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Endocrine abnormalities and renal complications

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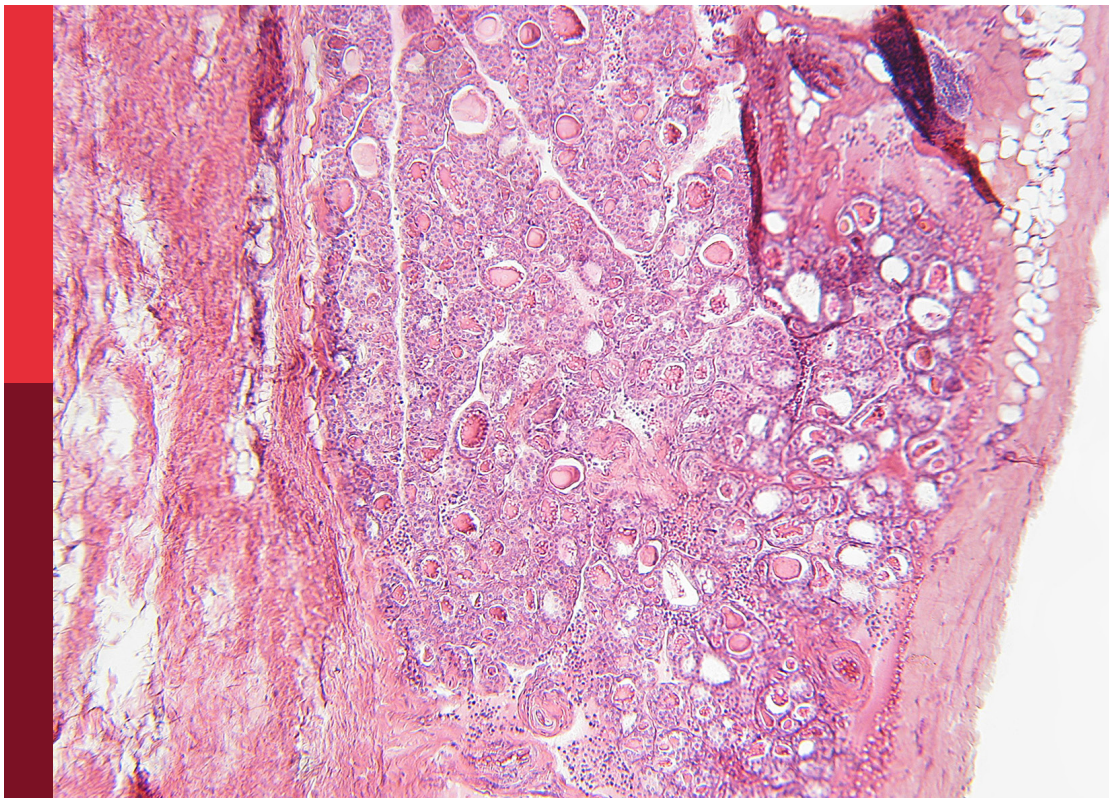
Published in
Frontiers in Endocrinology
Frontiers in Medicine



ISSN 1664-8714
ISBN 978-2-8325-3416-8
DOI 10.3389/978-2-8325-3416-8

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Endocrine abnormalities and renal complications

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Citation

Pasupulati, A. K., Kilari, S., Sahay, M., eds. (2023). *Endocrine abnormalities and renal complications*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3416-8

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frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 21 August 2023
DOI 10.3389/fendo.2023.1274669

OPEN ACCESS

EDITED AND REVIEWED BY
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Heidelberg University, Germany

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RECEIVED 08 August 2023
ACCEPTED 10 August 2023
PUBLISHED 21 August 2023

CITATION
Pasupulati AK, Kilari S and Sahay M (2023) Editorial: Endocrine abnormalities and renal complications.
Front. Endocrinol. 14:1274669.
doi: 10.3389/fendo.2023.1274669

Editorial: Endocrine abnormalities and renal complications

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KEYWORDS

kidney, hormones, chronic kidney disease, acute kidney injury, biomarkers, end-stage kidney disease

Editorial on the Research Topic
Endocrine abnormalities and renal complications

The principal function of the kidneys is to keep the blood clean and chemically balanced by filtering out waste products of metabolism and maintaining water, electrolyte, and acid-base balance. Kidneys help retain glucose, amino acids, vitamins, hormones, albumin, antibodies and other vital components in the blood while eliminating urea and creatinine. The kidneys' ability to elicit selective filtration and tight regulation of the composition of urine is accomplished by the glomerulus and tubular region of the nephron, the functional unit of the kidney. The severity of impairment of renal function is characterized by decreased glomerular filtration rate (GFR) and albuminuria, which are markers for kidney excretory function and glomerular barrier dysfunction, respectively. Further, impairment of renal function is characterized by elevated serum creatinine (SCr), cystatin C or blood urea nitrogen (BUN). Several endocrine factors secreted by kidneys regulate various physiological functions. Kidneys synthesize renin to maintain arterial blood pressure, whereas excess renin secreted by the injured kidney contributes to hypertension. Erythropoietin secreted by the kidney stimulates the production of RBC by the bone marrow, and in conditions of reduced kidney function, insufficient erythropoietin results in anemia. Notably, decreased kidney function is often presented with anemia and is associated with a reduced quality of life and increased morbidity (1). 25-hydroxyvitaminD(3)-1alpha-hydroxylase (*CYP27B1*) produced by proximal tubular cells of the kidney catalyzes the synthesis of calcitriol, the most active form of vitamin D (1,25-dihydroxyvitamin D), which plays an integral role in calcium homeostasis. Because of their multiple roles, kidneys are considered life-sustaining organs. The articles on this topic provide a comprehensive understanding of kidney function in various endocrine abnormalities, discuss the intricate role of intrarenal and systemic endocrine factors on kidney function, and investigate strategies for early and specific diagnosis of renal complications.

Kidney diseases are a modern-day epidemic, a direct cause of morbidity and mortality and a significant risk factor for cardiovascular disease (CVD) (2). Acute kidney injury (AKI) and chronic kidney disease (CKD) are two kinds of kidney complications. AKI is

presented with an abrupt decline in GFR (hrs to days) and is diagnosed with any one of the following: an increase in SCr by 0.3 mg/dl within 48hrs or an increase in SCr to 1.5 times baseline within the prior seven days, or urine volume less than 0.5 ml/kg/hr over 6 hrs (3). Some common causes of AKI include exposure to nephrotoxins, ischemia, pre-renal diseases such as hypotension, hypovolemic states, urinary tract obstruction, and viral infection such as COVID-19. AKI is frequent among hospitalized patients in the intensive care unit setting. Though AKI is asymptomatic in many patients, it is associated with thirst, dehydration, low or no urine volume, hematuria, edema, and shortness of breath. On the contrary, CKD is a long-term condition with a gradual loss of kidney function, as evidenced by a five-stage (1-5) progressive decline in GFR (4). As per Kidney Disease Improving Global Outcomes (KDIGO) guidelines, an individual is considered to have CKD if abnormalities of kidney structure or function persist for more than three months (5). End-stage kidney disease (ESKD) is the worst CKD stage and a significant public health concern. CKD is irreversible and eventually requires permanent dialysis or kidney transplant. Mortality increases with decreasing GFR and increasing albuminuria. CKD is associated with symptoms such as anemia, hypertension, edema, lethargy, persistent headaches, lower back pain, and growth delay in children.

Several factors can contribute to the pathogenesis of CKD. Hypertension, diabetic kidney disease (DKD), obesity, and aging are the most common causes, whereas factors such as HIV, exposure to toxins, and heavy metals contribute to the burden of CKD. In some areas of developing countries where CKD is endemic, precise causal factors remain to be established. These scenarios contribute to different factors that manifest in long-term glomerular and tubular damage and ultimately manifest in ESKD. In diabetes, elevated pituitary growth hormone (GH) circulatory levels are implicated in impaired renal function. Receptors for GH and its mediator IGF-1 are abundantly expressed in glomerular and tubular cells. GH can act directly on the kidneys or via circulating or paracrine-synthesized IGF-1. The GH/IGF-1 system regulates glomerular hemodynamics, tubular sodium and water, phosphate, calcium handling, and renal gluconeogenesis. The GH/IGF-1 system governs klotho synthesis, a coreceptor of the phosphaturic hormone fibroblast growth factor 23 (FGF-23) in the renal tubule. Although recombinant GH is widely used in treating short stature in children, including those with CKD, studies from experimental animals and acromegalic patients demonstrate that GH excess can have harmful effects on the kidney, including glomerular hyperfiltration, renal hypertrophy, and glomerulosclerosis (6). In addition, elevated GH in patients with poorly controlled type 1 diabetes mellitus was thought to induce podocyte injury and contribute to diabetic nephropathy. The direct action of GH mediates these adverse effects predominantly in the glomerulus, and by contrast, IGF-1 excess results in tubular hypertrophy only. The pleiotropic effects of GH on podocytes include expression of pro-sclerotic TGF-β, pro-inflammatory TNF-α, activation of epithelial-mesenchymal activation, and cell death by mitotic catastrophe (7–10).

Although the thyroid hormones (TH) contribute to the development of the kidney, both hypo- and hyperthyroidisms affect GFR, renal blood flow, tubular function, and electrolyte balance. Hypothyroidism is associated with a reversible increase in serum creatinine, reduced GFR and renal plasma flow. If hypothyroidism can be corrected with levothyroxine therapy, some individuals' blood creatinine levels can return to normal (Zhang et al.). In comparison, hyperthyroidism is associated with increased GFR and renal plasma flow. In patients with CKD, low T3 levels are independent predictors for all-cause mortality in euthyroid patients with ESKD. TH-TH receptor (TH-TR) axis alterations are critically involved in the pathogenesis of DKD. Despite low T3 levels, patients of DKD are presented with reexpression of fetal isoform TRα1 in podocytes and are concomitant with maladaptive cell-cycle induction/arrest (11). Interestingly, T3 treatment reduced TRα1 expression and mitigated podocytes' maladaptive response (11).

Abnormalities in the synthesis of estrogen and progesterone are common in women with CKD (12). Abnormal menstrual cycles with amenorrhea, anovulation, and early menopause are often observed in women with CKD. Diagnosis and management of menopausal symptoms and postmenopausal osteoporosis in CKD remain challenging. Testosterone deficiency and testicular dysfunction are frequent among men with CKD. Testosterone levels decline as CKD progresses with further reductions in GFR. Combined evaluation of the GFR and circulating testosterone improves mortality risk. Both men and women with CKD also suffer from decreased fertility. The relationship between sex hormones and kidney stone formation is a topic of debate because urolithiasis was observed more in men compared with women. It was largely believed that testosterone is the main reason for urolithiasis that observed predominantly among men. Huang et al. reported that serum testosterone levels were inversely associated with the prevalence of kidney stones in men over 40. The association of endogenous sex hormones and sex hormone-binding globulin (SHBG) with CKD was investigated by Lau et al. Among men, no associations were observed between androgens, eGFR, and CKD. In women, a higher T/DHT (Testosterone/dihydrotestosterone) ratio was associated with higher CKD prevalence and that higher circulating levels of free DHT were associated with a lower incidence of CKD.

The complex interplay among parathyroid hormone (PTH), calcitriol, and FGF-23 help regulate the normal serum calcium (Ca) and phosphorous (P) levels. Kidneys play an instrumental role in maintaining serum Ca and P levels by regulating these three hormones. CKD-related mineral bone disorder (MBD) represents a complex disease with elevated PTH and FGF-23, reduced levels of calcitriol and klotho (13). This adaptive endocrine response maintains serum levels of Ca and P in the normal range until the advanced stages of CKD, where hypocalcemia, hyperphosphatemia, renal osteodystrophy, and vascular calcification are evident. Lee et al. demonstrated deficiency of serum 25-hydroxy vitamin D levels was significantly associated with only severe CKD stage (4&5) among the Korean cohort. Vitamin D receptor agonists, nutritional vitamin D, and calcimimetic agents to reduce parathyroid hormone are prescribed to treat CKD-MBD and to influence the survival rate in patients with ESKD. Notably, elevated serum PTH levels were significantly associated with an increased

risk of peritonitis in the Chinese cohort undergoing continuous ambulatory peritoneal dialysis (Zhao et al.). Further, Kee et al. evaluated the influence of residual kidney function in patients undergoing prevalent hemodialysis on the detrimental effect of serum FGF-23 levels in CVD development.

Dyslipidemia and abnormal lipid metabolism contribute to kidney cell injury, increasing the risk of CKD in obese individuals (14). Several obesity indices are available, including body mass index, waist circumference, and visceral adiposity index (VAI). Lin et al. report that higher CVAI is associated with an increased risk of renal damage (as assessed in terms of eGFR and proteinuria) in patients with hypertension and abnormal glucose metabolism. Furthermore, CVAI strongly predicts renal damage incidence compared with the above mentioned indices. Therefore, a simple assessment of visceral adiposity by calculating CVAI may be helpful for the early identification of high-risk individuals and for adopting strict BP and glucose management, thereby reducing the risk of renal damage. Serum apolipoprotein B (ApoB) levels had the strongest correlation with CKD among all lipid variables, and accumulation of ApoB levels might precede the occurrence of CKD (Xu et al.).

Adiponectin (A) and leptin (L) are two hormonally active molecules secreted by adipose tissue and are critical mediators of cardiometabolic risk in obesity (15). In healthy individuals, adiponectin elicits anti-inflammatory effects and is cardioprotective, whereas leptin is associated with obesity-related cardiovascular complications and pro-inflammatory activity. Nevertheless, paradoxically, in CKD patients, A, L, and the ratio of L/A are increased, independently of traditional CKD risk factors. In the settings of CKD, elevated adiponectin levels are associated with decreased bone mineral density, anemia, and hypertrophy of the left ventricle. At the same time, elevated leptin levels are associated with endothelial dysfunction and aortic stiffness; adiponectin and leptin contribute to a higher risk of CVD in CKD. Graňák et al. revealed A/L(< 0.5) as a predictor of acute rejection in the early post-transplant period after kidney transplantation.

Considering the magnitude of the global burden of kidney disease and the seriousness of morbidity and mortality of ESKD, we solicit biomarkers that help early diagnose the individuals at high risk of renal complications with endocrine abnormalities, in addition to intervention strategies. Cao et al. performed two independent cross-sectional studies to explore whether plasma levels of urea cycle-associated amino acids with risk of DKD. According to this study, plasma citrulline levels were significantly associated with the risk of DKD in type II diabetes in the Chinese population. The protein concentration of urinary extracellular vesicles (UEV) in diabetic individuals is higher than in healthy controls before and after adjusting the urinary creatinine (UCr) (Gu et al.). The ratio of uEV-to-UCr may better indicate the progression of diabetic renal complications over the urine protein–Cr ratio or albumin-Cr ratio. Since albuminuria is associated with high glycemic variability in type II diabetic patients, avoiding fluctuations of blood glucose levels using flash monitoring methods could help better manage renal health among the diabetic population in the Indian cohort (Nathiya et al.).

Author contributions

AP: Writing – original draft, Writing – review & editing. SK: Writing – review & editing. MS: Writing – review & editing.

Funding

AP is supported by the Indian Council of Medical Research and the University of Hyderabad-Institute of Eminence Scheme.

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 authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Portolés J, Martín L, Broseta JJ, Cases A. Anemia in chronic kidney disease: from pathophysiology and current treatments, to future agents. *Front Med (Lausanne)* (2021) 8:642296. doi: 10.3389/fmed.2021.642296

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(10225):709–33. doi: 10.1016/S0140-6736(20)30045-3

3. KDIGO clinical practice guideline for acute kidney injury. Section 2: AKI Definition. *Kidney Int Suppl* (2012) 2:19–36. doi: 10.1038/kisup.2011.32

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(6):344–58. doi: 10.1038/nrneph.2017.52

5. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Chapter 1: Definition and classification of CKD. *Kidney Int Suppl* (2013) 3:19–62. doi: 10.1038/kisup.2012.64

6. Haffner D, Grund A, Leifheit-Nestler M. Renal effects of growth hormone in health and in kidney disease. *Pediatr Nephrol* (2021) 36(8):2511–30. doi: 10.1007/s00467-021-05097-6

7. Kumar PA, Kotlyarevska K, Dejkhmaron P, Reddy GR, Lu C, Bhojani MS, et al. Growth hormone (GH)-dependent expression of a natural antisense transcript induces zinc finger E-box-binding homeobox 2 (ZEB2) in the glomerular podocyte: a novel action of gh with implications for the pathogenesis of diabetic nephropathy. *J Biol Chem* (2010) 285(41):31148–56. doi: 10.1074/jbc.M110.132332

8. Nishad R, Mukhi D, Singh AS, Motrapu M, Chintala K, Tammineni P, et al. Growth hormone induces mitotic catastrophe of glomerular podocytes and contributes to proteinuria. *Cell Death Dis* (2021) 12(4):342. doi: 10.1038/s41419-021-03643-6

9. Mukhi D, Kolligundla LP, Maruvada S, Nishad R, Pasupulati AK. Growth hormone induces transforming growth factor-β1 in podocytes: Implications in podocytopathy and proteinuria. *Biochim Biophys Acta Mol Cell Res* (2023) 1870 (2):119391. doi: 10.1016/j.bbamcr.2022.119391

10. Nishad R, Mukhi D, Kethavath S, Raviraj S, Paturi ASV, Motrapu M, et al. Podocyte derived TNF-alpha mediates monocyte differentiation and contributes to glomerular injury. *FASEB J* (2022) 36(12):e22622. doi: 10.1096/fj.202200923R

11. Benedetti V, Lavecchia AM, Locatelli M, Brizi V, Corna D, Todeschini M, et al. Alteration of thyroid hormone signaling triggers the diabetes-induced pathological growth, remodeling, and dedifferentiation of podocytes. *JCI Insight* (2019) 4(18): e130249. doi: 10.1172/jci.insight.130249

12. Ma HY, Chen S, Du Y. Estrogen and estrogen receptors in kidney diseases. *Ren Fail* (2021) 43(1):619–42. doi: 10.1080/0886022X.2021.1901739

13. Waziri B, Duarte R, Naicker S. Chronic kidney disease-mineral and bone disorder (CKD-MBD): current perspectives. *Int J Nephrol Renovasc Dis* (2019) 12:263–76. doi: 10.2147/IJNRD.S191156

14. Kochan Z, Szupryczynska N, Malgorzewicz S, Karbowska J. Dietary lipids and dyslipidemia in chronic kidney disease. *Nutrients* (2021) 13(9):3138. doi: 10.3390/nu13093138

15. Coimbra S, Rocha S, Valente MJ, Catarino C, Bronze-da-Rocha E, Belo L, et al. New insights into adiponectin and leptin roles in chronic kidney disease. *Biomedicines* (2022) 10(10):2642. doi: 10.3390/biomedicines10102642

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ORIGINAL RESEARCH
published: 02 May 2022
doi: 10.3389/fendo.2022.863675

Relationship Between Serum Testosterone Levels and Kidney Stones Prevalence in Men

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Edited by:
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Specialty section:
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 January 2022*
Accepted: *21 March 2022*
Published: *02 May 2022*

Citation:
Huang F, Li Y, Cui Y, Zhu Z, Chen J, Zeng F, Li Y, Chen Z and Chen H (2022) Relationship Between Serum Testosterone Levels and Kidney Stones Prevalence in Men. Front. Endocrinol. 13:863675. doi: 10.3389/fendo.2022.863675

Background: The role of serum testosterone levels in male renal stone formation remains controversial. This study aimed to evaluate the relationship between serum testosterone levels and kidney stone prevalence in males.

Methods: We conducted a cross-sectional study based on the data from the National Health and Nutrition Examination Survey 2011–2016, which included 6,633 male participants, to investigate the association between testosterone levels and the prevalence of kidney stones.

Results: In this study, using the highest quartile of serum testosterone as a reference, a logistic regression model adjusted for confounders in all participants showed that the first quartile (OR: 1.375, p = 0.016), the second quartile (OR: 1.348, p = 0.021), and the third quartile (OR: 1.472, p = 0.003) of testosterone significantly increased kidney stone risks. In the 41–60 age group, the ORs of kidney stone risk in the first, second, and third of serum testosterone were 1.904 (P = 0.005), 1.599 (P = 0.040), and 1.734 (P = 0.015), respectively. This trend can also be found in the 61–80-year group, except in the first quartile of serum testosterone (OR: 1.169, P = 0.436). Adjusted smoothed curves suggest a non-linear relationship between the 8 quantiles of serum testosterone and the risk of kidney stones in all participants and the 61–80 age group and a significant negative relationship in the 41–60 age group (OR: 0.921, P = 0.0193). But no correlation was seen in the 20–40 group.

Conclusions: Serum testosterone levels were significantly inversely associated with the prevalence of kidney stones in men over 40 years of age, but no correlation was seen in the 20–40 group. The role of testosterone in stone formation should be redefined, and its effect should be further verified.

Keywords: testosterone, kidney stones, prevalence, association, NHANES

INTRODUCTION

There exists a persistent male predominance among urinary stone formers, with a two to three times higher prevalence compared with females (1, 2). Based on the gender differences observed in the ecological data, it makes sense to think that testosterone is the main cause. Several small human studies also reported higher levels of testosterone in men who formed stones than in a control group (3–6). Meanwhile, it has been reported that castration can significantly reduce the rate of stone formation in stone-induced ethylene glycol-fed rat models (7). Despite all that, the relationship between serum testosterone and urolithiasis in men remains controversial. First, it is more difficult to relate these findings to clinically significant associations between testosterone and urolithiasis because the associations were primarily established in small populations and those studies did not strictly control for confounding factors associated with testosterone and urolithiasis, such as obesity, hypertension, cardiovascular disease, diabetes, etc. Second, several studies have also suggested the opposite view, reporting an association between low serum testosterone levels and a higher incidence of urolithiasis (8, 9) or no relationship (10). By defining testosterone levels as low (≤300 ng/dl), normal (>300 ng/dl and <1,000 ng/dl), and high groups (≥1,000 ng/dl) based on a diagnosis of hypogonadism in men (11), Sirpi Nackeeran et al. analyzed the relationship between testosterone and the incidence of kidney stones in men using the data in the National Health and Nutrition Examination Survey (NHANES) database from 2013 to 2016, which showed no relationship between testosterone and kidney stones. But using the broad range of 300–1,000 ng/dl as the normal testosterone population to analyze the relationship with kidney stone incidence may obscure the underlying relationship (12). Third, metabolic syndrome and osteoporosis are known to be associated with a high incidence of stone formation (13–15) and low levels of testosterone (16, 17), which contradicts the prevailing view that high levels of testosterone can significantly promote stone formation. Fourth, as lifestyle-associated risk factors change, the susceptibility of men to urinary stones seems to be gradually decreasing compared with women. For example, a study reported a significant change in the male:female ratio of nephrolithiasis prevalence from 1.7:1 in 1997 to 1.3:1 in 2002 (18). Therefore, a large, representative cross-sectional study is needed to fully assess the relationship between serum testosterone and kidney stone prevalence in men. Thus, this study aimed to investigate an association between serum testosterone and kidney stone prevalence using representative data from the NHANES 2011–2016.

METHODS

Study Design and Study Population

NHANES is a cross-sectional, nationally representative survey to collect health examination data using a stratified, multistage probability design to select a representative sample of the noninstitutionalized population of the United States. The data included health interviews, examination components, and laboratory tests administered by highly trained medical personnel. In this study, we included male participants who participated in the 2011–2012, 2013–2014, and 2015–2016 NHANES study cycles. The exclusion criteria are as follows (1): No data on self-reported kidney stones and no serum testosterone measured (2); Participants missing covariates, namely, age, race, education, BMI, hypertension, diabetes, asthma, gout, coronary heart, disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid (see **Figure 1** for detailed information of inclusion/exclusion criteria). Eventually, 6633 participants were enrolled in the study.

Variable Definitions and Data Collection

The dependent variable was kidney stones, which was defined by self-response to a question of a standardized questionnaire, "Have you ever had a kidney stone?" The serum total testosterone was measured using isotope dilution liquid chromatography-tandem mass spectrometry in the 2011–2012, 2013–2014, and 2015–2016 NHANES study cycles.

According to previous studies on factors affecting serum testosterone and the incidence of kidney stones, the following variables were included as covariates: age, race, education, body mass index (BMI), hypertension, diabetes, asthma, gout, coronary heart disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid. The race was categorized as Mexican-American, non-Hispanic whites, non-Hispanic blacks, and Other/multicultural. According to completed years of schooling, education was categorized as ≤8 years, 9–12 years, and ≥12 years. BMI was calculated as the weight in kilograms divided by the height in meters squared, and obesity was defined as a BMI of 30 or higher. A history of gout and hypertension was obtained from the self-reported responses to questions asking whether a doctor had ever informed the respondent that they had the listed conditions. Participants were considered to have a history of smoking if they answered "Yes" to the question "Have you smoked at least 100 cigarettes in your entire life?" A history of diabetes was defined by self-response to the question, "Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?" Serum total cholesterol, triglycerides, calcium, and uric acid were obtained from the Standard Biochemistry Profile.

Statistical Analysis

Initially, in the stone former and non-stone former groups, the chi-square test was used to analyze the proportion of categorical variables, and the Student's t-test was used to analyze numerical variables. Previously reported factors affecting kidney stone prevalence, which included age, race, BMI, hypertension, diabetes, gout, coronary heart disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid, were considered potential confounders and were incorporated into the stepwise forward multivariate logistic regression models to adjust the evaluation of the relationship between the quartile of serum testosterone and the

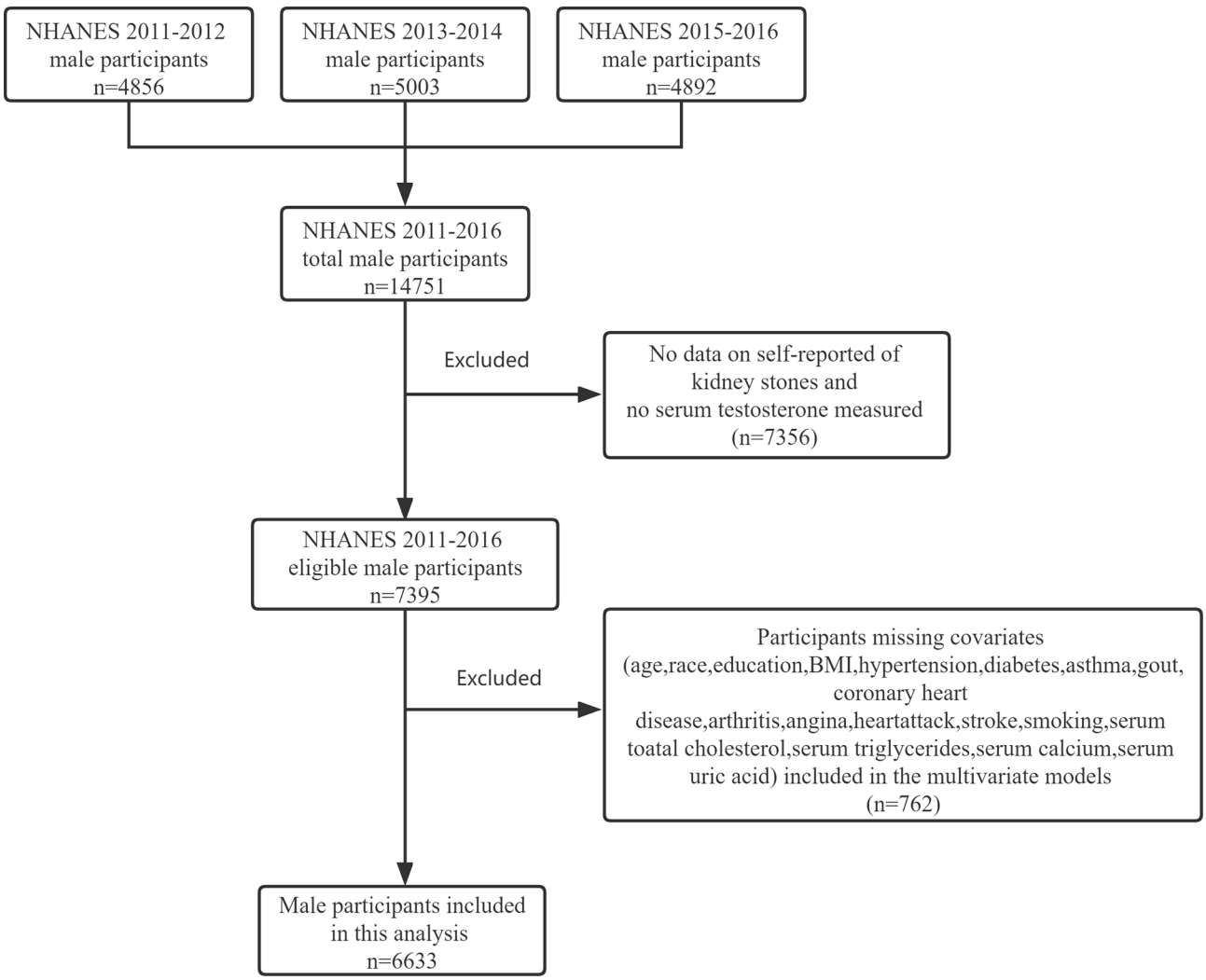

FIGURE 1 | Flowchart identifying process of the NHANES 2011–2016 male participants inclusion and exclusion.

prevalence of kidney stones.If serum testosterone was not eventually included in the stepwise forward multivariate logistic regression model, the enter logistic multivariate regression model (a model in which all the included independent variables are forced into the logistic regression equation in SPSS 22 software) was used. A test for linear trend was conducted with the use of quartiles of the total serum testosterone as a continuous variable.

We further applied a two-piecewise linear regression model to examine the threshold effect of the eight quantiles of serum testosterone on the prevalence of kidney stones using a smoothing function. The threshold level was determined using trial and error, including a selection of turning points along with a pre-defined interval and then choosing the turning point that gave the maximum model likelihood. A log-likelihood ratio test comparing the one-line linear regression model with a two-piecewise linear model was also conducted (19). This study was performed according to the guidelines of the NHANES and accounted for the complex survey design. All statistical analyses were performed with the statistical software EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) and SPSS 22 for Windows (Chicago, IL, USA), taking NHANES sampling weights into account. A p-value of ≤0.05 (two-sided) was considered statistically significant.

RESULTS

Data were available on kidney stones and serum testosterone levels in 6,633 male adult participants. **Table 1** shows the characteristics of the study participants according to kidney stones. The prevalence of kidney stones was 10.4% in the population and participants were more likely to be obese, smoker, diabetes, hypertension, gout, arthritis, coronary

TABLE 1 | Characteristics of the study population, according to stone formers (n = 6,633).

	Non-stone formers(n = 5,945)	Stone formers(n = 688)	P-value[a]
Number of participants (%)	89.6	10.4	–
Age (years), mean ± SD	47.9 ± 17.5	57.6 ± 15.7	<0.001
Race/ethnicity (%)			<0.001
Mexican-American	13.7	13.2	
Non-Hispanic white	37.2	51.2	
Non-Hispanic black	22.3	12.4	
Other/multicultural	26.9	23.3	
Education (%)			0.667
≤8 years	9.7	10.8	
9–12 years	36.6	36.3	
>12 years	53.7	52.9	
BMI, mean ± SD (kg/m^2)	28.4 ± 6.09	29.7 ± 6.05	<0.001
Hypertension (%)	33.6	50.0	<0.001
History of diabetes (%)	14.8	29.7	<0.001
History of asthma (%)	12.7	12.9	0.860
History of gout (%)	5.3	10.2	<0.001
History of arthritis (%)	18.5	33.7	<0.001
History of coronary heart disease (%)	4.1	11.5	<0.001
History of angina (%)	2.0	6.4	<0.001
History of heart attack (%)	4.2	10.2	<0.001
History of stroke (%)	3.1	4.9	0.017
History of high cholesterol level (%)	34.0	51.3	<0.001
Smoking>100	51.8	59.2	<0.001
Serum calcium, mean ± SD (mmol/l)	2.36 ± 0.09	2.34 ± 0.09	<0.001
Serum total cholesterol, mean ± SD (mg/dl)	4.87 ± 1.08	4.75 ± 1.11	0.005
Serum triglycerides, mean ± SD (mmol/l)	1.88 ± 1.54	2.05 ± 1.87	0.006
Serum uric acid, mean ± SD (mg/dl)	357.7 ± 77.2	357.7 ± 86.4	0.984
Serum total testosterone, mean ± SD (ng/dl)	419.2 ± 187.1	377.7 ± 175.7	<0.001
Testosterone quartile, % (range, ng/dl)			
Q1 (≤287.40)	24.3	31.5	<0.001
Q2 (287.41–386.00)	24.8	26.7	
Q3 (386.10–510.47)	25.0	25.1	
Q4 (>510.48)	26.0	16.6	

BMI, body mass index.

[a]*Student's t-test was used to compare the differences of continuous variables, and Chi-square test was performed to compare the differences of categorical variables.*

disease, angina, heart attack, and stroke. Participants with kidney stones had significantly lower serum testosterone levels than those without stones (377.7 ± 175.7 vs 419.2 ± 187.1 ng/dl, p <0.001, **Table 1**), and the incidence of kidney stones decreased with the increase of testosterone quartile (p <0.001, **Table 1**).

A strong negative correlation existed between serum testosterone quartiles and kidney stone history, with higher ORs of individuals reporting a stone history with lower testosterone quartiles in an unadjusted logistic regression model (**Table 2**). This trend is particularly pronounced in the 41–60 age group, and the OR value of the lowest serum testosterone quartile reporting a history of kidney stones was 2.391 times that of the highest serum testosterone quartile (P <0.001, **Table 2**). But it was statistically insignificant in the 20–40 age group (P = 0.178). The multivariate logistic regression analysis, controlling for factors known to impact kidney stones and serum testosterone, showed that the first quartile (OR 1.375, p = 0.016), the second quartile (OR 1.348, p = 0.021), and the third quartile (OR 1.472, p = 0.003) significantly increased the prevalence of kidney stones compared with the fourth quartile in all participants, but with P for trend equals 0.117 (**Table 2**). In subgroup analysis, using the highest quartile of serum testosterone as a reference in the 41–60 age group, the ORs of kidney stone risk in the first, second, and third of serum testosterone were 1.904 (95% CI: 1.212–2.991, P = 0.005), 1.599 (95% CI: 1.021–2.503, P = 0.040), and 1.734 (95% CI: 1.113–2.700, P = 0.015) and the trend test was statistically significant (P for trend = 0.001, **Table 2**). Although this trend was insignificant in the 61–80 group (P for trend = 0.966), the ORs for kidney stone risk were still higher in the second (OR: 1.471, 95% CI: 1.016–2.129, P = 0.041) and third (OR: 1.466, 95% CI: 1.020–2.106, P = 0.039) quartile of serum testosterone. However, the ORs of kidney stone risk in the first quartile of serum testosterone showed no significant difference compared to the highest quartile of serum testosterone in the 61–80 group (OR: 1.169, 95% CI: 0.789, 1.731, P = 0.436) (**Table 2**).

Adjusted smoothed curves suggest a non-linear relationship between the eight quantiles of serum testosterone and the risk of kidney stones (**Figures 2A, D**; **Table 3**) in all participants and the 61–80 age group, with a significant negative relationship in the 41–60 age group (**Figure 2C**, OR: 0.921, 95% CI: 0.860–0.987, P = 0.0193, **Table 3**). But no correlation was seen in the 20–40 age group (**Figure 2B**; **Table 3**). Using a two-piecewise regression adjusted model to evaluate the relationship between the 8 quantiles of serum testosterone and the risk of kidney stones, we found that the inflection point was Q5 (360–422 ng/dl) in all participants. When the serum testosterone ≥Q5, there was a

TABLE 2 | The association of the prevalence of kidney stones and testosterone evaluated by logistic regression analysis in subgroups stratified by age.

	Unadjusted, OR (95% CI)	P-value	Adjusted [b], OR (95% CI)	P-value
All, Testosterone[a]				
Q1 (≤287.40)	2.04 (1.607–2.585)	<0.001	1.375 (1.061–1.781)	0.016
Q2 (287.41–386.00)	1.691 (1.324–2.158)	<0.001	1.348 (1.041–1.739)	0.021
Q3 (386.10–510.47)	1.578 (1.233–2.020)	<0.001	1.472 (1.144–1.895)	0.003
Q4 (>510.48)	reference		reference	
P for trend		<0.001	0.117	
Age (20–40), Testosterone[a]				
Q1 (≤313.69)	1.531 (0.920–2.549)	0.101	–	–
Q2 (313.7–418.0)	1.038 (0.599–1.801)	0.893	–	–
Q3 (418.1–542.0)	1.282 (0.757–2.171)	0.355	–	–
Q4 (>542.01)	reference		–	–
P for trend		0.178		
Age (41–60), Testosterone[a]				
Q1 (≤280.31)	2.391 (1.565–3.654)	<0.001	1.904 (1.212–2.991)	0.005
Q2 (280.31–370.00)	1.785 (1.149–2.775)	0.010	1.599 (1.021–2.503)	0.040
Q3 (370.10–492.22)	1.827 (1.177–2.836)	0.007	1.734 (1.113–2.700)	0.015
Q4 (>492.23)	reference		reference	
P for trend		<0.001		0.001
Age (61–80), Testosterone[a]				
Q1 (≤268.0)	1.613 (1.134–2.296)	0.008	1.169 (0.789–1.731)	0.436
Q2 (268.1–360.9)	1.776 (1.252–2.520)	0.001	1.471 (1.016–2.129)	0.041
Q3 (361.0–492.0)	1.582 (1.110–2.254)	0.011	1.466 (1.020–2.106)	0.039
Q4 (>492.1)	reference		reference	
P for trend		0.008		0.966

CI, confidence interval; HR, hazard ratio.

[a]*Presented in quartiles (ng/dl).*

[b]*Adjusted for age, race, BMI, hypertension, diabetes, gout, coronary heart, disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid.*

significant negative correlation between serum testosterone and kidney stone risks (OR = 0.875, 95% CI: 0.731 to 0.981, p = 0.01). By contrast, there was no correlation when the serum testosterone<Q5 (**Table 3**). This trend was also found in the 61–80 age group. Serum testosterone exhibited a negative correlation with kidney stone risk when ≥Q5 (360–422 ng/dl) (OR = 0.831, 95% CI: 0.717–0.963, P = 0.014). When serum testosterone is less than the inflection point, there is no significant relationship (**Table 3**). In the 41–60 age group of the two-piecewise regression adjusted model, when testosterone ≥Q6 (427–492 ng/dl) (OR = 0.657, 95% CI: 0.487–0.886, P = 0.006), the negative correlation between serum testosterone and kidney stone risk was more significant than in Model I one-line (**Table 3**).

DISCUSSION

The relationship between sex hormones and urolithiasis has been widely discussed by urologists because of the gender difference in the incidence of urolithiasis (2). Most scholars believe that testosterone is the main reason for this observational data, but they seem to ignore the role of estrogen in the regulation of urolithiasis in women (1, 7, 20). Furthermore, whether androgens promote stone formation is still a controversial topic and lacks a large, representative cohort study (9).

Using a large cross-sectional study, we examined the association between serum testosterone and kidney stones in men. For all participants, the mean serum testosterone of patients with stones was significantly lower than that of normal participants, and in an unadjusted logistic regression model, the prevalence of kidney stones was inversely associated with the quartile of serum testosterone. Furthermore, in multivariate logistic regression, we adjusted for confounders, and the OR values of the first three quartiles involved in kidney stone risk were still 1.3–1.4 times that of the highest quartile, which was statistically significant. Notably, there is a nonlinear relationship between the eight quantiles of serum testosterone and the risk of kidney stones after fully adjusting for confounders. When the serum testosterone ≥Q5 (360–422 ng/dl), there was a significant negative correlation between serum testosterone and kidney stone risks. By contrast, there was no correlation when the serum testosterone <Q5.

In the introduction, we mentioned the NHANES (2013–2016) study by Sirpi Nackeeran et al. which showed that testosterone was not related to the incidence of kidney stones in men (12). Their results differ from ours because they grouped testosterone levels differently from our study. They classified testosterone into low (≤300 ng/dl), normal (>300 ng/dl and <1,000 ng/dl), and high groups high (≥1,000 ng/dl) based on diagnosis of hypogonadism in men (11). Their normal testosterone group almost covered the range of testosterone levels in the Q2–Q4 groups (**Tables 1**, **2**) in our study. Therefore, their findings may reflect only the relationship between male hypogonadism and kidney stone incidence, ignoring the potential association between different testosterone levels and kidney stone incidence. In our study, we added the data from the NHANES from 2011 to 2012 and adopted a more scientific quartile grouping method for testosterone to analyze the relationship between different testosterone levels and

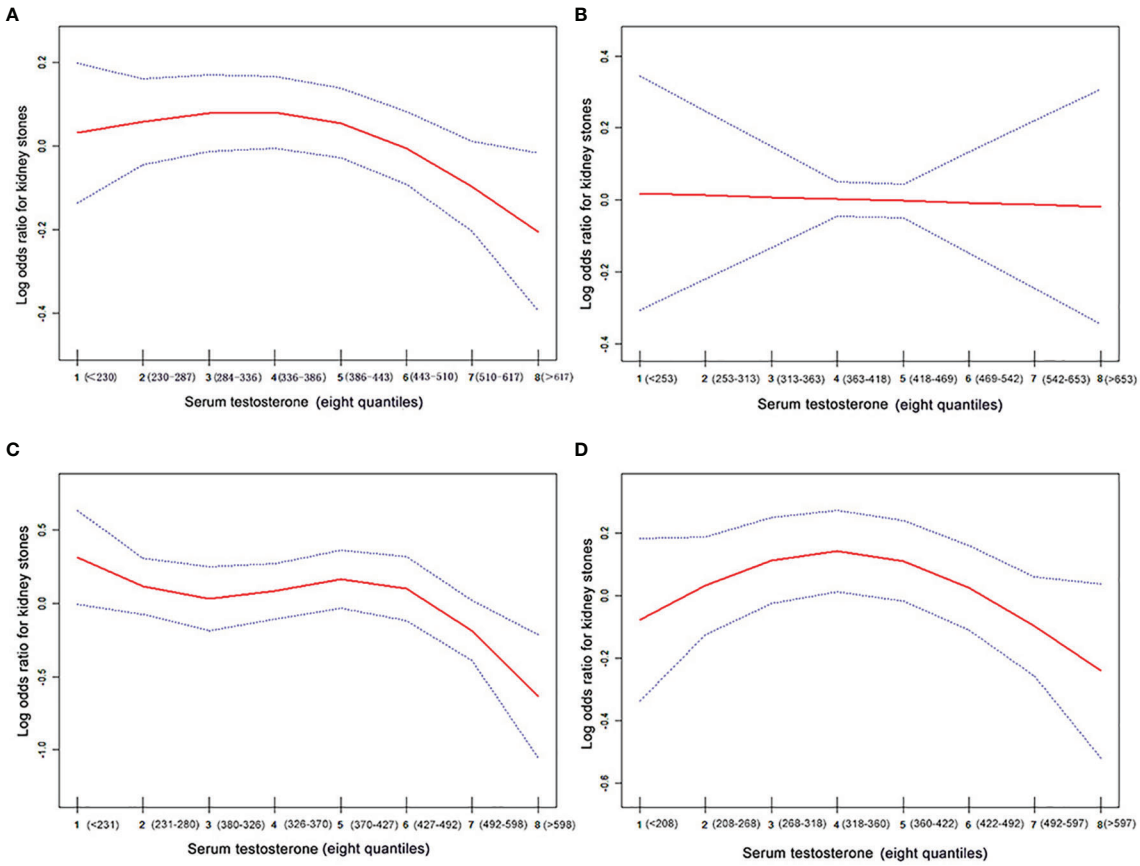

FIGURE 2 | The association curve between serum testosterone levels and log odds for kidney stones. The solid red line represents the smooth curve fit between variables. The blue line represents the 95% of confidence interval from the fit. The values have been adjusted for age, race, BMI, hypertension, diabetes, gout, coronary heart, disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid. **(A)** All participants; **(B)** the 20–40 age group; **(C)** the 41–60 age group, and **(D)** the 61–80 age group.

the incidence of male kidney stones. Therefore, our results may better reflect the potential relationship between testosterone levels and the incidence of kidney stones in men.

The results of our study seem to be inconsistent with the prevailing view, testosterone promotes stone formation, which is mainly based on small sample size studies (3–5) and some observations that men have a higher risk of developing kidney stones than women (2). As we mentioned before, explaining this sex difference in kidney stone incidence with androgens seems to ignore the protective effect of estrogen on stone formation in women. Furthermore, some studies have found that the incidence of kidney stones is lower in women than in men before age 50, but the gender difference in incidence narrows in people aged >50 years, suggesting that estrogen may be the main cause of gender differences in kidney stones (1, 21). In the rat ethylene glycol model of urolithiasis studies also have shown that testosterone can promote hyperoxaluria caused by increasing liver glycolate oxidase (GAO) levels, which results in calcium oxalate crystal deposition in the renal interstitium (22, 23). As far as we know, the rat ethylene glycol model of urolithiasis relies on artificial mechanisms that are not comparable with the pathophysiology of naturally occurring stone disease in humans (24). *Via* ingestion of ethylene glycol, it causes intratubular calcium oxalate (CaOx) crystallization and, in the setting of renal injury, can lead to discrete crystal formation in the renal interstitium (24, 25). However, in natural human stone formation, no evidence exists to show that stones form secondary to oxalate-induced renal injury and calcium oxalate crystals are deposited in the renal interstitium (24, 26, 27). Several studies have also reported a higher incidence of urolithiasis in men with low serum testosterone levels (8, 9). Although the results of our large, representative cross-sectional study are consistent with these smaller studies and are consistent with observations of high stone incidence and low testosterone levels in diseases closely associated with stones, such as metabolic syndrome, we cannot draw arbitrary conclusions at this time.

Interestingly, in subgroup analysis, after adjusting for confounders, we found no association between serum testosterone levels and stone incidence in the 20–40 age group, but a significant negative association in the 41–60 age group. Meanwhile, in the 61–80 group, serum testosterone exhibited a negative correlation with kidney stone risk when ≥Q5 (360–422

TABLE 3 | Threshold effect analysis of testosterone on the prevalence of kidney stones using piece-wise linear regression.

	Crude OR[b] (95% CI)	p-value		Adjusted OR[c] (95% CI)	p-value
All, Testosterone[a]					
Model I one-line	0.904 (0.873, 0.937)	<0.001		0.972 (0.935, 1.011)	0.159
Model II turning point: Q5 (386–443)			Model II turning point: Q5 (386–443)		
<Q5	0.942 (0.884, 1.002)	0.059	<Q5	1.033 (0.966, 1.105)	0.336
≥Q5	0.843 (0.764, 0.930)	<0.001	≥Q5	0.875 (0.731, 0.981)	0.010
Age (20–40) Testosterone[a]					
Model I one-line	0.953 (0.880, 1.031)	0.2271		0.995 (0.906, 1.092)	0.9118
Model II turning point: Q3 (313–363)			Model II turning point: Q3 (313–363)		
<Q3	0.807 (0.603, 1.080)	0.1499	<Q3	0.809 (0.598, 1.096)	0.1721
≥Q3	1.006 (0.891, 1.136)	0.9257	≥Q3	1.063 (0.932, 1.213)	0.3645
Age 41–60 Testosterone[a]					
Model I one-line	0.892 (0.839, 0.948)	<0.001		0.921 (0.860, 0.987)	0.0193
Model II turning point: Q5 (370–427)			Model II turning point: Q6 (427–492)		
<Q5	0.941 (0.844, 1.049)	0.2736	<Q6	0.995 (0.906, 1.092)	0.9084
≥Q5	0.810 (0.680, 0.964)	0.0179	≥Q6	0.657 (0.487, 0.886)	0.006
Age 61–80 Testosterone[a]					
Model I one-line	0.930 (0.883, 0.979)	0.005		0.979 (0.924, 1.038)	0.483
Model II turning point: Q3 (268–318)			Model II turning point: Q5 (360–422)		
<Q3	1.141 (0.938, 1.389)	0.187	<Q5	1.084 (0.979, 1.200)	0.120
≥Q3	0.872 (0.805, 0.943)	<0.001	≥Q5	0.831 (0.717, 0.963)	0.014

CI, confidence interval; HR, hazard ratio.

[a]*Presented in eight quantile (ng/dl) and was conducted the with eight quantiles of the total serum testosterone as a continuous variable.*

[b]*Adjusted for: none.*

[c]*Adjusted for: age, race, BMI, hypertension, diabetes, gout, coronary heart, disease, arthritis, angina, heart attack, stroke, smoking, serum total cholesterol, triglycerides, calcium, and uric acid.*

ng/dl). This result is consistent with the physiological cycle of male testosterone levels, which decline with age, gradually decreasing with each decade after the age of 40 years (28). There are a large number of comorbidities in men associated with low serum testosterone, namely, heart failure, vascular disease, osteoporosis, dyslipidemia, type 2 diabetes, metabolic syndrome, and obesity (28, 29), which are significantly associated with a higher incidence of kidney stones (13, 14, 30). Therefore, we have reason to believe that there is a direct or indirect relationship between testosterone and the occurrence of urinary stones in men after 40 years of age. In men aged 20–40 years, most individuals have normal gonadal function, so testosterone is in a stable balance, and the regulation of physiological metabolism is also in a stable state. Therefore, testosterone does not appear to be associated with kidney stone development in this age group. When men are over 40 years old, some gonadal dysfunctions cause a significant decrease in testosterone release and physiological metabolism disorders, resulting in metabolic diseases and kidney stones. Thus, testosterone in men over 40 years old shows the same protective effect on the occurrence of urinary stones as estrogen in postmenopausal women (1, 31). Testosterone levels were inversely associated with kidney stone incidence in men over 40 years of age, which may be associated with testosterone-related metabolic diseases such as hypertension, diabetes, and metabolic syndrome. But because the role of testosterone in human metabolism is too complex to be understood in a cross-sectional national design, further research is needed in the future.

The current large sample cross-sectional national study provided evidence that serum testosterone levels are associated with kidney stone risk and that testosterone may have a protective effect on kidney stones in men older than 40. According to rigorous sampling design, high-quality research measurements, and detailed quality control procedures, the NHANES selected representative populations from the national population of the United States for the study, which made our research conclusions more representative. At the same time, we adjusted for confounders that might be responsible, and fully demonstrated an independent correlation between testosterone levels and the prevalence of kidney stones. There are a lot of limitations in our study. Most importantly, this is a cross-sectional observational study, and the fundamental flaw is that it can only assess correlation, not causation; Secondly, as self-reported data based on questionnaires are used in this study, the results are easily misclassified; Third, although we strictly adjusted multiple covariates, our findings might have been affected by residual confounding factors. Fourth, as mentioned before, some studies suggest that estrogen may play a role in the development of kidney stones in women. But in the incidence of kidney stones in men, one study suggested that estrogen may not play a role in stone formation (12). Since estrogen was not included in the NHANES Database from 2011 to 2012, this study did not include estrogen for analysis.

CONCLUSION

Serum testosterone levels were significantly inversely associated with the prevalence of kidney stones in men over 40 years of age, but no correlation was seen in the 20–40 age group. The role of testosterone in stone formation should be redefined and its effect should be further verified.

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 below: https://www.cdc.gov/nchs/nhanes/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Wake Forest School of Medicine Institutional Review Board deemed this study of publically available, de-identified data exempt from human subjects research. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FH: Conceptualization, Visualization, Methodology, Writing original draft. YoL: Investigation, Resources. YC: Validation, Data curation, Investigation. ZZ: Investigation, Resources. JC: Writing review & editing. FZ: Investigation, Resources. YaL: Methodology, Software, Formal analysis. ZC: Methodology, Software, Formal analysis. HC: Conceptualization, Project administration, Supervision. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study was supported by the National Natural Science Foundation of China (82170781).

