* (2015) 8:330–4.
64. Expert group on early detection, diagnosis and treatment system construction of chronic kidney disease in Shanghai. Guideline for screening, diagnosis, prevention and treatment of chronic kidney disease. *Chin J Prac Intern Med.* (2017) 37:28–34.
65. Schernthaner G, Ritz E, Schernthaner GH. Strict glycaemic control in diabetic patients with CKD or ESRD: Beneficial or deadly? *Nephrol Dial Transplant.* (2010) 25:2044–7. doi: 10.1093/ndt/gfq199
66. Gamarra E, Baffoni C, Borretta G, Feola M, Tassone F. Reduction of insulin Requirement After Starting Treatment With Sacubitril/Valsartan in a Patient with Diabetes Treated With Continuous Subcutaneous Insulin Infusion (CSII): A case report. *J Diabetes Sci Technol.* (2018) 12:1254–5. doi: 10.1177/1932296818785644
67. Jordan J, Stinkens R, Jax T, Engeli S, Blaak EE, May M, et al. Improved insulin sensitivity with angiotensin receptor neprilysin inhibition in individuals with obesity and hypertension. *Clin Pharmacol Ther.* (2017) 101:254–63. doi: 10.1002/cpt.455
68. De Vecchis R, Soreca S, Ariano C. Anti-hypertensive effect of Sacubitril/Valsartan: a meta-analysis of randomized controlled trials. *Cardiol Res.* (2019) 10:24–33. doi: 10.14740/cr813
69. Kario K, Tamaki Y, Okino N, Gotou H, Zhu M, Zhang J. LCZ696, a first-in-class angiotensin receptor-neprilysin inhibitor: The first clinical experience in patients with severe hypertension. *J Clin Hypertens (Greenwich).* (2016) 18:308–14. doi: 10.1111/jch.12667
70. Desai AS, Vardeny O, Claggett B, McMurray JJ, Packer M, Swedberg K, et al. Reduced risk of hyperkalemia during treatment of heart failure with mineralocorticoid receptor antagonists by use of Sacubitril/Valsartan compared with enalapril: a secondary analysis of the PARADIGM-HF trial. *JAMA Cardiol.* (2017) 2:79–85. doi: 10.1001/jamacardio.2016.4733
71. Feng Y, Yin Y, Deng R, Li H. Renal safety and efficacy of angiotensin receptor-neprilysin inhibitor: a meta-analysis of randomized controlled trials. *J Clin Pharm Ther.* (2020) 45:1235–43. doi: 10.1111/jcpt.13243
72. Eloisa Trina Cesante, et al. Presented at ASN Congress (2020). Available online at: https://www.asn-online.org/education/kidneyweek/2020/program-abstract.aspx?controlId=3449363
73. Pontremoli R, Borghi C, Perrone Filardi PP. Renal protection in chronic heart failure: Focus on sacubitril/valsartan. *Eur Heart J Cardiovasc Pharmacother.* (2021) 7:445–52. doi: 10.1093/ehjcvp/pvab030
74. Feng Z, Wang X, Zhang L, Apaer R, Xu L, Ma J et al. Pharmacokinetics and pharmacodynamics of SacubitrilValsartan in maintenance hemodialysis patients with heart failure. *Blood Purif.* (2022) 51:270–9. doi: 10.1159/000519643
75. Lee S, Oh J, Kim H, Ha J, Chun KH, Lee CJ, et al. Sacubitril/valsartan in patients with heart failure with reduced ejection fraction with end-stage of renal disease. *ESC Heart Fail.* (2020) 7:1125–9. doi: 10.1002/ehf2.12659
76. Fu S, Xu Z, Lin B, Chen J, Huang Q, Xu Y, et al. Effects of Sacubitril-Valsartan in heart failure with preserved ejection fraction in patients undergoing peritoneal dialysis. *Front Med (Lausanne).* (2021) 8:657067. doi: 10.3389/fmed.2021.657067
77. Lin J, Ding XQ, Lin P, Zou JZ, Teng J, Zhang JY, et al. A multi-center survey of hypertension and its treatment in patients with maintenance hemodialysis in Shanghai. *Zhonghua Nei Ke Za Zhi.* (2010) 49:563–7.
78. Zhang W, Shi W, Liu Z, Gu Y, Chen Q, Yuan W, et al. A nationwide cross-sectional survey on prevalence, management and pharmacoepidemiology patterns on hypertension in Chinese patients with chronic kidney disease. *Sci Rep.* (2016) 6:38768. doi: 10.1038/srep38768
79. Maruyama T, Takashima H, Abe M. Blood pressure targets and pharmacotherapy for hypertensive patients on hemodialysis. *Expert Opin Pharmacother.* (2020) 21:1219–40. doi: 10.1080/14656566.2020.1746272
80. Lihua WCL, Chen H, Wei F, Jiang A. Use of angiotensin receptor neprilysin inhibitor in patients on maintenance hemodialysis with reduced cardiac ejection fraction, real-world experience from a single center. *Iran J Kidney Dis.* (2021) 15:288–99. doi: 10.52547/ijkd.5875
81. Judge P, Haynes R, Landray MJ, Baigent C. Neprilysin inhibition in chronic kidney disease. *Nephrol Dial Transplant.* (2015) 30:738−43. doi: 10.1093/ndt/gfu269

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

BRIEF RESEARCH REPORT
published: 22 July 2022
doi: 10.3389/fmed.2022.937122

Association Between *NPHS2* p.R229Q and Focal Segmental Glomerular Sclerosis/Steroid-Resistant Nephrotic Syndrome

Qiongxiu Zhou[1,2†], Qinjie Weng[1†], Xiaoyan Zhang[1], Yunzi Liu[1], Jun Tong[1], Xu Hao[1], Hao Shi[1], Pingyan Shen[1], Hong Ren[1], Jingyuan Xie[1] and Nan Chen[1*]*

[1] Department of Nephrology, Institute of Nephrology, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [2] Department of Nephrology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

OPEN ACCESS

Edited by:
Xu-jie Zhou,
Peking University, China

Reviewed by:
Yuan-yuan Qi,
First Affiliated Hospital of Zhengzhou University, China
Fujun Lin,
Shanghai Jiao Tong University, China

***Correspondence:**
Jingyuan Xie
nephroxie@163.com
Nan Chen
cnrj100@126.com

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 05 May 2022
Accepted: 01 June 2022
Published: 22 July 2022

Citation:
Zhou Q, Weng Q, Zhang X, Liu Y, Tong J, Hao X, Shi H, Shen P, Ren H, Xie J and Chen N (2022) Association Between NPHS2 p.R229Q and Focal Segmental Glomerular Sclerosis/Steroid-Resistant Nephrotic Syndrome. Front. Med. 9:937122. doi: 10.3389/fmed.2022.937122

Aim: *NPHS2* is the coding gene of podocin. This study aims to investigate the association between *NPHS2* p.R229Q (rs61747728), the most frequently reported missense variant of *NPHS2*, and focal segmental glomerular sclerosis (FSGS) or steroid-resistant nephrotic syndrome (SRNS) based on typing the variant in a Chinese FSGS/SRNS cohort and conducting a meta-analysis.

Method: We recruited patients with FSGS or SRNS and healthy individuals. To conduct a meta-analysis, all studies on p.R229Q and FSGS/SRNS were searched from public databases.

Results: In total, we enrolled 204 patients with FSGS, 61 patients with SRNS [46 with FSGS, 9 with minimal change disease (MCD), and six patients with IgA nephropathy (IgAN)], and 100 healthy controls. Unexpectedly, p.R229Q was absent in the patients from our cohort. By meta-analysis of 21 studies including 2,489 patients with FSGS/SRNS and 6,004 healthy controls, we confirmed that the A allele of p.R229Q was significantly associated with increased risk of FSGS/SRNS (allelic OR = 1.9, 95% CI = 1.44-2.52, $P < 0.001$). However, the subgroup analysis showed that the association between p.R229Q and FSGS/SRNS was true only in Caucasians (allelic OR = 2.14, 95%CI = 1.54-2.98, $P < 0.001$) and in early-onset patients (allelic OR: 2.13, 95% CI = 1.21-3.76, $P = 0.009$).

Conclusion: *NPHS2* p.R229Q may play an important role in enhancing the susceptibility of FSGS/SRNS, especially in ethnicity of Caucasian and age of early-onset patients.

Keywords: focal segmental glomerular sclerosis, *NPHS2*, p.R229Q, steroid resistant nephrotic syndrome, meta-analysis

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is clinically characterized by edema, massive proteinuria, hypoalbuminemia, and hyperlipidemia. Although steroid is widely used for treatment of INS, about 10-20% of children (1) and 50% of adults patients (2) have steroid-resistant nephrotic syndrome (SRNS). Among different pathologic types of SRNS, focal segmental glomerulosclerosis (FSGS) is the most common cause in children (3), accounting for 60-70% (4, 5), and it also frequently occurs in adults (6).

Over the past decades, dozens of podocyte-related genes had been identified in several monogenic forms of hereditary FSGS/SRNS (7–9). Among these genes, mutations in podocin (*NPHS2*) had been found to play a significant role in SRNS, consisting of approximately 20 to 40% of familial and 10% of idiopathic childhood SRNS in different regions of the world (10–13). Podocin is composed of 383 amino acids localizing specifically from the insertion of the slit diaphragm (SD) to the podocyte cytoplasm (7, 14). It is required for the structural organization and regulation of the glomerular filtration barrier by interacting with other important SD molecules such as nephrin and CD2-associated protein (CD2AP) (15–18). rs61747728 (p.R229Q, G686A) is one of the most commonly reported variants in podocin. A study conducted by Tsukaguchi et al. (19) found that p.R229Q caused a decrease in the ability of podocin to bind to nephrin and was usually associated with secondary *NPHS2* mutation, which enhanced the susceptibility to develop FSGS. Meanwhile, some studies reported that heterozygous p.R229Q polymorphisms were associated with SRNS when compared to a healthy population (20, 21). Furthermore, p.R229Q-podocin was found to be related with microalbuminuria in the general population (10). In contrast with these findings, p.R229Q was found not associated with SRNS or urinary albumin-creatinine ratio (ACR) in some other studies performed on White or Black individuals (10, 20, 22). These controversial findings might be due to underpowered study design or different study populations. In addition, few studies were performed on Asian population although the prevalence of SRNS was relatively higher in Asians and African Americans (3, 23, 24). The aim of this study was to investigate the association between p.R229Q and FSGS/SRNS. We first conducted a screening study on our cohort composed of adult patients who were of Chinese descent and with FSGS/SRNS. To enhance the power of this study, we performed a meta-analysis by pooling data driven from our cohort and from bodies of literature obtained from public databases.

METHODS

Screening Study

Patients and Controls

Inclusion criteria were as follows: (1) age between 18 to 65 years old, (2) newly diagnosed nephrotic syndrome without previous usage of immunosuppressive agents within 1 month before the recruitment, and 3) renal biopsy-proved FSGS, minimal change disease (MCD), or IgA nephropathy (IgAN). We included patients with IgAN treated with full-dose prednisone as their initial therapy. SRNS was defined as less than 50%reduction in urine protein compared to baseline or urine protein higher than 3.5 g/d after treatment with a full dose of prednisone (1 mg/kg, maximum 80 mg/day) for at least 12 weeks. Healthy controls were defined as having serum creatinine bellow 100 umol/L without proteinuria or hematuria by urine routine test. All the individuals recruited in this study self-reported as having a Chinese Han origin. Informed consent was obtained from all the study participants before enrollment. The study protocol was reviewed and approved by Ruijin Hospital Human Research Ethics Committee.

Mutation Analysis

Genomic DNA was extracted and purified from peripheral leukocytes in peripheral blood cells of all the included subjects. All the eight coding regions, exon-intron boundaries, 5′UTR, and 3′UTR of *NPHS2* were amplified by polymerase chain reaction (PCR) (7). Primers were designed with the primier5 software or based on published information (19) (**Supplementary Table 1**). The PCR products were sequenced by Invitrogen. Sequence chromatograms were analyzed with the SEQUENCHER^{TM} software (Gene Code Corp., Ann Arbor, MI, United States) by comparing with the reference sequence of *NPHS2* downloaded from NCBI[1].

Systemic Review and Meta-Analysis

Search Strategy and Inclusion Criteria

Relevant studies published until 1 March 2021 were searched through electronic databases of PubMed, SCOPUS, Cochrane Library, and Web of Science and using the search terms "R229Q," "*NPHS2*," "rs61747728," "686G > A," and "Arg229Gln." Additional studies were also searched by reviewing the references cited in the retrieved articles.

Studies eligible for inclusion in our meta-analysis had to fulfill the following criteria: (1) studies discussing about the association between p.R229Q and SRNS (or FSGS) (e.g., SRNS group vs. control group or FSGS group vs. control group), (2) original data of genotype frequencies available, (3) SRNS was defined as patients who do not achieve complete remission within 12 weeks of glucocorticoid treatment, (4) early onset was defined as onset age $\leq$18 years, late onset was defined as onset age >18 years. Studies were excluded from our analysis if: (1) there was no control group, (2) genotype data were not available, (3) they were a duplicate of previous publication, and (4) they were reviews, editorials, or unpublished reports.

Data Extraction

All the data were extracted independently by two reviewers (Qiongxiu Zhou and Xiaoyan Zhang) according to a standard protocol. The following information was extracted from the eligible studies: first author's surname, year of publication, ethnicity of subjects, sample size, age, and distribution of p.R229Q genotype and allele frequencies in case and control groups.

[1]http://www.ncbi.nlm.nih.gov/

Statistical Analyses

In the current meta-analysis, the associations between p.R229Q and FSGS/SRNS was analyzed by calculating the pooled odds ratios (ORs) and their 95% confidence intervals (CIs) using a random-effects model. Three genetic models were used for the association study: the allelic model (A vs. G), the dominant model (GA + AA vs. GG), and the recessive model (AA vs. GG + GA) (25, 26). Subgroup analyses were conducted according to subject ethnicity and age of onset.

Hardy-Weinberg Equilibrium (HWE) was tested by chi-square test (χ^2) for goodness of fit to compare the observed and expected genotype frequencies with controls using a previous meta-analysis as reference, and a P-value < 0.01 was considered as significant deviation from HWE. As deviation from HWE in subjects may bias the estimates of genetic effects in a meta-analysis, a sensitivity analysis was conducted by removing one study and recalculating the pooled OR and 95% CI to assess the stability of the results. Potential publication bias was estimated by Begg's funnel plot. All statistical tests were performed with the STATA 11.0 software (Stata Corp., College Station, TX, United States). Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Screening Study on the Present Cohort

In total, there were 61 patients with SRNS and 204 patients with FSGS recruited in the screening study. Among the 61 patients with SRNS, 46 (75%) had FSGS, 9 (15%) had MCD and 6 (10%) had IgAN; 4 of the 6 patients with IgAN had FSGS changes in renal pathologic findings. The baseline characteristics of patients with SRNS and FSGS enrolled are shown in **Supplementary Table 2**. We also recruited 100 healthy individuals as a control group. Four synonymous single nucleotide polymorphisms (SNPs), c.187A > G (p.G34G) in exon1, c.288C > T (p.S96S) in exon2, and c.954C > T (p.A138A) and c.1038A > G (p.L346L) in exon8 of *NPHS2*, were identified by Sanger sequencing among all the individuals recruited in the screening study. However, p.R229Q was absent in all the patients with SRNS and FSGS.

TABLE 1 | Characteristics of studies evaluating the association between R229Q polymorphism and SRNS/FSGS risk.

Comparison	Study	Onset age	Race	No. of case /control	Genotype frequencies (GG/GA/AA)		
					case	control	P (HWE)
SRNS vs. control	Mishra (40)	early-onset	Asian	20/50	14/6/0	37/13/0	0.29
	Fotouhi (41)	late-onset	Asian	25/35	25/0/0	35/0/0	1.00
	Abid (42)	early-onset	Asian	145/100	143/0/2	99/1/0	0.00
	Reiterova (43)	late-onset	Caucasian	36/300	32/4/0	270/29/1	0.81
	Tsygin (44)	early-onset	Caucasian	59/15	48/11/0	13/2/0	0.78
	Santin (45)	all age	Caucasian	139/227	126/12/1	218/9/0	0.76
	Megremis (46)	early-onset	Caucasian	22/100	20/2/0	97/3/0	0.88
	Machuca et al. (36)	all age	Caucasian	214/308	179/32/3	292/16/0	0.64
		all age	Caucasian	47/70	31/16/0	69/1/0	0.95
		all age	African	40/95	35/3/2	88/7/0	0.71
		all age	NA	34/14	31/3/0	14/0/0	1.00
	Mao et al. (37)	early-onset	Asian	22/30	22/0/0	30/0/0	1.00
	Weber et al. (20)	early-onset	Caucasian	319/320	294/18/7	308/12/0	0.73
	Ruf et al. (10)	early-onset	Mix	190/80	177/11/2	71/9/0	0.59
	Caridi et al. (32)	early-onset	Caucasian	120/100	110/7/3	95/5/0	0.80
	Karle et al. (31)	early-onset	Caucasian	31/100	28/3/0	94/6/0	0.76
	Zaki (47)	early-onset	African	22/53	7/15/0	51/2/0	0.27
	Baylarov (48)	early-onset	Asian	21/21	20/0/1	21/0/0	0.00
	Our study (2022)	late-onset	Asian	61/100	61/0/0	100/0/0	1.00
FSGS vs. control	Fotouhi (43)	late-onset	Asian	25/35	25/0/0	35/0/0	1.00
	Abid (42)	early-onset	Asian	48/100	47/0/1	99/1/0	0.96
	Reiterova (43)	late-onset	Caucasian	50/300	44/6/0	270/29/1	0.81
	Tsygin (44)	early-onset	Caucasian	59/15	48/11/0	13/2/0	0.78
	Tonna et al. (30)	all age	Mix	371/2596	330/40/1	2420/169/7	0.03
	McKenzie (49)	late-onset	African	247/634	242/5/0	618/16/0	0.75
		late-onset	Caucasian	129/271	117/12/0	250/21/0	0.51
	He (50)	late-onset	Caucasian	63/54	58/5/0	51/3/0	0.83
	Aucella et al. (34)	late-onset	Caucasian	33/124	30/3/0	117/7/0	0.75
	Tsukaguchi et al. (19)	late-onset	Mix	91/257	NA	NA	NA
	Our study (2022)	late-onset	Asian	204/100	204/0/0	100/0/0	1.00

Meta-Analysis

Searching Studies for Meta-Analysis

There were 21 studies investigating the association between p.R229Q and SRNS/FSGS, including the present study. A flow chart shows the literature search for relevant studies on the association between p.R229Q and SRNS/FSGS (**Supplementary Figure 1**). The studies contained 2,489 cases and 6,004 controls with 12 comparisons on Caucasians, 6 on Asians, and 3 on Africans. Characteristics of these studies evaluating the association between R229Q polymorphism and SRNS/FSGS risk are shown in **Table 1**. The frequency distributions of genotypes were consistent with HWE.

Association Between p.R229Q and SRNS/FSGS

The minor allele frequency (MAF) (A allele) of p.R229Q was.06 in the patients with SRNS/FSGS and.03 in the healthy controls. An increased risk for p.R229Q in SRNS/FSGS was confirmed by the allelic model (OR = 1.9, 95% CI 1.44-2.52, $P < 0.001$, **Figure 1**), the recessive model (OR = 3.9, 95% CI 1.56-9.77, $P = 0.004$, **Supplementary Figure 2**) and the dominant model (OR = 1.91, 95% CI 1.37-2.73, $P < 0.001$, **Supplementary Figure 3**).

A subgroup analysis was performed based on disease phenotype: SRNS or FSGS. A significant increased risk of SRNS and FSGS in patients with R229Q was determined based on the allelic model (OR = 2.6, 95% CI 1.68-4.03, $P = 0.024$ and OR = 1.44, 1.12-1.85, 95%CI P <0.001, respectively, **Figure 2**). When all the patients were stratified by ethnicity, significant risks were found among Caucasians either in the allele-based analysis (OR = 2.14, 95% CI 1.54-2.98, $P < 0.001$, **Figure 3**) or in the genotype-based analysis (dominant model: OR = 2.11, 95% CI: 1.48-3, $P < 0.001$; recessive model: OR = 6.56, 95% CI 1.69-25.5, $P = 0.007$). However, no association was found in the African and Asian populations. In the subgroups stratified by onset age, the p.R229Q variant increased the risk of SRNS among the early-onset patients in the allelic (OR: 2.13, 95% CI = 1.21-3.76, $P = 0.009$, **Figure 2**), recessive (OR = 4.85,

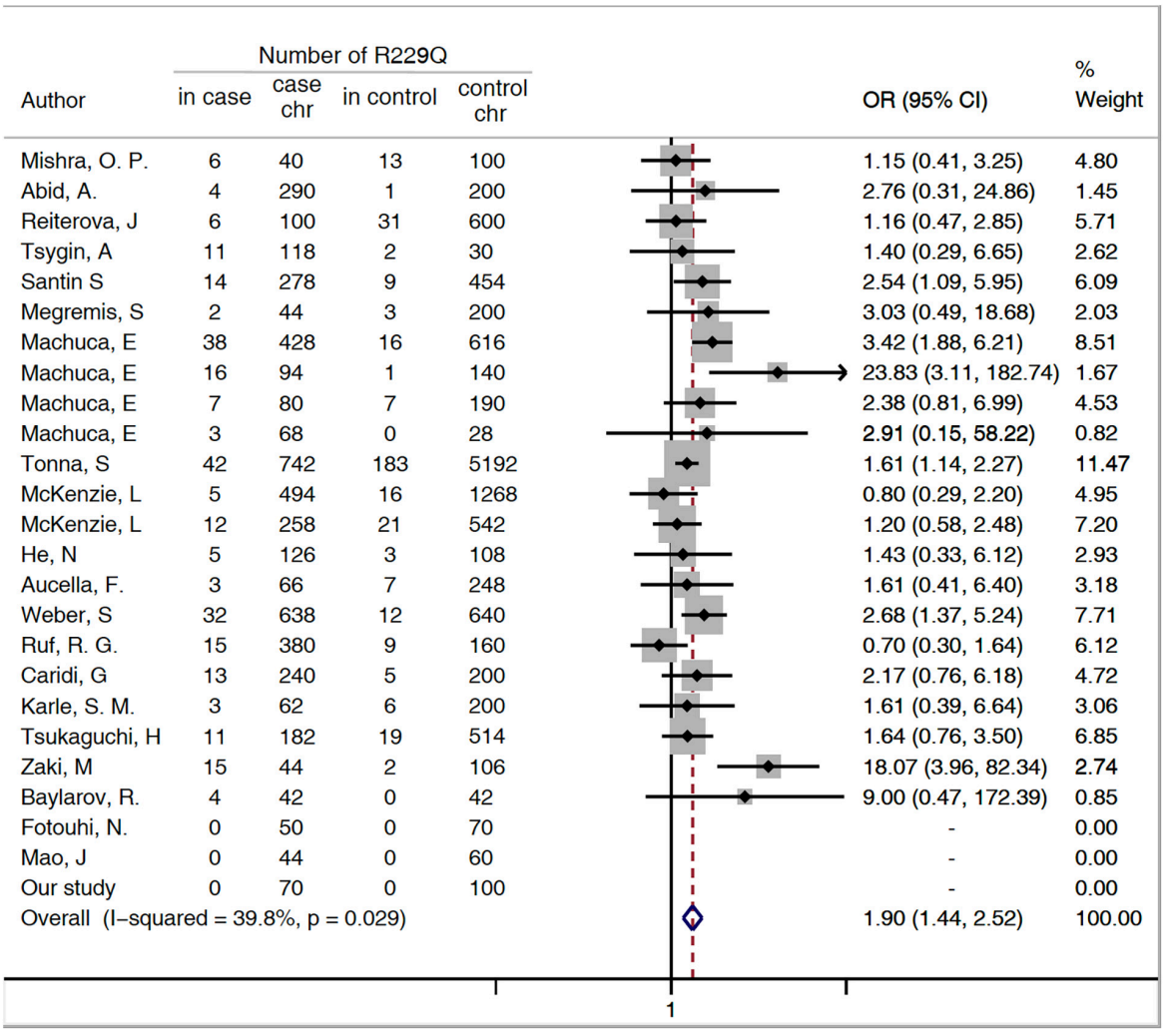


FIGURE 1 | Forest plots of meta-analysis of association between p.R229Q and FSGS/SRNS in the allelic model. Chr, chromosome; CI, confidence interval; OR, odds risk; SRNS, steroid-resistant nephrotic syndrome; and FSGS, focal segmental glomerular sclerosis.

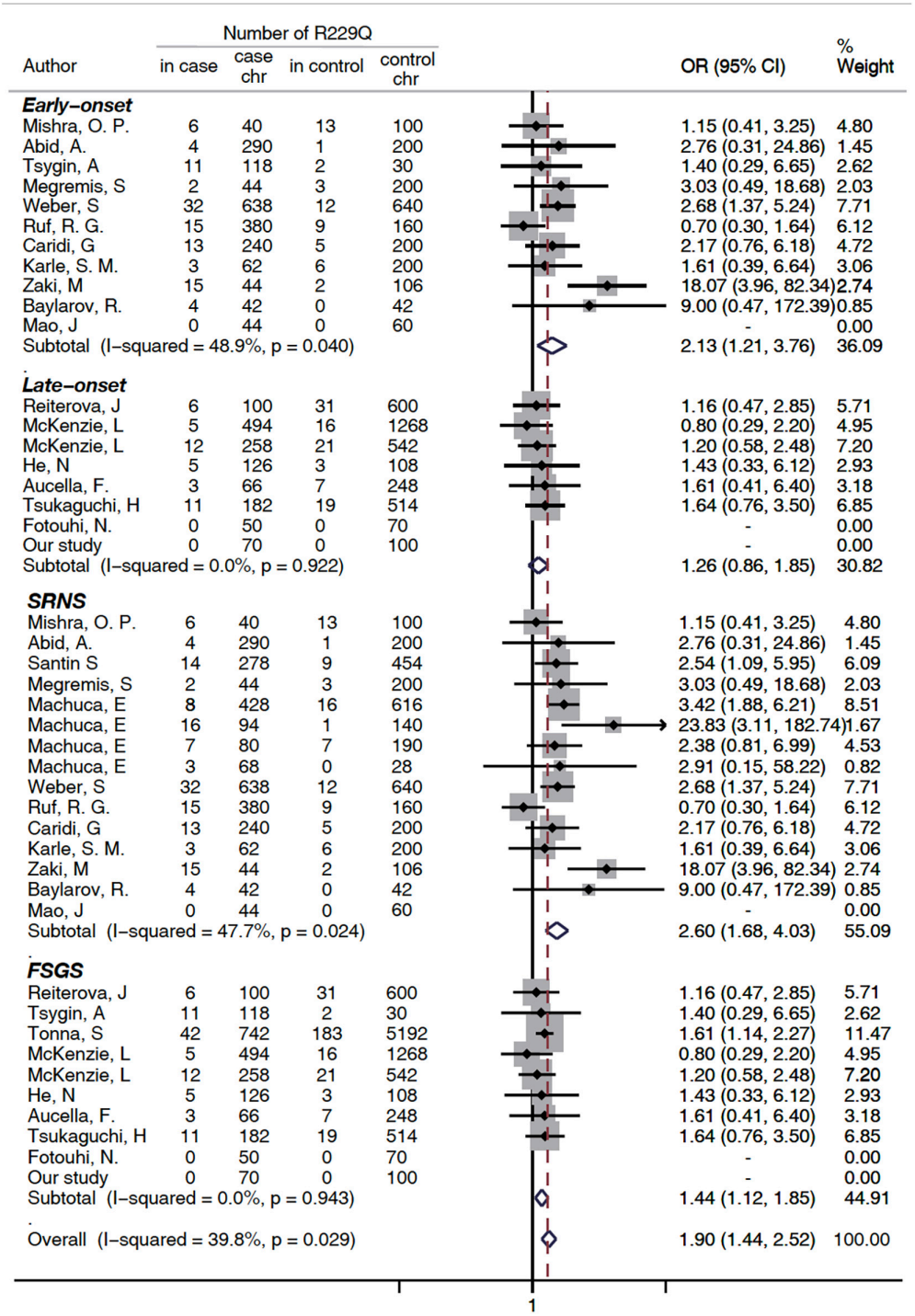


FIGURE 2 | Subgroup analysis of association between FSGS/SRNS and the p.R229Q variant based on age of onset and disease types (allelic model). CI, confidence interval; OR, odds risk; chr, chromosome; SRNS, steroid-resistant nephrotic syndrome; and FSGS, focal segmental glomerular sclerosis. SRNS, steroid-resistant nephrotic syndrome; FSGS, focal segmental glomerular sclerosis; HWE, Hardy Weinberg Equilibrium; NA, not available (i.e., not stated).

95% CI = 1.25-18.81, $P = 0.02$), and dominant (OR: 2.09, 95% CI = 1.04-4.21, $P = 0.04$) models, while no significant association was found in all the three genetic models among the late-onset patients.

Sensitivity Analysis

In this meta-analysis, significant heterogeneity ($I^2 = 52.1\%$, $P = 0.003$) was observed in the included dominant model, so a sensitivity analysis performed. The combined ORs were similar

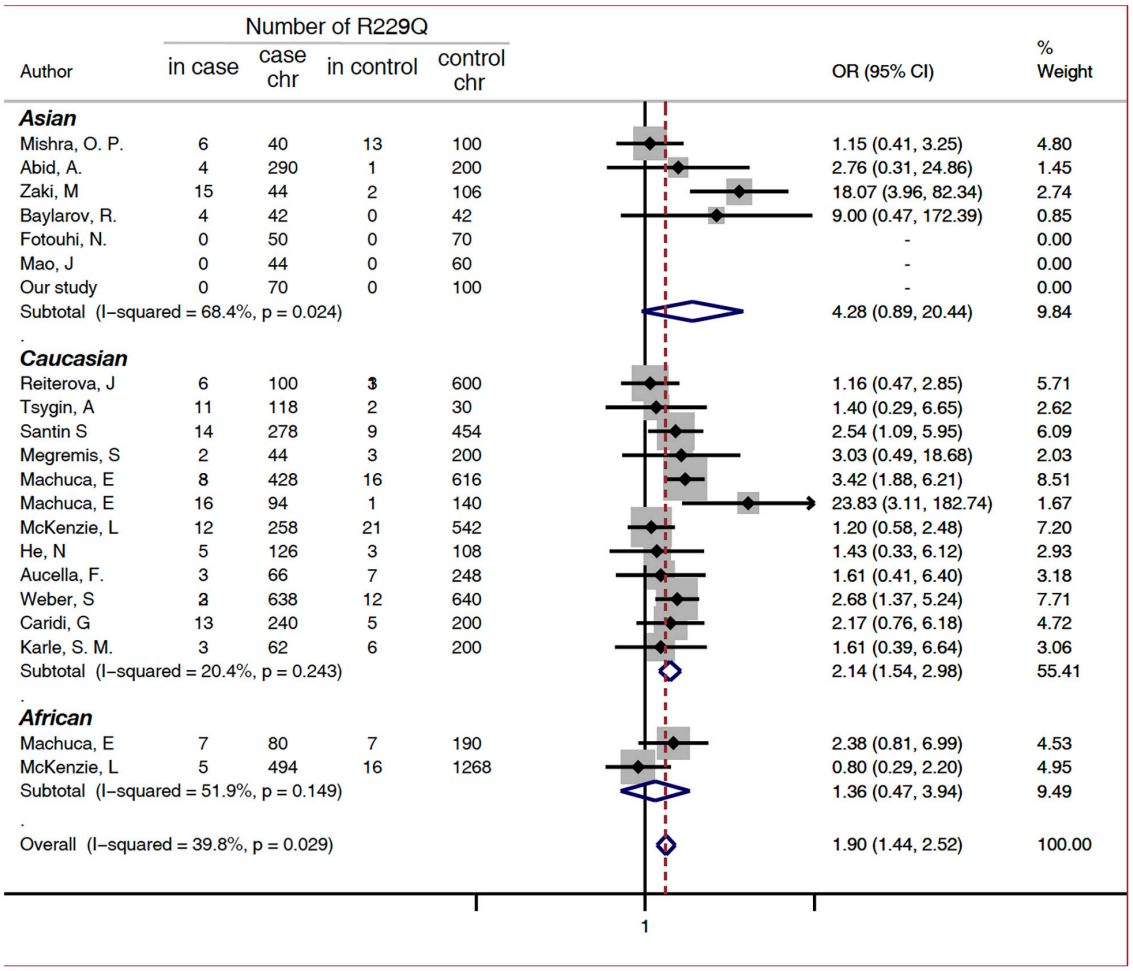


FIGURE 3 | Subgroup analysis of association between FSGS/SRNS and the p.R229Q variant based on ethnicity (allelic model). CI, confidence interval; OR, odds risk; chr, chromosome; SRNS, steroid-resistant nephrotic syndrome; and FSGS, focal segmental glomerular sclerosis.

with one another, with a narrow range from 1.72 (95% CI: 1.32-2.26) to 2.08 (95%CI: 1.49-2.91) (**Supplementary Figure 4**). It indicated that our results were stable.

Publication Bias

The publication bias of the individual studies was evaluated by Begg's test. No significant difference was found (P = 0.15). It indicated that there was no significant publication bias in our meta-analysis (**Supplementary Figure 5**).

DISCUSSION

NPHS2 mutations were initially described in patients with NS from birth to 6 years of age (20, 27), while they were infrequently detected in children with non-familial SRNS (28, 29). A study on adults with FSGS/SRNS has not been fully performed. In our study, we examined the frequency of *NPHS2* mutation in a cohort of adult Chinese patients with SRNS or FSGS. Four known synonymous variations (G34G, S96S, A318A, and L346L) were identified. However, the distribution of genotypes among our patients and the controls were similar, which suggested that the SNPs did not increase the risk of SRNS or FSGS. We did not find non-synonymous variations in *NPHS2*, and no p.R229Q was observed in patients with either SRNS or FSGS. It suggested that adult-onset *NPHS2* mutation was rare in Chinese patients, and that p.R229Q had no effect on the patients, at least in our population.

In the current meta-analysis, we found that the frequency of the p.R229Q variant was significantly higher in the patients with SRNS/FSGS than in the healthy populations in the allelic (OR = 1.9, 95% CI 1.44-2.52), dominant (OR = 1.91, 95% CI 1.37-2.73), and recessive (OR = 3. 0, 95% CI 1.56-9.77) models. Furthermore, it was significant in the subgroups of either SRNS (OR 2.6, P = 0.024) or FSGS (OR 1.44, P <0.001). As FSGS was a common cause of SRNS in both children and adults, we could hypothesize that p.R229Q plays a pathogenic role in FSGS and SRNS. These findings were consistent with previous studies, suggesting that the p.R229Q allele may be a disease-causing variant, which could enhance the susceptibility of FSGS,

and FSGS patients with podocin mutations were more likely to be p.R229Q in heterozygous state with one pathogenic mutation (10, 19, 20, 30–34).

The results from our meta-analysis were different from Lu Lu's study (35), which reported no association between p.R229Q and FSGS. First, we recruited more cases, with 2,489 patients with SRNS/FSGS and 6,004 controls, and there were more comparisons on different ethnicity, with 12 comparisons on Caucasians, 6 on Asians, and 3 on Africans. Secondly, Lu L's study excluded the studies that all variant individuals are compound heterozygotes, considering that the excessive possible affecting SNPs in the *NPHS2* gene related to FSGS was difficult to identify. We agree with this concern. Therefore, in our study, we constructed genetic models, the allelic (A vs. G), dominant (GA + AA vs. GG), and recessive (AA vs. GG + GA) models, to explain the allele frequency and genotype-phenotype correlation of p.R229Q in these studies. We concluded that p.R229Q might play a pathogenic role in developing SRNS/FSGS in the state of compound heterozygotes (36).

Additionally, we found that the frequency of p.R229Q was various throughout different populations. The frequency was higher in Caucasians than in Asians and Africans (36). For the Caucasians, p.R229Q increased the risk of FSGS/SRNS (allelic model: OR = 2.14, 95% CI 1.54-6.64; dominant model: OR = 2.11, 95% CI 1.48-3; recessive model: OR = 3.9, 95% CI 1.56-9.76). However, the associations with other populations were not significant. In our screening study, we did not find the p.R229Q variant in Chinese patients with adult-onset SRNS and FSGS, which was in agreement with a previous study conducted on Chinese patients with childhood-onset SRNS (37). This suggested that p.R229Q allele distribution in different races was uneven, although one explanation for this discrepancy might be that relatively small studies and low frequencies of p.R229Q in these populations could have limited the statistical power for analysis. Besides, significant results were also observed in patients with early-onset FSGS/SRNS in the allelic (OR: 2.13, 95% CI 1.21-3.76), dominant (OR: 2.11, 95% CI 1.48-3) and recessive (OR: 6.56, 95% CI 1.69-25.5) models compared with the healthy controls. We could hypothesize that p.R229Q was more likely to act as a disease modifier to increase the risk for patients with early-onset FSGS/SRNS.

Even though many studies and meta-analyses had been conducted on the genetic role of the p.R229Q variant in the previous years, most of them were performed on Caucasians. Limited studies were available on African and Asian populations. This limited our ability to reach a strong conclusion and investigate a potential function on the basis of race. Moreover, p.R229Q was recently discovered pathogenic only when trans-associated to specific mutations, and this could not be analyzed by the current meta-analysis (38, 39).

In conclusion, our study indicated that *NPHS2* mutations were rare in Asian patients with sporadic adult-onset FSGS/SRNS and p.R229Q was undetectable in our cohort. *NPHS2* p.R229Q may play an important role in enhancing susceptibility to FSGS/SRNS, especially in Caucasian and early-onset patients. Further studies and international multi-ethnicity approaches are needed to distinguish a pathogenic and benign p.R229Q genotype-phenotype correlation for clinical assessment.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are publicly available. This data can be found here: GenBank, accessions ON470453 - ON470576.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ruijin Hospital Human Research Ethics Committees. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QZ and XZ completed the screening test and extracted all the data that were needed for the meta-analysis. QZ and QW drafted the original manuscript. YL, JT, and XH helped in polishing the manuscript and were responsible for the figures. JX and NC created the overall design of this study. HS, PS, and HR revised the article. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the National Key Research and Development Program of China (2016YFC0904100), National Natural Science Foundation of China (Grant Nos: 81870460, 81570598, and 81370015), Science and Technology Innovation Action Plan of Shanghai Science and Technology Committee (Grant No: 17441902200), Shanghai Municipal Education Commission, Gaofeng Clinical Medicine Grant (Grant No: 20152207), Shanghai Jiao Tong University School of Medicine, Multi-Center Clinical Research Project (Grant No: DLY201510), and Shanghai Health and Family Planning Committee Hundred Talents Program (Grant No: 2018BR37).

ACKNOWLEDGMENTS

We thank the Department of Nephrology of Ruijin Hospital for providing the clinical data for this study. We would also like to thank Jian Liu and Yuanmeng Jin for helping us get in touch with patients.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.937122/full#supplementary-material

REFERENCES

1. Mekahli D, Liutkus A, Ranchin B, Yu A, Bessenay L, Girardin E, et al. Long-term outcome of idiopathic steroid-resistant nephrotic syndrome: a multicenter study. *Pediatr Nephrol.* (2009) 24:1525–32. doi: 10.1007/s00467-009-1138-5
2. Korbet SM. Treatment of primary focal segmental glomerulosclerosis. *Kidney Int.* (2002) 62:2301–10. doi: 10.1046/j.1523-1755.2002.00674.x
3. Kim JS, Bellew CA, Silverstein DM, Aviles DH, Boineau FG, Vehaskari VM. High incidence of initial and late steroid resistance in childhood nephrotic syndrome. *Kidney Int.* (2005) 68:1275–81. doi: 10.1111/j.1523-1755.2005.00524.x
4. D'Agati VD. Pathobiology of focal segmental glomerulosclerosis: new developments. *Curr Opin Nephrol Hypertens.* (2012) 21:243–50. doi: 10.1097/MNH.0b013e32835200df
5. Chesney R. The changing face of childhood nephrotic syndrome. *Kidney Int.* (2004) 66:1294–302. doi: 10.1111/j.1523-1755.2004.00885.x
6. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney.* (1997) 30:621–31. doi: 10.1016/s0272-6386(97)90485-6
7. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet.* (2000) 24:349–54. doi: 10.1038/74166
8. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, et al. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet.* (2000) 24:251–6. doi: 10.1038/73456
9. Kim JM, Wu H, Green G, Winkler CA, Kopp JB, Miner JH, et al. CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science.* (2003) 300:1298–300. doi: 10.1126/science.1081068
10. Ruf RG, Lichtenberger A, Karle SM, Haas JP, Anacleto FE, Schultheiss M, et al. Patients with mutations in NPHS2 (podocin) do not respond to standard steroid treatment of nephrotic syndrome. *J Am Soc Nephrol.* (2004) 15:722–32. doi: 10.1097/01.ASN.0000113552.59155.72
11. Caridi G, Bertelli R, Carrea A, Di Duca M, Catarsi P, Artero M, et al. Prevalence, genetics, and clinical features of patients carrying podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. *J Am Soc Nephrol.* (2001) 12:2742–6. doi: 10.1681/ASN.V12122742
12. Pollak MR. The genetic basis of FSGS and steroid-resistant nephrosis. *Semin Nephrol.* (2003) 23:141–6. doi: 10.1053/snep.2003.50014
13. Niaudet P. Podocin and nephrotic syndrome: implications for the clinician. *J Am Soc Nephrol.* (2004) 15:832–4. doi: 10.1097/01.asn.0000118135.00519.b0
14. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attié T, et al. Podocin localizes in the kidney to the slit diaphragm area. *Am J Pathol.* (2002) 160:131–9. doi: 10.1016/S0002-9440(10)64357-X
15. Huber TB, Schermer B, Muller RU, Bartram M, Calixto A, Hagmann H, et al. Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. *Proc Natl Acad Sci U.S.A.* (2006) 103:17079–86. doi: 10.1073/pnas.0607465103
16. Huber TB, Schermer B, Benzing T. Podocin organizes ion channel-lipid supercomplexes: implications for mechanosensation at the slit diaphragm. *Nephron Exp Nephrol.* (2007) 106:e27–31. doi: 10.1159/000101789
17. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, et al. Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest.* (2001) 108:1621–9. doi: 10.1172/JCI12849
18. Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, et al. A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science.* (2005) 308:1801–4. doi: 10.1126/science.1106215
19. Tsukaguchi H, Sudhakar A, Le TC, Nguyen T, Yao J, Schwimmer JA, et al. NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele. *J Clin Invest.* (2002) 110:1659–66. doi: 10.1172/JCI16242
20. Weber S, Gribouval O, Esquivel EL, Morinière V, Tête MJ, Legendre C, et al. NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. *Kidney Int.* (2004) 66:571–9. doi: 10.1111/j.1523-1755.2004.00776.x
21. Franceschini N, North KE, Kopp JB, McKenzie L, Winkler C. NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: a HuGE review. *Genet Med.* (2006) 8:63–75. doi: 10.1097/01.gim.0000200947.09626.1c
22. Köttgen A, Hsu CC, Coresh J, Shuldiner AR, Berthier-Schaad Y, Gambhir TR, et al. The association of podocin R229Q polymorphism with increased albuminuria or reduced estimated GFR in a large population-based sample of US adults. *Am J Kidney Dis.* (2008) 52:868–75. doi: 10.1053/j.ajkd.2008.02.306
23. Sorof JM, Hawkins EP, Brewer ED, Boydstun II, Kale AS, Powell DR. Age and ethnicity affect the risk and outcome of focal segmental glomerulosclerosis. *Pediatr Nephrol.* (1998) 12:764–8. doi: 10.1007/s004670050542
24. Bonilla-Felix M, Parra C, Dajani T, Ferris M, Swinford RD, Portman RJ, et al. Changing patterns in the histopathology of idiopathic nephrotic syndrome in children. *Kidney Int.* (1999) 55:1885–90. doi: 10.1046/j.1523-1755.1999.00408.x
25. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. *Int J Epidemiol.* (2005) 34:1319–28. doi: 10.1093/ije/dyi169
26. Bush WS, Moore JH. Chapter 11: genome-wide association studies. *PLoS Comput Biol.* (2012) 8:e1002822. doi: 10.1371/journal.pcbi.1002822
27. Hinkes B, Vlangos C, Heeringa S, Mucha B, Gbadegesin R, Liu J, et al. Specific podocin mutations correlate with age of onset in steroid-resistant nephrotic syndrome. *J Am Soc Nephrol.* (2008) 19:365–71. doi: 10.1681/ASN.2007040452
28. Chernin G, Heeringa SF, Gbadegesin R, Liu J, Hinkes BG, Vlangos CN, et al. Low prevalence of NPHS2 mutations in African American children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol.* (2008) 23:1455–60. doi: 10.1007/s00467-008-0861-7
29. Lovric S, Fang H, Vega-Warner V, Sadowski CE, Gee HY, Halbritter J, et al. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol.* (2014) 9:1109–16. doi: 10.2215/CJN.09010813
30. Tonna SJ, Needham A, Polu K, Uscinski A, Appel GB, Falk RJ, et al. NPHS2 variation in focal and segmental glomerulosclerosis. *BMC Nephrol.* (2008) 9:13. doi: 10.1186/1471-2369-9-13
31. Karle SM, Uetz B, Ronner V, Glaeser L, Hildebrandt F, Fuchshuber A. Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol.* (2002) 13:388–93. doi: 10.1681/ASN.V132388
32. Caridi G, Bertelli R, Di Duca M. Dagnino M. Emma F, Muda A, et al. Broadening the spectrum of diseases related to podocin mutations. *J Am Soc Nephrol.* (2003) 14:1278–86. doi: 10.1097/01.ASN.0000060578.79050.E0
33. Schultheiss M, Ruf RG, Mucha BE, Wiggins R, Fuchshuber A, Lichtenberger A, et al. No evidence for genotype/phenotype correlation in NPHS1 and NPHS2 mutations. *Pediatr Nephrol.* (2004) 19:1340–8. doi: 10.1007/s00467-004-1629-3
34. Aucella F, De Bonis P, Gatta G, Muscarella LA, Vigilante M, di Giorgio G, et al. Molecular analysis of NPHS2 and ACTN4 genes in a series of 33 Italian patients affected by adult-onset nonfamilial focal segmental glomerulosclerosis. *Nephron Clin Pract.* (2005) 99:c31–6. doi: 10.1159/000082864
35. Lu L, Wan H, Yin Y, Feng WJ, Wang M, Zou YC, et al. The p.R229Q variant of the NPHS2 (podocin) gene in focal segmental glomerulosclerosis and steroid-resistant nephrotic syndrome: a meta-analysis. *Int Urol Nephrol.* (2014) 46:1383–93. doi: 10.1007/s11255-014-0676-3
36. Machuca E, Hummel A, Nevo F, Dantal J, Martinez F, Al-Sabban E, et al. Clinical and epidemiological assessment of steroid-resistant nephrotic syndrome associated with the NPHS2 R229Q variant. *Kidney Int.* (2009) 75:727–35. doi: 10.1038/ki.2008.650
37. Mao J, Zhang Y, Du L, Dai Y, Gu W, Liu A, et al. NPHS1 and NPHS2 gene mutations in Chinese children with sporadic nephrotic

syndrome. *Pediatr Res.* (2007) 61:117–22. doi: 10.1203/01.pdr.0000250041.19306.3d
38. Miko A. Menyhárd M, Kaposi A, Antignac C, Tory K. The mutation-dependent pathogenicity of NPHS2 p.R229Q: a guide for clinical assessment. *Hum Mutat.* (2018) 39:1854–60. doi: 10.1002/humu.23660
39. Tory K, Menyhard DK, Woerner S, Nevo F, Gribouval O, Kerti A, et al. Mutation-dependent recessive inheritance of NPHS2-associated steroid-resistant nephrotic syndrome. *Nat Genet.* (2014) 46:299–304. doi: 10.1038/ng.2898
40. Mishra OP, Kakani N, Singh AK, Narayan G, Abhinay A, Prasad R, et al. NPHS2 R229Q polymorphism in steroid resistant nephrotic syndrome: is it responsive to immunosuppressive therapy? *J Trop Pediatr.* (2014) 60:231–7. doi: 10.1093/tropej/fmu006
41. Fotouhi N, Ardalan M, Jabbarpour Bonyadi M, Abdolmohammadi R, Kamalifar A, Nasri H, et al. R229Q polymorphism of NPHS2 gene in patients with late-onset steroid-resistance nephrotic syndrome: a preliminary study. *Iran J Kidney Dis.* (2013) 7:399–403.
42. Abid A, Khaliq S, Shahid S, Lanewala A, Mubarak M, Hashmi S, et al. A spectrum of novel NPHS1 and NPHS2 gene mutations in pediatric nephrotic syndrome patients from Pakistan. *Gene.* (2012) 502:133–7. doi: 10.1016/j.gene.2012.04.063
43. Reiterova J, Safrankova H, Obeidova L, Stekrova J, Maixnerova D, Merta M, et al. Mutational analysis of the NPHS2 gene in Czech patients with idiopathic nephrotic syndrome. *Folia Biol.* (2012) 58:64–8.
44. Tsygin A, Kornienko V, Vashurina T, Zrobok O, Matveeva M, Leonova L, et al. Prevalence of R229Q polymorphysm of NPHS2 gene in Russian children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol.* (2011) 26:1662.
45. Santin S, Tazon-Vega B, Silva I, Cobo MA, Gimenez I, Ruiz P, et al. Clinical value of NPHS2 analysis in early- and adult-onset steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol.* (2011) 6:344–54. doi: 10.2215/CJN.03770410
46. Megremis S, Mitsioni A, Mitsioni AG, Fylaktou I, Kitsiou-Tzelli S, Stefanidis CJ, et al. Nucleotide variations in the NPHS2 gene in Greek children with steroid-resistant nephrotic syndrome. *Genet Test Mol Biomark.* (2009) 13:249–56. doi: 10.1089/gtmb.2008.0083
47. Zaki M, El-Shaer S, Rady S, El-Salam MA, Abd-El-Salam R, Alkashlan IA, et al. Analysis of NPHS2 gene mutations in Egyptian children with nephrotic syndrome. *Open Access Maced J Med Sci.* (2019) 7:3145–8. doi: 10.3889/oamjms.2019.700
48. Baylarov R, Senol O, Atan M, Berdeli A. NPHS2 gene mutations in Azerbaijani children with steroid-resistant nephrotic syndrome. *Saudi J Kidney Dis Transpl.* (2020) 31:144–9. doi: 10.4103/1319-2442.279934
49. McKenzie LM, Hendrickson SL, Briggs WA, Dart RA, Korbet SM, Mokrzycki MH, et al. NPHS2 variation in sporadic focal segmental glomerulosclerosis. *J Am Soc Nephrol.* (2007) 18:2987–95. doi: 10.1681/ASN.2007030319
50. He N, Zahirieh A, Mei Y, Lee B, Senthilnathan S, Wong B, et al. Recessive NPHS2 (Podocin) mutations are rare in adult-onset idiopathic focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol.* (2007) 2:31–7. doi: 10.2215/CJN.02690806

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer FL declared a shared parent affiliation with the authors JX, QW, JT, XH, HS, XZ, YL, PS, HR, and NC to the handling editor at the time of review.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 28 July 2022
DOI 10.3389/fmed.2022.911737

OPEN ACCESS

EDITED BY
Hong Liu,
Central South University, China

REVIEWED BY
L. J. Muhammad,
Federal University Kashere, Nigeria
Björn Tampe,
University Medical Center Göttingen, Germany

*CORRESPONDENCE
Yafeng Li
dr.yafengli@gmail.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 03 April 2022
ACCEPTED 04 July 2022
PUBLISHED 28 July 2022

CITATION
Song W, Zhou X, Duan Q, Wang Q, Li Y Li A, Zhou W, Sun L, Qiu L, Li R and Li Y (2022) Using random forest algorithm for glomerular and tubular injury diagnosis.
Front. Med. 9:911737.
doi: 10.3389/fmed.2022.911737

Using random forest algorithm for glomerular and tubular injury diagnosis

Wenzhu Song[1], Xiaoshuang Zhou[2], Qi Duan[3], Qian Wang[3], Yaheng Li[3], Aizhong Li[3], Wenjing Zhou[4], Lin Sun[5], Lixia Qiu[1], Rongshan Li[2,3] and Yafeng Li[2,3,6,7]*

[1]School of Public Health, Shanxi Medical University, Taiyuan, China, [2]Department of Nephrology, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan, China, [3]Shanxi Provincial Key Laboratory of Kidney Disease, Taiyuan, China, [4]School of Medical Sciences, Shanxi University of Chinese Medicine, Jinzhong, China, [5]College of Traditional Chinese Medicine and Food Engineering, Shanxi University of Chinese Medicine, Jinzhong, China, [6]Core Laboratory, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan, China, [7]Academy of Microbial Ecology, Shanxi Medical University, Taiyuan, China

Objectives: Chronic kidney disease (CKD) is a common chronic condition with high incidence and insidious onset. Glomerular injury (GI) and tubular injury (TI) represent early manifestations of CKD and could indicate the risk of its development. In this study, we aimed to classify GI and TI using three machine learning algorithms to promote their early diagnosis and slow the progression of CKD.

Methods: Demographic information, physical examination, blood, and morning urine samples were first collected from 13,550 subjects in 10 counties in Shanxi province for classification of GI and TI. Besides, LASSO regression was employed for feature selection of explanatory variables, and the SMOTE (synthetic minority over-sampling technique) algorithm was used to balance target datasets, i.e., GI and TI. Afterward, Random Forest (RF), Naive Bayes (NB), and logistic regression (LR) were constructed to achieve classification of GI and TI, respectively.

Results: A total of 12,330 participants enrolled in this study, with 20 explanatory variables. The number of patients with GI, and TI were 1,587 (12.8%) and 1,456 (11.8%), respectively. After feature selection by LASSO, 14 and 15 explanatory variables remained in these two datasets. Besides, after SMOTE, the number of patients and normal ones were 6,165, 6,165 for GI, and 6,165, 6,164 for TI, respectively. RF outperformed NB and LR in terms of accuracy (78.14, 80.49%), sensitivity (82.00, 84.60%), specificity (74.29, 76.09%), and AUC (0.868, 0.885) for both GI and TI; the four variables contributing most to the classification of GI and TI represented SBP, DBP, sex, age and age, SBP, FPG, and GHb, respectively.

Conclusion: RF boasts good performance in classifying GI and TI, which allows for early auxiliary diagnosis of GI and TI, thus facilitating to help alleviate the progression of CKD, and enjoying great prospects in clinical practice.

KEYWORDS

random forest, machine learning, auxiliary diagnosis, glomerular injury, tubular injury

Introduction

Chronic kidney disease (CKD) is a common chronic condition worldwide, with a prevalence of 13.4% (1). Due to its imperceptible symptoms at the initial stages, it may progress into end-stage renal disease, which requires kidney transplantation, posing a substantial financial burden to the society leading to a lower quality of life and higher mortality rate. Additionally, it's highly associated with such complications as cardiovascular disease (2), emerging as another "silent killer" that threatens human life after tumors and diabetes. Renal injury is the prerequisite for CKD, including glomerular injury (GI) and tubular injury (TI), and thus early diagnosis of GI and TI is of practical significance to alleviate the progression of CKD. How to better make an early diagnosis of renal injury is now a topic running into the forefront of research.

In 2012, Luxia Zhang employed logistic regression to predict CKD, but it comes with drawbacks (3). The first one concerns its sensitivity to multicollinearity; the second one is that maximum likelihood estimation does not fit the true distribution of the data well. A better model is needed. With artificial intelligence springing up, data-driven algorithms pick up pace, and have become a research hotspot in the life sciences, enjoying great popularity in cardiovascular diseases (4), tumors (5), immune diseases (6), and neurological diseases (7). Also, its application in renal diseases is on the rise, from acute kidney injury prediction (8) to kidney transplantation outcome prediction (9), interstitial fibrosis, and tubular atrophy detection (10). One of the well-known algorithms represents Random forest (RF), which has been shown a powerful tool in disease auxiliary diagnosis (11, 12). However, it has not yet been determined in glomerular and tubular injury.

Feature selection is of great necessity in constructing classifiers, since the presence of irrelevant features may be responsible for the poor model performance (13). L1 regularization, Absolute Shrinkage and Selection Operator (LASSO) regression is a welcome choice. It is characterized by the inclusion of an L1 regularization penalty term in fitting generalized linear regression, which makes the sum of the absolute values of the regression coefficients of the model lower than a particular value. It aims to minimize the sum of squared residuals, forcing the regression coefficients of variables that contribute less to the model to be compressed to zero and achieving a feature sparse process (14, 15). Also, it could eliminate predictors with autocorrelation or redundancy, allowing for automated variable selection within the model, and significantly contributing to the performance of classification models (16, 17). Another headwind in the development of classifiers relates to imbalanced datasets. It is not unusual in medical research, because the number of non-patients is extremely larger than that of patients, which serves as an obstacle to predictive performance (18). RF is sensitive to response variables with unbalanced data, and imbalances in classes in the data tend to tend to larger classes in the output of the model, resulting in some classification errors, resulting in lower classification accuracy (19). It has been documented that machine learning methods with data balancing techniques represent effective approach for stroke prediction with imbalanced data. As such, it's crucial to balance the data prior to model construction (20).

In this study, we aimed to (1) employ LASSO algorithm to conduct feature selection for GI and TI; (2) use the classical and widely accepted SMOTE (Synthetic Minority Over-sampling Technique) algorithm to handle the imbalanced classes of GI and TI; (3) employ the mature machine learning algorithm, Random Forest (RF) to make a classification of GI and TI, respectively, and compare its performance with logistic regression (LR) and Naive Bayes (NB), thus achieving the auxiliary diagnosis of GI and TI and providing a new idea for clinical practice in delaying the progression of CKD.

Participants and methods

Study participants

Shanxi Provincial People's Hospital conducted CKD screening for permanent residents aged ≥ 40 years in the northern region of Shanxi Province (Ningwu County), the central regions (Yu County, Yangqu County, Lin County, Shouyang County), and the southern regions (Zezhou County, Huozhou City, Hejin City, Linyi County, and Ruicheng County) from April 2019 to November 2019. A total of 13,550 residents volunteered for this screening, and 12,285 were eventually enrolled in the study, including 5,206 men and 7,079 women aged 41–91 years.

Inclusion criteria: (1) residents aged 40 years or older; (2) conscious participants without communication impairment; (3) participants understanding the significance of the study and willing to sign a written informed consent; (4) participants with no cognitive impairment or mental illness; (5) more than 1 year of local residents as of the survey date. Exclusion criteria: (1) Severely incomplete information recorded; (2) poor compliance; (3) pregnant women or those with a history of substance abuse.

Data collection

Questionnaires, physical examination, and laboratory analysis were used to collect data. (1) The questionnaire comprised demographic information (including age, sex, annual income, educational levels), lifestyle (including smoking, alcohol consumption, diet, and exercise). The questionnaire was administered online and completed by the subjects themselves

or their family members. (2) Physical examination included height, weight and blood pressure (systolic blood pressure, diastolic blood pressure), which were measured twice and then the mean value was calculated. All data were measured by a medical professional. Body mass index (BMI) was calculated by weight in kilograms divided by the square of height in meters.

(3) Fasting venous blood was collected from subjects for fasting blood glucose (FPG), glycated hemoglobin (GHb), homocysteine (Hcy), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein (HDL-C). (4) Morning urine specimens were collected from subjects. After centrifugation at 3,000 r/min for 10 min, the supernatant was extracted (low-speed centrifuge Anhui Zhongke Zhongjia SC3616), and α1-microglobulin (α1MG), urinary creatinine (UCr), and microalbuminuria (MAU) were determined by latex turbidimetry, sarcosine oxidase, and immunoturbidimetry, respectively.

Variable assignments

Information on the annual income, educational levels, health history, and lifestyle of the study participants was obtained from the questionnaire. Annual income was defined as < 5K yuan, 5K–10K yuan, 10K–20K yuan, > 20K yuan; education levels were defined as ≤ primary school, ≤ middle school, ≤ high school, ≥ bachelor's degree; smoking was classified as yes or no; alcohol consumption was classified

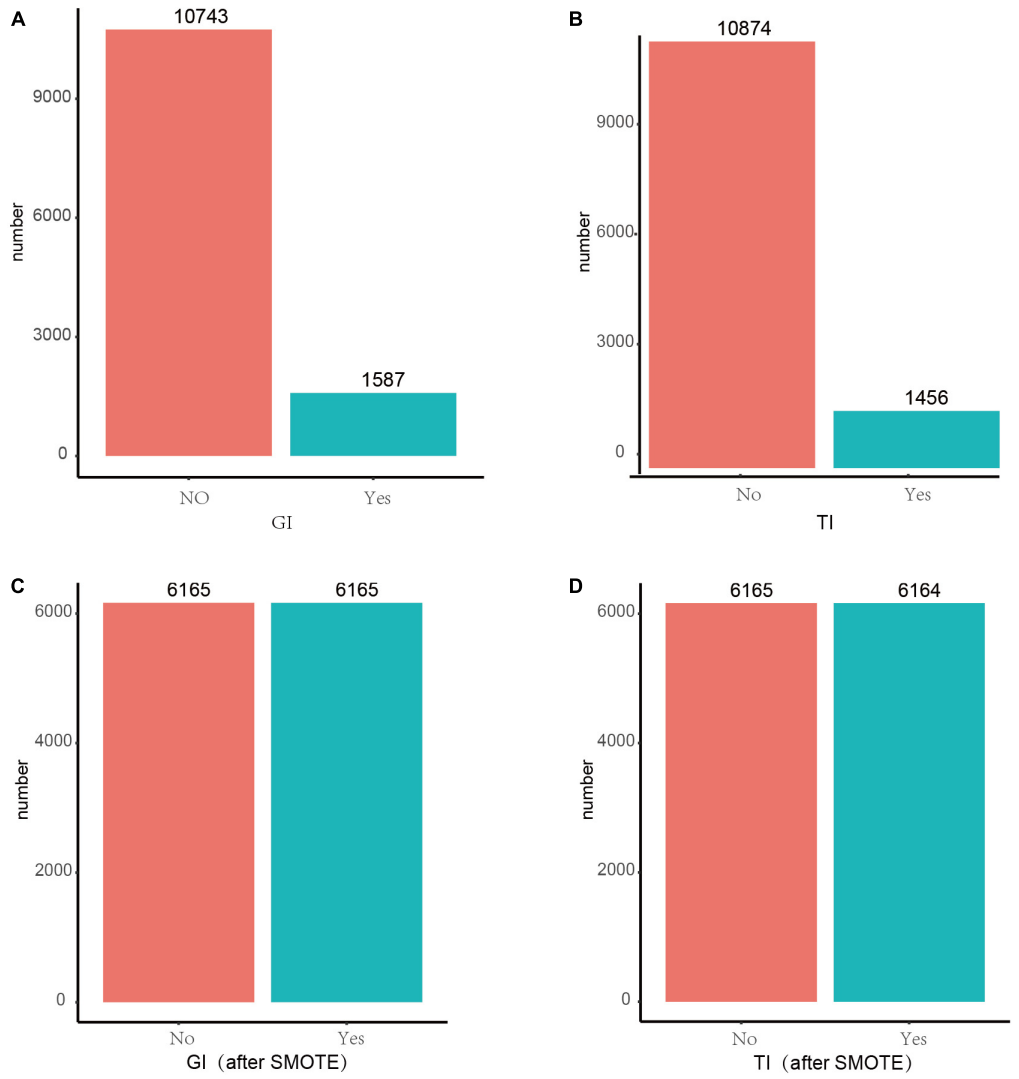


FIGURE 1
Before and after SMOTE of response variables for GI and TI. SMOTE, Synthetic Minority Over-Sampling Technique. It's a good and powerful way to handle imbalanced data, and it was conducted under the parameters of k = 5, C.perc = "balance," dist = "Overlap." **(A)** GI before SMOTE; **(B)** TI before SMOTE; **(C)** GI after SMOTE; **(D)** TI after SMOTE.

as always (>100 g/time and 3 times/week), sometimes (<3 times/week or < 100 g/time) and rarely; exercise was classified as "none or a little" or "regular" (≥3 times/week, ≥ 30 min/time). BMI was defined as underweight (<18.5 kg/m^2), normal weight (18.5–24.0 kg/m^2), overweight (24.0–28.0 kg/m^2), obesity (≥28 kg/m^2). ACR was defined as urinary microalbumin divided by urinary creatinine multiplied 8.84; MCR was defined as urinary microglobulin divided by urinary creatinine multiplied 8.84.

Explanatory variables

(1) Questionnaire: demographic information (age, sex, educational levels, annual income, residence, etc.); lifestyle (smoking, alcohol, exercise, salt consumption, diet). (2) Morning blood: HDL, LDL, TG, TC, Hcy, FPG, GHb. (3) Physical examination: SBP, DBP, BMI. 20 variables in total.

Response variables

ACR ≥ 30 mg/g was defined as GI; MCR > 23 mg/g was defined as TI. The presence of GI, TI was assigned 1; otherwise, they were defined as 0. In this study, we employed RF, LR and NB to make a classification of GI and TI, respectively.

L1 regularization, absolute shrinkage and selection operator regression

Absolute Shrinkage and Selection Operator (LASSO) is one of the common methods for feature selection. It is characterized by the inclusion of an L1 regularization penalty term in fitting generalized linear regression, which makes the sum of the absolute values of the regression coefficients of the model lower than a particular value. It aims to minimize the sum of squared residuals, forcing the regression coefficients of variables that contribute less to the model to be compressed to zero and achieving a feature sparse process (14, 15). LASSO was used to select the collected explanatory variables, and to determine those more relevant to the response variables.

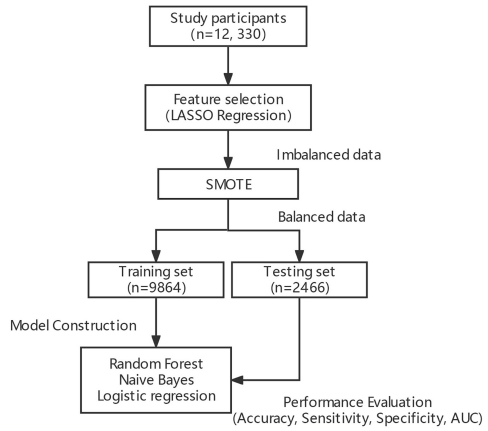


FIGURE 2
Workflow of the model construction.

Synthetic minority over-sampling technique algorithm

The Synthetic Minority Over-Sampling Technique (SMOTE) is an oversampling technique that is an effective algorithm for dealing with imbalances between data classes (21). It's employed to synthetically enlarge the minority class using K-nearest neighors to obtain a balanced data set (22) and has been shown good performance in such fields as network intrusion detection systems and disease detection. In this study, there is a serious imbalance in the response variables, GI and TI (**Figures 1A,B**). SMOTE was used to balance the classes to facilitate the machine learning models to better learn the inter-data features, thus making the best classification judgment.

Random forest

RF, a data-driven integrated learning algorithm, could obtain multiple new training data by an autonomous sampling of the training set, constructing multiple classification trees based on the parallelization of these new data, and achieving de-correlation between the trees by introducing the selection of independent variables. By doing so, the diversity of the classification trees originates from both sample and independent variable perturbations to achieve the effect of reducing the model variance, and finally to vote on the classification results of multiple trees to obtain the final classification results (23, 24). The workflow of the model construction is shown in **Figure 2**.

Statistical methods

Statistical description

Qualitative data are expressed as percentages (%), and quantitative data are expressed as mean ± standard deviation (M ± *SD*) or median ± interquartile [(Median(P25, P75))], as appropriate.

TABLE 1 Clinical parameters of study subjects (quantitative ones).

Variables	$\bar{x} \pm s$	Variables	$\bar{x} \pm s$
Age(y)	58.75 ± 9.49	TC (mmol/L)	4.43 ± 0.95
LDL (mmol/L)	2.35 ± 0.84	TG (mmol/L)	1.73 ± 0.82
HDL (mmol/L)	1.30 ± 0.37	Hcy (mmol/L)	22.98 ± 14.26
FPG (mmol/L)	4.97 ± 1.36	SBP (mmHg)	136.10 ± 18.39
GHb (mmol/L)	5.54 ± 1.09	DBP (mmHg)	82.84 ± 10.76

TABLE 2 Clinical parameters of study subjects (qualitative ones).

Variables	Percentage (%)	Variables	Percentage (%)	Variables	Percentage (%)
Education		**BMI**		**Income (Yuan)**	
≤Primary	32.7	Underweight	1.7	<5k	41.8
≤Junior	50.9	Normal	39.5	5k–10k	25.5
≤Senior	11.9	Overweight	42.6	10k–20k	10.3
≥Bachelor	4.5	Obesity	16.3	>20k	22.4
Salt consumption		**Alcohol**		**Diet**	
Light	26.3	Rarely	84.7	Vegetable	33.5
Moderate	60.5	Sometimes	13.2	Balanced	61.9
Salty	13.1	Always	2.1	Meat	4.6
Exercise		**Smoking**		**Sex**	
Regular	41.7	No	76.2	Male	42.4
None or a little	58.3	Yes	23.8	Female	57.6

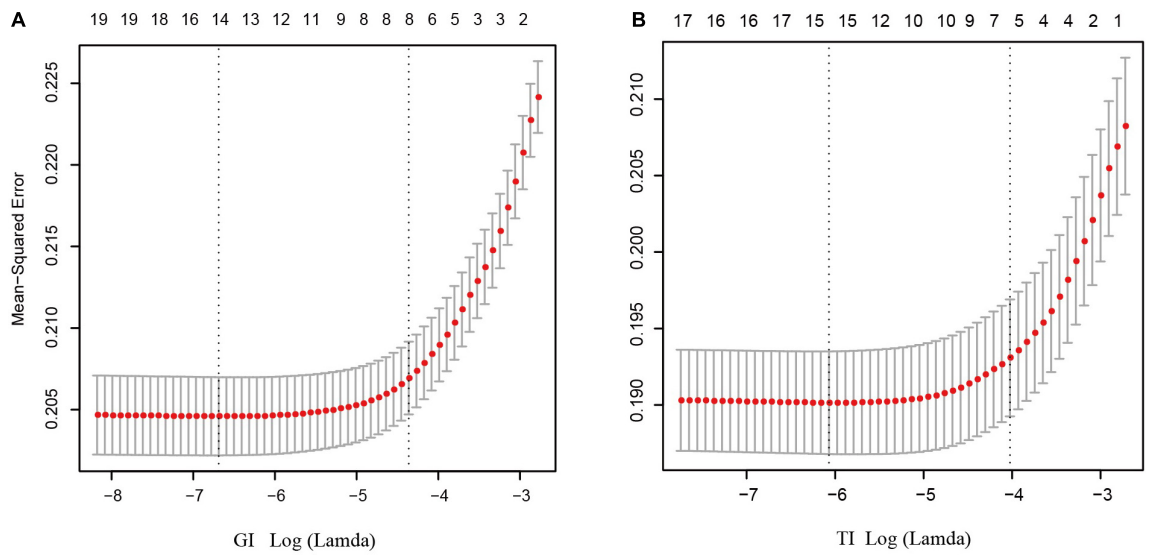


FIGURE 3
Results of feature selection using LASSO. When Lamda is minimum, corresponding features were taken into model construction (14 features for GI, and 15 feature for TI). **(A)** Feature selection for GI; **(B)** feature selection for TI.