REFERENCES

1. Heller HJ, Sakhaee K, Moe OW, Pak CY. Etiological Role of Estrogen Status in Renal Stone Formation. *J Urol* (2002) 168(5):1923–7. doi: 10.1097/01.ju.0000033907.21910.be
2. Scales CD Jr., Smith AC, Hanley JM, Saigal CS. Prevalence of Kidney Stones in the United States. *Eur Urol* (2012) 62(1):160–5. doi: 10.1016/j.eururo.2012.03.052
3. Kuczera M, Kiersztejn M, Kokot F, Klin M. Behavior of Sex Hormone and Gonadotropin Secretion in Men With Active Nephrolithiasis. *Endokrynologia Polska* (1993) 44(4):539–47.
4. Li JY, Zhou T, Gao X, Xu C, Sun Y, Peng Y, et al. Testosterone and Androgen Receptor in Human Nephrolithiasis. *J Urol* (2010) 184(6):2360–3. doi: 10.1016/j.juro.2010.08.009
5. Naghii MR, Babaei M, Hedayati M. Androgens Involvement in the Pathogenesis of Renal Stones Formation. *PloS One* (2014) 9(4):e93790. doi: 10.1371/journal.pone.0093790
6. Gupta K, Gill GS, Mahajan R. Possible Role of Elevated Serum Testosterone in Pathogenesis of Renal Stone Formation. *Int J Appl Basic Med Res* (2016) 6(4):241–4. doi: 10.4103/2229-516x.192593
7. Lee YH, Huang WC, Huang JK, Chang LS. Testosterone Enhances Whereas Estrogen Inhibits Calcium Oxalate Stone Formation in Ethylene Glycol Treated Rats. *J Urol* (1996) 156(2 Pt 1):502–5. doi: 10.1097/00005392-199608000-00071
8. Polat EC, Ozcan L, Otunctemur A, Ozbek E. Relation of Urinary Stone Disease With Androgenetic Alopecia and Serum Testosterone Levels. *Urolithiasis* (2016) 44(5):409–13. doi: 10.1007/s00240-016-0888-3
9. Otunctemur A, Ozbek E, Cakir SS, Dursun M, Polat EC, Ozcan L, et al. Urolithiasis Is Associated With Low Serum Testosterone Levels in Men. *Arch Ital Urol Androl organo ufficiale [di] Societa italiana di ecografia urol e nefrologica* (2015) 87(1):83–6. doi: 10.4081/aiua.2015.1.83
10. Knoedler JJ, Krambeck AE, Astorne W, Bergstralh E, Lieske J. Sex Steroid Hormone Levels May Not Explain Gender Differences in Development of Nephrolithiasis. *J Endourol* (2015) 29(12):1341–5. doi: 10.1089/end.2015.0255
11. Bhasin S, Basaria S. Diagnosis and Treatment of Hypogonadism in Men. *Best Pract Res Clin Endocrinol Metab* (2011) 25(2):251–70. doi: 10.1016/j.beem.2010.12.002
12. Nackeeran S, Katz J, Ramasamy R, Marcovich R. Association Between Sex Hormones and Kidney Stones: Analysis of the National Health and Nutrition Examination Survey. *World J Urol* (2021) 39(4):1269–75. doi: 10.1007/s00345-020-03286-w
13. Taylor EN, Stampfer MJ, Curhan GC. Obesity, Weight Gain, and the Risk of Kidney Stones. *Jama* (2005) 293(4):455–62. doi: 10.1001/jama.293.4.455
14. Taylor EN, Stampfer MJ, Curhan GC. Diabetes Mellitus and the Risk of Nephrolithiasis. *Kidney Int* (2005) 68(3):1230–5. doi: 10.1111/j.1523-1755.2005.00516.x
15. Lee YC, Huang SP, Juan YS, Huang TY, Liu CC. Impact of Metabolic Syndrome and its Components on Kidney Stone in Aging Taiwanese Males. *Aging Male Off J Int Soc Study Aging Male* (2016) 19(3):197–201. doi: 10.1080/13685538.2016.1174987
16. Kupelian V, Hayes FJ, Link CL, Rosen R, McKinlay JB. Inverse Association of Testosterone and the Metabolic Syndrome in Men Is Consistent Across Race and Ethnic Groups. *J Clin Endocrinol Metab* (2008) 93(9):3403–10. doi: 10.1210/jc.2008-0054
17. Zitzmann M. Testosterone Deficiency, Insulin Resistance and the Metabolic Syndrome. *Nat Rev Endocrinol* (2009) 5(12):673–81. doi: 10.1038/nrendo.2009.212
18. Scales CDJr., Curtis LH, Norris RD, Springhart WP, Sur RL, Schulman KA, et al. Changing Gender Prevalence of Stone Disease. *J Urol* (2007) 177(3):979–82. doi: 10.1016/j.juro.2006.10.069
19. Yu X, Cao L, Yu X. Elevated Cord Serum Manganese Level Is Associated With a Neonatal High Ponderal Index. *Environ Res* (2013) 121:79–83. doi: 10.1016/j.envres.2012.11.002
20. Peerapen P, Thongboonkerd V. Protective Cellular Mechanism of Estrogen Against Kidney Stone Formation: A Proteomics Approach and Functional Validation. *Proteomics* (2019) 19(19):e1900095. doi: 10.1002/pmic.201900095
21. Wilson C. Risk Factors: Estrogen and Kidney Stones. *Nat Rev Endocrinol* (2011) 7(1):3. doi: 10.1038/nrendo.2010.206
22. Yagisawa T, Ito F, Osaka Y, Amano H, Kobayashi C, Toma H. The Influence of Sex Hormones on Renal Osteopontin Expression and Urinary Constituents in Experimental Urolithiasis. *J Urol* (2001) 166(3):1078–82.
23. Fan J, Chandhoke PS, Grampsas SA. Role of Sex Hormones in Experimental Calcium Oxalate Nephrolithiasis. *J Am Soc Nephrol JASN* (1999) 10(Suppl 14):S376–80.
24. Alford A, Furrow E, Borofsky M, Lulich J. Animal Models of Naturally Occurring Stone Disease. *Nat Rev Urol* (2020) 17(12):691–705. doi: 10.1038/s41585-020-00387-4
25. Khan SR. Animal Models of Kidney Stone Formation: An Analysis. *World J Urol* (1997) 15(4):236–43. doi: 10.1007/bf01367661
26. Evan AP, Coe FL, Rittling SR, Bledsoe SM, Shao Y, Lingeman JE, et al. Apatite Plaque Particles in Inner Medulla of Kidneys of Calcium Oxalate Stone Formers: Osteopontin Localization. *Kidney Int* (2005) 68(1):145–54. doi: 10.1111/j.1523-1755.2005.00388.x
27. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, et al. Randall's Plaque of Patients With Nephrolithiasis Begins in Basement Membranes of Thin Loops of Henle. *J Clin Invest* (2003) 111(5):607–16. doi: 10.1172/jci17038
28. Kaufman JM, Vermeulen A. The Decline of Androgen Levels in Elderly Men and its Clinical and Therapeutic Implications. *Endocr Rev* (2005) 26(6):833–76. doi: 10.1210/er.2004-0013
29. Livingston M, Kalansooriya A, Hartland AJ, Ramachandran S, Heald A. Serum Testosterone Levels in Male Hypogonadism: Why and When to

Check-A Review. *Int J Clin Pract* (2017) 71(11):e12995. doi: 10.1111/ijcp.12995
30. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney Stones. *Nat Rev Dis Primers* (2016) 2:16008. doi: 10.1038/nrdp.2016.8
31. Dey J, Creighton A, Lindberg JS, Fuselier HA, Kok DJ, Cole FE, et al. Estrogen Replacement Increased the Citrate and Calcium Excretion Rates in Postmenopausal Women With Recurrent Urolithiasis. *J Urol* (2002) 167 (1):169–71.

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ORIGINAL RESEARCH
published: 14 July 2022
doi: 10.3389/fendo.2022.910329

Chinese Visceral Adiposity Index Is Associated With Incident Renal Damage in Patients With Hypertension and Abnormal Glucose Metabolism: A Longitudinal Study

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OPEN ACCESS

Edited by:
Daisuke Koya,
Kusatsu General Hospital, Japan

Reviewed by:
Dafeng Liu,
Public Health and Clinical Center of Chengdu, China
Xinxia Chang,
Fudan University, China

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Specialty section:
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *01 April 2022*
Accepted: *15 June 2022*
Published: *14 July 2022*

Citation:
Lin M, Li N, Heizhati M, Gan L, Zhu Q, Yao L, Li M and Yang W (2022) Chinese Visceral Adiposity Index Is Associated With Incident Renal Damage in Patients With Hypertension and Abnormal Glucose Metabolism: A Longitudinal Study. Front. Endocrinol. 13:910329. doi: 10.3389/fendo.2022.910329

Objective: To evaluate the association between Chinese visceral adiposity index (CVAI) and incident renal damage and compared its predictive power with that of other visceral obesity indices in patients with hypertension and abnormal glucose metabolism (AGM).

Methods: This retrospective cohort consecutively included patients with hypertension and AGM who did not have renal damage at baseline. Renal damage was defined using the estimated glomerular filtration rate (eGFR) and urine protein. Multivariable Cox regression analysis was used to evaluate the association between CVAI and incident renal damage. Restricted cubic splines were used to determine the shape of the association. The predictive power of the CVAI was examined and directly compared with other indices, including the VAI, body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WHtR), using the area under the receiver operating characteristic curve (AUC) and C-index.

Results: In total, 2,033 patients with hypertension and AGM were included. During a median follow-up of 2.6 years, the incidence of renal damage was 31.5, 48.9, 56.8, and 67.5/1,000 person-years across the quartiles of CVAI. Compared with the first quartile, the risk of renal damage was higher in the second (hazard ratio (HR) = 1.36 [95% CI: 0.93 –1.97]), third (HR = 1.57 [95% CI: 1.09–2.27]), and fourth (HR = 1.65 [95% CI: 1.11 –2.44]) quartiles (p for trend = 0.011). A linear dose–response association was observed. Sensitivity and subgroup analyses confirmed the robustness and consistency of the results. In terms of predictive power, the CVAI had the highest AUC and C-index values.

Conclusions: CVAI is positively associated with renal damage risk in a linear dose–response pattern and has the best performance in predicting incident renal damage in patients with hypertension and AGM. The CVAI may serve as a reliable indicator for identifying patients at a high risk of renal damage.

Keywords: diabetes, hypertension, Chinese visceral adiposity index, renal damage, cohort

INTRODUCTION

Chronic kidney disease (CKD) has been recognized as a major public health issue due to its high prevalence and strong association with cardiovascular events and premature death (1). The prevalence and incidence of CKD are increasing as an ongoing epidemic of metabolic diseases, such as hypertension, abnormal glucose metabolism (AGM), and obesity (2). We recently found in a population-based study that the prevalence of kidney dysfunction in patients with hypertension and diabetes was higher than that in those with either hypertension or diabetes alone (3). Notably, the prevalence of AGM, including diabetes (12.4%) and prediabetes (38.1%), is more than 50% among Chinese adults (4). Given the synergistic effect of hypertension and hyperglycemia on renal damage (5), it would be beneficial for disease management to focus on patients with hypertension and AGM (6). However, traditional risk factors fail to fully explain the increased risk of renal damage in this patient population (7).

Studies have shown that visceral obesity is associated with organ injury, resulting in an increased risk of hypertension, carotid atherosclerosis, diabetes, and kidney disease (8–10). MRI and CT are the two most sensitive methods for measuring visceral fat. However, the use of both procedures for screening large populations is infeasible because of expensive equipment and ionizing radiation (11). Recently, Xia et al. established a Chinese visceral adiposity index (CVAI) to estimate visceral adiposity and predict metabolic disorders (12). CVAI has been shown to outperform other visceral obesity indices in predicting prediabetes, diabetes, and carotid plaque in the Chinese population (9, 13). In addition, several studies have reported an association between obesity and CKD, with visceral obesity appearing to be more closely related to kidney impairment (14–16). However, the association of CVAI with the risk of renal damage has not been reported, especially in patients with hypertension and AGM, a high-risk group for kidney disease.

Therefore, this study aimed to evaluate the association between CVAI and the risk of renal damage in patients with hypertension and AGM, based on a longitudinal cohort. We also compared the predictive power of the CVAI with other indices to determine whether the CVAI could be a better indicator for identifying high-risk individuals.

METHODS

Study Population

The study population was recruited from the Hypertension Center of the People's Hospital of Xinjiang Uygur Autonomous Region between January 2012 and May 2019. Inpatients aged ≥18 years with hypertension and AGM were consecutively included. Exclusion criteria were diagnosis of secondary hypertension (primary aldosteronism, adrenal tumor, Cushing syndrome, pheochromocytoma, and polycystic ovary syndrome), history of cardiovascular events within the last 3 months (including myocardial infarction, heart failure, stroke, unstable angina, coronary revascularization, and coronary bypass surgery), or malignant tumor. In addition, patients with CKD at baseline were also excluded. A total of 2,459 patients with hypertension and AGM and free of CKD at baseline were initially identified, and 2,033 of them completed follow-up at least once and were finally analyzed. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region.

Data Collection

Baseline information was extracted from the medical electronic system, including age, sex, height, weight, waist circumference (WC), cigarette consumption (yes or no), alcohol intake (yes or no), blood pressure (BP), fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), uric acid (UA), serum creatinine (Scr), duration of hypertension, type of AGM (prediabetes or diabetes), plasma aldosterone concentration (PAC), plasma renin activity (PRA), and medication use (antihypertensive, lipid lowering, and hypoglycemic drugs). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The waist-to-height ratio (WHtR) was calculated as the WC divided by height.

Seated BP at the time of hospitalization was measured in the upper arm after patients rested quietly for at least 10 min with a mercury sphygmomanometer using international recommendations (17). The mean values of two measurements were recorded and used for the analysis. WC was measured at the midway level between the lower rib margin and the iliac crest in the midaxillary line, with the participants standing with their feet 25–30 cm apart.

Definition of Diseases and Obesity Indices

Hypertension was defined as systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg or the use of antihypertensive drugs. AGM includes prediabetes and diabetes. Prediabetes was defined as FPG ranging from 6.1 to <7.0 mmol/L or 2-h postprandial glucose ranging from 7.8 to <11.0 mmol/L. Diabetes was defined if there was a previously confirmed diagnosis, or FPG was ≥7.0 mmol/L, or 2-h postprandial glucose was ≥11.1 mmol/L. The estimated glomerular filtration rate (eGFR) was calculated using the simplified modification of diet in renal disease (MDRD) equation based on data from Chinese adults (18). Urine protein levels were determined using urine dipstick results (−, ±, 1+, 2+, and 3+). Renal damage was defined as an eGFR < 60 ml/min/1.73 m^2 and/or the presence of proteinuria (≥1+). CVAI and visceral adiposity index (VAI) were calculated as follows (12, 19):

$$\mathrm{CVAI(men)} = -267.93 + 0.68 \times \mathrm{age} + 0.03 \times \mathrm{BMI} + 4.00 \times \mathrm{WC} + 22.00 \times \log_{10}\mathrm{TG} - 16.32 \times \mathrm{HDL} - \mathrm{C}.$$

$$\text{CVAI(women)} = -187.32 + 1.71 \times \text{age} + 4.23 \times \text{BMI} + 1.12 \times \text{WC} + 39.76 \times \log_{10}\text{TG} - 11.66 \times \text{HDL} - \text{C}.$$

$$\text{VAI(men)} = (\text{WC}/39.68 + [1.88 \times \text{BMI}]) \times (\text{TG}/1.03) \times (1.31/\text{HDL}).$$

$$\text{VAI(women)} = (\text{WC}/36.58 + [1.89 \times \text{BMI}]) \times (\text{TG}/0.81) \times (1.52/\text{HDL}).$$

Follow-Up and Outcome

The outcome of this study was new-onset renal damage during follow-up. Follow-up data were obtained using annual health checkups or hospital readmissions. An examination time ≥3 months after baseline was considered valid. Only the first outcome was used for the analysis if a participant experienced the outcomes more than once during the follow-up period.

Statistical Analysis

Baseline characteristics were described according to CVAI quartiles. Continuous variables were presented as mean ± SD or median (interquartile range [IQR]) according to the normality test results and compared between groups using analysis of variance or non-parametric Kruskal–Wallis H test. Categorical variables were summarized as numbers and percentages and compared between groups using Pearson's chi-square test.

The cumulative incidence of renal damage was estimated using the Kaplan–Meier method and compared using the log-rank test. Three Cox proportional hazards regression models were constructed to determine the independent predictive value of CVAI for renal damage. Model 1 was adjusted for age and sex. Model 2 was adjusted for variables with significant differences among CVAI quartile groups, including age, sex, smoking status, drinking status, SBP, baseline eGFR, duration of hypertension, types of AGM, antidiabetic drugs, antihypertensive drugs, HbA1c, BUN, and hyperuricemia. Model 3 was adjusted for all included factors, including PAC, which has been recently shown to be independently associated with incident renal damage in hypertensives with AGM (20). Hazard ratios (HRs) for outcomes were calculated for quartiles CVAI (with the first quartile as reference), high CVAI (with the group below the median of CVAI as reference), and each SD increase of CVAI. The tolerance and VIF were used for collinearity testing among the included variables.

To evaluate the robustness of the results, sensitivity analyses were performed by excluding patients with a follow-up time of less than 12 months. Furthermore, interaction terms were introduced into the multivariable model to evaluate whether the association between CVAI and renal damage differed according to age (<60 or ≥60 years), sex (men or women), types of AGM (prediabetes or diabetes), SBP (<140 or ≥140 mmHg), DBP (<90 or ≥90 mmHg), BMI (<28 or ≥28 kg/m^2), and medication use (antihypertensive, lipid-lowering, and hypoglycemic drugs).

The lack of repeated renal function measurements may have overestimated the outcomes of renal damage. Therefore, we used more stringent criteria to redefine the outcome as "overt renal damage" (eGFR < 50 ml/min/1.73 m^2 and/or urine protein ≥ 2+), and repeated analyses were performed using the abovementioned procedure.

To describe the shape of the association between CVAI and incident renal damage, we used restricted cubic splines incorporated into the Cox models. In addition, the predictive power of the CVAI was examined and directly compared with other indices, including the VAI, BMI, WC, and WHtR, using the area under the receiver operating characteristic curve (AUC) and C-index. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows (SPSS Inc., Chicago, IL, USA) and R version 4.0.3.

RESULTS

Characteristics of the Study Population

In total, 2,033 participants with hypertension and AGM were finally included in the analysis. The mean age of the study population was 55 ± 11 years, and 884 (43.5%) patients were women. The mean SBP and DBP levels were 149 ± 21 and 88 ± 15 mmHg, respectively. The baseline eGFR was 118 ± 30 ml/min/1.73 m^2. The median CVAI score was 154 (IQR: 129-182). Details of the baseline characteristics across the CVAI quartiles are shown in **Table 1**. Participants with higher CVAI levels tended to have higher BMI, WC, BP, HbA1c, TG, and BUN levels. In addition, with an increase in CVAI, there was an increased proportion of men, smokers, drinkers, hyperuricemia, and use of antidiabetic and antihypertensive drugs.

With a median follow-up of 2.6 (IQR: 1.5–4.2) years, the incidence of renal damage was 31.5, 48.9, 56.8, and 67.5/1,000 person-years across quartiles of CVAI. Regarding the outcome of overt renal damage (eGFR < 50 and/or urine protein ≥ 2+), a similar trend was observed (**Table 1**). The cumulative incidence of renal damage significantly increased with increasing CVAI (**Figure 1**), similar to the outcome of overt renal damage.

Baseline Chinese Visceral Adiposity Index and Risk of Renal Damage

Table 2 shows that the risk of renal damage significantly increased with increasing CVAI quartiles. After adjustment for potential confounders in Model 2, there was a significantly increased risk of incident renal damage for quartile 3 and quartile 4 of CVAI, with HRs and 95% CIs of 1.60 (1.11–2.31) and 1.70 (1.15–2.51), respectively. When all variables were adjusted (model 3), including PAC and PRA, consistent results were observed. Reanalyses by redefining the outcome as overt renal damage showed a stronger association (**Table 3**). The HRs (95% CIs) for overt renal damage in quartiles 2, 3, and 4 of CVAI were 1.85 (0.93–3.67), 2.36 (1.21–4.60), and 2.94 (1.47–5.89), respectively. Each SD increase in CVAI (SD = 42) had a 38% increased risk of overt renal

TABLE 1 | Baseline characteristics of study population across CVAI quartiles.

Characteristics	Q1 (*n* = 508) 38.63–128.92	Q2 (*n* = 508) 128.93–154.03	Q3 (*n* = 508) 154.11–181.42	Q4 (*n* = 509) 181.56–376.25	*p*-Value
Age (year)	53.4 ± 10.2	56.8 ± 11.0	56.4 ± 11.5	55.5 ± 11.1	<0.001
Women, *n* (%)	344 (67.7)	272 (53.5)	182 (35.8)	86 (16.9)	<0.001
Ethnicity, *n* (%)					
Han	370 (72.8)	325 (64.0)	310 (61.0)	210 (41.3)	<0.001
Others	138 (27.2)	183 (36.0)	198 (39.0)	299 (58.7)	
BMI (kg/m^2)	24.9 ± 2.5	27.0 ± 2.7	28.5 ± 3.0	31.8 ± 3.7	<0.001
WC (cm)	90.0 ± 7.2	97.4 ± 5.6	103.4 ± 6.0	113.6 ± 8.4	<0.001
Duration of HTN (year)	5.0 (2.0–10.0)	8.0 (3.0–13.0)	7.0 (2.0–13.0)	8.0 (3.0–13.0)	<0.001
SBP (mmHg)	146.9 ± 22.0	146.9 ± 21.1	147.8 ± 20.1	152.5 ± 21.1	<0.001
DBP (mmHg)	86.7 ± 14.3	85.6 ± 14.3	87.6 ± 14.3	91.7 ± 15.5	<0.001
AGM types, *n* (%)					
Prediabetes	260 (51.2)	216 (42.5)	183 (36.0)	193 (37.9)	<0.001
Diabetes	248 (48.8)	292 (57.5)	325 (64.0)	316 (62.1)	
HbA1c	6.7 ± 1.2	6.9 ± 1.3	7.0 ± 1.3	7.1 ± 1.4	<0.001
Smoking, *n* (%)	95 (18.7)	115 (22.6)	160 (31.5)	222 (43.6)	<0.001
Alcohol drinking, *n* (%)	82 (16.1)	115 (22.6)	155 (30.5)	187 (36.7)	<0.001
Total cholesterol (mmol/L)	4.44 ± 1.13	4.45 ± 1.05	4.37 ± 1.09	4.48 ± 1.12	0.489
Triglyceride (mmol/L)	1.40 (1.06–1.92)	1.70 (1.26–2.45)	1.72 (1.22–2.50)	1.91 (1.45–2.70)	<0.001
HDL-C (mmol/L)	1.07 ± 0.28	0.99 ± 0.23	0.94 ± 0.20	0.89 ± 0.19	<0.001
LDL-C (mmol/L)	2.67 ± 0.86	2.59 ± 0.85	2.60 ± 0.84	2.64 ± 0.88	0.449
Serum creatinine (μmol/L)	61.3 ± 15.1	63.9 ± 14.4	67.8 ± 16.1	71.2 ± 15.3	<0.001
Baseline eGFR (ml/min/1.73 m^2)	119.6 (103.0–139.3)	115.7 (98.9–136.2)	112.9 (94.5–134.9)	110.7 (93.2–130.4)	<0.001
Blood urea nitrogen (mmol/L)	4.88 ± 1.35	5.05 ± 1.40	5.25 ± 1.52	5.24 ± 1.29	<0.001
Uric acid (μmol/L)	299.9 ± 77.3	331.0 ± 80.3	343.6 ± 85.2	354.4 ± 88.3	<0.001
Hyperuricemia, *n* (%)	69 (13.6)	116 (22.8)	120 (23.6)	131 (25.7)	<0.001
PAC (ng/dl)	13.5 (11.5–19.7)	13.9 (11.6–20.5)	13.6 (11.7–18.9)	13.6 (11.7–19.9)	0.627
PRA (ng/ml/h)	1.31 (0.51–2.47)	1.52 (0.57–2.67)	1.22 (0.45–2.64)	1.40 (0.60–2.69)	0.088
Antidiabetic drugs	233 (45.9)	284 (55.9)	306 (60.2)	315 (61.9)	<0.001
Lipid-lowering drugs	399 (78.5)	427 (84.1)	417 (82.1)	424 (83.3)	0.104
Anti-hypertensive drugs					
ACEI/ARB	261 (51.4)	292 (57.5)	293 (57.7)	329 (64.6)	<0.001
CCB	398 (78.3)	418 (82.3)	417 (82.1)	444 (87.2)	0.003
Beta-blocker	79 (15.6)	112 (22.0)	104 (20.5)	148 (29.1)	<0.001
Diuretics	159 (31.3)	170 (33.5)	190 (37.4)	197 (38.7)	0.049
Follow-up time (person-years)	1587	1431	1496	1436	–
Outcome incidence, number (incidence per 1000 person-years of follow-up)					
Renal damage	50 (31.5)	70 (48.9)	85 (56.8)	97 (67.5)	<0.001
Overt renal damage	14 (8.8)	25 (17.5)	32 (21.4)	46 (32.0)	<0.001

Data are presented as the mean ± SD, n (%), or median (interquartile range).

CVAI, Chinese visceral adiposity index; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; AGM, abnormal glucose metabolism; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers.

damage. Consistent trends were observed in diabetes and prediabetes groups (**Tables S1, S2**), as well as separated by sex (**Table S3**). No obvious collinearity was detected among the variables in the fully adjusted models (**Table S4**).

By excluding participants with a follow-up time of less than 12 months, sensitivity analysis confirmed the robustness of the results (**Table S5**). In model 3, quartiles 3 and 4 of CVAI had 50% and 55% increased risks of incident renal damage, respectively (both $p < 0.05$). In addition, restricted cubic splines showed a linear dose–response association between CVAI and renal damage ($p_{\text{nonlinearity}} > 0.05$, **Figure 2**).

Subgroup Analyses and Prediction Power

Subgroup analyses were performed by age, sex, type of AGM, SBP, DBP, BMI, and medication use (antihypertensive, lipid-lowering, and antidiabetic drugs) to further evaluate the association between CVAI and renal damage. The results showed consistent trends in all subgroups for overt renal damage (**Figure 3**) and renal damage (**Figure S1**). In addition, none of the variables significantly modified the association (p for interaction >0.05), except for age (p for interaction < 0.05), and the association between CVAI and renal damage was stronger in older adults.

The AUC and C-index values of CVAI, VAI, BMI, WC, and WHtR for predicting incident renal damage are shown in **Table S6**. Among these obesity indices, the CVAI had the highest AUC and C-index, significantly higher than BMI, WC, and WHtR. By using ROC analysis, the best cutoff value for CVAI to distinguish individuals with and without incident renal damage was 149. In addition, when the VAI was compared with other indicators (BMI, WC, and WHtR), there was no significant difference ($p > 0.05$).

DISCUSSION

In the present study, with a longitudinal design, CVAI was positively associated with incident renal damage in a linear

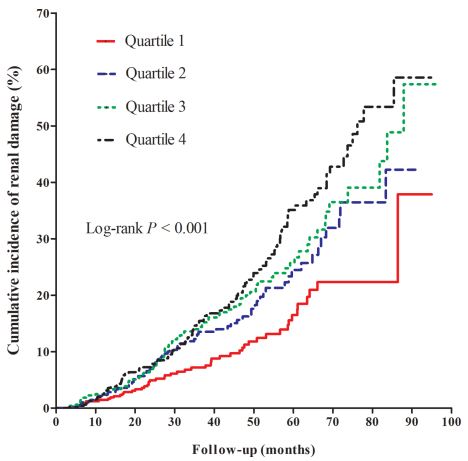

FIGURE 1 | Kaplan–Meier curve of cumulative incidence of renal damage across quartiles of Chinese visceral adiposity index.

dose–response pattern in patients with hypertension and AGM. Furthermore, CVAI had the best performance in predicting incident renal damage compared with other obesity indices, including VAI, BMI, WC, and WHtR. Patients with hypertension and AGM tend to have a higher risk of kidney disease; however, most patients are already in an irreversible stage at the time of detection for CKD and usually have complications, and some of them even need renal replacement therapy (21). According to our study, for those with hypertension and AGM, a simple assessment of visceral adiposity by calculating CVAI may be helpful for the early identification of high-risk individuals. It is necessary to pay close attention to the high risk of renal damage when CVAI is greater than 149 and to adopt strict BP and glucose management, thereby reducing the risk of renal damage.

Obesity, especially visceral obesity, is associated with the occurrence and development of kidney disease (22, 23). Non-alcoholic fatty liver disease development and fibrosis progression have recently been shown to be associated with incident CKD (24). Although MRI and CT are the gold standard for measuring

TABLE 2 | Multivariable Cox regression for the association between CVAI and incident renal damage.

CVAI	Crude model HR (95% CI)	*p*-Value	Model 1 HR (95% CI)	*p*-Value	Model 2 HR (95% CI)	*p*-Value	Model 3 HR (95% CI)	*p*-Value
Quartile groups								
Quartile 1	Ref.		Ref.		Ref.		Ref.	
Quartile 2	1.61 (1.12–2.31)	0.010	1.55 (1.08–2.24)	0.018	1.42 (0.97–2.06)	0.069	1.36 (0.93–1.97)	0.111
Quartile 3	1.83 (1.29–2.60)	0.001	1.79 (1.25–2.56)	0.002	1.60 (1.11–2.31)	0.012	1.57 (1.09–2.27)	0.016
Quartile 4	2.16 (1.54–3.04)	<0.001	2.10 (1.46–3.03)	<0.001	1.70 (1.15–2.51)	0.008	1.65 (1.11–2.44)	0.013
p for trend		<0.001		<0.001		0.008		0.011
Dichotomous groups								
Lower (<154.1)	Ref.		Ref.		Ref.		Ref.	
Higher (≥ 154.1)	1.56 (1.23–1.96)	<0.001	1.51 (1.18–1.93)	0.001	1.34 (1.04–1.73)	0.024	1.34 (1.04–1.73)	0.025
Each SD increase	1.23 (1.11–1.38)	<0.001	1.22 (1.08–1.38)	0.002	1.13 (0.98–1.29)	0.085	1.12 (0.98–1.28)	0.112

Results are shown as hazard ratios (95% CIs) derived from Cox proportional hazards models. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, ethnicity, smoking status, drinking status, SBP, baseline eGFR, duration of hypertension, type of AGM, duration of AGM, antidiabetic drugs, antihypertensive drugs, HbA1c, BUN, and hyperuricemia. Model 3 was adjusted for variables in model 2 plus TC, LDL-C, lipid-lowering drugs, Ln PAC, and Ln PRA.
CVAI, Chinese visceral adiposity index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; AGM, abnormal glucose metabolism; HbA1c, glycosylated hemoglobin; BUN, blood urea nitrogen; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

TABLE 3 | Multivariable Cox regression for the association between CVAI and incident overt renal damage.

CVAI	Crude model HR (95% CI)	*p*-Value	Model 1 HR (95% CI)	*p*-Value	Model 2 HR (95% CI)	*p*-Value	Model 3 HR (95% CI)	*p*-Value
Quartile 1	Ref.		Ref.		Ref.		Ref.	
Quartile 2	2.05 (1.07–3.95)	0.032	2.05 (1.06–3.96)	0.033	1.98 (1.00–3.92)	0.050	1.85 (0.93–3.67)	0.079
Quartile 3	2.45 (1.31–4.60)	0.005	2.57 (1.35–4.90)	0.004	2.39 (1.23–4.64)	0.010	2.36 (1.21–4.60)	0.012
Quartile 4	3.64 (2.00–6.63)	<0.001	3.97 (2.10–7.51)	<0.001	3.01 (1.53–5.95)	0.002	2.94 (1.47–5.89)	0.002
p for trend		<0.001		<0.001		0.002		0.002
Dichotomous groups								
Lower (<154.1)	Ref.		Ref.		Ref.		Ref.	
Higher (≥ 154.1)	2.04 (1.39–3.00)	<0.001	2.10 (1.39–3.16)	<0.001	1.75 (1.15–2.68)	0.010	1.77 (1.15–2.72)	0.009
Each SD increase	1.46 (1.23–1.73)	<0.001	1.51 (1.25–1.83)	<0.001	1.39 (1.12–1.71)	0.002	1.38 (1.15–1.71)	0.003

Results are shown as hazard ratios (95% CIs) derived from Cox proportional hazards models. Overt renal damage was defined as an eGFR < 50 and/or urine protein ≥ 2+. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, ethnicity, smoking status, drinking status, SBP, baseline eGFR, duration of hypertension, type of AGM, duration of AGM, antidiabetic drugs, antihypertensive drugs, HbA1c, BUN, and hyperuricemia. Model 3 was adjusted for variables in model 2 plus TC, LDL-C, lipid-lowering drugs, Ln PAC, and Ln PRA.
CVAI, Chinese visceral adiposity index; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; AGM, abnormal glucose metabolism; HbA1c, glycosylated hemoglobin; BUN, blood urea nitrogen; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

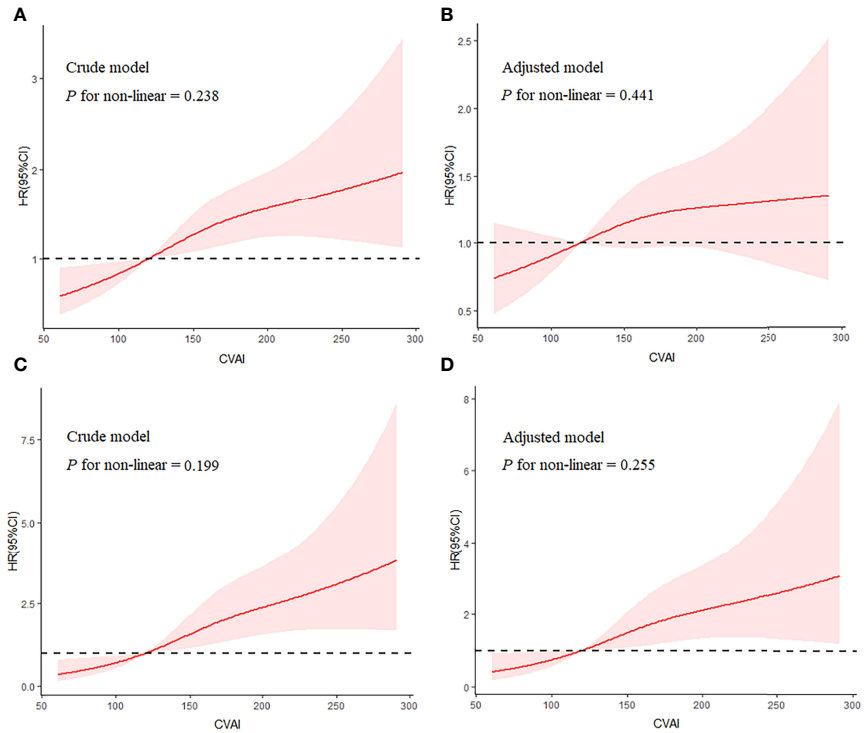

FIGURE 2 | Shape of the association of CVAI with renal damage **(A, B)** and overt renal damage **(C, D)** by restricted cubic spline. Adjusted model included variables of age, sex, smoking status, drinking status, SBP, baseline eGFR, duration of hypertension, types of GMD, glucose metabolism disorders; antidiabetic drugs, antihypertension drugs, HbA1c, BUN, and hyperuricemia. CVAI, Chinese visceral adiposity index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; BUN, blood urea nitrogen.

visceral fat, these techniques are rarely available in daily practice because of the limitations of equipment and cost. Several simple indicators such as BMI, WC, WHtR, and VAI are commonly used to assess obesity and fat distribution (25). However, body fat distribution varies by race, and it has been reported that the Asian population seems to be more inclined to visceral fat accumulation at a lower BMI (26). Previous studies have shown that VAI is not superior to BMI or WC in estimating visceral adipose tissue and predicting type 2 diabetes in the Chinese population (27). Similar results were observed in our study, with no significant difference in the predictive power of VAI for renal damage compared with BMI, WC, and WHtR. CVAI was initially established as a reliable indicator for evaluating metabolic health in the Chinese population and was further confirmed to be a strong and independent predictor of diabetes in Chinese adults (28, 29). In a recent cross-sectional study, CVAI showed the strongest association with cardiovascular disease among the commonly used abdominal obesity indices (30). Similarly, several other studies have demonstrated that CVAI is related to cardiovascular risk or its risk factors, such as carotid atherosclerosis (31–33). Our study extends this field by demonstrating an association between CVAI and incident renal damage.

The association between obesity and kidney disease has been reported in the general population and the population without diabetes (10, 14, 15, 34); however, as a reliable measure of visceral fat, the association between CVAI and kidney disease remains to be verified. To our knowledge, this is the first longitudinal study to evaluate the association between CVAI and the risk of renal damage in patients with hypertension and AGM. Based on previous studies and our analysis, several underlying mechanisms may be involved. First, it is mediated by BP and glucose. Given the association between visceral fat and other risk factors for kidney disease, higher CVAI may increase the risk of renal damage by exacerbating these factors, such as BP and glucose, especially in patients with hypertension and AGM. Second, a chronic inflammatory reaction may be involved. A higher CVAI represents an increased accumulation of visceral fat, which produces a variety of pro-inflammatory factors, such as tumor necrosis factor α, interleukin-6, and interleukin-8, resulting in the occurrence of renal damage (35, 36). Third, fat had a direct effect. The infiltration and accumulation of adipokines, produced by visceral adipose tissue, may induce structural and functional changes in podocytes and proximal tubule cells that contribute to renal damage (37, 38). Fourth, there are synergistic effects of multiple factors. High TG and WC and low HDL-C levels have been associated with kidney disease (39–41). Therefore, the stronger association between CVAI and renal damage may be partly explained by the synergistic effects of these factors.

By comparing the CVAI with other commonly used obesity indicators (BMI, WC, WHtR, and VAI), we found that the CVAI

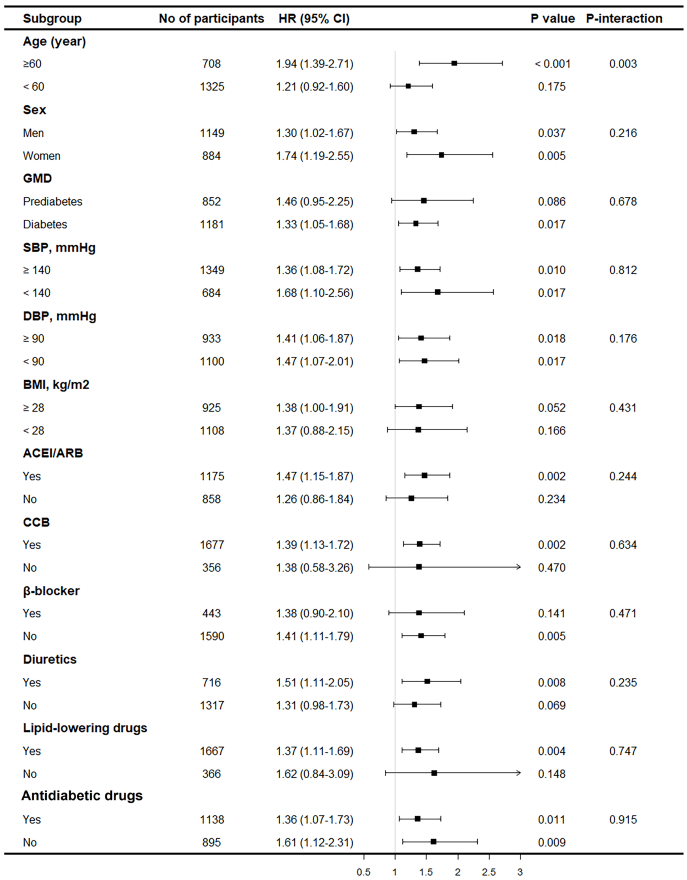

FIGURE 3 | Subgroup analysis on the association between CVAI and overt renal damage. Results were derived from multivariable Cox regression adjusted for age, sex, smoking status, drinking status, SBP, baseline eGFR, duration of hypertension, types of GMD, glucose metabolism disorders; antidiabetic drugs, anti-hypertension drugs, HbA1c, BUN, and hyperuricemia and presented as hazard ratio for each SD increment of CVAI and the corresponding 95% CIs. CVAI, Chinese visceral adiposity index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; BUN, blood urea nitrogen.

had the highest predictive power for renal damage. It has been reported that BMI cannot adequately discriminate between body fat mass and lean tissues or identify regional body fat distribution (42, 43). WC and WHtR can better reflect abdominal obesity than BMI but have limitations in distinguishing subcutaneous from visceral adipose tissue (44, 45). Interestingly, in our study, although no significant differences in AUC and C-index were observed between CVAI and VAI, the performance of VAI was not significantly improved when compared with the other three indices (BMI, WC, and WHtR, $p > 0.05$). This may also reconfirm that CVAI is more suitable than VAI for the Chinese population.

The present study has several strengths. First, a longitudinal design with a large sample size and a series of confounder adjustments yielded relatively stable and reliable results. Second, our study consisted of a sample of individuals at a high risk of renal damage, and the results may contribute to the prevention and treatment of kidney disease. However, several limitations of this study warrant discussion. First, single measurements of serum creatinine and urine protein without repeated examinations may have resulted in the misclassification of individuals with renal damage. Also, proteinuria was examined through qualitative but not quantitative methods. However, analyses by redefining the outcome as overt renal damage (eGFR < 50 and/or urine protein ≥ 2+) confirmed the robustness of the results. Second, although a wide range of confounders were adjusted, residual confounding factors were not considered, such as dietary and inflammation indicators. Also, future studies with larger sample sizes are needed to assess the association between diabetes and prediabetes separately. Third, the study was conducted in a single center, although it was conducted in a regional center for patients with hypertension of a large age range and ethnic groups. Fourth, using a retrospective design, we were unable to evaluate the association between the dynamic changes in CVAI and renal damage.

In conclusion, higher CVAI is associated with an increased risk of renal damage in patients with hypertension and AGM. Furthermore, CVAI has the best performance in predicting incident renal damage as compared to other obesity indices. Therefore, a simple assessment of visceral adiposity by calculating CVAI may be helpful for the early identification of

high-risk individuals and adopting strict BP and glucose management, thereby reducing the risk of renal damage.

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 People's Hospital of Xinjiang Uygur Autonomous Region. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MYL contributed to the study design and statistical analysis. MYL, NL, MH, and LG analyzed the data together and drafted the manuscript. QZ, LY, ML, and WY participated in the data collection. All authors have read and approved the final manuscript.

FUNDING

This work was supported by the Non-Profit Central Research Institute Fund of the Chinese Academy of Medical Sciences (grant number 2020-RW330-002).

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.cn) for the English language editing. We thank Yidu Cloud Technology Co., Ltd., Beijing, and all the members of the Statistical Analysis Team, for generously sharing their experience and technology.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.910329/full#supplementary-material

REFERENCES

1. Herzog CA, Asinger RW, Berger AK, Charytan DM, Díez J, Hart RG, et al. Cardiovascular Disease in Chronic Kidney Disease. A Clinical Update From Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* (2011) 80 (6):572–86. doi: 10.1038/ki.2011.223
2. 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
3. Lin M, Heizhati M, Wang L, Gan L, Li M, Yang W, et al. Prevalence and Associated Factors of Kidney Dysfunction in Patients With Hypertension and/or Diabetes Mellitus From a Primary Care Population in Northwest China. *Int J Gen Med* (2021) 14:7567–78. doi: 10.2147/IJGM.S329604
4. Wang L, Peng W, Zhao Z, Zhang M, Shi Z, Song Z, et al. Prevalence and Treatment of Diabetes in China, 2013-2018. *Jama* (2021) 326(24):2498–506. doi: 10.1001/jama.2021.22208
5. Wang Z, do Carmo JM, Aberdein N, Zhou X, Williams JM, da Silva AA, et al. Synergistic Interaction of Hypertension and Diabetes in Promoting Kidney Injury and the Role of Endoplasmic Reticulum Stress. *Hypertension* (2017) 69 (5):879–91. doi: 10.1161/HYPERTENSIONAHA.116.08560
6. Hoerger TJ, Wittenborn JS, Segel JE, Burrows NR, Imai K, Eggers P, et al. A Health Policy Model of CKD: 2. The Cost-Effectiveness of Microalbuminuria Screening. *Am J Kidney Dis* (2010) 55(3):463–73. doi: 10.1053/j.ajkd.2009.11.017
7. Lin M, Heizhati M, Gan L, Yao L, Yang W, Li M, et al. Development and Validation of a Prediction Model for 5-Year Risk of Kidney Dysfunction in Patients With Hypertension and Glucose Metabolism Disorder. *Risk Manag Healthc Policy* (2022) 15:289–98. doi: 10.2147/RMHP.S345059
8. Rallidis LS, Baroutsi K, Zolindaki M, Karagianni M, Varounis C, Dagres N, et al. Visceral Adipose Tissue Is a Better Predictor of Subclinical Carotid Atherosclerosis Compared With Waist Circumference. *Ultrasound Med Biol* (2014) 40(6):1083–8. doi: 10.1016/j.ultrasmedbio.2013.12.017
9. Han M, Qin P, Li Q, Qie R, Liu L, Zhao Y, et al. Chinese Visceral Adiposity Index: A Reliable Indicator of Visceral Fat Function Associated With Risk of Type 2 Diabetes. *Diabetes Metab Res Rev* (2021) 37(2):e3370. doi: 10.1002/dmrr.3370
10. Xu X, Zhao Y, Zhao Z, Zhu S, Liu X, Zhou C, et al. Correlation of Visceral Adiposity Index With Chronic Kidney Disease in the People's Republic of China: To Rediscover the New Clinical Potential of an Old Indicator for Visceral Obesity. *Ther Clin Risk Manage* (2016) 12:489–94. doi: 10.2147/TCRM.S96340
11. Wang H, Chen YE, Eitzman DT. Imaging Body Fat: Techniques and Cardiometabolic Implications. *Arterioscler Thromb Vasc Biol* (2014) 34 (10):2217–23. doi: 10.1161/ATVBAHA.114.303036
12. Xia MF, Chen Y, Lin HD, Ma H, Li XM, Aleteng Q, et al. A Indicator of Visceral Adipose Dysfunction to Evaluate Metabolic Health in Adult Chinese. *Sci Rep* (2016) 6:38214. doi: 10.1038/srep38214
13. Bi H, Zhang Y, Qin P, Wang C, Peng X, Chen H, et al. Association of Chinese Visceral Adiposity Index and Its Dynamic Change With Risk of Carotid Plaque in a Large Cohort in China. *J Am Heart Assoc* (2022) 11(1):e022633. doi: 10.1161/JAHA.121.022633
14. Dai D, Chang Y, Chen Y, Chen S, Yu S, Guo X, et al. Visceral Adiposity Index and Lipid Accumulation Product Index: Two Alternate Body Indices to Identify Chronic Kidney Disease Among the Rural Population in Northeast China. *Int J Environ Res Public Health* (2016) 13(12):1231. doi: 10.3390/ijerph13121231
15. Xiao H, Xiong C, Shao X, Gao P, Chen H, Ning J, et al. Visceral Adiposity Index and Chronic Kidney Disease in a Non-Diabetic Population: A Cross-Sectional Study. *Diabetes Metab Syndr Obes* (2020) 13:257–65. doi: 10.2147/DMSO.S231656
16. Betzler BK, Sultana R, Banu R, Tham YC, Lim CC, Wang YX, et al. Association Between Body Mass Index and Chronic Kidney Disease in Asian Populations: A Participant-Level Meta-Analysis. *Maturitas* (2021) 154:46–54. doi: 10.1016/j.maturitas.2021.09.005
17. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, et al. Human Blood Pressure Determination by Sphygmomanometry. *Circulation* (1993) 88 (5 Pt 1):2460–70. doi: 10.1161/01.CIR.88.5.2460
18. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified Glomerular Filtration Rate Estimating Equation for Chinese Patients With Chronic Kidney Disease. *J Am Soc Nephrol* (2006) 17(10):2937–44. doi: 10.1681/ASN.2006040368

19. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, et al. Visceral Adiposity Index: A Reliable Indicator of Visceral Fat Function Associated With Cardiometabolic Risk. *Diabetes Care* (2010) 33(4):920–2. doi: 10.2337/dc09-1825
20. Lin M, Heizhati M, Gan L, Yao X, Luo Q, Zhang D, et al. Higher Aldosterone Is Associated With Increased Renal Impairment Risk in Patients With Hypertension and Abnormal Glucose Metabolism: A Longitudinal Study. *J Hypertens* (2022) 40(3):561–9. doi: 10.1097/HJH.0000000000003049
21. 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 (9888):260–72. doi: 10.1016/S0140-6736(13)60687-X
22. Madero M, Katz R, Murphy R, Newman A, Patel K, Ix J, et al. Comparison Between Different Measures of Body Fat With Kidney Function Decline and Incident CKD. *Clin J Am Soc Nephrol* (2017) 12(6):893–903. doi: 10.2215/CJN.07010716
23. Biyik Z, Guney I. Lipid Accumulation Product and Visceral Adiposity Index: Two New Indices to Predict Metabolic Syndrome in Chronic Kidney Disease. *Eur Rev Med Pharmacol Sci* (2019) 23(5):2167–73. doi: 10.26355/eurrev_201903_17262
24. Zuo G, Xuan L, Xin Z, Xu Y, Lu J, Chen Y, et al. New Nonalcoholic Fatty Liver Disease and Fibrosis Progression Associate With the Risk of Incident Chronic Kidney Disease. *J Clin Endocrinol Metab* (2021) 106(10):e3957–e68. doi: 10.1210/clinem/dgab425
25. Mousapour P, Barzin M, Valizadeh M, Mahdavi M, Azizi F, Hosseinpanah F, et al. Predictive Performance of Lipid Accumulation Product and Visceral Adiposity Index for Renal Function Decline in Non-Diabetic Adults, an 8.6-Year Follow-Up. *Clin Exp Nephrol* (2020) 24(3):225–34. doi: 10.1007/s10157-019-01813-7
26. Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, et al. The Relationship of Waist Circumference and BMI to Visceral, Subcutaneous, and Total Body Fat: Sex and Race Differences. *Obes (Silver Spring)* (2011) 19(2):402–8. doi: 10.1038/oby.2010.248
27. Deurenberg P, Deurenberg-Yap M, Guricci S. Asians are Different From Caucasians and From Each Other in Their Body Mass Index/Body Fat Per Cent Relationship. *Obes Rev* (2002) 3(3):141–6. doi: 10.1046/j.1467-789X.2002.00065.x
28. Wu J, Gong L, Li Q, Hu J, Zhang S, Wang Y, et al. A Novel Visceral Adiposity Index for Prediction of Type 2 Diabetes and Pre-Diabetes in Chinese Adults: A 5-Year Prospective Study. *Sci Rep* (2017) 7(1):13784. doi: 10.1038/s41598-017-14251-w
29. Wei J, Liu X, Xue H, Wang Y, Shi Z. Comparisons of Visceral Adiposity Index, Body Shape Index, Body Mass Index and Waist Circumference and Their Associations With Diabetes Mellitus in Adults. *Nutrients* (2019) 11(7):1580. doi: 10.3390/nu11071580
30. Wan H, Wang Y, Xiang Q, Fang S, Chen Y, Chen C, et al. Associations Between Abdominal Obesity Indices and Diabetic Complications: Chinese Visceral Adiposity Index and Neck Circumference. *Cardiovasc Diabetol* (2020) 19(1):118. doi: 10.1186/s12933-020-01095-4
31. Huang YC, Huang JC, Lin CI, Chien HH, Lin YY, Wang CL, et al. Comparison of Innovative and Traditional Cardiometabolic Indices in Estimating Atherosclerotic Cardiovascular Disease Risk in Adults. *Diag (Basel)* (2021) 11(4):603. doi: 10.3390/diagnostics11040603
32. Shao MJ, Luo JY, Shi J, Liu F, Shan CF, Luo F, et al. Association of Visceral Obesity-Related Indices With Coronary Collateralization in Patients With Chronic Total Occlusion. *Front Cardiovasc Med* (2021) 8:742855. doi: 10.3389/fcvm.2021.742855
33. Li B, Lai X, Yan C, Jia X, Li Y. The Associations Between Neutrophil-to-Lymphocyte Ratio and the Chinese Visceral Adiposity Index, and Carotid Atherosclerosis and Atherosclerotic Cardiovascular Disease Risk. *Exp Gerontol* (2020) 139:111019. doi: 10.1016/j.exger.2020.111019
34. Seong JM, Lee JH, Gi MY, Son YH, Moon AE, Park CE, et al. Gender Difference in the Association of Chronic Kidney Disease With Visceral Adiposity Index and Lipid Accumulation Product Index in Korean Adults: Korean National Health and Nutrition Examination Survey. *Int Urol Nephrol* (2021) 53(7):1417–25. doi: 10.1007/s11255-020-02735-0
35. Kawai T, Autieri MV, Scalia R. Adipose Tissue Inflammation and Metabolic Dysfunction in Obesity. *Am J Physiol Cell Physiol* (2021) 320(3):C375–c91. doi: 10.1152/ajpcell.00379.2020
36. Anthony SR, Guarnieri AR, Gozdiff A, Helsley RN, Phillip Owens A, Tranter M, et al. Mechanisms Linking Adipose Tissue Inflammation to Cardiac Hypertrophy and Fibrosis. *Clin Sci (Lond)* (2019) 133(22):2329–44. doi: 10.1042/CS20190578
37. Wahba IM, Mak RH. Obesity and Obesity-Initiated Metabolic Syndrome: Mechanistic Links to Chronic Kidney Disease. *Clin J Am Soc Nephrol* (2007) 2 (3):550–62. doi: 10.2215/CJN.04071206
38. Gai Z, Wang T, Visentin M, Kullak-Ublick GA, Fu X, Wang Z. Lipid Accumulation and Chronic Kidney Disease. *Nutrients* (2019) 11(4):722. doi: 10.3390/nu11040722
39. You A, Li Y, Tomlinson B, Yue L, Zhao K, Fan H, et al. Association Between Renal Dysfunction and Low HDL Cholesterol Among the Elderly in China. *Front Cardiovasc Med* (2021) 8:644208. doi: 10.3389/fcvm.2021.644208
40. Wang X, Chen H, Shao X, Xiong C, Hong G, Chen J, et al. Association of Lipid Parameters With the Risk of Chronic Kidney Disease: A Longitudinal Study Based on Populations in Southern China. *Diabetes Metab Syndr Obes* (2020) 13:663–70. doi: 10.2147/DMSO.S229362
41. Chen D, Sun H, Lu C, Chen W, Guo VY. The Association Between Hypertriglyceridemic-Waist Phenotype and Chronic Kidney Disease: A Cohort Study and Meta-Analysis. *Sci Rep* (2022) 12(1):1935. doi: 10.1038/s41598-022-05806-7
42. Okorodudu DO, Jumean MF, Montori VM, Romero-Corral A, Somers VK, Erwin PJ, et al. Diagnostic Performance of Body Mass Index to Identify Obesity as Defined by Body Adiposity: A Systematic Review and Meta-Analysis. *Int J Obes (Lond)* (2010) 34(5):791–9. doi: 10.1038/ijo.2010.5
43. Nevill AM, Stewart AD, Olds T, Holder R. Relationship Between Adiposity and Body Size Reveals Limitations of BMI. *Am J Phys Anthropol* (2006) 129 (1):151–6. doi: 10.1002/ajpa.20262
44. Pou KM, Massaro JM, Hoffmann U, Lieb K, Vasan RS, O'Donnell CJ, et al. Patterns of Abdominal Fat Distribution: The Framingham Heart Study. *Diabetes Care* (2009) 32(3):481–5. doi: 10.2337/dc08-1359
45. Chen C, Xu Y, Guo ZR, Yang J, Wu M, Hu XS, et al. The Application of Visceral Adiposity Index in Identifying Type 2 Diabetes Risks Based on a Prospective Cohort in China. *Lipids Health Dis* (2014) 13:108. doi: 10.1186/1476-511X-13-108

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.