Model construction

The datasets were divided into training set (80%) and testing set (20%). The former ones were used for models training, i.e., RF, NB, and LR, while the latter ones were employed for evaluation of model performance. All analyses were implemented in R software (version 4.0.3).

Evaluation parameters

The evaluation parameters comprised Accuracy (1), Specificity (2), Sensitivity (3) and area under the receiver operating curve (AUC). The predicted result was defined as True Positive (TP) when patients with renal conditions were classified as patients and True Negative (TN) when healthy ones were classified as healthy. Besides, the predicted result was False Positive (FP) if healthy subjects are considered patients; similarly, False Negative (FN) if patients are considered healthy subjects. Accuracy is to evaluate how accurate the machine learning algorithms are to detect what it is supposed to measure. Specificity is the ability to correctly exclude those without renal conditions and Sensitivity is to correctly identify those with renal conditions.

$$Accuracy = \frac{(TN + TP)}{(TP + TN + FP + FN)} \times 100\% \quad (1)$$

$$Specificity = \frac{TN}{(TN + FP)} \times 100\% \quad (2)$$

$$Sensitivity = \frac{TP}{(TP + FN)} \times 100\% \quad (3)$$

Results

Baseline characteristics

A total of 12,330 people were included in this study, 5,230 men and 7,100 women, the number of GI was 1,587 (12.8%) and the number of TI was 1,456 (11.8%). Besides, the number of participants with both GI and TI was 2,439 (19.7%). Other parameters are detailed in **Tables 1**, **2**.

Feature selection and results of sampling technique

As shown in **Figures 3A,B**, after LASSO feature selection, 14 and 15 explanatory variables remained in the two datasets, respectively, in which data 1 with GI as the response variable excluded six variables of annual income, residence, LDL, HDL, smoking, and exercise; while data 2 with TI as the response variable excluded five variables of TC, LDL, HDL, exercise, and salt consumption. The remained variables are comparable for GI (except TG and sex) and TI (**Supplementary Tables 1, 2**).

As shown in **Figures 1C,D**, after resampling by SMOTE, the number of patients and normal ones were 6,165, 6,165 for GI, and 6,165, 6,164 for TI, espectively.

Model performance

When constructing model for GI, the number of GI and non-GI in the training set were both 4,932, and 1,233 in the testing set, respectively. When constructing model for GI, the number of TI and non-TI in the training set were 4,973 and 4,891, respectively, and 1,273 and 1,192 in the testing set. The accuracy, sensitivity, specificity and AUC of RF for classification of GI and TI performed better than NB and LR in both the training set and testing set, which shows that RF does have a high diagnostic value for classification (**Tables 3**, **4** and **Figure 4**).

TABLE 3 Performance evaluation of the three classifiers on the training set (GI/TI).

Model	Accuracy (%)	Sensitivity (%)	Specificity (%)
RF	99.90/99.92	99.96/99.94	99.84/99.90
NB	65.39/67.06	52.08/54.26	78.71/79.65
LR	66.40/68.52	64.90/66.94	67.90/70.08

TABLE 4 Performance evaluation of the three classifiers on the testing set (GI/TI).

Model	Accuracy(%)	Sensitivity(%)	Specificity(%)
RF	78.14/80.49	82.00/84.60	74.29/76.09
NB	65.17/65.68	52.23/53.34	78.10/78.86
LR	66.87/67.51	64.23/66.06	69.51/69.04

Feature importances

We indicated the contribution of the explanatory variables to the model by %IncMSE, and the larger the %IncMSE, the more important the variables were for the RF model. The four variables that contributed most to the classification of GI in the RF model represented SBP, DBP, sex, and age. The four variables that were most important for the classification of TI constituted age, SBP, FPG, and GHb (**Figure 5**).

Discussion

In this study, ACR and MCR levels were used as screening indicators for GI and TI, and ACR $\geq$ 30 mg/g was considered GI; MCR $>$ 23 mg/g was considered TI. Besides, the machine learning model RF was used to classify them, and we compared its classification performance with NB, and LR.

This study suggested that the accuracy, sensitivity, specificity and AUC of the RF algorithm outperformed other classifiers in both the training set and testing set. Yet its performance in the testing set was comparatively lower than that in the training set, because the classification performance based on the training set was prone to overfitting (25), while the results of the testing set could better reflect the classification performance of the model, which proves their potential applications in GI and TI-aided diagnosis.

Of note, the RF model is sensitive to response variables with unbalanced data. Imbalanced classes in the data would leave the output of the model tending to larger classes, causing some classification errors, and leading to a less accurate classification performance (20). As such, SMOTE algorithm was employed to resample the data set with GI and TI as response variables, respectively, before performing the classification task to achieve balanced classes. By doing so, the learning capability of the model could be maximized, and a more accurate predictive performance could be achieved.

Since RF model is data-driven, a visible functional equation is unavailable to determine the extent to which the explanatory variables contribute to the model based on the regression coefficients. However, a more intuitive alternative for the model is that by outputting feature importances, the model could explain the importance of the variables on the explanatory variables. This study demonstrated that the four explanatory variables with the greatest output weight of RF classifier for GI

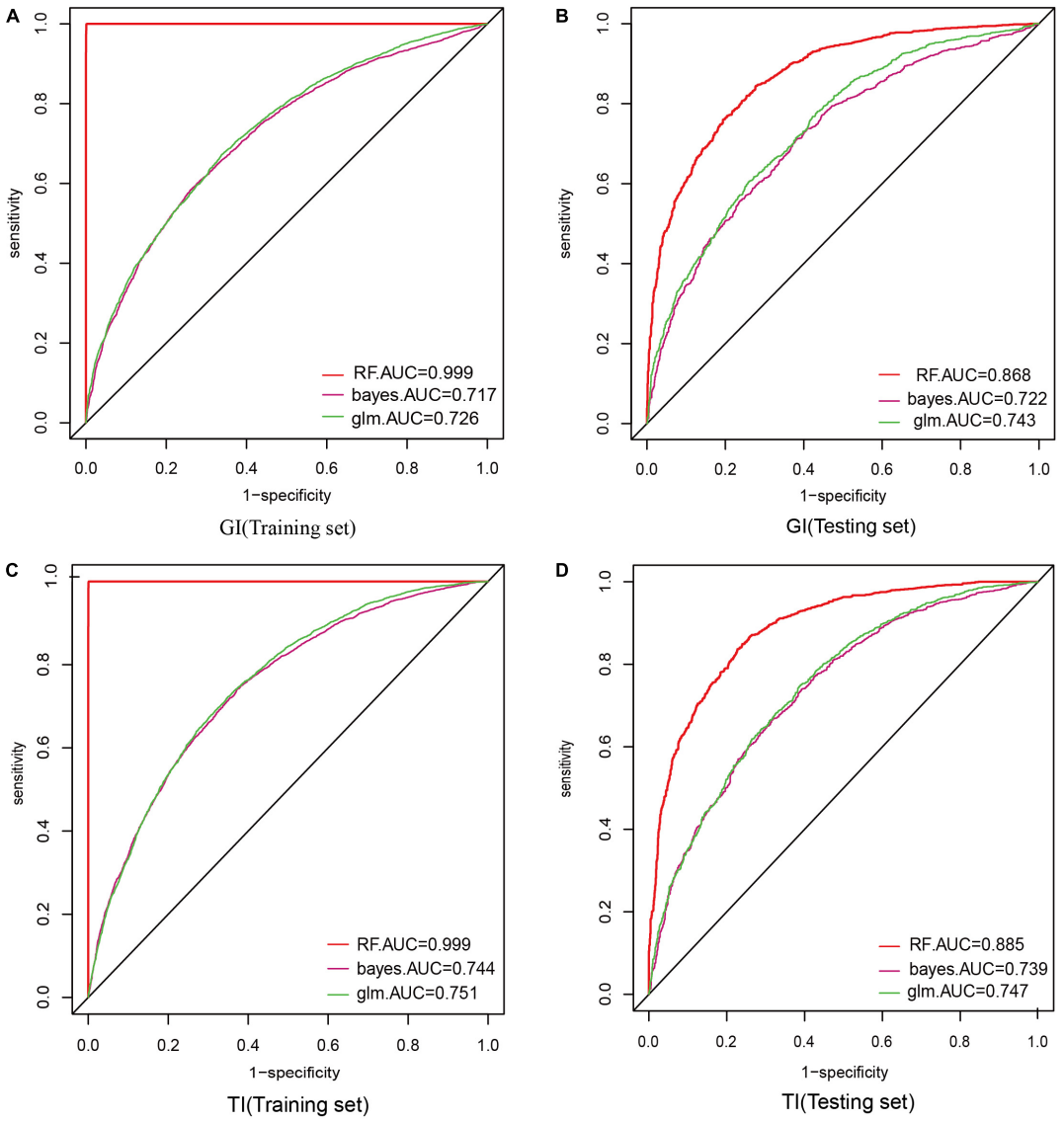


FIGURE 4
Comparison of the ROC curve areas of the three model classifiers. In model construction, 70% of samples were randomly divided as training set, and the rest 30% were as testing set. AUC (area under curve) was used to evaluate the performance of these three classifiers. **(A)** AUC of GI in the training set; **(B)** AUC of GI in the testing set; **(C)** AUC of TI in the training set; **(D)** AUC of TI in the testing set.

represented SBP, DBP, sex, and age; and the four explanatory variables for TI constituted age, SBP, FPG, and GHb.

In a hypertensive state, abnormal glomerular hemodynamics, spasmodic constriction of renal arteries would reduce renal blood flow, leading to renal ischemia and long-term hyperperfusion and hyperfiltration of glomerular capillaries, resulting in damage to glomerular vascular endothelial cells and podocytes (26, 27). Meanwhile, renal ischemia caused by hypertension activates the renin-angiotensin-aldosterone system, leading to constriction of the inlet and outlet arteries and a further increase in glomerular pressure, which aggravates renal ischemia. The high pressure causes damage to endothelial cells, podocytes and tubular epithelial cells, leading to the destruction of the filtration barrier and dysfunction of reabsorption, thus, resulting in proteinuria occurrence (28, 29). Additionally, hypertension can lead to thickening of glomerular duct wall hardening, renal parenchymal ischemia, which would further increase the production of vasoactive substances, stimulate interstitial collagen deposition, and eventually leading to glomerular sclerosis and kidney injury (30).

It has been documented that kidney disease in China is more prevalent in male patients, suggesting that sex is also one contributor to degenerative changes in kidney structure and function. The prevalence of CKD has been reported to be

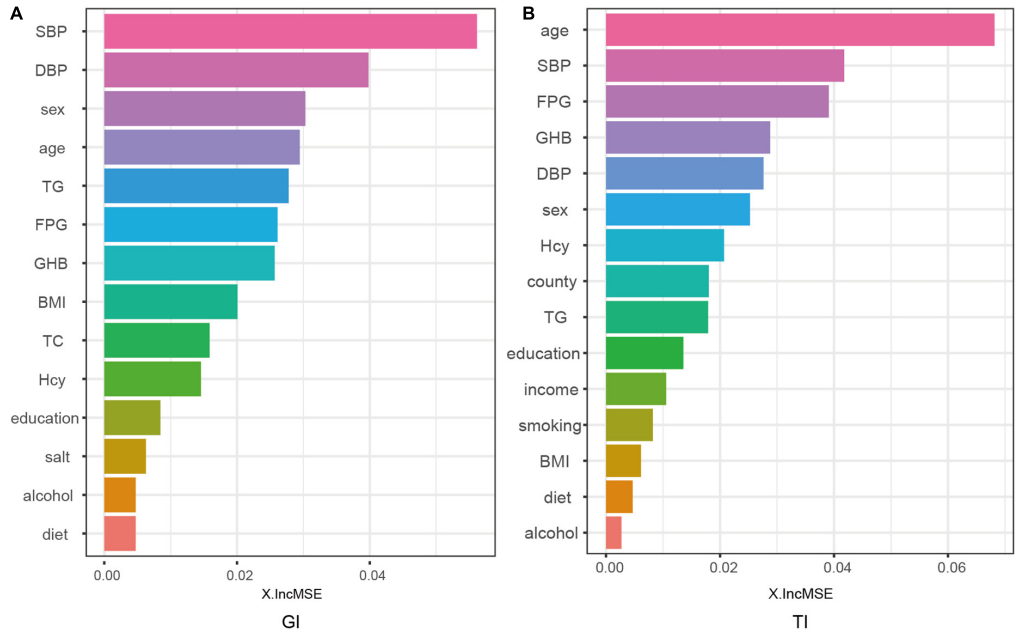


FIGURE 5
Contributions of explanatory variables to the random forest model. The "%IncMSE" is the increase in mean squared error, where the error of the model prediction is increased by randomly replacing the value of each predictor variable if it is more important. Therefore, a larger value indicates that the variable is more important. **(A)** feature importances for GI; **(B)** feature importances for TI.

higher in women than in men. A representative study pooling 33 population-based studies worldwide evaluated the global prevalence of stage 1–5 CKD at 10.4% among men and 11.8% among women aged 20 years and older (31). The reasons for these differences are unclear, and although the GFR estimation equation includes a gender correction factor, a single threshold value of < 60 ml/min per 1.73 m^2 for the definition of CKD may lead to overdiagnosis of CKD in women (32). A follow-up of Swedish patients with CKD not on dialysis in the national registry showed that male patients had a faster decline in eGFR, more rapid CKD progression and higher all-cause mortality compared to women (33). Also. the results of a study are consistent with experimental data showing the protective effect of estrogen and the potentially deleterious effect of testosterone on non-diabetic CKD (34). The effect of gender on CKD incidence, prevalence, and progression needs further study, and the development of gender-specific CKD markers is also a hot topic of current research.

After the age of 40, the glomerular filtration rate decreases at a rate of 1 ml/min/1.73 per year, resulting in stiffening of the renal vessel wall, glomerular atrophy, sclerosis, tubular atrophy, and interstitial fibrosis, which eventually lead to renal hypofunction (35). In diabetic patients, high blood glucose concentration would cause glucose metabolism disorder, hemodynamic changes, oxidative stress, which induce renal tubular epithelial cell hypertrophy, tubular basement membrane destruction, interstitial cell infiltration, and renal tubular interstitial fibrosis, contributing to reabsorption dysfunction (36, 37). Therefore, the present study shows that RF has some clinical practice combined with feature selection by LASSO.

There are also some limitations in this paper. Firstly, the study constructed the models with data from Shanxi Province, and no other external datasets are available to validate the model performance. Our ongoing work is to collect samples from other areas, to validate the generalization capabilities of the model. Secondly, this study was initially considered for cost-effectiveness and other indicators reflecting CKD were not collected, such as blood creatinine, which will also be the focus of our next step. Additionally, as CKD was more prevalent in people aged $\geq$ 40 years, this study centered on those over 40 years. In the future, we consider surveys on those aged 18–40 years to improve the prediction model in younger groups. Finally, GI and TI were defined only by surrogate parameters, ACR and MCR, which may not well accurately reflect the renal conditions. In our future work, we would conduct a follow-up for those with positive urine protein.

In short, as early manifestations of CKD, GI and TI have emerged as a global public health issue; their early diagnosis and corresponding treatment are of great importance. Our results demonstrate the potential value of machine learning algorithms in GI and TI-assisted diagnosis, which facilitates reducing the workload of doctors, while achieving automated diagnosis and treatment decisions, and thus could be promoted in clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanxi Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WS was responsible for the data analysis and the writing of the manuscript. XZ, QD, QW, and YahL helped polish the manuscript. AL, WZ, LS, LQ, and RL gave precious advice on the statistical methods. YafL was responsible for the conception and design of the research. All authors read and approved the final draft.

Funding

This work was supported by the Key Laboratory Project of Shanxi Province (201805D111020) and the Key Laboratory Construction Plan Project of Shanxi Provincial Health Commission (2020SYS01).

Acknowledgments

We appreciate all the authors and patients participating in this study. We're also indebted to those who helped us a lot during our writing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.911737/full#supplementary-material

References

1. Lv JC, Zhang LX. Prevalence and disease burden of chronic kidney disease. *Adv Exp Med Biol.* (2019) 1165:3–15. doi: 10.1007/978-981-13-8871-2_1

2. Wilson S, Mone P, Jankauskas SS, Gambardella J, Santulli G. Chronic kidney disease: definition, updated epidemiology, staging, and mechanisms of increased cardiovascular risk. *J Clin Hypertens.* (2021) 23:831–4. doi: 10.1111/jch.14186

3. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet.* (2012) 379:815–22. doi: 10.1016/S0140-6736(12)60033-6

4. Zheng X, Wang F, Zhang J, Cui X, Jiang F, Chen N, et al. Using machine learning to predict atrial fibrillation diagnosed after ischemic stroke. *Int J Cardiol.* (2021) 347:21–7. doi: 10.1016/j.ijcard.2021.11.005

5. Ruini C, Schlingmann S, Jonke Ž, Avci P, Padrón-Laso V, Neumeier F. Machine learning based prediction of squamous cell carcinoma in *ex vivo* confocal laser scanning microscopy. *Cancers.* (2021) 13:5522. doi: 10.3390/cancers13215522

6. Chen Y, Liao R, Yao Y, Wang Q, Fu L. Machine learning to identify immune-related biomarkers of rheumatoid arthritis based on WGCNA network. *Clin Rheumatol.* (2021) 41:1057–68. doi: 10.1007/s10067-021-05960-9

7. Yang S, Bornot JMS, Fernandez RB, Deravi F, Wong-Lin K, Prasad G. Integrated space-frequency-time domain feature extraction for MEG-based Alzheimer's disease classification. *Brain Inform.* (2021) 8:24. doi: 10.1186/s40708-021-00145-1

8. Le S, Allen A, Calvert J, Palevsky PM, Braden G, Patel S, et al. Convolutional neural network model for intensive care unit acute kidney injury prediction. *Kidney Int Rep.* (2021) 6:1289–98. doi: 10.1016/j.ekir.2021.02.031

9. Coorey CP, Sharma A, Muller S, Yang JYH. Prediction modeling-part 2: using machine learning strategies to improve transplantation outcomes. *Kidney Int.* (2021) 99:817–23. doi: 10.1016/j.kint.2020.08.026

10. Ginley B, Jen KY, Han SS, Rodrigues L, Jain S, Fogo AB, et al. Automated computational detection of interstitial fibrosis, tubular atrophy, and glomerulosclerosis. *J Am Soc Nephrol.* (2021) 32:837–50. doi: 10.1681/ASN.2020050652

11. Yang L, Wu H, Jin X, Zheng P, Hu S, Xu X, et al. Study of cardiovascular disease prediction model based on random forest in eastern China. *Sci Rep.* (2020) 10:5245. doi: 10.1038/s41598-020-62133-5

12. Heo J, Yoon JG, Park H, Kim YD, Nam HS, Heo JH. Machine learning-based model for prediction of outcomes in acute stroke. *Stroke.* (2019) 50:1263–5. doi: 10.1161/STROKEAHA.118.024293

13. Sreejith S, Khanna Nehemiah H, Kannan A. Clinical data classification using an enhanced SMOTE and chaotic evolutionary feature selection. *Comput Biol Med.* (2020) 126:103991. doi: 10.1016/j.compbiomed.2020.103991

14. Mullah MAS, Hanley JA, Benedetti A. LASSO type penalized spline regression for binary data. *BMC Med Res Methodol.* (2021) 21:83. doi: 10.1186/s12874-021-01234-9

15. Kang J, Choi YJ, Kim IK, Lee HS, Kim H, Baik SH, et al. LASSO-based machine learning algorithm for prediction of lymph node metastasis in T1 colorectal cancer. *Cancer Res Treat.* (2021) 53:773–83. doi: 10.4143/crt.2020.974

16. Wang J, Zhang H, Wang J, Pu Y, Pal NR. Feature selection using a neural network with group lasso regularization and controlled redundancy. *IEEE Trans Neural Netw Learn Syst.* (2021) 32:1110–23. doi: 10.1109/TNNLS.2020.2980383

17. Jiang L, Greenwood CMT, Yao W, Li L. Bayesian hyper-LASSO classification for feature selection with application to endometrial cancer RNA-seq data. *Sci Rep.* (2020) 10:9747. doi: 10.1038/s41598-020-66466-z

18. Geetha R, Sivasubramanian S, Kaliappan M, Vimal S, Annamalai S. Cervical cancer identification with synthetic minority oversampling technique and PCA analysis using random forest classifier. *J Med Syst.* (2019) 43:286. doi: 10.1007/s10916-019-1402-6

19. Blagus R, Lusa L. SMOTE for high-dimensional class-imbalanced data. *BMC Bioinformatics.* (2013) 14:106. doi: 10.1186/1471-2105-14-106

20. Wu Y, Fang Y. Stroke prediction with machine learning methods among older Chinese. *Int J Environ Res Public Health.* (2020) 17:1828. doi: 10.3390/ijerph17061828

21. Chen PN, Lee CC, Liang CM, Pao SI, Huang KH, Lin KF. General deep learning model for detecting diabetic retinopathy. *BMC Bioinformatics.* (2021) 22:84. doi: 10.1186/s12859-021-04005-x

22. Wang K, Tian J, Zheng C, Yang H, Ren J, Li C, et al. Improving risk identification of adverse outcomes in chronic heart failure using SMOTE+ENN and machine learning. *Risk Manag Healthc Policy.* (2021) 14:2453–63. doi: 10.2147/RMHP.S310295

23. Sarica A, Cerasa A, Quattrone A. Random forest algorithm for the classification of neuroimaging data in Alzheimer's disease: a systematic review. *Front Aging Neurosci.* (2017) 9:329. doi: 10.3389/fnagi.2017.00329

24. Song M, Jung H, Lee S, Kim D, Ahn M. Diagnostic classification and biomarker identification of Alzheimer's disease with random forest algorithm. *Brain Sci.* (2021) 11:453. doi: 10.3390/brainsci11040453

25. Kang J, Chen T, Luo H, Luo Y, Du G, Jiming-Yang M. Machine learning predictive model for severe COVID-19. *Infect Genet Evol.* (2021) 90:104737. doi: 10.1016/j.meegid.2021.104737

26. Kalaitzidis RG, Elisaf MS. Treatment of Hypertension in Chronic Kidney Disease. *Curr Hypertens Rep.* (2018) 20:64. doi: 10.1007/s11906-018-0864-0

27. Ku E, Lee BJ, Wei J, Weir MR. Hypertension in CKD: core curriculum 2019. *Am J Kidney Dis.* (2019) 74:120–31. doi: 10.1053/j.ajkd.2018.12.044

28. Hsu CN, Tain YL. Targeting the renin-angiotensin-aldosterone system to prevent hypertension and kidney disease of developmental origins. *Int J Mol Sci.* (2021) 22:2298. doi: 10.3390/ijms22052298

29. Almeida LF, Tofteng SS, Madsen K, Jensen BL. Role of the renin-angiotensin system in kidney development and programming of adult blood pressure. *Clin Sci.* (2020) 134:641–56. doi: 10.1042/CS20190765

30. Pugh D, Gallacher PJ, Dhaun N. Management of Hypertension in Chronic Kidney Disease. *Drugs.* (2019) 79:365–79. doi: 10.1007/s40265-019-1064-1

31. Mills KT, Xu Y, Zhang W, Bundy JD, Chen CS, Kelly TN, et al. A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. *Kidney Int.* (2015) 88:950–7. doi: 10.1038/ki.2015.230

32. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl.* (2022) 12:7–11. doi: 10.1016/j.kisu.2021.11.003

33. Swartling O, Rydell H, Stendahl M, Segelmark M, Trolle Lagerros Y, Evans M. CKD progression and mortality among men and women: a nationwide study in Sweden. *Am J Kidney Dis.* (2021) 78:190–9.e1. doi: 10.1053/j.ajkd.2020.11.026

34. Silbiger SR, Neugarten J. The impact of gender on the progression of chronic renal disease. *Am J Kidney Dis.* (1995) 25:515–33. doi: 10.1016/0272-6386(95)90119-1

35. Zhou XJ, Saxena R, Liu Z, Vaziri ND, Silva FG. Renal senescence in 2008: progress and challenges. *Int Urol Nephrol.* (2008) 40:823–39. doi: 10.1007/s11255-008-9405-0

36. Thomas MC. Targeting the pathobiology of diabetic kidney disease. *Adv Chronic Kidney Dis.* (2021) 28:282–9. doi: 10.1053/j.ackd.2021.07.001

37. Chagnac A, Zingerman B, Rozen-Zvi B, Herman-Edelstein M. Consequences of glomerular hyperfiltration: the role of physical forces in the pathogenesis of chronic kidney disease in diabetes and obesity. *Nephron.* (2019) 143:38–42. doi: 10.1159/000499486

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 02 August 2022
DOI 10.3389/fmed.2022.917603

OPEN ACCESS

EDITED BY
Li Zuo,
Peking University People's Hospital, China

REVIEWED BY
Tomasz Porazko,
Opole University, Poland
Naya Huang,
The First Affiliated Hospital of Sun Yat-sen University, China
Huiping Zhao,
Peking University People's Hospital, China

*CORRESPONDENCE
Yinghong Liu
liuyingh2002@csu.edu.cn

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 11 April 2022
ACCEPTED 06 July 2022
PUBLISHED 02 August 2022

CITATION
Song P, Yang D, Li J, Zhuo N, Fu X, Zhang L, Zhang H, Liu H, Sun L and Liu Y (2022) Dynamic serum albumin and outcome of peritoneal dialysis patients: A retrospective study in China.
Front. Med. 9:917603.
doi: 10.3389/fmed.2022.917603

Dynamic serum albumin and outcome of peritoneal dialysis patients: A retrospective study in China

Panai Song, Dong Yang, Jine Li, Ning Zhuo, Xiao Fu, Lei Zhang, Hongqing Zhang, Hong Liu, Lin Sun and Yinghong Liu*

Department of Nephrology, The Second Xiangya Hospital of Central South University, Hunan Key Laboratory of Kidney Disease and Blood Purification, Changsha, China

Introduction: Serum albumin levels at a single time point have been shown to predict mortality in peritoneal dialysis (PD) patients. However, we believe that the dynamic change in albumin after PD may be more significant. In this study, we investigated the relationship between dynamic serum albumin and the clinical outcome of patients undergoing continuous ambulatory peritoneal dialysis (CAPD).

Methods: The participants in this study enrolled 586 patients who underwent CAPD at the peritoneal dialysis center of Second Xiangya Hospital in China. We retrospectively reviewed medical records from January 1, 2010, to December 31, 2019. Baseline serum albumin (Alb), time-averaged albumin level (TA-ALB) and serum albumin reach rate (SR: defined as the percentage of serum albumin measurements that reached $\geq$ 35 g/L) were applied as the predictor variables. All-cause mortality and cardiovascular mortality were used as the outcome variables. Hazard function of all-cause mortality and cardiovascular mortality in the study participants were examined by using Cox proportional hazard regression models.

Results: Age (HR = 1.03, 95% CI 1.00–1.05), cardiovascular disease (HR = 1.80, 95% CI 1.07–3.03) and TA-ALB (HR = 0.92, 95% CI 0.85–0.99) were independent risk factors for all-cause mortality in PD patients. Patients with TA-ALB of <33 g/L (HR = 2.33, 95% CI 1.17–4.62) exhibited a higher risk for all-cause mortality than those with TA-ALB $\geq$ 36 g/L. Stratified SR showed a similar trend. Patients with a <25% SR exhibited a significantly increased risk for all-cause mortality (HR = 2.72, 95% CI, 1.24–5.96) by fully adjusted analysis. However, neither TA-ALB nor SR were associated with the risk of cardiovascular mortality after adjusted analysis.

Conclusion: This study demonstrated that age, cardiovascular disease, and TA-ALB were independent risk factors for all-cause mortality in PD patients.

TA-ALB and SR can better predict the prognosis of PD patients than baseline Alb. Dynamic changes in Alb are more clinically significant than baseline Alb in predicting mortality risk.

KEYWORDS

time-averaged albumin, serum albumin reach rate, peritoneal dialysis, all-cause mortality, cardiovascular mortality

Introduction

Peritoneal dialysis (PD) is an important kidney replacement therapy. It has been reported that PD protected residual renal functions better and had other advantages in many studies (1). Therefore the number of PD patients is increasing year by year. It is estimated that there are more than 272,000 PD patients worldwide, accounting for around 11% of the dialysis population (2). Despite the technology and treatments for PD have improved over the years, many PD patients still develop metabolic disorders, such as hypokalemia and hypoproteinemia, leading to an increased risk of mortality (3).

Protein-energy wasting (PEW) is a common metabolic disorder in PD patients (4) 4, and serum albumin (Alb) is an important index to evaluate PEW. The causes of hypoalbuminemia in PD patients are complex, including protein loss during peritoneal dialysis, inflammation, decreased protein intake, chronic acidosis, and psychosocial factors (5). Clear evidence has shown that hypoalbuminemia was closely related to all-cause mortality and cardiovascular(CV) mortality in PD patients (6, 7). However, Olga et al. (8) have reported that baseline peritoneal loss and albumin clearance was not a determinant of survival. Another study (9) has demonstrated that albumin trajectories after PD was better than initial serum albumin level in predicting mortality risk. Therefore, it may be more meaningful to analyze the association of dynamic serum albumin changes with the long-term survival of dialysis patients.

The aim of this cohort study was to investigate whether serum albumin changes over time are predictors of changes in survival in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). We measured 3 predictor variables, target baseline albumin (Alb), time-averaged albumin (TA-ALB) levels, and albumin standard reach rates (SR), to predict all-cause mortality and CV mortality.

Materials and methods

Study population

In this study, as shown in **Figure 1**, a total of 1,003 patients catheterized and received CAPD treatment from January 1, 2010 to December 31, 2019 at PD center of Second Xiangya Hospital, Central South University in China were reviewed. We excluded 403 patients because of incomplete demographic and laboratory data, and 586 patients were considered eligible for the survival analysis. The exclusion criteria were as follows: (1) age $<$ 18 years, (2) incomplete clinical information, (3) duration of PD was less than 3 months, (4) cirrhosis or malignancy, (5) serum albumin was measured less than 2 times, (6) kidney transplantation, and (7) no regular follow-up was performed 1–3 months after catheterization. Approval of the study by the research ethics committee of Central South University and informed consents were provided prior to their inclusion in the study.

Stratified serum albumin levels

We used Alb, TA-ALB level, and SR as the predictor variables. All-cause mortality and CV mortality were used as the outcome variables. Two serum albumin stratifications were used for analyses.

TA-ALB: according to the trapezoidal area method (10), the area under the curve is formed by albumin values divided by the follow-up time. The formula is as follows: where B is serum albumin values, T is the follow-up time (the month after catheterization), and N is the frequency of follow-up ($n \geq 2$).

$$TA-ALB = \frac{(B_1 + B_2)(T_2 - T_1) + (B_2 + B_3)(T_3 - T_2) + \ldots + (B_{n-1} + B_n)(T_n - T_{n-1})}{2(T_n - T_1)}$$

And according to the rule of tertiles, TA-ALB was divided into three groups (A1 $\leq$ 33 g/L, A2:33 g/L-36 g/L, A3 $\geq$ 36 g/L).

SR: SR is the percentage of albumin measurements reaching $\geq$ 35 g/L. We stratified the albumin reach rate into 4 groups depending on the serum albumin levels that reached 35 g/L: S1: SR $<$ 25%, S2: 25% $\leq$ SR $<$ 50%, S3: 50% $\leq$ SR $<$ 75%, S4 $\geq$ 75%, 25% albumin reach rate indicated that a quarter of serum albumin measurements had reached 35 g/L, 50% indicated that half of serum albumin measurements had reached 35 g/L, and 75% indicated that three-quarters of serum albumin measurements had reached 35 g/L.

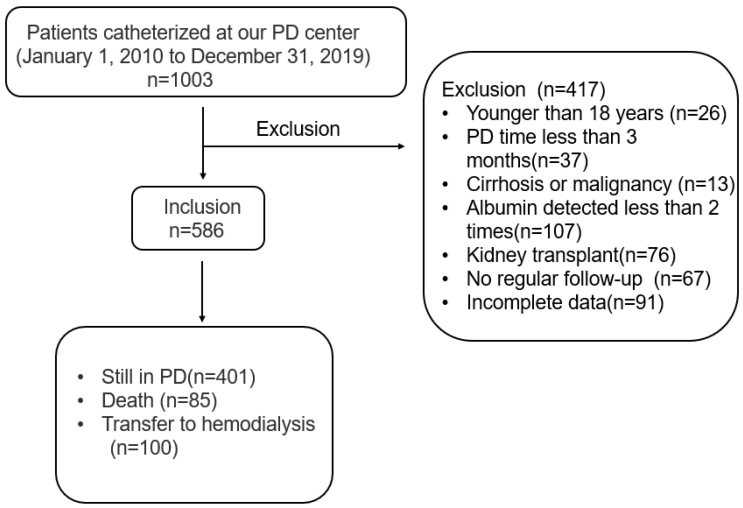


FIGURE 1
Flow chart for the study participants enrollment and outcomes.

Outcome measures

All-cause mortality and cardiovascular mortality were recorded as endpoint events. Cardiovascular mortality was defined as cardiac arrest, myocardial infarction, arrhythmia, heart failure, cerebrovascular accident, and other heart-related diseases. All-cause mortality is defined as death from any cause.

Statistical analysis

Results were expressed as count and percentage or median and interquartile range, as appropriate. Categorical variables were compared using the chi-squared test and Fisher's exact test. Independent risk factors of all-cause mortality in PD patients were analyzed by univariate and multivariate Cox regression analysis. The correlations between Alb, TA-ALB, SR and all-cause mortality, and cardiovascular mortality were evaluated by Cox proportional hazards model analysis. Survival curves were generated using the Kaplan–Meier method. SPSS 26.0 was used for all statistical analyses, and the P-value of <0.05 was considered statistically significant.

Results

Clinical characteristics of different time-averaged albumin level groups

A total of 586 patients were analyzed. During the 10 years, 203 patients had higher albumin levels (A3: TA-ALB $>$ 36 g/l), whereas 196 patients had low albumin levels (A1: TA-ALB $<$ 33 g/l) and 187 patients had intermediate albumin levels (A2: 33 g/l $<$ TA-ALB $<$ 36 g/l). Patients who were older, male, diabetic, and had high use rate of diuretics had lower serum albumin. Moreover, patients with low albumin had lower rates of glomerular disease, lower residual renal function, and higher rates of ACEI use, cardiovascular disease, all-cause mortality, and cardiovascular mortality. Data of blood parameters showed that patients with lower albumin exhibited significantly higher C-reactive protein(CRP), lower Hb, albumin uric acid(UA), calcium(Ca), phosphorus(P), and magnesium(Mg) values than those with higher albumin. However, the ratio of monocytes to lymphocytes was significantly higher in patients with lower albumin (Table 1).