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TYPE Original Research
PUBLISHED 08 September 2022
DOI 10.3389/fendo.2022.983747

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 01 July 2022
ACCEPTED 12 August 2022
PUBLISHED 08 September 2022

CITATION
Cao P, Huang B, Hong M, Jiang Y, Cao R, Chi C, Cao Y and Li S (2022) Association of amino acids related to urea cycle with risk of diabetic nephropathy in two independent cross-sectional studies of Chinese adults.
Front. Endocrinol. 13:983747.
doi: 10.3389/fendo.2022.983747

Association of amino acids related to urea cycle with risk of diabetic nephropathy in two independent cross-sectional studies of Chinese adults

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Objective: To investigate the association between amino acids related to the urea cycle and diabetic nephropathy (DN) in two independent cross-sectional studies.

Methods: We obtained the medical records of 145 individuals with DN and 596 individuals without DN who attended an annual health examination at Liaoning Medical University First Affiliated Hospital (LMUFAH), China, from May 2015 to August 2016. From April 2018 to April 2019, we collected medical records of another 741 individuals: 338 individuals with DN and 403 individuals without DN from the Second Affiliated Hospital of Dalian Medical University (DALIAN), China. Binary logistic regression was used to obtain the odds ratio (OR) and 95% confidence interval (CI).

Results: In two independent cross-sectional studies, we observed that citrulline was consistently associated with DN risk [OR (95% CI) of per standard deviation (SD) increase for citrulline in the LMUFAH population: 1.200 (1.006, 1.432); OR (95% CI) of per SD increase for citrulline in the DALIAN population: 1.189 (1.012, 1.396); pooled effect size for citrulline: 1.194 (1.060, 1.345)]. However, ornithine, arginine, and the ratio of arginine to ornithine were consistently unrelated to DN risk, and the ratios of other amino acids in the urea cycle were inconsistently associated with DN risk.

Conclusions: Citrulline was consistently associated with DN risk in two independent cross-sectional studies in Chinese adults.

KEYWORDS

diabetic nephropathy, urea cycle, amino acids, association, cross-sectional studies

Introduction

When the pancreas does not produce enough insulin or the body is unable to use insulin effectively, symptoms such as excessive urine excretion (polyuria), thirst (polydipsia), frequent hunger pangs, weight loss, loss of vision, and fatigue may occur; we call this disease diabetes (1–3). Diabetes can be classified according to its cause into type 1 diabetes (insufficient insulin production, common in children and adolescents) and type 2 diabetes (T2D; insulin resistance, common in obese adults), with over 95% of people with diabetes experiencing T2D. In recent years, diabetes has become a severe, worldwide public health problem: in 2019, diabetes was the ninth leading cause of death worldwide, directly causing an estimated 1.5 million deaths, and 48% of diabetes deaths occur before the age of 70 years (4). This means that diabetes-related research is imperative and significant.

Diabetic nephropathy (DN) is a result of a complex interaction between metabolic processes, is a result of long-term tubular (5, 6) and glomerular damage, and is an important cause of renal failure (7) and inflammatory and hemodynamic change (3). Its key risk factor is insulin resistance.

Early studies have found that 5%–40% of T2D patients eventually develop DN (8), which is the leading cause of end-stage renal disease (ESRD) worldwide (9, 10). The increased risk of cardiovascular death in patients with diabetes is mainly associated with the presence of DN (11). In addition, research has shown that DN has become the main cause of chronic kidney disease in China (12), and it is important to note that once the disease is established it can only progress; it cannot be cured (13). All in all, DN poses a major burden on healthcare systems and the global economy (14).

It is well known that disease leads to changes in the pathophysiological processes of the body, which ultimately cause corresponding changes in metabolites. Furthermore, metabolites reflect the environment in which the cells live, which in turn is closely related to the nutritional status of the cells, the effects of drugs and environmental pollutants, and the influence of other external factors. Therefore, analyzing certain metabolites and comparing them with those of non-diseased individuals to find biomarkers of the disease will provide a better approach to disease diagnosis.

Considering the seriousness of the dangers of DN, we urgently need to find biomarkers that can identify populations at high risk of DN and predict this disease at an early stage. In recent decades many novel biomarkers related to diabetes and its complications were found through metabolomics (1). Experimental evidence developed in murine models and cell culture suggests that urea reduces insulin sensitivity and suppresses insulin secretion (15). Moreover, a recent study showed that higher levels of blood urea nitrogen were associated with an increased risk of developing diabetes mellitus (16). Arginine, citrulline, and ornithine belong to urinary metabolites and substrates. Some studies showed that amino acids related to the urea cycle were associated with inflammatory markers and oxidative stress (17, 18). Our previous study found that amino acids related to the urea cycle were associated with T2D in Chinese adults (19). However, whether amino acids related to the urea cycle are associated with the risk of incident DN remains unclear.

In this study, we aimed to explore whether plasma levels of amino acids related to the urea cycle were associated with DN risk using two independent cross-sectional surveys.

Material and methods

Subjects

Liaoning Medical University First Affiliated Hospital (LMUFAH) is a national comprehensive tertiary class A hospital in China. By retrieving electronic medical records, data from 741 T2D patients (596 non-DN and 145 DN patients) were collected from May 2015 to August 2016. The Second Affiliated Hospital of Dalian Medical University (DALIAN) is the largest comprehensive grade 3A hospital in the Southern Liaoning province of China, with nearly 2 million emergency department visits per year. In accordance with the inclusion and exclusion criteria below, we collected data from 741 T2D patients (403 non-DN and 338 DN patients) to address our research questions. Diabetes patients were diagnosed in accordance with the World Health Organization's criteria from 1998 (20).

In both cross-sectional studies, the inclusion criteria were as follows: (1) for patients in the non-DN group, a diagnosis of T2D without DN, and (2) for patients in the DN group, a diagnosis of T2D with DN (21). The exclusion criteria were as follows: (1) a lack of information on triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels; (2) a lack of information on metabolites; and (3) age under 18 years. The specific process is shown in Figure 1.

We obtained the data from the hospitals' computer systems, including demographic characteristics, anthropometric measurements, relevant clinical measurements, and metabolism measurements. Demographic characteristics data included age, gender, current smoking status, and current drinking status. Anthropometric measurements included weight, height, and blood pressure. Clinical measurements on lipid profiles were collected. Metabolism measurements included amino acids related to the urea cycle (i.e., ornithine, arginine, citrulline). Anti-diabetic measures included oral anti-diabetic agents and insulin. The statuses "current smoker" and "current

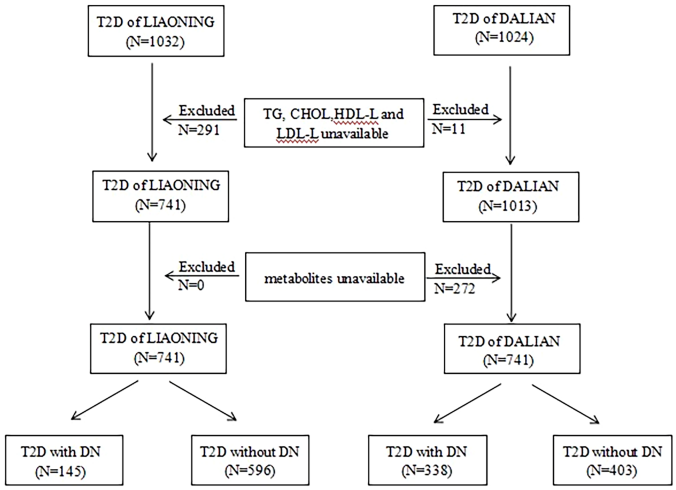

FIGURE 1
Flow chart of selection of participant in this study. T2D, type 2 diabetics; LMUFAH, Liaoning Medical University First Affiliated Hospital; DALIAN, Second Affiliated Hospital of Dalian Medical University; TG, triglyceride; CHOL, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; DN, diabetic nephropathy.

drinker" indicate patients who currently smoke and currently drink alcohol, respectively, regardless of whether they have reduced their consumption. There were no major changes in treatment and mainstream medications for people with diabetes, with negligible differences over time.

The protocol of the study was approved by the Ethics Committee for Clinical Research of the LMUFAH and the Ethics Committee for Clinical Research of the DALIAN. Informed consent was waived because of the nature of the retrospective study, which was in accordance with the Helsinki Declaration of 1964 and its later amendments.

Data collection

Height, weight, and blood pressure were measured by specially trained doctors and nurses using standardized methods. Patients had to remove heavy clothing and shoes while weight and height measurements were obtained. Weight and height measurements were accurate to one decimal point. Blood pressure was not measured until patients had been resting in a sitting position for at least 10 minutes. Body mass index (BMI) was calculated by dividing weight in kilograms by squared height in meters.

After a continuous fasting period of at least 8 hours, patients' blood samples were collected. Lipid profiles were assayed by an automatic biochemical analyzer, including TG, CHOL, HDL-C, and LDL-C. Metabolomic profiles of amino acids including ornithine, arginine, and citrulline were assayed.

Measurement of plasma amino acids related to urea cycle

Methods for the measurement of plasma amino acids have already been discussed in detail in previous publications (22). Briefly, each patient had capillary whole blood drawn after at least 8 hours of fasting; the sample was stored as a dried blood spot and used in the assay of metabolomics. Metabolites in a dried blood spot were measured by direct infusion using mass spectrometry technology and the AB Sciex 4000 QTrap System (AB Sciex, Framingham, MA, USA). We used high-purity water and acetonitrile from Thermo Fisher Scientific (Waltham, MA, USA) as a diluting agent and mobile phase. Samples were 1-butanol and acetyl chloride from Sigma-Aldrich (St Louis, MO, USA). Isotope-labeled internal standard samples of 12 amino acids (NSK-A) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA) and standard samples of the amino acids were purchased from Chromsystems Instruments & Chemicals GmbH (Gräfelfing, Germany).

Statistical analysis

Continuous data of normal distribution were expressed as mean ± standard deviation (SD); if normal distributions were not accepted, the data were expressed as the median (interquartile range). Qualitative data were presented as a number (percentile). Mann–Whitney *U*-tests or Student's *t*-tests were used to compare differences in continuous data.

Chi-squared tests were used to compare the differences in qualitative data. Binary logistic regression was used to obtain odds ratios (ORs) and their 95% confidence intervals (CIs). A structured adjustment scheme was used to control for confounders: model 1, unadjusted; model 2, adjusted for gender and age; model 3, adjusted for covariates in model 2 plus BMI and systolic blood pressure (SBP); model 4, adjusted for covariates in model 3 plus TG, CHOL, HDL-C, and LDL-C; and model 5, adjusted for covariates in model 4 plus anti-diabetic measures. Metabolites' associations with DN risk were pooled with an inverse variance-weighted fixed-effect meta-analysis. Before being introduced into regression models, amino acids related to the urea cycle and their ratios were scaled to SD concentrations. All statistical analyses were performed using the Statistical Analysis System 9.4 (SAS Institute Inc., Cary, NC, USA). A two-tailed p-value <.05 was considered statistically significant.

Results

Characteristics of the population

The clinical characteristics and the baseline demographics of the study individuals from the two populations are presented in Table 1. The LMUFAH patients had a median age of 59.00 years (interquartile range: 50.00 to 68.00 years) and a median BMI of 25.10 kg/m^2 (interquartile range: 22.85 to 27.46 kg/m^2). Levels of SBP, HDL-C, anti-diabetic measures, citrulline, and citrulline/ornithine for those in the DN group were significantly higher than for those in the non-DN group. The arginine–citrulline ratio for those in the DN group was significantly lower than for those in the non-DN group. However, there was no significant difference in gender, age, current smokers, current drinkers, BMI, diastolic blood pressure (DBP), TG, CHOL, LDL-C, ornithine, arginine, or arginine–ornithine ratio between the DN and non-DN groups.

The DALIAN patients had a median age of 61.00 years (interquartile range: 53.00 to 68.00 years) and a median BMI of 26.22 kg/m^2 (interquartile range: 24.10 to 29.06 kg/m^2). HDL-C levels for those in the DN group were significantly lower than for those in the non-DN group. Age, BMI, SBP, and anti-diabetic measures for those in the DN group were significantly higher than for those in the non-DN group. Moreover, gender; current smoking status; current drinking status; levels of DBP, TG, CHOL, LDL-C, ornithine, arginine, and citrulline; and the ratios of arginine to ornithine, citrulline to ornithine, and arginine to citrulline in both the DN and non-DN groups were not significantly different.

In the LMUFAH population and DALIAN population, SBP and anti-diabetic measures for those in the DN groups were significantly higher than for those in the non-DN groups, and HDL-C levels in both the DN and non-DN groups were significantly different.

Associations of amino acids with diabetic nephropathy

In the LMUFAH population, citrulline was positively associated with DN risk in the univariate analyses. The association was still significant after further adjustment for traditional risk factors [OR (95% CI) of per SD increase for citrulline: 1.200 (1.006, 1.432)]. However, the associations of ornithine and arginine with DN risk were not significant.

In the DALIAN population, citrulline was positively associated with DN risk in the univariate analyses. After further adjustment for traditional risk factors, the association was still significant [OR (95% CI) of per SD increase for citrulline: 1.189 (1.012, 1.396)]. However, the associations of ornithine and arginine with DN risk were not significant.

In the fixed-effect pooled analysis, the associations of amino acids related to the urea cycle with DN risk were in accordance with findings in the LMUFAH and DALIAN populations. Citrulline was still positively associated with DN risk. The OR (95% CI) of per SD increase was 1.252 (1.121, 1.398) for citrulline in the univariate analyses. After further adjustment for traditional risk factors, the pooled effect size was 1.194 (1.060, 1.345) for citrulline. The associations of ornithine and arginine with DN risk were not significant (Table 2).

Associations between ratios of amino acids and diabetic nephropathy risk

The relationship between ratios of amino acids in the urea cycle and DN is presented in Table 3. In the LMUFAH population, the ratio of arginine to citrulline was significantly associated with DN risk in the univariate analyses and in the multivariate analyses [the OR (95% CI) of per SD increase for arginine–citrulline: 0.716 (0.571, 0.898)]. The ratio of citrulline to ornithine was associated with DN risk in the univariate analyses. After adjustment for confounders, the association was changed [the OR (95% CI) of per SD increase for citrulline–ornithine: 1.141 (0.950, 1.370)]. However, the association between the ratio of arginine to ornithine and DN risk was not significantly different.

In the DALIAN population, the association between the ratio of citrulline to ornithine and DN risk was significantly different in the univariate analyses and in the multivariate analyses [the OR (95% CI) of per SD increase for citrulline–ornithine: 1.192 (1.021, 1.392)]. However, the ratios of arginine to ornithine and arginine to citrulline were not associated with DN risk.

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TABLE 1 Clinical and biological characteristics of all patients.

	LMUFAH (N = 741)			DALIAN (N = 741)		
	Non-DN (N = 596)	DN (N = 145)	p-value	Non-DN (N = 403)	DN (N = 338)	p-value
Gender (male)	319 (53.52%)	72 (49.66%)	0.403	218 (54.09%)	167 (49.41%)	0.204
Age (years)	58.5 (50.0, 67.0)	59.0 (50.0, 69.0)	0.359	60.0 (51.0, 66.0)	63.0 (53.0, 70.0)	0.000
Current smoker, n (%)	193 (32.38%)	44 (30.34%)	0.637	78 (19.35%)	57 (16.86%)	0.382
Current drinker, n (%)	164 (27.52%)	41 (28.28%)	0.855	41 (10.17%)	31 (9.17%)	0.646
BMI (kg/m^2)	25.0 (22.7, 27.4)	25.7 (22.9, 27.7)	0.316	25.7 (23.9, 29.0)	26.6 (24.4, 29.2)	0.039
SBP (mmHg)	138.0 (122.0, 154.0)	142.0 (126.0, 162.0)	0.010	144.0 (131.0, 154.0)	151.0 (136.0, 169.0)	0.000
DBP (mmHg)	81.0 (74.0, 90.0)	82.0 (73.0, 91.0)	0.710	81.0 (73.0, 89.0)	80.0 (74.0, 89.0)	0.888
TG (mmol/l)	1.64 (1.10, 2.38)	1.72 (1.21, 2.39)	0.396	1.56 (1.04, 2.10)	1.61 (1.15, 2.74)	0.050
CHOL (mmol/l)	4.62 (3.84, 5.27)	4.80 (3.92, 5.57)	0.077	4.99 (4.24, 5.67)	4.92 (4.06, 5.88)	0.420
HDL-C(mmol/l)	1.00 (0.84, 1.24)	1.06 (0.90, 1.30)	0.018	1.18 (1.00, 1.39)	1.14 (0.95, 1.33)	0.046
LDL-C(mmol/l)	2.78 (2.19, 3.37)	2.79 (2.27, 3.49)	0.205	2.58+0.81	2.48+0.88	0.105
Anti-diabetic measures	538 (90.27%)	144 (99.31%)	0.000	320 (79.40%)	302 (89.35%)	0.000
Orn, μmol/l	17.63 (13.18, 23.73)	17.45 (12.63, 23.41)	0.820	11.31 (8.56, 14.92)	11.29 (8.28, 14.46)	0.716
Arg, μmol/l	9.77 (5.43, 17.17)	10.08 (5.70, 15.98)	0.628	2.79 (1.75, 4.34)	3.11 (1.78, 4.42)	0.113
Cit, μmol/l	19.56 (15.20, 25.33)	22.03 (16.98, 27.50)	0.002	22.57 (17.45, 27.69)	22.78 (17.00, 30.58)	0.194
Arg/Orn	0.57 (0.28, 0.98)	0.57 (0.29, 0.90)	0.818	0.24 (0.15, 0.37)	0.26 (0.18, 039)	0.038
Cit/Orn	1.10 (0.77, 1.50)	1.25 (0.94, 1.69)	0.002	2.03 (1.45, 2.68)	2.10 (1.54, 2.83)	0.051
Arg/Cit	0.53 (0.30, 0.85)	0.44 (0.26, 0.70)	0.010	0.13 (0.08, 0.18)	0.13 (0.08, 0.20)	0.587

Orn, ornithine; Arg, arginine; Cit, citrulline; Arg/Orn, arginine–ornithine ratio; Cit/Orn, citrulline–ornithine ratio; Arg/Cit, arginine–citrulline ratio.
Data are mean (standard deviation), median (interquartile range) to n (%).
p-values <.05 were considered statistically significant.

TABLE 2 The difference of amino acids related to the urea cycle by logistic regression in two independent cross-sectional studies.

	Univariate		Multivariate							
	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
LMUFAH, per 1 SD										
Orn, μmol/l	0.924 (0.757, 1.129)	0.441	0.925 (0.758, 1.130)	0.446	0.932 (0.760, 1.142)	0.496	0.943 (0.768, 1.157)	0.573	0.957 (0.774, 1.183)	0.682
Arg, μmol/l	0.896 (0.739, 1.085)	0.261	0.891 (0.732, 1.084)	0.249	0.862 (0.707, 1.051)	0.141	0.861 (0.705, 1.052)	0.144	0.853 (0.697, 1.043)	0.122
Cit, μmol/l	1.278 (1.082, 1.508)	0.004	1.261 (1.065, 1.493)	0.007	1.233 (1.039, 1.463)	0.017	1.202 (1.009, 1.432)	0.039	1.200 (1.006, 1.432)	0.042
DALIAN, per 1 SD										
Orn, μmol/l	0.960 (0.829, 1.111)	0.580	0.961 (0.829, 1.115)	0.603	0.964 (0.829, 1.121)	0.634	0.962 (0.826, 1.121)	0.619	0.953 (0.816, 1.114)	0.548
Arg, μmol/l	1.096 (0.948, 1.266)	0.215	1.092 (0.944, 1.264)	0.237	1.096 (0.944, 1.272)	0.227	1.099 (0.945, 1.279)	0.221	1.086 (0.933, 1.265)	0.285
Cit, μmol/l	1.232 (1.062, 1.428)	0.006	1.188 (1.019, 1.383)	0.027	1.162 (0.994, 1.359)	0.060	1.187 (1.012, 1.393)	0.035	1.189 (1.012, 1.396)	0.035
Pooled, per 1 SD										
Orn, μmol/l	0.947 (0.842, 1.066)	0.369	0.948 (0.842, 1.068)	0.380	0.953 (0.844, 1.075)	0.432	0.955 (0.845, 1.080)	0.463	0.954 (0.842, 1.082)	0.466
Arg, μmol/l	1.019 (0.908, 1.144)	0.751	1.016 (0.903, 1.142)	0.794	1.005 (0.892, 1.132)	0.936	1.006 (0.891, 1.135)	0.927	0.995 (0.881, 1.123)	0.933
Cit, μmol/l	1.252 (1.121, 1.398)	0.000	1.220 (1.090, 1.367)	0.001	1.194 (1.064, 1.340)	0.003	1.194 (1.061, 1.343)	0.003	1.194 (1.060, 1.345)	0.003

Orn, ornithine; Arg, arginine; Cit, citrulline.
Model 1, unadjusted.
Model 2, adjusted for gender and age.
Model 3, adjusted for covariates in model 2 plus BMI and SBP.
Model 4, adjusted for covariates in model 3 plus TG, CHOL, HDL-C, and LDL-C.
Model 5, adjusted for covariates in model 4 plus anti-diabetic measures.

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TABLE 3 Ratios of amino acids related to the urea cycle for the risk of diabetic nephropathy.

	Univariate		Multivariate							
	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
LMUFAH, per 1 SD										
Arg/Orn,	0.918 (0.760, 1.109)	0.376	0.913 (0.753, 1.106)	0.354	0.884 (0.729, 1.072)	0.209	0.880 (0.724, 1.071)	0.202	0.876 (0.718, 1.068)	0.189
Cit/Orn	1.212 (1.023, 1.435)	0.026	1.191 (1.002, 1.415)	0.047	1.170 (0.983, 1.392)	0.077	1.137 (0.951, 1.361)	0.160	1.141 (0.950, 1.370)	0.158
Arg/Cit	0.732 (0.592, 0.905)	0.004	0.737 (0.594, 0.913)	0.005	0.714 (0.574, 0.887)	0.002	0.722 (0.579, 0.901)	0.004	0.716 (0.571, 0.898)	0.004
DALIAN, per 1 SD										
Arg/Orn	1.095 (0.947, 1.265)	0.221	1.094 (0.946, 1.266)	0.225	1.088 (0.939, 1.262)	0.262	1.090 (0.939, 1.266)	0.258	1.080 (0.930, 1.254)	0.316
Cit/Orn	1.213 (1.047, 1.405)	0.010	1.179 (1.016, 1.369)	0.030	1.147 (0.985, 1.334)	0.077	1.173 (1.006, 1.368)	0.042	1.192 (1.021, 1.392)	0.026
Arg/Cit	1.024 (0.886, 1.183)	0.746	1.051 (0.908, 1.217)	0.504	1.064 (0.917, 1.235)	0.412	1.054 (0.907, 1.225)	0.493	1.041 (0.894, 1.211)	0.607
Pooled, per 1 SD										
Arg/Orn	1.026 (0.915, 1.151)	0.663	1.024 (0.912, 1.150)	0.687	1.008 (0.896, 1.133)	0.901	1.007 (0.895, 1.134)	0.903	1.001 (0.889, 1.128)	0.984
Cit/Orn	1.213 (1.085, 1.355)	0.001	1.184 (1.058, 1.326)	0.003	1.157 (1.032, 1.297)	0.012	1.158 (1.030, 1.301)	0.014	1.170 (1.040, 1.317)	0.009
Arg/Cit	0.921 (0.817, 1.037)	0.175	0.939 (0.832, 1.060)	0.309	0.937 (0.829, 1.059)	0.299	0.935 (0.826, 1.059)	0.291	0.927 (0.817, 1.051)	0.238

Arg/Orn, arginine/ornithine; Cit/Orn, citrulline/ornithine; Arg/Cit, arginine/citrulline; Arg/Orn, arginine–ornithine ratio; Cit/Orn, citrulline–ornithine ratio; Arg/Cit, arginine–citrulline ratio.
Model 1, unadjusted.
Model 2, adjusted for gender and age.
Model 3, adjusted for covariates in model 2 plus BMI and SBP.
Model 4 adjusted for covariates in model 3 plus TG, CHOL, HDL-C, and LDL-C.
Model 5, adjusted for covariates in model 4 plus anti-diabetic measures.

In the fixed-effect pooled analysis, the ratio of citrulline to ornithine was associated with DN risk in the univariate analyses and in the multivariate analyses [OR (95% CI) of per SD increase: 1.170 (1.040, 1.317)]. The associations between the ratios of other amino acids and DN risk were not significantly different.

Discussion

One of the serious complications of T2D is DN; patients with DN have an increased risk of developing cardiovascular and cerebrovascular diseases (23, 24). However, discovering a single diagnostic marker of DN is still in the exploration stage. Thus, we urgently need biomarkers for the early diagnosis of DN. In this study, we tested associations between amino acids related to the urea cycle and DN risk. We observed that citrulline was consistently associated with DN risk in two independent cross-sectional studies.

A small study of 78 Japanese participants showed that the correlation coefficients for citrulline were significantly associated with the urinary albumin–creatinine ratio and estimated glomerular filtration rate (25). Consistent with this finding, we found that citrulline was associated with DN. Citrulline is efficiently taken up by the proximal renal tubules, where it is converted to urea *via* arginine (26). Urea is a major end product of nitrogen metabolism. Researchers have found a significant accumulation of urea cycle intermediates in patients with ESRD (27). Owing to the important role kidneys play in the conversion of citrulline to arginine, we speculate that increased serum levels of citrulline in individuals with DN could be related to the degradation of this function. Moreover, citrulline is involved in the synthesis of nitric oxide (NO) in the citrulline–NO cycle (26). NO is identified as an important regulator of renal function and morphology (28). Asymmetric dimethylarginine can be metabolized to citrulline, which potentially prevents the inhibition of endothelial nitric oxide synthase by asymmetric dimethylarginine (29). The elevation level of citrulline in DN patients may be related to dysregulation of the renal NO-producing system. Interestingly, the ratio of citrulline to ornithine was inconsistently associated with DN risk in two independent cross-sectional studies, whereas the pooled result was associated with DN risk. The findings need to be further explored in a broader population, and an analysis of serum NA levels will be helpful in figuring this out.

There are several limitations to this study. First, estimated glomerular filtration rates (eGFRs) have not been evaluated in the present study; therefore, direct evidence about the association between amino acids related to the urea cycle and DN is lacking. Second, the data we collected mainly focused on DN patients in Jinzhou and Dalian, which cannot necessarily be generalized to all DN patients in China. Third, our patients with T2D and DN were in-patients. Their condition might be more serious than that of general patients with T2D and DN. Finally, this study is cross-sectional design: experimental values of blood urea nitrogen,

serum ammonia, serum creatinine, proteinuria, albuminuria, and dietary factors were not included in this study, so we cannot derive a causal relationship between amino acids related to the urea cycle and DN. In summary, the findings of the study need to be evaluated and validated in a broader population.

The present study has important implications for public health. DN is one of the major public health problems worldwide, and it has caused a huge burden on the global health system. Moreover, the incidence of progression of DN to ESRD is increasing (30). Therefore, it is very important to accurately predict the occurrence and development of DN. This study suggests that citrulline might be a candidate marker for future DN risk scoring if these findings can be replicated in cohort studies, especially in China.

In conclusion, citrulline was consistently associated with DN risk in two independent cross-sectional studies of amino acids related to the urea cycle and DN risk. As this was a cross-sectional study, it is necessary to confirm the findings through studies in other populations.

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 for Clinical Research of the LMUFAH and the Ethics Committee for Clinical Research of the DALIAN. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SL and YC conceived the project and designed the experiments. PC wrote the manuscript. BH analyzed data. MH, YJ, RC, and CC collected the information and contributed to the writing of this manuscript. All authors edited the final version of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Key Research and Development Program of China (2019YFA0802302, 2019YFA0802300), the Special Fund of State Key Joint Laboratory of Environment Simulation and Pollution Control, the Development and application of clinical mass spectrometry detection technology for important small molecule metabolites (2020JH2/10300116), the Key R&D Program of Liaoning Province and the Dalian Laboratory Medicine Mass Spectrometry Technology Innovation Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors thank all doctors, nurses, and research staff at the Liaoning Medical University in Jinzhou and the Second Affiliated Hospital of Dalian Medical University for their participation in 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. Bain JR. Targeted metabolomics finds its mark in diabetes research. *Diabetes* (2013) 62:349–51. doi: 10.2337/db12-1189

2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2019. *Diabetes Care* (2019) 42:S13–s28. doi: 10.2337/dc19-S002

3. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* (2001) 414:813–20. doi: 10.1038/414813a

4. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* (2018) 138:271–81. doi: 10.1016/j.diabres.2018.02.023

5. Collins KS, Eadon MT, Cheng YH, Barwinska D, Melo Ferreira R, McCarthy TW, et al. Alterations in protein translation and carboxylic acid catabolic processes in diabetic kidney disease. *Cells* (2022) 11:1166. doi: 10.3390/cells11071166

6. An Y, Xu F, Le W, Ge Y, Zhou M, Chen H, et al. Renal histologic changes and the outcome in patients with diabetic nephropathy. *Nephrol Dial Transplant* (2015) 30:257–66. doi: 10.1093/ndt/gfu250

7. Saran R, Li Y, Robinson B, Ayanian J, Balkrishnan R, Bragg-Gresham J, et al. US Renal data system 2014 annual data report: Epidemiology of kidney disease in the united states. *Am J Kidney Dis* (2015) 66:Svii, S1–305. doi: 10.1053/j.ajkd.2015.05.001

8. Papadopoulou-Marketou N, Kanaka-Gantenbein C, Marketos N, Chrousos GP, Papassotiriou I. Biomarkers of diabetic nephropathy: A 2017 update. *Crit Rev Clin Lab Sci* (2017) 54:326–42. doi: 10.1080/10408363.2017.1377682

9. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, et al. US Renal data system 2018 annual data report: Epidemiology of kidney disease in the united states. *Am J Kidney Dis* (2019) 73:A7–a8. doi: 10.1053/j.ajkd.2019.01.001

10. Kurokawa K, Nangaku M, Saito A, Inagi R, Miyata T. Current issues and future perspectives of chronic renal failure. *J Am Soc Nephrol* (2002) 13 Suppl 1:S3–6. doi: 10.1681/ASN.V13suppl_1s3

11. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* (2010) 375:2073–81. doi: 10.1016/s0140-6736(10)60674-5

12. Zhang L, Wang H, Long J, Shi Y, Bai K, Jiang W, et al. China Kidney disease network (CK-NET) 2014 annual data report. *Am J Kidney Dis* (2017) 69:A4. doi: 10.1053/j.ajkd.2016.06.011

13. Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J. Oxidative stress in diabetic nephropathy with early chronic kidney disease. *J Diabetes Res* (2016) 2016:7047238. doi: 10.1155/2016/7047238

14. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA* (2009) 301:2129–40. doi: 10.1001/jama.2009.726

15. D'Apolito M, Du X, Zong H, Catucci A, Maiuri L, Trivisano T, et al. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. *J Clin Invest* (2010) 120:203–13. doi: 10.1172/jci37672

16. Xie Y, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z. Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. *Kidney Int* (2018) 93:741–52. doi: 10.1016/j.kint.2017.08.033

17. Pietzner M, Kaul A, Henning AK, Kastenmüller G, Artati A, Lerch MM, et al. Comprehensive metabolic profiling of chronic low-grade inflammation among generally healthy individuals. *BMC Med* (2017) 15:210. doi: 10.1186/s12916-017-0974-6

18. Carracedo J, Merino A, Briceño C, Soriano S, Buendía P, Calleros L, et al. Carbamylated low-density lipoprotein induces oxidative stress and accelerated senescence in human endothelial progenitor cells. *FASEB J* (2011) 25:1314–22. doi: 10.1096/fj.10-173377

19. Cao YF, Li J, Zhang Z, Liu J, Sun XY, Feng XF, et al. Plasma levels of amino acids related to urea cycle and risk of type 2 diabetes mellitus in Chinese adults. *Front Endocrinol (Lausanne)* (2019) 10:50. doi: 10.3389/fendo.2019.00050

20. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetes Med* (1998) 15:539–53. doi: 10.1002/(sici)1096-9136(199807)15:7<539::Aid-dia668>3.0.Co;2-s

21. Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes Obes Metab* (2020) 22 Suppl 1:3–15. doi: 10.1111/dom.14007

22. Wang Q, Sun T, Cao Y, Gao P, Dong J, Fang Y, et al. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. *Onco Targets Ther* (2016) 9:1389–98. doi: 10.2147/ott.S95862

23. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primers* (2015) 1:15018. doi: 10.1038/nrdp.2015.18

24. Chou CA, Lin CN, Chiu DT, Chen IW, Chen ST. Tryptophan as a surrogate prognostic marker for diabetic nephropathy. *J Diabetes Investig* (2018) 9:366–74. doi: 10.1111/jdi.12707

25. Hirayama A, Nakashima E, Sugimoto M, Akiyama S, Sato W, Maruyama S, et al. Metabolic profiling reveals new serum biomarkers for differentiating diabetic nephropathy. *Anal Bioanal Chem* (2012) 404:3101–9. doi: 10.1007/s00216-012-6412-x

26. Morris SMJr. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* (2002) 22:87–105. doi: 10.1146/annurev.nutr.22.110801.140547

27. Chuang CK, Lin SP, Chen HH, Chen YC, Wang TJ, Shieh WH, et al. Plasma free amino acids and their metabolites in Taiwanese patients on hemodialysis and continuous ambulatory peritoneal dialysis. *Clin Chim Acta* (2006) 364:209–16. doi: 10.1016/j.cccn.2005.07.001

28. Nakagawa T, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, et al. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. *J Am Soc Nephrol* (2007) 18:539–50. doi: 10.1681/asn.2006050459

29. Lai YL, Aoyama S, Ohata M, Otsuka N, Shiokawa H, Tomono S, et al. Dysregulation of dimethylargininedimethylaminohydrolase/asymmetric dimethylarginine pathway in rat type II diabetic nephropathy. *J Clin Biochem Nutr* (2012) 51:143–9. doi: 10.3164/jcbn.11-33

30. Ahmad J. Management of diabetic nephropathy: Recent progress and future perspective. *Diabetes Metab Syndr* (2015) 9:343–58. doi: 10.1016/j.dsx.2015.02.008

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 18 November 2022
DOI 10.3389/fendo.2022.1011411

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Renal Endocrinology,
a section of the journal
Frontiers in Endocrinology

RECEIVED 04 August 2022
ACCEPTED 31 October 2022
PUBLISHED 18 November 2022

CITATION
Nathiya D, Singh M, Suman S,
Bareth H, Pal N, Jain A and Tomar BS
(2022) Albuminuria, glycemic variability
and effect of flash glucose monitoring
based decision making on short term
glycemic variability in Indian type 2
diabetes patients: Indi-GlyVar study.
Front. Endocrinol. 13:1011411.
doi: 10.3389/fendo.2022.1011411

Albuminuria, glycemic variability and effect of flash glucose monitoring based decision making on short term glycemic variability in Indian type 2 diabetes patients: Indi-GlyVar study

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Aim and scope: Glycemic variability (GV) denotes the fluctuations in the glucose values around the baseline. High glycemic variability is associated with a higher risk of diabetes-associated complications. In this study, we sought to determine the impact of therapeutic interventions based on flash glucose monitoring on rapid, short-term glycemic variability. We also studied the prevalent albuminuria in diabetic kidney disease and its effect on glycemic variability.

Methods: In a 14-day, single-center, prospective intervention study, we measured the GV indices at baseline (days 1–4) and ten days after ambulatory glucose profile-based intervention using flash glucose monitoring (Abbott Libre Pro, Abbott Diabetes Care, Alameda, California, USA) in patients with type 2 diabetes. An EasyGV calculator was used to estimate the flash glucose monitoring (FGM)-derived measures of GV. The primary outcome was to assess the impact of FGMS-based therapeutic interventions on glycemic variability markers: SD, mean amplitude of glycemic excursion [MAGE], continuous overall net glycemic action [CONGA], absolute means of daily differences [MODD], *M* value, and coefficient of variance [%CV], AUC below 70 mg/dl, low blood glucose index, AUC above 180 mg/dl [AUC >180], high blood glucose index [HBGI], and J index. Time-related matrices (time in range (%), time above range (%), and time below range (%) were also calculated from the ambulatory glucose profile. Renal function parameters (serum creatinine, estimated glomerular filtration

rate, urine albumin excretion) were calculated. The GV with regard to albumin excretion rate was compared.

Results: Fifty-eight T2DM patients (63.8%, males) with a mean age of 51.5 ± 11.9 years were studied. When compared with baseline (days 1–4), on day 14, there was a significant improvement in mean sensor glucose (mg/dl) median (IQR) [155 (116–247) *vs* 131 (103–163) (p ≤0.001)], JINDEX [15,878 (7,706–28,298) *vs* 8,812 (5,545–14,130) (p ≤0.001)], HBGI [361 (304–492) *vs* 334 (280–379) (p ≤0.001)], MAGE (mg/dl) [112 (8–146) *vs* 82 (59–109) (p ≤0.001)], M-value [2,477 (1,883–3,848) *vs* 2,156 (1,667–2,656) (p ≤ 0.001)], MAG (mg/dl) [111 (88–132) *vs* 88 (69–102) (p ≤ 0.001)]. Patients with albuminuria at baseline had high mean sensor glucose (mg/dl) median (IQR) [190 (131–200) *vs* 131 (112–156) (p = 0.001)], CONGA (mg/dl) median (IQR) [155 (101–165) *vs* 108 (83–120) (p = 0.001)], JINDEX, HBGI, MAGE (mg/dl), and M-value are, median (IQR) [20,715 (10,970–26,217 *vs* 91,118 (6,504–15,445)) (p ≤ 0.01)], [415 (338–423) *vs* 328 (292–354) (p = 0.001)], [125 (102–196) *vs* 103 (74–143) (p ≤ 0.01)], [3,014 (2,233–3,080) *vs* 2,132 (1,788–2,402) (p ≤0.01)], respectively.

Conclusion: In type 2 diabetes, flash glucose monitoring-guided therapeutic interventions can reduce glycemic variability in a brief span (10 days) of time. Also, albuminuria in type 2 diabetes is associated with high glycemic variability. Reduced diabetes complications may ultimately result from this reduced glycemic variability.

KEYWORDS

glycemic variability, type 2 diabetes, FGMS, albuminuria, diabetic kidney disease

Introduction

In 2021, the International Diabetes Federation (IDF, 10th edition Atlas) estimated the prevalence of diabetes among adults (20–79 years) at around 9%, with 537 million in total. By 2045, this could reach 784 million, with a disproportionately high burden in the Southeast region and middle- to low-income countries (68% *vs.* 46%) (1). If type 2 diabetes is not controlled, diabetic complications include chronic kidney disease (CKD) (40%), ischemic heart disease (30%), cataracts (20%), retinopathy (15.4%), peripheral vascular disease (11.5%), and cerebrovascular accidents (CVAs) (6.9%), will develop in long-term (2–4).

Glycated hemoglobin (HbA1c), a marker of glycemic control, does not account for the fluctuation in blood glucose values. Glycemic variability (GV) matrices are believed to be a more accurate indicator of glycemic control. The American Diabetes Association also recommended time measures (time in range, time above range, and time below range) to evaluate glycemic control (5). The GV was added to the control parameter because variations in glucose levels raise reactive oxygen species (ROS) and impair endothelial function (6, 7). This endothelial dysfunction increases the prevalence of renal (albuminuria, CKD) and atherosclerotic vascular diseases. For the various parts of the glycemic spectrum, several GV measures are utilized, including SD, MAGE, MAG, JINDEX, LBGI, and HBGI.

The ambulatory glucose profile (AGP) and glycemic variability (GV) are frequently generated using retrospective flash glucose monitoring (FGM). The therapy of type 2 diabetes is frequently determined by several clinical factors (age, comorbidities, life expectancy, and risk of hypoglycemia) without considering baseline glycemic variability. This blinded approach may not correct the glycemic variability. Even in cases of well-controlled diabetes, this untreated GV may be the root of diabetic complications. The glycemic variability is influenced by diet, exercise, and drugs. The data regarding the single use of ambulatory glucose and GV-based therapeutic amendments have not yet been studied.

In this study, we tried to see the effect of retrospective FGMS-based decision-making on short-term glycemic variability.

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Material and methods

Study design

This single-center prospective intervention study was conducted from March 2021 to August 2021 in the endocrinology outpatient department (OPD) of the National Institute of Medical Sciences and Research Hospital, Rajasthan, Jaipur, India. The protocol was developed under the principles of the Declaration of Helsinki and approved by the Institutional Ethics Committee (IEC No. NIMSUR/IEC/2021/0112). We obtained informed consent from the study participants.

Study population

The study enrolled patients aged 18 to 70 with type 2 diabetes mellitus and HbA1c levels ranging from 6.5 to 11.5%. We excluded patients with advanced diabetic complications. The following were the exclusion criteria: Serum creatinine >1.5 mg/dl, eGFR 30 ml, severe NPDR (Non-Proliferative Diabetic Retinopathy) or higher, including PDR (Proliferative Diabetic Retinopathy), uncontrolled CAD (coronary artery disease), angina, and heart failure. We also excluded immunocompromised individuals, patients with active malignant disease, and patients with chronic conditions such as heart failure, cognitive disorders, dementia, amnesia, autoimmune diseases, drug addiction, pregnancy, and breastfeeding females.

Collection of the demographic details and clinical data

We obtained demographic information about the patients, such as their age, gender, place of residence, and social status. We also obtained clinical data on diabetes duration, anti-diabetic medicines, comorbidities, and complications.

Anthropometric measurements

We measured height and weight and calculated the body mass index (BMI). The waist circumference and hip circumference were measured using flexible fiberglass tape. The waist–hip ratio (WHR) was determined using the above two measures.

Blood investigations

Glycosylated hemoglobin (HPLC, Bio-Rad 2, Alfred Noble Drive, Hercules, California, USA) was used to assess glycemic control. We measured blood urea, serum creatinine, urine albumin creatinine ratio (UACR), serum bilirubin, serum albumin, serum AST, and ALT (HUMAN analyzer, Gesellschaft für Biochemical and Diagnostic GmbH Wiesbaden, Germany) to diagnose diabetes complications and end-organ dysfunction. We performed a direct fundus examination to rule out diabetic retinopathy. We performed a fasting lipid profile (HUMAN analyzer, Gesellschaft für Biochemical and Diagnostic GmbH Wiesbaden, Germany) to assess diabetes-associated dyslipidemia.

The patients were categorized as the urine albumin creatinine ratio. If the fasting morning spot is done, then albumin excretion <30 mg/g of creatinine is considered normal.

Flash glucose monitoring system

The Abbott FreeStyle Libre Pro Flash Glucose Monitoring System (FGMS) (Abbott Diabetic Care, Alameda, California, USA) was used to calculate glycemic variability. Abbott FreeStyle Libre Pro is a retrospective tool that can be used for 14 days. The Abbott Libre Pro's MARD (mean absolute relative difference) is 10.1% (80–180 mg/dl—10.7%, >180 mg/dl—8.7%, 70 mg/dl—14%). Compared to the YSI reference, 99.9% of the glucose values were in the Consensus Error Grid Zones A and B.

Sensor insertion and GV calculation

After obtaining the patient's consent, we inserted the sensor. Patients were advised to continue with the same diet, exercise, and diabetes medications for four days before returning to the OPD. Patients were asked to come on the fifth day after four days to collect baseline GV data. The variables of the glycemic variability were also derived by the excel-based calculator (EasyGV, Nuffield Primary Care Department, University of Oxford, Radcliffe Primary Care Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford, OX2 6GG, United Kingdom). These variables were MAGE (mean average glycemic excursion), SD (standard deviation), HBGI (high blood glucose index), LBGI (low blood glucose index), M-value, CONGA (continuous overall net glycemic action), MAG (mean absolute glucose), MODD (mean of daily differences), J-index, and LI (lability index). We discussed ambulatory glucose profile patterns with the patients. The AGP guided the therapeutic changes (diet, exercise, and medicines). The patients were counseled about nutrition using a food frequency questionnaire. They were given a standard diabetic diet chart using the plate technique adapted from the ICMR Nutritive Value of Indian Foods, National Institute of Nutrition, Hyderabad.

FGMS-based therapeutic amendments and follow up

On day five, the following interventions were based on the ambulatory glucose profile and guided by the diet and exercise log.

A. Meal and post-meal excursions were analyzed. The meal pattern was altered if the glycemic variability was caused by an irregular diet, such as poor meal timing or content.
B. The anti-diabetic medication was modified based on the ambulatory glucose profile and trends.
C. If physical activity is associated with increased glycemic variability, the kind, frequency, and intensity of the exercise were adjusted as needed. Mid-activity snacks were introduced to prevent hypoglycemia during exercising.

We asked the patients to return after the flash glucose monitoring system was completed (after 14 days). At the time, we downloaded the sensor data and used the EasyGV calculator to calculate the GV matrices (version 9.0.R2 2).

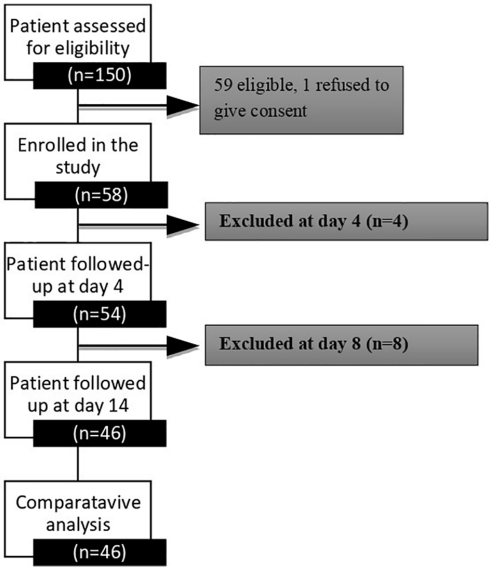

FIGURE 1
A schematic study plan in consideration of the patients enrolled in the study.

Statistical analysis

The software IBM Statistical Package for Social Sciences (SPSS) v22 was used for data entry and statistical analysis (IBM Corp. Version 22, Chicago, Illinois, USA).

The mean ± standard deviation (SD) or median (IQR) was used to represent continuous variables, depending on whether the data distribution was parametric or non-parametric, as determined by the Shapiro–Wilk test. The paired t-test was applied to compare pre-post means in parametric data. In non-parametric data, the Wilcoxon signed rank test was applied to compare pre-post means. Pearson's and Spearman's correlations were used to determine the association between GV parameters and their determinants. Multiple logistic regression was used to determine the association between GV and UACR. The level of statistical significance was set at $p < 0.05$.

Results

One hundred fifty patients were screened at the Nims Hospital and Research Centre's Endocrine Outpatient Department, and fifty-nine were found to be eligible. The study flow sheet is depicted in Figure 1. Fifty-eight patients were enrolled in the study. Thirteen patients were excluded from the study for a variety of reasons; including patient withdrawal related to logistical issues (n−3), therapeutic modifications (n−3), sensor dysfunction (n−6), and patient refusal to consent (n−1). We have a complete data set of 46 (79.3%) patients at the end of this study.

Baseline demographics

Fifty-eight T2DM patients (63.8% were males) with a mean age of 51.5 ± 11.9 years were studied. Twenty-five (43.2%) were unemployed or retired, 25 (43.2%) were active smokers, and nine (17.3%) were alcoholics. The detailed demographic variables are shown in Table 1.

TABLE 1 Baseline demographic variables of the patients, showing profession and social status.

Variables	Values
Age [mean ± Std. Error] (yrs)	51.47 ± 11.89
Gender [% (n)]	
Male	63.8 (37)
Female	36.2 (21)
Occupation [% (n)]	
Professional	10.3 (6)
Semi-professional	8.6 (5)
Clerical, Shop owner farmer	17.3 (10)

(Continued)

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TABLE 1 Continued

Variables	Values
Skilled worker	10.3 (6)
Semi-Skilled worker	6.9 (4)
Unskilled Worker	3.4 (2)
Unemployed	43.2 (25)
Social Economic Status [% (n)]	
Lower Class	5.2 (3)
Upper Lower Class	22.4 (13)
Lower Middle Class	39.7 (23)
Upper Middle Class	22.4 (13)
Upper Class	10.3 (6)
Education [% (n)]	
Graduate/Postgraduate	7.2 (10)
Intermediate/Post-high School diploma	10.3 (6)
High School	10.3 (6)
Middle School	12.2(7)
Primary School	17.2 (10)
Illiterate	32.8 (19)

Glycemic variability and albumin excretion

We compared the glycemic variability in patients with normal and abnormal UACR and found that mean sensor glucose (mg/dl) [median (IQR)] [130 (110–150) *vs* 189 (138–195) (p = 0.001)], CONGA (mg/dl) [median (IQR)] [104 (81–118) *vs* 152 (109–164) (p ≤0.01)], JINDEX [median (IQR)] [9,118 (6,504–15,445) *vs* 20,715 (10,970–26,217) (p ≤0.01)], HBGI [median (IQR)] [328 (292–354) *vs* 415 (338–423) (p = 0.001)], MAGE (mg/dl) [median (IQR)] [103 (74–143) *vs* 125 (102–196) (p ≤0.01)], and M-value [median (IQR)] [2,132 (1,788–2,402) *vs* 3,014 (2,233–3,080) (p ≤0.01)] were significantly higher in the patients with abnormal UACR. Table 2 illustrates this.