Independent risk factors analysis of all-cause mortality in peritoneal dialysis patients

In univariate Cox regression analysis, age, diabetes, UA, Alb, TA-ALB, serum potassium, and cardiovascular disease (CVD) were chosen for adjustment for multivariate Cox proportional-hazards model analysis. We found that age(HR = 1.03, 95%CI 1.00–1.05, p = 0.012), CVD(HR = 1.80, 95%CI 1.07–3.02, p = 0.028), and TA-ALB(HR = 0.92, 95%CI 0.85–0.99, p = 0.028)were independent risk factors for all-cause mortality (Table 2).

Correlations between albumin, time-averaged albumin levels, standard reach rates, and all-cause mortality and cardiovascular mortality

Based on Cox regression analysis, Table 3 shows the association between different albumin stratification levels and

TABLE 1 Baseline characteristics and clinical features of the study population according to different TA-ALB groups.

	A1 (N = 196) TA-ALB < 33 g/L	A2 (N = 187) TA-ALB 33–36 g/L	A3 (N = 203) TA-ALB ≥ 36 g/L	p
Age(year)	53.8 ± 14.0	50.1 ± 12.5*	45.4 ± 12.1*	<0.001
Gender(male)	113 (57.7%)	81 (43.3%)*	88 (43.3%)*	0.005
Primary disease				
Glomerulonephritis	99 (50.5%)	102 (54.5%)	146 (71.9%)*Δ	<0.001
Diabetic nephropathy	32 (16.3%)	19 (10.2%)	8 (3.94%)*Δ	<0.001
Hypertensive nephropathy	36 (18.4%)	26 (13.9%)	22 (10.8%)	0.098
Medication				
ACEI	73 (37.2%)	55 (29.4%)	48 (23.6%)*	0.012
β-blockers	91 (46.4%)	79 (42.2%)	85 (41.9%)	0.600
CCB	173 (88.3%)	170 (90.9%)	168 (83.2%)	0.064
Diuretics	45 (23.0%)	24 (12.8%)*	25 (12.3%)*	0.005
Complications				
Cardiovascular disease	43 (21.9%)	25 (13.4%)	15 (7.39%)*	<0.001
Diabetes mellitus	47 (24.0%)	23 (12.3%)*	14 (6.90%)*	<0.001
Outcomes				
Peritonitis	49 (25.0%)	44 (23.5%)	49 (24.1%)	0.944
All-cause mortality	44 (22.4%)	26 (13.9%)	15 (7.39%)*	<0.001
Cardiovascular death	28 (14.3%)	16 (8.56%)	7 (3.45%)*	0.001
Hemodialysis	39 (19.9%)	31 (16.6%)	30 (14.8%)	0.388
Laboratory findings				
White blood cell (10^9/L)	6.0(4.8,7.4)	6.0(5.0,7.5)	6.1(5.1,7.4)	0.748
Hemoglobin (g/L)	95 ± 19	98 ± 17	104 ± 18*Δ	<0.001
Platelets (10^9/L)	196(154,243)	191(151,246)	195(162,250)	0.771
Neutrophils (10^9/L)	3.9(3.1,5.3)	4.0(3.3,5.3)	4.1(3.3,5.1)	0.658
M/L	0.25(0.19,0.34)	0.23(0.18,0.30)*	0.21(0.17,0.28)*	0.001
Urea nitrogen (mmol/L)	19.0(15.2,23.3)	19.7(16.6,26.3)	20.0(16.2,25.3)	0.064
Creatinine (umol/L)	730(567,974)	777(653,986)	749(624,974)	0.186
Uric acid (umol/L)	411 ± 95.8	442 ± 99.9*	455 ± 103*	<0.001
Calcium (mmol/L)	2.00(1.86,2.09)	2.10(1.95,2.19)*	2.11(1.97,2.25)*	<0.001
Phosphorus (mmol/L)	1.49(1.28,1.83)	1.60(1.37,1.92)*	1.60(1.34,1.94)*	0.029
Sodium(mmol/L)	141(139,143)	140(139,142)	141(139,142)	0.518
Potassium (mmol/L)	4.09(3.60,4.57)	4.05(3.67,4.62)	4.00(3.60,4.52)	0.409
Magnesium (mmol/L)	0.89(0.79,0.98)	0.95(0.88,1.05)*	0.99(0.88,1.08)*	<0.001
Albumin (g/L)	30.9 ± 4.04	35.1 ± 3.38*	38.0 ± 3.49*	<0.001
Total protein (g/L)	58.5 ± 6.76	64.0 ± 7.20*	66.7 ± 6.97*	<0.001
CRP (mg/dL)	3.25(1.41,8.15)	2.16(0.92,6.28) *	1.81(0.68,3.94) *Δ	<0.001
iPTH (mg/dL)	26.9(17.5,38.6)	29.1(15.6,43.4)	28.7(16.8,45.1)	0.824
BMI (kg/m^2)	21.6(19.6,23.8)	21.8(19.8,24.0)	21.3(19.1,23.5)	0.651
Total Kt/v	1.8(1.5,2.2)	1.8(1.6,2.3)	1.9(1.5,2.4)	0.095
Total Ccr	59.9(47.3,80.2)	60.6(49.2,77.7)	64.1(49.8,77.2)	0.712
eGFR (mL/min/1.73 m^2)	3.1(1.5,5.1)	3.1(1.7,4.9)	3.7(2.3,5.4)*Δ	0.015

BMI, body mass index; Total Kt/V, total urea clearance index; Total Ccr, total creatinine clearance rate; eGFR, glomerular filtration rate; ACEI, angiotensin enzyme inhibitor; CCB, calcium channel blocker; M/L, monocytes/lymphocytes; CRP, C-reactive protein; iPTH, ionization parathyroid hormone; *, compared to group A1, $P < 0.05$; Δ, compared to group A2, $P < 0.05$.

TABLE 2 Independent risk factors analysis of all-cause mortality in PD patients.

Variable	Univariate Cox regression analysis			Multivariate Cox regression analysis		
	HR	95%CI	*p*	HR	95%CI	*p*
Age (year)	1.06	1.04–1.07	<0.001	1.03	1.00–1.05	0.012
Primary disease						
Glomerulonephritis	0.58	0.31–1.11	0.101			
Diabetic nephropathy	2.07	1.00–4.26	0.049			
Hypertensive nephropathy	1.82	0.92–3.60	0.085			
Other	1					
Uric acid (umol/L)	0.99	0.99–1.00	0.005			
Albumin (g/L)	0.93	0.89–0.97	0.002			
TA-ALB(g/L) Potassium(mmol/L)	0.87 0.68	0.83–0.92 0.49–0.95	<0.001 0.022	0.92	0.85–0.99	0.028
Cardiovascular disease	2.44	1.55–3.85	<0.001	1.80	1.07–3.03	0.028

TA-ALB: Time averaged albumin; HR: hazard ratio, CI: confidence interval.

all-cause mortality and cardiovascular mortality. Baseline Alb, TA-ALB, and SR were inversely associated with the risk of all-cause mortality and cardiovascular mortality. Adjusted analysis showed that the risk of all-cause mortality in the participants increased parallel to low serum albumin. Participants with TA-ALB levels < 33 g/L exhibited a higher risk for all-cause mortality as compared with those with the reference level (≥ 36 g/L) (HR = 2.33, 95% CI 1.17–4.62). Stratified SR showed a similar trend. Participants with a < 25% SR had the highest statistically significant risk for all-cause mortality (HR = 2.72, 95% CI, 1.24–5.96). However, neither TA-ALB nor SR were associated with the risk of cardiovascular mortality after fully adjusted analysis.

Cumulative survival

Taking all-cause mortality of the CAPD patients as the endpoint, cumulative survival was analyzed. The 1-, 3-, and 5-year survival rates of the A1 group were 94.1, 83.6, and 67.4%; A2 group were 99.1, 89.2, and 85.1%; A3 group were 99.0, 95.8, and 90.8%, respectively. **Figure 2A** describes the cumulative survival of three groups of TA-ALB using the Kaplan-Meier analysis. The cumulative survival of each group was significantly different. Taking CV mortality as the endpoint, the 1-, 3-, and 5-year survival rates of the A1 group were 98.7, 87.4, and 76.1%; A2 group were 99.1, 93.5, and 89.4%; A3 group were 99.5, 97.2, and 95.2%, respectively. Kaplan-Meier analysis also showed a significant difference in cumulative survival among the three groups (**Figure 2B**).

Discussion

This retrospective cohort study examined the association of baseline Alb, TA-ALB, and SR with all-cause and cardiovascular mortality in PD patients. The results showed that TA-ALB is an independent risk factor for all-cause mortality in PD patients, a decrease in TA-ALB and a low SR were associated with an increased risk of all-cause mortality in PD patients. Dynamic changes in Alb can better predict the risk of all-cause mortality in PD patients.

Age (11), gender (12), and comorbidities (13) have been shown to influence the prognosis of dialysis patients. This study found that age, CVD, and TA-ALB were independent risk factors for all-cause death in PD patients. We also found that patients with hypoalbuminemia were older and had higher rates of diuretic use, male and DM. The weakened immunity of elderly patients and the susceptibility to infection caused by improper operation, higher peritoneal transport and peritoneal permeability in DM patients than non-diabetic patients (14), and more leakage of Alb may explain the above findings. It has been reported that female patients receiving dialysis have an increased risk of infection (15), which is more likely to lead to malnutrition. However, our study found that the proportion of males in the low serum albumin group was higher, which may be related to the influence of regional differences.

The decline of RRF is closely related to inflammation, malnutrition, and death in PD patients (16, 17). Alb can reflect inflammation and malnutrition, which explains the higher CRP, lower eGFR and lower Hb, UA, Ca, P, Mg in the low albumin group. In this study, the use of diuretics and ACEIs was higher in the A1 group, which may be related to the increased edema and increased volume load caused by low Alb, leading to increased blood pressure. M/L are novel indicators of baseline inflammatory response (18). We speculated that the higher M/L in A1 group might be related to the inflammatory state.

PD patients' prognosis has always been a concern of clinicians. Initial or single-time point serum albumin is commonly considered to be important in predicting the prognosis of dialysis patients (19–21). Sharma et al. (20) proposed that Alb at the start of PD was a better predictor

TABLE 3 Correlations between Alb, TA-ALB, SR, and all-cause mortality, cardiovascular mortality in the study population.

	Model 1		Model 2		Model 3	
	HR(95%CI)	*P*	HR(95%CI)	*P*	HR(95%CI)	*P*
All-cause mortality						
Alb(g/L)	0.93(0.89,0.97)	0.002	0.99(0.93,1.05)	0.700	0.99(0.93,1.06)	0.830
TA-ALB(g/L)	0.87(0.83,0.92)	<0.001	0.94(0.88,1.00)	0.046	0.93(0.87,1.00)	0.043
A3	Reference		Reference		Reference	
A2	2.06(1.09,3.89)	0.026	1.52(0.78,2.94)	0.217	1.51(0.77,2.94)	0.223
A1	3.79(2.10,6.82)	<0.001	2.20(1.13,4.30)	0.020	2.29(1.15,4.58)	0.019
SR						
S4	Reference		Reference		Reference	
S3	2.47(1.16,5.26)	0.019	2.07(0.96,4.48)	0.064	1.91(0.88,4.18)	0.103
S2	2.88(1.30,6.42)	0.009	2.17(0.94,4.97)	0.068	2.25(0.97,5.25)	0.059
S1	4.64(2.31,9.34)	<0.001	2.78(1.29,6.00)	0.009	2.72(1.24,5.96)	0.013
Cardiovascular mortality						
Alb(g/L)	0.91(0.86,0.97)	0.001	0.97(0.90,1.06)	0.508	0.97(0.89,1.06)	0.494
TA-ALB(g/L)	0.87(0.81,0.93)	<0.001	0.97(0.89,1.06)	0.465	0.97(0.88,1.06)	0.480
A3	Reference		Reference		Reference	
A2	2.71(1.11,6.59)	0.028	1.80(0.71,4.56)	0.217	1.76(0.68,4.55)	0.243
A1	5.14(2.24,11.80)	<0.001	2.38(0.93,6.08)	0.071	2.35(0.89,6.21)	0.086
SR						
S4	Reference		Reference		Reference	
S3	1.92(0.70,5.28)	0.208	1.50(0.53,4.23)	0.448	1.43(0.50,4.08)	0.508
S2	2.56(0.89,7.39)	0.081	1.78(0.58,5.41)	0.311	1.62(0.51,5.15)	0.416
S1	5.30(2.18,12.89)	<0.001	2.56(0.96,6.84)	0.062	2.56(0.92,7.07)	0.071

Univariate and multivariate Cox proportional hazard regression models. Model 1: unadjusted.Model 2: adjusted for demographic variables including age, sex, diabetes mellitus and laboratory variables including White blood cell, hemoglobin, platelet, M/L, neutrophil, blood calcium, blood phosphorus, blood potassium, blood sodium, blood magnesium, urea nitrogen, creatinine, uric acid, C-reactive protein, iPTH.Model 3: adjusted for all demographic and laboratory variables including Total KT/V, Total CCR, eGFR, ACEI, β-blockers, CCB, diuretics and peritonitis. HR, hazard ratio, CI, confidence interval.

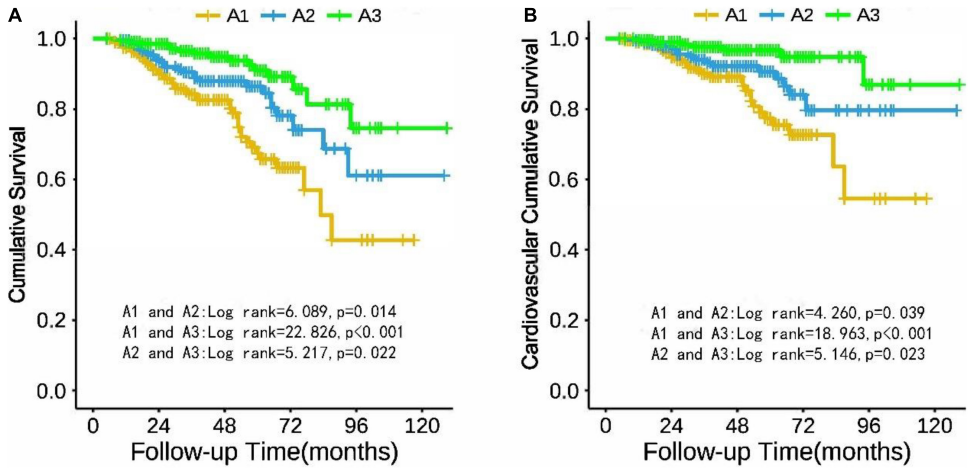


FIGURE 2
Cumulative survival of PD patients in TA-ALB groups (A1, A2, and A3) with all-cause mortality **(A)** and cardiovascular mortality **(B)** as end points.

of mortality than Alb after PD initiation. However, in recent years, some studies considered time-varying changes in serum albumin levels in examining the relationship of serum albumin levels with mortality in dialysis patients (22, 23). Wang et al. (9) demonstrated that albumin trajectories after PD was better than initial serum albumin level in predicting mortality risk.

In this study, we assumed the dynamic change and trend of albumin after PD was essential and investigated the relationship of TA-ALB and SR with mortality in PD patients. We found that PD patients with lower TA-ALB and lower SR demonstrated higher all-cause mortality. The results demonstrated the long-term effect of serum albumin levels on the mortality risk of PD patients and showed that a sustained serum albumin level is crucial for maintaining survival benefits in PD patients.

Studies have shown that Alb is also a predictor of cardiovascular death in PD patients. One study suggested that after adjusting for other risk factors, the risk of cardiovascular death increased by more than 10-fold in Alb < 35 g/L patients (24). Another study showed that when Alb < 30 g/L, the risk of cardiovascular mortality was increased in both PD and HD patients (25). Our study showed that cardiovascular mortality was increased when PD patients with TA-ALB < 33 g/L. After adjusting for related factors, the risk of cardiovascular mortality remained higher in patients with low TA-ALB, although there was no statistically significant difference ($P = 0.067$), which may have been influenced by the small number of cardiovascular death endpoint events in this study.

Our study is rare in comparing the effects of baseline Alb, TA-ALB, and SR on all-cause mortality and CV mortality in PD patients. We find that TA-ALB and SR can better reflect the dynamic changes of Alb over time and predict the survival outcome of PD patients. Of course, there are still some limitations in this study. As a retrospective cohort study, we could not prove the causal relationship between TA-ALB, SR, and all-cause mortality, so more prospective studies are needed to confirm this in the future. Inflammation indicators such as CRP and IL-6 were not included in the model when we adjusted for confounding factors, which may impact the results. In addition, this study is a single-center study with a small sample size, and a multi-center randomized controlled study is needed to verify this conclusion.

Conclusion

This study demonstrated that age, cardiovascular disease and TA-ALB were independent risk factors for all-cause mortality in PD patients. TA-ALB and SR can better predict the prognosis of PD patients than baseline Alb. Dynamic changes in Alb are more clinically significant than baseline Alb in predicting mortality risk. Increasing the albumin level over time can improve the prognosis of PD patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Second Xiangya Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PS collected the clinical data and drafted and revised the manuscript. JL, DY, and HZ collected the clinical data and searched the relative literatures. NZ searched the relative literatures, made analysis, and revised the English of the manuscript. XF, LZ, HL, and LS provided with the clinical assistance and contributed to the acquisition of these data. YL revised the manuscript and takes responsibility for the work. All authors have read and approved the final version.

Funding

This work was supported by the National Natural Science Foundation of China (81800649), the Changsha Municipal Natural Science Foundation (kq2202401 and kq2014235), and the Natural Science Foundation of Hunan Province (2022JJ30835 and 2021JJ30942).

Acknowledgments

We appreciate all the participants and their families. We also thank the members of the study team from the Department of Nephrology of Second Xiangya Hospital Affiliated to Central South University for their assistance in completing this project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Tokgoz B. Clinical advantages of peritoneal dialysis. *Perit Dial Int.* (2009) 29(Suppl. 2):S59–61.doi: 10.1177/089686080902902S11

2. Li PK, Chow KM, Van de Luijtgaarden MW, Johnson DW, Jager KJ, Mehrotra R, et al. Changes in the worldwide epidemiology of peritoneal dialysis. *Nat Rev Nephrol.* (2017) 13:90–103.doi: 10.1038/nrneph.2016.181

3. Han SH, Han DS. Nutrition in patients on peritoneal dialysis. *Nat Rev Nephrol.* (2012) 8:163–75.doi: 10.1038/nrneph.2012.12

4. Kang DH, Kang EW, Choi SR, Yoon SY, Han DS. Nutritional problems of Asian peritoneal dialysis patients. *Perit Dial Int.* (2003) 23(Suppl. 2):S58–64.doi: 10.1177/089686080302302s13

5. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis.* (2020) 76:S1–107.doi: 10.1053/j.ajkd.2020.05.006

6. Spiegel DM, Breyer JA. Serum albumin: a predictor of long-term outcome in peritoneal dialysis patients. *Am J Kidney Dis.* (1994) 23:283–5.doi: 10.1016/S0272-6386(12)80985-1

7. Kalantar-Zadeh K, Kilpatrick RD, Kuwae N, McAllister CJ, Alcorn H Jr, Kopple JD, et al. Revisiting mortality predictability of serum albumin in the dialysis population: time dependency, longitudinal changes and population-attributable fraction. *Nephrol Dial Transplant.* (2005) 20:1880–8. doi: 10.1093/ndt/gfh941

8. Balafa O, Halbesma N, Struijk DG, Dekker FW, Krediet RT. Peritoneal albumin and protein losses do not predict outcome in peritoneal dialysis patients. *Clin J Am Soc Nephrol.* (2011) 6:561–6.doi: 10.2215/CJN.05540610

9. Wang X, Han Q, Wang T, Tang W. Serum albumin changes and mortality risk of peritoneal dialysis patients. *Int Urol Nephrol.* (2020) 52:565–71.doi: 10.1007/s11255-020-02389-y

10. Artigas A, Wernerman J, Arroyo V, Vincent JL, Levy M. Role of albumin in diseases associated with severe systemic inflammation: pathophysiologic and clinical evidence in sepsis and in decompensated cirrhosis. *J Crit Care.* (2016) 33:62–70. doi: 10.1016/j.jcrc.2015.12.019

11. Franco MRG, Bastos MG, Qureshi AR, Schreider A, Bastos KA, Divino-Filho JC, et al. Incident elderly patients on peritoneal dialysis: epidemiological characteristics and modality impact on survival time. *Saudi J Kidney Dis Transpl.* (2017) 28:782–91.

12. Ros S, Remón C, Qureshi AR, Quiros P, Lindholm B, Carrero JJ. Increased risk of fatal infections in women starting peritoneal dialysis. *Perit Dial Int.* (2013) 33:487–94. doi: 10.3747/pdi.2012.00243

13. Portolés J, Del Peso G, Fernández-Reyes MJ, Bajo MA, López-Sánchez P. Previous comorbidity and lack of patient free choice of technique predict early mortality in peritoneal dialysis. *Perit Dial Int.* (2009) 29:150–7.doi: 10.1177/089686080902900208

14. Nakamoto H, Suzuki H. Hypoproteinemia in patients with diabetes undergoing continuous ambulatory peritoneal dialysis is attributable to high permeability of peritoneal membrane. *Perit Dial Int.* (2003) 23(Suppl. 2):S72–8.doi: 10.1177/089686080302302s16

15. Ahmed SB, Ramesh S. Sex hormones in women with kidney disease. *Nephrol Dial Transplant.* (2016) 31:1787–95.doi: 10.1093/ndt/gfw084

16. Wang AY, Woo J, Wang M, Sea MM, Sanderson JE, Lui SF, et al. Important differentiation of factors that predict outcome in peritoneal dialysis patients with different degrees of residual renal function. *Nephrol Dial Transplant.* (2005) 20:396–403. doi: 10.1093/ndt/gfh331

17. Liao CT, Chen YM, Shiao CC, Hu FC, Huang JW, Kao TW, et al. Rate of decline of residual renal function is associated with all-cause mortality and technique failure in patients on long-term peritoneal dialysis. *Nephrol Dial Transplant.* (2009) 24:2909–14. doi: 10.1093/ndt/gfp056

18. Ji H, Li Y, Fan Z, Zuo B, Jian X, Li L, et al. Monocyte/lymphocyte ratio predicts the severity of coronary artery disease: a syntax score assessment. *BMC Cardiovasc Disord.* (2017) 17:90. doi: 10.1186/s12872-017-0507-4

19. Yun T, Ko YE, Kim SJ, Kang DH, Choi KB, Oh HJ, et al. The additional benefit of weighted subjective global assessment (SGA) for the predictability of mortality in incident peritoneal dialysis patients: a prospective study. *Medicine.* (2017) 96:e8421. doi: 10.1097/MD.0000000000008421

20. Sharma AP, Gupta A, Sharma RK, Agarwal DK, Sural S, Wardhe DJ. Does serum albumin at start of continuous ambulatory peritoneal dialysis (CAPD) or its drop during CAPD determine patient outcome? *Adv Perit Dial.* (2000) 16:119–22.

21. Gamba G, Mejía JL, Saldívar S, Peña JC, Correa-Rotter R. Death risk in CAPD patients. The predictive value of the initial clinical and laboratory variables. *Nephron.* (1993) 65:23–7. doi: 10.1159/000187435

22. Chen JB, Cheng BC, Yang CH, Hua MS. An association between time-varying serum albumin level and the mortality rate in maintenance haemodialysis patients: a five-year clinical cohort study. *BMC Nephrol.* (2016) 17:117. doi: 10.1186/s12882-016-0332-5

23. Hao N, Cheng BC, Yang HT, Wu CH, Lei YY, Chao MC, et al. Time-varying serum albumin levels and all-cause mortality in prevalent peritoneal dialysis patients: a 5-year observational study. *BMC Nephrol.* (2019) 20:254. doi: 10.1186/s12882-019-1433-8

24. Jiang J, Wang LH, Fei YY, Zhou XW, Peng L, Lan L, et al. Serum albumin at start of peritoneal dialysis predicts long-term outcomes in anhui han patients on continuous ambulatory peritoneal dialysis: a retrospective cohort study. *Kidney Dis.* (2018) 4:262–8. doi: 10.1159/000492426

25. Mehrotra R, Duong U, Jiwakanon S, Kovesdy CP, Moran J, Kopple JD, et al. Serum albumin as a predictor of mortality in peritoneal dialysis: comparisons with hemodialysis. *Am J Kidney Dis.* (2011) 58:418–28.doi: 10.1053/j.ajkd.2011.03.018

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 25 August 2022
DOI 10.3389/fmed.2022.911998

OPEN ACCESS

EDITED BY
Gian Marco Ghiggeri,
Giannina Gaslini Institute (IRCCS), Italy

REVIEWED BY
Emanuele De Simone,
Azienda Sanitaria Locale "Città di Torino", Italy
Sree Bhushan Raju,
Nizam's Institute of Medical Sciences, India

*CORRESPONDENCE
Yu Yan
yanmail1004@163.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 03 April 2022
ACCEPTED 29 July 2022
PUBLISHED 25 August 2022

CITATION
Wang Y, Yan Y, Dong B, Zou W, Li X, Shao C, Jiang L, Wang M and Zuo L (2022) Clinicopathological manifestations of coexistent monoclonal immunoglobulin deposition disease and immunotactoid glomerulopathy.
Front. Med. 9:911998.
doi: 10.3389/fmed.2022.911998

Clinicopathological manifestations of coexistent monoclonal immunoglobulin deposition disease and immunotactoid glomerulopathy

Yina Wang[1], Yu Yan[1]*, Bao Dong[1], Wanzhong Zou[2], Xin Li[1], Chunying Shao[1], Lei Jiang[3], Mei Wang[1] and Li Zuo[1]

[1]Department of Nephrology, Peking University People's Hospital, Beijing, China, [2]Department of Pathology, Peking University Health Science Center, Beijing, China, [3]Electron Microscope Laboratory, Peking University People's Hospital, Beijing, China

Combination of monoclonal immunoglobulin deposition disease (MIDD) and immunotactoid glomerulopathy (ITG) is a rare form of monoclonal immunoglobulin (MIg)-associated renal disease. We retrospectively reviewed the native kidney biopsy specimens at Peking University People's Hospital from 2011 to 2020. Five patients were diagnosed as MIDD + ITG. Their clinical and pathological characteristics were studied. The typical clinical features were nephritic syndrome and renal dysfunction with prominent anemia, but hematuria was mild. Unlike single MIDD and single ITG, on light microscopy, segmentally distributed mesangial nodular sclerosis on the basis of mesangial matrix hyperplasia was the major lesion. Others including membranoproliferative glomerulonephritis (MPGN)-like lesion, glomerular basement membrane thickness, and mild to moderate mesangial and endothelial proliferations might presented at the same time and in the same glomeruli. On immunofluorescence, MIg, usually monoclonal light chains, deposited along glomerular basement membranes and tubular basement membranes, while the intact MIg or monoclonal heavy chain deposited in the mesangial regions. Corresponding to the depositions on immunofluorescence, punctate "powdery" deposits along glomerular basement membranes and tubular basement membranes under electronic microscopy indicated the presence of MIDD. Microtubular substructures (diameters of 20–50 nm) exhibiting hollow cores arranged in parallel arrays in mesangial regions indicated the presence of ITG. Patients treated with bortezomib-based regimen seemed to have better outcomes. In conclusion, MIDD + ITG is a rare combination form of MIg-associated renal disease. Accurate diagnosis requires the comprehensive pathological investigations.

KEYWORDS

monoclonal immunoglobulin deposition disease, immunotactoid glomerulopathy, pathology, glomerulonephritis, multiple myeloma, renal biopsy

Introduction

Monoclonal immunoglobulin (MIg)-associated renal disease has heterogeneous morphologic forms (1). It usually presented in one form (2). Some of literatures reported combinations with two or more different pathologic forms (3–5). The most common pathologic form is monoclonal immunoglobulin deposition disease (MIDD) coexisting with light chain cast nephropathy (LCCN) (4, 6–9). However, the combination of two forms of glomerular diseases, especially the co-deposition of organized and non-organized structures was rare (3, 10). The presentation of this combination was not simply the add-on, but had unique characteristics. Up to date, no case of MIDD + ITG was reported. Here, we report the clinicopathological features, treatments and outcomes of 5 patients with MIDD + ITG, and help to understand the characteristics of this pattern.

Materials and methods

All 11,767 native kidney biopsy specimens from 2011 to 2020 at Peking University People's Hospital were reviewed from patients' medical records. Patients fulfilled the diagnostic criteria of both MIDD and immunotactoid glomerulopathy (ITG) were enrolled in the study. MIDD and ITG were diagnosed according to previous literatures (2, 11, 12). Briefly, the patients were diagnosed as MIDD and ITG when there were typical depositions on EM ("powdery" deposits along basement membrane for MIDD and microtubular substructure exhibiting hollow cores arranged in parallel arrays for ITG) with IF proved MIg deposition.

Demographic and clinical information including age, gender, clinical symptoms, past histories of hypertension and diabetes, blood pressure, hemoglobin, urinalysis, urine protein output, serum albumin, serum creatinine were collected at the time of biopsy. MIg was detected by serum and/or urine immunofixation electrophoresis (IEF) and free light chains (FLCs) (Freelite, Binding Site, United Kingdom) test. Treatment and follow-up data were also obtained from the patients' medical records.

All kidney biopsy samples were processed for light microscopy (LM), immunofluorescence (IF) and electronic microscopy (EM) examination using standard techniques. IF was performed on cryosections (5 μm) using polyclonal fluorescein isothiocyanate (FITC)-conjugated antibodies against IgG, IgM, IgA, C3, C1q, κ and λ light chains (Dako, Denmark), respectively. Determination of the IgG subclasses was performed using monoclonal FITC-conjugated antibodies to IgG1, IgG2, IgG3, and IgG4 (SouthernBiotech, United States). For LM, kidney biopsy specimens were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Masson's trichrome, periodic acid-silver methenamine, respectively. Also, Congo red and immunohistochemical (IHC) staining (CD38, CD 138, CD3, and CD20) were performed. Ultrastructural evaluation was performed using a transmission electron microscope (Thermo Scientific, TECNAI SPIRIT, United States).

This research was carried out in accordance with International ethical guidelines for biomedical research involving human subjects (CIOMS) and Helsinki Declaration. This research was approved by the Ethics Committee of Peking University People's Hospital (2121PHB-84-001). Informed consent was obtained from all participants.

Statistical analysis

Continuous variables with normal distribution were expressed as mean ± *SD* and variables with non-normal distribution were expressed as median (Q_{25}, Q_{75}). Categorical variables were expressed as numbers or percentages.

Results

Patient characteristics

The incidence of MIDD + ITG was quite low, accounting for only 0.04% of biopsied patients in our center. Five patients were identified. The demographic characteristics are shown in **Table 1**. There were three males (60%) and two females with an average age of 61.0 ± 4.6 years at kidney biopsy.

Clinical characteristics

The clinical characteristics of the five patients are shown in **Table 1**. Foamy urine, dark urine, fatigue and weight loss were the main initial symptoms. The median duration from onset to diagnosis was 6 (6, 21) months (range 6–24 months). Microscopic hematuria was seen in all patients. Proteinuria level was 1.85 ± 1.32 g/d. Serum albumin was 34.1 ± 5.9 g/L. Four patients suffered renal insufficiency as chronic kidney disease (CKD) in three and acute kidney injury (AKI) in one. Patient 4 was dialysis-dependent before renal biopsy. Only one patient (patient 2) had normal renal function. The median serum creatinine level was 142 (range 101–656) μmol/L. Three patients (60%) had hypertension, and none had diabetes.

As shown in **Table 1**, two patients fulfilled the established diagnostic criteria for MM and three were monoclonal gammopathy of renal significance (MGRS). All patients had prominent anemia with hemoglobin level of 85.0 ± 5.1 g/L.

TABLE 1 Demographics and clinical characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender/age	F/68	F/61	M/58	M/56	M/62
Initial symptoms	Weight loss	Fatigue	Foamy urine	Foamy urine	Dark urine
Time from initial symptom to kidney biopsy (months)	6	24	18	6	6
Hypertension	N	N	Y	Y	Y
Edema	N	N	Y	N	N
Hepatosplenomegaly	Y	N	N	N	N
Other manifestation	N	N	N	N	Osteolysis
Hb (g/L)	87	86	80	80	92
Urine RBC/μl	38	70	27	556	43
Proteinuria (g/d)	0.53	0.93	1.28	3.15	3.38
Alb (g/L)	43.4	34.3	34.6	28.4	29.6
Scr (μmol/L)	142	101	206	656	137
SIFE/UIFE	IgA κ	Neg	κ	IgA λ	IgG κ
Serum FLC ratio (κ/λ)	NA	322.5/38.3 (8.39)	NA	52.2/179.25 (0.29)	136/13.5 (10.07)
Hematologic condition	MM	MGRS	MGRS	MGRS	MM

SIFE, serum immunofixation electrophoresis; UIFE, urine immunofixation electrophoresis; FLC, free light chain; NA, not applicable; MM, multiple myeloma; MGRS, monoclonal gammopathy of renal significance; N, no; Y, yes.

TABLE 2 Renal pathological findings.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Pathologic diagnosis	HCDD + ITG	LCDD + ITG	LCDD + ITG + ATIN	LCDD + ITG	LCDD + ITG
LM					
Major lesion patterns	MNS, MsHP	MNS, MPGN	MsHP, MPGN	MNS, MsHP	MNS, MsHP
IF					
Heavy chains	α++, GBM/TBM (linear), MG (coarse granular)	γ1++, MG (coarse granular)	α++, MG (coarse granular)	α++, MG (coarse granular)	γ2++, MG (coarse granular)
Light chains	Neg	κ+, GBM/TBM (linear), MG (coarse granular)	κ++, GBM/TBM (linear), MG (coarse granular)	λ++, GBM/TBM (linear), MG (coarse granular)	κ++, GBM/TBM (linear), MG (coarse granular)
EM					
Powdery electron dense deposits	GBM, TBM	GBM, TBM	GBM, TBM	GBM, TBM	GBM, TBM
Microtubular deposits	MG, Sub-Endo	MG	MG	MG	MG, Sub-Endo

HCDD, heavy chain deposition disease; LCDD, light chain deposition disease; ITG, immunotactoid glomerulopathy; ATIN, acute tubular-interstitial nephropathy; LM, light microscopy; MsHP, mesangial hyperplasia; MPGN, membranoproliferative glomerulonephritis; MNS, mesangial nodular sclerosis; IF, immunofluorescence; EM, electron microscopy; GBM, glomerular basement membrane; TBM, tubular basement membrane; MG, mesangium; Sub-Endo, subendothelial.

Serum and/or urine IEF confirmed the presence of MIg in four patients except patient 2. She was proved to have MIg for the high κ/λ ratio of 8.39, despite the negative results of serum and urine IEF. The complement levels (C3 and C4) were normal in all the five patients. The results of autoantibodies, hepatitis B and C, and serum cryoglobulin were all negative.

Pathologic findings

The pathologic findings are shown in Table 2 and Figure 1. Mesangial nodular sclerosis was the major lesion on LM usually distributing segmentally, formed on the basis of mesangial matrix hyperplasia lesions. There were also mesangial and segmental endocapillary proliferation. Some manifested membranoproliferative glomerulonephritis (MPGN)-like lesions due to mesangial interposition resulted in double-contour or multicontour. However, the lesion only distributed focally and segmentally and the GBMs were thickened mainly at the site of double-contour or multicontour. The GBMs in the non-sclerotic area were not thickened. Congo red staining were negative.