The UACR correlated with the mean sensor glucose (mg/dl) (0.527) (p = 0.001), CONGA (mg/dl) (0.501) (p = 0.01), JINDEX (0.529) (p = 0.001), HBGI (0.521) (p = 0.001), MAGE (mg/dl) (0.502) (p = 0.01), M-value (0.528) (p = 0.001). UACR and GV analysis are shown in Table 3.

TABLE 2 A comparison of glycemic variability in patients with normal and abnormal urine albumin excretion ratios revealed that patients with high albumin creatinine ratios had poor glycemic variability.

Determinants	Urine albumin excretion rate (mg/day)			Urine albumin creatinine ratio (mg/g)		
	Normal [median (IQR)]	Abnormal [median (IQR)]	*p-Value*	Normal [median (IQR)]	Abnormal [median (IQR)]	*p-Value*
AGE (Years)	51 ± 12	**51** ± 9	0.53	53.1 ± 12	**49** ± 10	0.14
MALE [% (n)]	36 (n = 21)	22 (n = 13)	0.83	32 (n = 19)	**25 (n = 15)**	0.84
BMI (kg/m^2)	25.8 ± 4.7	26.9 ± 4.7	0.33	26.4 ± 4.3	26 ± 5.3	0.96
WHR	0.92 ± 0.09	0.97 ± 0.07	0.20	0.93 ± 0.08	0.95 ± 0.08	0.56
MEAN (mg/dl)	**131 (112–156)**	**190 (131–200)**	**0.01***	**130 (110–150)**	**189 (138–195)**	**0.001****
SD (mg/dl)	43 (36–65)	57 (39–76)	0.08	**41 (35–64)**	**63 (43–89)**	**<0.01****
CV (%)	34 (27–40)	33 (32–38)	0.87	32 (26–39)	36 (32–45)	0.87
CONGA (mg/dl)	**108 (83–120)**	**155 (101–165)**	**0.02***	**104 (81–118)**	**152 (109–164)**	**<0.01****
LI	2,711 (1,842–4,690)	3,185 (2,140–7,105)	0.23	**2,705 (1,619–4,490)**	**3,596 (2,543–7,399)**	**0.01***
JINDEX	**16,447 (9,264–26,898)**	**19,499 (9,468–26,431)**	**0.03***	**9,118 (6,504–15,445)**	**20,715 (10,970–26,217)**	**<0.01****
HBGI (mg/dl)	**332 (295–354)**	**415 (329–429)**	**<0.01****	**328 (292–354)**	**415 (338–423)**	**0.001****
MAGE (mg/dl)	110 (81–150)	115 (89–174)	0.14	**103 (74–143)**	**125 (102–196)**	**<0.01****
M-VALUE	**2,162 (1,813–2,469)**	**3,029 (2,133–3,154)**	**<0.01****	**2,132 (1,788–2,402)**	**3,014 (2,233–3,080)**	**<0.01****
MAG	88 (79–104)	89 (77–112)	0.29	**87 (78–101)**	**94 (80–117)**	**0.01***
TIR (%)	74 (46–87)	52 (31–77)	0.05	**76 (58–88)**	**52 (30–76)**	**<0.01****
TAR (%)	**35 (12–68)**	**48 (16–66)**	**0.01***	**11 (4.5–33.7)**	**48 (18–66)**	**<0.01****
TBR (%)	**15 (9–27)**	**0.5 (0–6)**	**0.01***	8.5 (1–13.5)	1 (0–6)	0.16
HbA1C (%)	7.6 (6.2–9.5)	8.0 (6.7–11.7)	0.05	**7.5 (6.1–9.8)**	**8.6 (6.8–11.4)**	**<0.01****
NET AUC>180 (mg/dl/min)	90,735 (30,975–375,075)	545,407 (74,032–874,241)	0.06	**73,245 (27,427–346,106)**	**607,245 (97,920–827,685)**	**<0.01****
NET AUC<70 (mg/dl/min)	**16,860 (1,387–33,382)**	**1,432 (78–7,353)**	**0.01***	15,232 (435–68,296)	2,010 (690–12,135)	0.26

The bold values represent the significant values, we have used them to highlight values. *p-value<0.05; **p-value <0.01.

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TABLE 3 Correlation of the spot urine albumin creatinine ratio (mg/g) with glycemic variability variables at baseline (days 1–4).

	MEAN (mg/dl)	SD(mg/dl)	CV (%)	CONGA (mg/dl)	LI	JINDEX	HBGI	MAGE (mg/dl)	MVALUE	MAG (mg/dl)	TIR (%)	TAR (%)	TBR (%)	HbA1C (%)
UUrine albumin creatinine ratio (mg/g)	**<0.001*** (0.527)**	**<0.01** (0.511)**	**00.08 (0.270)**	**<0.01** (0.501)**	**<0.01** (0.447)**	**<0.001*** (0.529)**	**<0.001*** (0.521)**	**<0.01** (0.502)**	**<0.001*** (0.528)**	**<0.01** (0.442)**	**<0.01** (-0.496)**	**<0.001*** (0.518)**	00.33 (-0.150)	**00.01* (0.352)**

The bold values represent the significant values, we have used them to highlight values. *p-value <0.05; **p-value <0.01; ***p-value <0.001.

Effect of FGMS-based therapeutic amendments on short-term glycemic variability

We assessed glycemic variability at baseline (days 1–4), generated (diet, exercise, and drugs) on day 5, and subsequently measured GV on day 14. The glycemic variability between day fourteen (after the intervention) and baseline (days 1–4 before the intervention) was compared. There was a significant improvement in mean sensor glucose (mg/dl) median (IQR) [155 (116–247) *vs* 131 (103–163) (p ≤0.001)], JINDEX [15,878 (7,706–28,298) *vs* 8,812 (5,545–14,130) (p ≤0.001)], HBGI [361 (304–492) *vs* 334 (280–379) (p ≤0.001)], MAGE (mg/dl) [112 (8–146) *vs* 82 (59–109) (p ≤0.001)], M-value [2,477 (1,883–3,848) *vs* 2,156 (1,667–2,656) (p ≤0.001)], MAG (mg/dl) [111 (88–132) *vs* 88 (69–102) (p ≤0.001)]. CV, on the other hand, was numerically lower but failed to achieve statistical significance. This is shown in Table 4 and Figure 2.

Safety profile and product compliance

During the study, no adverse drug reactions were reported. The given products were well received by all patients.

Discussion

The effect of retrospective flash glucose monitoring-based decision-making on glycemic variability was investigated in this study. The therapeutic changes based on flash glucose monitoring reduced glycemic variability in a very short period. Patients with high glycemic variability also had a high albumin excretion rate.

Poor GV carries a high risk of diabetic complications, specifically cardiovascular disease. Twenty to forty percent of type 2 diabetes patients have albuminuria (8–10). Patients with obesity, hypertension, and dyslipidemia are more likely to have albuminuria. Additionally, albuminuria has been linked to a higher risk of cardiovascular disease (11). This synergy exists because glycemic variability is associated with endothelial dysfunction, and albuminuria reflects endothelial dysfunction. In one study, patients with a stroke and a high J-index at the time of admission had an increased risk of cardiovascular death and 3-P MACE (12). GV is associated with myocardial damage and predicts mortality in patients with myocardial infarction (13). This proves that endothelial dysfunction plays a central role in cardiovascular and renal diseases.

The flash glucose monitoring system improves doctors' therapeutic decision-making (drugs, diet, and exercise) over traditional blood glucose self-monitoring by producing the ambulatory glucose profile and glycemic patterns. In an Indian

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TABLE 4 The effect of FGMS-based therapeutic decision making on short-term glycemic variability.

Glycemic Variability Indices	Baseline (Days 1–4)(Pre-intervention[#]) Median (IQR)	Day 14 (Post-intervention)Median (IQR)	p-Value
Mean sensor glucose (mg/dl)	155 (116–247)	131 (103–163)	**<0.001*****
Standard deviation of glucose (mg/dl)	46 (32–62)	32 (24–51)	**<0.01****
Coefficient of Variation (%)	28 (23–32)	27 (21–34)	0.109
Continuous overall net glycemic action (CONGA) (mg/dl)	117 (82–201)	103 (76–132)	**<0.01****
Lability Index (LI)	4,061 (2,067–6,631)	1,869 (1,171–4,520)	**<0.01****
JINDEX	15,878 (7,706–28,298)	8,812 (5,545–14,130)	**<0.001*****
High blood glucose index	361 (304–492)	334 (280–379)	**<0.001*****
Mean amplitude of glycemic excursion (mg/dl)	112 (84–146)	82 (59–109)	**<0.001*****
M-value	2,477 (1,883–3,848)	2,156 (1,667–2,656)	**<0.001*****
Mean absolute glucose (mg/dl)	111 (88–132)	88 (69–102)	**<0.001*****

**p-value <0.01, ***p-value <0.001.
[#]The ambulatory glucose profile and glycemic trends were discussed with the patients on day five.

multi-centric study, 181 patients with type 2 diabetes were studied using iPro-2 retrospective CGMS. Although the glycemic variability matrix was not studied, they demonstrated that the therapeutic change based on the overlay resulted in diabetes improvement (14). On the other hand, we used retrospective FGMS and adjusted therapy accordingly, resulting in rapid control of GV.

This is the first study of its kind to investigate glycemic variability and the effect of ambulatory glucose profile-based decision-making (diet, exercise, and medicines) on short-term GV. MAGE, which indicates postprandial excursion, is associated with long-term cardiovascular disease. In our study, MAGE improved within ten days. This could lead to improved cardiovascular outcomes.

The study did have some limitations, most of which were due to COVID-19. The sample size was small, and the study was brief. There is also a lack of long-term data to assess the impact on diabetic complications. However, we expect the complications to decrease over time as glycemic variability decreases. A more comprehensive study is needed to determine the impact on diabetic complications.

Conclusions

High glycemic variability is linked to albuminuria in type 2 diabetes or vice versa. In a very short period, a treatment intervention based on flash glucose monitoring decreased the

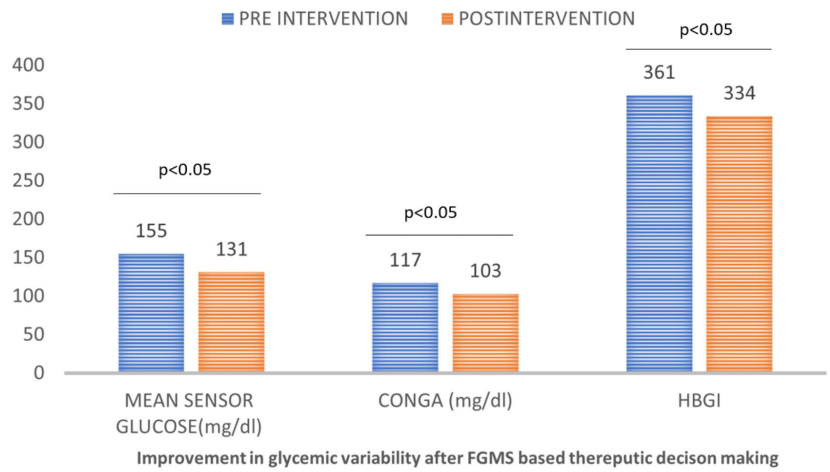

FIGURE 2
Effect of FGMS based therapeutic decision making on short-term glycemic variability.

glycemic variability (10 days). A longer follow-up is needed to see the effect on diabetic complications.

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 Institutional Ethical Committee, Nims University Rajasthan. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MS: Conceptualization, methodology, investigation, validation, and original draft writing. DN: Methodology, project administration, and supervision. SS: Investigation, writing, reviewing, and editing. HB: Investigation, methodology, reviewing, and editing. NP: Investigation, formal analysis, original draft writing. AJ: Investigation, data interpretation, formal analysis, and original draft writing. BT: Conceptualization, supervision, resources, funding, and critical review. All authors contributed to the article and approved the submitted version.

Funding

The funding for FGMS was provided by the NIMS University Rajasthan, Jaipur, India. There was no other funding received from any other sources.

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.

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References

1. IDF diabetes atlas 2021. In: *IDF diabetes atlas.* (Atlas: The International Diabetes Federation (IDF)). Available at: https://diabetesatlas.org/atlas/tenth-edition/.

2. Vaz NC, Ferreira AM, Kulkarni MS, Vaz FS, Pinto NR. Prevalence of diabetic complications in rural goa, India. *Indian J Community Med* (2011) 36(4):283. doi: 10.4103/0970-0218.91330

3. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, et al. Diabetic kidney disease: A report from an ADA consensus conference. *Diabetes Care* (2014) 37(10):2864–83. doi: 10.2337/dc14-1296

4. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* (2017) 88–98. doi: 10.1038/nrendo.2017.151

5. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care* (2017) 40(12):1631–40. doi: 10.2337/dc17-1600

6. Saisho Y. Glycemic variability and oxidative stress: A link between diabetes and cardiovascular disease? *Int J Mol Sci* (2014) Vol. 15:18381–406. doi: 10.3390/ijms151018381

7. Zhang ZY, Miao LF, Qian LL, Wang N, Qi MM, Zhang YM, et al. Molecular mechanisms of glucose fluctuations on diabetic complications. *Front Endocrinol (Lausanne)* (2019) :10: 640. doi: 10.3389/fendo.2019.00640

8. Varghese A, Deepa R, Rema M, Mohan V. Prevalence of microalbuminuria in type 2 diabetes mellitus at a diabetes centre in southern India. *Postgrad Med J* (2001) 77(908):399–402. doi: 10.1136/pmj.77.908.399

9. Sana MA, Chaudhry M, Malik A, Iqbal N, Zakiuddin A, Abdullah M. Prevalence of microalbuminuria in type 2 diabetes mellitus. *Cureus* (2020) 12 (12):266–9. doi: 10.7759/cureus.12318

10. Shikata K, Kodera R, Utsunomiya K, Koya D, Nishimura R, Miyamoto S, et al. Prevalence of albuminuria and renal dysfunction, and related clinical factors in Japanese patients with diabetes: The Japan diabetes complication and its prevention prospective study 5. *J Diabetes Investig* (2020) 11(2):325–32. https://onlinelibrary.wiley.com/doi/full/10.1111/jdi.13116. doi: 10.1111/jdi.13116

11. Fangel MV, Nielsen PB, Kristensen JK, Larsen TB, Overvad TF, Lip GY, et al. Albuminuria and risk of cardiovascular events and mortality in a general population of patients with type 2 diabetes without cardiovascular disease: A Danish cohort study. *Am J Med* (2020) 133(6):e269–79. doi: 10.1016/j.amjmed.2019.10.042

12. Yoon JE, Sunwoo JS, Kim JS, Roh H, Ahn MY, Woo HY, et al. Poststroke glycemic variability increased recurrent cardiovascular events in diabetic patients. *J Diabetes Complications* (2017) 31(2):390–4. doi: 10.1016/j.jdiacomp.2016.11.014

13. Oka S, Deyama J, Umetani K, Harama T, Shimizu T, Makino A, et al. Glycemic variability is associated with myocardial damage in nondiabetic patients with ST-elevation myocardial infarction. *Cardiovasc Endocrinol Metab* (2018) 7 (2):47. doi: 10.1097/xce.0000000000000145

14. Mohan V, Kesavadev J, Jain S, Chawla M, Mutha A, Viswanathan V, et al. Use of retrospective continuous glucose monitoring for optimizing management of type 2 diabetes in India primary prevention of diabetes view project effect of psychological counseling in overcoming fear of self injecting and self testing among type 2 diabetic patients on insulin initiation view project use of retrospective continuous glucose monitoring for optimizing management of type 2 diabetes in India. *Article J Assoc Physicians India* (2016) 64:16–21.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 19 December 2022
DOI 10.3389/fendo.2022.1000650

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 22 July 2022
ACCEPTED 01 December 2022
PUBLISHED 19 December 2022

CITATION
Lau LHY, Nano J, Prehn C, Cecil A, Rathmann W, Zeller T, Lechner A, Adamski J, Peters A and Thorand B (2022) Associations of endogenous androgens and sex hormone-binding globulin with kidney function and chronic kidney disease. *Front. Endocrinol.* 13:1000650. doi: 10.3389/fendo.2022.1000650

Associations of endogenous androgens and sex hormone-binding globulin with kidney function and chronic kidney disease

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Introduction: The role of endogenous androgens in kidney function and disease has not been extensively explored in men and women.

Research design and methods: We analyzed data from the observational KORA F4 study and its follow-up examination KORA FF4 (median follow-up time 6.5 years) including 1293 men and 650 peri- and postmenopausal women, not using exogenous sex hormones. We examined the associations between endogenous androgens (testosterone [T], dihydrotestosterone [DHT], free T [fT], free DHT [fDHT], and T/DHT), with estimated glomerular filtration rate (eGFR) at baseline and follow-up, prevalent, and incident chronic kidney disease (CKD) adjusting for common CKD risk factors.

Results: At baseline, 73 men (5.7%) and 54 women (8.4%) had prevalent CKD. Cross-sectionally, no significant associations between androgens and kidney function were observed among men. In women, elevated T (β=-1.305, [95%

CI -2.290; -0.320]) and fT (β=-1.423, [95% CI -2.449; -0.397]) were associated with lower eGFR. Prospectively, 81 men (8.8%) and 60 women (15.2%) developed incident CKD. In women, a reverse J-shaped associations was observed between DHT and incident CKD ($P_{non\text{-}linear}$=0.029), while higher fDHT was associated with lower incident CKD risk (odds ratio per 1 standard deviation=0.613, [95% CI 0.369; 0.971]. Among men, T/DHT (β=-0.819, [95% CI -1.413; -0.226]) and SHBG ($P_{non\text{-}linear}$=0.011) were associated with eGFR at follow-up but not with incident CKD. Some associations appeared to be modified by type 2 diabetes (T2D).

Conclusion: Suggestive associations are observed of androgens and SHBG with kidney impairment among men and women. However, larger well-phenotyped prospective studies are required to further elucidate the potential of androgens, SHBG, and T2D as modifiable risk factors for kidney function and CKD.

KEYWORDS

testosterone (T), dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), chronic kidney disease, type 2 diabetes, kidney function

1 Introduction

Age-related kidney function decline can lead to CKD (1, 2). CKD development is accelerated by increasing prevalence of its risk factors such as obesity, smoking, hypertension and pre-eminently, T2D (2). As a well-known independent risk factor for cardiovascular and all-cause mortality, CKD represents a burgeoning silent epidemic straining healthcare systems (3). As CKD progresses faster in men (2), more focus has recently been placed on understanding the role of androgens in CKD development.

Aberrant androgen levels are a characteristic manifestation of CKD. Hypogonadism (a condition characterized by low T levels) is prevalent among men with CKD. Among women with CKD, androgen profiles are unclear (4), although women with metabolic syndrome show elevated T levels (5). Epidemiological reports in this context are inconsistent. Some observational cross-sectional (6) and prospective studies (7) report reduced kidney function with lower T levels in men, while others showed no differences (8, 9). In women, an observational prospective (9) and a Mendelian randomization (MR) study (10) found no relationships between T and kidney function. Further, despite being implicated in cardiovascular disease (CVD) (11) and sodium reabsorption (12), the role of DHT in kidney function has been barely evaluated (9, 13).

While bound to sex hormone-binding globulin (SHBG), a protein involved in sex hormone transportation, androgens are inactive. Meanwhile, free (unbound) androgens exert their effects in target tissues through androgen receptor binding (14). SHBG was prospectively-associated with better kidney function (9) and causally associated with lower CKD risk among men, but not among women (15). Few studies (9, 15) investigated the association between SHBG and kidney function in women. Therefore, additional investigations are needed.

Diabetic kidney disease (DKD) comprises 30-50% of CKD cases (16). T2D, representing an additional disease burden, could influence the relationship between androgens and kidney function. While sex-specific differences in androgen levels are evident in T2D (5), it remains unclear whether the putative associations between androgens and kidney function differ between person with and without T2D.

Therefore, the present study aimed to evaluate cross-sectional and prospective associations between levels of endogenous androgens and SHBG with measures of kidney function, as estimated by eGFR, in men and peri-/postmenopausal women from the general population. In addition, we aimed to assess whether any putative associations between androgens and kidney function were modified by the presence of T2D at baseline.

2 Methods

2.1 Study population

Data were obtained from the Cooperative Health Research in the Region of Augsburg (KORA) baseline (F4) (2006-2008)

and follow-up (FF4) studies (2013-2014). Both studies are follow-up examinations of the KORA S4 study (1999-2001) conducted in Augsburg, Southern Germany, and two surrounding counties. The study design has been described previously in detail (17).

The KORA F4 study included 3080 participants aged between 32 and 81 years, of which 2161 participated in KORA FF4. Three participants who withdrew consent and 570 premenopausal women were excluded from the analyses. After further exclusions as described in Figure 1, the final sample for the cross-sectional analyses comprised 1943 participants (1293 men and 650 peri-/postmenopausal women), while the prospective analyses sample comprised 1349 participants (933 men and 416 peri-/postmenopausal women) for follow-up eGFR and 1294 participants for incident CKD after exclusion of 55 participants with prevalent CKD.

2.2 Kidney function

Glomerular filtration rates were estimated based on serum creatinine concentrations using the Chronic Kidney Disease Epidemiology Collaboration formula (18). In the main analysis, prevalent CKD was defined as an eGFR <60 ml/min/1.73m² at baseline. Incident CKD was defined as eGFR <60 ml/min/1.73m² at follow-up in participants without prevalent CKD at baseline. Serum creatinine was measured in fresh serum with a modified Jaffe test (KREA Flex, Dade Behring) in F4 and the first part of FF4, whilst the standard Jaffe method was used in the second part of FF4 (Roche Cobas 8000 instrument). All measurements were calibrated to IDMS standards.

2.3 Androgen and SHBG quantification

Serum samples at baseline were collected and stored at -80°C until the analysis of androgens and SHBG. T and DHT were quantified in serum using liquid chromatography-electrospray ionization-tandem mass spectrometry and the AbsoluteIDQ Stero17 Kit (BIOCRATES Life Sciences, Austria) (19). The calibration, imputation, and normalization of sex hormone measurements are described in detail in the Supplementary Methods and Materials 1. For T measurements the intra-assay CV was 10.3%, the lower limit of quantification (LLOQ) was 0.35 nmol/L, and the upper limit of quantification (ULOQ) was 34.7 nmol/L. For DHT respective values were as follows: intra-assay CV: 11.1%, LLOQ: 0.04 nmol/L, ULOQ: 10.2 nmol/L. Intra-assay CVs were calculated using the means from five quality

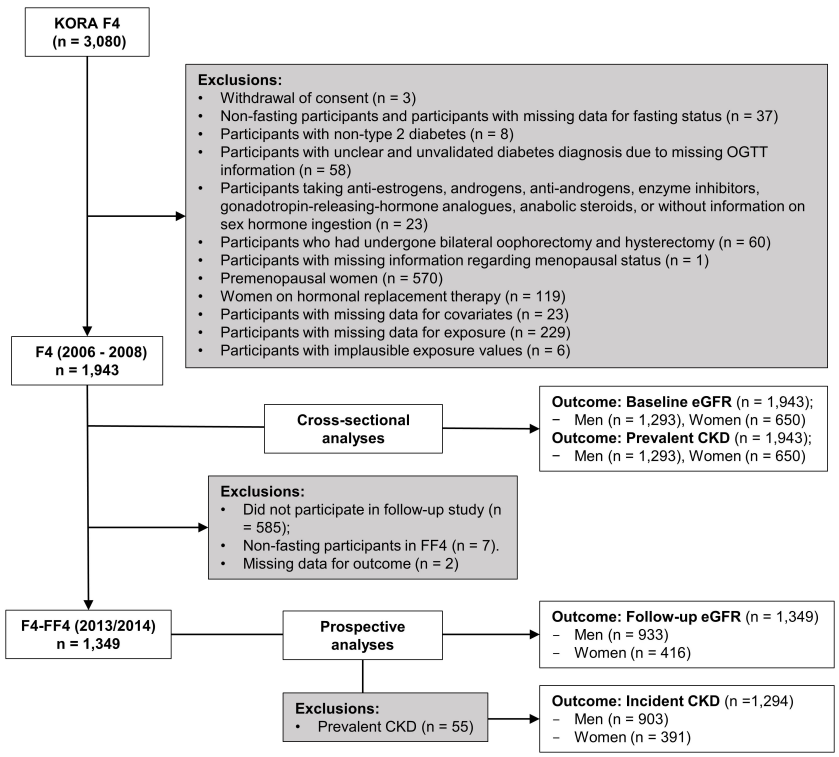

FIGURE 1
Exclusion flowchart.

control samples and means over thirty-nine plates. T/DHT was calculated by dividing T concentrations by those of DHT. fT and fDHT were calculated based on measured T, DHT, SHBG, and serum albumin using the formula derived by Mazer (20). SHBG in serum was quantified using the ARCHITECT SHBG assay, a chemilumineschent microparticle immunoassay (Abbott Laboratories, USA). SHBG samples had an intra-assay CV of 4.3%. Serum albumin was quantified using immunophelometry (ALB Flex; Dade Behring, Germany).

2.4 Covariates

At baseline, non-high-density lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from total cholesterol to account for all LDL cholesterol types. Total cholesterol and HDL-C were measured in fresh serum by enzymatic methods (CHOL Flex and AHDL Flex, Dade Behring). C reactive protein (CRP) was quantified from frozen plasma using a high-sensitivity latex-enhanced nephelometric assay (BN II Analyzer, Dade Behring). Thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) were quantified using immunochemiluminescent procedures (Dimension Vista System, Siemens, Germany).

Information on age, sex, waist circumference, prevalent cardiovascular diseases (CVD), anti-hypertensive medication usage, lipid-lowering medication usage, blood pressure, smoking status, alcohol consumption, and physical activity were assessed using a standardized interview, performed by trained medical staff (17). A participant was considered to have prevalent CVD if they had a history of either myocardial infarction, stroke, or angina pectoris. Participants' medication use within seven days before the examination was assessed by trained medical staff using the IDOM database (21). Smoking status was categorized as never smokers, former smokers, and current smokers (≥1 cigarette a day). Physical activity was estimated through two separate four-category interview questions regarding the time spent per week on sport activities in summer and winter. Possible answers were (1) >2 hours, (2) 1-2 hours, (3) <1 hour, and (4) none. Participants who had a total score of <5, obtained by summing the numbers (1)-(4) relating to winter and summer, were considered to be 'physically active'. Alcohol consumption was categorized into three groups: no consumption (0 g/day), moderate consumption (men 0.1-39.9 g/day, women 0.1-19.9 g/day), and high consumption (men ≥40 g/day, women ≥20 g/day) (22).

Previously known T2D was defined as a self-reported T2D diagnosis that was validated by a physician or medical chart review, or as self-reported current use of glucose-lowering mediation. Participants without known T2D obtained a standard 75g oral glucose tolerance test. Blood samples were taken without stasis after an overnight fast of ≥8 hours and 2 hours after glucose solution ingestion. Serum glucose was measured using hexokinase-G6PD (GLUFlex, Dade Behring, USA). Normoglycemia (NGT) (fasting glucose (FG) <6.1 mmol/L and 2 hour-glucose (2hG) <7.8 mmol/L), prediabetes [6.1 ≤ FG <7.0 mmol/L and 2hG <7.8 mmol/L (isolated impaired fasting glucose (IFG)] or FG <6.1 mmol/L and 7.8 ≤ 2hG <11.1 mmol/L (isolated impaired glucose tolerance (IGT)), or both (IFG and IGT)), and newly-diagnosed diabetes (FG ≥7.0 mmol/L or 2hG ≥11.1 mmol/L) were defined according to the 1999/2006 WHO criteria (23).

2.5 Statistical analyses

For baseline characteristics, categorical variables were presented as proportions (%), and continuous variables reported as mean (SD) or median (25^{th} and 75^{th} percentiles) for variables with normal and skewed distributions, respectively (Table 1). Natural log (ln)-transformations were applied to skewed variables to improve normality. Men and women were analyzed separately. Linear regression models were used to examine associations of baseline androgen and SHBG levels with continuous baseline and follow-up eGFR measures. Additionally, we examined the relationship between androgen and SHBG levels with prevalent and incident CKD using logistic regression. Exact time-to-event information regarding CKD manifestation during follow-up was unavailable, therefore logistic regression was used rather than survival analyses. Models with incident CKD as the outcome included men (n=903) and women (n=391) without prevalent CKD at baseline.

To enable sex-specific comparisons of association strengths across different sex hormones, effect estimates were calculated for a sex-specific one standard deviation (SD) increase in hormone concentrations. Models were adjusted for known risk factors for CKD: Age, waist circumference, systolic blood pressure, antihypertensive medication usage (yes/no), non-HDL-C, prevalent CVD (yes/no), lipid-lowering medication usage (yes/no), smoking status (never/former/current), physical activity (active/inactive), alcohol consumption (no/moderate/high), baseline diabetes status (NGT/prediabetes/T2D), and ln(CRP). Models with eGFR at follow-up and incident CKD as the outcome were additionally adjusted for baseline eGFR. Non-linearity was evaluated by including a non-linear term of hormone measurements in the models, and was visualized using restricted cubic splines. We evaluated the interaction between sex hormones and baseline diabetes status (i.e. NGT and prediabetes vs. T2D).

We performed additional sensitivity analyses: (1) We used a 3 SD cut-off for exclusion of participants with extreme androgen concentrations to ascertain the impact of extreme values on our estimates. (2) We further adjusted the models for TSH, fT3, and fT4 since thyroid hormones may impact androgen receptor expression and steroidogenesis (24, 25) and have been

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TABLE 1 Baseline characteristics.

	Men (n = 1293)	Women (n = 650)
Age (years)[a]	56 (13)	63 (9)
BMI (kg/m²)[a]	27.9 (4.2)	28.5 (5.3)
Waist circumference	98.5 (91.4, 106.1)	91.2 (82.5, 100.2)
Systolic BP (mmHg)[a]	128 (17.4)	120.8 (18.4)
Diastolic BP (mmHg) [a]	77.7 (10.1)	73.5 (9.4)
Antihypertensive medication (%)	411 (31.8)	265 (40.8)
Hypertension (%)	469 (36.3)	278 (42.8)
Prevalent cardiovascular diseases (%)	123 (9.5)	70 (10.8)
Total cholesterol (mmol/L)[a]	5.52 (0.99)	5.97 (1.02)
HDL-cholesterol (mmol/L)[a]	1.30 (0.32)	1.58 (0.37)
Non-HDL cholesterol (mmol/L)[a]	4.21 (0.98)	4.40 (1.00)
LDL-cholesterol (mmol/L)[a]	3.56 (0.86)	3.75 (0.93)
Triglycerides (nmol/L)[b]	1.33 (0.93, 1.94)	1.21 (0.87, 1.63)
C-reactive protein (mg/L)[b]	1.09 (0.55, 2.39)	1.50 (0.75, 3.06)
Lipid-lowering medication (%)	187 (14.4)	102 (15.7)
Smoking status (%)		
Never	393 (30.4)	374 (57.5)
Former	639 (49.4)	195 (30.0)
Current	261 (20.2)	81 (12.5)
Physically active (%)	709 (54.8)	357 (54.9)
Alcohol consumption (%)		
None	256 (19.8)	263 (40.5)
Moderate [c]	773 (59.8)	281 (43.2)
High [c]	264 (20.4)	106 (16.3)
Baseline diabetes status (%)		
Normal glucose tolerance	896 (69.3)	426 (65.8)
Prediabetes	232 (18.0)	137 (21.1)
Type 2 diabetes	165 (12.7)	85 (13.0)
Kidney function		
Baseline eGFR (ml/min/1.73m²) [a]	88.3 (16.2)	82.4 (15.7)
Follow-up eGFR (ml/min/1.73m²) [a]	81.0 (16.4)	75.6 (16.1)
eGFR change (ml/min/1.73m²/year) [b]	-1.18 (-2.22, -0.37)	-1.26 (-2.53, -0.41)
Baseline UACR (mg/g)	4.92 (3.14, 11.0)	7.23 (4.70, 12.9)
Follow-up UACR (mg/g)	4.12 (2.71, 8.30)	6.23 (4.08, 11.7)
Prevalent CKD [d] (%)	73 (5.7)	54 (8.4)
Incident CKD [e] (%)	81 (8.8)	60 (15.2)

(Continued)

TABLE 1 Continued

	Men (n = 1293)	Women (n = 650)
Sex hormones		
Total T (nmol/L) [b]	14.6 (11.4, 18.6)	0.62 (0.42, 0.88)
Free T (pmol/L) [b]	191 (152, 228)	6.23 (4.20, 9.58)
DHT (nmol/L) [b]	1.25 (0.90, 1.71)	0.18 (0.10, 0.29)
Free DHT (pmol/L) [b]	6.85 (5.12, 8.81)	0.75 (0.41, 1.22)
T/DHT (unit) [b]	11.9 (9.47, 14.4)	3.47 (2.18, 5.97)
SHBG (nmol/L) [b]	48.2 (35.2, 65.4)	67.3 (48.3, 95.8)

[a]Measure of central tendency is presented as mean with corresponding standard deviation.
[b]Measure of central tendency is presented as median with corresponding 25th and 75th percentiles.
[c]Alcohol consumption defined as follows: moderate alcohol consumption (males 0.1-39.9 g/day and females 0.1-19.9 g/day), and high alcohol consumption (males ≥40 g/day and females ≥20 g/day).
[d]Defined as having an eGFR of <60ml/min/1.73m² at baseline.
[e]Defined as having an eGFR of <60 ml/min/1.73m² at follow-up. Those with prevalent CKD (n = 130; 73 males and 54 peri-/postmenopausal females) were excluded.
BMI, Body mass index; CKD, Chronic kidney disease; DHT, Dihydrotestosterone; eGFR, Estimated glomerular rate; HDL, High density lipoprotein; LDL, Low density lipoprotein; SHBG, Sex hormone-binding globulin; T, Testosterone; UACR, Urinary albumin to creatinine ratio.

associated with kidney function (26–28). (3) We adjusted further with T in SHBG models to ascertain the independency of SHBG on assessed outcomes. (4) We excluded perimenopausal women as sex hormone levels can fluctuate during this phase.

Significance levels were based on two-sided tests, where p-values of <0.05 were considered to be statistically significant. Statistical analyses were performed using R (v4.0.5).

3 Results

Baseline characteristics of the study participants are presented in Table 1. At baseline, 127 (6.5%) participants [73 (5.7%) men, 54 (8.4%) women] had prevalent CKD. During a median follow-up time of 6.5 (25^{th}, 75^{th} percentiles: 6.3, 6.6) years, 141 (7.2%) participants [81 (8.8%) men, 60 (15.2%) women] developed incident CKD. Due to the exclusion of premenopausal women in the present study, women were older than men; potentially explaining some baseline characteristic differences between men and women. Men had on average higher eGFR at baseline and follow-up, while women had steeper eGFR decline. As expected, total and free androgen levels as well as the ratio of T/DHT were considerably higher in men, while SHBG concentrations were higher in women.

In fully-adjusted models, among men, we did not observe any significant associations between T, DHT, and SHBG with baseline eGFR and prevalent CKD. Excluding extreme values (>3 SD) in a sensitivity analysis did not discernibly change these observations (Tables 2, S2, S4). Prospectively, higher T/DHT was associated with lower follow-up eGFR ($\beta_{T/DHT}$=-0.819, [-1.413; -0.226]). When extreme values were excluded, this association was attenuated to non-significance $\beta_{T/DHT}$=-1.181 [-2.685; 0.322] (Tables 3, S6). Also, a U-shaped association between baseline SHBG and follow-up eGFR was observed (β_{SHBG}=0.015, [-0.687; 0.717], β_{SHBG}^2 = 0.592, [0.133; 1.051]), $P_{non\text{-}linear}$=0.011) (Tables 3, S6, Figure 2A). Further adjustment with T did not discernibly change the results (β_{SHBG}=0.218, [-0.590; 1.026], β_{SHBG}^2 = 0.618, [0.156; 1.079]), $P_{non\text{-}linear}$=0.009). Among 933 men, 921 had fT3, fT4, and TSH measurements. This association remained significant after further adjustment for thyroid hormones (β_{SHBG}=0.051, [-0.655; 0.757], β_{SHBG}^2 = 0.696, [0.102; 1.037]), $P_{non\text{-}linear}$=0.017). Exclusion of extreme SHBG values (Table S6) did not discernibly alter this association. No significant associations between androgens, SHBG, and incident CKD were detected among men (Table S8).

Among women, elevated T and fT were inversely associated with baseline eGFR (β_T=-1.305, [-2.290; -0.320], β_{fT}=-1.423, [-2.449; -0.397]). When extreme T and fT values were excluded, these associations did not persist (β_T=-0.770, [-2.104; 0.565], β_{fT}=-0.721, [-2.117; 0.675]) (Tables 2, S3). Additionally, a non-linear association was observed between fDHT and prevalent CKD (β_{fDHT}=0.549, [0.324; 0.890], β_{fDHT}^2 = 1.151, [1.051; 1.291]), $P_{non\text{-}linear}$=0.007). Ultimately this non-linear association did not persist after excluding extreme fDHT values ($P_{non\text{-}linear}$=0.963) (Tables 2, S3). Instead, we observed a significant linear association (OR_{fDHT}=0.571, [0.328; 0.931]) (Table S5). A significant positive association between T/DHT and prevalent CKD ($OR_{T/DHT}$= 2.305, 1.069; 4.529) was observed only after excluding extreme values (Tables 2, S5). Prospectively, no significant associations were observed between androgens, SHBG, and follow-up eGFR among women (Tables 3, S7). A reverse J-shaped association was seen between DHT and incident CKD (β_{DHT}=0.579, [0.350; 0.938], β_{DHT}^2 = 1.230, [0.976; 1.450]), $P_{non\text{-}linear}$=0.025) (Table 3; Figure 2B). DHT values below the mean were inversely associated with incident CKD, while no association or possibly a very weak positive association was suggested for values

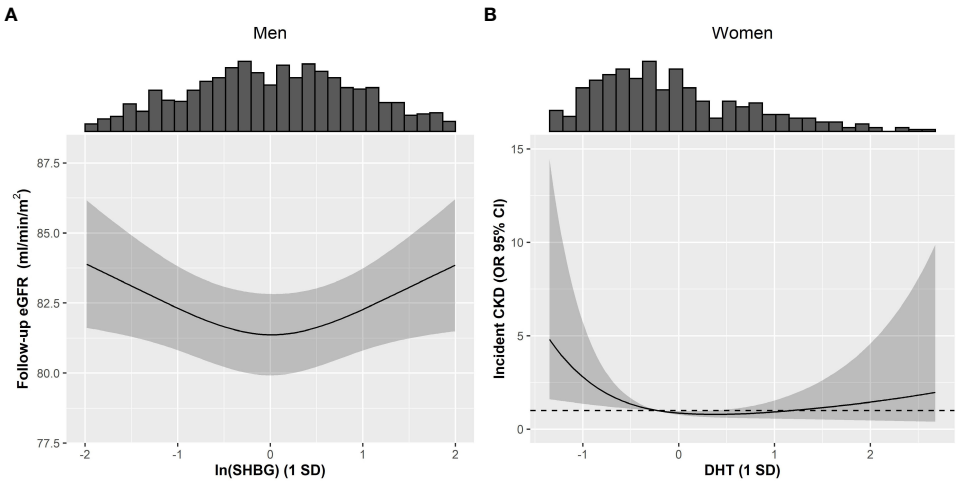

FIGURE 2
Restricted cubic splines describing non-linear associations between androgens or SHBG with kidney function in men and women of the KORA F4/FF4 study. Non-linear associations between **(A)** ln(SHBG) and follow-up eGFR in men, plus **(B)** between DHT and incident CKD in women. Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Interaction between diabetes and DHT or SHBG was assessed using a 3-way interaction (i.e. DHT or SHBG*diabetes + (DHT or SHBG)^2*diabetes). Non-linear associations were not modified by diabetes ($P_{interaction}$: **(A)** 0.920 and **(B)** 0.948). Solid line represents the estimated spline function for follow-up eGFR and incident CKD, and the grey shaded area represents the respective 95% CI spline estimation. The histograms provide insight to the population density along ln(SHBG) and DHT values. Models were adjusted using Model 2, comprising of baseline eGFR, baseline age, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular disease, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, and ln(CRP). CKD: Chronic kidney disease, CI: Confidence interval, CRP: C-reactive protein, DHT: Dihydrotestosterone, eGFR: Estimated glomerular filtration rate, HDL: High-density lipoprotein, OR: Odds ratio, SD: Standard deviation.

above the mean (Figure 2B). This association remained significant after exclusion of extreme DHT values (β_{DHT}=0.551, [0.334; 0.894], β_{DHT}^2 = 1.689, [1.156; 2.491]), $P_{non\text{-}linear}$=0.007) (Table S9). Among 391 women, 375 had fT3, fT4, and TSH measurements. The association between DHT and incident CKD did not persist after accounting for thyroid hormones (β_{DHT}=0.588, [0.343; 0.986], β_{DHT}^2 = 1.188, [0.868; 1.429]), $P_{non\text{-}linear}$=0.122). Additionally, an inverse association between fDHT and incident CKD was observed (OR_{fDHT}=0.613, [0.369; 0.971]) – which did not appreciably change after excluding extreme fDHT values (Tables 3, S9). Among 386 women, 370 women had fT3, fT4, and TSH measurements. The association between fDHT and incident CKD remained significant after further adjustment for thyroid hormones (OR_{fDHT}=0.613, [0.359; 0.993]). Notably, all observations among women did not significantly change when perimenopausal women were excluded.

Diabetes status was a significant effect modifier in several models. Among men, diabetes modified the association between T and follow-up eGFR ($P_{T\times diabetes}$=0.041). In men without diabetes, eGFR decreases ($\beta_{T(No\ diabetes)}$=-0.425, [-1.130; 0.279]), whereas in men with diabetes, eGFR increases ($\beta_{T(Diabetes)}$=1.709, [-0.679; 4.097]) as T levels increased. Among women, diabetes modified the association between T and baseline eGFR ($P_{T\times diabetes}$=0.014) and between DHT and prevalent CKD ($P_{DHT\times diabetes}$=0.001). Compared to women without diabetes, those with diabetes have steeper eGFR decline (β=-2.978, [-5.342; -0.614]) vs. β=-0.739, [-1.838; 0.361]) as T levels increased. However, due to the small sample size, we observed exceedingly wide CIs of the interaction term between DHT and diabetes status for prevalent CKD among women. Thus, stratified analysis was not performed.

4 Discussion

In the present study, we found several suggestive associations linking androgens and SHBG to kidney health among men and women. Among men, while no associations were observed between androgens, eGFR, and CKD, SHBG showed a U-shaped association with follow-up eGFR that was independent of T and not modified by diabetes status. In women, DHT showed a reverse J-shaped association with incident CKD, while elevated fDHT was significantly associated with lower CKD prevalence and incidence. Additionally, a higher T/DHT ratio was associated with higher CKD prevalence. Taken together, these findings suggest involvement of endogenous androgens and SHBG in CKD pathophysiology.

Our cross-sectional results in men regarding the lack of association of T and DHT with eGFR and CKD agree with the Diabetes Prevention Program Outcomes Study (DPPOS) that

TABLE 2 Cross-sectional associations of endogenous androgens and SHBG with baseline eGFR and prevalent CKD.

Exposure [a]	Baseline eGFR β (95% CI)		Prevalent CKD OR (95% CI)	
	Men (n = 1,293)	Women (n = 650)	Men (n = 1,293)	Women (n = 650)
T	0.210 (-0.501 – 0.920)	**-1.305 (-2. 290 – -0.320)** [b]	0.810 (0. 597 – 1.081)	1.114 (0. 846 – 1.446)
fT	-0.034 (-0. 750 – 0.682)	**-1.423 (-2. 449 – -0.397)** [b]	0.765 (0.549 – 1.054)	1.166 (0. 874 – 1.539)
DHT	0.285 (-0. 395 – 0.965)	-0.197 (-1. 195 – 0.802)	0.964 (0. 663 – 1.327)	0.767 (0.484 – 1.132)
fDHT $fDHT^2$	0.065 (-0. 611 – 0.740)	-0.358 (-1. 364 – 0.648)	0.910 (0. 571 – 1.353)	**0.549 (0. 324 – 0.890)** **1.151 (1.051 – 1.291)** [b]
T/DHT	0.430 (-0. 227 – 1.086)	-0.405 (-1. 382 – 0.572)	0.790 (0. 486 – 1.098)	1.213 (0.976 – 1.473) [b]
ln(SHBG)	0.372 (-0. 364 – 1.107)	-0.076 (-1. 190 – 1.037)	1.071 (0. 774 – 1.484)	1.027 (0.715 – 1.478)

[a]Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Here, only significant (P<0.05) terms are reported. Quadratic terms, which were significant, were presented in this table along with corresponding linear terms (i.e. fDHT and $fDHT^2$ with prevalent CKD in women).
[b]Number of participants after exclusion of androgen or SHBG measurements >3 SD above/below the mean; Men: T (n = 1,273), fT(n = 1270), DHT (n = 1,268), fDHT (n = 1,271), T/DHT (n = 1,279), and ln(SHBG) (n = 1,289). Women: T (n = 636), fT(n = 638), DHT (n = 637), fDHT (n = 642), T/DHT (n = 640), and ln(SHBG) (n = 648). Following estimates were revealed for baseline eGFR: β_T = -0. 770 (-2. 104 – 0.565), β_{fT} = -0.721 (-2. 117 – 0.675), OR_{fDHT} = 0. 571 (0.328 – 0.931), and for prevalent CKD: $OR_{T/DHT}$ = 2.305 (1. 069 - 4.529). β-estimates are per 1 sex-specific SD and adjusted using Model 2, comprising of baseline age, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular diseases, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, ln(CRP). Interaction by diabetes status was assessed by entering the interaction as a multiplicative term (i.e. androgen or SHBG*diabetes) in Model 2. For models with a significant quadratic term (i.e. $fDHT^2$), interaction was assessed using a 3-way interaction (i.e. fDHT*diabetes + $fDHT^2$*diabetes). Prevalent CKD was defined as a baseline eGFR <60 ml/min/1.73m^2. Bold values indicate statistical significance ($p < 0.05$).
CKD, Chronic kidney disease; CI, Confidence interval; CRP, C-reactive protein; DHT, Dihydrotestosterone; eGFR, Estimated glomerular filtration rate; HDL, High-density lipoprotein; SD, Standard deviation; SHBG, Sex hormone-binding globulin; T, Testosterone; T/DHT, T to DHT ratio.

assessed the associations between endogenous androgens and kidney measures over 11 years in 2170 participants (889 men, 1281 women) (9). Similar observations were made in 1470 men from the Third National Health and Nutrition Examination Survey (8). The tendency towards an inverse association of T (and fT) with follow-up eGFR in the present study was concordant with a randomized controlled trial in 48 hypogonadal men, which showed that 3-month and 6-month T treatment lowered eGFR (29). In contrast to the above investigations, Kurita et al. (6) reported that elevated endogenous T was cross-sectionally associated with higher eGFR among Japanese men. Differing T levels among men attributed to genetic differences could explain this (30).

In the present study, women with extreme T and fT levels appear to have driven the inverse cross-sectional relationship with eGFR. The null association between T, fT, and baseline eGFR after excluding women with extreme T and fT levels is consistent to observations from Kim et al. (9). Hyperandrogenism is common among women with polycystic ovarian syndrome (PCOS) and T2D (5, 31). Higher BMI and waist circumference, as well as lower eGFR levels among women with extreme T and fT levels in the current study (data not shown) are consistent to previous reports of higher adiposity (32–34) and higher risk of PCOS-associated (35) and non-PCOS-associated kidney dysfunction (35–38). Androgen excess is associated with visceral fat accumulation (33, 39–41) and endothelial dysfunction (42–45), both of which can drive kidney dysfunction (46). This potentially explains our finding regarding the initial inverse association between T levels and eGFR; particularly for the extreme T and fT levels among women. Even though there is evidence that T may compromise kidney function in women (12, 47), the link between androgens and kidney function has not been extensively investigated. Thus, additional studies are required to better understand these associations among women.

We additionally observed during sensitivity analyses, that an elevated T/DHT ratio (higher T levels in regards to DHT) was associated with higher CKD prevalence among women, and that higher circulating levels of fDHT were associated with a lower prevalence and incidence of CKD. Considering that T and DHT levels, as well as T/DHT ratios are maintained at least partially by 5α-reductase (an enzyme responsible for converting T to DHT) (14), the possibility of sex-specific changes in the expression or activity of 5α-reductases during endocrine disorders merits further investigation.

The positive association between higher SHBG levels and eGFR at follow-up in men with SHBG levels above the mean, together with the tendency for an inverse association between SHBG and incident CKD was partially consistent to findings from an observational study (9) and an MR study that reported

TABLE 3 Prospective associations between endogenous androgens and SHBG with follow-up eGFR and incident CKD.

Exposure [a]	Follow-up eGFR β (95% CI)		Incident CKD OR (95% CI)	
	Male (n = 933)	Female (n = 416)	Male (n = 903)	Female (n = 391)
T	-0.252 (-0.931 – 0.427)	-0.097 (-1.148 – 0.953)	0.851 (0.597 – 1.198)	0.932 (0.593 –1.432)
fT	-0.521 (-1.192 – 0.150)	-0.224 (-1.324 – 0.877)	0.875 (0. 594 – 1.272)	0.745 (0.429 – 1.231)
DHT DHT^2	0.476 (-0.169 – 1.122)	0.310 (-0.755 – 1.376)	0.731 (0. 452 – 1.150)	**0.579** **(0. 350 – 0.938)** **1.230** **(0. 976 - 1.450)** [c]
fDHT	0.156 (-0.486 – 0.797)	0.285 (-0.777 – 1.348)	0.828 (0. 475 – 1.394)	**0.613** **(0. 369 – 0.971)**
T/DHT	**-0.819** **(-1.413- -0.226)** [b]	-0.215 (-1.190 – 0.759)	1.290 (0.662 – 2.519)	1.559 (0.692 – 3.605)
ln(SHBG) $ln(SHBG)^2$	**0.015** **(-0. 687 – 0.717)** **0.592** **(0.133 - 1.051)**	0.558 (-0.620 – 1.736)	0.845 (0. 604 – 1.179)	1.207 (0.804 – 1.836)

[a]Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Here, only significant quadratic terms (P<0.05) are reported. Quadratic terms which were significant were presented in this table along with corresponding linear terms (i.e. ln(SHBG) and $ln(SHBG)^2$ with follow-up eGFR in men, as well as DHT and DHT^2 with incident CKD in women). No other quadratic associations were observed in the main analyses.

[b]Number of participants after exclusion of sex hormone or SHBG measurements 3 SDs above/below the mean for follow-up eGFR; Men: T (n = 920), fT(n = 920), DHT (n = 913), fDHT (n = 915), T/DHT (n = 923), and ln(SHBG) (n = 931). Women: T (n = 408), fT(n = 410), DHT (n = 408), fDHT (n = 411), T/DHT (n = 410), and ln(SHBG) (n = 415). For incident CKD; Men: T (n = 890), fT(n = 891), DHT (n = 884), fDHT (n = 885), T/DHT (n = 893), and ln(SHBG) (n = 901). Women, T (n = 384), fT (n = 386), DHT (n = 383), fDHT (n = 386), T/DHT (n = 387), and ln(SHBG) (n = 390). After exclusion of measurements >3 SD, the association between T/DHT and follow-up eGFR in men was attenuated to non-significance (β = -1.181, [-2.685; 0.322]).

[c]Of 391 women, 375 had fT3, fT4, and TSH measurements. After adjusting for thyroid hormones, the non-linear association between DHT and incident CKD was attenuated to non-significance (β_{DHT}=0.588, [0.343; 0.986], β_{DHT^2} = 1.188, [0.868; 1.429]), $P_{non\text{-}linear}$=0.122)

ORs are per 1 sex-specific SD and adjusted using Model 2, comprising of baseline age, baseline eGFR, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular disease, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, and ln(CRP). Interaction by diabetes status was assessed by entering the interaction as a multiplicative term (i.e. androgen/SHBG*diabetes) in Model 2. For models with a significant quadratic term (i.e. DHT^2 and $ln(SHBG)^2$), interaction was assessed using a 3-way interaction (i.e. DHT or ln(SHBG)*diabetes + DHT^2 or $ln(SHBG)^2$*diabetes). Incident CKD was defined as a follow-up eGFR <60 ml/min/1.73m^2 in participants without prevalent CKD.