Except for the glomerular lesions, there were various degrees of tubulointerstitial injury, with tubular atrophy and multifocal inflammatory infiltration. Lymphocytes ($CD3^+$ or $CD20^+$) and monocytes were seen without eosinophils according to the HE

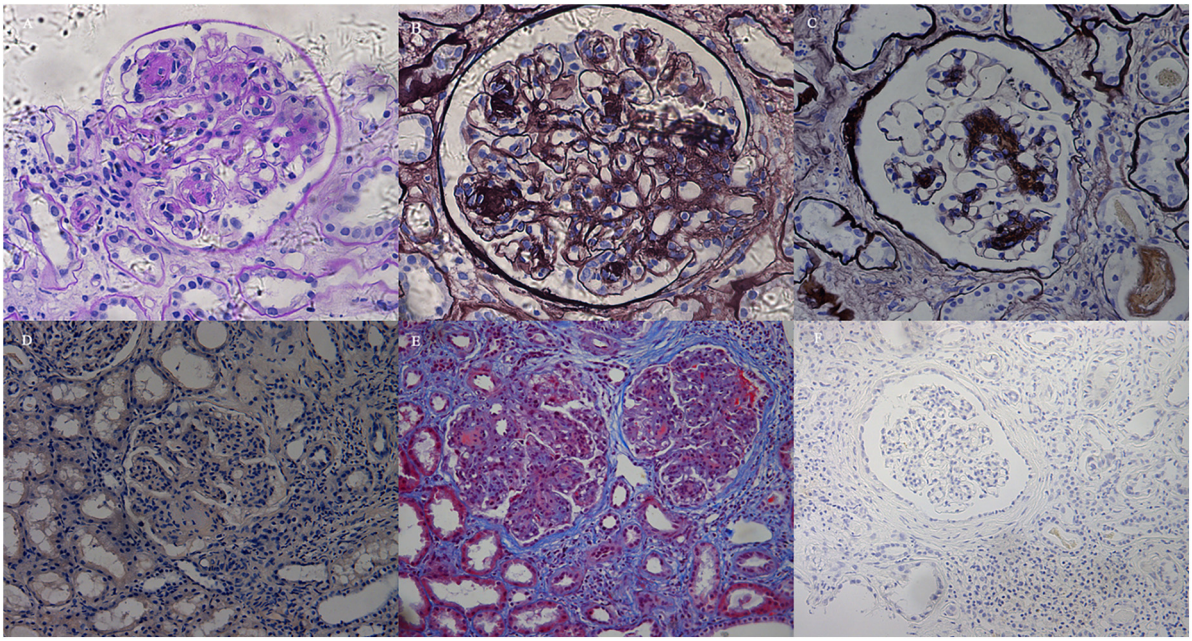


FIGURE 1
Light microscopy findings. **(A)** The glomerulus exhibited mesangial expansion and extensive proliferation of mesangial cells and matrix, with mesangial nodular sclerosis. The nodules and tubular basement membranes were periodic acid–Schiff (PAS) positive (Patient 3, PAS; × 200). **(B)** Diffuse membranoproliferative-like features revealed GBM duplication and mesangial interposition with double-contour or multicontour appearances (Patient 3, PASM; × 400). **(C)** The glomerulus exhibited segmental nodular sclerosis with mild mesangial hypercellularity. The GBMs in non-sclerotic areas were not thickened (Patient 4, PASM; × 200). **(D)** Congo red staining was negative (Patient 2, Congo red staining; × 100). **(E)** The mesangium expanded with diffuse hypercellularity and mesangial matrix proliferation. There was tubular atrophy and multifocal infiltration of lymphocytes and monocytes in the interstitium with fibrosis (Patient 2, Masson; × 100). **(F)** Lymphocytes and monocytes infiltrating the interstitium were CD38-negative by immunohistochemical staining (Patient 3, CD38; × 100).

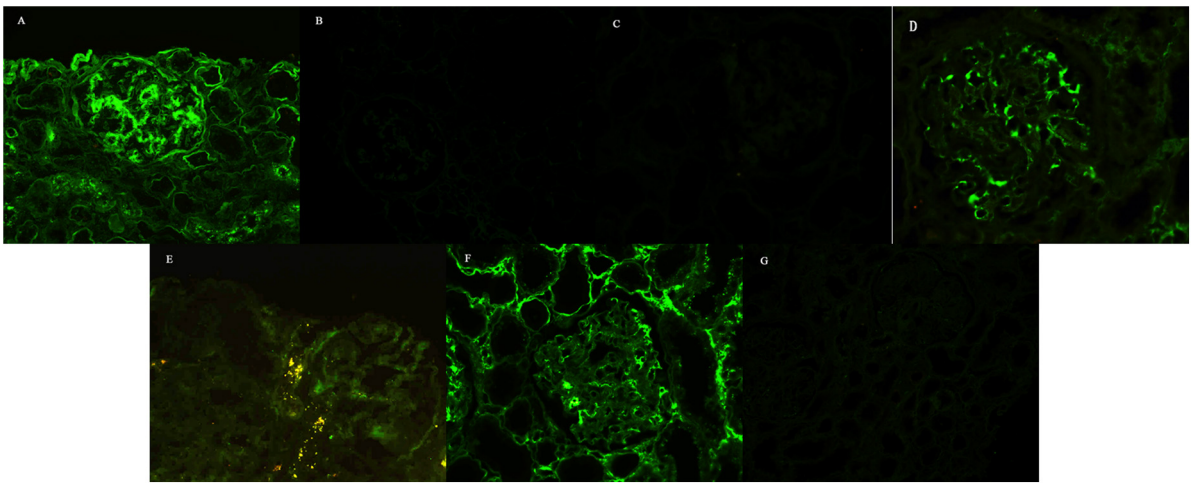


FIGURE 2
Immunofluorescence findings. **(A)** IgA linearly deposited along the GBMs and segmentally along the TBMs, while it was coarsely granularly deposited in the mesangial regions, with 3 + intensity (Patient 1, ×100). Staining for κ-chain **(B)** and λ-chain **(C)** was negative (Patient 1, ×100). Staining for IgG **(D)** and IgG2 **(E)** revealed coarse granular deposition only in the mesangial regions, without deposition along GBMs and TBMs (Patient 5, × 200). Restrictive κ-chain **(F)** was linearly deposited along the GBMs, segmentally deposited along the TBMs, and granularly deposited in the mesangial regions, while staining for λ-chain **(G)** was negative (Patient 5, × 100).

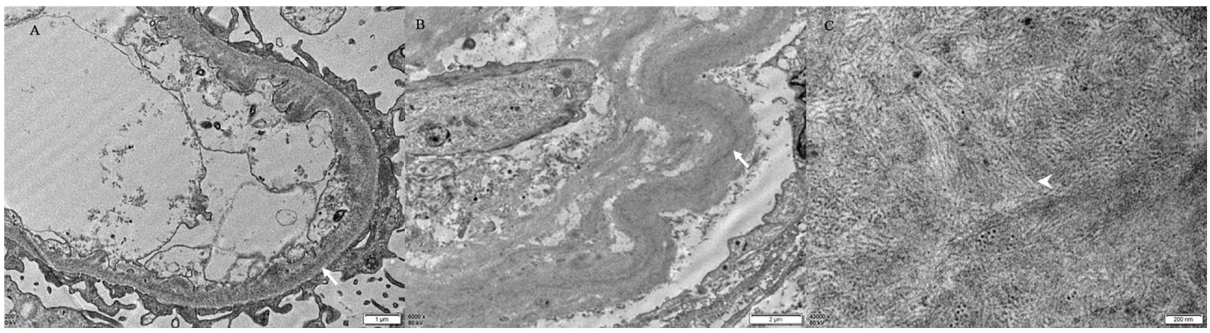


FIGURE 3
Electron microscopy findings. There are finely punctate "powdery" electron-dense deposits (white arrow) involving the inner aspect of the GBM **(A)** and the outer aspect of the TBM [white arrow, **(B)**] [Patient 4, **(A)** × 200, **(B)** × 6,000]. **(C)** The mesangial deposits comprised microtubular substructures (diameters of 20–50 nm) with hollow cores (arrowhead) arranged at least focally in parallel arrays (Patient 4 × 43,000).

staining. In IHC staining, $CD38^+$ or $CD138^+$ cells were rarely seen indicating no plasma cells infiltration (shown in **Figure 1**). No PAS-negative casts were found in the tubules.

On IF, immune depositions with monoclonal light chain and/or heavy chain were consistent with the type of serum/urine MIg (**Table 2**). Monoclonal heavy chain without light chain was found in one patient, while the deposits of monoclonal light chain + heavy chain were found in other four patients. κ chain was the major type of light chain, while α and γ chains were the major types of heavy chains. In all the patients, light chains or heavy chain α deposited in mesangial region, along the GBMs and segmentally along the TBMs. Heavy chains were found mainly in mesangial region without deposition along GBMs and TBMs in four patients besides patient 1 (shown in **Figure 2**).

Punctate "powdery" electron-dense deposits were seen in mesangial regions and along the GBMs and/or TBMs on EM. Concurrently, coarse granular deposits were found in the mesangial regions with a microtubular substructure (diameters of 20–50 nm) exhibiting hollow cores arranged in parallel arrays (shown in **Table 2** and **Figures 2**, **3**).

Treatment and outcomes

Follow-up data were available for four patients (except patient 1) with a median duration of 31 (20, 54) months (range 18–60 months). Patients 2 and 5 received bortezomib-based therapy with or without autologous stem cell transplantation. They achieved complete remission of hematological and renal symptoms with improved renal function. Patient 3 was treated with steroids combined with cyclophosphamide and lenalidomide. Four months later, he advanced to end stage renal failure albeit hematological complete remission was achieved. After 60 months of follow-up, he remained dialysis-dependent. Patient 4 refused any chemotherapy and was treated with hemodialysis. After 18 months of follow-up, he also remained dialysis-dependent.

Discussion

Although the incidence of single MIDD or ITG patients was relatively low (6, 13, 14), here we described a rarer series of patients with MIDD + ITG. Their characteristic pathological features had great diagnostic value, despite the non-specific clinical presentations. Segmental mesangial nodular sclerosis on the basis of mesangial matrix hyperplasia was the main lesion. MPGN-like lesion, GBM thickness and mild to moderate mesangial and endothelial proliferations presented at the same time and in the same glomeruli. EM manifestations of "powdery" deposits along GBM and/or TBM, and microtubular substructures with hollow cores arranged in parallel arrays in mesangial regions indicated the presence of both MIDD and ITG. At the same time, IF proved MIg depositions.

Clinical features of MIDD + ITG

The patients in this study were all in the middle aged or older. It was similar to that previously reported in single MIDD or ITG patients (6, 13, 14). However, due to the small sample size in this study, we could not determine whether there was a gender difference in MIDD + ITG. The duration from onset to diagnosis was relatively long due to atypical early symptoms and late renal biopsy. Nephritic syndrome with chronic renal dysfunction was the prominent presentation of MIDD + ITG, in accordance with most MIg-associated renal diseases. The remarkable anemia incompatible with the patients' renal function indicated the possible existence of hematologic diseases. The results of serum/urine IEF and/or FLCs implied the possible diagnosis of MIg associated disease. Our study was

consistent with the reports in the literature that not all the patients have positive M-spike on serum/urine IEF, and serum FLCs assay can make up for this deficiency (6, 12, 15, 16).

The prognosis of these patients was heterogeneous. Similar with previous studies (17, 18), patients treated with bortezomib-based regimen seemed to have better outcomes. Further study containing more patients with longer follow up is needed to confirm this hypothesis.

Unique pathological features of MIDD + ITG in contrast with each of monoclonal immunoglobulin deposition disease or immunotactoid glomerulopathy

As the non-specificity of the clinical manifestations, the accurate diagnosis relied on renal pathology. The manifestations on LM displayed the heterogeneity of lesions including mesangial nodular sclerosis forming on the basis of mesangial matrix hyperplasia, MPGN-like lesions, GBM thickness and etc. This was a unique feature of MIDD + ITG. Although mesangial nodular sclerosis could be seen in both single MIDD and single ITG, there were prominent differences compared with that in MIDD + ITG (6, 11, 19). Mesangial nodular sclerosis distributed focally and segmentally in MIDD + ITG in contrast to the diffusely and globally distribution in single MIDD. This was consistent with the literature reports that the combination of LCDD with other forms of disease had lower presence of nodular sclerosis and was only diagnosed through the powdery deposits on EM (20). Considering ITG, although mesangial nodular sclerosis might present in the late stage, mesangial and endothelial proliferation leading to MPGN-like lesions were the typical pathological features (12, 14, 21–23). However, in MIDD + ITG, less proliferation and only segmental MPGN-like lesions were seen. At the same time, the partial thickened GBMs resulted from mesangial interposition with a double-contour or multicontour appearance was more comparable with the feature of ITG, rather than that of MIDD mainly presenting as diffuse thickness of GBMs. So, we speculated that ITG might play more roles in the formation of glomerular lesions in our patients. At the same time, due to the non-homogeneous distribution of mesangial nodular sclerosis lesions and negative result of Congo red staining, amyloidosis could be excluded.

On EM, there were characteristic features to differentiate the components of MIDD + ITG. The punctate "powdery" deposits along the GBMs and/or TBMs was the feature of MIDD, while the microtubular deposits with hollow cores in mesangial region indicated the presence of ITG (6, 15, 23). Although cryoglobulinemic glomerulonephritis (Cryo GN) also produce microtubular structures, there were several differences to ITG. In ITG, the microtubules were relatively thicker than Cryo GN. On cross-section, microtubules of Cryo GN have 8–12 spokes emanating from the perimeter, making the cross-sectional outer diameter about 33 nm (24). No EM features relating to Cryo GN with the negative results of serum cryoglobulin have excluded the diagnosis. Thus, the coexistence of both "powdery" deposits along the GBMs and/or TBMs and microtubular deposits with hollow cores in mesangial region confirmed the diagnosis when there was evidence of MIg deposition in the kidney.

On IF, κ was the major light chain in patients with MIDD + ITG, consisting with the immune type of single MIDD and single ITG (2, 6, 13–15). γ and α heavy chains were both commonly seen, while γ1 and γ2 were the main γ subclasses. This was in accordance with the immune deposition types of ITG in the literature (13). The features of MIg deposition indicated the existence of different forms of the disease. In this cohort, heavy chains mainly deposited in mesangial regions, and light chains deposited along GBMs and TBMs, as well as in mesangial regions. This suggested that intact MIg might be the component of ITG deposits while restricted light chains were the component of MIDD. Thus, the four patients except patient 1 were diagnosed as LCDD + ITG. For patient 1, α heavy chain participated in both MIDD and ITG deposits indicating the diagnosis of HCDD + ITG.

Various degrees of tubular interstitial lesions incompatible with glomerular lesions were also visible in four patients except patient 2 in this study. These might explain the renal dysfunction presented in the patients. Pathologically, according to IHC results, the infiltrated inflammatory cells were mainly $CD3^+$ T cells and $CD20^+$ B cells. Together with manifestations of routine staining, there was no evidence of allergic nephritis, LCCN, myeloma infiltration, or any tubulointerstitial damage caused by MM or MGRS. Considering the significant immune deposition along TBMs, renal dysfunction presented either chronically or acutely in patients with MIDD + ITG, may be related to the involvement of renal tubules and interstitium of MIDD. Taken together, careful and thorough investigations with the combined application of IF, EM, LM and IHC could provide an accurate diagnosis of MIDD + ITG.

Possible mechanisms of MIDD + ITG

We reported a rare MIg-associated renal disease in which MIg deposited in both organized and non-organized ultrastructures. According to the pathogenesis reported (10), MIDD and ITG might resulted from immunoglobulins (MIgs) of different origins with unusual or abnormal structures. The deposition may be the consequence of the acquired defects in podocyte functions relating the clearance of the filtrated and retained immunoglobulin, which created the unique environment for deposition (19, 25). According to the immune phenotype, we hypothesize that in MIDD + ITG, intact MIgs with abnormal structures might result in organized deposits. At the same time, light chains or heavy chains were more likely to deposit on GBM, due to the abnormal physicochemical properties of MIg. We presumed that this might be one

of the reasons to cause the acquired defects in podocyte functions. These defects promoted the retaining and deposition of organized intact MIgs with abnormal structures, leading to the formation of MIDD + ITG.

At the same time, whether the organized and non-organized deposits resulted from the same MIg of different conformations remained to be elucidated. In the series of LCDD + AL reported by Said, through the method of laser microdissection-assisted liquid chromatography-tandem mass spectrometry (LC-MS/MS), the combination was thought to be caused by pathological light chains produced by subclones stemming from one immunoglobulin light chain rearrangement, with a distinct mutated complementary determining region. Despite the lack of LC-MS/MS exam, we speculated that MIDD + ITG in this study was also caused by the same MIg for the following reasons. Firstly, MIDD and ITG deposits on IF had the same light chain or heavy chain isotype. Secondly, none of the patients had two different MIgs in the serum or urine. However, the accurate evidence needs to be obtained from LC-MS/MS.

In this study, the extraordinary combination of MIDD + ITG were described. The major limitation of the current study was that because of the limited number of cases, it was difficult to accurately summarize the prognostic factors for the outcomes. More cases are needed to further confirm our findings and speculations.

Conclusion

MIDD + ITG is a rare form of MIg-associated renal disease. Clinically it mainly presents as nephritic syndrome and renal dysfunction with prominent anemia. Serum/urine IEF or serum FLCs may prove the existence of MIg. The accurate diagnosis relies on the comprehensive pathologic investigations. Larger cohort would help to determine the best choice of treatment of MIDD + ITG. The accurate evidence of whether the coexisted organized and non-organized deposits came from the same MIg, needs to be obtained from LC-MS/MS.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YW, YY, and BD concepted and designed the study. WZ, XL, CS, and LJ analyzed and interpreted the data. YW and YY drafted the article and revised the article. MW and LZ provided intellectual content of critical importance to the work described. YY final approved the version to be published. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the National Natural Science Foundation of China (81000296) and the Specialized Research Fund for the Doctoral Program of Higher Education (20090001120098).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Motwani SS, Herlitz L, Monga D, Jhaveri KD, Lam AQ. Paraprotein-related kidney disease: glomerular diseases associated with paraproteinemias. *Clin J Am Soc Nephrol.* (2016) 11:2260–72. doi: 10.2215/CJN.02980316

2. Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nat Rev Nephrol.* (2019) 15:45–59. doi: 10.1038/s41581-018-0077-4

3. Adapa S, Konala VM, Naramala S, Nast CC. Multiple morphological phenotypes of monoclonal immunoglobulin disease on renal biopsy: significance of treatment. *Clin Nephrol Case Stud.* (2020) 8:17–24. doi: 10.5414/CNCS110052

4. Qian Q, Leung N, Theis JD, Dogan A, Sethi S. Coexistence of myeloma cast nephropathy, light chain deposition disease, and nonamyloid fibrils in a patient with multiple myeloma. *Am J Kidney Dis.* (2010) 56:971–6. doi: 10.1053/j.ajkd.2010.06.018

5. Sundaram S, Mainali R, Norfolk ER, Shaw JHT, Zhang PL. Fibrillary glomerulopathy secondary to light chain deposition disease in a patient with monoclonal gammopathy. *Ann Clin Lab Sci.* (2007) 37:370–4.

6. Nasr SH, Valeri AM, Cornell LD, Fidler ME, Sethi S, D'Agati VD, et al. Renal monoclonal immunoglobulin deposition disease: a report of 64 patients from a single institution. *Clin J Am Soc Nephrol.* (2012) 7:231–9. doi: 10.2215/CJN.08640811

7. Zand L, Nasr SH, Gertz MA, Dispenzieri A, Lacy MQ, Buadi FK, et al. Clinical and prognostic differences among patients with light chain deposition disease, myeloma cast nephropathy and both. *Leuk Lymphoma.* (2015) 56:3357–64. doi: 10.3109/10428194.2015.1040011

8. Lin J, Markowitz GS, Valeri AM, Kambham N, Sherman WH, Appel GB, et al. Renal monoclonal immunoglobulin deposition disease: the disease spectrum. *J Am Soc Nephrol.* (2001) 12:1482–92. doi: 10.1681/ASN.V1271482

9. Joly F, Cohen C, Javaugue V, Bender S, Belmouaz M, Arnulf B, et al. Randall-type monoclonal immunoglobulin deposition disease: novel insights from a nationwide cohort study. *Blood.* (2019) 133:576–87. doi: 10.1182/blood-2018-09-872028

10. Said SM, Best Rocha A, Valeri AM, Paueksakon P, Dasari S, Theis JD, et al. The characteristics of patients with kidney light chain deposition disease concurrent with light chain amyloidosis. *Kidney Int.* (2021) 101:152–63. doi: 10.1016/j.kint.2021.10.019

11. Charles JJ, Olson JL, Silva FG, D'Agati VD. *Heptinstall's Pathology of the Kidney*. (2014). Available online at: https://shop.lww.com/Heptinstall-s-Pathology-of-the-Kidney/p/9781451144116# (accessed August 27, 2014).

12. Bridoux F, Leung N, Hutchison CA, Touchard G, Sethi S, Fermand JP, et al. Diagnosis of monoclonal gammopathy of renal significance. *Kidney Int.* (2015) 87:698–711.

13. Nasr SH, Kudose SS, Said SM, Santoriello D, Fidler ME, Williamson SR, et al. Immunotactoid glomerulopathy is a rare entity with monoclonal and polyclonal variants. *Kidney Int.* (2021) 99:410–20. doi: 10.1016/j.kint.2020.07.037

14. Nasr SH, Fidler ME, Cornell LD, Leung N, Cosio FG, Sheikh SS, et al. Immunotactoid glomerulopathy: clinicopathologic and proteomic study. *Nephrol Dial Transplant.* (2012) 27:4137–46. doi: 10.1093/ndt/gfs348

15. Li XM, Rui HC, Liang DD, Xu F, Liang SS, Zhu XD, et al. Clinicopathological characteristics and outcomes of light chain deposition disease: an analysis of 48 patients in a single Chinese center. *Ann Hematol.* (2016) 95:901–9. doi: 10.1007/s00277-016-2659-1

16. Sethi S, Rajkumar SV, D'Agati VD. The Complexity and heterogeneity of monoclonal immunoglobulin-associated renal diseases. *J Am Soc Nephrol.* (2018) 29:1810–23. doi: 10.1681/ASN.2017121319

17. Cohen C, Royer B, Javaugue V, Szalat R, El Karoui K, Caulier A, et al. Bortezomib produces high hematological response rates with prolonged renal survival in monoclonal immunoglobulin deposition disease. *Kidney Int.* (2015) 88:1135–43. doi: 10.1038/ki.2015.201

18. Bridoux F, Javaugue V, Bender S, Leroy F, Aucouturier P, Debiais-Delpech C, et al. Unravelling the immunopathological mechanisms of heavy chain deposition disease with implications for clinical management. *Kidney Int.* (2017) 91:423–34. doi: 10.1016/j.kint.2016.09.004

19. Korbet SM, Schwartz MM, Lewis EJ. Immuotactoid glomerulopathy (fibrillary glomerulonephritis). *Clin J Am Soc Nephrol.* (2006) 1:1351–6. doi: 10.2215/CJN.01140406

20. Lin ZS, Zhang X, Li DY, Yu XJ, Qin AB, Dong Y, et al. Clinicopathological features and outcomes of coexistent light chain cast nephropathy and light chain deposition disease in patients with newly diagnosed multiple myeloma. *J Clin Pathol.* (2021) [Online ahead of print]. doi: 10.1136/jclinpath-2021-207449

21. Jain A, Haynes R, Kothari J, Khera A, Soares M, Ramasamy K. Pathophysiology and management of monoclonal gammopathy of renal significance. *Blood Adv.* (2019) 3:2409–23. doi: 10.1182/bloodadvances.2019031914

22. Batko K, Malyszko J, Jurczyszyn A, Vesole DH, Gertz MA, Leleu X, et al. The clinical implication of monoclonal gammopathies: monoclonal gammopathy of undetermined significance and of renal significance. *Nephrol Dial Transplant.* (2019) 34:1440–52. doi: 10.1093/ndt/gfy259

23. Kanzaki G, Okabayashi Y, Nagahama K, Ohashi R, Tsuboi N, Yokoo T, et al. Monoclonal immunoglobulin deposition disease and related diseases. *J Nippon Med Sch.* (2019) 86:2–9.

24. Cameron C. Diagnostic electron microscopy. A Text/Atlas (2nd edn). G. Richard Dickersin. Springer-Verlag, New York, 2000. No. of pages: 1005 (894 illustrations). ISBN: 0 387 98388 0. *J Pathol.* (2001) 194:137–8. doi: 10.1002/1096-9896(200105)194:1<137::AID-PATH841>3.0.CO;2-9

25. Ohashi A, Kumagai J, Nagahama K, Fujisawa H. Case of immunotactoid glomerulopathy showing high responsiveness to steroids therapy despite severe pathological features. *BMJ Case Rep.* (2019) 12:e229751. doi: 10.1136/bcr-2019-229751

frontiers | Frontiers in Medicine

TYPE Clinical Trial
PUBLISHED 30 August 2022
DOI 10.3389/fmed.2022.949108

OPEN ACCESS

EDITED BY
Jingyuan Xie,
Shanghai Jiao Tong University, China

REVIEWED BY
Qiong Qiong Yang,
Sun Yat-sen Memorial Hospital, China
Ying Yao,
Huazhong University of Science and Technology, China
Chen Yu,
Tongji University, China

*CORRESPONDENCE
Shu Rong
goldenxc@126.com
Weijie Yuan
ywj4169@163.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 20 May 2022
ACCEPTED 25 July 2022
PUBLISHED 30 August 2022

CITATION
Zhang YY, Gu LJ, Wang L, Rong S and Yuan WJ (2022) Low protein diet supplemented with ketoacids on muscle wasting in chronic kidney disease: A clinical trial.
Front. Med. 9:949108.
doi: 10.3389/fmed.2022.949108

Low protein diet supplemented with ketoacids on muscle wasting in chronic kidney disease: A clinical trial

Yueyue Zhang, Lijie Gu, Ling Wang, Shu Rong* and Weijie Yuan*

Division of Nephrology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Aim: Nutrition is an important part of the care of patients with chronic kidney disease (CKD). However, there is limited clinical research on the skeletal muscle nutrition of patients with CKD. We carried out this study to find out whether a low-protein diet supplemented with ketoacids (LPD + KA) could improve muscle wasting in patients with CKD.

Methods: Patients were enrolled in this non-blind, parallel-group, randomized trial assessing the nutritional status of CKD, randomly assigned to either the LPD + KA group or conventional LPD group. Blood samples such as Hemoglobin, Cystatin C, Creatinine, BUN, Albumin, Pre- Albumin, Glycerin Trilaurate, and Cholesterol were measured at baseline and every 3 months. The parameters of skeletal muscle and other body composition were assessed before and after dietary intervention for 12 months.

Results: A total of 58 patients with CKD completed the study and were available for further analysis. The hemoglobin and albumin were observed to be markedly improved in the LPD + KA group during the follow-up as compared to baseline. Body mass index and total body water index of both groups were increased upon follow-up but the increase in the LPD + KA group was comparatively higher. Moreover, an increase in body fat%, skeletal muscle mass index, and appendicular skeletal muscle mass index was observed in both groups between baseline and follow-up, but it was statistically insignificant.

Conclusion: This study did not find a significant improvement of KAs on muscle wasting, and a long time or more indices study may need to find the effects of the LPD + KA diets.

Clinical trial registration: [www.ClinicalTrials.gov], identifier [NCT02568020].

KEYWORDS

chronic kidney disease, low protein diet, ketoacids, skeletal muscle wasting, body composition analysis

Introduction

Chronic kidney disease (CKD) makes complex metabolic processes alter and affect muscular homeostasis, leading to a loss of muscle mass and, ultimately leading to muscle atrophy (1, 2). There are about 16–54% of patients with CKD are dystrophic (3). The syndromic uremic dystrophy has been nominated as protein-energy wasting (PEW) (4), is the main characteristic of PEW, indicating the simultaneous losses in protein and energy storage (5), increasing as CKD progresses, and ultimately increasing the risk of mortality (6, 7).

Nutrition is an important part of the care of patients with CKD. For decades, protein restriction has been used to improve complications such as abnormal glucose metabolism and hypertension in patients with CKD and protect remnant kidneys' function. As in long-term protein restriction, decreased amino-acid supply may lead to decreased protein synthesis and malnutrition, we often prescribed a low-protein diet (LPD) together with ketoacids (KAs), a nitrogen-free substitution for the essential amino acids, to patients with advanced CKD (8, 9).

Ketoacids supplementation may be protective against muscle atrophic in animal models (10–13). In 5/6th nephrectomy rats, LPD supplemented with ketoacids (LPD + KAs) was able to inhibit the activation of the ubiquitin-proteasome system and protect skeletal muscle from atrophy and oxidative damage when compared with LPD alone. KAs can prevent the decreased activity of the mitochondrial electron transport chain complexes and increase mitochondrial respiration (10, 12, 13). LPD + KAs decreased muscle autophagy markers, but no difference in inflammation in CKD skeletal muscle (11).

Although the reviewed evidence seems to suggest that KAs supplementation can be expected to bring positive results, there is limited clinical research on the skeletal muscle nutrition of patients with CKD. We, therefore, carried out this study, and divided subjects into the LPD group or LPD + KAs group, following up and testing their blood assay and body composition analysis.

Materials and methods

Study population

Between 26 October 2016 and 13 May 2020, subjects were enrolled from the Nephrology department of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, as part of this non-blind, parallel-group, randomized trial assessing the nutritional status of CKD (This trial was registered at www.ClinicalTrials.gov as NCT02568020). All individuals came from the outpatient clinics of Shanghai General Hospital. Once identified, they continued routine medical care in their clinics and had an investigative follow-up. Only those who finished the follow-up were further analyzed. Demographics of the patients were obtained from medical records or patient interviews.

Inclusion criteria: patients agree to participate in this study; age $\geq$ 18 years and $<$ 70 years; renal function measured with creatinine clearance [by Modification of Diet in Renal Disease equation (10)] $<$ 60 and $>$ 15 ml/min (3 monthly consecutive measurements); at least 6 months of follow up at our clinic before recruitment and haven't received any diet intervention.

Exclusion criteria: pregnant patients; diabetes; heart or liver failure; a recent myocardial infarction (in the last 12 months); long-term immobilization; chronic respiratory failure; cancer; any pharmacological treatment that could modify muscle structure or function such as glucocorticoids or insulin; contraindications of Ketosteril, such as hypersensitivity to the active substances or to any of the excipients, hypercalcemia, disturbed amino acid metabolism to the study protocol. Furthermore, patients who were $\geq$ 150 kg body weight, had a physical disability, or have metal implants were excluded to make sure the prerequisites of body composition were monitored.

All protocols were approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Shanghai General Hospital. Written informed consent was obtained from all patients or their closest family members. This research was conducted in adherence to the principles of the Helsinki Declaration of 1975 as revised in October 2013.

Treatment regimen

Patients were randomly assigned to either conventional LPD (LPD group, 30 patients) or LPD + KAs (LPD + KAs group, 30 patients). All patients were treated with an LPD containing 0.6 g protein/kg body weight per day and 120–125 kJ/kg body weight per day. Besides, the LPD + KAs group will be supplemented with keto-amino acids (Ketosteril®, Fresenius Kabi) at a dosage of one tablet/5 kg ideal body weight/day, divided into three doses taken during meals.

Follow-up

We obtained the subjects' dietary and medication intake through questionnaires every 3 months, operated a computer-based nutritional evaluation with diet software, and reminded the patients to have a 0.6 g protein/kg protein diet.

Abbreviations: ASMI, appendicular skeletal muscle mass index; BF, body fat; BMI, body mass index; CKD, Chronic kidney disease; KAs, ketoacids; LPD, low-protein diet; PEW, protein-energy wasting; SMMI, Skeletal Muscle Mass Index; TBWI, total body water index.

The patients were followed up for 1 year. During follow-up, three patients were lost to follow-up, in which two cases were for epidemic and traffic reasons, with one case was for myocardial infarction, which were thought to have nothing to do with medication. Then one case was included to make up the number and finally completed the study. The methodological process of the current study is described in Figure 1.

Biochemical data

Blood samples were collected at baseline and every 3 months. All the patients were told to fast overnight and were collected 5 ml of blood the next morning between 7:00 and 7:30 a.m. Hemoglobin, Cystatin C, Creatinine, BUN, Albumin, Pre-Albumin, Glycerin Trilaurate, and Cholesterol were measured at local sites by using standard techniques.

Body composition analysis

The parameters of body composition were assessed at baseline and at the end of follow-up. Body composition was determined through the Donghuayuan body composition monitor (DBA-550). It works on the principle of bioelectrical impedance. Variables such as sex, age, and height were input into the monitor. Patients were asked to stand barefoot on the foot electrodes of the measurement platform, with knees and back upright and straight without moving, then hold the grip electrodes firmly with their hands and extend their arms straight at a 45° angle to their body. As the measurement was completed, body mass index (BMI), percent body fat (BF%), skeletal muscle mass, in intracellular water (ICW), extracellular water (ECW), and total body water (TBW) appeared on the report. Appendicular skeletal muscle mass index (ASMI), Skeletal Muscle Mass Index (SMMI), and total body water index (TBWI) were calculated. ASMI was calculated as the sum of lean mass for the arms and legs (kilograms)/height^2 (meters^2) (14). Accordingly, SMMI was calculated as the sum of lean mass for the body, arms, and legs (kilograms)/height^2 (meters^2), and TBWI was calculated as the total body water (kilograms)/height^2 (meters^2).

Study endpoint

The endpoint is the arrival point or the patient is converted to dialysis. The initiation of chronic dialysis was obtained through participant self-reporting during the follow-up period and was verified by the clinical and hospital records at the local site.

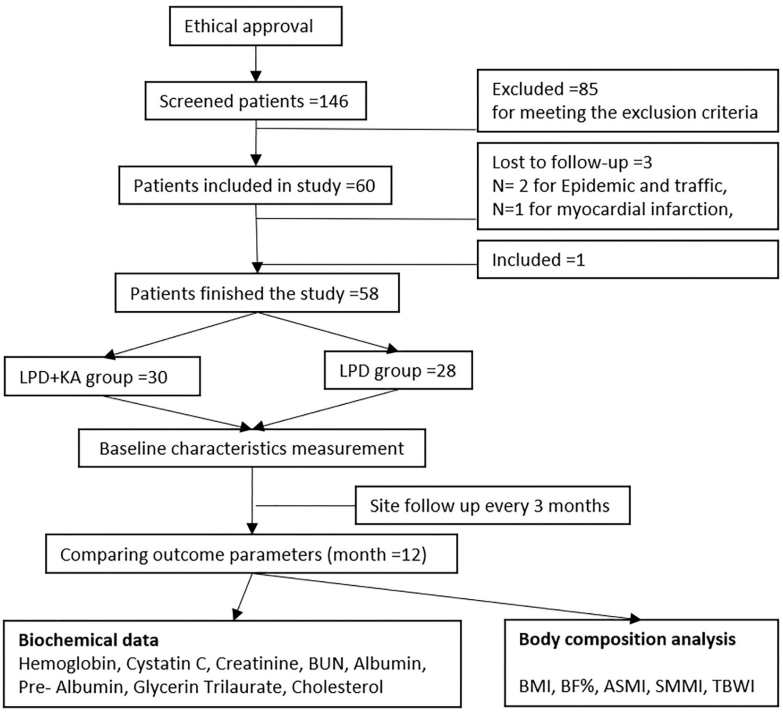


FIGURE 1
Study methodological flow diagram. KAs, ketoacids; LPD, low-protein diet; BMI, body mass index; BF, body fat; SMMI, Skeletal Muscle Mass Index; ASMI, appendicular skeletal muscle mass index; TBWI, total body water index.