CKD, Chronic kidney disease; CI, Confidence interval; CRP, C-reactive protein; DHT, Dihydrotestosterone; eGFR, Estimated glomerular filtration rate; HDL, High-density lipoprotein; SD, Standard deviation; SHBG, Sex hormone-binding globulin; T, Testosterone; T/DHT, T to DHT ratio.

significant associations between genetically-predicted higher SHBG concentrations and lower CKD risk, independent of T (15). In women, a report (9) regarding SHBG was also consistent to our results as no association between SHBG levels and kidney function were observed. Notably, the abovementioned MR study (15) also did not find an association between genetically-predicted higher SHBG and CKD risk among women. Despite limited studies providing mechanistic insights linking SHBG and CKD, inflammation and insulin resistance could mediate this link. An *in vitro* experiment showed that SHBG suppresses inflammation - an effect not altered by simultaneous T or E2 supplementation (48). Based on further MR reports, genetically-predicted higher SHBG may lower insulin resistance and T2D risk in men and women (49–51). Moreover, in men, elevated fasting insulin has been linked to impaired kidney function, but not vice versa (52). Further studies that can provide sex-specific mechanistic insights to the SHBG-kidney relationship are warranted.

In the present study, we noted that adjustment for lipid levels and prevalent CVD consistently attenuated measures of association. Some plausible mechanisms linking androgens to kidney impairment include mediation through CV risk factors such as inflammation, hypertension (53), and hyperlipidemia (54). In patients with CKD, these risk factors are highly prevalent and contribute to atherosclerotic vascular disease, accounting for a majority of lesions that cause blood flow disruption to renal arteries (55). T can worsen atherosclerosis (56, 57), through its proinflammatory effects on the vascular system (58, 59). Hence, progression to kidney failure is accelerated. However, RCTs that attempted to assess CV safety associated with testosterone replacement therapy (TRT) in hypogonadal men (60–62) and postmenopausal women (63–65), showed no conclusive evidence that T supplementation is associated with increased CV risk (66). Albeit, these findings until now are derived from underpowered trials. Evidence concerning DHT is sparse. The few trials assessing the effects of DHT supplementation usually focused on DHT effects on prostate (67–69), rather than CV effects. Nevertheless, exogenous DHT has been shown to lower total and LDL-cholesterol, indicating favorable effects of DHT on traditional CVD risk factors in men (69).

RCTs to date have not investigated kidney outcomes following reinstatement of physiological androgen levels *via* TRT. Additionally, investigations in women are still lacking. Thus, further large-scale RCTs or population-based studies should assess the effects of androgens on kidney health in both sexes.

In the present study, baseline eGFR increases with higher T levels among men with T2D. Although T (and fT) have been inversely associated with T2D risk in epidemiological studies in men (70), glomerular hyperfiltration has been observed during early CKD stages among individuals with T2D (71); potentially explaining the opposing effects seen between eGFR and T among men with and without T2D. Moreover, diabetes-associated tubular hyperplasia and hypertrophy, as well as proximal tubular hyper-reabsorption reduces pressure in Bowman's space. This perpetuates hyperfiltration (72). As shown in the current study population, women with T2D showed steeper eGFR decline compared to their counterparts without diabetes, and had higher T levels (5). As oxidative stress is more apparent in the hyperglycemic state (73), the cumulative effect of higher T levels and increased oxidative stress burden could accelerate the deterioration of kidney function. However, the link between sex hormones and DKD remains ambiguous and further investigations are needed.

To our knowledge, this is the first epidemiological study evaluating the role DHT in kidney function among women. Strengths of the current study include the prospective design, allowing the examination of prospective associations between endogenous androgens, SHBG, and changes in kidney function. As our study population was well-characterized, we were able to adjust for various relevant confounders. Further, mass spectrometry was used for androgen quantification. However, this study also has its limitations. Instead of a clinical CKD diagnosis, eGFR was used to define CKD. Sex hormone measurements were available only at baseline so we could not monitor the changes over time and evaluate their contribution to our outcomes of interest. However, single T measurements have been shown to be an adequate representation of the mean annual T levels (74). Next, we could not identify women with PCOS in our dataset as the information is unavailable. PCOS symptoms persist in postmenopausal women and could cause perturbations in sex hormone concentrations. Furthermore, we performed multiple comparisons as various associations between androgens, SHBG, and kidney function were examined. Due to the hypotheses-generating nature of our study, we did not adjust for multiple testing (75). Finally, despite adjusting for multiple risk factors for kidney disease, we cannot rule out any residual confounding or unmeasured confounders in the investigated associations.

Conclusion

The results of the present study suggest that androgens and SHBG could be markers of kidney function impairment within a general population. Specifically, in men, we observed a U-shaped association between SHBG and follow-up eGFR, whereas in women, those with lower DHT levels may have an increased CKD risk. Conditions that are associated with abnormal hormonal profiles, such as T2D could alter the link between androgens and kidney function. Larger well-phenotyped prospective studies are required to further elucidate the potential of androgens, SHBG, and T2D as modifiable risk factors for kidney function.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Data are available upon reasonable request. Data collection in the KORA study is done in cooperation with the University Hospital of Augsburg. The data are subject to national data protection laws and restrictions were imposed by the ethics committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with the Cooperative Health Research in the Region of Augsburg (KORA) *via* the online portal KORA.passt (https://helmholtz-muenchen.managed-otrs.com/external). Please contact the corresponding author, Barbara Thorand, in case of further questions. Requests to access these datasets should be directed to https://helmholtz-muenchen.managed-otrs.com/external.

Ethics statement

The studies involving human participants were reviewed and approved by Bavarian Chamber of Physicians (Ethical Approval Number 06068). The patients/participants provided their written informed consent to participate in this study.

Author contributions

LHYL and BT designed the study. CP, AC, TZ, WR, JA, AP, and BT contributed data. LHYL performed all data analyses with guidance from JN and BT, and is the guarantor of this work. Result interpretation was done by LHYL, JN, and BT. LHYL wrote the manuscript with guidance from JN and BT. All authors contributed to the article and approved the submitted version.

Funding

This study was supported in part by a research grant within the German Center for Cardiovascular Research (DZHK) shared

expertise program (DZHK B 17-035 SE). LHYL is supported through the International Helmholtz Research School for Diabetes, funded by the Helmholtz Association - Initiative and Networking Fund. TZ is supported by the German Center for Cardiovascular Research (DZHK 81Z1710101, FKZ 81Z0710102, and FKZ 81X2710160). The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria.

Acknowledgments

We thank the members of the Metabolomics and Proteomics Core Facility, Helmholtz Zentrum München, Germany for their excellent technical work in sample preparation and quantification. We also extend our gratitude to all members of the Institute of Epidemiology, Helmholtz Zentrum München, and the KORA field staff in Augsburg who planned and conducted the 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1000650/full#supplementary-material

References

1. Nitsch D, Grams M, Sang Y, Black C, Cirillo M, Djurdjev O, et al. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. *BMJ Br Med J* (2013) 346: f324. doi: 10.1136/bmj.f324

2. Neugarten J, Golestaneh L. Influence of sex on the progression of chronic kidney disease. *Mayo Clinic Proc* (2019) 94(7):1339–56. doi: 10.1016/j.mayocp.2018.12.024

3. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease - a systematic review and meta-analysis. *PLoS One* (2016) 11(7):e0158765. doi: 10.1371/journal.pone.0158765

4. Wang C, Zhang W, Wang Y, Wan H, Chen Y, Xia F, et al. Novel associations between sex hormones and diabetic vascular complications in men and postmenopausal women: a cross-sectional study. *Cardiovasc Diabetol* (2019) 18 (1):97. doi: 10.1186/s12933-019-0901-6

5. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* (2006) 295(11):1288–99. doi: 10.1001/jama.295.11.1288

6. Kurita N, Horie S, Yamazaki S, Otani K, Sekiguchi M, Onishi Y, et al. Low testosterone levels and reduced kidney function in Japanese adult men: The locomotive syndrome and health outcome in aizu cohort study. *J Am Med Directors Assoc* (2016) 17(4):371.e371–371.e376. doi: 10.1016/j.jamda.2016.01.011

7. Amiri M, Ramezani Tehrani F, Rahmati M, Amanollahi Soudmand S, Behboudi-Gandevani S, Sabet Z, et al. Low serum testosterone levels and the incidence of chronic kidney disease among male adults: A prospective population-based study. *Andrology* (2020) 8(3):575–82. doi: 10.1111/andr.12728

8. Yi S, Selvin E, Rohrmann S, Basaria S, Menke A, Rifai N, et al. Endogenous sex steroid hormones and measures of chronic kidney disease (CKD) in a nationally representative sample of men. *Clin Endocrinol (Oxf)* (2009) 71(2):246–52. doi: 10.1111/j.1365-2265.2008.03455.x

9. Kim C, Ricardo AC, Boyko EJ, Christophi CA, Temprosa M, Watson KE, et al. Sex hormones and measures of kidney function in the diabetes prevention program outcomes study. *J Clin Endocrinol Metab* (2019) 104(4):1171–80. doi: 10.1210/jc.2018-01495

10. Zhao JV, Schooling CM. The role of testosterone in chronic kidney disease and kidney function in men and women: a bi-directional mendelian randomization study in the UK biobank. *BMC Med* (2020) 18(1):122. doi: 10.1186/s12916-020-01594-x

11. Shores MM, Biggs ML, Arnold AM, Smith NL, Longstreth WT Jr, Kizer JR. Testosterone, dihydrotestosterone, and incident cardiovascular disease and mortality in the cardiovascular health study. *J Clin Endocrinol Metab* (2014) 99 (6):2061–8. doi: 10.1210/jc.2013-3576

12. Yanes LL, Sartori-Valinotti JC, Reckelhoff JF. Sex steroids and renal disease. *Hypertension* (2008) 51(4):976–81. doi: 10.1161/HYPERTENSIONAHA.107.105767

13. Tomaszewski M, Charchar FJ, Maric C, Kuzniewicz R, Gola M, Grzeszczak W, et al. Inverse associations between androgens and renal function: The young men cardiovascular association (YMCA) study. *Am J Hypertension* (2009) 22 (1):100–5. doi: 10.1038/ajh.2008.307

14. Swerdloff RS, Dudley RE, Page ST, Wang C, Salameh WA. Dihydrotestosterone: Biochemistry, physiology, and clinical implications of elevated blood levels. *Endocr Rev* (2017) 38(3):220–54. doi: 10.1210/er.2016-1067

15. Zhao JV, Schooling CM. Sex-specific associations of sex hormone binding globulin with CKD and kidney function: A univariable and multivariable mendelian randomization study in the UK biobank. *J Am Soc Nephrol* (2021) 32 (3):686. doi: 10.1681/ASN.2020050659

16. Maric-Bilkan C. Sex differences in diabetic kidney disease. *Mayo Clinic Proc* (2020) 95(3):587–99. doi: 10.1016/j.mayocp.2019.08.026

17. Rathmann W, Haastert B, Icks A, Löwel H, Meisinger C, Holle R, et al. High prevalence of undiagnosed diabetes mellitus in southern Germany: Target populations for efficient screening. *KORA survey 2000. Diabetologia* (2003) 46 (2):182–9. doi: 10.1007/s00125-002-1025-0

18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro III AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Internal Med* (2009) 150(9):604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

19. Breier M, Wahl S, Prehn C, Ferrari U, Sacco V, Weise M, et al. Immediate reduction of serum citrulline but no change of steroid profile after initiation of metformin in individuals with type 2 diabetes. *J Steroid Biochem Mol Biol* (2017) 174:114–9. doi: 10.1016/j.jsbmb.2017.08.004

20. Mazer NA. A novel spreadsheet method for calculating the free serum concentrations of testosterone, dihydrotestosterone, estradiol, estrone and cortisol: with illustrative examples from male and female populations. *Steroids* (2009) 74 (6):512–9. doi: 10.1016/j.steroids.2009.01.008

21. Mühlberger N, Behrend C, Stark RG, Holle R. *Datenbankgestützte online-erfassung von arzneimitteln im rahmen gesundheitswissenschaftlicher studien -*

erfahrungen mit der IDOM-software Vol. vol. 34. . München: Urban & Fischer (2003).

22. Meisinger C, Thorand B, Schneider A, Stieber J, Döring A, Löwel H. Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA augsburg cohort study. *Arch Intern Med* (2002) 162(1):82–9. doi: 10.1001/archinte.162.1.82

23. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetes Med* (1998) 15(7):539–53. doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

24. Panno ML, Sisci D, Salerno M, Lanzino M, Pezzi V, Morrone EG. Thyroid hormone modulates androgen and oestrogen receptor content in the sertoli cells of peripubertal rats. *J Endocrinol* (1996) 148(1):43–50. doi: 10.1677/joe.0.1480043

25. Maran RR. Thyroid hormones: their role in testicular steroidogenesis. *Arch Androl* (2003) 49(5):375–88. doi: 10.1080/01485010390204968

26. Kim SH, Min HK, Lee SW. Relationship between thyroid and kidney function: Analysis from the Korea national health and nutrition examination survey between 2013 and 2015. *Kidney Blood Pressure Res* (2020) 45(3):442–54. doi: 10.1159/000507290

27. Ellervik C, Mora S, Ridker PM, Chasman DI. Hypothyroidism and kidney function: A mendelian randomization study. *Thyroid* (2020) 30(3):365–79. doi: 10.1089/thy.2019.0167

28. Rhee CM. The interaction between thyroid and kidney disease: an overview of the evidence. *Curr Opin Endocrinol Diabetes Obes* (2016) 23(5):407–15. doi: 10.1097/MED.0000000000000275

29. Pedersen L, Christensen LL, Pedersen SM. et al: Reduction of calprotectin and phosphate during testosterone therapy in aging men: a randomized controlled trial. *J Endocrinological Invest* (2017) 40(5):529–38. doi: 10.1007/s40618-016-0597-3

30. Ohlsson C, Wallaschofski H, Lunetta KL, Stolk L, Perry JR, Koster A, et al. Genetic determinants of serum testosterone concentrations in men. *PLoS Genet* (2011) 7(10):e1002313. doi: 10.1371/journal.pgen.1002313

31. Schmidt J, Landin-Wilhelmsen K, Brännström M, Dahlgren E. Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-year controlled follow-up study. *J Clin Endocrinol Metab* (2011) 96(12):3794–803. doi: 10.1210/jc.2011-1677

32. Sowers MF, Beebe JL, McConnell D, Randolph J, Jannausch M. Testosterone concentrations in women aged 25–50 years: Associations with lifestyle, body composition, and ovarian status. *Am J Epidemiol* (2001) 153(3):256–64. doi: 10.1093/aje/153.3.256

33. Janssen I, Powell LH, Kazlauskaite R, Dugan SA. Testosterone and visceral fat in midlife women: the study of women's health across the nation (SWAN) fat patterning study. *Obes (Silver Spring)* (2010) 18(3):604–10. doi: 10.1038/oby.2009.251

34. Cao Y, Zhang S, Zou S, Xia X. The relationship between endogenous androgens and body fat distribution in early and late postmenopausal women. *PLoS One* (2013) 8(3):e58448. doi: 10.1371/journal.pone.0058448

35. Song Y, Ye W, Ye H, Xie T, Shen W, Zhou L. Serum testosterone acts as a prognostic indicator in polycystic ovary syndrome-associated kidney injury. *Physiol Rep* (2019) 7(16):e14219. doi: 10.14814/phy2.14219

36. de Boer IH, Katz R, Fried LF, Ix JH, Luchsinger J, Sarnak MJ, et al. Obesity and change in estimated GFR among older adults. *Am J Kidney Dis* (2009) 54 (6):1043–51. doi: 10.1053/j.ajkd.2009.07.018

37. Jhee JH, Joo YS, Han SH, Yoo TH, Kang SW, Park JT. High muscle-to-fat ratio is associated with lower risk of chronic kidney disease development. *J Cachexia Sarcopenia Muscle* (2020) 11(3):726–34. doi: 10.1002/jcsm.12549

38. Chen YY, Fang WH, Wang CC, Kao TW, Chang YW, Yang HF, et al. Changes of percent body fat as a useful surrogate for risk of declined renal function. *Sci Rep* (2018) 8(1):17289. doi: 10.1038/s41598-018-35601-2

39. Loh NY, Humphreys E, Karpe F, Tomlinson JW, Noordam R, Christodoulides C. Sex hormones, adiposity, and metabolic traits in men and women: a mendelian randomisation study. *Eur J Endocrinol* (2022) 186(3):407–16. doi: 10.1530/EJE-21-0703

40. Mongraw-Chaffin ML, Anderson CAM, Allison MA, Ouyang P, Szklo M, Vaidya D, et al. Association between sex hormones and adiposity: Qualitative differences in women and men in the multi-ethnic study of atherosclerosis. *J Clin Endocrinol Metab* (2015) 100(4):E596–600. doi: 10.1210/jc.2014-2934

41. Lovejoy JC, Bray GA, Bourgeois MO, Macchiavelli R, Rood JC, Greeson C, et al. Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women–a clinical research center study. *J Clin Endocrinol Metab* (1996) 81(6):2198–203. doi: 10.1210/jcem.81.6.8964851

42. Mathews L, Subramanya V, Zhao D, Ouyang P, Vaidya D, Guallar E, et al. Endogenous sex hormones and endothelial function in postmenopausal women and men: The multi-ethnic study of atherosclerosis. *J Womens Health (Larchmt)* (2019) 28(7):900–9. doi: 10.1089/jwh.2018.7441

43. Usselman CW, Yarovinsky TO, Steele FE, Leone CA, Taylor HS, Bender JR, et al. Androgens drive microvascular endothelial dysfunction in women with polycystic ovary syndrome: role of the endothelin b receptor. *J Physiol* (2019) 597(11):2853–65. doi: 10.1113/JP277756

44. Georgiopoulos GA, Lambrinoudaki I, Athanasouli F, Armeni E, Rizos D, Kazani M, et al. Free androgen index as a predictor of blood pressure progression and accelerated vascular aging in menopause. *Atherosclerosis* (2016) 247:177–83. doi: 10.1016/j.atherosclerosis.2016.02.021

45. Thurston RC, Bhasin S, Chang Y, Barinas-Mitchell E, Matthews KA, Jasuja R, et al. Reproductive hormones and subclinical cardiovascular disease in midlife women. *J Clin Endocrinol Metab* (2018) 103(8):3070–7. doi: 10.1210/jc.2018-00579

46. Nowak KL, Jovanovich A, Farmer-Bailey H, Bispham N, Struemph T, Malaczewski M, et al. Vascular dysfunction, oxidative stress, and inflammation in chronic kidney disease. *Kidney360* (2020) 1(6):501. doi: 10.34067/KID.0000962019

47. Reckelhoff JF, Yanes LL, Iliescu R, Fortepiani LA, Granger JP. Testosterone supplementation in aging men and women: possible impact on cardiovascular-renal disease. *Am J Physiology-Renal Physiol* (2005) 289(5):F941–8. doi: 10.1152/ajprenal.00034.2005

48. Yamazaki H, Kushiyama A, Sakoda H, Fujishiro M, Yamamotoya T, Nakatsu Y, et al. Protective effect of sex hormone-binding globulin against metabolic syndrome: *In vitro* evidence showing anti-inflammatory and lipolytic effects on adipocytes and macrophages. *Mediators Inflammation* (2018) 2018:3062319–3062319. doi: 10.1155/2018/3062319

49. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, et al. Sex hormone–binding globulin and risk of type 2 diabetes in women and men. *New Engl J Med* (2009) 361(12):1152–63. doi: 10.1056/NEJMoa0804381

50. Perry JR, Weedon MN, Langenberg C, Jackson AU, Lyssenko V, Sparsø T, et al. Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum Mol Genet* (2010) 19(3):535–44. doi: 10.1093/hmg/ddp522

51. Wang Q, Kangas AJ, Soininen P, Tiainen M, Tynkkynen T, Puukka K, et al. Sex hormone-binding globulin associations with circulating lipids and metabolites and the risk for type 2 diabetes: observational and causal effect estimates. *Int J Epidemiol* (2015) 44(2):623–37. doi: 10.1093/ije/dyv093

52. Zhao JV, Schooling CM. Sex-specific associations of insulin resistance with chronic kidney disease and kidney function: a bi-directional mendelian randomisation study. *Diabetologia* (2020) 63(8):1554–63. doi: 10.1007/s00125-020-05163-y

53. Alexander RW. Hypertension and the pathogenesis of atherosclerosis. *Hypertension* (1995) 25(2):155–61. doi: 10.1161/01.HYP.25.2.155

54. Ross R, Harker L. Hyperlipidemia and atherosclerosis. *Science* (1976) 193 (4258):1094–100. doi: 10.1126/science.822515

55. Lao D, Parasher PS, Cho KC, Yeghiazarians Y. Atherosclerotic renal artery stenosis–diagnosis and treatment. *Mayo Clinic Proc* (2011) 86(7):649–57. doi: 10.4065/mcp.2011.0181

56. Mäkinen J, Järvisalo MJ, Pöllänen P, Perheentupa A, Irjala K, Koskenvuo M, et al. Increased carotid atherosclerosis in andropausal middle-aged men. *J Am Coll Cardiol* (2005) 45(10):1603–8. doi: 10.1016/j.jacc.2005.01.052

57. Svartberg J, von Mühlen D, Mathiesen E, Joakimsen O, Bønaa KH, Stensland-Bugge E. Low testosterone levels are associated with carotid atherosclerosis in men. *J Intern Med* (2006) 259(6):576–82. doi: 10.1111/j.1365-2796.2006.01637.x

58. Soisson V, Brailly-Tabard S, Empana JP, Féart C, Ryan J, Bertrand M, et al. Low plasma testosterone and elevated carotid intima-media thickness: importance of low-grade inflammation in elderly men. *Atherosclerosis* (2012) 223(1):244–9. doi: 10.1016/j.atherosclerosis.2012.05.009

59. Skogastierna C, Hotzen M, Rane A, Ekström L. A supraphysiological dose of testosterone induces nitric oxide production and oxidative stress. *European J Preventive Cardiol* (2014) 21(8):1049–54. doi: 10.1177/2047487313481755

60. Basaria S, Harman SM, Travison TG, Hodis H, Tsitouras P, Budoff M, et al. Effects of testosterone administration for 3 years on subclinical atherosclerosis progression in older men with low or low-normal testosterone levels: A randomized clinical trial. *JAMA* (2015) 314(6):570–81. doi: 10.1001/jama.2015.8881

61. Snyder PJ, Ellenberg SS, Cunningham GR, Matsumoto AM, Bhasin S, Barrett-Connor E, et al. The testosterone trials: Seven coordinated trials of testosterone treatment in elderly men. *Clin Trials* (2014) 11(3):362–75. doi: 10.1177/1740774514524032

62. Budoff MJ, Ellenberg SS, Lewis CE, Mohler ER 3rd, Wenger NK, Bhasin S, et al. Testosterone treatment and coronary artery plaque volume in older men with low testosterone. *JAMA* (2017) 317(7):708–16. doi: 10.1001/jama.2016.21043

63. Iellamo F, Volterrani M, Caminiti G, Karam R, Massaro R, Fini M, et al. Testosterone therapy in women with chronic heart failure. *J Am Coll Cardiol* (2010) 56(16):1310–6. doi: 10.1016/j.jacc.2010.03.090

64. Schwartz JB, Volterrani M, Caminiti G, Marazzi G, Fini M, Rosano GM, et al. Effects of testosterone on the q-T interval in older men and older women with chronic heart failure. *Int J Androl* (2011) 34(5 Pt 2):e415–421. doi: 10.1111/j.1365-2605.2011.01163.x

65. Spoletini I, Vitale C, Pelliccia F, et al. Androgens and cardiovascular disease in postmenopausal women: a systematic review. *Climacteric* (2014) 17(6):625–34. doi: 10.3109/13697137.2014.887669

66. European Medicines Agency. No consistent evidence of an increased risk of heart problems with testosterone medicines. (2014). Available at: https://www.ema.europa.eu/documents/press-release/no-consistent-evidence-increased-risk-heart-problems-testosterone-medicines_en.pdf.

67. Idan A, Griffiths KA, Harwood DT, Seibel MJ, Turner L, Conway AJ, et al. Long-term effects of dihydrotestosterone treatment on prostate growth in healthy, middle-aged men without prostate disease. *Ann Internal Med* (2010) 153(10):621–32. doi: 10.7326/0003-4819-153-10-201011160-00004

68. Kunelius P, Lukkarinen O, Hannuksela ML, Itkonen O, Tapanainen JS. The effects of transdermal dihydrotestosterone in the aging Male: A prospective, randomized, double blind study. *J Clin Endocrinol Metab* (2002) 87(4):1467–72. doi: 10.1210/jcem.87.4.8138

69. Ly LP, Jimenez M, Zhuang TN, Celermajer DS, Conway AJ, Handelsman DJ, et al. A double-blind, placebo-controlled, randomized clinical trial of transdermal dihydrotestosterone gel on muscular strength, mobility, and quality of life in older men with partial androgen deficiency. *J Clin Endocrinol Metab* (2001) 86(9):4078–88. doi: 10.1210/jcem.86.9.7821

70. Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, et al. Type 2 diabetes mellitus and testosterone: a meta-analysis study. *Int J Androl* (2011) 34(6 Pt 1):528–40. doi: 10.1111/j.1365-2605.2010.01117.x

71. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: Mechanisms, clinical significance, and treatment. *J Am Soc Nephrol* (2017) 28(4):1023–39. doi: 10.1681/ASN.2016060666

72. Vallon V, Komers R. Pathophysiology of the diabetic kidney. *Compr Physiol* (2011) 1(3):1175–232. doi: 10.1002/cphy.c100049

73. Vasavada N, Agarwal R. Role of oxidative stress in diabetic nephropathy. *Adv Chronic Kidney Dis* (2005) 12(2):146–54. doi: 10.1053/j.ackd.2005.01.001

74. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* (1999) 84(10):3666–72. doi: 10.1210/jcem.84.10.6079

75. Ranstam J. Hypothesis-generating and confirmatory studies, bonferroni correction, and pre-specification of trial endpoints. *Acta Orthop* (2019) 90 (4):297–7. doi: 10.1080/17453674.2019.1612624

frontiers | Frontiers in Medicine

TYPE Case Report
PUBLISHED 10 January 2023
DOI 10.3389/fmed.2022.1020096

OPEN ACCESS

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

RECEIVED 15 August 2022
ACCEPTED 12 December 2022
PUBLISHED 10 January 2023

CITATION
Brunerova L, Remes O, Zoubkova V and Votypka P (2023) Case report: Two heterozygous pathogenic variants of CYP24A1: A novel cause of hypercalcemia and nephrocalcinosis in adulthood. *Front. Med.* 9:1020096. doi: 10.3389/fmed.2022.1020096

Case report: Two heterozygous pathogenic variants of CYP24A1: A novel cause of hypercalcemia and nephrocalcinosis in adulthood

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Background and aims: Vitamin D 24-hydroxylase is an enzyme encoded by the CYP24A1 gene, which inhibits the activation of vitamin D to form inactive metabolites. More than 20 currently described pathogenic variants (usually biallelic) of this gene are responsible for idiopathic infantile hypercalcemia manifested typically in childhood (often in newborns) with hypercalcemia, hypercalciuria, and nephrocalcinosis. However, a few patients (mostly with monoallelic heterozygous pathogenic variants) can develop mild symptoms in adulthood.

Case description: We present the case of a 43-year-old male patient with hypertension and heterozygous Leiden mutation, with mural thrombi in the common iliac artery, who was sent by a nephrologist to endocrinological examination due to hypoparathyroidism, progressive hypercalcemia, hypercalciuria, and CKDG2A1. Complete laboratory and imaging methods (including PET-CT) excluded PTH-related peptide-mediated hypercalcemia and granulomatosis. Finally, the genetic analysis of the CYP24A1 gene revealed the presence of a novel combination of two heterozygous pathogenic variants: CYP24A1: c. 443T>C p.(Leu148Pro) and c.1186C>T p.(Arg396Trp).

Conclusion: Differential diagnosis of patients with hypercalciuria, nephrocalcinosis, and hypercalcemia related to vitamin D exposure should include the CYP24A1 gene mutation. To the best of our knowledge, this is the first case of the novel combination of two heterozygous pathogenic variants of CYP24A1.

KEYWORDS

CYP24A1 mutation, hypercalcemia, nephrocalcinosis, adult, low PTH

Introduction

Calcium–phosphate metabolism is regulated by three key hormones—active vitamin D (1,25OHD), parathormone (PTH), and calcitonin—acting on three major players in calcium–phosphate homeostasis (kidney, bone, and small intestine) (1). Hypercalcemia represents a potentially life-threatening condition with a wide differential diagnosis. The causes of hypercalcemia comprise of two major groups: PTH-mediated (primary or tertiary hyperparathyroidism) and non-PTH mediated. Non-PTH mediated hypercalcemia includes PTH-related peptide-associated hypercalcemia (in malignancies), vitamin D-associated hypercalcemia (in benign and malignant granulomatous diseases), medication-associated hypercalcemia (associated with lithium, thiazides, etc.), hypercalcemia associated with other endocrine diseases (e.g., hyperthyroidism), hypercalcemia due to genetic causes (typically familial hypocalciuric hypercalcemia caused by mutation of the calcium-sensing receptor), and others (e.g., immobilization with high bone turnover) (2).

A minority of genetic causes is represented by mutations of the CYP24A1 (cytochrome-P450 family 24 subfamily A member 1) gene. In 2011, Schlingmann et al. identified CYP24A1 as a candidate gene for autosomal recessive infantile hypercalcemia (IHH, OMIM 143880). Loss-of-function mutations of CYP24A1 result in hypercalcemia after vitamin D exposure (3). The CYP24A1 gene encodes the mitochondrial inner membrane P450 24-hydroxylase enzyme, which inhibits the activation of vitamin D metabolites (25-hydroxyvitamin D/25OHD and 1α,25-dihydroxyvitamin D/1,25OHD) to the inactive or less active ones (to 24,25-dihydroxyvitamin D/24,25OHD and 1α,24,25 trihydroxyvitamin D/1,24,25OHD) (3–5). The phenotype of IIH includes a wide spectrum of clinical scenarios (6), from severe forms, diagnosed early in infancy [severe hypercalcemia manifested as failure to thrive, dehydration, vomiting, and nephrocalcinosis (7)] to milder forms, often diagnosed in adulthood during a workout for recurrent nephrolithiasis (8). However, most patients with CYP24A1 mutation (particularly monoallelic heterozygous) remain unrecognized, as only a few hundred cases have been reported up to date (9, 10).

Herein, we present the case of an adult male patient with hypercalciuria, nephrocalcinosis, and intermittent progressive hypercalcemia caused by two heterozygous pathogenic variants of CYP24A1. The patient agreed with the anonymous presentation of his case, and he provided signed informed consent.

Case presentation

A 43-year-old male patient with marfanoid habitus (190 cm, 73 kg with long extremities) has been treated for hypertension since 2013 (ramipril 5 mg daily and felodipine 5 mg daily). Ultrasonography revealed nephrocalcinosis, and a CT scan described atherosclerotic aorta and common iliac arteries with mural thrombi. A geneticist excluded Marfan syndrome and found a heterozygous factor V. (Leiden) mutation. A cardiologist started treatment with aspirin 100 mg daily and atorvastatin 10 mg daily. A nephrologist diagnosed slightly decreased renal function (CKDG2) with normal albumin/creatinine (ACR) and protein/creatinine (PCR) ratio, normal urine analysis, and hypercalciuria and initiated the treatment with hydrochlorothiazide (12.5 mg daily). The patient was referred to an endocrinologist for possible hypoparathyroidism (serum calcium levels at the lower limit of the normal range and low PTH) in 2019.

The patient denied any history of renal stones and fractures. His father had hypertension and died of colorectal cancer, and his mother, siblings, and two children were healthy. There was no history of nephrolithiasis in the family.

At the endocrinological examination, the physical finding (except for marfanoid habitus) was normal, and no bone abnormalities were observed. The laboratory test revealed hypoparathyroidism but did not confirm hypocalcemia (serum calcium/S-Ca 2.50 mmol/l). Genetic analysis of the calcium-sensing receptor (Ca-SR) gene, performed due to low PTH and initially lower serum calcium, did not find any pathogenic variants.

Serum calcium levels progressively increased during the follow-up, and thus, hydrochlorothiazide was stopped in 2020, with practically no effect on calcium levels. The patient kept visiting both his nephrologist and his endocrinologist, however, very irregularly, and he missed his appointment several times; thus, the parameters were quite sparse (in overview in Table 1).

The highest level of serum calcium (3 mmol/l) was observed during the summer of 2021. In between, the detailed work-up was carried out for the exclusion of PTH-related peptide-associated hypercalcemia and granulomatosis-associated hypercalcemia. Complete laboratory tests showed normal serum protein electrophoresis including immunofixation, normal serum level of ACE/angiotensin-converting enzyme, and normal level of 1,25OHD. The markers of bone turnover were within the normal range (cross laps 331 ng/l: normal range 182–801; and P1NP 27.6 μg/l: normal range 15–80).

There were normal findings in densitometry (Z-score of lumbar spine 1.1; Z-score of proximal femur −0.1 and trabecular bone score 1.485), and ^{99m}Tc/technetium scintigraphy and ^{18}FDG-PET/CT (fluorodeoxyglucose positron emission tomography/computed tomography) did not reveal any pathology.

In the end, in January 2022, the genetic examination for CYP24A1 was performed by massively parallel sequencing using a Clinical Exome Solution (CES, Sophia Genetics, Switzerland) comprising ∼5,000 Mendelian disease-related genes. DNA library was sequenced on the NextSeq platform (Illumina, USA).

TABLE 1 Laboratory finding throughout the time of follow-up.

Parameter/ year	2018	2019	2020	2021	2022
S-Ca (2.2–2.6 mmol/l)	2.2–2.5–2.66	2.69	2.63	2.8–3	2.69
S-Ca-i (1.0–1.4 mmol/l)	x–x—1.28				
S-P (0.7–1.5 mmol/l)	0.88–x—0.9	1.26		0.9–x	0.88
S-Mg (0.7–1.0 mmol/l)	0.69–x—0.8	0.77		0.88–x	
PTH (1.6–6.9 pmol/l)	0.58–x—0.3	0.47		0.3–x	
25OHD (75–150 nmol/l)	85–x—141	135	75.4	141–x	
1.25OHD (19.9–79.3 ng/l)	x	61		67–x	
S-crea (64–104 μmol/l)	102–115–126	134	137	136–x	135
DU-Ca (2–5 mmol/day)	8.5–x–x	6.1		6.1–7.7	5.5

The data comes from both—the nephrological and the endocrinological examinations. S-Ca, serum calcium; S-Ca-i, serum ionized calcium; S-P, serum phosphate; S-Mg, serum magnesium; PTH, parathormon; 25OHD, 25-hydroxyvitamin D; 1,25OHD, 1,25-dihydroxyvitamin D; S-crea, serum creatinin; DU-Ca, total amount of urine calcium/24 h; x, unmeasured parameter at certain time point.

Sequencing data were processed and analyzed by Sophia DDM software. Variant prioritization was also performed by Sophia DDM software and supported by Integrative Genomics Viewer (IGV; Broad Institute), Alamut Visual (Interactive Biosoftware), and VarSome Clinical software. Variant prioritization was carried out on the basis of frequency in the general population (gnomAD and dbSNP databases), presence and frequency in clinical databases (i.e., ClinVar), interspecies conservation of the residue and coherence, and *in silico* predictions using tools integrated into VarSome Clinical software (DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, REVEL, PolyPhen, and SIFT). The pathogenicity of the detected variants was classified into five categories according to the evidence criteria proposed by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines (11). We identified two pathogenic variants in the CYP24A1 gene: NM_000782.5(CYP24A1):c.443T>C p.(Leu148Pro) and NM_000782.5(CYP24A1):c.1186C>T p.(Arg396Trp). The ACMG classification of these pathogenic variants is as follows: NM_000782.5(CYP24A1):c.443T>C p.(Leu148Pro)—class 5 (PM2, PP2, PP3, PP5, and PS3) and NM_000782.5(CYP24A1):c.1186C>T p.(Arg396Trp)—class 5 (PM2, PP2, PP3, PP5, and PM5). Detected variants were validated by Sanger DNA sequencing.

We strongly recommended the genetic analysis of the patient's relatives; however, neither of them has undergone the blood sampling yet. Nonetheless, we advised the patient to inform his relatives that vitamin D supplementation and excessive calcium intake could be risky in terms of developing severe hypercalcemia if CYP24A1 mutation is present.

The patient was instructed to maintain higher fluid intake, not to be expose to vitamin D (including sun exposure if possible), and to avoid excessive calcium intake. During further follow-up, calcium levels remained stable and slightly above the upper normal range.

Discussion

Loss-of-function mutations of the CYP24A1 gene lead to increased levels of active metabolites of vitamin D causing hyperabsorptive hypercalcemia, hypercalciuria, nephrocalcinosis, and nephrolithiasis, typically diagnosed in childhood as IHH. Although rare in their occurrence (12), they should be considered as the cause of hypercalcemia, particularly if associated with nephrocalcinosis and/or nephrolithiasis, even in adults. In our patient, the laboratory findings (hypercalcemia, hypercalciuria, and suppressed PTH levels) were typical for the carriers of loss-of-function mutations in CYP24A1. However, normal levels of 1α,25OHD and negative family history did not fit into the typical clinical picture; thus, we first focused on the exclusion of other (more common) causes of hypercalcemia with suppressed PTH levels. The levels of 1α,25OHD were measured two times during the follow-up and were surprisingly at the upper limit of the normal range but not elevated as would be expected in the case of CYP24A1 loss-of-function mutations. However, the heterogeneity of 1,25OH levels is very high in patients with mild forms of IHH (and their adult relatives), ranging from near-normal levels to highly elevated ones (13). Furthermore, in the Molin's et al. study (14) of patients with hypersensitivity to vitamin D, the levels of 1,25OHD did not significantly differ among patients with or without CYP24A1 mutation. We can just speculate that the combination of these pathogenic variants is not necessarily associated with elevated levels of 1,25OHD (although no functional studies were done to confirm this hypothesis and since it is the first described case of this combination of pathogenic variants, no literature data are available).

During the sequencing, two pathogenic variants in the CYP24A1 gene were identified, NM_000782.5(CYP24A1):c.443T>C p.(Leu148Pro) and NM_000782.5(CYP24A1):c.1186C>T p.(Arg396Trp), and validated by Sanger DNA sequencing. The former is known as missense mutation resulting in a 25–50% decrease in the activity of CYP24A1 (15–17). The latter is also a missense mutation (18–25); however, to the best of our knowledge, no case of the

common occurrence of two heterozygous pathogenic variants, p.(Leu148Pro) and p.(Arg396Trp), has been described yet.

The blood samples of our patient were analyzed repeatedly throughout the year with the highest levels of calcium found during the summer period (the patient was an active football player massively exposed to sunlight). The fluctuations of serum calcium levels related to sun exposure in the p.(Arg396Trp) mutation carriers were described previously (21, 24).

Bone densitometry found normal age- and gender-related BMD, and markers of bone turnover were also normal. Similar findings were observed in a small study of heterozygous (Arg396Trp) mutation carriers, which did not find any major differences in bone health if compared with the wild type (23). However, in preclinical studies, mice with CYP24A1 mutations (complete deficiency of the enzyme) revealed impaired intramembranous bone mineralization due to the accumulation of osteoid caused by elevated 1,25OHD levels (26). No data on bone health in p.(Leu148Pro) mutation carriers have (to the best of our knowledge) been described yet. Based on our case, we suggest that the carrier of two heterozygous pathogenic variants, p.(Leu148Pro) and p.(Arg396Trp), does not seem to have a negative impact on bone health (although it could be modified by intensive physical activity of our patient).

In terms of non-pharmacologic treatment, it is reasonable to avoid exogenous vitamin D supplementation and excessive sunlight exposure; however, the efficacy of these approaches has to be confirmed (9). In our patient, this approach seemed to be quite effective (particularly the avoidance of un-protective sunlight exposure).

Hypercalcemia can be managed with intravenous 0.9% saline and frusemide, but steroids are not effective since functional CYP24A1 is required for its action (27, 28). Other therapeutic options described in the literature include the use of azoles (reducing the synthesis of 1,25OHD by inhibiting CYP27B1) (29–31), rifampin (inducer of CYP3A4 enzyme that catalyzes non-specific hydroxylation of 1,25OHD to inactive metabolite) (32), supplementation with L-methionine for urinary acidification as the prevention of calcium stone formation (33), or bisphosphonates in severe cases of IHH (25).

No association between the factor V. Leiden mutation and the mutations pertaining to vitamin D metabolism was found in the literature.

It would be proper to perform a segregation analysis of the proband's parents in order to confirm the variants being on both the alleles—that is, trans-heterozygous (as considered); however, his father is unavailable, and his mother and siblings have not been willing to undergo the blood sampling yet.

Conclusion

Biallelic homozygous mutations of the CYP24A1 gene lead to the typical phenotype of IHH; however, even two heterozygous pathogenic variants can result in hypercalcemia, hypercalciuria, and nephrocalcinosis/nephrolithiasis, but due to relatively mild symptoms, it can be diagnosed (in comparison with IHH) late in adulthood. In the presented case of a young male, the novel common occurrence of two heterozygous pathogenic variants p.(Leu148Pro) and p.(Arg396Trp) has been identified. Genetic analysis of CYP24A1 should be considered in patients with hypercalcemia and suppressed PTH, particularly if associated with hypercalciuria and nephrocalcinosis/nephrolithiasis, despite normal levels of 1,25OHD.

Data availability statement

The datasets presented in this article are not readily available to protect patient privacy and confidentiality. Requests to access the datasets should be directed to the corresponding author(s).

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. 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

LB prepared the manuscript. OR, VZ, and PV revised the manuscript. All the authors contributed in the clinical and genetic diagnosis of the patient. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Cooperatio 37—Metabolic diseases a MH CZ—DRO (Kralovske Vinohrady University Hospital—FNKV, 00064173).

Acknowledgments

The authors would like to thank Michael Allen for the English editing.

Conflict of interest

OR was employed by Nefromed.

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

References

1. DiMeglio LA, Imel EA. Chapter 13 - calcium and phosphate: hormonal regulation and metabolism. In: Burr DB, Allen MR, editors. *Basic and Applied Bone Biology*. San Diego, CA: Academic Press (2014). p. 261–82. doi: 10.1016/B978-0-12-416015-6.00013-7

2. Carroll MF, Schade DS. A practical approach to hypercalcemia. *Am Fam Physician*. (2003) 67:1959–66.

3. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med*. (2011) 365:410–21. doi: 10.1056/NEJMoa1103864

4. Carpenter TO. CYP24A1 loss of function: Clinical phenotype of monoallelic and biallelic mutations. *J Steroid Biochem Mol Biol*. (2017) 173:337–40. doi: 10.1016/j.jsbmb.2017.01.006

5. De Paolis E, Scaglione GL, De Bonis M, Minucci A, Capoluongo E. CYP24A1 and SLC34A1 genetic defects associated with idiopathic infantile hypercalcemia: from genotype to phenotype. *Clin Chem Lab Med*. (2019) 57:1650–67. doi: 10.1515/cclm-2018-1208

6. O'Keeffe DT, Tebben PJ, Kumar R, Singh RJ, Wu Y, Wermers RA. Clinical and biochemical phenotypes of adults with monoallelic and biallelic CYP24A1 mutations: evidence of gene dose effect. *Osteoporosis Int*. (2016) 27:3121–5. doi: 10.1007/s00198-016-3615-6

7. Lightwood R, Stapleton T. Idiopathic hypercalcaemia in infants. *Lancet*. (1953) 262:255–6. doi: 10.1016/S0140-6736(53)90187-1

8. Jacobs TP, Kaufman M, Jones G, Kumar R, Schlingmann K-P, Shapses S, et al. A lifetime of hypercalcemia and hypercalciuria, finally explained. *J Clin Endocrinol Metab*. (2014) 99:708–12. doi: 10.1210/jc.2013-3802

9. Jones G, Kottler ML, Schlingmann KP. Genetic diseases of vitamin D metabolizing enzymes. *Endocrinol Metab Clin North Am*. (2017) 46:1095–117. doi: 10.1016/j.ecl.2017.07.011

10. Molin A, Baudoin R, Kaufmann M, Souberbielle JC, Ryckewaert A, Vantyghem MC, et al. CYP24A1 mutations in a cohort of hypercalcemic patients: evidence for a recessive trait. *J Clin Endocrinol Metab*. (2015) 100:E1343–52. doi: 10.1210/jc.2014-4387

11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. (2015) 17:405–24. doi: 10.1038/gim.2015.30

12. Cappellani D, Brancatella A, Kaufmann M, Minucci A, Vignali E, Canale D, et al. Hereditary hypercalcemia caused by a homozygous pathogenic variant in the CYP24A1 gene: a case report and review of the literature. *Case Rep Endocrinol*. (2019) 2019:4982621. doi: 10.1155/2019/4982621

13. Lenherr-Taube N, Young EJ, Furman M, Elia Y, Assor E, Chitayat D, et al. Mild idiopathic infantile hypercalcemia-part 1: biochemical and genetic findings. *J Clin Endocrinol Metab*. (2021) 106:2915–37. doi: 10.1210/clinem/dgab431

14. Molin A, Lemoine S, Kaufmann M, Breton P, Nowoczyn M, Ballandonne C, et al. overlapping phenotypes associated with *CYP24A1*, *SLC34A1*, and *SLC34A3* mutations: a cohort study of patients with hypersensitivity to vitamin D. *Front Endocrinol*. (2021) 12:736240. doi: 10.3389/fendo.2021.736240

15. Nesterova G, Malicdan MC, Yasuda K, Sakaki T, Vilboux T, Ciccone C, et al. 1,25-(OH)2D-24 hydroxylase (CYP24A1) deficiency as a cause of nephrolithiasis. *Clin J Am Soc Nephrol*. (2013) 8:649–57. doi: 10.2215/CJN.05360512

16. Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys*. (2012) 523:9–18. doi: 10.1016/j.abb.2011.11.003

17. Kaufmann M, Prosser DE, Jones G. Bioengineering anabolic vitamin D-25-hydroxylase activity into the human vitamin D catabolic enzyme, cytochrome P450 CYP24A1, by a V391L mutation. *J Biol Chem*. (2011) 286:28729–37. doi: 10.1074/jbc.M111.236679

18. Fencl F, Bláhová K, Schlingmann KP, Konrad M, Seeman T. Severe hypercalcemic crisis in an infant with idiopathic infantile hypercalcemia caused by mutation in CYP24A1 gene. *Eur J Pediatr*. (2013) 172:45–9. doi: 10.1007/s00431-012-1818-1

19. Wolf P, Müller-Sacherer T, Baumgartner-Parzer S, Winhofer Y, Kroo J, Gessl A, et al. A case of "late-onset" idiopathic infantile hypercalcemia secondary to mutations in the CYP24A1 gene. *Endocr Pract*. (2014) 20:e91–5. doi: 10.4158/EP13479.CR

20. Dinour D, Davidovits M, Aviner S, Ganon L, Michael L, Modan-Moses D, et al. Maternal and infantile hypercalcemia caused by vitamin-D-hydroxylase mutations and vitamin D intake. *Pediatr Nephrol*. (2015) 30:145–52. doi: 10.1007/s00467-014-2889-1

21. Figueres ML, Linglart A, Bienaime F, Allain-Launay E, Roussey-Kessler G, Ryckewaert A, et al. Kidney function and influence of sunlight exposure in patients with impaired 24-hydroxylation of vitamin D due to CYP24A1 mutations. *Am J Kidney Dis*. (2015) 65:122–6. doi: 10.1053/j.ajkd.2014.06.037

22. Shah AD, Hsiao EC, O'Donnell B, Salmeen K, Nussbaum R, Krebs M, et al. Maternal hypercalcemia due to failure of 1,25-dihydroxyvitamin-D3 catabolism in a patient with CYP24A1 mutations. *J Clin Endocrinol Metab*. (2015) 100:2832–6. doi: 10.1210/jc.2015-1973

23. Cools M, Goemaere S, Baetens D, Raes A, Desloovere A, Kaufman JM, et al. Calcium and bone homeostasis in heterozygous carriers of CYP24A1 mutations: a cross-sectional study. *Bone*. (2015) 81:89–96. doi: 10.1016/j.bone.2015.06.018

24. Jiráčková J, Hyšpler R, Alkanderi S, Pavlíková L, Palicka V. Novel CYP24A1 mutation in a young male patient with nephrolithiasis: case report. *Kidney Blood Press Res*. (2019) 44: 870–7. doi: 10.1159/000500922

25. Skalova S, Cerna L, Bayer M, Kutilek S, Konrad M, Schlingmann KP. Intravenous pamidronate in the treatment of severe idiopathic infantile hypercalcemia. *Iran J Kidney Dis*. (2013) 7:160–4.

26. St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, et al. Deficient mineralization of intramembranous bone in vitamin D-24-hydroxylase-ablated mice is due to elevated 1,25-dihydroxyvitamin D and not to the absence of 24,25-dihydroxyvitamin D. *Endocrinology*. (2000) 141:2658–66. doi: 10.1210/endo.141.7.7579

27. Colussi G, Ganon L, Penco S, Korneyeva S, Poole L, Saha G, et al. Chronic hypercalcaemia from inactivating mutations of vitamin D 24-hydroxylase (CYP24A1): Implications for mineral metabolism changes in chronic renal failure. *Nephrol Dial Transplant*. (2014) 29:636–43. doi: 10.1093/ndt/gft460

28. St-Arnaud R. CYP24A1-deficient mice as a tool to uncover a biological activity for vitamin D metabolites hydroxylated at position 24. *J Steroid Biochem Mol Biol*. (2010) 121:254–6. doi: 10.1016/j.jsbmb.2010.02.002

29. Nguyen M, Boutignon H, Mallet E, Linglart A, Guillozo H, Jehan F, et al. Infantile hypercalcemia and hypercalciuria: new insights into a vitamin D-dependent mechanism and response to ketoconazole treatment. *J Pediatr*. (2010) 157:296–302. doi: 10.1016/j.jpeds.2010.02.025s

30. Sayers J, Hynes AM, Srivastava S, Dowen F, Quinton R, Datta HK, et al. Successful treatment of hypercalcaemia associated with a CYP24A1 mutation with fluconazole. *Clin Kidney J*. (2015) 8:453–5. doi: 10.1093/ckj/sfv028

31. Tebben PJ, Milliner DS, Horst RL, Harris PC, Singh RJ, Wu Y, et al. Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to CYP24A1 mutations: effects of ketoconazole therapy. *J Clin Endocrinol Metab*. (2012) 97:E423–7. doi: 10.1210/jc.2011-1935

32. Hawkes CP, Li D, Hakonarson H, Meyers KE, Thumme KE, Levine MA. CYP3A4 Induction by Rifampin: an alternative pathway for Vitamin D inactivation in patients with CYP24A1 mutations. *J Clin Endocrinol Metab*. (2017) 102:1440–6. doi: 10.1210/jc.2016-4048

33. Siener R, Struwe F, Hesse A. Effect of L-methionine on the risk of phosphate stone formation. *Urology*. (2016) 98:39–43. doi: 10.1016/j.urology.2016.08.007

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 February 2023
DOI 10.3389/fendo.2023.1031895

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 30 August 2022
ACCEPTED 30 January 2023
PUBLISHED 14 February 2023

CITATION
Liu H, Cao M, Jin Y, Jia B, Wang L, Dong M, Han L, Abankwah J, Liu J, Zhou T, Chen B, Wang Y and Bian Y (2023) Network pharmacology and experimental validation to elucidate the pharmacological mechanisms of Bushen Huashi decoction against kidney stones.
Front. Endocrinol. 14:1031895.
doi: 10.3389/fendo.2023.1031895

Network pharmacology and experimental validation to elucidate the pharmacological mechanisms of Bushen Huashi decoction against kidney stones

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Introduction: Kidney stone disease (KS) is a complicated disease with an increasing global incidence. It was shown that Bushen Huashi decoction (BSHS) is a classic Chinese medicine formula that has therapeutic benefits for patients with KS. However, its pharmacological profile and mechanism of action are yet to be elucidated.