The primary efficacy variable will be ASMI. Secondary efficacy variables will be BMI, BF%, and SMMI.

Safety variables will be serum creatinine.

Statistical analysis

The normally distributed measurement data were expressed as mean ± standard deviation (*SD*), the comparison between two groups was performed by *t*-test, and the comparison among multiple groups was performed by analysis of variance. The differences between the same periods comparing the two groups were analyzed using an independent Student's *t*-test. The same group between baseline and 12 months was compared by Paired Student's *t*-test. The sample size calculation assumes that the primary variable is the percentage change from baseline ASMI. With 30 patients per group, a one-sided *t*-test at a significance level of 2.5% has a power of 80% to detect an improvement of 7.36% in the LPD + KA group vs. the LPD group. $P < 0.05$ was considered statistically significant, and SPSS 20.0 statistical software was used for analysis.

Results

A total of 58 patients with CKD, 28 in the LPD group and 30 in the LPD + KAs group, completed the study and were available for further analysis. Characteristics and blood assays of the study participants at baseline and at the end of 1-year follow-up are shown in Tables 1, 2, and no participant converted to dialysis during the follow-up.

It could be seen from Table 2 and Figure 2 that, a progression was observed in cystatin C, creatinine, and blood urea nitrogen between baseline and 1-year follow-up in both groups but it was statistically insignificant ($p > 0.05$). It could be seen that, in LPD group, pre-Albumin was markedly improved ($p < 0.05$), hemoglobin, albumin, and cholesterol was improved statistically insignificant ($p > 0.05$), while glycerin trilaurate progressed but statistically insignificant ($p > 0.05$). In LPD + KA group, the hemoglobin and albumin were observed to be markedly improved at the end of 1-year followed up as compared to the baseline ($p < 0.05$). Although an improvement was beheld in pre-Albumin, glycerin trilaurate, cholesterol of LPD + KA group this change was statistically insignificant (Table 2 and Figure 2, $p > 0.05$).

TABLE 1 Patient demographics and characteristics.

Characteristic	LPD + KAs group	LPD group
Gender (male%)	21/30	18/28
Ages	56.16 ± 12.39	54.39 ± 8.18
Cause of chronic kidney disease		
Hypertension	1/30	1/28
Glomerular disease	1/30	0/28
Presumed chronic glomerular disease	20/30	20/28
IgA Nephrology	1/30	0/28
Others	7/30	7/28
Complication and medical history		
Hypertension	18/30	20/28
Coronary artery disease	5/30	4/28
Hyperuricemia	10/30	10/28

KAs, ketoacids; LPD, low-protein diet.

Body composition analysis at baseline and the end of 1-year follow-up is shown in Table 3. BMI and TBWI in the LPD + KA group were comparatively higher and increased upon follow-up ($p < 0.05$). Moreover, an increase in BF%, SMMI, and ASMI were observed in both groups between baseline and follow-up, but it was statistically insignificant ($p > 0.05$).

Discussion

Nutrition is a key component of care during CKD. There were findings suggesting that patients with CKD inhibiting protein intake can obtain the adaptation of muscle protein metabolism through the combined response to protein degradation and the recycling efficiency of protein breakdown amino acids. KAs supplementation has been proposed to reduce the risk of nutritional disorders of a very low-protein diet (VLPD), and VLPD + KAs were included as part of the clinical recommendations for patients with CKD in KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update (15): for CKD adults without diabetes, with an eGFR < 20 mL/min/1.73 m^2 (before dialysis), a VLPD with 0.28 to 0.43 g protein/kg plus KAs added to meet protein requirements were recommended.

In this study, a progression was observed in renal function (cystatin C, creatinine, blood urea nitrogen) between baseline and 1-year follow-up in both groups but it was statistically insignificant. This may be because reducing protein intake can delay hyalinosis, and reduce proteinuria and glomerular hyperfiltration (15). In the LPD group, pre-Albumin was markedly improved, but hemoglobin, albumin, and cholesterol improved statistically insignificant, while glycerin trilaurate progressed but was statistically insignificant. While in the LPD + KA group, hemoglobin and albumin were markedly improved, similar to the previous reference (16), but the improvement of pre-Albumin, glycerin trilaurate, and cholesterol were statistically insignificant. As in a *post hoc* analysis of the MDRD Study (17), the authors compared the various outcomes of LPD and VLPD + KAs related to nutritional status, and found the safety of dietary protein restriction over 2–3 years in patients with moderate to advanced CKD. Serum

TABLE 2 Biochemical data of study participants at baseline and at the end of follow-up.

	Baseline			1 year-follow up			Pair-comparison-LPD + KAs group	Pair-comparison-LPD group
	LPD + KAs group	LPD group	Sig	LPD + KAs group	LPD group	Sig	Sig	Sig
Hemoglobin (g/l)	121.99 ± 21.41	126.21 ± 16.94	0.395	133.41 ± 28.64	127.44 ± 23.1	0.386	0.001	0.685
Cystatin C(mg/l)	1.88 ± 0.67	1.72 ± 0.49	0.317	2.75 ± 1.3	2.08 ± 0.7	0.171	0.305	0.351
Creatinine (μmol/l)	181.17 ± 100.85	139.5 ± 62.84	0.058	220.76 ± 199.31	165.35 ± 155.36	0.230	0.107	0.316
Blood urea nitrogen (mmol/l)	10.46 ± 5.27	8.9 ± 3.12	0.167	12.36 ± 9.58	10.94 ± 7.78	0.533	0.146	0.089
Albumin (g/l)	40.77 ± 4.44	39.83 ± 3.07	0.340	42.37 ± 4.43	41 ± 4.54	0.246	0.005	0.161
Pre-Albumin (mg/l)	297.87 ± 109.58	257.9 ± 65.56	0.097	298.69 ± 66.01	299.19 ± 87.6	0.981	0.901	0.029
Glycerin Trilaurate (mmol/l)	1.89 ± 1.08	1.63 ± 0.72	0.290	2.2 ± 1.95	1.76 ± 0.78	0.454	0.367	0.445
Cholesterol (mmol/l)	4.49 ± 0.77	4.74 ± 1.28	0.371	4.74 ± 0.91	4.68 ± 1.34	0.822	0.093	0.566

KAs, ketoacids; LPD, low-protein diet.

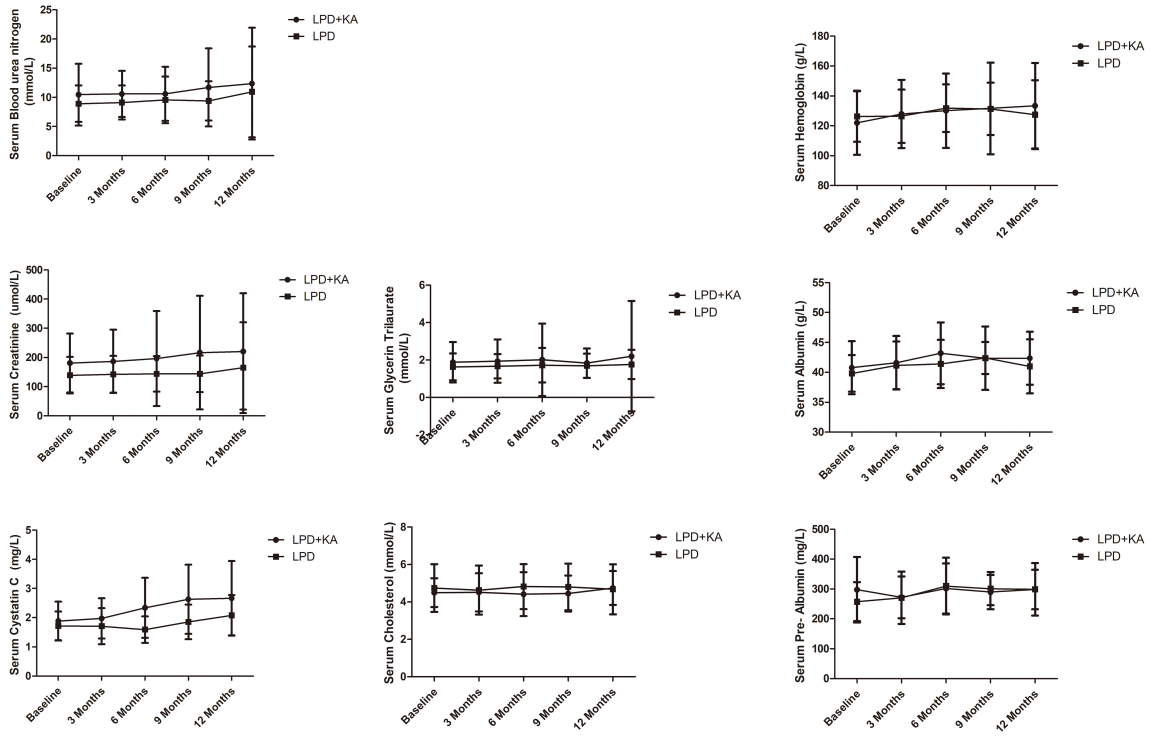


FIGURE 2
Biochemical data of study participants at baseline and every 3 months during follow-up. KA, ketoacids; LPD, low-protein diet.

albumin levels were elevated, while serum transferrin levels and urine creatinine excretion were decreased in LPD and VLPD groups. Also, the review by Koppe et al. showed that VLPD + KAs appears to reduce the production of uremic toxins, acidosis, phosphorous, and possible sodium intake while providing an adequate calcium intake (18).

As to body composition in this study, BMI and TBWI in the LPD + KA group were significantly increased, but the increase of BF%, SMMI, and ASMI was not statistically significant, indicating that the increase in BMI may be related to the change in TBWI. According to analysis in the MDRD study (17), body weight, BF%, and arm muscle area were decreased in LPD and VLPD groups. As reported in a longitudinal study looking at body composition, a VLPD + KA caused a small decline in lean body mass, accompanied by an increase in fat mass, mainly during the first 3 months (16). These

TABLE 3 Body composition analysis of study participants at baseline and at the end follow up.

	Baseline			1 year-follow up			Pair-comparison-LPD + KAs group	Pair-comparison-LPD group
	LPD + KAs group	LPD group	Sig	LPD + KAs group	LPD group	Sig	Sig	Sig
BMI (kg/m^2)	23.92 ± 3.67	24.3 ± 3.65	0.683	24.52 ± 4.05	24.69 ± 4.19	0.873	0.045	0.239
BF% (%)	25.03 ± 8.29	27.36 ± 7.68	0.255	25.06 ± 8.59	27.45 ± 8.1	0.265	0.894	0.755
SMMI (kg/m^2)	15.23 ± 2.17	14.9 ± 1.64	0.506	15.31 ± 2.42	14.98 ± 1.96	0.565	0.633	0.614
ASMI (kg/m^2)	7.42 ± 1.11	7.12 ± 0.91	0.263	7.50 ± 1.40	7.25 ± 1.23	0.453	0.513	0.313
TBWI (kg/m^2)	13 ± 1.76	12.77 ± 1.3	0.561	13.31 ± 1.93	13.02 ± 1.66	0.530	0.028	0.127

KAs, ketoacids; LPD, low-protein diet; BMI, body mass index; BF, body fat; SMMI, Skeletal Muscle Mass Index; ASMI, appendicular skeletal muscle mass index.

parameters were subsequently stabilized and even improved slightly thereafter.

There were other short-term studies that did not show a significant effect of LPDs and VLPDs + KAs on nutritional parameters. And there are also some studies that observed the small anthropometric measurement declines which may be for the prolonged use of LPDs and VLPDs + KAs in routine practice and the adverse effect of end-stage kidney disease. That is why physicians who prescribe LPDs must regularly monitor patients for their protein and energy intake, body weight, and nutritional status. Although this diet is not associated with wasting in carefully monitored studies, attention should focus on energy intake, which may decrease over time and lead to weight loss and consumption.

There were many limitations in this study, although there was a significant change in serum hemoglobin and albumin, neither of them was a direct indicator of muscle wasting. As for the completeness of the low-protein diet or the daily protein intake, it is not guaranteed. But it was documented that medication adherence is strongly associated with patient medication knowledge (19, 20), and it can be speculated that the same is true for dietary adherence. As to the study, we may need more time and more indices to find the effects of the LPD + KA diets in the following studies, and more research is needed to examine the effectiveness of KAs, especially on muscle wasting.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Shanghai General Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YYZ explored the data and wrote the manuscript. LJG and LW collected the data. SR reviewed this manuscript. WJY designed this study and reviewed this manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Ketosteril® Research Award 2014 and the Shanghai Youth Science and Technology Excellence Plan Project 2019 (grant no. 19YF1439100).

Acknowledgments

We thank all the participants for their contribution to this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Schardong J, Marcolino MAZ, Plentz RDM. Muscle atrophy in chronic kidney disease. *Adv Exp Med Biol.* (2018) 1088:393–412. doi: 10.1007/978-981-13-1435-3_18

2. Thome T, Salyers ZR, Kumar RA, Hahn D, Berru FN, Ferreira LF, et al. Uremic metabolites impair skeletal muscle mitochondrial energetics through disruption of the electron transport system and matrix dehydrogenase activity. *Am J Physiol Cell Physiol.* (2019) 317:C701–13. doi: 10.1152/ajpcell.00098.2019

3. Garg AX, Blake PG, Clark WF, Clase CM, Haynes RB, Moist LM. Association between renal insufficiency and malnutrition in older adults: Results from the NHANES III. *Kidney Int.* (2001) 60:1867–74. doi: 10.1046/j.1523-1755.2001.00001.x

4. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. *Kidney Int.* (2008) 73:391–8. doi: 10.1038/sj.ki.5002585

5. Carrero JJ, Stenvinkel P, Cuppari L, Ikizler TA, Kalantar-Zadeh K, Kaysen G, et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: A consensus statement from the international society of renal nutrition and metabolism (ISRNM). *J Ren Nutr.* (2013) 23:77–90. doi: 10.1053/j.jrn.2013.01.001

6. Kovesdy CP, Kopple JD, Kalantar-Zadeh K. Management of protein-energy wasting in non-dialysis-dependent chronic kidney disease: Reconciling low protein intake with nutritional therapy. *Am J Clin Nutr.* (2013) 97:1163–77. doi: 10.3945/ajcn.112.036418

7. Kanazawa Y, Nakao T, Murai S, Okada T, Matsumoto H. Diagnosis and prevalence of protein-energy wasting and its association with mortality in Japanese haemodialysis patients. *Nephrology.* (2017) 22:541–7. doi: 10.1111/nep.12814

8. Walser M. Nutritional effects of nitrogen-free analogues of essential amino acids. *Life Sci.* (1975) 17:1011–20. doi: 10.1016/0024-3205(75)90320-3

9. Cupisti A, Bolasco P. Keto-analogues and essential aminoacids and other supplements in the conservative management of chronic kidney disease. *Panminerva Med.* (2017) 59:149–56. doi: 10.23736/S0031-0808.16.03288-2

10. Wang D, Wei L, Yang Y, Liu H. Dietary supplementation with ketoacids protects against CKD-induced oxidative damage and mitochondrial dysfunction in skeletal muscle of 5/6 nephrectomised rats. *Skelet Muscle.* (2018) 8:18. doi: 10.1186/s13395-018-0164-z

11. Zhang YY, Huang J, Yang M, Gu LJ, Ji JY, Wang LJ, et al. Effect of a low-protein diet supplemented with keto-acids on autophagy and inflammation in 5/6 nephrectomized rats. *Biosci Rep.* (2015) 35:e00263. doi: 10.1042/BSR20150069

12. Wang DT, Lu L, Shi Y, Geng ZB, Yin Y, Wang M, et al. Supplementation of ketoacids contributes to the up-regulation of the Wnt7a/Akt/p70S6K pathway and the down-regulation of apoptotic and ubiquitin-proteasome systems in the muscle of 5/6 nephrectomised rats. *Br J Nutr.* (2014) 111:1536–48. doi: 10.1017/S0007114513004091

13. Huang J, Wang J, Gu L, Bao J, Yin J, Tang Z, et al. Effect of a low-protein diet supplemented with ketoacids on skeletal muscle atrophy and autophagy in rats with type 2 diabetic nephropathy. *PLoS One.* (2013) 8:e81464. doi: 10.1371/journal.pone.0081464

14. Sharma D, Hawkins M, Abramowitz MK. Association of sarcopenia with eGFR and misclassification of obesity in adults with CKD in the United States. *Clin J Am Soc Nephrol.* (2014) 9:2079–88. doi: 10.2215/CJN.02140214

15. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis.* (2020) 76:S1–107. doi: 10.1053/j.ajkd.2020.05.006

16. Chauveau P, Barthe N, Rigalleau V, Ozenne S, Castaing F, Delclaux C, et al. Outcome of nutritional status and body composition of uremic patients on a very low protein diet. *Am J Kidney Dis.* (1999) 34:500–7. doi: 10.1016/S0272-6386(99)70078-8

17. Kopple JD, Levey AS, Greene T, Chumlea WC, Gassman JJ, Hollinger DL, et al. Effect of dietary protein restriction on nutritional status in the modification of diet in renal disease study. *Kidney Int.* (1997) 52:778–91. doi: 10.1038/ki.1997.395

18. Koppe L, Cassani de Oliveira M, Fouque D. Ketoacid analogues supplementation in chronic kidney disease and future perspectives. *Nutrients.* (2019) 11:2071. doi: 10.3390/nu11092071

19. Khokhar A, Khan YH, Mallhi TH, Khan HM, Alotaibi NH, Alzarea AI, et al. Effectiveness of pharmacist intervention model for chronic kidney disease patients; a prospective comparative study. *Int J Clin Pharm.* (2020) 42:625–34. doi: 10.1007/s11096-020-00982-w

20. Zullig LL, Peterson ED, Bosworth HB. Ingredients of successful interventions to improve medication adherence. *JAMA.* (2013) 310:2611–2. doi: 10.1001/jama.2013.282818

frontiers | Frontiers in Medicine

TYPE Clinical Trial
PUBLISHED 27 September 2022
DOI 10.3389/fmed.2022.911205

OPEN ACCESS

EDITED BY
Giuseppe Regolisti,
University of Parma, Italy

REVIEWED BY
Michele Provenzano,
University of Bologna, Italy
Sui Zhun,
Peking University People's Hospital, China

*CORRESPONDENCE
Xiaoling Zhou
zxlingsky@163.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 02 April 2022
ACCEPTED 07 September 2022
PUBLISHED 27 September 2022

CITATION
Wang G, Ma K, Ma Z, Guo X, Wang Y, Ma L, Qi C, Li Y and Zhou X (2022) Short-term blood pressure variability and outcomes in non-dialysis chronic kidney disease. *Front. Med.* 9:911205. doi: 10.3389/fmed.2022.911205

Short-term blood pressure variability and outcomes in non-dialysis chronic kidney disease

Ge Wang[1], Kai Ma[2], Zhilan Ma[3], Xiaoyan Guo[3], Yan Wang[3], Lan Ma[3], Chenchen Qi[4], Yan Li[5] and Xiaoling Zhou[3]*

[1]Department of Nephrology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [2]Department of Chest Surgery, Xi'an International Medical Center Hospital, Xi'an, China, [3]Department of Nephrology, General Hospital of Ningxia Medical University, Yinchuan, China, [4]Department of Nephrology, NO215.Hospital of Shaanxi Nuclear Industry, Xianyang, China, [5]Department of Nephrology, The First People's Hospital of Yinchuan, Yinchuan, China

Background: Blood pressure variability (BPV) is associated with cardiovascular and all-cause mortality, and has been demonstrated in dialysis patients, but has been poorly studied and remains controversial in non-dialysis chronic kidney disease (CKD) patients. We investigated the effect of short-term BPV on prognosis in this population.

Methods: A total of 245 stage 1–4 CKD patients with 24-h ambulatory blood pressure recordings were recruited. BPV was evaluated by standard deviation, coefficient of variation, and variation independent of the mean, respectively. All subjects were followed up to the composite end-point event or until January 15, 2020. Patients were divided into two groups based on 24-h median variation independent of the mean, and demographics, laboratory indicators and echocardiogram results were compared. Logistic regression was used to analyze the risk factors for increased BPV. Multivariate Cox regression and Kaplan-Meier survival analysis were used to explore the relationship between BPV and renal prognosis and major cardiovascular events.

Results: The mean age was 42.07 ± 12.66 years, with 141 males (57.55%). Multivariate Logistic regression analysis showed that high BMI (OR 1.110, $P = 0.017$), hyperkalemia (OR 2.227, $P = 0.040$), increased left ventricular end-diastolic diameter (OR 1.103, $P = 0.010$) and hypertension (OR 2.525, $P = 0.002$) were independent risk factors for high BPV. Kaplan-Meier survival analysis showed that renal and cardiovascular outcomes were better in the low BPV group than in the high BPV group ($P = 0.006$; $P = 0.002$). After adjusting for age, sex and traditional kidney related risk factors, BPV were not independently associated with renal outcomes. High BPV (HR 4.662, $P = 0.017$) was the main independent risk factor for major cardiovascular events in CKD.

Conclusions: In non-dialysis CKD, short-term BPV was associated with major cardiovascular disease but not renal progression. BMI, hypertension, potassium balance, and left ventricular end-diastolic diameter influenced short-term BPV.

KEYWORDS

chronic kidney disease, blood pressure variability, cardiovascular disease, renal outcome, ambulatory blood pressure monitoring

Introduction

Problems associated with aging are becoming more and more severe throughout the world and high blood pressure (BP) is prevalent in the elderly. Epidemiological data indicate that the global prevalence of CKD is approximately 8–16% and increasing from year to year (1). In 2010, a cross-sectional study conducted in China revealed that the prevalence of CKD in China was 10.8%, or about 119.5 million adults (2).

Poor control of hypertension can result in kidney damage or worsen the initial kidney, or cardiovascular disease. A study in China showed that the awareness rate of hypertension in CKD patients was 80.7%, the treatment rate was 95.6%, but the control rate was only 57.1% (3). BP fluctuates greatly in CKD patients and is difficult to control due to abnormal activation of the renin-angiotensinogen-aldosterone system, water and sodium retention, insulin resistance and other factors, it is often necessary to take three or more antihypertensive drugs (4, 5). As a result, stability monitoring and accurate BP assessment are essential to patients with CKD.

The fluctuation of BP over a certain period of time is called blood pressure variability (BPV), including long term (visit-to-visit), mid-term (day-by-day), short term (within 24h) and very short term (beat-by-beat) BPV. Prior studies have demonstrated that BPV independent of mean BP is associated with the occurrence of cardiovascular disease (6, 7) as well as incident stroke in people with hypertension (8). Sarafidis et al. conducted a large, cross-sectional study and found that BPV increased with decreasing estimated glomerular filtration rate (eGFR) (9). In hemodialysis patients, some studies documented that BPV was related to cardiovascular, cerebrovascular and all-cause death (10, 11). However, in CKD patients who do not yet require dialysis, the influence of BPV on renal disease and its value in determining long-term prognosis remains to be elucidated (12). For patients with stage 1–4 CKD, it is of great importance to protect residual renal function, delay the need for renal replacement therapy, reduce the occurrence of CVD, and improve the prognosis. In stage 1-4 CKD patients who have not yet started renal replacement therapy, does this relationship still exist? We conducted a single-center retrospective study on this issue.

Materials and methods

Subjects

This was a retrospective, longitudinal, observational study performed in the Department of Nephrology, General Hospital of Ningxia Medical University on all non-dialysis CKD patients who were diagnosed and followed up regularly from January 1, 2012 to December 31, 2018. The inclusion criteria were age >18 but <70, those with a follow-up time of ≥1 year, and those who completed 24-h ambulatory blood pressure monitoring (ABPM). We excluded patients who had undergone renal replacement therapy, including hemodialysis, peritoneal dialysis, kidney transplantation, or patients with a history of clearly diagnosed cardiovascular and cerebrovascular diseases, including coronary heart disease, myocardial infarction, malignant arrhythmia, cerebral infarction, cerebral hemorrhage and those with any tumor or those with missing follow-up data. All patients voluntarily participated in this study and signed an informed consent form. This project was approved by the Ethics Committee of Ningxia Medical University.

Study design

Patients were divided into two groups based on the median 24 h systolic blood pressure VIM at baseline. (1) high BPV group (VIM >11.96) and (2) low BPV group (VIM≤11.96). The demographic information collected on patients included age, gender, smoking history, BMI (weight/height^2) and previously diagnosed chronic diseases. Laboratory tests performed on the enrolled patients included blood potassium, sodium, calcium, phosphorus, albumin, hemoglobin, 24-h urine protein quantification, uric acid, blood urea nitrogen, creatinine, and eGFR (calculated by CKD-EPI formula), The composite endpoints included the occurrence of renal progression (creatinine doubling, initiation of maintenance hemodialysis or peritoneal dialysis), major cardiovascular events (coronary heart disease, myocardial infarction, heart failure, malignant arrhythmia, cerebral infarction, and cerebral hemorrhage) and death. The study termination was January 15, 2020, or the occurrence of any of the endpoint events for individual patients. Follow-up was done on an outpatient basis and by telephone every 1–3 months.

Short-term BPV measurement

The Welch Allyn ABPM 6,100 non-invasive ambulatory blood pressure monitor was used and the cuff was worn on the left upper arm. ABPM started at around 8.am and readings were taken every 30 min during the day and every 1 h during the night, and was stopped after 24 h on the next day. During the monitoring period, patients were told to avoid strenuous activities and emotional agitation, but otherwise maintain their normal lifestyle. We used standard deviation (SD), coefficient of variation (CV = SD / mean), and variation independent of the mean (VIM) to quantify the 24 h blood pressure variation range. The VIM was calculated according to the following formula:

$VIM = k \times SD/mean^X$, where ^{X} is the curve–fitting coefficient of each patient's SD (dependent variable) and *mean* is the BP mean (independent variable); and $k = M^X$, where M is the mean blood pressure of all subjects (13).

TABLE 1 Baseline demographics, clinical characteristics, and 24-h ambulatory BP parameters, in the study cohort composed of 245 patients with CKD.

Variable	Overall	VIM ≤ 11.96(123)	VIM > 11.96(122)	*P*-value
Age (yrs)	42.1 ± 12.7	40.27 ± 12.26	43.85 ± 12.86	0.028
Men, *n* (%)	141 (58%)	64 (52.03%)	77 (63.11%)	0.079
BMI (kg/m2)	24.7 ± 3.6	23.8 ± 3.29	25.62 ± 3.66	<0.001
Smoking history, *n* (%)	69 (28%)	39 (31.71%)	36 (29.51%)	0.641
Diabetes, *n* (%)	31 (21%)	8 (6.5%)	23 (18.85%)	0.004
Hypertension, *n* (%)	135 (55%)	49 (39.84%)	86 (70.49%)	<0.001
Potassium (mmol/l)	4.1 ± 0.4	4.06 ± 0.36	4.18 ± 0.38	0.008
Calcium (mmol/l)	2.1 ± 0.2	141.41 ± 2.35	141.27 ± 2.49	0.647
Phosphorous (mmol/l)	1.2 ± 0.2	2.14 ± 0.2	2.1 ± 0.18	0.107
Creatinine (umol/l)	91.4 (69,128.6)	89.1 (68.6,117.4)	93.5 (70.53,139.75)	0.163
Uric acid (umol/L)	370.13 ± 99.79	355.49 ± 98.95	384.9 ± 98.85	0.021
Proteinuria (g/d)	1.9 (0.7,3.8)	1.61 (0.62,3.02)	2.43 (0.84,4.16)	0.014
Blood glucose (mmol/l)	4.8 (4.44,5.32)	4.76 (4.44,5.25)	4.83 (4.41,5.35)	0.931
Hemoglobin (g/l)	138.6 ± 22.2	140.52 ± 22.39	136.7 ± 21.97	0.179
Total cholesterol (mmol/l)	5.0 (4.0,6.64)	4.82 (3.87,6.45)	5.05 (4.09,7.03)	0.413
24h SBP (mmHg)	120 (111,133.5)	114 (107,121.5)	129 (118,141)	<0.001
24h DBP (mmHg)	77 (70,84)	73 (66.5,81)	78.5 (74,86)	<0.001
24h PP (mmHg)	44 (38.5,51)	41 (37,46)	48 (41,56)	<0.001
24h SBPSD	11.81 (9.58,14.27)	9.59 (8.45,10.8)	14.27 (12.96,17.08)	<0.001
24h DBPSD	9.7 (8.2,11.46)	8.25 (7.5,9.39)	11.37 (10.04,12.84)	<0.001
24h SBPCV	9.8 (8.2,11.7)	8.23 ± 1.52	11.58 (10.24,13.34)	<0.001
24h DBPCV	12.9(11.2,15.3)	11.73 ± 2.38	14.6 (12.76,16.57)	<0.001
Morning BP surge (mmHg)	25.59 ± 14.32	20.37 ± 9.78	30.86 ± 16.17	<0.001
LVST (mm)	9 (8,9)	9 (8,9)	9 (8,9)	0.029
LVPW (mm)	9 (8,9)	9 (8,9)	9 (8,9)	0.026
LVDD (mm)	47.69 ± 3.92	46.8 ± 3.67	48.6 ± 3.97	<0.001
LVEF (%)	68.68 ± 4.98	68.9 ± 4.05	68.45 ± 5.77	0.480
LVM (g)	144.57 ± 30.88	136.8 ± 26.25	152.4 ± 33.23	<0.001
LVMI (g/m)	82.36 ± 16.64	79.45 ± 13.25	85.28 ± 19.09	0.006

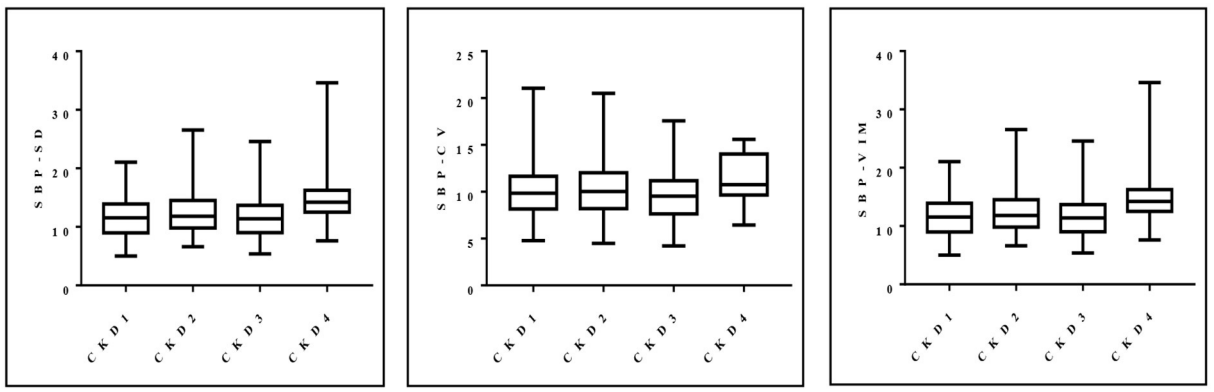


FIGURE 1
Distribution of SD, CV and VIM of systolic blood pressure in patients with stages 1–4 CKD.

Echocardiography

Left ventricular septal thickness (LVST), left ventricular posterior wall thickness (LVPWT), left ventricular end diastolic diameter (LVDD), left ventricular ejection fraction (LVEF) and body surface area (BSA) were recorded to calculate left ventricular mass (LVM) and left ventricular mass index (LVMI).

Devereux (14) LVM(g) = 0.832 × [(LVDD+LVST+LVPWT)^3 - LVDD^3]+0.6

LVMI (g/m) = LVW/BSA

BSA (15) = 0.0061 × height (cm) + 0.0128 × weight (kg)−0.1529

Statistical analyses

The data are expressed as mean ± standard deviation for normal distribution, and the non-normal data are expressed as median (interquartile interval, M, as (Q1, Q3, etc.). *T*-test was used to compare the two groups with normal data, and a non-parametric test was used to compare the two groups with non-normal data. The chi-squared test was used to compare the counting data between groups. The Kaplan-Meier method was used to analyze the relationship between BPV and kidney prognosis and major cardiovascular events. We used multiple Logistic regression analysis to explore the factors of BPV. A multivariate Cox regression model was used to analyze the risk factors of renal prognosis and cardiovascular and cerebrovascular events. The difference was statistically significant at $P < 0.05$), and $\alpha = 0.05$ was the test level.

Results

This study investigated 271 patients with non-dialysis CKD who underwent 24 h ABPM. We excluded 12 patients with incomplete data, four patients with previously diagnosed cerebrovascular disease, and two patients older than 70 years. Eight patients were excluded because of lack of follow-up. Ultimately, we included 245 non-dialysis stage 1–4 CKD patients to study the relationship between short-time BPV and the outcomes. There were 141 males (57.55%) and the average age was 42.07 ± 12.66 years. There were 135 patients with hypertension (55.1%) and 31 patients with diabetes (12.65%). With respect to etiology, there were 212 patients (86.53%) with primary glomerular disease, 13 (5.31%) with diabetic nephropathy, 10 (4.08%) with hypertensive nephropathy and 10 with other diseases. Renal biopsy was performed on 185 subjects, among which 78 (42%) had IgA nephropathy, 55 (30%) had membranous nephropathy and 18 (10%) patients had glomerular microlesions as the main pathological types, while 34 patients exhibited other pathological types. Table 1 presents the baseline characteristics for all subjects, as well as differences in baseline data between the higher and lower BPV groups. The median eGFR was 81.77 mL /min/1.73m^2, with 102 (41.63%) patients in stage CKD1, 67 (27.35%) in stage CKD2, 59 (24.08%) in stage CKD3, and 17 (6.94%) in stage CKD4.

TABLE 2 Factors that contribute to the increase of BPV.

Variable	*OR*	95% *CI*	*P*–value
BMI (kg/m^2)	1.110	1.019–1.209	0.017
Hypertension	2.525	1.420–4.491	0.002
Potassium (mmol/l)	2.227	1.038–4.777	0.040
LVDD (mm)	1.103	1.023–1.189	0.010

Blood pressure variability (VIM = 15.03) in patients with CKD4 was significantly greater than that in patients with CKD1 (VIM = 11.89) ($P = 0.002$). This trend persisted even when SD and CV were used as quantitative indicators of blood pressure variability ($P = 0.002$; $P = 0.032$) (Figure 1).

Patients in the higher BPV group were older, had higher BMI, and higher average BP. There was no difference in the distribution of BPV between men and women or between the low and high groups ($P = 0.079$). In terms of clinical indices, the blood potassium, uric acid and urinary protein were higher in the high BPV group, but there was no significant difference in serum creatinine distribution. In addition, the patients in the higher BPV group had greater left ventricular thickness and left ventricular mass, which also meant they had larger hearts and limited diastolic and systolic function.

Risk factors for increased BPV

We included the above variables ($P < 0.1$) in the multivariate logistic regression analysis to determine the risk factors for increased BPV. High BMI (OR 1.110, $P = 0.017$), hyperkalemia (OR 2.227, $P = 0.040$), increased left ventricular end-diastolic diameter (OR 1.103, $P = 0.010$) and hypertension (OR 2.525, $P = 0.002$) were all statistically significant as risk factors for elevated BPV (Table 2).