Methods: The present study used a network pharmacology approach to characterize the mechanism by which BSHS affects KS. Compounds were retrieved from corresponding databases, and active compounds were selected based on their oral bioavailability (≥30) and drug-likeness index (≥0.18). BSHS potential proteins were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, whereas KS potential genes were obtained from GeneCards and OMIM, TTD, and DisGeNET. Gene ontology and pathway enrichment analysis were used to determine potential pathways associated with genes. The ingredients of BSHS extract were identified by the ultra-high-performance liquid chromatography coupled with quadrupole orbitrap mass spectrometry (UHPLC-Q/Orbitrap MS). The network pharmacology analyses predicted the potential underlying action mechanisms of BSHS on KS, which were further validated experimentally in the rat model of calcium oxalate kidney stones.

Results: Our study found that BSHS reduced renal crystal deposition and improved renal function in ethylene glycol(EG)+ammonium chloride(AC)-induced rats, and also reversed oxidative stress levels and inhibited renal tubular epithelial cell apoptosis in rats. BSHS upregulated protein and mRNA expression of E2, ESR1, ESR2, BCL2, NRF2, and HO-1 in EG+AC-induced rat kidney while downregulating BAX protein and mRNA expression, consistent with the network pharmacology results.

Discussion: This study provides evidence that BSHS plays a critical role in anti-KS *via* regulation of E2/ESR1/2, NRF2/HO-1, and BCL2/BAX signaling pathways, indicating that BSHS is a candidate herbal drug for further investigation in treating KS.

KEYWORDS

Bushen Huashi decoction, kidney stone, network pharmacology, Chinese medicine formula, crystal deposition

1 Introduction

Kidney stone disease is a urinary system disease caused by the kidney's abnormal accumulation of crystalline material such as calcium, oxalic acid, uric acid, and cystine (1). With a prevalence of 7% to 13% in North America, 5-9% in Europe, and 1-5% in Asia, it is one of the most common diseases affecting populations all over the world (1). Recent data from the United States showed that the prevalence of stones in the United States was 8.8%, with 10.6% and 7.1% reported for their prevalence in men and women, respectively (2). Primary hyperparathyroidism (3), obesity (4), diabetes (5, 6), and hypertension (7, 8) are some of the risk factors for kidney stone formation. Patients with kidney stones are also at high risk of hypertension (7), chronic kidney disease (CKD) (9, 10), and end-stage renal disease (ESRD) (10, 11). Kidney stones always lead to several complications, such as urinary tract obstruction, hydronephrosis, infection, local damage to the kidney, and renal dysfunction (12). The formation of kidney stones is a complex process involving urinary supersaturation, nucleation, growth, aggregation, and retention of urinary stone components within the renal tubular cells (13). Surgical treatment for the removal of kidney and ureteral stones has already achieved mature development over the past several decades (14). Currently, the most common kidney stone treatments include shock wave lithotripsy, ureteroscopic fragmentation and removal, and percutaneous nephrolithotomy (14). Although surgical therapies have greatly resolved patients' pain, postoperative adverse effects and a high recurrence rate of stones are vexing. Therefore, scientists are focusing on exploring new targets and new drugs, which is crucial to reduce the incidence and recurrence rate of kidney stones.

Traditional Chinese medicine (TCM) has been effectively used in treating diverse diseases for a long time in China and is also traditionally used to treat kidney stones (15). Different from the single-target concept of Western medicine, TCM emphasizes the concept of the body as an organic whole. Generally, a TCM prescription is composed of several herbs. Each herb typically has multiple active compounds that simultaneously act on multi-targets. Due to the complexity of the TCM components, conventional pharmacology research methods are complex to fully elucidate the potential molecular mechanism of TCM compounds in disease treatment.

BSHS is an experienced TCM prescription of the National Famous TCM Doctor, Professor Baogui Chen, commonly used to treat kidney stones and has shown remarkable effectiveness in clinical practice. BSHS is mainly composed of eight Chinese herbs, including Lysimachiae Herba, Lygodii Spora, Plantaginis Semen, Clerodendranthus Spicatus, Cibotii Rhizoma, Dipsaci Radix, Malva verticillata seed, and Licorice. It remains relatively unclear, however, what the bioactive components of THCQ are as well as their pharmacological mechanisms. During the past few years, system biology, polypharmacology, and system biology-based network pharmacology have boomed due to the increase in biomedical data. With network pharmacology, target molecules, biological functions, and bioactive compounds can be combined to form complex interaction networks, which are precisely in line with the natural characteristics of TCM and can improve our understanding of TCM's mechanisms of action (16). A network pharmacology approach can contribute to our understanding of the multicomponent, multitarget, and multi-pathway nature of TCM. This study aimed to decipher the mechanism of action of BSHS in suppressing kidney stones by integrating network pharmacology and pharmacological evaluation.

2 Materials and methods

2.1 Reagents and materials

All medicinal plants were provided by Wuqing Hospital of Traditional Chinese Medicine affiliated to Tianjin University of Traditional Chinese Medicine (Tianjin, China). The Paishi granule (PSG) was purchased from Nanjing Tongrentang Pharmaceutical Co., Ltd. (Nanjing, China). The Urea (BUN) Assay Kit, Creatinine (Cr) Assay kit, Calcium (Ca) Assay Kit, Alkaline phosphatase (ALP) assay kit, Magnesium (Mg) Assay Kit, Superoxide Dismutase (SOD) assay kit, and Malondialdehyde (MDA) assay kit were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). The oxalate content detection kit and BCA Protein Assay Kit were purchased from Beijing Suolaibao Technology Co., Ltd. (Beijing, China), whiles Von Kossa dye and HE dye were purchased from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China). The ELISA assay kits for estradiol (E2) were purchased from cloud-clone Corp. Wuhan (Wuhan, China). The anti-estrogen receptor alpha (ERα) antibody, anti-NRF2 antibody, anti-BCL-2 antibody, and anti-Bax antibody were purchased from Abcam (USA). The Caspase-3 Antibody and Cleaved Caspase-3 Antibody were purchased from CST (USA). The Estrogen receptor beta (ERβ) Rabbit pAb and HO-1 Rabbit pAb were purchased from Abclonal (Wuhan, China). The

HPLC grade acetonitrile and formic acid was purchased from Fisher Chemicals (Fisher Scientific, Waltham, MA, USA). The purified water was purchased from Guangzhou Watsons Food and Beverage Co., Ltd (Guangzhou, China).

2.2 Analysis of BSHS by network pharmacology

2.2.1 Screening bioactive components and action targets of BSHS

We used the TCMSP database (http://www.tcmspw.com/tcmsp.php) to search for BSHS constituent medicines (Lysimachiae Herba, Lygodii Spora, Plantaginis Semen, Dipsaci Radix, and Licorice) and their chemical and pharmacological data. The remaining herbs, such as Clerodendranthus spicatus, Cibotii Rhizoma, and Malva verticillata seed were not retrieved from the database; however, their active compounds were retrieved by reviewing the literature. As parameters for screening the compounds collected, oral bioavailability (OB) and drug-like quality (DL) were selected. The OB represents the percentage of unchanged drug that reaches the systemic circulation after oral administration. DL indexes can be used to optimize pharmacokinetic and pharmaceutical properties, such as solubility and chemical stability (17). Here, we set OB ≥ 30 and DL ≥ 0.18 as criteria to screen for biologically active compounds. TCMSP was further used to screen the targets of the active ingredients of BSHS, and Uniprot was used to correct and deduplicate the drug targets. (https://www.uniprot.org/).

2.2.2 The construction of the drug-active ingredient-target interaction network

The obtained active ingredients and cross-targets were sorted using Microsoft Excel worksheet. After the data were imported into Cytoscape 3.7.2 software, a "drug-active ingredient-target" network model was constructed, in which the nodes represent herbs, ingredients, and targets, while the edges represent the relationship role among the three nodes. We calculated the 'degree' value according to the number of associations between each node.

2.2.3 Screening of potential targets for KS

We used terms such as kidney calculus, kidney stones, Nephrolithiasis, Renal calculus, and Renal stones as keywords related to screened potential targets from Genecards (https://www.genecards.org), Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/ttd/ttd.asp), Online Mendelian Inheritance in Man (OMIM, https://www.genecards.org), and DisGeNET (https://www.disgenet.org/home/) databases. After eliminating repetitive targets, the potential targets that correlated with KS were obtained. In order to determine the intersection between BSHS and KS targets, we drew a Venn diagram.

2.2.4 Construction of the protein-protein interaction network and screening of key targets

In order to clarify the functional interactions between the screened potential proteins, we constructed a protein-protein interaction (PPI) network using the STRING database (https://string-db.org/). The PPI network was inputted into Cytoscape 3.7.2 software using the CytoNCA software to analyze the topology of the intersection network. Further, we took the nodes with the 'degree' value greater than twice the median as the basis for screening the key targets and finally got the critical targets of BSHS for treating kidney stone disease.

2.2.5 KS-related target gene ontology and KEGG pathway enrichment analysis for BSHS

The analysis of GO enrichment and KEGG pathways was conducted by DAVID Bioinformatics Resources 6.8 (http://david.ncifcrf.gov). For functional annotation clustering, terms with thresholds of Count ≥ 2 and Expression Analysis Systematic Explorer (EASE) scores ≤ 0.05 were selected.

2.3 Experimental verification

2.3.1 Preparation of BSHS

Eight raw herbs of BSHS were provided from the pharmacy department of the Wuqing Hospital of Traditional Chinese Medicine affiliated with Tianjin University of Traditional Chinese Medicine(Tianjin, China) (Supplementary Figure 1). Lysimachiae Herba, Lygodii Spora, Plantaginis Semen, Clerodendranthus Spicatus, Cibotii Rhizoma, Dipsaci Radix, Malva verticillata seed, and Licorice were mixed in proportions of 3:1.5:1.5:3:1.5:1.5:1.5:1(w/w) respectively and then soaked in 12 times the volume of distilled water (v/m) for 1 h, decocted twice, at 1.5 h per decoction. After concentrating the decoction to 1 g/mL, it was stored at -20°C until used.

2.3.2 UHPLC-Q/Orbitrap MS analysis of SM ethanol extract

2.3.2.1 Preparation of test solution

Weigh 20 mg of BSHS extract in a 1.5 mL centrifuge tube, add 1 mL of pure water, vortex for 2 min, sonicate for 10 min, dilute 10 times with pure water, centrifuge at 1,4000 rpm for 20 min to extract the supernatant and leave for measurement.

2.3.2.2 Chromatographic conditions

The chromatographic column was a Waters ACQUITY UPLC® BEH C18 column (1.8 μm, 2.1 × 100 mm); the mobile phase was 0.1% formic acid in water (A) -acetonitrile (B). Gradient elution (0-2 min, 3% B; 2-6 min, 3-23% B; 6-10 min, 23-23.5% B; 10-10.5 min, 23.5-35% B; 10.5-11 min, 35-40% B; 11-15 min, 40-45% B; 15-16 min, 45-100% B; 16-17 min, 100% B; 17.01 min, 3% B; 18 min; 3% B); Flow rate: 0.4 mL/min. Column temperature: 40°C; The injection volume was 5.0 μL.

2.3.2.3 Mass spectrometry conditions

The HESI (heated electrospray ionization probe) parameters were as follows: spray voltage, -3.0 kV/+3.5 kV; sheath gas, (N2) 35 L/h; auxiliary gas (N2), 10 L/h; purge gas (N2), 0 L/h; capillary temperature, 350 °C; auxiliary gas heater temperature, 350 °C. The Full MS/dd-MS^2 scan method is used, with simultaneous detection in both positive and negative ion modes. The MS^1 full Scan Range was

m/z 100-1500 with a resolution of 70000; MS^2 mass spectrometry scan was dynamic mass range with a resolution of 17500; automatic gain control (AGC) for MS^1 and MS^2 were set to 3×10^6 and 1×10^5 respectively; maximum injection time was defined as 100 ms and 50 ms for MS^1 and MS^2, respectively; collision energy (HCD) was performed at a normalised collision energy (NCE) of 10/30/50 V; isolation width was set to 4.0 *m/z*; dynamic exclusion time was 10 s.

2.3.2.4 Data processing

The raw mass spectrometry data files of BSHS extracts were imported into Compound Discover software for automatic identification according to the Natural Product database, followed by processing of the analytical results from Compound Discover. Xcalibur software was used to identify and characterize the chemical components of BSHS.

2.4 Establishment and grouping of a rat model of calcium oxalate kidney stones

Male SD rats (n=40) weighing 180-220 g were purchased from Beijing Viton Lever Laboratory Animal Technology Ltd. (Beijing, China). All animals were housed under standard laboratory conditions with free access to water and food. After a 7-day environmental adaptation period, the rats were randomly divided into four groups, i.e., the normal control group, model group, PSG group, and BSHS group, with 10 rats in each group. The rats in the normal control group were given free water and were intragastrically injected with saline (2 ml/d) after two weeks. The rats in the model group were given 0.75% EG (v/v) + 0.75% AC (w/v) free water for two weeks and were intragastrically injected with saline (2 ml/d) after two weeks. The rats in the PSG group were given 0.75% EG (v/v) + 0.75% AC (w/v) free water for two weeks and were intragastric injected with PSG (6.17 g/kg/d) after two weeks. The rats in the BSHS group were given 0.75% EG (v/v) + 0.75% AC (w/v) free water for two weeks and were intragastric injected with BSHS herbal concentrate (11.6 g/kg/d) after two weeks. A day before the execution, 24 h urine samples were collected from all the rats and stored at -80°C. On day 28, the animals were treated with anesthesia, and blood samples were taken from the abdominal aorta and centrifuged at 3500 rpm for 15 min at 4°C. The serum supernatant was aspirated and stored at -80°C. The left kidney was fixed in 4% paraformaldehyde and embedded in paraffin for hematoxylin and eosin (H&E) staining, Von Kossa staining, and TUNEL staining. After snap-freezing in liquid nitrogen, the right kidney was stored at -80°C. All animal experiments were performed according to the requirements of the Experimental Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine.

2.5 Renal pathological examination and crystal deposition assay

The left kidney was fixed in 4% paraformaldehyde, dehydrated in gradient alcohol and embedded in paraffin as described by Qian et al (18). Longitudinal 4-μm paraffin sections were prepared for the H & E and Von Kossa staining. These sections were observed under the fluorescence microscope (Olympus IX2-UCB, Japan) to confirm the presence of crystals in the stained materials. The formed crystals were evaluated using professional image analysis software (ImageJ, U.S.A). Each section was photographed with 20 randomly selected fields of view under a 200× microscope. The calculations of the stone area were determined for each section using Image J software to obtain the sum of stone area under 20 fields of view and the percentage of stone area, i.e., percentage of stone area = $\frac{\text{stone area}}{\text{longitudinal section area of kidney}} * 100$.

2.6 Urine volume and renal/body weight index in rats

On day 28, we fed rats in metabolic cages and collected 24 h of urine using 0.02% sodium azide to prevent bacterial growth and recording of 24 h urine volume in rats. The kidneys were removed bilaterally, stripped of peritoneum and fat, and then weighed. Renal /body weight index = $\frac{\text{renal weight}}{\text{body weight}} * 100$.

2.7 Renal biochemical examination

The right kidneys were placed in ice-cold phosphate-buffered saline, pH 7.4, and homogenized using a tissue homogenizer. Commercial kits were used strictly according to the manufacturer's instructions to determine the levels of malondialdehyde (MDA), superoxide dismutase (SOD), oxalate, and calcium (Ca) in the tissue.

2.8 Urine biochemical examination

On day 28, we fed rats in metabolic cages and collected 24 h of urine using 0.02% sodium azide to prevent bacterial growth. The levels of urinary oxalate, Ca, phosphorus (P), and magnesium (Mg) were examined by utilizing the commercial kits on the microplate reader (Varioskan Flash, Thermo Scientific) following the instructions of the manufacturers.

2.9 Serum biological parameters analysis

After 2 hours at room temperature, blood samples were centrifuged for 15 minutes at 3500 rpm (4°C). The supernatants were collected and stored at −80°C until use. The levels of serum Ca, P, Mg, creatinine (Cr), and urea nitrogen (BUN) were examined by utilizing the commercial kits in the microplate reader following the instructions of the manufacturers.

2.10 Terminal deoxynucleotidyl transferase dUTP nick-end labeling staining

We used a TUNEL assay kit according to the instructions of the manufacturer to assay renal apoptosis in left kidney tissues embedded in paraffin and cut into 4mm thick sections. Cells positive for TUNEL were counted in 5 randomly selected fields (400x magnification) under a fluorescence microscope (Olympus IX2-UCB, Japan). The rate of apoptotic cells was analyzed using Image J (USA).

2.11 ELISA assay

After 2 hours at room temperature, blood samples were centrifuged for 15 minutes at 3500 rpm (4°C). The supernatants were collected and stored at -80°C until used. The levels of E2 were measured with commercial ELISA kits following the protocols of the manufacturer.

2.12 Quantitative real time polymerase chain reaction

Total RNA from frozen right renal tissue was isolated using RNA simple Total RNA Kit (Tiangen, Beijing, China) and then reverse-transcribed to cDNA with a Reverse Transcription Kit (Tiangen, Beijing, China). The qPCR was performed using Bio-Rad IQ5 (Bio-Rad, USA) and according to the manufacturer's protocols for the setup procedure. The housekeeping gene GAPDH was used for normalization. The fold changes were calculated using the method of $2^{-\Delta\Delta Ct}$. All primer sequences used in this study have been shown in Table 1.

2.13 Western blot analysis

RIPA buffer (Solarbio Co., Ltd. Beijing, China) was used to extract proteins from kidney tissues. Protein needs to be denatured by boiling it in a metal bath at 100°C for 10 minutes. The total protein concentration was determined using the BCA protein assay kit (Solarbio, Beijing, China). An equal amount of protein (20 μg) was separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrophoretically transferred onto PVDF (Millipore. Billerica, MA, USA). The membranes were blotted with 5% fat-free milk in tris buffer saline with tween 20 (TBST) buffer for 2 h at room temperature and then incubated at 4°C overnight with primary antibodies: anti-GAPDH (1:1,0000), anti-ERα (1:1,000), anti- ERβ (1:1,000), anti-NRF2 (1:1,000), anti-HO-1 (1:1,000), anti-BCL2 (1:1,000), anti-BAX (1:1,0000), anti-Caspase-3 (1:1,000) and anti-Cleaved Caspase-3 (1:1,000). Afterward, the membranes were incubated with HRP-conjugated anti-rabbit/mouse IgG. The blots were imaged under an enhanced chemiluminescence (ECL) system. The target band molecular weights and net optical density were analyzed using the multifunctional imager (Jena UVP Chem studio, Germany).

TABLE 1 Primer sequences.

Genes	Primer sequence(5′-3′)
β-actin	Forward : CCTCTATGCCAACACAGTGC
	Reverse : CCTGCTTGCTGATCCACATC
Esr1	Forward : GCACCATCGATAAGAACCGG
	Reverse : TTCGGCCTTCCAAGTCATCT
Esr2	Forward : AGGATGTACCACCGAATGCCAAGT
	Reverse : TCCAAGTGGGCAAGGAGACAGAAA
Nrf2	Forward : GCCTTCCTCTGCTGCCATTAGTC
	Reverse : TGCCTTCAGTGTGCTTCTGGTTG
Ho-1	Forward : TATCGTGCTCGCATGAACACTCTG
	Reverse : GTTGAGCAGGAAGGCGGTCTTAG
Bcl2	Forward : CTTCAGGGATGGGGTGAACT
	Reverse : ATCAAACAGAGGTCGCATGC
Bax	Forward : GACGCATCCACCAAGAAGCTGAG
	Reverse : GCTGCCACACGGAAGAAGACC

2.14 Statistical analysis

All data are presented as mean values ± SD, and graphs were created and analyzed using Prism Software (GraphPad Prism 7). The one-way analysis of variance (ANOVA) was used to evaluate the differences among the groups. It was deemed statistically significant when the $p<0.05$.

3 Results

In this study, we identified BSHS-related active compounds, critical therapeutic targets, and the molecular mechanism of action of BSHS in kidney stone disease treatment by network pharmacology, functional gene pathway analysis, network analysis, and other comprehensive methods. Finally, we predicted the potential molecular mechanisms of BSHS and validated *in vivo* experiments. A flowchart of this research is shown in Figure 1.

3.1 Screening of BSHS bioactive ingredients and therapeutic targets for KS

We obtained 10 active ingredients of Lysimachiae Herba, 10 active compounds of Lygodii Spora, 9 active ingredients of Plantaginis Semen, 8 active ingredients of Dipsaci Radix, 92 active ingredients of Licorice, 6 active ingredients of Clerodendranthus spicatus, 8 active ingredients of Cibotii Rhizoma, and 1 active ingredient of Malva verticillata seed. After removing duplicate entries, a total of 126 active ingredients and 244 ingredient action targets were obtained. A total of 1970 targets related to KS treatment were obtained from four databases: Genecards, OMIM, DisGeNET, and TTD. Using the online Venn diagram editing website (http://jvenn.toulouse.inra.fr/app/example.html), 140 potential target genes were identified for KS treatment by BSHS by importing the potential targets for KS and the targets for BSHS active ingredients (Figure 2A).

3.2 Construction of drug-compound-target networks

We used Cytoscape 3.7.2 software to construct the drug-compound-target network diagram. The purple circle nodes represented the 8 traditional Chinese medicines of BSHS, the hexagons nodes represented the compounds, the A1 and A2 nodes

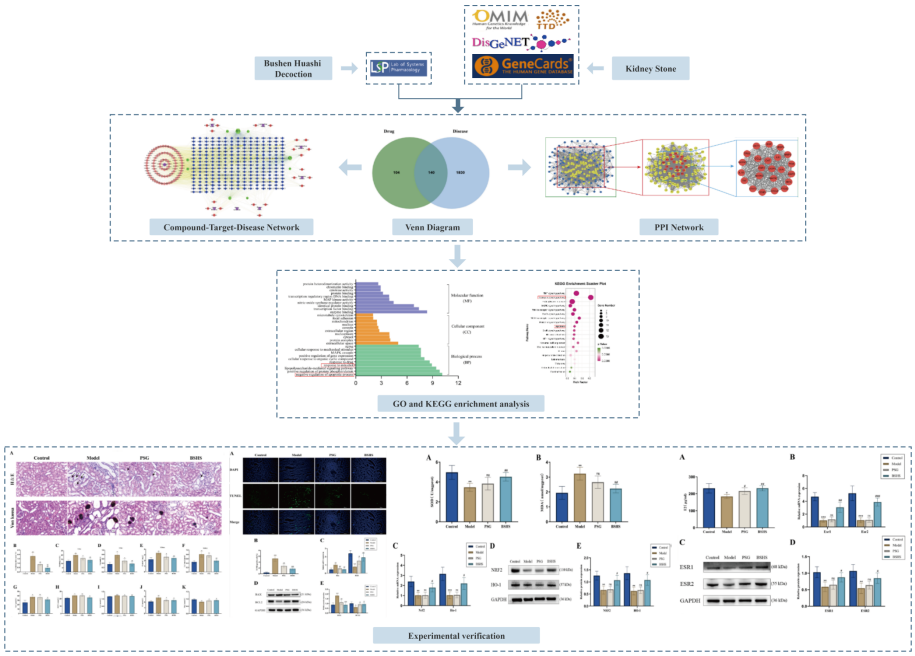

FIGURE 1
The flowchart illustrates the mechanism of BSHS in KS, from target identification, network construction, and enrichment analysis to experimental validation.

represented the common compounds of Lysimachiae Herba and Lygodii Spora, the A3 node represented the joint compound of Lysimachiae Herba and Licorice, the A4 node represented the joint compound of Lysimachiae Herba and Plantaginis Semen. The A5 and A6 nodes represented the common compounds of Licorice and Cibotii Rhizoma, the B1 node represented the joint compound of Lysimachiae Herba, Plantaginis Semen, and Licorice. The C1 node represented the joint compound of Lysimachiae Herba, Plantaginis Semen, Dipsaci Radix, and Licorice. The C2 node represented the joint compound of Malva verticillata seed, Dipsaci Radix, Lygodii Spora, and Cibotii Rhizoma. The D1 node represented the joint compound of Lysimachiae Herba, Lygodii Spora, Licorice, Clerodendranthus spicatus, and Cibotii Rhizoma, while the blue quadrilateral nodes represented the targets. The drug-compound-target network diagram included 365 nodes and 2713 edges (Figure 2B). The top five active compounds in BSHS, ranked according to the degree value, were quercetin, kaempferol, naringenin, β-sitosterol, and baicalein, which may play an essential role in treating kidney stones.

3.3 PPI network analysis and screening of key targets

In order to obtain the key proteins of BSHS in the treatment of KS, we constructed a PPI network with 239 nodes and 4557 edges based on a string database (Figure 2C). Based on the 'degree' value of topological parameters calculated by CytoNCA, 23 pivotal proteins were filtered out, including AKT1, IL6, MAPK3, TP53, VEGFA, CASP3, JUN, TNF, PTGS2, EGF, MAPK8, EGFR, STAT3, MYC, MMP9, MAPK1, ESR1, CXCL8, IL1β, CCND1, CAT, FOS, CCL2, which were strongly linked to KS (Figures 2D, E).

3.4 GO enrichment analysis and KEGG pathway analysis of key targets

We used the DAVID database to perform GO enrichment analysis of the 23 key targets for the identification of the relevant biological functions of BSHS against KS. The analysis uncovered 251 biological pathways, 22 cell localizations, and 35 molecular functions. As shown in Figure 2F, the top 10 terms in the biological process (BP), cellular component (CC), and molecular function (MF) categories that are significantly enriched are demonstrated. BP was found to be primarily associated with the negative regulation of apoptotic process, positive regulation of protein phosphorylation, lipopolysaccharide-mediated signaling pathway, and response to estradiol. The CC mainly included the extracellular space, protein complex, cytosol, nucleoplasm, etc. The MF mainly included enzyme binding, transcription factor binding, identical protein binding, etc.

In order to determine the potential pathway for BSHS in the treatment of KS, we performed a KEGG pathway enrichment analysis and found 102 signal pathways related to BSHS. A total of 20 pathways related to KS were screened, mainly including Tumour Necrosis Factor (TNF), estrogen, Mitogen-Activated Protein Kinase (MAPK), and Toll-like receptor signaling pathways. The enrichment pathway was visualized according to the size of the p-value (Figure 2G). In the pathways with the highest enrichment levels, estrogen and apoptosis signaling pathways were most closely related to KS. In addition, CAT is a key target of BSHS anti-kidney stones according to 2.4.3, which protects cells from oxidative stress by scavenging hydrogen peroxide produced by cellular metabolism (19). Multiple studies have also shown the harmful effects of oxidative stress on kidney stones (20, 21). Therefore, we predict that oxidative stress is also a key signaling pathway in the treatment of kidney stones by BSHS.

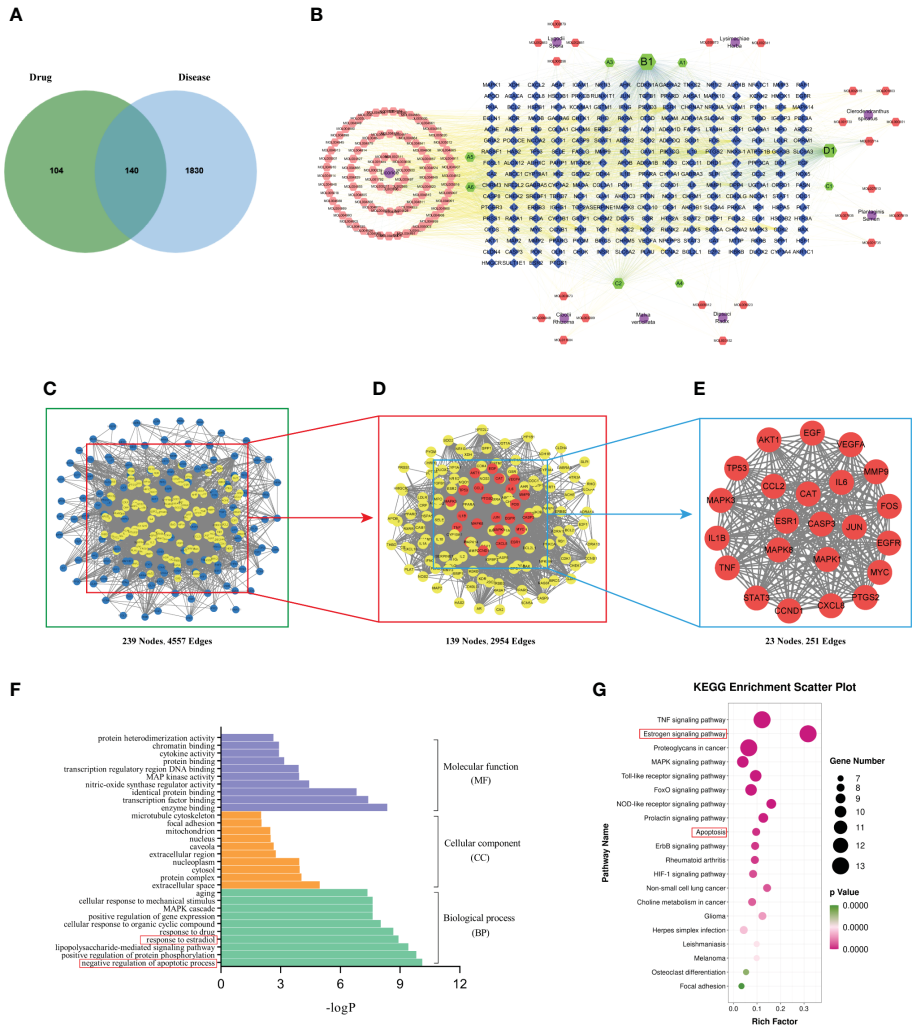

FIGURE 2
Network pharmacology analysis. **(A)** Target intersections between BSHS and KS. **(B)** The network of drug-compound-target included 8 kinds of herbs, 126 active components, and 244 target genes. Purple circle: drug, orange and green hexagon: active ingredients of BSHS; blue quadrilateral: targets. **(C)** A PPI network of predicted BSHS targets against KS. **(D)** A list of significant proteins from the PPI network was derived from **(C)**. **(E)** A list of 23 key proteins of BSHS in KS treatment was derived from **(D)**. **(F)** Based on the GO enrichment analysis, these are the top 10 indicators of BP, CC, and MF. **(G)** The top 20 signaling pathways were identified according to KEGG.

3.5 Experimental validation

3.5.1 Identification and characterization of BSHS components

Total ion flow maps for the BSHS positive and negative ion scan modes were obtained from the data acquisition (Supplementary Figures 2A, B). The acquired data was processed with Xcailbur software. Matches were made in the HMDB and PubChem databases according to retention times, the mass information of the quasi-molecular and fragment ions, while keeping the quasi-molecular ions within ±5 ppm. A total of 52 compounds were eventually identified (Supplementary Table 1).

3.5.2 BSHS inhibits the formation of calcium oxalate crystals

We investigated the impact of BSHS on renal injury and calcium oxalate crystal deposition after EG+AC induction *in vivo*. H&E and Von Kossa staining of kidney sections revealed renal tubule severe dilation, tubule destruction, and epithelial cell desquamation induced by EG+AC. A large amount of calcium oxalate crystal deposition was noticed after EG+AC induction, whereas coadministration of BSHS could protect the EG+AC-injured kidney tissue from inflammatory damage and calcium oxalate crystal deposition (Figures 3A, B).

3.5.3 BSHS increases 24 h urine volume and improves renal/body weight index in rats

On day 28, we recorded 24h urine volume and weighed the body weight and renal weight of rats. The results showed that there were significantly decreased 24 h urine volume and increased renal/body weight index in the model group rats compared to the normal control group. Simultaneous treatment with BSHS resulted in a remarkable decrease in renal/body weight index, as well as an increase in 24 h urine volume in the rat (Supplementary Figures 3A, B).

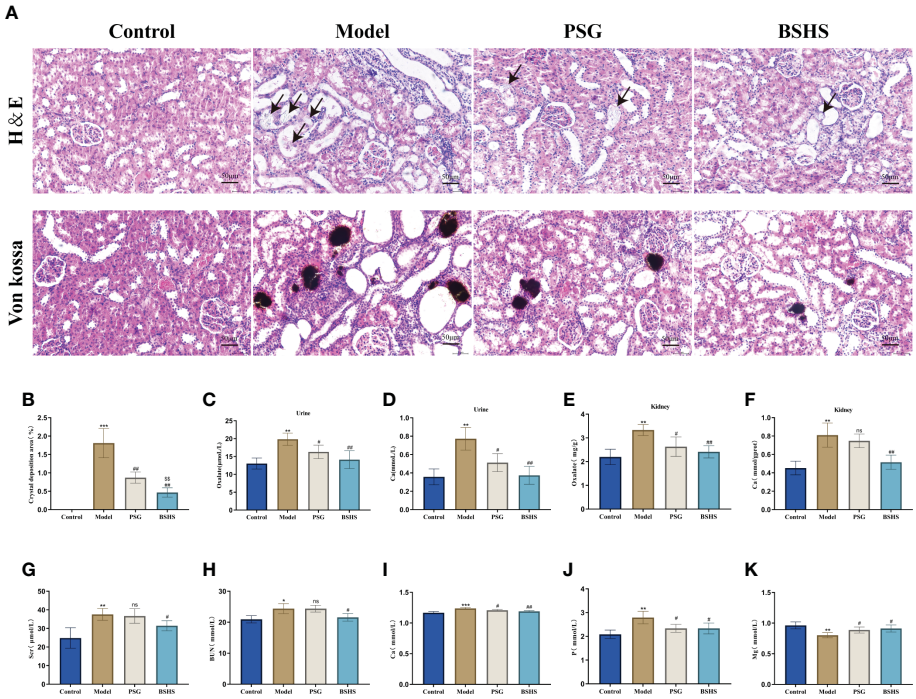

FIGURE 3
The therapeutic effect of BSHS on KS. **(A)** The H&E and Von Kossa staining of kidney sections from each group revealed tissue injury and CaOx crystal deposition. **(B)** The Image J (USA) software calculated the percentage of area positively stained for crystal deposition in each kidney section based on 20 random views at 200× magnification. **(C)** Oxalate content in the rat urine. **(D)** Ca content in the rat urine. **(E)** Oxalate content in the rat kidneys. **(F)** Ca content in the rat kidneys. **(G)** Cr content in the rat serum. **(H)** Blood urea nitrogen (BUN) content in the rat serum. **(I)** Ca content in the rat serum. **(J)** P content in the rat serum. **(K)** Mg content in the rat serum. All values were expressed as mean ± SD. $^{*}p<0.05$ *vs.* the normal control group, $^{**}p < 0.01$ *vs.* the normal control group, $^{***}p < 0.001$ *vs.* the normal control group, $^{\#}p < 0.05$ *vs.* the model group, $^{\#\#}p < 0.01$ *vs.* the model group, $^{\$\$}p < 0.01$ *vs.* the PSG group, ns for $p>0.05$ *vs.* the model group.

3.5.4 BSHS regulated urine and renal biological parameters in rats

In this study, we investigated whether BSHS inhibited the formation of oxalate, Ca, and P in an animal model, and increased the level of Mg. As expected, there was a significant increase ($p<0.05$) of oxalate and calcium contents in both the kidneys and urine of rats in the model group compared to those in the normal control group. Simultaneous treatment with BSHS resulted in a significant decrease ($p<0.05$) in oxalate and calcium levels in the kidney of rats (Figures 3C–F). BSHS also decreased the level of P and increased the level of Mg in the urine of rats compared to the model group (Supplementary Figures 3C, D).

3.5.5 BSHS regulated serum biological parameters in rats

Meanwhile, to examine the effect of BSHS on the renal function and serum biological parameters of rats, we further examined the levels of Cr, BUN, Ca, P, and Mg in the serum of rats. The results showed that there were significantly decreased Mg content and increased Ca, P, Cr, and BUN contents in the serum of model group rats compared to the normal control group. Simultaneous treatment with BSHS resulted in a remarkable decrease in Ca, P, Mg, Cr, and BUN levels, as well as an increase in Mg levels in the rat serum. Worthy of note, there was no significant trend of lowering Cr and BUN in the PSG group compared to the model group, suggesting a better effect of BSHS in improving renal function (Figures 3G–K).

3.5.6 BSHS inhibited apoptosis induced by EG+AC in rat

Network pharmacological analysis indicated that apoptosis might be involved in BSHS treatment of kidney stones. Several studies have also demonstrated that apoptosis is crucial in kidney stone formation (22–24). Therefore, our study examined the apoptotic effects of BSHS on KS in animal models. In the model group, TUNEL-positive cells were significantly higher than in the normal control group, according to the TUNEL staining results. Contrary to what was observed in the model group, BSHS groups showed fewer apoptotic cells (Figures 4A, B). The qRT-PCR results indicated that *Bax* levels were significantly increased and *Bcl2* was decreased in the model group, while treatment with BSHS reversed the increase of *Bax* levels and restored *Bcl2* expression (Figure 4C). The Western bolting results indicated that BAX and Cleaved Caspase-3/Caspase-3 levels were significantly increased and BCL2 was decreased in the model group, while treatment with BSHS reversed the increase of BAX and Cleaved Caspase-3/Caspase-3 levels and restored BCL2 expression (Figures 4D, E) (Supplementary Figures 4A, B).

3.5.7 BSHS improves the imbalance of estrogen levels induced by EG+AC in rat

Network pharmacological analysis suggested estrogen signaling pathways may be involved in BSHS treating kidney stones. Interestingly, lower estrogen levels have also been shown to be strongly associated with the formation of kidney stones (25–27). As

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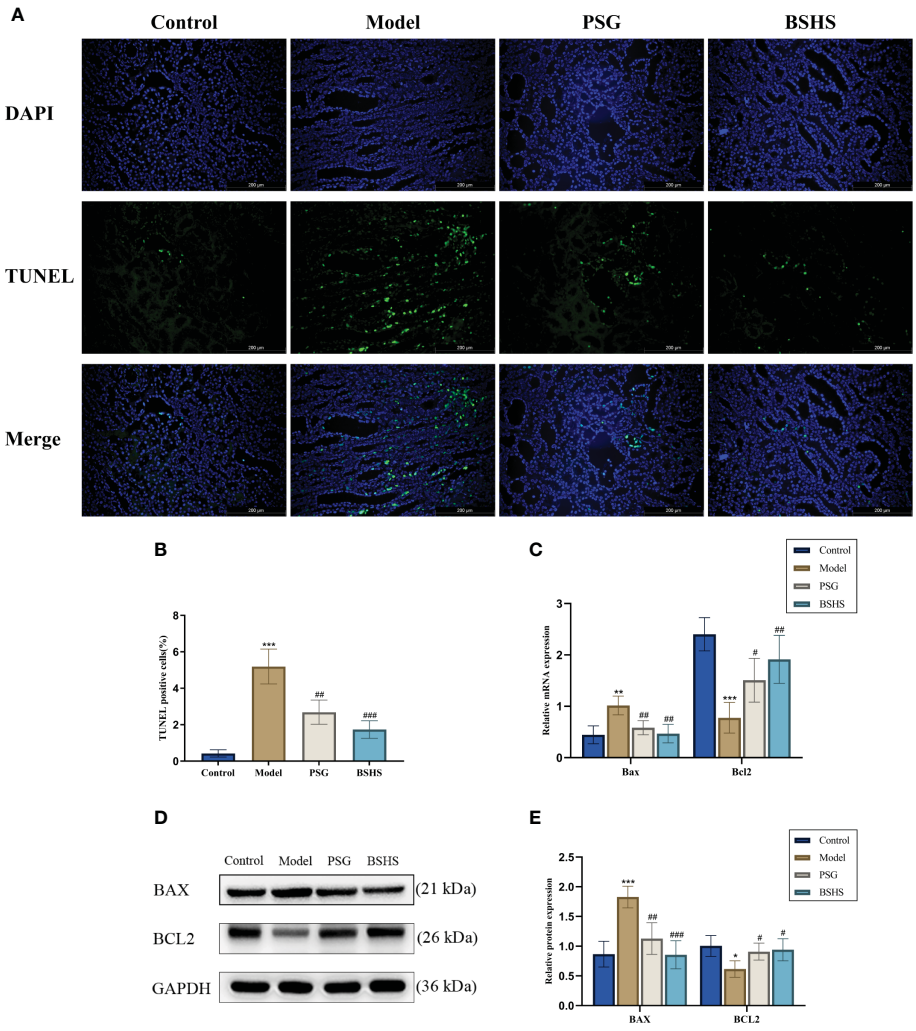

FIGURE 4
Effect of BSHS on EG+AC-induced apoptosis in rat kidney tissue. **(A)** TUNEL staining was used to assess renal apoptosis. **(B)** Images J was used to count the percentages of TUNEL-positive cells (green) to total cells (blue). **(C)** The expression of apoptosis-related genes was evaluated by qRT-PCR. **(D)** The expression of apoptosis-related proteins was evaluated by Western bolting. **(E)** A graph showing the semi-quantitative analysis of BAX and BCL2. Data are presented as the mean ± SD and density normalized to GAPDH. *p<0.05 *vs*. the normal control group, **p < 0.01 *vs*. the normal control group, ***p < 0.001 vs. the normal control group, $^{\#}p$ < 0.05 *vs*. the model group, $^{\#\#}p$ < 0.01 *vs*. the model group, $^{\#\#\#}p$ < 0.001 *vs*. the model group.

part of our study, we examined the effect of BSHS on estrogen and estrogen receptors in this animal model. E2 serum levels were found to be lower in the model group compared with the control group in the ELISA experiment. In contrast, it more dramatically increased in the BSHS groups than in the model group (Figure 5A). The qRT-PCR results illustrated that *Esr1* and *Esr2* mRNA levels were remarkably decreased in the model group, while treatment with BSHS reversed the decrease in *Esr1* and *Esr2* levels (Figure 5B). The Western blotting results showed the same trend (Figures 5C, D).

3.5.8 BSHS alleviated EG+AC-induced oxidative stress in rats

According to growing evidence, oxidative stress may play an essential role in hyperoxaluria-induced kidney injury, resulting in renal CaOx crystallization (28–30). Herein, we examined the potential antioxidative properties of BSHS in this animal model. As expected, compared to the normal control group, rats in the model group significantly increased MDA content and decreased SOD activity in the kidneys. The simultaneous treatment of rats with BSHS reduced MDA levels in the kidneys and increased SOD activity (Figures 6A, B). The qRT-PCR results illustrated that *Nrf2* and *Ho-1* mRNA levels were remarkably decreased in the model group, while treatment with BSHS reversed the decrease in *Nrf2* and *Ho-1* levels (Figure 6C). The Western blotting results showed a similar trend (Figures 6D, E). Interestingly, the PSG group did not show significant antioxidant effects, while the BSHS group had great antioxidant capacity.

4 Discussion

Kidney stones are a common and frequently-occurring disease of the urinary system, and their incidence increases annually (31). According to the most recent epidemiological study conducted in China, kidney stones are prevalent in approximately 5.8% of the population (32). It is estimated that 12% of men and 6% of women in the world population will have kidney stones at least once in their

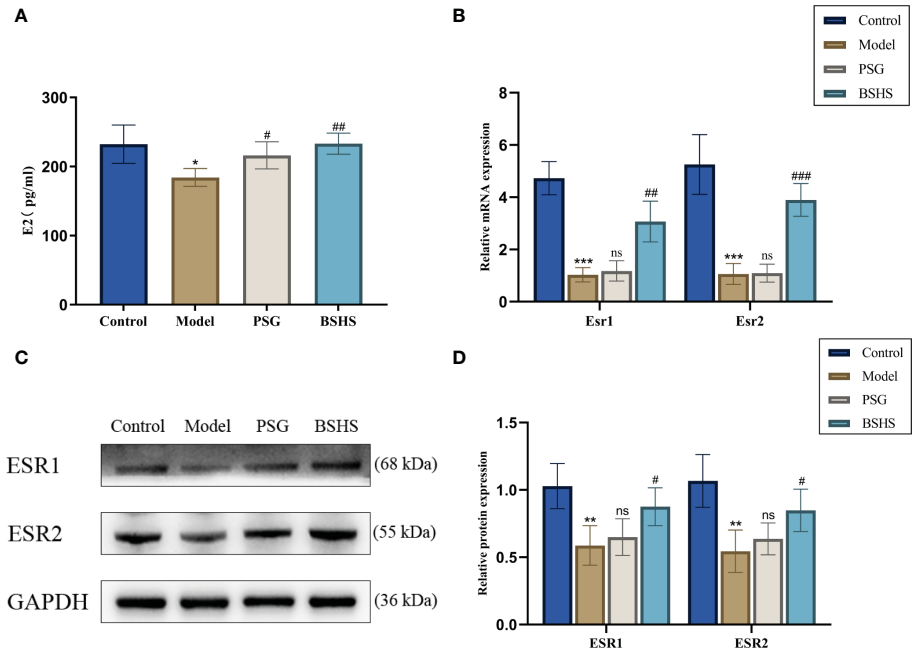

FIGURE 5
The effect of BSHS on the imbalance of estrogen levels induced by EG+AC in the rat. **(A)** ELISA evaluated the expression of estrogen levels. **(B)** The expression of estrogen receptor-related genes was evaluated by qRT-PCR. **(C)** The expression of estrogen receptor-related proteins was examined by Western blotting. **(D)** A semi-quantitative analysis of ESR1 and ESR2 is shown. Data are presented as the mean ± SD and density normalized to GAPDH. *p<0.05 *vs*. the normal control group, **p < 0.01 *vs*. the normal control group, ***p < 0.001 *vs*. the normal control group, $^{\#}p$ < 0.05 *vs*. the model group, $^{\#\#}p$ < 0.01 *vs*. the model group, $^{\#\#\#}p$< 0.001 *vs*. the model group, ns for p > 0.05 *vs*. the model group.

lifetime, with recurrence rates of 70–80% for men and 47–60% for women (33). Among them, calcium oxalate stones are the most common kidney stones (34, 35), accounting for over 80% of them (36). Although people have an in-depth understanding of crystallization and stone formation, there is currently a lack of effective treatment methods and drugs due to the slow progress in determining the pathophysiology of stone formation. Therefore, kidney stone disease must be given sufficient attention. The expansion of the treatment model of kidney stone disease based on TCM can provide a reliable solution for the pathogenesis of kidney stone disease that is difficult to cure and easy to relapse. Although under the guidance of the holistic view of TCM, Chinese herbal compound has an excellent therapeutic effect on diseases. Due to their complex components, multi-target, and multi-channel treatment characteristics, it is not easily accessible for an in-depth study of its internal mechanism. In recent years, network pharmacology has

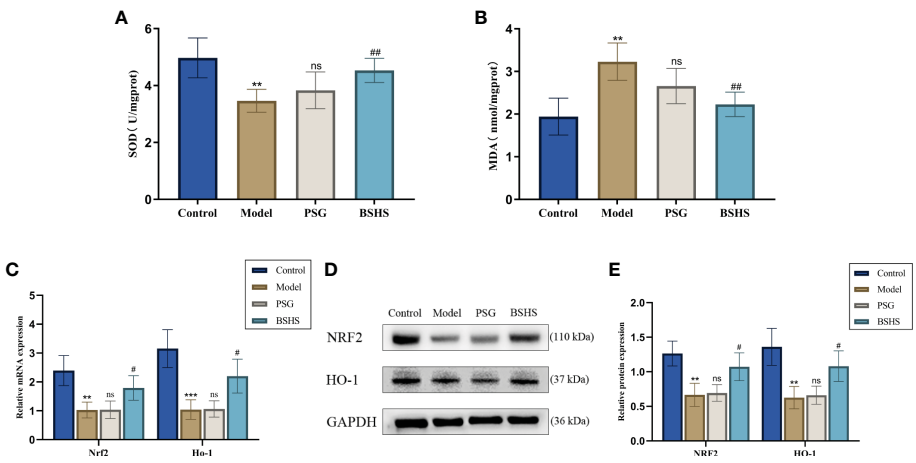

FIGURE 6
A comparison of oxidative stress levels in kidneys among different groups. **(A)** SOD activity in the kidneys of rats. **(B)** MDA content in the kidneys of rats. **(C)** qRT-PCR was used to evaluate the expression of genes related to oxidative stress. **(D)** The expression of oxidative stress-related proteins was evaluated by Western blotting. **(E)** A semi-quantitative analysis of NRF2 and HO-1 is shown. Data are presented as the mean ± SD and density normalized to GAPDH. **p < 0.01 *vs*. the normal control group, ***p < 0.001 *vs*. the normal control group, $^{\#}p$ < 0.05 *vs*. the model group, $^{\#\#}p$ < 0.01 *vs*. the model group, ns for p > 0.05 *vs*. the model group.

become a popular technique for analyzing the mechanism of action of complex TCM prescriptions (37). The combination of network pharmacology and experimental verification was used in this study in order to clarify the pharmacological mechanism of BSHS against kidney stones.

It is well known that the formation of kidney stones is a complex process involving urinary supersaturation, nucleation, growth, aggregation, and retention of urinary stone components within the renal tubular cells (38). Multiple studies have shown that kidney stone formation could be attributed to higher supersaturation of urine because of low urine volume and increased secretion of calcium, phosphates, oxalates, uric acid, and cysteine in urine (39–41). The elevated urinary excretion of calcium (hypercalciuria) and oxalate (hyperoxaluria) are the most common risk factor for CaOx kidney stones (42, 43). However, some scholars supported that oxalate in the urine combines with free calcium to form insoluble CaOx, which induces kidney stones and can lead to a decrease in urinary calcium (44, 45). Interestingly, we discovered that BSHS can decrease urine oxalates and calcium excretion which may be related to the fact that BSHS increase urinary magnesium levels. Studies have shown that magnesium can compete with calcium to bind oxalate and form insoluble solutes that are excreted in the urine (46). Low urinary oxalate concentrations lead to a reduction in urinary calcium levels, therefore BSHS can treat kidney stones by reducing urinary oxalate, urinary calcium and increasing urinary magnesium levels.

TCM compounds that lacked proper pharmacokinetic properties would not reach their target organs to exert their biological effects (47). It has been demonstrated that compounds with OB ≥30% and DL index ≥0.18 may be absorbed and distributed in the human body and are thus considered pharmacokinetically active (48, 49). Compounds with high-degree may explain the significant therapeutic effects of BSHS on kidney stones in the compound-key targets network. According to this study, quercetin was the most significant compound, followed by kaempferol, naringenin, β-sitosterol, and baicalein. It is reported that quercetin, a natural flavonoid, has efficient antioxidant properties and can be used to inhibit oxidative damage in renal tubular cells and tissues (50). In addition, quercetin can inhibit the formation of urinary tract stones induced by oxalate (51). Kaempferol is one of the most common glycoside forms of aglycon flavonoids, which can increase the level of coenzyme Q in kidney cells to play an antioxidant role (52). As a naturally occurring flavanone, naringenin inhibits oxidative stress in the kidneys and improves kidney function (53). β-Sitosterol is a phytosterol reported in ancient medicinal history for treating nephritis and prostatitis (54). β-sitosterol has been reported to inhibit nephrotoxicity and anti-kidney oxidation properties (55, 56). Baicalein is a member of the flavonoid family, and modern pharmacology proves that baicalein can inhibit inflammation by activating the Nrf2 signaling pathway, thereby alleviating lupus nephritis (57). Oxidative stress-induced apoptosis of renal tubular epithelial cells is a risk factor for stone formation (20). All these works demonstrate that BSHS has excellent anti-kidney oxidation and renal protection.

It has been revealed that BSHS acts on multiple targets using multiple signaling pathways when we integrate the topological network parameters with all the network analyses. We finally identified estrogen, apoptosis, and oxidative stress as crucial mechanisms for BSHS treatment of kidney stones based on network pharmacology analysis. Multiple clinical studies have suggested that estrogen has a protective effect during the formation of kidney stones (58, 59). However, the Women's Health Initiative Study and the Nurses' Health Study found no positive correlation between hormone replacement therapy and the prevention of kidney stones (59, 60). These results have caused scholars to question the relationship between estrogen and kidney stones. For this result, some researchers suggested that the long-term estrogen decline caused by menopause may aggravate the deterioration of normal physiological estrogen receptor function in the kidney (60). Therefore, the poor effect of hormone replacement therapy on renal calculi may be due to the reduced protein expression of estrogen receptors or its cofactors in these women (60). We validated the effects of BSHS on E2 and estrogen receptors *in vivo*. The results showed that BSHS could not only increase the level of E2 but also increase the levels of ESR1 and ESR2. There are growing numbers of studies demonstrating that the adhesion or endocytosis of renal tubular epithelial cells to crystals plays an essential role in forming stones (61, 62). Moreover, crystal adhesion can be enhanced by injured renal tubular epithelial cells, which can promote kidney stones (34). Interestingly, the damage to renal tubular epithelial cells is closely related to oxidative stress (63). As an essential antioxidant pathway for endogenous anti-oxidative stress in cells (64, 65), the NRF2/HO-1 signaling pathway is vital in improving oxidative stress in kidney diseases (66–68). Many studies have confirmed that the inhibitory effect of the estrogen signaling pathway on oxidative stress is closely related to the activation of the NRF2/HO-1 antioxidant pathway (69, 70). Interestingly, our *in vivo* studies showed that BSHS could not only increase the expression of NRF2 and HO-1 proteins and genes but also increase SOD activity and decrease MDA levels in the rat kidney. It is suggested that BSHS has a good anti-oxidative stress effect on the kidney. It has been reported that renal tubular epithelial cell apoptosis is an essential factor that causes crystals to adhere to renal tubular epithelial cells (22). Some scholars suggested that oxidative stress is a risk factor for apoptosis (71). BCL2/BAX signaling pathway is a pivotal way to regulate cell apoptosis. Our studies demonstrated that BSHS increases the expression of BCL2 and reduces the expression of BAX, thereby reducing the level of apoptosis of renal tubular epithelial cells. Therefore, the therapeutic effect of BSHS on the calcium oxalate stone model in rats may be related to the increase of estrogen receptor levels and the inhibition of apoptosis.

Our findings suggest that BSHS may inhibit kidney stone formation mainly by regulating estrogen and estrogen receptor levels, inhibiting oxidative stress processes, reversing apoptosis, and decreasing CaOx crystals deposition through E2/ESR1/ESR2, NRF2/HO-1, and BCL2/BAX signaling pathways. Overall, BSHS ameliorated KS progression through a multi-ingredient, multi-target, and multi-pathway mode, which is different from chemical drugs that work on a distinct and single target. The understanding of complex interactions between disease and chemical ingredients in TCM could be well accomplished by identifying network targets and signaling pathways. It is important to note, however, that this study has some limitations. First, the results may have been slightly skewed since we only validated part of the core pathways and targets of BSHS. Therefore, further validation of other relevant targets and signaling pathways predicted by network pharmacology would be required in

future experiments. Secondly, our study did not demonstrate an association between estrogen, oxidative stress, and apoptotic signaling pathways. In a follow-up experiment, we will examine their connection through *in-vitro* experiments.

5 Conclusions

In summary, network pharmacology analysis coupled with experimental validation was performed to decipher the molecular mechanisms of BSHS in the treatment of KS. The network pharmacology analysis revealed that BSHS exerted anti-KS effects *via* multi-ingredients, multi-targets, and multi-pathways. The experimental results verified that BSHS improved CaOx crystal deposition in KS by modulating the E2/ESR1/ESR2, NRF2/HO-1, and BCL2/BAX signaling pathways. This study could provide an optimized method to elucidate the pharmacological mechanisms of BSHS and supply a novel candidate for treating KS.

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 animal study was reviewed and approved by the experimental animal ethics committee of the Tianjin University of Traditional Chinese Medicine.

Author contributions

YB and YW conceived this project. HL and MC designed the study, wrote the manuscript, and performed the experiments. YJ performed the network pharmacology and data analysis. BJ, MD, and LH edited the manuscript. LW performed the UHPLC-Q/Orbitrap MS experiments. JA, JL, and TZ revision of the manuscript. BC provides drug prescriptions. All authors contributed to the article and approved the submitted version.

Funding

This work was by supported by the National Key R&D Program of China (No. 2018YFC1706500), Postgraduate Science Research Fund of Tianjin (No. 2021YJSB293), and Postgraduate Science Research Fund of Tianjin University of Traditional Chinese Medicine (No. YJSKC-20211019).

Acknowledgments

We thank YW and YB for their assistance with conceptualization and helpful discussion. We are also grateful to the National Key R&D Program of China, the Postgraduate Science Research Fund of Tianjin, and the Postgraduate Science Research Fund of Tianjin University of Traditional Chinese Medicine for the funding support.

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/fendo.2023.1031895/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Drug composition of BSHS.

SUPPLEMENTARY FIGURE 2
BSHS total ion flow diagram. **(A)** negative; **(B)** positive.

SUPPLEMENTARY FIGURE 3
The therapeutic effect of BSHS on KS. **(A)** 24 h urine volume. **(B)** Renal/body weight index. **(C)** Mg content in the rat urine. **(D)** P content in the rat urine. All values were expressed as mean ± SD. $^{*}p<0.05$ *vs*. the normal control group, $^{**}p<0.01$ *vs*. the normal control group, $^{***}p<0.001$ *vs*. the normal control group, $^{\#}p<0.05$ *vs*. the model group, $^{\#\#}p<0.01$ *vs*. the model group, ns for $p>0.05$ *vs*. the model group.

SUPPLEMENTARY FIGURE 4
Effect of BSHS on EG+AC-induced apoptosis in rat kidney tissue. **(A)** The expression of Caspase-3 and Cleaved Caspase-3 proteins was evaluated by Western bolting. **(B)** A graph showing the semi-quantitative analysis of Cleaved Caspase-3/Caspase-3. Data are presented as the mean ± SD and density normalized to GAPDH. $^{**}p<0.01$ *vs*. the normal control group, $^{\#}p<0.05$ *vs*. the model group, $^{\#\#}p<0.01$ *vs*. the model group.

References

1. Sorokin I, Mamoulakis C, Miyazawa K, Rodgers A, Talati J, Lotan Y. Epidemiology of stone disease across the world. *World J Urol* (2017) 35:1301–20. doi: 10.1007/s00345-017-2008-6

2. Scales CD, Smith AC, Hanley JM, Saigal CS. Prevalence of kidney stones in the united states. *Eur Urol* (2012) 62:160–5. doi: 10.1016/j.eururo.2012.03.052

3. Mollerup CL, Vestergaard P, Frøkjaer VG, Mosekilde L, Christiansen P, Blichert-Toft M. Risk of renal stone events in primary hyperparathyroidism before and after parathyroid surgery: Controlled retrospective follow up study. *Br Med J* (2002) 325:807. doi: 10.1136/bmj.325.7368.807

4. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. *JAMA* (2005) 293:455–62. doi: 10.1001/jama.293.4.455

5. Aune D, Mahamat-Saleh Y, Norat T, Riboli E. Body fatness, diabetes, physical activity and risk of kidney stones: a systematic review and meta-analysis of cohort studies. *Eur J Epidemiol* (2018) 33:1033–47. doi: 10.1007/s10654-018-0426-4

6. Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int* (2005) 68:1230–5. doi: 10.1111/j.1523-1755.2005.00516.x

7. Strazzullo P, Barba G, Vuotto P, Farinaro E, Siani A, Nunziata V, et al. Past history of nephrolithiasis and incidence of hypertension in men: a reappraisal based on the results of the Olivetti prospective heart study. *Nephrol dialysis Transplant* (2001) 16:2232–5. doi: 10.1093/ndt/16.11.2232

8. Obligado SH, Goldfarb DS. The association of nephrolithiasis with hypertension and obesity: A review. *Am J Hypertens* (2008) 21:257–64. doi: 10.1038/ajh.2007.62

9. Rule AD, Krambeck AE and L. JC: Chronic kidney disease in kidney stone formers. *Clin J Am Soc Nephrol* (2011) 6:2069–75. doi: 10.2215/CJN.10651110

10. Shoag J, Halpern J, Goldfarb DS, Eisner BH. Risk of chronic and end stage kidney disease in patients with nephrolithiasis. *J Urol* (2014) 192:1440–5. doi: 10.1016/j.juro.2014.05.117

11. El-Zoghby ZM, Lieske JC, Foley RN, Bergstralh EJ, Li X, Rd MLJ, et al. AD: Urolithiasis and the risk of ESRD. *Clin J Am Soc Nephro* (2012) 77:1409–15. doi: 10.2215/CJN.03210312

12. Singh P, Harris PC, Sas DJ, Lieske JC. The genetics of kidney stone disease and nephrocalcinosis. *Nat Rev Nephrol* (2022) 18:224–40. doi: 10.1038/s41581-021-00513-4

13. Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: Molecular mechanism of renal stone formation and the critical role played by modulators. *BioMed Res Int* (2013) 2013:1–21. doi: 10.1155/2013/292953

14. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. *Nat Rev Dis Primers* (2016) 2:16008. doi: 10.1038/nrdp.2016.8

15. Du H, Lei Y, Gao AN, Sun XM, Song R, Yu XD, et al. Traditional Chinese medicine combined with conventional therapy for female kidney stone: A protocol for systematic review. *Med (Baltimore)* (2020) 99:e19611. doi: 10.1097/MD.0000000000019611

16. Luo T, Lu Y, Yan S, Xiao X, Rong X, Guo J. Network pharmacology in research of Chinese medicine formula: Methodology, application and prospective. *Chin J Integr Med* (2020) 26:72–80. doi: 10.1007/s11655-019-3064-0

17. Zhou S, Ai Z, Li W, You P, Wu C, Li L, et al. Deciphering the pharmacological mechanisms of taohe-chengqi decoction extract against renal fibrosis through integrating network pharmacology and experimental validation *In vitro* and in vivo. *Front Pharmacol* (2020) 11:425. doi: 10.3389/fphar.2020.00425

18. Liu Q, Cai BY, Zhu LX, Xin X, Wang X, An ZM, et al. Liraglutide modulates gut microbiome and attenuates nonalcoholic fatty liver in db/db mice. *Life Sci* (2020) 15:118457. doi: 10.1016/j.lfs.2020.118457

19. Yang F, Pei R, Zhang Z, Liao J, Yu W, Qiao N, et al. Copper induces oxidative stress and apoptosis through mitochondria-mediated pathway in chicken hepatocytes. *Toxicol Vitro* (2019) 54:310–6. doi: 10.1016/j.tiv.2018.10.017

20. Xi J, Jing J, Zhang Y, Liang C, Hao Z, Zhang L, et al. SIRT3 inhibited the formation of calcium oxalate-induced kidney stones through regulating NRF2/HO-1 signaling pathway. *J Cell Biochem* (2018) 120:8259–71. doi: 10.1002/jcb.28109

21. Qin B, Wang Q, Lu Y, Li C, Hu H, Zhang J, et al. Losartan ameliorates calcium oxalate-induced elevation of stone-related proteins in renal tubular cells by inhibiting NADPH oxidase and oxidative stress. *Oxid Med Cell Longev* (2018) 2018:1271864. doi: 10.1155/2018/1271864

22. Ye QL, Wang DM, Wang X, Zhang ZQ, Tian QX, Feng SY, et al. Sirt1 inhibits kidney stones formation by attenuating calcium oxalate-induced cell injury. *Chem Biol Interact* (2021) 347:109605. doi: 10.1016/j.cbi.2021.109605

23. Sun Y, Kang J, Tao Z, Wang X, Liu Q, Li D, et al. Effect of endoplasmic reticulum stress-mediated excessive autophagy on apoptosis and formation of kidney stones. *Life Sci* (2020) 244:117232. doi: 10.1016/j.lfs.2019.117232

24. Yu L, Gan X, Bai Y, An R. CREB1 protects against the renal injury in a rat model of kidney stone disease and calcium oxalate monohydrate crystals-induced injury in NRK-52E cells. *Toxicol Appl Pharmacol* (2021) 413:115394. doi: 10.1016/j.taap.2021.115394

25. Peerapen P, Thongboonkerd V. Protective cellular mechanism of estrogen against kidney stone formation: A proteomics approach and functional validation. *Proteomics* (2019) 19:e1900095. doi: 10.1002/pmic.201900095

26. Peerapen P, Boonmark W, Thongboonkerd V. Trigonelline prevents kidney stone formation processes by inhibiting calcium oxalate crystallization, growth and crystal-cell adhesion, and downregulating crystal receptors. *Biomed Pharmacother* (2022) 149:112876. doi: 10.1016/j.biopha.2022.112876

27. Heller HJ, Sakhaee K, Moe OW, Pak CY. Etiological role of estrogen status in renal stone formation. *J Urol* (2002) 168:1923–7. doi: 10.1097/01.ju.0000033907.21910.be

28. Li Z, Chang L, Ren X, Hu Y, Chen Z. Modulation of rat kidney stone crystallization and the relative oxidative stress pathway by green tea polyphenol. *ACS Omega* (2021) 6:1725–31. doi: 10.1021/acsomega.0c05903

29. Park JH, Jo YI, Lee JH. Renal effects of uric acid: hyperuricemia and hypouricemia. *Korean J Intern Med* (2020) 35:1291–304. doi: 10.3904/kjim.2020.410

30. Khan SR, Canales BK, Dominguez-Gutierrez PR. Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation. *Nat Rev Nephrol* (2021) 17:417–33. doi: 10.1038/s41581-020-00392-1

31. Li DF, Gao YL, Liu HC, Huang XC, Zhu RF, Zhu CT. Use of thiazide diuretics for the prevention of recurrent kidney calculi: a systematic review and meta-analysis. *J Transl Med* (2020) 18:106. doi: 10.1186/s12967-020-02270-7

32. Zhang D, Li S, Zhang Z, Li N, Yuan X, Jia Z, et al. Urinary stone composition analysis and clinical characterization of 1520 patients in central China. *Sci Rep* (2021) 11:6467. doi: 10.1038/s41598-021-85723-3

33. Queiroz MF, Melo K, Sabry DA, Sassaki GL, Rocha H, Costa LS. Gallic Acid-chitosan conjugate inhibits the formation of calcium oxalate crystals. *Molecules* (2019) 24:2074. doi: 10.3390/molecules24112074

34. Tsujihata M. Mechanism of calcium oxalate renal stone formation and renal tubular cell injury. *Int J Urol* (2008) 15:115–20. doi: 10.1111/j.1442-2042.2007.01953.x

35. Ivanovski O, Drüeke TB. A new era in the treatment of calcium oxalate stones? *Kidney Int* (2013) 83:998–1000. doi: 10.1038/ki.2013.41

36. Huang Y, Zhang YH, Chi ZP, Huang R, Huang H, Liu G, et al. The handling of oxalate in the body and the origin of oxalate in calcium oxalate stones. *Urol. Int* (2020) 104:167–76. doi: 10.1159/000504417

37. Wang Y, Shi Y, Zou J, Zhang X, Liang Y, Tai J, et al. Network pharmacology exploration reveals a common mechanism in the treatment of cardio-cerebrovascular disease with salvia miltiorrhiza burge. and carthamus tinctorius l. *BMC Complement Med Ther* (2020) 20:351–68. doi: 10.1186/s12906-020-03026-y

38. Chen JY, Sun XY, Ouyang JM. Modulation of calcium oxalate crystal growth and protection from oxidatively damaged renal epithelial cells of corn silk polysaccharides with different molecular weights. *Oxid Med Cell Longev* (2020) 2020:6982948–6982966. doi: 10.1155/2020/6982948

39. Flannigan R, Choy WH, Chew B, Lange D. Renal struvite stones–pathogenesis, microbiology, and management strategies. *Nat Rev Urol* (2014) 11:333–41. doi: 10.1038/nrurol.2014.99

40. Mehde AA, Mehdi WA, Yusof F, Raus RA, Abidin Z, Ghazali H, et al. Determination of alpha-2-MRAP gene polymorphisms in nephrolithiasis patients. *Int J Biol Macromol* (2017) 105:1324–7. doi: 10.1016/j.ijbiomac.2017.07.167

41. Heneghan JF, Alper SL. This, too, shall pass–like a kidney stone: a possible path to prophylaxis of nephrolithiasis? focus on "Cholinergic signaling inhibits oxalate transport by human intestinal T84 cells". *Am J Physiol Cell Physiol* (2012) 302:C18–20. doi: 10.1152/ajpcell.00389.2011

42. Perry GM, Nehrke KW, Bushinsky DA, Reid R, Lewandowski KL, Hueber P, et al. Sex modifies genetic effects on residual variance in urinary calcium excretion in rat (Rattus norvegicus). *Genetics* (2012) 191:1003–13. doi: 10.1534/genetics.112.138909

43. Zhu W, Liu Y, Lan Y, Li X, Luo L, Duan X, et al. Dietary vinegar prevents kidney stone recurrence *via* epigenetic regulations. *EBioMedicine* (2019) 45:231–50. doi: 10.1016/j.ebiom.2019.06.004

44. Li X, Wang W, Su Y, Yue Z, Bao J. Inhibitory effect of an aqueous extract of radix paeoniae alba on calcium oxalate nephrolithiasis in a rat model. *Ren Fail* (2017) 39:120–9. doi: 10.1080/0886022X.2016.1254658

45. Karabulut D, Kaymak E, Yalçin B, Ulger H, Keti DB. A different perspective on the filtration barrier after kidney stone formation: An immunohistochemical and biochemical study. *Urolithiasis* (2021) 49:201–10. doi: 10.1007/s00240-020-01227-1

46. Wang Z, Zhang Y, Zhang J, Deng Q, Liang H. Recent advances on the mechanisms of kidney stone formation (Review). *Int J Mol Med* (2021) 48:149–58. doi: 10.3892/ijmm.2021.4982

47. Mao Y, Peng L, Kang A, Xie T, Xu J, Shen C, et al. Influence of jiegeng on pharmacokinetic properties of flavonoids and saponins in gancao. *Molecules* (2017) 22:1587. doi: 10.3390/molecules22101587

48. Xu X, Zhang W, Huang C, Li Y, Yu H, Wang Y, et al. A novel chemometric method for the prediction of human oral bioavailability. *Int J Mol Sci* (2012) 13:6964–82. doi: 10.3390/ijms13066964

49. Tao W, Xu X, Wang X, Li B, Wang Y, Li Y, et al. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal radix curcumae formula for application to cardiovascular disease. *J Ethnopharmacol* (2013) 145:1–10. doi: 10.1016/j.jep.2012.09.051

50. Al-Rasheed NM, Faddah LM, Mohamed AM, Abdel BN, Al-Rasheed NM, Mohammad RA. Potential impact of quercetin and idebenone against immuno-inflammatory and oxidative renal damage induced in rats by titanium dioxide nanoparticles toxicity. *J Oleo Sci* (2013) 62:961–71. doi: 10.5650/jos.62.961

51. Park HK, Jeong BC, Sung M, Park M, Choi EY, Kim BS, et al. Reduction of oxidative stress in cultured renal tubular cells and preventive effects on renal stone formation by the bioflavonoid quercetin. *J Urol* (2008) 179:1620–6. doi: 10.1016/j.juro.2007.11.039

52. Fernández-Del-Río L, Nag A, Gutiérrez CE, Ariza J, Awad AM, Joseph AI, et al. Kaempferol increases levels of coenzyme q in kidney cells and serves as a biosynthetic ring precursor. *Free Radic Biol Med* (2017) 110:176–87. doi: 10.1016/j.freeradbiomed.2017.06.006

53. Khan TH, Ganaie MA, Alharthy KM, Madkhali H, Jan BL, Sheikh IA. Naringenin prevents doxorubicin-induced toxicity in kidney tissues by regulating the oxidative and inflammatory insult in wistar rats. *Arch Physiol Biochem* (2020) 126:300–7. doi: 10.1080/13813455.2018.1529799

54. Choi S, Kim KW, Choi JS, Han ST, Park YI, Lee SK, et al. Angiogenic activity of beta-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian gerbil. *Planta Med* (2002) 68:330–5. doi: 10.1055/s-2002-26750

55. Sharmila R, Sindhu G, Arockianathan PM. Nephroprotective effect of beta-sitosterol on n-diethylnitrosamine initiated and ferric nitrilotriacetate promoted acute nephrotoxicity in wistar rats. *J Basic Clin Physiol Pharmacol* (2016) 27:473–82. doi: 10.1515/jbcpp-2015-0085

56. Koc K, Geyikoglu F, Cakmak O, Koca A, Kutlu Z, Aysin F, et al. The targets of β-sitosterol as a novel therapeutic against cardio-renal complications in acute renal ischemia/reperfusion damage. *Naunyn Schmiedebergs Arch Pharmacol* (2021) 394:469–79. doi: 10.1007/s00210-020-01984-1

57. Li D, Shi G, Wang J, Zhang D, Pan Y, Dou H, et al. Baicalein ameliorates pristane-induced lupus nephritis *via* activating Nrf2/HO-1 in myeloid-derived suppressor cells. *Arthritis Res Ther* (2019) 21:105. doi: 10.1186/s13075-019-1876-0

58. Mattix KH, Grodstein F, Stampfer MJ, Curhan GC. Menopause and postmenopausal hormone use and risk of incident kidney stones. *J Am Soc Nephrol* (2003) 14:1272–7. doi: 10.1097/01.asn.0000060682.25472.c3

59. Maalouf NM, Sato AH, Welch BJ, Howard BV, Cochrane BB, Sakhaee K, et al. Postmenopausal hormone use and the risk of nephrolithiasis: results from the women's health initiative hormone therapy trials. *Arch Intern Med* (2010) 170:1678–85. doi: 10.1001/archinternmed.2010.342

60. Turner RJ, Kerber IJ. Renal stones, timing hypothesis, and eu-estrogenemia. *Menopause* (2012) 19:104–8. doi: 10.1097/gme.0b013e318221be9b

61. Gill WB, Jones KW, Ruggiero KJ. Protective effects of heparin and other sulfated glycosaminoglycans on crystal adhesion to injured urothelium. *J Urol* (1982) 127:152–4. doi: 10.1016/s0022-5347(17)53650-2

62. Lieske JC, Leonard R, Toback FG. Adhesion of calcium oxalate monohydrate crystals to renal epithelial cells is inhibited by specific anions. *Am J Physiol* (1995) 268: F604–612. doi: 10.1152/ajprenal.1995.268.4.F604

63. Khan SR. Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: evidence from clinical and experimental investigations. *J Urol* (2013) 189:803–11. doi: 10.1016/j.juro.2012.05.078

64. Liang F, Fang Y, Cao W, Zhang Z, Pan S, Xu X. Attenuation of tert-butyl hydroperoxide (t-BHP)-Induced oxidative damage in HepG2 cells by tangeretin: Relevance of the Nrf2-ARE and MAPK signaling pathways. *J Agric Food Chem* (2018) 66:6317–25. doi: 10.1021/acs.jafc.8b01875

65. Ali T, Kim T, Rehman SU, Khan MS, Amin FU, Khan M, et al. Natural dietary supplementation of anthocyanins *via* PI3K/Akt/Nrf2/HO-1 pathways mitigate oxidative stress, neurodegeneration, and memory impairment in a mouse model of alzheimer's disease. *Mol Neurobiol* (2018) 55:6076–93. doi: 10.1007/s12035-017-0798-6

66. Liu M, Grigoryev DN, Crow MT, Haas M, Yamamoto M, Reddy SP, et al. Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. *Kidney Int* (2009) 76:277–85. doi: 10.1038/ki.2009.157

67. Ma JQ, Ding J, Xiao ZH, Liu CM. Puerarin ameliorates carbon tetrachloride-induced oxidative DNA damage and inflammation in mouse kidney through ERK/Nrf2/ARE pathway. *Food Chem Toxicol* (2014) 71:264–71. doi: 10.1016/j.fct.2014.06.017

68. Ruiz S, Pergola PE, Zager RA, Vaziri ND. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int* (2013) 83:1029–41. doi: 10.1038/ki.2012.439

69. Torihata Y, Asanuma K, Iijima K, Mikami T, Hamada S, Asano N, et al. Estrogen-dependent Nrf2 expression protects against reflux-induced esophagitis. *Dig Dis Sci* (2018) 63:345–55. doi: 10.1007/s10620-017-4885-3

70. Chu C, Gao X, Li X, Zhang X, Ma R, Jia Y, et al. Involvement of estrogen receptor-α in the activation of Nrf2-antioxidative signaling pathways by silibinin in pancreatic β-cells. *Biomol Ther (Seoul)* (2020) 28:163–71. doi: 10.4062/biomolther.2019.071

71. An R, Wang X, Yang L, Zhang J, Wang N, Xu F, et al. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. *Toxicology* (2021) 449:152665. doi: 10.1016/j.tox.2020.152665

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 16 February 2023
DOI 10.3389/fmed.2023.1017459

OPEN ACCESS

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

RECEIVED 12 August 2022
ACCEPTED 31 January 2023
PUBLISHED 16 February 2023

CITATION
Lee J, Bae EH, Kim SW, Chung W, Kim YH, Oh YK, Kim Y-S, Oh K-H and Park SK (2023) The association between vitamin D deficiency and risk of renal event: Results from the Korean cohort study for outcomes in patients with chronic kidney disease (KNOW-CKD). *Front. Med.* 10:1017459. doi: 10.3389/fmed.2023.1017459

The association between vitamin D deficiency and risk of renal event: Results from the Korean cohort study for outcomes in patients with chronic kidney disease (KNOW-CKD)

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Backgrounds: Some observational studies have suggested a possible association between vitamin D deficiency and CKD. However, in most studies, the causality between low levels of vitamin D and risk of renal events could not be explained. We investigated the relationship between vitamin D deficiency and risk of severe CKD stage and renal event in a large-scale prospective cohort study.

Methods: We used data from a prospective cohort of 2,144 patients with available information on serum 25-hydroxyvitamin D (25(OH)D) levels at baseline from KNOW-CKD, 2011-2015 were included. Vitamin D deficiency was defined as serum 25(OH)D levels < 15 ng/mL. We performed a cross-sectional analysis to elucidate the relationship between 25(OH)D and CKD stage using baseline CKD patient data. We further examined a cohort analysis to clarify the association between 25(OH)D and risk of renal event. Renal event was a composite of the first occurrence of a 50% decline in eGFR from the baseline value or the onset of CKD stage 5 (initiation of dialysis or kidney transplantation) across the follow-up period. We also investigated the associations of vitamin D deficiency with risk of renal event according to diabetes and overweight status.

Results: Vitamin D deficiency were significantly associated with an increased risk of severe CKD stage – 1.30-fold (95% CI: 1.10-1.69) for 25(OH)D. Deficiency of 25(OH)D with 1.64-fold (95% CI: 1.32-2.65) was related to renal event compared with the reference. Furthermore, vitamin D deficiency patients with presence of

DM and overweight status also displayed higher risk than non-deficient patients for risk of renal event.

Conclusion: Vitamin D deficiency is associated with significantly increased risk of severe CKD stage and renal event.

KEYWORDS

vitamin D deficiency (VDD), renal event, CKD stage, propensity score match, cohort study [or longitudinal study]

Introduction

Chronic kidney disease (CKD) is defined as the existence of structural or functional abnormalities of the kidney, with or without decreased estimated glomerular filtration rate (eGFR < 60 mL/min/1.73m^2), lasting over three months (1); it is considered a significant worldwide health problem (2). The estimated prevalence of CKD in Korean adults is 8.2% according to the Korean National Health and Nutritional Examination Surveys (KNHANES) (3). In addition, Vitamin D deficiency is high among Korean adults (4), both physicians and patients are questioning whether supplement of vitamin D are needed.

Vitamin D, a steroid hormone known for its importance in bone metabolism, can be supplemented with diet, supplement, or produced in the body by dermal synthesis with UV from the precursor (5). Vitamin D deficiency has been related to numerous conditions such as fracture risk, diabetes, fractures, renal disease, cardiovascular disease, auto-immune disease, depression, and cancer (6, 7).

The causal relationship between vitamin D deficiency and decreased kidney function remains debates. Decreased kidney function is related to lower enzyme 1a-hydroxylase activity in the proximal tubule, which leads to decreased activation of 25(OH)D to 1,25(OH)$_2$D (8). However, increasing evidence from scientific approaches has shown an association between low vitamin D levels and decreased estimated glomerular filtration (eGFR) (9, 10). Vitamin D is known to play an important role in maintaining homeostasis, which is associated with the renin-angiotensin system (RAS) (11). Several studies have shown that activation of the RAS system due to low vitamin D level leads to kidney disease, hypertension, cardiovascular disease, insulin resistance, and increased mortality (12, 13). In fact, 2017 Kidney Disease Improving Global Outcomes (KDIGO) experts carefully suggested (level of evidence 2C) checking and supplementing low vitamin D levels in CKD and dialysis patients (14).

Some observational studies and guidelines have suggested a possible association between low vitamin D level and CKD (15–17). However, in most studies and guidelines, the causality between low vitamin D levels and risk of renal event could not be explained because this relationship was analyzed through a cross-sectional study or focused on dialysis patients, or did not distinguish the stage of CKD (early versus advanced stage) (15–17).

The purpose of this study was twofold. First, to evaluate the relationship between patients with 25(OH)D deficiency and CKD stages at baseline. Second, to analyze longitudinal follow-up data to elucidate the association between patients with 25(OH)D deficiency and risk of renal event, which is defined as a 50% decline in eGFR from the baseline value or the onset of CKD stage 5 across the follow-up. Further, controlling the effects of confounding on vitamin D levels using propensity score matching analysis (PSM), we evaluated the relationship between patients with 25(OH)D deficiency and severe CKD stage and renal event. We also investigated the associations of patients with 25(OH)D deficiency and risk of renal event according to diabetes and overweight status.

Materials and methods

Study design and subjects

We collected baseline data for 2,238 non-dialysis dependent patients with CKD from stage G1 to 5, who were enrolled in a prospective cohort study [KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease] from 2011 to 2015 in collaboration with a multicenter, patient-based, prospective-cohort study. The detailed design and methods were previously published (18). Among 2,238 (KNOW-CKD) cohort study patients, a total of 2,144 patients with serum 25(OH)D was included in this study (**Supplementary Figure 1**). This study was conducted according to guidelines established by the Declaration of Helsinki. All patients gave written informed consent for inclusion before they participated in the study. The study protocol was approved by the institutional review board of each participating clinical center: Seoul National University Hospital (1104-089-359), Seoul National University Bundang Hospital (B-1106/129–008), Kangbuk Samsung Medical Center (2011–01-076), Yonsei University Severance Hospital (4-2011-0163), Seoul St. Mary's Hospital (KC11OIMI0441), Eulji General Hospital (201105-01), Gil Hospital (GIRBA2553), Pusan Paik Hospital (11-091), Chonnam National University Hospital (CNUH-2011-092) in 2011.

Data collection

Data regarding personal and family history, anthropometric measurements, cardiac evaluation, radiological imaging, and baseline laboratory results were extracted from the electronic data management system (PhactaX: http://www.phactaX.org) with assistance from the Division of data management at Seoul National University Medical Research Collaborating Center. Serum samples were harvested and sent immediately to the central laboratory of Lab

TABLE 1 Association between serum vitamin D levels and moderate (Stage 3A/3B) and severe CKD (Stage 4/5) stage in the Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) study, 2011-2015.

Vitamin D biomarkers	CKD Stage 1/2	CKD Stage 3A/3B		CKD Stage 4/5	
	N (%)	N (%)	OR (95% CI) [1]	N (%)	OR (95% CI) [1]
Total cohort					
25(OH)D					
≥ 15 *ng/mL*	474 (62.8)	500 (62.5)	1.00	320 (54.3)	1.00
< 15	281 (37.2)	300 (37.5)	1.06 (0.84-1.33)	269 (45.7)	1.30 (1.01-1.69)
3T (≥ 19.4 *ng/mL*)	244 (32.3)	286 (35.7)	1.00	184 (31.2)	1.00
2T (14.1 – 19.3)	266 (35.2)	264 (33.0)	0.98 (0.74-1.26)	188 (31.9)	1.04 (0.77-1.41)
1T (< 14.1)	245 (32.4)	250 (31.2)	0.95 (0.72-1.25)	217 (36.8)	1.15 (0.84-1.57)
PS matching cohort [2]					
25(OH)D					
≥ 15 *ng/mL*	158 (55.8)	130 (49.4)	1.00	85 (42.5)	1.00
< 15	125 (44.2)	133 (50.6)	1.21 (0.86-1.71)	115 (57.5)	1.44 (0.98-2.13)

Chronic kidney disease (CKD); 25-hydroxyvitamin (25(OH)D); 1,25-dihydroxyvitamin (1,25$(OH)_2$D); Propensity score (PS) matching; Systolic blood pressure (SBP); Fibroblast growth factor 23 (FGF23); Urine albumin creatinine ratio (UACR); Diabetes mellitus (DM); Angiotensin receptor blockers (ARB) medication.

[1]Logistic regression model for total cohort was adjusted for age, sex, systolic blood pressure, serum phosphorus, Fibroblast growth factor 23, klotho, cause of chronic kidney disease, UACR, Diabetes mellitus, use of vitamin supplements and ARB medications. Logistic regression model for PS matching cohort were adjusted for baseline eGFR, SBP, DM, 24 h-Urine protein, UACR, and use of ARB medications.

[2]For propensity score (PS) matching cohort, PS matching was performed using the variables such as low vitamin D and high vitamin D biomarker levels.

Genomics, Seongnam, Republic of Korea. Urine samples were also immediately sent to the central laboratory for KNOW-CKD. The reliability of biomarker analysis was previously reported (18).

Main exposure variables and other biomarker

Serum 25(OH)D level was measured by electrochemiluminescence immunoassay (ECLIA), using an ADVIA Centaur Vitamin D Total assay reagents (Siemens, NY, USA). 25(OH)D deficiency was defined as level < 15 ng/mL, as considered in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines (19). Non-deficient levels of 25(OH)D was ≥ 15 ng/mL. Limit of detection for 25(OH)D level was 3.20 ng/mL (18).

Serum c-terminal FGF23 level was measured using enzyme-linked immunosorbent assay (ELISA; Immunotopics, San Clemente, CA, USA). Serum levels of klotho were measured using an ELISA kit (Immuno-Biological Laboratories Co., Gunma, Japan). (18).

Outcome variables

Serum creatinine was measured by an IDMS-traceable method at a central laboratory and eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) (20). Mild CKD stage (Stage 1/2) was defined as eGFR ≥ 60 mL/min/1.73 m^2. Moderate CKD stage (Stage 3A/3B) was defined as 30 ≤ eGFR < 60 mL/min/1.73 m^2 and severe CKD stage (Stage 4/5) was defined as eGFR of less than 30 mL/min/1.73 m^2.

Renal event was a composite of the first occurrence of a 50% decline in eGFR from the baseline value or the onset of CKD stage 5 (initiation of dialysis or kidney transplantation) across the follow-up period.

Statistical analysis

Characteristics of patients are described by binary of 25(OH)D, before and after propensity score matching analysis. Continuous variables are expressed as mean and standard deviation for t-tests, and categorical variables are reported as number of patients and percentage for the chi-squared test. To examine the association of 25(OH)D with CKD stages and risk of renal event, polytomous logistic regression and Cox proportional hazard models were constructed to calculate odds ratio (ORs) and hazard ratio (HRs), using non-deficiency vitamin D levels as the reference. Patients who were lost to follow-up were censored at the date of the last hospital examination. This study was adjusted for clinically important confounding factors: age, sex, baseline eGFR, serum phosphorus levels, serum intact parathyroid hormone (iPTH), serum fibroblast growth factor 23 (FGF23), klotho, random urine albumin-to-creatinine ratio (UACR), 24-h urine protein, diabetes mellitus (DM), cause of CKD, use of vitamin D supplements, and angiotensin converting enzyme inhibitors(ACEi)/angiotensin receptor blocker (ARBs) medications. Further, controlling the effects of confounding on vitamin D levels using propensity score matching analysis (PSM), we evaluated the relationship between vitamin D deficiency and severe CKD stage and risk of renal event. Confounding variables affecting age, sex, PTH, serum phosphorus, serum klotho, FGF23, and cause of CKD were matched in the two study groups using 1:1 propensity score matching analysis (PSM) and a caliper width of 0.01. We also examined multi-collinearity between independent variables with Pearson correlation coefficients and variance inflation factor. We also performed stratified analysis by DM (No vs. Yes), and overweight (BMI < 23 kg/m^2 *vs.* BMI ≥ 23 kg/m^2) status using Cox regression models. P-interaction term was calculated to determine interaction between DM, overweight and each vitamin D level. The spline model was adjusted for age, sex, and confounding factors. The survival probability was calculated with the Kaplan-Meier (KM) method. To analyze the association with vitamin D on the risk of

TABLE 2 Association between serum vitamin D levels and renal event in the Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) study, 2011-2015.

Vitamin D biomarkers	CKD cohort		Renal event[1]	
	N	PY	N	HR (95% CI)[2]
Total cohort				
25(OH)D				
≥ 15 *ng/mL*	1,294	3,891	171	1.00
< 15	850	2,432	171	1.64 (1.32-2.05)
3T (≥ 19.4 *ng/mL*)	630	2,078	84	1.00
2T (14.1 – 19.3)	604	2,269	114	1.25 (0.94-1.66)
1T (< 14.1)	568	1,977	144	1.82 (1.38-2.41)
PS matching cohort [3]				
25(OH)D				
≥ 15 *ng/mL*	373	607	19	1.00
< 15	373	621	42	1.52 (0.88-2.65)

Chronic kidney disease (CKD); 25-hydroxyvitamin (25(OH)D); 1,25-dihydroxyvitamin (1,25(OH)$_2$D); Propensity score (PS) matching; Systolic blood pressure (SBP); Fibroblast growth factor 23 (FGF23); Urine albumin creatinine ratio (UACR); Diabetes mellitus (DM); Angiotensin receptor blockers (ARB) medication.

[1]Renal events were a composite of the first occurrence of a 50% decline in eGFR from the baseline value or the onset of ESRD (the initiation of dialysis or kidney transplantation) during follow-up period.

[2]Cox proportional hazards model were adjusted for age, sex, baseline eGFR, SBP, serum phosphorus, FGF23, klotho, cause of CKD, 24h-Urine protein, UACR, Diabetes mellitus, use of vitamin supplements and ARB medications. Cox proportional hazards model for PS matching cohort were adjusted for baseline eGFR, SBP, DM, 24 h-Urine protein, UACR, and use of ARB medications.

[3]For propensity score (PS) matching cohort, PS matching was performed using the variables such as low vitamin D and high vitamin D biomarker levels.

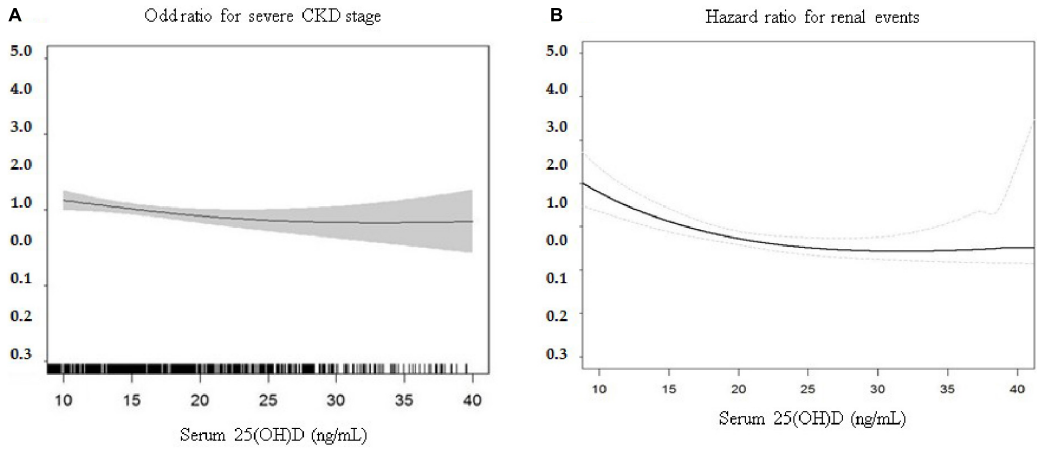

FIGURE 1
Multivariate association of continuously measured 25(OH)D levels (ng/mL) and **(A)** severe CKD stages and **(B)** risk of renal events. The relationship between serum vitamin D levels (continuous scale) and risk of renal event were non-linear after multivariate adjustment. CKD, Chronic kidney disease; 25(OH)D, 25-hydroxyvitamin D.

renal events, a Cox regression model was constructed by controlling the following 8 risk factors, which were statistically significant in each univariable analysis: age, sex, baseline eGFR, cause of CKD, 24-h proteinuria, intact PTH and serum klotho. Each model was constructed by backward Cox regression model. In order to observe the effect of vitamin D on the risk of renal events under adjustment for 25(OH)D levels and other risk factors, we constructed a vitamin D-epidemiologic-clinical model which was a full multivariable model. In addition, we evaluated the discriminatory accuracy of the only vitamin D model, vitamin D-epidemiological model, and a vitamin D-epidemiologic-clinical model in predicting risk of renal events using Harrell's C index.

P-value less than 0.01 were considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and spline plots were drawn by R version 4.2.1 (http://www.r-project.org). *P*-values < 0.05 were considered statistically significant.

Results

Baseline characteristics of the study subjects

Baseline characteristics of the study population are described Supplementary Table 1. Compared with non-deficient vitamin D patients, patients with deficiency were more likely to be male; have

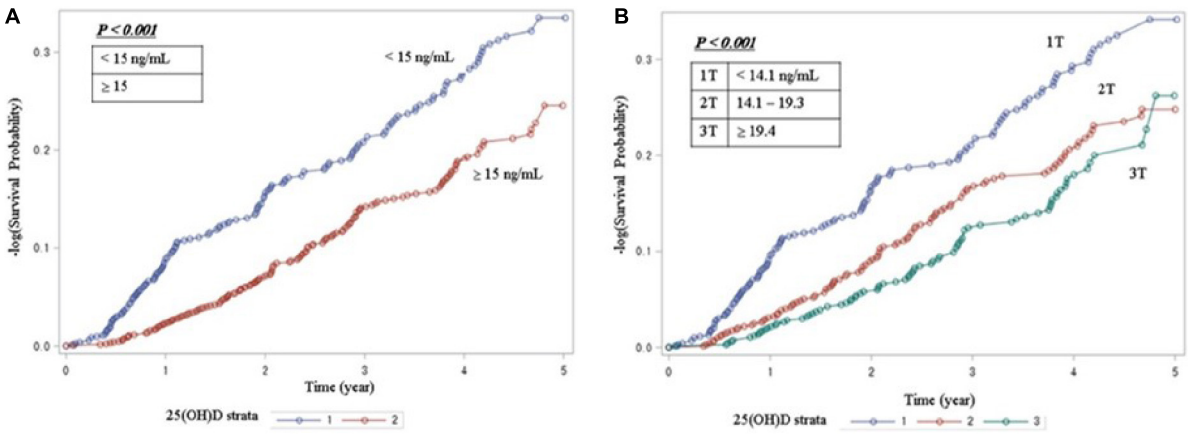

FIGURE 2
Kaplan-Meier survival analysis for serum vitamin D levels **(A)** Survival curve for renal event by binary of 25(OH)D. **(B)** Survival curve for renal event by tertiles of 25(OH)D. The Kaplan-Meier curves show that the patient with vitamin D deficiency group had a significantly higher cumulative incidence of renal events ($P < 0.001$). 25(OH)D, 25-hydroxyvitamin D.

higher SBP, DBP, UACR, UPCR, 24-h urine protein, serum creatinine, phosphorus, potassium, total cholesterol, i-PTH, FGF23, klotho, hepcidin, angiotensin levels; have lower eGFR, hemoglobin, uric acid, albumin, calcium and sodium levels. The PSM study showed that all variables were not statistically different according to vitamin D levels.

Association between vitamin D deficiency and severe CKD stage

Table 1 presents the associations of patients with 25(OH)D deficiency with severe CKD stage. We found increased odds for severe CKD stage in patients with vitamin D deficiency levels compared to patients with non-deficient levels (OR = 1.30, 95% CI = 1.01-1.69). In addition, Table 1 shows the likelihood of having severe CKD stage relative to mild CKD stage by serum levels of vitamin D biomarkers using PSM. Patients with 25(OH)D deficiency levels were not statistically significant associated with increased odds of severe CKD stage compared to patients with non-deficient levels (OR = 1.44, 95% CI = 0.98-2.13).

Association between vitamin D deficiency and renal event

Table 2 shows that 342 patients reached the composite outcome of renal event. Patients with 25(OH)D deficiency levels had a significantly increased risk of renal events compared to patients with non-deficient levels (HR = 1.64, 95% CI = 1.32-2.05). Table 2 also shows the risk of renal event according to serum levels of vitamin D biomarkers using PSM. After PSM, patients with 25(OH)D deficiency levels had no significant association with renal event (HR = 1.52, 95% CI = 0.88-2.65). The relationship between serum vitamin D levels (continuous scale) and severe CKD stage and risk of renal event were non-linear after multivariate adjustment (Figure 1). The Kaplan-Meier survival curves show statistically significant difference in renal event probability among the binary and tertiles of serum 25(OH)D levels ($p < 0.001$) (Figure 2). Thus, this model has a certain practicability and reliability in evaluating prognosis.

Association between vitamin D deficiency and severe CKD stage according to diabetes and overweight

Compared to the non-DM, the diabetic CKD showed stronger associations between CKD stage 4 and 5 and patients with 25(OH) deficiency (OR = 1.59, 95% CI = 1.13-2.25, p-interaction = 0.02).

In this study, compared to the non-overweight (BMI < 23), the patients with overweight (BMI ≥ 23) status demonstrated stronger associations between CKD stage 4 and 5 and patients with 25(OH) deficiency (OR = 1.41, 95% CI = 1.03-1.92, p-interaction = 0.30). On the other hand, none of the patients with 25(OH)D deficiency showed a significant association with the risk of CKD stage 3A and 3B according to DM and overweight status (Table 3).

Association between vitamin D deficiency and renal event according to diabetes and overweight

As shown in Table 4, patients with DM, and overweight (BMI ≥ 23) status appeared to have a greater risk for renal event in the context of vitamin D deficiency. The associations of 25(OH)D deficiency with risk of renal event were stronger in patients with DM (HR = 1.81, 95% CI = 1.38-2.38, $p < 0.01$ for interaction between 25(OH)D and DM for renal event; and overweight status (HR = 1.89, 95% CI = 1.44-2.48, $p < 0.01$ for interaction between 25(OH)D and overweight for renal event.

Discriminatory accuracy of models on predicting the risk of renal events

With the ability to discriminate renal events for the epidemiological model, a multivariate model controlled by 25(OH)D levels and other risk factors, the Harrell's C-index at long term follow up was as follow: Model 1 (vitamin D only) was constructed with 1 variable and the Harrell's C-index was 0.5822. Model 2 (vitamin

TABLE 3 Association between serum vitamin D levels and moderate (Stage 3A/3B) and severe CKD (Stage 4/5) stage according to diabetes and overweight status in the Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) study, 2011-2015.

Vitamin D biomarkers	CKD Stage 1/2	CKD Stage 3A/3B		CKD Stage 4/5		CKD Stage 1/2	CKD Stage 3A/3B		CKD Stage 4/5		P-interaction[2]
	N (%)	N (%)	OR (95% CI) [1]	N (%)	OR (95% CI) [1]	N (%)	N (%)	OR (95% CI) [1]	N (%)	OR (95% CI) [1]	
	DM					Non-DM					
25(OH)D											
≥ 15 *ng/mL*	243 (64.5)	303 (60.9)	1.00	197 (50.7)	1.00	231 (61.1)	197 (65.0)	1.00	123 (61.2)	1.00	
< 15	134 (35.3)	194 (39.0)	1.20 (0.88-1.64)	191 (49.2)	1.59 (1.13-2.25)	147 (38.9)	106 (35.0)	0.91 (0.64-1.30)	78 (38.8)	0.85 (0.55-1.30)	0.02
3T (≥ 19.4 *ng/mL*)	135 (35.8)	178 (35.8)	1.00	111 (28.6)	1.00	109 (28.8)	108 (35.6)	1.00	73 (36.3)	1.00	
2T (14.1 – 19.3)	118 (31.3)	152 (30.6)	0.99 (0.69-1.43)	118 (30.4)	1.21 (0.80-1.84)	148 (39.1)	112 (36.9)	0.88 (0.59-1.32)	70 (34.8)	0.75 (0.46-1.21)	
1T (< 14.1)	124 (32.9)	167 (33.6)	1.06 (0.73-1.53)	159 (40.9)	1.39 (0.92-2.10)	121 (32.0)	83 (27.4)	0.75 (0.48-1.16)	58 (38.9)	0.59 (0.35-1.01)	
	BMI ≥ 23 kg/m^2					BMI < 23 kg/m^2					
25(OH)D											
≥ 15 *ng/mL*	311 (63.7)	369 (64.2)	1.00	201 (51.9)	1.00	160 (61.3)	127 (58.3)	1.00	118 (58.7)	1.00	
< 15	177 (36.3)	206 (35.8)	0.97 (0.73-1.28)	186 (48.1)	1.41 (1.03-1.92)	101 (38.7)	91 (41.7)	1.38 (0.89-2.13)	83 (41.3)	1.29 (0.81-2.05)	0.30
3T (≥ 19.4 *ng/mL*)	160 (32.8)	199 (34.6)	1.00	119 (30.7)	1.00	83 (31.8)	84 (38.5)	1.00	64 (31.8)	1.00	
2T (14.1 – 19.3)	175 (35.9)	201 (35.0)	1.00 (0.73-1.37)	115 (29.7)	0.95 (0.65-1.38)	89 (34.1)	62 (28.4)	0.90 (0.54-1.50)	73 (36.3)	1.39 (0.80-2.39)	
1T (< 14.1)	153 (31.4)	175 (30.4)	0.92 (0.66-1.29)	153 (39.5)	1.14 (0.78-1.67)	89 (34.1)	72 (33.0)	0.97 (0.58-1.62)	64 (31.8)	1.13 (0.65-1.99)	

Chronic kidney disease (CKD); 25-hydroxyvitamin (25(OH)D); Systolic blood pressure (SBP); Fibroblast growth factor 23 (FGF23); Urine albumin creatinine ratio (UACR); Diabetes mellitus (DM); Angiotensin receptor blockers (ARB) medication.

[1]Logistic regression model for total cohort was adjusted for age, sex, systolic blood pressure, serum phosphorus, Fibroblast growth factor 23, klotho, cause of chronic kidney disease, UACR, use of vitamin supplements and ARB medications.

[2]P-interaction term was calculated to determine interaction between DM, overweight and each vitamin D level.

10.3389/fmed.2023.1017459

TABLE 4 Association between serum vitamin D levels and renal event according to diabetes and overweight status in the Korean Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) study, 2011-2015.

Vitamin D Biomarkers	Renal event [1]		Renal event [1]		
	N	HR (95% CI)	N	HR (95% CI)	P-interaction [4]
Stratification by DM[2]	DM		Non-DM		
25(OH)D					
≥ 15 *ng/mL*	101	1.00	70	1.00	
< 15	128	1.81 (1.38-2.38)	43	1.12 (0.75-1.67)	< 0.01
Stratification by BMI[3]	BMI ≥ 23 kg/m^2		BMI < 23 kg/m^2		
25(OH)D					
≥ 15 *ng/mL*	105	1.00	66	1.00	
< 15	120	1.89 (1.44-2.48)	51	1.08 (0.73-1.60)	< 0.01

Chronic kidney disease (CKD); 25-hydroxyvitamin (25(OH)D); Propensity score (PS) matching; Systolic blood pressure (SBP); Fibroblast growth factor 23 (FGF23); Urine albumin creatinine ratio (UACR); Diabetes mellitus (DM); Body mass index (BMI); Angiotensin receptor blockers (ARB) medication.
[1]Renal events were a composite of the first occurrence of a 50% decline in eGFR from the baseline value or the onset of ESRD (the initiation of dialysis or kidney transplantation) during follow-up period.
[2]Adjusted for age, sex, baseline GFR, SBP, serum phosphorus, FGF23, klotho, cause of CKD, 24h-Urine protein, UACR, use of vitamin supplements and ARB medications.
[3]Adjusted for age, sex, baseline GFR, SBP, serum phosphorus, FGF23, klotho, cause of CKD, 24h-Urine protein, UACR, Diabetes mellitus, use of vitamin supplements and ARB medications.
[4]P-interaction term was calculated to determine interaction between DM, overweight and each vitamin D level.