Outcomes

A cohort of 245 patients with non-dialysis CKD were enrolled, with a maximum follow-up time of 94 months and a median follow-up time of 64 months. A total of 44 patients had multiple endpoints, among which 25 had renal endpoints, 12 had major cardiovascular events and 7 patients died. We investigated the effects of BPV on kidney disease progression and major cardiovascular events in patients with non-dialysis CKD patients. Figure 2 shows the renal progression, incidence of major cardiovascular events all-cause deaths, and comparison of the incidence of different end-points in each group. We

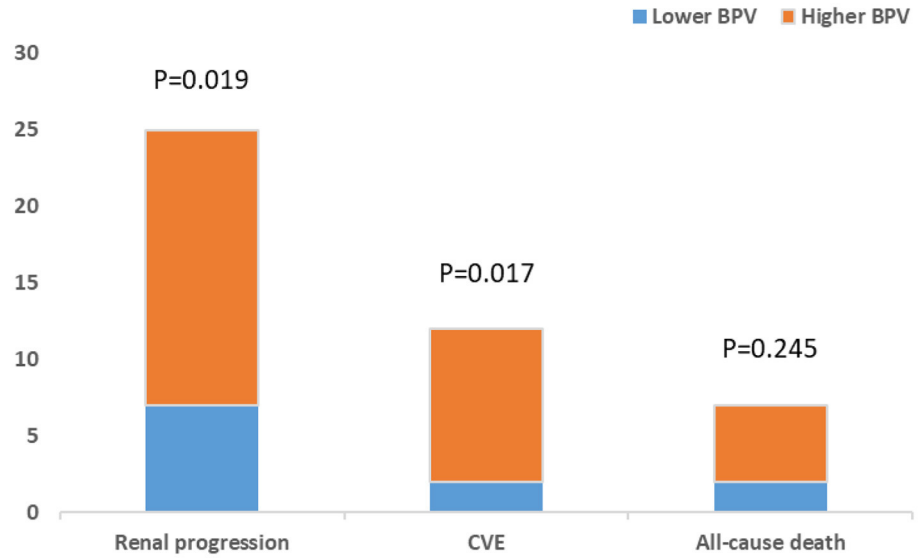


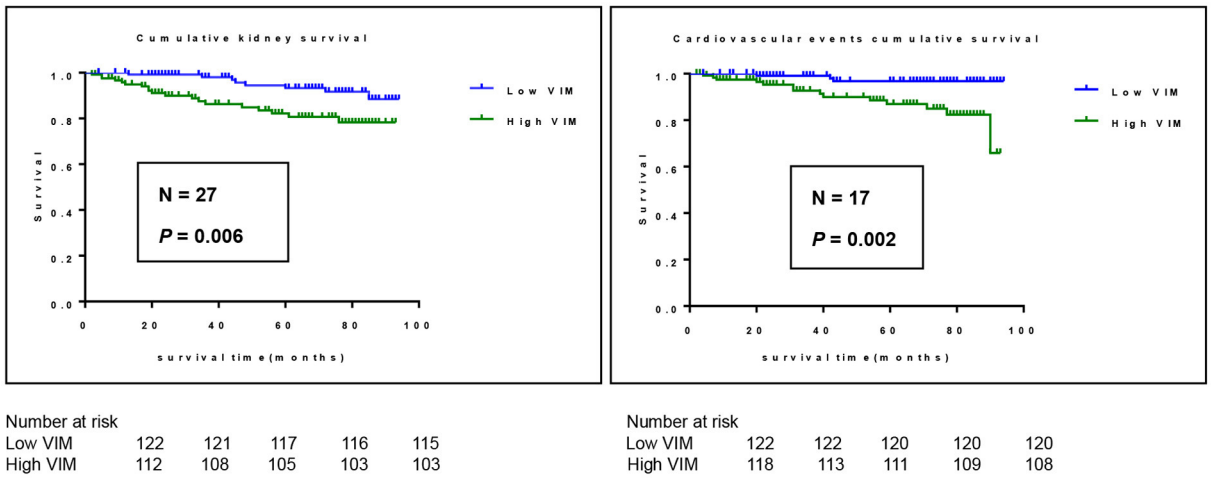
FIGURE 2
Comparison of renal progression, cardiovascular events and all-cause death between higher BPV and lower BPV group.

observed an obvious increase in renal progression and incidence of cardiovascular events in the higher BPV group ($P < 0.05$), but there was no statistical difference in all-cause mortality ($P > 0.05$). After Kaplan-Meier survival analysis, renal prognosis in the low BPV group was significantly greater than that in the high BPV group (log rank = 7.444, $P = 0.006$). Similar results were also seen in major cardiovascular prognosis. Patients in the low BPV group had better cardiovascular outcomes than those in the high BPV group during follow-up (log rank = 10.03, $P = 0.002$) (Figure 3). The association between 24 h SBP-VIM and the risk of renal progression and major cardiovascular events was further investigated with the Cox proportional hazard model. High SBP-VIM (> VIM 11.96) was positively correlated with renal progression without adjustment (HR = 2.998, $P = 0.009$). However, the association disappeared in fully adjusted models. High SBP-VIM (> VIM 11.96) was always associated with the occurrence of cardiovascular events in both unadjusted and fully adjusted models (HR = 4.704, $P = 0.022$) (Table 3).

Discussion

The purpose of this study was to analyze blood pressure variability in patients with non-dialysis CKD, identify the risk factors that may increase BPV, and explore the relationship between BPV and prognosis. During the follow-up of this study (longest follow-up period, 94 months; median follow-up period, 64 months), a total of 44 end-point events occurred. With the Kaplan-Meier survival analysis, we observed that the high VIM group with low VIM had worse renal prognosis ($P = 0.006$) and higher risk of major cardiovascular events in ($P = 0.002$). We also demonstrated that high SBP-VIM (> VIM 11.96) was independently associated with major cardiovascular events but not renal progression in patients with pre-dialysis CKD. Most studies have shown that long-term and short-term BPV were associated with target organ damage (such as heart, kidney, and brain) and all-cause death in the general population (16–18) and in people with hypertension (19, 20), however, some studies, like ours, have not found an association with renal prognosis after full adjustment (21–23). Therefore, we consider that BPV is strongly associated with major cardiovascular events and all-cause mortality, but further prospective studies of renal progression with larger sample sizes are needed.

We selected those factors that may contribute to increased BPV subjected them to multifactorial logistic regression analysis, and determined that high BMI, hyperkalemia, increased left ventricular end-diastolic diameter and hypertension were the main risk factors responsible for BPV increase. CKD patients with high BMI, excess blood volume, and a sedentary lifestyle with lack of exercise were prime candidates for increased blood pressure fluctuations. They also showed a tendency to abnormal lipid metabolism, often accompanied by high blood pressure and high blood sugar, in addition to insulin resistance and other factors leading to atherosclerosis and loss of vascular elasticity. Chen et al. found a positive association between BMI and average real variability (ARV) of systolic BPV (24). In a study of risk factors for BPV in hemodialysis patients, Feng at al. found that age and weight gain during hemodialysis were independent risk factors for BPV (25). Hyperactivity of sympathetic nerves, increased catecholamine concentration, activation of the RAAS system, increased cardiac afterload, progressive left ventricular



Number at risk					
Low VIM	122	121	117	116	115
High VIM	112	108	105	103	103

Number at risk					
Low VIM	122	122	120	120	120
High VIM	118	113	111	109	108

FIGURE 3
Kaplan-Meier survival curves for outcome of cardiovascular events and renal progression in participants stratified according to 24 h SBP-VIM. Cardiovascular events include all cerebrovascular and cardiovascular events.

TABLE 3 Multivariate Cox proportional hazard model showing association of high SBP–VIM with renal progression and cardiovascular events.

Model	HR	95% CI	*P*–value
Risk of renal progression			
Unadjusted	2.998	1.309–6.866	0.009
Adjusted for age and sex	2.886	1.246–6.683	0.013
Adjusted for age, sex, and baseline eGFR–EPI	2.67	1.128–6.321	0.025
Adjusted for age, sex, baseline eGFR–EPI, DM, SBP, and DBP	1.739	0.686–4.408	0.243
Risk of cardiovascular disease			
Unadjusted	5.898	1.693–20.542	0.005
Adjusted for age and sex	4.671	1.330–16.401	0.016
Adjusted for age, sex, BMI, SBP and DBP	4.663	1.241–17.520	0.023
Adjusted for age, sex, BMI, SBP, DBP and HB	4.236	1.120–16.024	0.033
Adjusted for age, sex, BMI, SBP, DBP, HB, LVM and LVMI	4.704	1.253–17.658	0.022

HB, Hemoglobin; DM, diabetes mellitus.

hypertrophy and increased end diastolic diameter of the left ventricle in patients with CKD, is associated with the dysregulation of cardiac BP control and increased fluctuation of BP. Persistent high BP can lead to increased pressure in the glomeruli, glomerular fibrosis or atrophy, impaired regulation of body fluid balance and decreased production of active vascular compounds, and metabolic disorders, aggravating the severity of hypertension and BPV even further. When patients with CKD develop hypertension, sympathetic nerves become hyperactive, the concentration of catecholamine increases, the RAAS system is activated, cardiac afterload increases, and left ventricular hypertrophy gradually occurs, while the left ventricular end diastolic diameter increases, the regulatory effect of the heart on blood pressure is weakened, and BPV increases. Low urinary potassium excretion was independently associated with high BPV in a Korean study of 1,860 patients with pre-dialysis chronic kidney disease and a median follow-up of approximately 5.6 years (26).This may suggest that low urinary potassium excretion is an important mechanism of high BPV in CKD patients. We may be the first to find a relationship between blood potassium levels and BPV that is not yet supported by the literature. However, studies on the effects of potassium intake levels on BPV as well as the cardiovascular system suggest that a certain level of potassium intake (90–120 mmol/day) may be a protective factor for blood pressure and cardiovascular events in patients with CKD (27). However, this may also have the risk of hyperkalemia. At present, there have been few reports about the correlation between serum potassium and BPV, and further prospective studies with large samples are needed to test this hypothesis.

It is clear that the definition of BPV is not unequivocal. In the case of traditional 'dippers' (fall in nighttime systolic and diastolic BP >10% from day-time BP), non- dippers (fall in nighttime BP <10%), inverted dippers (night-time BP fall of 10%-20%) and extreme dippers (night-time BP fall >20%) current methods can only represent the variation of BP at night. They have no capacity for quantifying the variation of BP throughout the whole 24 h or even during observation. Several studies have confirmed that the prevalence of the non-dipper BP pattern in patients with CKD is higher than that in patients with essential hypertension and is related to damage of the kidney, cardiovascular system and other target organs in patients with CKD (28–30). Researchers began to use statistical parameters such as SD, CV, weighted standard deviation (wSD), VIM and ARV to calculate BP fluctuation within a period of events. However, SD, CV and wSD were always based on average blood pressure and could not independently explain the correlation between BPV and prognosis. ARV averages the difference between successive BP readings over a specific time period and is widely used in many clinical studies of BPV, especially in long-term BPV (31). VIM is derived from more complex calculations such as curve fitting of SD and mean BP and has greater value in scientific research (32). Several studies found that when VIM was used as a measure of BPV, it was considered a better parameter than certain others because it was independent of mean BP (33). In this study, SD, CV and VIM were selected to evaluate BPV. In addition, ambulatory BP monitoring was more representative than clinical readings, and VIM was ultimately selected for grouping and as the main indicator.

It was noted that some prognostic studies also found numbers of biomarkers associated with end-stage renal disease (ESRD) or cardiovascular events in patients with CKD. Some of these biomarkers may have analyzed the etiological and predictive links demonstrated with clinical results. Cystatin C is a low molecular weight protein produced by nucleated cells that is freely filtered through the glomerulus and not secreted by renal tubules. It is completely reabsorbed. Shin et al. found that cystatin C was a better predictor of cardiovascular events and mortality than creatinine or Egfr (34). FGF-23 is a widely studied biomarker, which has been proved to be closely related to atherosclerosis, calcium and phosphorus metabolism disorder and renal function progression in patients with CKD (35). At present, some markers, such as hypersensitive troponin and NT-pro BNP, have been widely used in clinical practice, which are considered by clinicians to be closely related to acute myocardial infarction and heart failure. GDF-15, a member of the TGF-β family of cytokines, has been found to be involved in apoptosis repair and growth. GDF-15 may be a predictor of incidence of CKD, eGFR decline (36) and CVD independent of traditional CV risk factors, renal function, and other biomarkers (C-reactive protein, B-type natriuretic peptide, cardiac troponin) (37). There are still some controversial biomarkers to be further studied, which is of great significance for clinicians to improve the early detection of the prognosis and complications of CKD.

Limitations

The limitations of this study are mainly in the following aspects. First, we are not able to clarify the casual relation between high BPV and the kidney outcome and major cardiovascular events in non-dialysis CKD patients. In this study, the patients' conditions were relatively mild at enrollment. 169(69%) patients in stage 1 and 2 CKD, and the number of endpoint events observed was small. Thus, our study may be underpowered to detect adverse kidney outcomes within the specific follow-up time. Second, single-center retrospective assessments with small sample size may have bias that is difficult to account for. Third, although we found that BPV was independently associated with several factors such as larger BMI and hyperkalemia in our study, the mechanisms between them are not particularly well defined. In particular, the pathophysiological mechanisms between blood potassium levels and BPV need to be confirmed in animal models or in randomized controlled trials. In the future, large-sample, multi-center, prospective clinical studies are needed to further explore the impact of BPV on the prognosis of CKD patients, and whether lowering BPV can delay the progression of non-dialysis CKD and improve the prognosis. Lastly, due to the limitation of time and data, we only analyzed the effect of BPV at baseline on prognosis of enrolled patients. However, over the long course of CKD, there are many factors affecting BPV that cause it to change dynamically, and how to detect and manage them will require much future effort.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

GW conceived, designed, and coordinated the writing of the whole manuscript. KM, XG, and YL revised literature. ZM and LM provided clinical study of this work and collected clinical data. YW and CQ made statistical analysis. XZ took part in study design and responsible for the final draft. All the authors

contributed to this manuscript and approved the submitted version.

Funding

This paper was supported by Ningxia Science Foundation (Project number: 2019A0288) and Foundation for Returned Scholars of Ningxia (Project number: 2017659).

Acknowledgments

The authors thanks each member of the team for their contribution and the great efforts of teachers from the Department of Kidney Medicine at Ningxia Medical University.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* (2013) 382:260–72. doi: 10.1016/S0140-6736(13)60687-X

2. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet.* (2012) 379:815–22. doi: 10.1016/S0140-6736(12)60033-6

3. Yan Z, Wang Y, Li S, Wang J, Zhang L, Tan H, et al. Hypertension control in adults with Ckd in China: baseline results from the chinese cohort study of chronic kidney disease (C-Stride). *Am J Hypertens.* (2018) 31:486–94. doi: 10.1093/ajh/hpx222

4. Teo BW, Chua HR, Wong WK, Haroon S, Subramanian S, Loh PT, et al. Blood pressure and antihypertensive medication profile in a multiethnic asian population of stable chronic kidney disease patients. *Singapore Med J.* (2016) 57:267–73. doi: 10.11622/smedj.2016089

5. Sarafidis PA, Ruilope LM. Aggressive blood pressure reduction and renin-angiotensin system blockade in chronic kidney disease: time for re-evaluation? *Kidney Int.* (2014) 85:536–46. doi: 10.1038/ki.2013.355

6. Pierdomenico SD, Di Nicola M, Esposito AL, Di Mascio R, Ballone E, Lapenna D, et al. Prognostic value of different indices of blood pressure variability in hypertensive patients. *Am J Hypertens.* (2009) 22:842–7. doi: 10.1038/ajh.2009.103

7. Wan EYF Yu EYT, Chin WY, Fong DYT, Choi EPH, Lam CLK. Association of visit-to-visit variability of systolic blood pressure with cardiovascular disease, chronic kidney disease and mortality in patients with hypertension. *J Hypertens.* (2020) 38:943–53. doi: 10.1097/HJH.0000000000002347

8. Men X, Sun W, Fan F, Zhao M, Huang X, Wang Y, et al. China stroke primary prevention trial: visit-to-visit systolic blood pressure variability is an independent predictor of primary stroke in hypertensive patients. *J Am Heart Assoc.* (2017) 6:4350. doi: 10.1161/JAHA.116.004350

9. Sarafidis PA, Ruilope LM, Loutradis C, Gorostidi M. de la Sierra A, de la Cruz JJ, et al. Blood pressure variability increases with advancing chronic kidney disease stage: a cross-sectional analysis of 16 546 hypertensive patients. *J Hypertens.* (2018) 36:1076–85. doi: 10.1097/HJH.0000000000001670

10. Liao R, Li J, Xiong Y, Lin L, Wang L, Sun S, et al. Association of peridialysis blood pressure and its variability with cardiovascular events in hemodialysis patients. *Kidney Blood Press Res.* (2018) 43:1352–62. doi: 10.1159/000492595

11. Sarafidis PA, Loutradis C, Karpetas A, Tzanis G, Bikos A, Raptis V, et al. The association of interdialytic blood pressure variability with cardiovascular events and all-cause mortality in haemodialysis patients. *Nephrol Dial Transplant.* (2019) 34:515–23. doi: 10.1093/ndt/gfy247

12. Li H, Xue J, Dai W, Chen Y, Zhou Q, Chen W. Visit-to-Visit blood pressure variability and risk of chronic kidney disease: a systematic review and meta-analyses. *PLoS ONE.* (2020) 15:e0233233. doi: 10.1371/journal.pone.0233233

13. Dolan E, O'Brien E. Blood pressure variability: clarity for clinical practice. *Hypertension.* (2010) 56:179–81. doi: 10.1161/HYPERTENSIONAHA.110.154708

14. Xie L, Wang Z. Correlation between echocardiographic left ventricular mass index and electrocardiographic variables used in left ventricular hypertrophy criteria in Chinese hypertensive patients. *Hellenic J Cardiol.* (2010) 51:391–401. doi: 10.1097/01.hjh.0000379687.09826.63

15. Mallamaci F, Tripepi G. Blood pressure variability in chronic kidney disease patients. *Blood Purif.* (2013) 36:58–62. doi: 10.1159/000351004

16. Basson MD, Klug MG, Hostetter JE, Wynne J. Visit-to-Visit variability of blood pressure is associated with hospitalization and mortality in an unselected adult population. *Am J Hypertens.* (2018) 31:1113–9. doi: 10.1093/ajh/hpy088

17. Sible IJ, Yew B, Dutt S, Bangen KJ Li Y, Nation DA, et al. Visit-to-Visit blood pressure variability and regional cerebral perfusion decline in older adults. *Neurobiol Aging.* (2021) 105:57–63. doi: 10.1016/j.neurobiolaging.2021.04.009

18. Yoo JE, Shin DW, Han K, Kim D, Lee SP, Jeong SM, et al. Blood pressure variability and the risk of dementia: a nationwide cohort study. *Hypertension.* (2020) 75:982–90. doi: 10.1161/HYPERTENSIONAHA.119.14033

19. Cuspidi C, Carugo S, Tadic M. Blood pressure variability and target organ damage regression in hypertension. *J Clin Hypertens (Greenwich).* (2021) 23:1159–61. doi: 10.1111/jch.14208

20. Hung MH, Huang CC, Chung CM, Chen JW. 24-H ambulatory blood pressure variability and hypertensive nephropathy in han chinese hypertensive patients. *J Clin Hypertens (Greenwich).* (2021) 23:281–8. doi: 10.1111/jch.14108

21. Di Iorio B, Pota A, Sirico ML, Torraca S, Di Micco L, Rubino R, et al. Blood pressure variability and outcomes in chronic kidney disease. *Nephrol Dial Transplant.* (2012) 27:4404–10. doi: 10.1093/ndt/gfs328

22. Borrelli S, Garofalo C, Mallamaci F, Tripepi G, Stanzione G, Provenzano M, et al. Short-Term blood pressure variability in nondialysis chronic kidney disease patients: correlates and prognostic role on the progression of renal disease. *J Hypertens.* (2018) 36:2398–405. doi: 10.1097/HJH.0000000000001825

23. Okada T, Matsumoto H, Nagaoka Y, Nakao T. Association of home blood pressure variability with progression of chronic kidney disease. *Blood Press Monit.* (2012) 17:1–7. doi: 10.1097/MBP.0b013e32834f7125

24. Chen H, Zhang R, Zheng Q, Yan X, Wu S, Chen Y. Impact of body mass index on long-term blood pressure variability: a cross-sectional study in a cohort of Chinese adults. *BMC Public Health.* (2018) 18:1193. doi: 10.1186/s12889-018-6083-4

25. Feng Y, Li Z, Liu J, Sun F, Ma L, Shen Y, et al. Association of short-term blood pressure variability with cardiovascular mortality among incident hemodialysis patients. *Ren Fail.* (2018) 40:259–64. doi: 10.1080/0886022X.2018.1456456

26. Suh SH, Song SH, Oh TR, Choi HS, Kim CS, Bae EH, et al. Association of urinary potassium excretion with blood pressure variability and cardiovascular

outcomes in patients with pre-dialysis chronic kidney disease. *Nutrients.* (2021) 13:124443. doi: 10.3390/nu13124443

27. Gritter M, Wouda RD, Yeung SMH, Wieers MLA, Geurts F, de Ridder MAJ, et al. Effects of short-term potassium chloride supplementation in patients with Ckd. *J Am Soc Nephrol.* (2022) 33:1779–89. doi: 10.1681/ASN.2022020147

28. Cha RH, Lee H, Lee JP, Kang E, Song YR, Kim YS, et al. Changes of blood pressure patterns and target organ damage in patients with chronic kidney disease: results of the aprodite-2 study. *J Hypertens.* (2017) 35:593–601. doi: 10.1097/HJH.0000000000001185

29. Abdalla M, Caughey MC, Tanner RM, Booth JN, 3rd Diaz KM, Anstey DE, et al. Associations of blood pressure dipping patterns with left ventricular mass and left ventricular hypertrophy in blacks: the jackson heart study. *J Am Heart Assoc.* (2017) 6:4847. doi: 10.1161/JAHA.116.004847

30. Cuspidi C, Facchetti R, Quarti-Trevano F. Dell'Oro R, Tadic M, Gherbesi E, et al. Clinical correlates and subclinical cardiac organ damage in different extreme dipping patterns. *J Hypertens.* (2020) 38:858–63. doi: 10.1097/HJH.0000000000002351

31. Sethna CB, Meyers KEC, Mariani LH, Psoter KJ, Gadegbeku CA, Gibson KL, et al. Blood pressure and visit-to-visit blood pressure variability among individuals with primary proteinuric glomerulopathies. *Hypertension.* (2017) 70:315–23. doi: 10.1161/HYPERTENSIONAHA.117.09475

32. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet.* (2010) 375:895–905. doi: 10.1016/S0140-6736(10)60308-X

33. Asayama K, Kikuya M, Schutte R, Thijs L, Hosaka M, Satoh M, et al. Home blood pressure variability as cardiovascular risk factor in the population of ohasama. *Hypertension.* (2013) 61:61–9. doi: 10.1161/HYPERTENSIONAHA.111.00138

34. Shin MJ, Song SH, Kwak IS, Lee SB, Lee DW, Seong EY, et al. Serum cystatin c as a predictor for cardiovascular events in end-stage renal disease patients at the initiation of dialysis. *Clin Exp Nephrol.* (2012) 16:456–63. doi: 10.1007/s10157-011-0583-1

35. Song T, Fu Y, Wang Y, Li W, Zhao J, Wang X, et al. Fgf-23 correlates with endocrine and metabolism dysregulation, worse cardiac and renal function, inflammation level, stenosis degree, and independently predicts in-stent restenosis risk in coronary heart disease patients underwent drug-eluting-stent pci. *BMC Cardiovasc Disord.* (2021) 21:24. doi: 10.1186/s12872-020-01839-w

36. Bao X, Xu B, Borné Y, Orho-Melander M, Melander O, Nilsson J, et al. Growth differentiation factor-15 and incident chronic kidney disease: a population-based cohort study. *BMC Nephrol.* (2021) 22:351. doi: 10.1186/s12882-021-02558-w

37. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem.* (2017) 63:140–51. doi: 10.1373/clinchem.2016.255174

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 09 January 2023
DOI 10.3389/fmed.2022.930541

OPEN ACCESS

EDITED BY
Li Zuo,
Peking University People's Hospital, China

REVIEWED BY
Guisen Li,
Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, China
Eswaran Perumal,
Alagappa University, India

*CORRESPONDENCE
Rongshan Li
✉ rongshanli13@163.com
Xiaoshuang Zhou
✉ xiaoshuangzhou66@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 28 April 2022
ACCEPTED 19 December 2022
PUBLISHED 09 January 2023

CITATION
Song W, Liu Y, Qiu L, Qing J, Li A, Zhao Y, Li Y, Li R and Zhou X (2023) Machine learning-based warning model for chronic kidney disease in individuals over 40 years old in underprivileged areas, Shanxi Province.
Front. Med. 9:930541.
doi: 10.3389/fmed.2022.930541

Machine learning-based warning model for chronic kidney disease in individuals over 40 years old in underprivileged areas, Shanxi Province

Wenzhu Song[1†], Yanfeng Liu[2†], Lixia Qiu[1], Jianbo Qing[2], Aizhong Li[3], Yan Zhao[2], Yafeng Li[2,3,4,5], Rongshan Li[2,3*] and Xiaoshuang Zhou[2*]

[1]School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China, [2]Department of Nephrology, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan, China, [3]Shanxi Provincial Key Laboratory of Kidney Disease, Taiyuan, China, [4]Core Laboratory, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan, China, [5]Academy of Microbial Ecology, Shanxi Medical University, Taiyuan, China

Introduction: Chronic kidney disease (CKD) is a progressive disease with high incidence but early imperceptible symptoms. Since China's rural areas are subject to inadequate medical check-ups and single disease screening programme, it could easily translate into end-stage renal failure. This study aimed to construct an early warning model for CKD tailored to impoverished areas by employing machine learning (ML) algorithms with easily accessible parameters from ten rural areas in Shanxi Province, thereby, promoting a forward shift of treatment time and improving patients' quality of life.

Methods: From April to November 2019, CKD opportunistic screening was carried out in 10 rural areas in Shanxi Province. First, general information, physical examination data, blood and urine specimens were collected from 13,550 subjects. Afterward, feature selection of explanatory variables was performed using LASSO regression, and target datasets were balanced using the SMOTE (synthetic minority over-sampling technique) algorithm, i.e., albuminuria-to-creatinine ratio (ACR) and α1-microglobulin-to-creatinine ratio (MCR). Next, Bagging, Random Forest (RF) and eXtreme Gradient Boosting (XGBoost) were employed for classification of ACR outcomes and MCR outcomes, respectively.

Results: 12,330 rural residents were included in this study, with 20 explanatory variables. The cases with increased ACR and increased MCR represented 1,587 (12.8%) and 1,456 (11.8%), respectively. After conducting LASSO, 14 and 15 explanatory variables remained in these two datasets, respectively. Bagging, RF, and XGBoost performed well in classification, with the AUC reaching 0.74, 0.87, 0.87, 0.89 for ACR outcomes and 0.75, 0.88, 0.89, 0.90 for MCR outcomes. The five variables contributing most to the classification of ACR

outcomes and MCR outcomes constituted SBP, TG, TC, and Hcy, DBP and age, TG, SBP, Hcy and FPG, respectively. Overall, the machine learning algorithms could emerge as a warning model for CKD.

Conclusion: ML algorithms in conjunction with rural accessible indexes boast good performance in classification, which allows for an early warning model for CKD. This model could help achieve large-scale population screening for CKD in poverty-stricken areas and should be promoted to improve the quality of life and reduce the mortality rate.

KEYWORDS

machine learning, chronic kidney disease, albuminuria-to-creatinine ratio, α1-microglobulin-to-creatinine ratio, auxiliary diagnosis, warning model

Introduction

Chronic kidney disease (CKD) is defined as renal structural or functional abnormalities for 3 months, with a prevalence of 13.4% worldwide (1). In recent years, CKD has gradually moved away from the perception of "disease of the elderly," with a growing trend toward age groups. Yet, imperceptible symptoms at the initial stages and lower public awareness are often responsible for the missing of golden treatment time (2). More seriously, it may develop into end-stage renal disease that requires renal replacement therapy, leading to a decrease in quality of life and an increase in mortality. What's worse, it's highly related to complications such as cardiovascular disease (3), becoming another "silent killer" after tumours and diabetes. As such, its early screening enables patients and their families to plan ahead, consult professional doctors for treatment, and discuss lifestyle modifications, which contribute to the alleviation of CKD.

Accurate diagnosis is closely related to the detection of CKD. Undoubtedly, albuminuria and α1-microglobulin serve as a good approach for early CKD screening in tertiary hospitals. Yet, when it comes to the rural areas in China, health care workers, critical care units, emergency facilities, health services and medical examinations are not necessarily guaranteed (4–6). In such a situation, it is not practical for primary health care institutions to make an early CKD screening using urine protein (7). Therefore, it is worth considering how to maximise the accessible medical parameters to construct a warning model for CKD in rural areas under the existing health care conditions.

Previous studies demonstrated that some risk factors, such as demographic, lifestyle, and blood biochemical parameters could be used to predict disease occurrence (8, 9). Also, it has been documented that the machine-learning approach allows for higher accuracy of disease risk prediction using routine clinical data, facilitating better decision support for clinicians (10). Accordingly, it is of great significance to combine machine learning algorithms with those easily accessible medical indexes to construct a warning model for CKD in poverty-stricken areas.

In this study, we aimed to employ machine learning algorithms for early screening of CKD and developed a warning model targeted at poverty-stricken areas using demographic, blood biochemical and physical examinations data from ten rural areas in Shanxi Province, intending to achieve a larger-scale CKD screening programme at a lower cost (reduced financial expenditure, reduced burden on medical staff) where possible, thus shifting forward its treatment window and improving quality of life.

Participants and methods

Study participants

From April 2019 to November 2019, Shanxi Provincial People's Hospital carried out opportunistic screening for chronic kidney disease for residents over 40 years old in Ningwu County, Lu County, Yangqu County, Linxian County, Shouyang County, Zezhou County, Huozhou City, Hejin City, Linyi County and Ruicheng County in Shanxi Province. Up to 13,550 residents participated in this screening and 12,285 eventually enrolled in the study, including 5,206 men and 7,079 women aged 41–91 years. All study subjects signed informed consent forms and it was approved by the Shanxi Provincial Hospital Ethics Committee (No. 2021213).

Inclusion criteria: (1) Residents aged 40 years and above; (2) Participants without communication barriers; (3) Participants understanding the study significance and willing to sign a written informed consent; (4) participants without cognitive impairment or mental illness. Exclusion criteria: (1) incomplete information recorded; (2) poor compliance; (3) pregnant women or those with a history of drug abuse.

Data collection

Data is collected through questionnaires, physical examinations, and laboratory analysis. The questionnaire included demographic information (including age, sex, annual income, educational levels), lifestyle (including smoking, alcohol consumption, diet and exercise). Questionnaires were conducted online and were completed by the subjects themselves or their family members. Physical examination comprised height, weight and systolic blood pressure (SBP), diastolic blood pressure (DBP), measured twice and averaged. All data were measured by medical professionals. Body mass index (BMI) was calculated by weight in kilograms divided by the square of height in meters.

Fasting venous blood was collected from subjects for fasting blood glucose (FPG), glycated haemoglobin (GHb), homocysteine (Hcy), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein (HDL-C). Urine specimens were collected from subjects. After centrifugation of 3,000 r/min for 10 min, the supernatant (low-speed centrifuge Anhui Zhongke Zhongke A SC3616) was extracted, and latex turbidity, sarcosine oxidase and immunoturbidimetry were employed for detection of α_1-microglobulin (α_1MG), urine creatinine (UCr) and microalbuminuria (MAU), respectively.

Variable assignments

Study participants' annual income, educational levels, health history, and lifestyle information was obtained from the questionnaire. Annual income was defined as <5K yuan, 5–10K yuan, 10–20K yuan, >20K yuan; educational attainment was defined as ≤ primary school, ≤ middle school, ≤ high school, ≥ college; smoking was classified as yes or no; alcohol consumption was classified as always (>100 g/time and 3 times/week), sometimes (<3 times/week or < 100 g/time) and rarely; exercise was classified as "none or a little" or "regular" (≥3 times/week, ≥30 min/time). BMI was defined as underweight (<18.5 kg/m^2), normal (18.5–24.0 kg/m^2), overweight (24.0–28.0 kg/m^2), obesity (≥28 kg/m^2). ACR was defined as MAU divided by UCr multiplied by 8.84; MCR was defined as α_1MG divided by UCr multiplied by 8.84.

Explanatory variables include demographic information (age, sex, educational levels, annual income, residence), lifestyle (smoking, alcohol, exercise, salt consumption, diet), blood biochemistry (HDL, LDL, TG, TC, Hcy, FPG, GHb), physical examination indexes (SBP, DBP, BMI), a total of 20 variables. The response variables were defined as ACR outcomes or MCR outcomes with two classes (increased ACR or normal ACR and increased MCR or normal MCR) ACR ≥ 30 mg/g was defined as increased ACR; MCR > 23 mg/g was defined as increased MCR. The increased ones were assigned 1, and the normal ones were assigned as 0.

Data preprocessing

Since missing data is not an issue of great severity in this study, we excluded those samples with missing values, without making a data imputation. Then, Absolute Shrinkage and Selection Operator (LASSO) was used to select features that are more relevant to the response variables, the increased ACR and the increased MCR. Afterward, Synthetic Minority Over-Sampling Technique (SMOTE) was utilized to balance the classes to enable the machine learning models to better learn the data features, thus making the best classification. The workflow was shown in Figure 1.

LASSO represents one of the commonly used feature selection methods. It is characterized by adding L1 regularization penalty term when fitting generalized linear regression, so that the sum of absolute values of regression coefficients is less than a certain value. Its purpose is to minimize the sum of squares of residuals, and force the regression coefficients of variables that contribute less to the model to compress to zero, enabling a feature sparsity process (11, 12). It could eliminate predictors with autocorrelation or redundancy, allowing for automated variable selection within

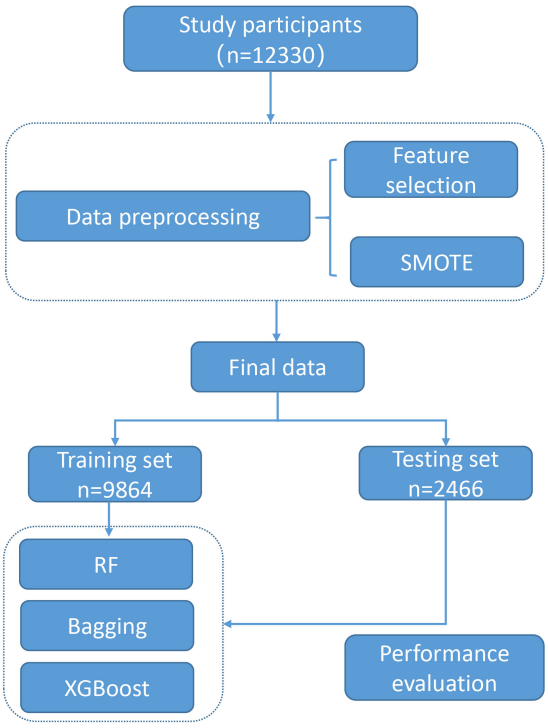


FIGURE 1
Workflow of the model construction.