D-epidemiological model) was constructed with 4 variables and the Harrell's C-index was 0.8386. Model 3 (vitamin D-epidemiologic-clinical model) was constructed with 8 variables and the Harrell's C-index was 0.8916. A full multivariable model shows the highest differential accuracy among the three models predicting the risk of renal events. The C-index of the model was significantly different from that of the vitamin D only model (p-value < 0.01). (**Figure 3**).

Discussions

In this study, we found that patients with 25(OH)D deficiency levels was significantly associated with severe CKD stage and increased risk of renal event. Furthermore, vitamin D deficiency patients with presence of DM and overweight status (≥ 23 kg/m^2) also displayed higher risk than non-deficient patients for severe CKD stage and risk of renal event. None of the patients with 25(OH)D deficiency showed a significant association with the risk of CKD stage 3A and 3B.

Our findings are consistent with a number of previous studies. A cross-sectional studies conducted in France and Thailand showed that 25(OH)D deficiency was related to decreased GFR and developing ESRD (15, 16). A cohort study in the United States found that low 25(OH)D level was associated with development of ESRD (21). Similarly, another cohort study in Italy reported that low 25(OH)D level is an independent predictor of CKD progression (22).

On the other hand, previous studies found no association between low 25(OH)D level and CKD progression. A longitudinal patient cohort study reported that African Americans with low 25(OH)D levels were not associated with ESRD or doubling of serum creatinine (23). The Framingham Offspring cohort study showed similar results (24). The biggest difference between the former studies (15, 16, 21, 22) including our study and the latter studies (23, 24), is that the former studies (15, 16, 21, 22) reported that 25(OH)D deficiency is associated with risk of renal event while the latter studies (23, 24) showed that 25(OH)D deficiency is not associated with the risk of ESRD in the general population, where incidence of ESRD is less common. These inconsistencies in results can be attributed to various reasons, including differences in the general characteristics of the study participants, outcome variables, and confounding variables.

In addition, we found interactions between vitamin D deficiency and DM or between vitamin D deficiency and overweight for the risk of renal event. DM is one of the most common causes of CKD. Low vitamin D levels are related to insulin resistance and impaired pancreatic islet B-cell function (25). The effect is on upregulation of the insulin receptor gene and calcium and phosphorus metabolism (26). Also, an adequate vitamin D levels suppresses RAS system by reducing the expression AT1 receptors and by inhibiting renin synthesis in the kidney. It could lead to nephron-protective effects by reducing proteinuria and decreasing CKD progression (27). Previous studies reported that 25(OH)D deficiency is associated with type 2 DM (28). Since DM can induce increased risk of renal event, patients with DM may be more susceptible to vitamin D deficiency with regards to CKD than those without DM. Also, high BMI is one of the strongest risk factors for CKD (29). Previous study has shown that adipose tissue may increase the secretion of inflammatory cytokines or chemokine (MCP-1, IL-6, IL-1beta, TNF-a) and its plausible influence on the course of CKD (30). Overweight or obesity status is directly related to hypertension and diabetes status, the metabolic disorders responsible for the ESRD (31). One animal study presents that association between vitamin D deficiency and obesity impairs the renal function, hemodynamics, and metabolic parameters in the High-fat vitamin D deficient (H + VDD) rats. Obesity associated to vitamin D deficiency aggravated the renal inflammation associated to renal progression (32).

While the exact mechanism for vitamin D deficiency and risk of renal event is unclear, several biological mechanisms have been proposed. Vitamin D is converted enzymatically in the liver to 25(OH)D, the major circulating form, and then in the kidney to 1,25(OH)$_2$D, the active form of vitamin D (33). Low levels of 1,25(OH)$_2$D levels in the body can lead to deterioration of the RAS system by inducing abnormal metabolic profiles such as increasing renin, proteinuria, blood pressure, insulin resistance, and renal injury (34–36). For example, vitamin D supplementation decreases renin receptor and renin expression in rat models of CKD (37). Another possible mechanism is that low levels of 25(OH)D may

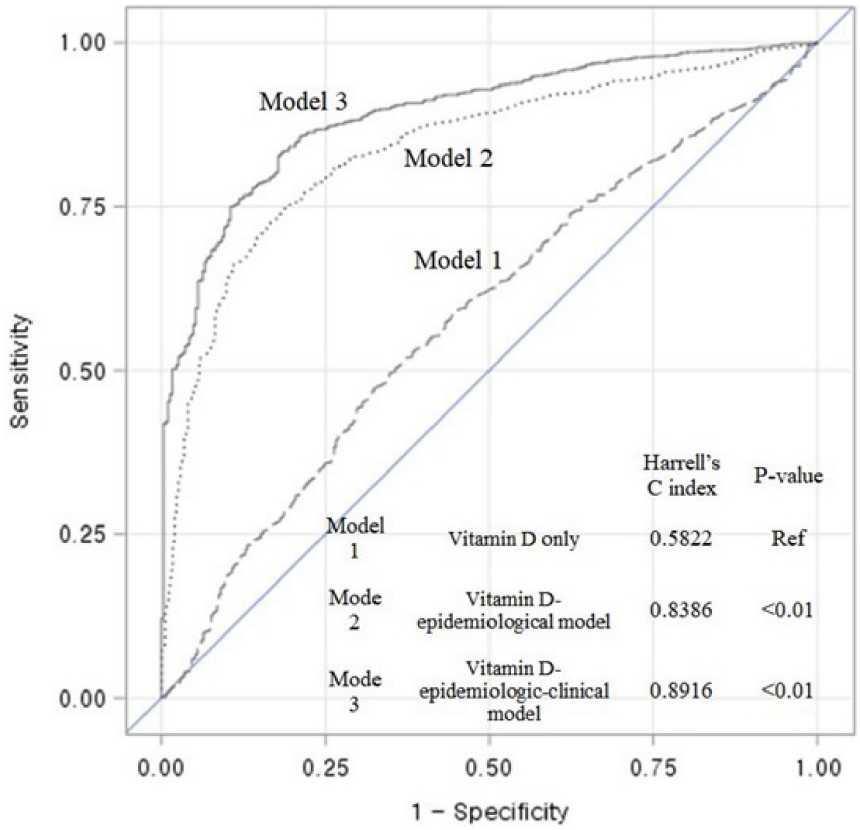

FIGURE 3
Receiver operating characteristic (ROC) curves and Harrell's C-index showing the discriminant accuracy of each model for the ability to distinguish renal events in entire cohort; Model 1 (vitamin D model): Function**(Y)** = β1[25(OH)D], Function(Y) = Log ((〚 Hazard 〛_Exposed)/〚 Hazard 〛_(Non-Exposed)); Model 2 (Vitamin D-epidemiological model): Function(Y) = β1[25(OH)D] + β2[Age] + β3[Sex] + β4[Baseline eGFR]; Model 3 (Vitamin D-epidemiologic -clinical model): Function(Y) = β1[25(OH)D] + β2[Age] + β3[Sex] + β4[Baseline eGFR] + β5[Cause of CKD] + β6[24-h proteinuria] + β7[intact PTH] + β8 [Klotho]; A full multivariable model shows the highest differential accuracy among the three models predicting the risk of renal events. The C-index of the model was significantly different from that of the vitamin D only model (p-value < 0.01). 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration.

modulate inflammation and oxidative stress and reduce fibroblast activation (13). In addition, CKD progression has been related to lower 25(OH)D reuptake and reduction of intracrine $1,25(OH)_2D$ levels in the renal proximal tubules (38). Also, $1,25(OH)_2D$ levels are known to reduce expression of the nuclear factor kB and promote a shift in T-helper cell response from T-helper 1 cell to T-helper 2 cell (39). Therefore, this reduces T-helper 1 cell-mediated tissue damage and increases production of T-helper 2 cell immunomodulatory cytokines (40). A genetic predisposition that affects higher vitamin D binding protein (DBP) was suggested as a plausible mechanism in the association between vitamin D level and risk of renal events (41). DBP is facilitated by receptor-mediated endocytosis. In the renal proximal tubule, cublin and megalin induce uptake of extracellular ligands. Deficiency of these proteins results in increased vitamin D excretion in the urine (42). More studies are needed to understand these effects in humans.

Several limitations should be considered when interpreting our results. First, serum vitamin D levels are generally affected by seasonal variation and the extent of UV exposure should be considered (43). However, our study could not be adjusted for seasonal variation. Second, we did not adjust our data for patient nutritional status. Nutritional status may also contribute to vitamin D status in CKD patients. Malnutrition is a typical finding in patients with CKD. Uremia may be related to impaired gastrointestinal absorption of vitamin D (44). Third, we conducted a single measurement of vitamin D levels at baseline. Our data in the present study did not have enough repeated measures to perform the analysis. Fifth, this study is an observational study. Therefore, it is possible that potential confounding factors were not correctly adjusted. To reduce this possibility, we conducted PSM analysis and found consistent results. Finally, the KNOW-CKD cohort consists only of Korean CKD patients and our findings may not be generalizable to other ethnic groups.

Despite these limitations, the present study has several strengths. First, our study used a well-designed patient-based prospective cohort (the KNOW-CKD) representative of Korean CKD patients. In addition, serum vitamin D levels can be easily obtained in the clinical setting and treatment of vitamin D deficiency/insufficiency is simple and inexpensive. This study should be taken into consideration by researchers and clinicians in order to improve CKD patient outcomes.

Conclusion

The present study suggests that vitamin D deficiency is significantly associated with risk of severe CKD stage and renal event.

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 Seoul National University Hospital (1104-089-359), Seoul National University Bundang Hospital (B-1106/129–008), Kangbuk Samsung Medical Center (2011–01-076), Yonsei University Severance Hospital (4-2011-0163), Seoul St. Mary's Hospital (KC11OIMI0441), Eulji General Hospital (201105-01), Gil Hospital (GIRBA2553), Pusan Paik Hospital (11-091), Chonnam National University Hospital (CNUH-2011-092). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL, EB, SK, K-HO, and SP designed the study. JL, K-HO, and SP conducted the data analysis and drafted the manuscript and wrote the manuscript. K-HO and SP provided the supervision and mentorship. All authors supported the interpretation of results, provided important intellectual content and revised the final version of the manuscript and also provided final approval of the version to be published.

Funding

This work was supported by the Research Program funded by the Korea Disease Control and Prevention Agency (2011E3300300, 2012E3301100, 2013E3301600, 2013E3301601, 2013E3301602, 2016E3300200, 2016E3300201, 2016E3300202, 2019E320100, 2019E320101, 2019E320102, and 2022-11-007). Funding sources had no role in the design and conduct of study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant no. HI16C1127). This study was supported by a grant from Seoul National University Hospital (2022).

Acknowledgments

We thank all the participants and researchers who participated in the KNOW-CKD study. We are grateful to all of those with whom we have had the pleasure to work during this article.

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.2023.1017459/full#supplementary-material

References

1. Levey A, Eckardt K, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from kidney disease: improving global outcomes (KDIGO). *Kidney Int.* (2005) 67:2089–100. doi: 10.1111/j.1523-1755.2005.00365.x

2. Levey A, Atkins R, Coresh J, Cohen E, Collins A, Eckardt K, et al. Chronic kidney disease as a global public health problem: approaches and initiatives–a position statement from kidney disease improving global outcomes. *Kidney Int.* (2007) 72:247–59. doi: 10.1038/sj.ki.5002343

3. Park J, Baek H, Jung H. Prevalence of chronic kidney disease in Korea: the Korean national health and nutritional examination survey 2011–2013. *J Korean Med Sci.* (2016) 31:915–23. doi: 10.3346/jkms.2016.31.6.915

4. Park J, Hong I, Chung J, Choi H. Vitamin D status in South Korean population: seven-year trend from the KNHANES. *Medicine.* (2018) 97:e11032. doi: 0.097/MD.0000000000011032

5. Heaney R. Vitamin D in health and disease. *Clin J Am Soc Nephrol.* (2008) 3:1535–41. doi: 10.2215/CJN.01160308

6. Holick M. Vitamin D deficiency. *N Engl J Med.* (2007) 357:266–81. doi: 10.1056/NEJMra070553

7. Plum L, DeLuca H. Vitamin D, disease and therapeutic opportunities. *Nat Rev Drug Discov.* (2010) 9:941–55. doi: 0.038/nrd3318

8. Bikle D. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol.* (2014) 21:319–29. doi: 10.1016/j.chembiol.2013.12.016

9. Shroff R, Wan M, Nagler E, Bakkaloğlu S, Fischer D, Bishop N, et al. Clinical practice recommendations for native vitamin D therapy in children with chronic kidney disease Stages 2–5 and on dialysis. *Nephrol Dial Transplant.* (2017) 32:1098–113. doi: 10.1093/ndt/gfx065

10. de Boer I, Katz R, Chonchol M, Ix J, Sarnak M, Shlipak M, et al. Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. *Clin J Am Soc Nephrol.* (2011) 6:2141–9. doi: 10.2215/CJN.02640311

11. Li Y. Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem.* (2003) 88:327–31. doi: 10.1002/jcb.10343

12. Fanari Z, Hammami S, Hammami M, Hammami S, Abdellatif A. Vitamin D deficiency plays an important role in cardiac disease and affects patient outcome: still a myth or a fact that needs exploration? *J Saudi Heart Assoc.* (2015) 27:264–71. doi: 10.1016/j.jsha.2015.02.003

13. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong J, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ.* (2014) 348:g1903. doi: 0.136/bmj.g1903

14. Wheeler D, Winkelmayer W. KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD) foreword. *Kidney Int Suppl.* (2017) 7:1–59. doi: 10.1016/j.kisu.2017.04.001

15. Urena-Torres P, Metzger M, Haymann J, Karras A, Boffa J, Flamant M, et al. Association of kidney function, vitamin D deficiency, and circulating markers of mineral and bone disorders in CKD. *Am J Kidney Dis.* (2011) 58:544–53. doi: 10.1053/j.ajkd.2011.04.029

16. Satirapoj B, Limwannata P, Chaiprasert A, Supasyndh O, Choovichian P. Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. *BMC Nephrol.* (2013) 14:206. doi: 10.1186/1471-2369-14-206

17. Koh J, Kwak I, Song S, Lee S, Rhee H, Seong E, et al. 25-hydroxyvitamin D status based on estimated glomerular filtration rate in patients with chronic kidney disease. *Korean J Med.* (2012) 83:740–51. doi: 10.3904/kjm.2012.83.6.740

18. Oh K, Park S, Park H, Chin H, Chae D, Choi K, 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

19. Levey A, Coresh J, Bolton K, Culleton B, Harvey K, Ikizler T, et al. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39:S1–266.

20. Levey A, Stevens L, Schmid C, Zhang Y, Castro A, Feldman H, 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

21. Melamed M, Astor B, Michos ED, Hostetter T, Powe N, Muntner P. 25-hydroxyvitamin D levels, race, and the progression of kidney disease. *J Am Soc Nephrol.* (2009) 20:2631–9. doi: 10.1681/ASN.2009030283

22. Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, et al. Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int.* (2009) 75:88–95. doi: 10.1038/ki.2008.501

23. Scialla J, Astor B, Isakova T, Xie H, Appel L, Wolf M. Mineral metabolites and CKD progression in African Americans. *J Am Soc Nephrol.* (2013) 24:125–35. doi: 10.1681/ASN.2012070713

24. O'Seaghdha C, Hwang S, Holden R, Booth S, Fox C. Phylloquinone and vitamin D status: associations with incident chronic kidney disease in the Framingham offspring cohort. *Am J Nephrol.* (2012) 36:68–77. doi: 10.1159/000339005

25. Martin T, Campbell R. Vitamin D and diabetes. *Diabetes Spectr.* (2011) 24:113–8. doi: 10.2337/diaspect.24.2.113

26. Nada A, Shaheen D. Cholecalciferol improves glycemic control in type 2 diabetic patients: a 6-month prospective interventional study. *Ther Clin Risk Manag.* (2017) 13:813–20. doi: 10.2147/TCRM.S132344

27. Lang C, Wang M, Chiang C, Lu K. Vitamin D and the immune system from the nephrologist's viewpoint. *ISRN Endocrinol.* (2014) 2014:105456. doi: 10.1155/2014/105456

28. Mattila C, Knekt P, Männistö S, Rissanen H, Laaksonen M, Montonen J, et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care.* (2007) 30:2569–70. doi: 10.2337/dc07-0292

29. Kovesdy C, Furth S, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Braz J Nephrol.* (2017) 39:1–10. doi: 10.5935/0101-2800.20170001

30. Mori J, Patel V, Ramprasath T, Alrob O, DesAulniers J, Scholey J, et al. Angiotensin 1–7 mediates renoprotection against diabetic nephropathy by reducing oxidative stress, inflammation, and lipotoxicity. *Am J Physiol Renal Physiol.* (2014) 306:F812–21. doi: 10.1152/ajprenal.00655.2013

31. Hall M, do Carmo J, da Silva A, Juncos L, Wang Z, Hall J. Obesity, hypertension, and chronic kidney disease. *Int J Nephrol Renovasc Dis.* (2014) 7:75–88. doi: 10.2147/IJNRD.S39739

32. Bernardo D, Carolina de Braganca A, Heloisa Massola Shimizu M, Carlos Seguro A, Moura Nascimento M, Canale D, et al. MO417: obesity associated with vitamin D deficiency impairs renal function, haemodynamics, metabolic parameters and aggravates the renal morphological damage in rats submitted to ischaemia-reperfusion injury. *Nephrol Dial Transplant.* (2022) 37(Suppl. 3):gfac070.31. doi: 10.1093/ndt/gfac070.031

33. Bouillon R, Norman A, Lips P. Vitamin D deficiency. *N Engl J Med.* (2007) 357:1980–1. doi: 10.1056/NEJMc072359

34. Santoro D, Caccamo D, Lucisano S, Buemi M, Sebekova K, Teta D, et al. Interplay of vitamin D, erythropoiesis, and the renin-angiotensin system. *Biomed Res Int.* (2015) 2015:145828. doi: 10.1155/2015/145828

35. Kim C, Kim S. Vitamin D and chronic kidney disease. *Korean J Intern Med.* (2014) 29:416–27. doi: 10.3904/kjim.2014.29.4.416

36. Vaidya A, Williams J. Vitamin D in the pathophysiology of hypertension, kidney disease, and diabetes: examining the relationship between vitamin D and the renin-angiotensin system in human diseases. *Metabolism.* (2012) 61:450–8. doi: 10.1016/j.metabol.2011.09.007

37. Freundlich M, Quiroz Y, Zhang Z, Zhang Y, Bravo Y, Weisinger J, et al. Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. *Kidney Int.* (2008) 74:1394–402. doi: 10.1038/ki.2008.408

38. Takemoto F, Shinki T, Yokoyama K, Inokami T, Hara S, Yamada A, et al. Gene expression of vitamin D hydroxylase and megalin in the remnant kidney of nephrectomized rats. *Kidney Int.* (2003) 64:414–20. doi: 10.1046/j.1523-1755.2003.00114.x

39. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Rheum Dis Clin North Am.* (2012) 38:125–39. doi: 10.1016/j.rdc.2012.03.012

40. Chen S, Sims G, Chen X, Gu Y, Chen S, Lipsky P. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol.* (2007) 179:1634–47. doi: 10.4049/jimmunol.179.3.1634

41. Brown A, Coyne D. Bioavailable vitamin D in chronic kidney disease. *Kidney Int.* (2012) 82:5–7. doi: 10.1038/ki.2012.135

42. Goldsmith D. Pro: should we correct vitamin D deficiency/insufficiency in chronic kidney disease patients with inactive forms of vitamin D or just treat them with active vitamin D forms? *Nephrol Dial Transplant.* (2016) 31:698–705. doi: 10.1093/ndt/gfw082

43. Klingberg E, Olerod G, Konar J, Petzold M, Hammarsten O. Seasonal variations in serum 25-hydroxy vitamin D levels in a Swedish cohort. *Endocrine.* (2015) 49:800–8. doi: 10.1007/s12020-015-0548-3

44. Cano A, Neil A, Kang J, Barnabas A, Eastwood J, Nelson S, et al. Gastrointestinal symptoms in patients with end-stage renal disease undergoing treatment by hemodialysis or peritoneal dialysis. *Am J Gastroenterol.* (2007) 102:1990–7. doi: 10.1111/j.1572-0241.2007.01321.x

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 17 February 2023
DOI 10.3389/fmed.2023.1117819

OPEN ACCESS

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

RECEIVED 06 December 2022
ACCEPTED 06 February 2023
PUBLISHED 17 February 2023

CITATION
Graňák K, Vnučák M, Beliančinová M, Kleinová P, Pytliaková M, Mokáň M and Dedinská I (2023) Adiponectin/leptin ratio as a predictor of acute rejection in early post-transplant period in patients after kidney transplantation.
Front. Med. 10:1117819.
doi: 10.3389/fmed.2023.1117819

Adiponectin/leptin ratio as a predictor of acute rejection in early post-transplant period in patients after kidney transplantation

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Introduction: Adipokines are largely involved in the regulation of immune system activity. While leptin is the main pro-inflammatory marker of adipose tissue, adiponectin is characterized by anti-inflammatory effects. The aim of our study was to determine the risk of acute graft rejection in protocol biopsy depending on the adiponectin/leptin (A/L) ratio in patients after kidney transplantation (KT).

Materials and methods: A total of 104 patients were included in the prospective analysis, in whom the levels of adipokines were examined pre-transplant, in the 3rd month after KT and the A/L ratio was calculated. In the 3rd month after KT, all patients underwent protocol biopsy of the graft and examination of donor-specific antibodies (DSA) using the Luminex method.

Results: After adjusting for differences in the basic characteristics of the donor and recipient, we identified a subgroup with A/L ratio < 0.5 pre-transplant [HR 1.6126, ($P = 0.0133$)] and 3 months after KT [HR 1.3150, ($P = 0.0172$)] as independent risk factor for acute graft rejection. In the subsequent specification of the rejection episode, we identified the risk ratio A/L < 0.5 before KT [HR 2.2353, ($P = 0.0357$)] and 3 months after KT [HR 3.0954, ($P = 0.0237$)] as independent risk factor for the development of acute humoral rejection with DSA positivity.

Conclusion: This is the first study to investigate the relationship between A/L ratio and immunological risk in terms of the development of rejection changes in patients after KT. In our study, we found that A/L ratio < 0.5 is an independent risk factor for the development of acute humoral rejection and *de novo* DSA production in the third month after KT.

KEYWORDS

adiponectin/leptin ratio, kidney transplantation, acute rejection, antibody-mediated rejection, adipose tissue hormones

Introduction

It is well known today that adipose tissue is involved in the production and secretion of a wide range of bioactive peptides, known as adipokines, which have a local (paracrine) but also a systemic (endocrine) effect. In addition to these efferent signals, adipose tissue receives signals from hormonal systems or the central nervous system through many receptors. Thanks to this interactive network, adipose tissue is directly involved in the coordination of biological processes, including energy metabolism and immune functions (1). Most molecules, especially those secreted by the non-adipocyte fraction of adipose tissue, have a dominant paracrine effect. Leptin and adiponectin are today generally accepted as the only endocrine hormones of adipose tissue with a defined effect on target organs (2). Their presence in the human body correlates with the amount of adipose tissue. Adipokines can be classified in different ways, but from the point of view of their impact on the immune system, we divide them into two groups - pro-inflammatory and anti-inflammatory (3).

Leptin is considered a major pro-inflammatory marker and shares structural homology with the interleukin (IL)-6 receptor. It participates in the activation and proliferation of granulocytes, monocytes, macrophages, dendritic cells, natural killer cells and leads to increased production of pro-inflammatory cytokines (IL-6, IL-12, tumor necrosis factor—TNF) (4). Leptin is directly involved in the regulation of activation and differentiation of T and B cells. It supports the proliferation of naïve and memory T cells and increases the secretion of Th1 and Th17 lymphocytes. Mechanism studies have shown that leptin activates the mTOR pathway, thereby having a positive effect on CD4 + CD25 + FOXP3 + effector T cells. In addition, it stimulates the formation, maturation and survival of thymic T cells by reducing their apoptosis (5). Its important role has also been demonstrated in the regulation of the development and function of B cells. Since the receptor for leptin is expressed on B cells, its direct effect is assumed. Deficiency of leptin signaling led to a reduction of B cells in the bone marrow and peripheral blood, and its reduced level was associated with a lower representation of pro-B and immature B cells in the bone marrow (6).

Adiponectin, as the main representative of the group of anti-inflammatory markers of adipose tissue, acts through two receptors, AdipoR1 and AdipoR2, which, among many other tissues, are also found on most cells of the immune system (7). Functionally, adiponectin reduces the ability of macrophages to phagocytose and secrete pro-inflammatory cytokines, while increasing the production of anti-inflammatory IL-10 (3). In endothelial cells, it blocks the expression of adhesive molecules, which results in a reduction of the diapedesis of circulating monocytes (4). Several studies have shown that adiponectin is a negative regulator of T cell activity. It has been shown to inhibit proliferation and cytokine production of T cells and promote their apoptosis. Recent data also suggest that it is involved in the inhibition of Th1 and Th17 lymphocyte differentiation (8). Even though the immunomodulatory effect of adiponectin on B lymphocytes is not completely clear, it has been shown to inhibit B lymphopoiesis in long-term bone marrow cultures. In addition, adiponectin stimulates B cells to secrete the peptide PEPITEM, which specifically inhibits the migration of CD4+ and CD8+ memory T cells (9).

Recent studies suggest that patients with adipose tissue dysfunction, characterized by lower adiponectin secretion compared to leptin levels, have an increased cardiometabolic risk. This fact results from an increase in systemic inflammation and oxidative stress, the occurrence of which is negatively correlated with the A/L ratio (10). A/L ratio can be a practical marker characterizing adipose tissue dysfunction. From the results on the general population, it follows that an A/L ratio >1 can be considered normal, an A/L ratio of 0.5–1 indicates a moderate risk and an A/L ratio <0.5 a high risk (11). Due to the significant involvement of adipokines in the regulation of immune system processes, we assume that the A/L ratio could be correlated with the occurrence of immune-related reactions in the transplanted population. If confirmed, it would be possible to identify recipients at increased risk of developing acute graft rejection. The aim of our study was to determine the risk of acute graft rejection in protocol biopsy depending on the A/L ratio in patients after KT.

Materials and methods

Adult patients who underwent primary KT at the Martin Transplantation Center in 2018–2019 were included in our prospective monocenter study. Patients with a history of diabetes mellitus (DM) type I or II, those who died during the study period, patients who suffered from infectious complications and those who did not undergo a protocol graft biopsy (poor anatomical conditions, recurrent infections) were excluded from the follow-up. A total of 104 patients completed our prospective follow-up.

All patients in the studied sample were set on the same immunosuppressive protocol. As part of the induction protocol, they were administered antithymocyte globulin in a cumulative dose of 3.5 mg/kg of body weight, which was divided into three doses (pre-transplantation, day 1 and day 2 after KT). A triple combination was used in the prophylactic immunosuppressive regimen: tacrolimus, mycophenolic acid and corticosteroids. Methylprednisolone was applied at a dose of 500 mg intravenously pretransplantation and on the first posttransplantation day followed by a change to oral prednisone (prednisone 20 mg until the second week after KT, prednisone 15 mg until the fourth week after KT, prednisone 10 mg until 16 weeks after KT and prednisone 7.5 mg up to 12 months after KT). Mycophenolic acid was used in a total daily dose of 1,440 mg until the first month after KT, in a daily dose of 1,080 mg until the third month after KT, and then continued with a daily dose of 720 mg.

We examined the basic level of leptin, adiponectin, IL-6, and IL-10 in KT recipients at the time of flow cytometry crossmatch

Abbreviations: A/L, adiponectin/leptin; ACR, acute cellular rejection; AMR, antibodies mediated rejection; ATN, acute tubular necrosis; BMI, body mass index; CIT, cold ischemia time; CKD-EPI, chronic kidney disease – epidemiology collaboration index; DGF, delayed graft function; DM, diabetes mellitus; DSA, donor specific antibody; ECD, donor with expanded criteria; FCXM, flow cytometry crossmatch; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HLA, histocompatibility antigen; IFG, impaired fasting glucose; IFTA, interstitial fibrosis tubular atrophy; IGT, impaired glucose tolerance; IL, interleukin; IRI, immunoreactivity insulin; KT, kidney transplantation; LDL, low-density lipoprotein; M, month; MFI, mean fluorescence intensity; NF-κB, nuclear factor-κB; PRA, panel reactive antibody; PTDM, post-transplant diabetes mellitus; TAC, tacrolimus; TNF, tumor necrosis factor; Y, year.

(FXCM), i.e., approximately 4–5 h before the transplantation. In the post-transplantation period, we examined their levels at 3 months, i.e., at the time of the protocol biopsy of the graft. We used Human Leptin Quantikine ELISA Kit, Human Total Adiponectin ELISA Kit, LEGEND MAX Human IL-6 ELISA Kit and LEGEND MAX Human IL-10 Kit to investigate the levels of adipokines and interleukins. The A/L ratio was calculated from the measured values. We consider an A/L ratio above 1.0 to be normal, an A/L ratio of 0.5–1.0 indicates a moderate risk, and an A/L ratio <0.5 a high metabolic risk (12).

At the time of KT, we recorded: basic characteristics of the donor (donor with extended criteria, cold ischemia time) and characteristics of the recipient (age, sex, length of dialysis treatment, underlying cause of kidney failure, delayed onset of graft function, panel of reactive antibodies, number of mismatches in class A, B, DR, and DQ). At 3 months after KT, we also determined anthropometric parameters (waist circumference, body mass index—BMI), glucose metabolism parameters (c-peptide and immunoreactive insulin levels), lipid profile (total cholesterol, low-density lipoprotein—LDL, high-density lipoprotein—HDL, triglycerides), vitamin D, tacrolimus level and parameters reflecting graft function as glomerular filtration rate determined using the CKD-EPI (Chronic Kidney Disease—Epidemiology Collaboration Index) formula and quantitative proteinuria from 24-h urine collection.

Protocol biopsy of the graft and examination of DSA was performed during a short hospitalization at 3 months after KT in all patients included in our study. Biopsy was performed under ultrasonographic control using an 18-gauge puncture needle. All samples were histologically evaluated by the same pathologist. We then divided the studied sample according to the result of the histological examination (based on the Banff classification from 2019) into a group with a negative result, with findings of interstitial fibrosis and tubular atrophy (IFTA), acute tubular necrosis (ATN), acute cellular rejection (ACR) including borderline changes and antibody-mediated rejection (AMR) with DSA positivity. The examination of DSA was carried out using the LUMINEX methodology, when a value of ≥500 mean fluorescence intensity (MFI) was considered a positive result.

In our study, we used a certified statistical program, MedCalc version 13.1.2. (MedCalc Software VAT registration number BE 0809 344,640, Member of International Association of Statistical Computing, Ostend, Belgium). Using parametric (Student's *t*-test) or non-parametric tests we compared continuous variables between groups; the χ^2 test and Fisher's exact test were used to analyze associations between categorical variables, as appropriate. For non-parametric tests, we used the Wilcoxon test in the first step (Table 1) to compare the group of patients before KT and 3 months after KT. In subsequent analysis (Tables 2, 3), we used the Mann–Whitney test to compare independent groups according to A/L ratio. To perform multivariate analysis, we used Cox regression Hazard model. A *P*-value <0.05 was considered to be statistically significant.

Results

A total of 170 patients after primary deceased donor KT were primarily included in the study. 66 patients were excluded from the follow-up meeting the exclusion criteria that we stated in the materials and methods section. Thus, 104 patients completed our prospective follow-up.

The serum level of tacrolimus was maintained during the monitored period in the range of 10–15 ng/L during the first month after KT, then in the range of 8.0–10 ng/L until the third month after KT. We did not observe significant differences in the level of tacrolimus between the individual studied subgroups. Likewise, there was no significant difference in the daily dose of prednisone.

TABLE 1 Basic study file characteristics.

n = 104	Base line	3M	*P*-value
Men (%)	63.5 (*n* = 66)	–	–
Age at the time of KT (Y)	45 ± 11	–	–
BMI (kg/m^2)	26 ± 4	25.8 ± 4.2	0.7255
Waist circumference (cm)	–	94 ± 12.4	–
ECD (%)	25 (*n* = 26)	–	–
CIT (min)	745 ± 388	–	–
DGF (%)	10.5 (*n* = 11)	–	–
Time of dialysis treatment (M)	27 (median 15)	–	–
PRA (%)	1.6 ± 0.7	–	–
Mismatch A	1.3 ± 0.7	–	–
Mismatch B	1.4 ± 0.6	–	–
Mismatch DR	1.3 ± 0.6	–	–
Mismatch DQ	1.1 ± 0.8	–	–
Leptin (ng/ml)	44.8 ± 27.8	53.6 ± 29.2	0.0271
Adiponectin (μ g/ml)	18.8 ± 8.8	15.9 ± 9	0.0197
Adiponectin/leptin ratio	0.41 ± 0.3	0.3 ± 0.3	0.0088
IL-6 (pg/ml)	26.3 ± 11.4	34.2 ± 18	0.0002
IL-10 (pg/ml)	5.6 ± 3.2	7.2 ± 4	0.0017
Glycemia (mmol/L)	–	5.7 ± 1.6	–
PTDM (%)	–	23.1 (*n* = 24)	–
IFG (%)	–	7.7 (*n* = 8)	–
IGT (%)	–	30.8 (*n* = 32)	–
HbA1c (%)	–	3.7 ± 0.9	–
C–peptid (μ g/L)	–	4.2 ± 2	–
IRI (mU/L)	–	8.1 ± 3.6	–
Cholesterol (mmol/L)	–	5.4 ± 1.3	–
LDL (mmol/L)	–	3.1 ± 1.1	–
HDL (mmol/L)	–	1.4 ± 0.5	–
Triglycerides (mmol/L)	–	2.5 ± 1.5	–
TAC value (ng/L)	–	8.8 ± 3.2	–
eGFR CKD-EPI (ml/min)	–	55.2 ± 21.7	–

KT, kidney transplantation; BMI, body mass index; ECD, expanded criteria donor; CIT, cold ischemia time; DGF, delayed graft function; PRA, panel reactive antibodies; IL, interleukin; PTDM, posttransplant diabetes mellitus; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; HbA1c, glycated hemoglobin; IRI, immunoreactive insulin; LDL, low density lipoprotein; HDL, high density lipoprotein; TAC, tacrolimus; eGFR CKD EPI, estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration Index; Y, year; M, month.

TABLE 2 Comparison of the observed groups based on A/L ratio baseline.

n = 104	A/L ratio < 0.5 *n* = 45	A/L ratio 0.5-1*n* = 21	A/L ratio > 1*n* = 38	*P*-value*	*P*-value**	*P*-value***
Men (%)	57.8	38.1	84.2	0.1388	0.0003	0.0094
Age at the time of KT (Y)	45.7 ± 13.4	44.6 ± 8.8	44.7 ± 10.8	0.7331	0.9712	0.7126
BMI (kg/m^2)	27.9 ± 3.9	25.1 ± 3.7	25 ± 4.4	0.0075	0.9300	0.0021
ECD (%)	33.3	19	18.4	0.2354	0.9552	0.1276
CIT (min)	928 ± 380	757 ± 364	550 ± 420	0.0893	0.0629	<0.0001
DGF (%)	13.3	9.5	7.9	0.6616	0.8340	0.4330
Time of dialysis treatment (M)	31.5 ± 17	30.2 ± 14	19.3 ± 13.1	0.7613	0.0042	0.0005
PRA (%)	2 ± 1	1.5 ± 0.7	1.3 ± 0.4	0.0431	0.4866	0.0001
Mismatch	1.3 ± 0.7	1.5 ± 0.4	1.2 ± 0.7	0.2282	0.0766	0.5185
IL-6 base line (pg/ml)	31.7 ± 13	22.8 ± 8.1	24.4 ± 13.1	0.0054	0.6137	0.0139
IL-10 base line (pg/ml)	4.3 ± 3.9	5.8 ± 2.5	6.7 ± 3.2	0.1121	0.2703	0.0033

KT, kidney transplantation; BMI, body mass index; ECD, expanded criteria donor; CIT, cold ischemia time; DGF, delayed graft function; PRA, panel reactive antibodies; IL, interleukin; A/L, adiponectin/leptin; Y, year; M, month.
*A/L ratio < 0.5 vs. A/L ratio 0.5-1.
**A/L ratio 0.5-1 vs. A/L ratio > 1.
***A/L ratio < 0.5 vs. A/L ratio > 1.

TABLE 3 Comparison of the observed groups based on A/L ratio at 3 months after kidney transplantation.

n = 104	A/L ratio < 0.5 *n* = 49	A/L ratio 0.5-1*n* = 17	A/L ratio > 1*n* = 38	*P*-value*	*P*-value**	*P*-value***
Men (%)	57.1	35.3	84.2	0.1241	0.0006	0.0003
Age at the time of KT (Y)	46.2 ± 13.8	43.8 ± 9.6	45 ± 9.6	0.5103	0.6701	0.6489
BMI 3M (kg/m^2)	28.6 ± 3.7	24.3 ± 5.8	23.7 ± 2.8	0.0008	0.6051	<0.0001
Waist circumference 3M (cm)	101.3 ± 10.9	89.3 ± 16.8	91.4 ± 9.8	0.0013	0.5622	<0.0001
ECD (%)	34.7	11.8	18.4	0.0746	0.4959	0.5446
CIT (min)	874 ± 382	694 ± 352	667 ± 430	0.0945	0.8215	0.0199
DGF (%)	14.3	11.8	5.3	0.7974	0.4475	0.3964
Time of dialysis treatment (M)	30.1 ± 16.1	30.2 ± 14	20.7 ± 14	0.9819	0.0239	0.0054
PRA (%)	2 ± 1	1.5 ± 0.7	1.3 ± 0.4	0.0617	0.1843	0.0001
Mismatch	1.3 ± 0.6	1.5 ± 0.4	1.2 ± 0.7	0.2065	0.1058	0.4755
IL-6 3M (pg/ml)	42.6 ± 24	33 ± 14	27 ± 16	0.1249	0.1882	0.0009
IL-10 3M (pg/ml)	6.2 ± 3.8	7.5 ± 4.4	7.5 ± 3.8	0.2477	1.0000	0.1172
TAC value (ng/ml)	8.5 ± 3.6	9.2 ± 4	8.7 ± 2	0.5044	0.5375	0.7593
eGFR CKD-EPI 3M (ml/min)	51.3 ± 21.7	50.1 ± 22.9	64.2 ± 20.5	0.8470	0.0271	0.0060
ACR 3M (%)	12.2	23.5	7.9	0.2660	0.1649	0.1119
AMR 3M (%)	14.3	17.6	0	0.7456	0.0156	0.0084

KT, kidney transplantation; BMI, body mass index; ECD, expanded criteria donor; CIT, cold ischemia time; DGF, delayed graft function; PRA, panel reactive antibodies; IL, interleukin; TAC, tacrolimus; eGFR CKD-EPI, estimated glomerular filtration rate by Chronic Kidney Disease – Epidemiology Collaboration Index; ACR, acute cellular rejection; AMR, antibody mediated rejection; A/L, adiponectin/leptin, Y, year; M, month.
*A/L ratio < 0.5 vs. A/L ratio 0.5-1.
**A/L ratio 0.5-1 vs. A/L ratio > 1.
***A/L ratio < 0.5 vs. A/L ratio > 1.

Table 1 summarizes the basic characteristics of the investigated file. Of the total number of patients, 63.5% were men and the average age was 45 ± 11 years. When comparing the average serum levels of adipokines and interleukins at the beginning and in the third month of follow-up, we found that in the third month after KT there was a significant increase in inflammatory markers (leptin, IL-6, IL-10) and, conversely, a significant decrease in anti-inflammatory marker (adiponectin). The A/L ratio decreased significantly during this period (Table 1).

We primarily divided the studied group into three subgroups based on the A/L ratio before transplantation and in the third month after KT: 1. A/L ratio <0.5, 2. A/L ratio 0.5–1.0, 3. A/L ratio

> 1.0. We compared the subgroups among themselves according to parameters related to the eventual development of graft rejection. Table 2 shows the comparison before KT. We found that there were significantly more men in the subgroup with A/L ratio > 1, on the other hand, there was no difference in the age structure between the individual subgroups. In the subgroup with high metabolic risk (A/L < 0.5), we found a significantly higher BMI value compared to subgroup with A/L ratio > 1, but also with A/L ratio 0.5–1.0. In the mentioned subgroup, patients also had a significantly higher panel reactive antibodies (PRA) value. In the subgroup with a normal A/L ratio, patients showed significantly shorter cold ischemia time and shorter time spent in the dialysis program. The level of IL-6 was significantly higher in the high-risk subgroup (A/L < 0.5), and the level of IL-10, on the other hand, was significantly lower in this subgroup compared to the subgroup with A/L ratio > 1.0. We did not notice a difference in the occurrence of delayed onset of graft function (DGF) or in the representation of donors with extended criteria (Table 2).

Table 3 summarizes a comparison of these subgroups in the third month after KT. As before transplantation, there were significantly more men in the subgroup with A/L ratio > 1.0 in the third month after KT and the age structure also did not change. Patients with an A/L ratio < 0.5 had a significantly higher BMI value, but also a waist circumference value compared to other subgroups. In this high-risk subgroup, patients had a higher PRA value. Patients in the subgroup with A/L ratio > 1.0 spent a significantly shorter time in the hemodialysis program and, compared to the subgroup with A/L ratio < 0.5, had a significantly shorter time of cold ischemia. The level of IL-6 was also significantly higher in the subgroup with A/L ratio < 0.5, on the other hand, the level of IL-10 did not show any significant differences between the subgroups. Using the eGFR value, a significantly better graft function was detected at 3 months after KT in the subgroup with a normal A/L ratio compared to the other subgroups. At the same time, we identified a significantly lower incidence of AMR in this subgroup (A/L > 1.0) compared to the high-risk (A/L < 0.5) and medium-risk (A/L ratio 0.5 – 1.0) subgroups. There were no differences in the incidence of ACR (Table 3).

In the multivariate analysis, we used the Cox regression hazard model. After adjusting for differences in the basic characteristics of the donor and recipient, we identified the length of dialysis treatment more than 24 months as an independent risk factor for A/L ratio < 0.5 pre-transplant [HR 2.2727, (P = 0.0386)] and BMI value > 30 kg/m^2 as independent risk factor for A/L ratio < 0.5 in the third month after KT [HR 3.8235, (P = 0.0386)] (Tables 4, 5).

In the next step, we used the Cox regression Hazard model to determine independent risk factors for the occurrence of acute rejection in protocol graft biopsy. After adjusting for differences in the baseline characteristics of the donor and recipient, we identified a subgroup with an A/L ratio < 0.5 pretransplant [HR 1.6126, (P = 0.0133)] and 3 months after KT [HR 1.3150, (P = 0.0172)] as an independent risk factor for the occurrence of acute graft rejection (ACR + AMR) (Table 6). In the subsequent specification of the rejection episode, we identified the risk ratio A/L < 0.5 before transplantation [HR 2.2353, (P = 0.0357)] and 3 months after KT [HR 3.0954, (P = 0.0237)] as an independent risk factor for the development of AMR with DSA positivity (Table 7). On the contrary, the investigated A/L ratios were not detected as independent risk or protective factors for the development of ACR (Table 8).

We evaluated the development of the A/L ratio (according to defined subgroups) during the three months of follow-up in all groups according to the histological findings in the protocol biopsy of the graft. In the individual groups, we did not find significant change in the A/L ratio before transplantation and 3 months after KT (Figure 1).

Finally, we performed the ROC curve analysis for A/L ratio month 3 as a predictor for AMR 3 months after KT with sensitivity 100, specificity 65.2 and criterion ≤ 0.89 (Figure 2).

Discussion

To our knowledge, this is the first study that investigated the A/L ratio in patients after KT in the context of the risk of developing acute graft rejection. In our work, we found that the A/L ratio < 0.5 pre-transplantation and 3 months after KT represents an independent risk factor for the finding of acute graft rejection in protocol biopsy. At the same time, we specified that the risk ratio A/L < 0.5 was significantly correlated with the development of AMR in protocol biopsy with *de novo* DSA production. This finding was clearly supported by the result of the ROC curve analysis with AUR 0.898, which confirmed significantly larger probability of developing AMR in the group with high-risk A/L ratio.

The A/L ratio can be considered as an indicator of adipose tissue dysfunction and the balance between these adipokines may very likely play an important role in the clinical outcome in this group of patients. Monitoring of adipose tissue hormones in the transplant population has only a short history, and until now they have been investigated as separate variables in correlation with cardiometabolic risk factors. In our recently published work, we found that a high-risk A/L ratio (<0.5) was significantly associated with the occurrence of post-transplant diabetes mellitus (PTDM) and pre-diabetic conditions 1 year after KT (13).

As a pro-inflammatory marker, leptin largely alters the adaptive immune system by activating CD4+ T lymphocytes and negative signaling for CD25+ T regulatory cells (14). Joffre et al. in their work, they assume that the inactivity of regulatory T cells can lead to graft loss, as stimulated CD4 + CD25 + Foxp3 regulatory T

TABLE 4 Cox proportional hazard model, end point: A/L ratio < 0.5 baseline.

A/L < 0.5 base line	HR	95% CI	*P*
Men	1.6825	0.5708–4.9599	0.3455
Age at the time of KT ≥ 50 Y	4.7406	2.1386–10.5059	0.9647
BMI base line ≥ 30 kg/m^2	1.5001	0.2744–8.1897	0.6396
Time of dialysis treatment > 24 M	2.2727	1.0439–4.9481	0.0386
PRA > 1%	1.0645	0.3675–3.0838	0.9083
hyper IL-6 base line	2.3703	1.0693–4.6525	0.9666
hypo IL-10 base line	0.8902	0.1825–4.3414	0.8859

A/L, adiponectin/leptin; Y, year; M, month; PRA, panel reactive antibodies; IL, interleukin. Hyper IL-6: IL-6 value > 7.5 pg/ml; Hypo IL-10: IL-10 value < 10 pg/ml; Age limit: based on the average age of the group; Time of dialysis treatment limit: based on the average time of the group; PRA limit: based on the average PRA value of the group.

TABLE 5 Cox proportional hazard model, end point: A/L ratio < 0.5 at 3 months after kidney transplantation.

A/L < 0.5 M3	HR	95% CI	*P*
Men	0.9545	0.3243–2.8096	0.9326
Age at the time of KT ≥ 50 Y	1.5986	0.5474–4.6680	0.3909
BMI 3M ≥ 30 kg/m^2	3.8235	1.5255–9.5834	0.0042
Waist circumference (men ≥ 94 cm, women ≥ 80 cm)	1.4519	0.2174–9.6962	0.1481
ECD	0.9976	0.1399–7.1119	0.9981
CIT ≥ 12 hod	1.3577	0.4909–3.7548	0.5558
DGF	1.2552	0.2569–6.1325	0.7788
Time of dialysis treatment > 24 M	1.3090	0.4757–3.6020	0.6021
PRA > 1%	0.8728	0.2765–2.7552	0.8165
hyper IL- 6 M3	2.7447	0.3165–23.8046	0.3596
hypo IL-10 M3	0.8961	0.2422–3.3150	0.8694
eGFR CKD-EPI M3 < 60 ml/min	2.5623	0.4236–15.4993	0.3056

A/L, adiponectin/leptin; Y, year; M, month; PRA, panel reactive antibodies; IL, interleukin; ECD, expanded criteria donor; CIT, cold ischemia time; DGF, delayed graft function; eGFR CKD-EPI, estimated glomerular filtration rate by Chronic Kidney Disease – Epidemiology Collaboration Index.
Hyper IL-6: IL-6 value > 7.5 pg/ml; Hypo IL-10: IL-10 value < 10 pg/ml; Age limit: based on the average age of the group; Time of dialysis treatment limit: based on the average time of the group; PRA limit: based on the average PRA value of the group; CIT limit: based on the average CIT value of the group; eGFR limit: based on the average eGFR value of the group; Waist circumference limit: based on the IDF criteria (men ≥ 94 cm, women ≥ 80 cm).

TABLE 6 Cox proportional hazard model, end point: acute rejection in protocol graft biopsy.

Rejection (ACR + AMR)	HR	95% CI	*P*
Hyper IL-6 base line	0.1795	0.0187–1.7247	0.1368
Hypo IL-10 base line	1.6744	0.2095–13.3851	0.6269
Adiponectin/leptin ratio base line < 0.5	1.6126	1.3530–1.9798	0.0133
Adiponectin/leptin ratio base line 0.5–1	1.3893	0.3730–2.5743	0.6240
Adiponectin/leptin ratio base line > 1	1.1250	0.5455–2.3200	0.7499
Hyper IL-6 3M	0.2317	0.0224–2.3953	0.2198
Hypo IL-10 3M	1.7000	0.4252–6.7972	0.4530
Adiponectin/leptin ratio 3M < 0.5	1.3150	1.0169–1.7094	0.0172
Adiponectin/leptin ratio 3M > 1	1.3969	0.3751–5.2016	0.6183

ACR, acute cellular rejection; AMR, antibody-mediated rejection; IL, interleukin; M, month.
Hyper IL-6: IL-6 value > 7.5 pg/ml; Hypo IL-10: IL-10 value < 10 pg/ml.

TABLE 7 Cox proportional hazard model, end point: antibody-mediated rejection in protocol graft biopsy.

AMR	HR	95% CI	*P*
Hyper IL-6 base line	0.5386	0.0337–8.6109	0.6617
Hypo IL-10 base line	6.7241	4.8469–8.3160	0.9510
Adiponectin/Leptin ratio base line < 0.5	2.2353	1.4094–3.2031	0.0357
Adiponectin/Leptin ratio base line 0.5–1	0.8710	0.1593–4.7404	0.8689
Adiponectin/Leptin ratio base line > 1	0.9027	0.7939–1.6399	0.8570
Hyper IL-6 3M	0.4617	0.0289–7.3812	0.5847
Hypo IL-10 3M	0.2160	0.0252–1.8489	0.1618
Adiponectin/Leptin ratio 3M < 0.5	3.0954	1.9346–6.8478	0.0237
Adiponectin/Leptin ratio 3M 0.5–1	0.2724	0.0672–1.9113	0.9584

AMR, antibody-mediated rejection; IL, interleukin; M, month.
Hyper IL-6: IL-6 value > 7.5 pg/ml; Hypo IL-10: IL-10 value < 10 pg/ml.

TABLE 8 Cox proportional hazard model, end point: acute cellular rejection in protocol graft biopsy.

ACR	HR	95% CI	*P*
Hyper IL-6 base line	0.7547	0.6034–0.9703	0.9639
Hypo IL-10 base line	0.6280	0.0653–6.0421	0.6871
Adiponectin/Leptin ratio base line < 0.5	1.1172	0.1574–7.9314	0.9118
Adiponectin/Leptin ratio base line 0.5–1	1.7365	0.2446–2.3281	0.5810
Adiponectin/Leptin ratio base line > 1	0.9649	0.1169–2.5643	0.0863
Hyper IL-6 3M	7.167	8.3882–10.8280	0.9630
Hypo IL-10 3M	5.2431	3.1036–9.3148	0.9255
Adiponectin/Leptin ratio 3M < 0.5	0.8505	0.1198–6.0380	0.8714
Adiponectin/Leptin ratio 3M > 1	0.9089	0.3180–1.9207	0.9660

ACR, acute cellular rejection; IL, interleukin; M, month.
Hyper IL-6: IL-6 value > 7.5 pg/ml; Hypo IL-10: IL-10 value < 10 pg/ml.

cells prevent rejection of the transplanted organ (12). However, this claim has not yet been confirmed by clinical studies. In previous years, worse graft survival was detected in obese patients with hyperleptinemia. Authors Moraes-Vieira et al. they searched for a possible immunological basis in mouse models. An interesting finding was that obese mice that were leptin-deficient showed better survival of skin grafts, which indicated that the transplant outcome observed in obese patients may not be directly related to obesity but to hyperleptinemia. The authors therefore focused on the immunological background and found that CD4+ T cells differentiated more efficiently into T regulatory lymphocytes and showed a lower degree of proliferation *in vivo*, which was ultimately highly likely the cause of better graft survival in these mice (15). No association between leptin and systemic inflammation in patients after KT has been found in observational studies conducted so far (16). On the contrary, in a study on the general population, the conclusions of which were published in