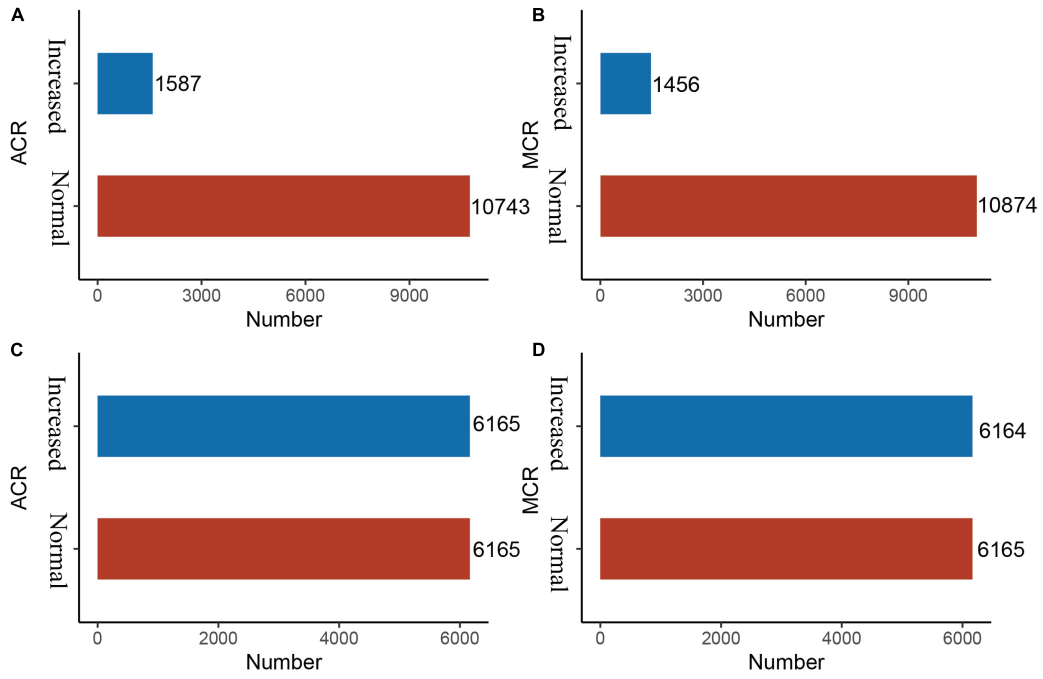


FIGURE 2
Before and after SMOTE of response variables for ACR and MCR outcomes. SMOTE, Synthetic Minority Over-Sampling Technique. It's a good and powerful way to handle imbalanced data, and it was conducted under the parameters of k = 5, C.perc = "balance", dist = "Overlap". **(A)** ACR outcomes (before SMOTE); **(B)** MCR outcomes (before SMOTE); **(C)** ACR outcomes (after SMOTE); and **(D)** MCR outcomes (after SMOTE).

the model, and significantly contributing to the performance of classification models (13, 14).

Imbalanced datasets are not unusual in medical research, because the number of non-patients is extremely larger than that of patients, which serves as an obstacle to predictive performance (15). The Synthetic Minority Over-Sampling Technique (SMOTE) is an oversampling technique that is an effective algorithm for handling imbalances between data classes (16). It uses k-neighbour synthesis to amplify minority classes to obtain a balanced data set (17) that exhibits good performance in areas such as network intrusion detection systems and disease detection. In this study, there is a serious imbalance in the response variables, ACR outcomes and MCR outcomes (**Figures 2A**, **B**).

Three classifiers and evaluation parameters

Three models, i.e., RF (18, 19), Bagging (20, 21) and XGBoost (22, 23) were employed to make a classification of ACR outcomes and MCR outcomes, respectively. More detailed information about the models could be obtained in the **Supplementary material**.

Evaluation parameters include Accuracy (1), Specificity (2), Sensitivity (3) and Area under the receiver operating curve (AUC). When patients with kidney disease are classified as patients, the prediction is defined as True Positive (TP), and when a healthy person is classified as healthy, the prediction is defined as True Negative (TN). In addition, if healthy subjects are considered patients, the prediction is False Positive (FP); Similarly, False Negative (FN) if patients are considered healthy subjects. Accuracy is to evaluate how accurate the machine learning algorithms are to detect what it is supposed to measure. Specificity is the ability to correctly exclude those without renal conditions and Sensitivity is to correctly identify those with renal conditions.

$$Accuracy = \frac{(TN+TP)}{(TP+TN+\mathrm{FP}+FN)} \times 100\% \quad (1)$$

$$Specificity = \frac{TN}{(TN+FP)} \times 100\% \quad (2)$$

$$Sensitivity = \frac{TP}{(TP+FN)} \times 100\% \quad (3)$$

Statistical methods

Qualitative data are described as percentages (%), and quantitative data are expressed as mean standard deviation (M $\pm$ SD) or median $\pm$ interquartile (P_{25}, P_{75}), as appropriate. Model construction: The datasets were divided into training

set (80%) and testing set (20%). The former is used for model training, i.e., e, RF, Bagging, and XGBoost, while the latter is used for model performance evaluation. The comparisons between training set and testing set were conducted using *t*-test or non-parameter test for quantitative variables, and Chi-square test for qualitative variables. All analyses were implemented in R software (version 4.0.3).

Results

Baseline characteristics

A total of 12,330 rural residents participated in this study, of whom 5,230 were men and 7,100 were women aged 40–91 years. There were 1,587 (12.8%) cases with increased ACR and 1,456

TABLE 1 Baseline characteristics of participants in this study.

Variables	Levels	Men (*N* = 5,230)	Women (*N* = 7,100)
Age	Median (IQR)	59.0 (52.0, 67.0)	57.0 (51.0, 65.0)
Education levels	≤Primary	1,394 (26.7%)	2,632 (37.1%)
	≤Junior	2,811 (53.7%)	3,470 (48.9%)
	≤Senior	761 (14.6%)	703 (9.9%)
	≥Bachelor	264 (5%)	295 (4.2%)
Annual income	<5K	1,563 (29.9%)	3,594 (50.6%)
	5–10K	1,396 (26.7%)	1,745 (24.6%)
	10–20K	641 (12.3%)	633 (8.9%)
	>20K	1,630 (31.2%)	1,128 (15.9%)
TG (mmol/L)	Median (IQR)	1.5 (1.1, 2.1)	1.6 (1.1, 2.2)
TC (mmol/L)	Median (IQR)	4.0 (3.5, 4.6)	4.6 (4.0, 5.2)
LDL (mmol/L)	Median (IQR)	2.1 (1.6, 2.6)	2.4 (1.9, 3.0)
HDL (mmol/L)	Median (IQR)	1.2 (1.0, 1.4)	1.3 (1.1, 1.5)
FPG (mmol/L)	Median (IQR)	4.6 (4.1, 5.1)	4.8 (4.4, 5.4)
GHB (mmol/L)	Median (IQR)	5.4 (5.0, 5.8)	5.4 (5.0, 5.8)
SBP (mmHg)	Median (IQR)	133.5 (123.0, 146.0)	134.0 (122.0, 148.5)
DBP (mmHg)	Median (IQR)	82.5 (77.5, 90.0)	81.0 (75.5, 88.5)
Hcy (mmol/L)	Median (IQR)	21.7 (15.5, 34.0)	16.6 (12.5, 23.7)
BMI	Underweight	80 (1.5%)	124 (1.7%)
	Normal	2,046 (39.1%)	2,828 (39.8%)
	Overweight	2,231 (42.7%)	3,017 (42.5%)
	Obesity	873 (16.7%)	1,131 (15.9%)
Smoking	No	2,415 (46.2%)	6,977 (98.3%)
	Yes	2,815 (53.8%)	123 (1.7%)
Alcohol consumption	Rarely	3,421 (65.4%)	7,024 (98.9%)
	Sometimes	1,549 (29.6%)	73 (1%)
	Always	260 (5%)	3 (0%)
Exercise	Regular	2,189 (41.9%)	2,952 (41.6%)
	None or a little	3,041 (58.1%)	4,148 (58.4%)
Salt consumption	Light	1,242 (23.7%)	2,002 (28.2%)
	Moderate	3,157 (60.4%)	4,308 (60.7%)
	Salty	831 (15.9%)	790 (11.1%)
Diet	Vegetable	1,541 (29.5%)	2,590 (36.5%)
	Balanced	3,322 (63.5%)	4,308 (60.7%)
	Meat	367 (7%)	202 (2.8%)
ACR	Normal	4,745 (90.7%)	5,998 (84.5%)
	Increased	485 (9.3%)	1,102 (15.5%)
MCR	Normal	4,473 (85.5%)	6,401 (90.2%)
	Increased	757 (14.5%)	699 (9.8%)

TABLE 2 Comparisons of quantitative clinical indexes between training set and testing set.

	ACR outcomes			MCR outcomes		
	Training (*N* = 9,864)	Testing (*N* = 2,466)	*P*	Training (*N* = 9,864)	Testing (*N* = 2,466)	*P*
Age (y)	59.00 (52.00,67.00)	59.00 (53.00, 67.00)	0.199	61.00 (54.00, 68.00)	60.00 (54.00, 68.00)	0.147
TG (mmol/L)	1.63 (1.17, 2.27)	1.69 (1.20, 2.34)	0.02	1.61 (1.15, 2.23)	1.66 (1.15, 2.34)	0.052
TC (mmol/L)	4.42 (3.78, 5.08)	4.39 (3.77, 5.04)	0.273			
FPG (mmol/L)	4.80 (4.30, 5.50)	4.80 (4.30, 5.60)	0.333	4.80 (4.30, 5.50)	4.80 (4.30, 5.50)	0.611
GHB (mmol/L)	5.40 (5.00, 5.90)	5.40 (5.00, 6.00)	0.17	5.40 (5.00, 6.00)	5.40 (5.10, 6.00)	0.749
SBP (mmHg)	137.50 (126.00, 152.00)	137.50 (125.00, 152.00)	0.948	137.00 (126.00, 151.00)	137.00 (125.00, 151.00)	0.813
DBP (mmHg)	83.00 (77.50, 91.00)	83.000 (77.50, 91.00)	0.529	82.00 (77.00, 90.00)	82.50 (77.50, 90.50)	0.113
Hcy (mmol/L)	18.60 (13.60,27.90)	18.55 (13.60, 28.13)	0.611	19.30 (14.00, 29.60)	19.10 (13.80, 29.05)	0.087

TABLE 3 Comparisons of qualitative clinical indexes between training set and testing set.

Variables	ACR outcomes			MCR outcomes		
	Training (*N* = 9,864)	Testing (*N* = 2,466)	*P*	Training (*N* = 9,864)	Testing (*N* = 2,466)	*P*
Education						
≤Primary	3,591 (36.4)	860 (34.9)	0.093	3,749 (38.0)	924 (37.5)	0.554
≤Junior	4,929 (50.0)	1,288 (52.2)		4,829 (49.0)	1,193 (48.4)	
≤Senior	969 (9.8)	243 (9.9)		974 (9.9)	262 (10.6)	
≥Bachelor	375 (3.8)	75 (3.0)		312 (3.2)	87 (3.5)	
Exercise						
Regular	4,215 (42.7)	1,026 (41.6)	0.312			
None or a little	5,649 (57.3)	1,440 (58.4)				
BMI						
Underweight	158 (1.6)	34 (1.4)	0.784	165 (1.7)	41 (1.7)	0.748
Normal	3,426 (34.7)	867 (35.2)		3,753 (38.0)	951 (38.6)	
Overweight	4,225 (42.8)	1,041 (42.2)		4,220 (42.8)	1,026 (41.6)	
Obesity	2,055 (20.8)	524 (21.2)		1,726 (17.5)	447 (18.1)	
Alcohol						
Rarely	8,677 (88.0)	2,129 (86.3)	0.05	8,329 (84.4)	2,095 (85.0)	0.09
Sometimes	1,009 (10.2)	294 (11.9)		1,327 (13.5)	304 (12.3)	
Always	178 (1.8)	43 (1.7)		208 (2.1)	66 (2.7)	
Smoking						
No	7,849 (79.6)	1,943 (78.8)	0.391	7,267 (73.7)	1,824 (74.0)	0.744
Yes	2,015 (20.4)	523 (21.2)		2,597 (26.3)	641 (26.0)	
Diet						
Vegetable	3,273 (33.2)	831 (33.7)	0.798	3,448 (35.0)	835 (33.9)	0.569
Balanced	6,188 (62.7)	1,530 (62.0)		6,024 (61.1)	1,534 (62.2)	
Meat	403 (4.1)	105 (4.3)		392 (4.0)	96 (3.9)	
Salt consumption						
Light	2,606 (26.4)	678 (27.5)	0.173			
Moderate	6,065 (61.5)	1,467 (59.5)				
Salty	1,193 (12.1)	321 (13.0)				
Sex						
Male	3,523 (35.7)	934 (37.9)	0.046	4,551 (46.1)	1,124 (45.6)	0.631
Female	6,341 (64.3)	1,532 (62.1)		5,313 (53.9)	1,341 (54.4)	

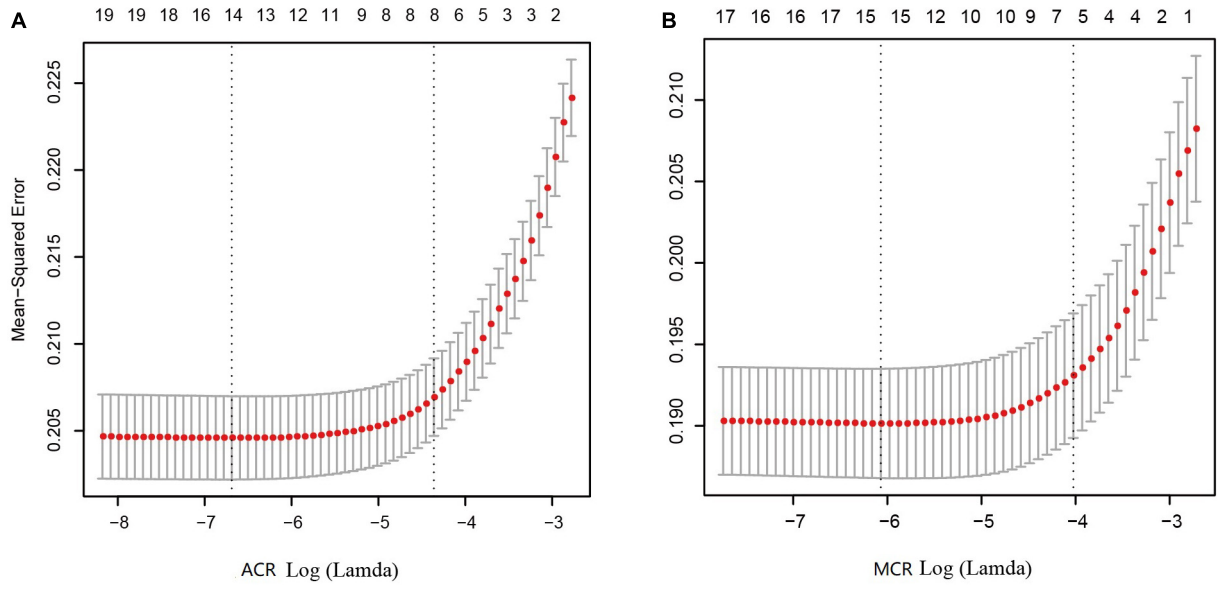


FIGURE 3
Results of feature selection using LASSO. When Lamda is minimum, corresponding features were taken into model construction, that is, 14 features for ACR outcomes **(A)** and 15 feature for MCR outcomes **(B)**.

(11.8%) cases with increased MCR, as shown in Table 1. Features in the training set and testing set are comparable for both ACR outcomes (except TG and sex) and MCR outcomes ($P < 0.05$), as shown in Tables 2, 3.

Feature selection and results of SMOTE

As shown in Figures 3A, B, after LASSO feature selection, 14 and 15 explanatory variables remained in the two datasets, respectively. Dataset with ACR outcomes as the response variable excluded six variables of annual income, residence, LDL, HDL, smoking, and exercise; while dataset with MCR outcomes as the response variable excluded five variables of TC, LDL, HDL, exercise, and salt consumption. As shown in Figures 2C, D, after SMOTE resampling, the number of patients and normal ones were 6,165, 6,165 for ACR outcomes, and 6,165, 6,164 for MCR outcomes, respectively.

Model performance

When constructing models for ACR outcomes, the number of increased ACR and normal ACR in the training set were both 4,932, and 1,233 in the testing set, respectively. When constructing model for MCR, the number of increased MCR and normal MCR in the training set were 4,973 and 4,891, respectively, and 1,273 and 1,192 in the testing set. The Accuracy, Sensitivity and Specificity of Bagging, RF and XGBoost reached over 99.00% in the training sets, and the AUC reached 0.99, as shown in Table 4. In the testing sets, XGBoost performed best, with Accuracy, Specificity and Sensitivity standing at 80.17, 77.05, and 83.29% for ACR outcomes and 82.27, 82.91, and 81.71% for MCR outcomes, and the AUC standing at 0.90. The performance of Bagging and RF is similar, as shown in Table 5.

Feature importances

Since XGBoost performs best in the classification, we indicated the contribution of the explanatory variables to the model by Gain, and the larger the Gain, the more important the variables were for the XGBoost model. The five variables that contributed most to the classification of ACR outcomes in the XGBoost model represented SBP, TG, TC, and Hcy, DBP. The five variables that were most important for the classification of MCR outcomes constituted age, TG, SBP, Hcy and FPG (Figure 4).

Discussion

To our knowledge, this study was the first one to employ machine learning algorithms in conjunction with existing indicators readily available in rural areas to construct an early warning model for CKD. In constructing the model, demographic information, physical examination and blood biochemical were taken as the explanatory variables; ACR (7) and MCR (24), early CKD screening parameters, collected and

TABLE 4 Performance evaluation of the three classifiers on the training set (ACR/MCR outcomes).

Model	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
Bagging	99.91/99.91	99.96/99.90	99.96/99.92	0.99/0.99
RF	99.90/99.89	99.96/99.90	99.84/99.88	0.99/0.99
XGBoost	99.89/99.59	99.96/99.82	99.82/99.38	0.99/0.99

TABLE 5 Performance evaluation of the three classifiers on the testing set (ACR/MCR outcomes).

Model	Accuracy (%)	Specificity (%)	Sensitivity (%)	AUC
Bagging	78.30/80.37	74.05/82.36	82.56/78.77	0.87/0.88
RF	78.14/80.20	74.29/81.88	82.00/78.84	0.87/0.89
XGBoost	80.17/82.27	77.05/82.91	83.29/81.71	0.89/0.90

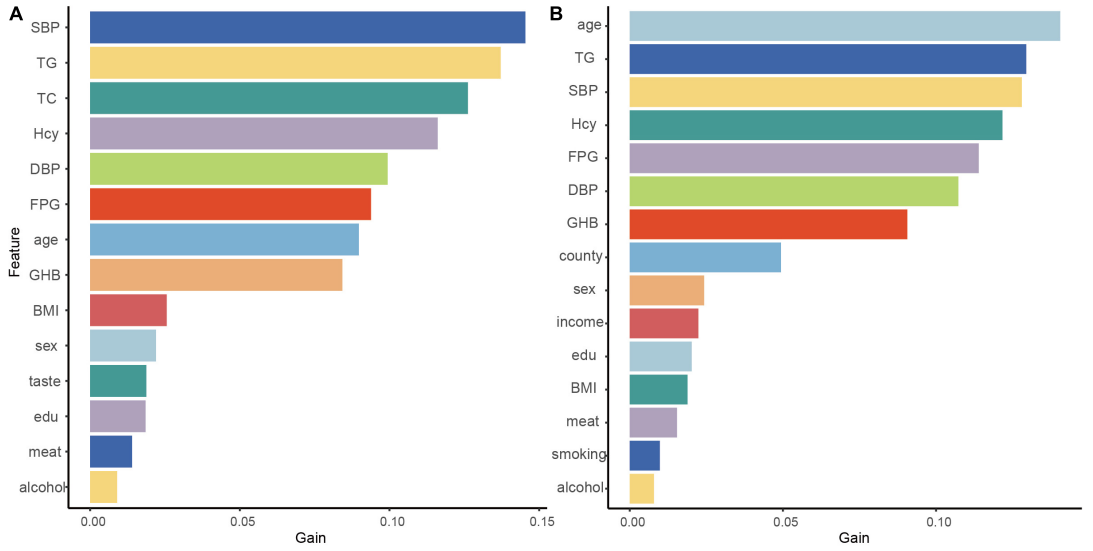


FIGURE 4
Contributions of explanatory variables to the XGBoost algorithm. The "Gain" means the relative contribution of the corresponding feature to the model calculated by taking the contribution of each feature to each tree in the model. The high value of this metric compared to other characteristics means that it is more important for generating predictions. Therefore, a larger value indicates that the variable is more important; ACR outcomes **(A)** and MCR outcomes **(B)**.

calculated from urine samples were taken as response variables. This study suggested that machine learning approaches show good performance in achieving early CKD-aided screening, as reflected by excellent Accuracy, Sensitivity, Specificity and AUC.

In 2012, Logistic Regression (LR) was employed for CKD prediction, but the model is accompanied by some defects (2), one of which concerns its sensitivity to multicollinearity. The second one relates to maximum likelihood estimation, unable to fit the true distribution of the data well. Recently, data-driven algorithms pick up pace, boasting great potential in cardiovascular diseases (25), tumors (26), immune diseases (27), and neurological diseases (28). Also, its application in renal diseases is increasing, ranging from acute kidney injury prediction (29) to kidney transplantation outcome prediction (30), interstitial fibrosis, and tubular atrophy detection (31).

In our previous work (32), we also employed LR, RF and Naive Bayes algorithms to make a classification of glomerular injury and tubular injury with the same population. The results suggested that RF performs best and could be employed as a novel auxiliary diagnostic approach for glomerular injury and tubular injury. To further compare the performance of other well-established algorithms, we, in this study, employed RF, Bagging and XGBoost to construct a warning model for CKD targeted at poverty-stricken areas with easily accessible parameters.

This study demonstrates that XGBoost represents the best classifier because the algorithm is a serial integrated learning algorithm based on Gradient Boosting Decision Tree that builds boosting trees in parallel by dependency generation (23). The objective function is improved by adding a regular term to

the original function, thus reducing overfitting and speeding up convergence (33). Its extraordinary classification power in other diseases has also been demonstrated (34, 35). Besides, the five explanatory variables with the greatest output weight of XGBoost classifier for ACR outcomes represented SBP, TG, TC, and Hcy, DBP; and the five explanatory variables for MCR outcomes constituted age, TG, SBP, Hcy and FPG.

In hypertension, the early glomerular filtration rate could remain normal, but when arterial pressure is constantly rising, exceeding the kidney's ability to self-regulate, it leads to glomerular hyperperfusion, which leads to damage to the visceral epithelial cells of the renal tubules, increasing the permeability of the glomerular basement membrane and thus causing proteinuria (36), while leading to glomerular duct wall hardening thickening, lumen stenosis, resulting in renal parenchymal ischemia, which eventually leads to glomerulosclerosis. Hyperhomocysteinemia is an important player involved in the progression of end-stage renal disease, acting directly on glomerular cells, inducing glomerular dysfunction and tubular fibrosis (37). Renal dyslipidemia is characterized by the accumulation of TG, which accelerates damage to the glomerular and tubular interstitials (38). Hyperglycemia levels not only enhance oxidative stress and hemodynamic factors such as activation of the renin-angiotensin-aldosterone system and impaired self-regulation due to systemic hypertension, but also increase the load of glucose delivered to the proximal tubules, triggering maladaptive hypertrophy (39) and hyperplasia of cortical tubules, and upregulation of glucose transport (40), and activation of globular feedback, leading to hyperfiltration of glomeruli and tubular fibrosis.

We think our early warning model is practical and down-to-earth in rural China. As the largest developing country confronted with underdeveloped healthcare systems and aging population (41), China is struggling to address the issue of health care coverage in rural areas. Despite the tremendous achievements in health care in rural China over the past 30 years, the problem of "difficult and expensive access to health care" still exists in the countryside. Promoting the establishment of a digital healthcare system in rural areas can greatly enhance the efficiency and accuracy of the public service system. Our study involves 12,330 participants from rural areas and how to better use such large-scale collected data to make a warning model for CKD is of great significance. Rather than considering these available indicators in isolation, holistically exploring their full potential, and seeking to explore a warning model for rural areas is a thing of great significance. Thus, early interventions tailored to CKD in improvised areas could be made in advance to lower its progression.

Some limitations also stand out in this manuscript. First, this study was based on a cross-sectional survey, and we did not conduct a follow-up for patients with proteinuria. Second, we constructed the models with data from Shanxi Province, and there is no other data for verification. Our next step is to collect more data from other regions to test the models' generalization. Additionally, cost-effectiveness was initially considered in this study, and other indicators reflecting CKD, such as blood creatinine, were not collected, which is our next focus. Besides, we did not collect a detailed history of smoking, alcohol consumption and dietary intake. More detailed information would allow for a more accurate and valid model.

In short, CKD has emerged as a global public health issue and its early diagnosis is of great importance. In rural China where primary health care service system and health education remain to be improved and strengthened, how to construct a warning model for CKD targeted at poverty-stricken areas is of great necessity and significance. In this study, we proposed a warning model using the available and accessible demographical, blood biochemical and lifestyle data in conjunction with machine learning approaches for rural areas. This model offers a leg-up for early CKD-assisted diagnosis in rural areas, which facilitates tailoring precise management and therapy for patients, thus, improving their quality of life and slowing the mortality rate.

Data availability statement

Data supporting the conclusions in this manuscript would be made available from the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Shanxi Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WS and YLu were responsible for the data analysis and the writing of the manuscript. LQ and JQ helped polish the manuscript. AL, YZ, and YLi gave precious advice on the statistical methods. RL and XZ were responsible for the conception and design of the research. All authors read and approved the final draft.

Funding

This work was supported by the Key Laboratory Project of Shanxi Province (201805D111020) and the Key Laboratory Construction Plan Project of Shanxi Provincial Health Commission (2020SYS01).

Acknowledgments

We appreciate all the authors and patients participating in this study. We are also indebted to my roommates, Fuliang Yi, Jiahui Ren, Jianguo Liang, and Zhenxing Zuo who encouraged me a lot during my writing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.930541/full#supplementary-material

References

1. Lv JC, Zhang LX. Prevalence and disease burden of chronic kidney disease. *Adv Exp Med Biol.* (2019) 1165:3–15. doi: 10.1007/978-981-13-8871-2_1

2. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet.* (2012) 379:815–22. doi: 10.1016/S0140-6736(12)60033-6

3. Wilson S, Mone P, Jankauskas SS, Gambardella J, Santulli G. Chronic kidney disease: definition, updated epidemiology, staging, and mechanisms of increased cardiovascular risk. *J Clin Hypertens.* (2021) 23:831–4. doi: 10.1111/jch.14186

4. Han J, Wu MC, Yang T. Challenge of China's rural health. *BMJ.* (2016) 353:i2003. doi: 10.1136/bmj.i2003

5. Song S, Yuan B, Zhang L, Cheng G, Zhu W, Hou Z, et al. Increased inequalities in health resource and access to health care in rural China. *Int J Environ Res Public Health.* (2018) 16:49. doi: 10.3390/ijerph16010049

6. Ma C, Song Z, Zong Q. Urban-rural inequality of opportunity in health care: evidence from China. *Int J Environ Res Public Health.* (2021) 18:7792. doi: 10.3390/ijerph18157792

7. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Internal Med.* (2013) 158:825–30. doi: 10.7326/0003-4819-158-11-201306040-00007

8. Wu Y, Fang Y. Stroke prediction with machine learning methods among older Chinese. *Int J Environ Res Public Health.* (2020) 17:1828. doi: 10.3390/ijerph17061828

9. Weng SF, Reps J, Kai J, Garibaldi JM, Qureshi N. Can machine-learning improve cardiovascular risk prediction using routine clinical data? *PLoS One.* (2017) 12:e0174944. doi: 10.1371/journal.pone.0174944

10. Kalafi EY, Nor NAM, Taib NA, Ganggayah MD, Town C, Dhillon SK. Machine learning and deep learning approaches in breast cancer survival prediction using clinical data. *Folia Biol.* (2019) 65:212–20.

11. Kang J, Choi YJ, Kim IK, Lee HS, Kim H, Baik SH, et al. LASSO-based machine learning algorithm for prediction of lymph node metastasis in T1 colorectal cancer. *Cancer Res Treatment.* (2021) 53:773–83. doi: 10.4143/crt.2020.974

12. Mullah MAS, Hanley JA, Benedetti A. LASSO type penalized spline regression for binary data. *BMC Med Res Methodol.* (2021) 21:83. doi: 10.1186/s12874-021-01234-9

13. Wang J, Zhang H, Wang J, Pu Y, Pal NR. Feature selection using a neural network with group lasso regularization and controlled redundancy. *IEEE Trans Neural Netw Learn Syst.* (2021) 32:1110–23. doi: 10.1109/TNNLS.2020.2980383

14. Jiang L, Greenwood CMT, Yao W, Li L. Bayesian hyper-LASSO classification for feature selection with application to endometrial cancer RNA-seq Data. *Sci Rep.* (2020) 10:9747. doi: 10.1038/s41598-020-66466-z

15. Geetha R, Sivasubramanian S, Kaliappan M, Vimal S, Annamalai S. Cervical cancer identification with synthetic minority oversampling technique and PCA analysis using random forest classifier. *J Med Syst.* (2019) 43:286. doi: 10.1007/s10916-019-1402-6

16. Chen PN, Lee CC, Liang CM, Pao SI, Huang KH, Lin KF. General deep learning model for detecting diabetic retinopathy. *BMC Bioinformatics.* (2021) 22(Suppl 5):84. doi: 10.1186/s12859-021-04005-x

17. Wang K, Tian J, Zheng C, Yang H, Ren J, Li C, et al. Improving risk identification of adverse outcomes in chronic heart failure using SMOTE+ENN and machine learning. *Risk Manage Healthcare Policy.* (2021) 14:2453–63. doi: 10.2147/RMHP.S310295

18. Sarica A, Cerasa A, Quattrone A. Random forest algorithm for the classification of neuroimaging data in Alzheimer's disease: a systematic review. *Front Aging Neurosci.* (2017) 9:329. doi: 10.3389/fnagi.2017.00329

19. Song M, Jung H, Lee S, Kim D, Ahn M. Diagnostic classification and biomarker identification of Alzheimer's disease with random forest algorithm. *Brain Sci.* (2021) 11:453. doi: 10.3390/brainsci11040453

20. Lin E, Lin CH, Lane HY. Applying a bagging ensemble machine learning approach to predict functional outcome of schizophrenia with clinical symptoms and cognitive functions. *Sci Rep.* (2021) 11:6922. doi: 10.1038/s41598-021-86382-0

21. Lin E, Lin CH, Lane HY. Prediction of functional outcomes of schizophrenia with genetic biomarkers using a bagging ensemble machine learning method with feature selection. *Sci Rep.* (2021) 11:10179. doi: 10.1038/s41598-021-89540-6

22. Davagdorj K, Pham VH, Theera-Umpon N, Ryu KH. XGBoost-based framework for smoking-induced noncommunicable disease prediction. *Int J Environ Res Public Health.* (2020) 17:6513. doi: 10.3390/ijerph17186513

23. Ogunleye A, Wang QG. XGBoost model for chronic kidney disease diagnosis. *IEEE/ACM Trans Comput Biol Bioinformat.* (2020) 17:2131–40. doi: 10.1109/TCBB.2019.2911071

24. Ishiwata S, Matsue Y, Nakamura Y, Dotare T, Sunayama T, Suda S, et al. Clinical and prognostic values of urinary alpha1-microglobulin as a tubular marker in acute heart failure. *Int J Cardiol.* (2021) 338:115–20. doi: 10.1016/j.ijcard.2021.06.041

25. Zheng X, Wang F, Zhang J, Cui X, Jiang F, Chen N, et al. Using machine learning to predict atrial fibrillation diagnosed after ischemic stroke. *Int J Cardiol.* (2021) 347:21–7. doi: 10.1016/j.ijcard.2021.11.005

26. Ruini C, Schlingmann S, Jonke Ž, Avci P, Padrón-Laso V, Neumeier F, et al. Machine learning based prediction of squamous cell carcinoma in Ex vivo confocal laser scanning microscopy. *Cancers.* (2021) 13:5522. doi: 10.3390/cancers13215522

27. Chen Y, Liao R, Yao Y, Wang Q, Fu L. Machine learning to identify immune-related biomarkers of rheumatoid arthritis based on WGCNA network. *Clin Rheumatol.* (2021) 41:1057–68. doi: 10.1007/s10067-021-05960-9

28. Yang S, Bornot JMS, Fernandez RB, Deravi F, Wong-Lin K, Prasad G. Integrated space-frequency-time domain feature extraction for MEG-based Alzheimer's disease classification. *Brain Informat.* (2021) 8:24. doi: 10.1186/s40708-021-00145-1

29. Le S, Allen A, Calvert J, Palevsky PM, Braden G, Patel S, et al. Convolutional neural network model for intensive care unit acute kidney injury prediction. *Kidney Int Rep.* (2021) 6:1289–98. doi: 10.1016/j.ekir.2021.02.031

30. Coorey CP, Sharma A, Muller S, Yang JYH. Prediction modeling-part 2: using machine learning strategies to improve transplantation outcomes. *Kidney Int.* (2021) 99:817–23. doi: 10.1016/j.kint.2020.08.026

31. Ginley B, Jen KY, Han SS, Rodrigues L, Jain S, Fogo AB, et al. Automated computational detection of interstitial fibrosis, tubular atrophy, and glomerulosclerosis. *J Am Soc Nephrol.* (2021) 32:837–50. doi: 10.1681/ASN.2020050652

32. Song W, Zhou X, Duan Q, Wang Q, Li Y, Li A, et al. Using random forest algorithm for glomerular and tubular injury diagnosis. *Front Med.* (2022) 9:911737. doi: 10.3389/fmed.2022.911737

33. Hong WS, Haimovich AD, Taylor RA. Predicting hospital admission at emergency department triage using machine learning. *PLoS One.* (2018) 13:e0201016. doi: 10.1371/journal.pone.0201016

34. Jiang YQ, Cao SE, Cao S, Chen JN, Wang GY, Shi WQ, et al. Preoperative identification of microvascular invasion in hepatocellular carcinoma by XGBoost and deep learning. *J Cancer Res Clin Oncol.* (2021) 147:821–33. doi: 10.1007/s00432-020-03366-9

35. Xu Y, Yang X, Huang H, Peng C, Ge Y, Wu H, et al. Extreme gradient boosting model has a better performance in predicting the risk of 90-day readmissions in patients with ischaemic stroke. *J Stroke Cerebrovasc Dis.* (2019) 28:104441. doi: 10.1016/j.jstrokecerebrovasdis.2019.104441

36. Zenker M, Machuca E, Antignac C. Genetics of nephrotic syndrome: new insights into molecules acting at the glomerular filtration barrier. *J Mol Med.* (2009) 87:849–57. doi: 10.1007/s00109-009-0505-9

37. Small DM, Bennett NC, Roy S, Gabrielli BG, Johnson DW, Gobe GC. Oxidative stress and cell senescence combine to cause maximal renal tubular epithelial cell dysfunction and loss in an in vitro model of kidney disease. *Nephron Exp Nephrol.* (2012) 122:123–30. doi: 10.1159/000350726

38. Attman PO, Samuelsson O, Alaupovic P. Progression of renal failure: role of apolipoprotein B-containing lipoproteins. *Kidney Int Suppl.* (1997) 63:S98–101.

39. Osterby R, Gundersen HJ. Glomerular size and structure in diabetes mellitus. I. Early abnormalities. *Diabetologia.* (1975) 11:225–9. doi: 10.1007/BF00422326

40. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes.* (2005) 54:3427–34. doi: 10.2337/diabetes.54.12.3427

41. Hu H, Jian W, Fu H, Zhang H, Pan J, Yip W. Health service underutilization and its associated factors for chronic diseases patients in poverty-stricken areas in China: a multilevel analysis. *BMC Health Services Res.* (2021) 21:707. doi: 10.1186/s12913-021-06725-5

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact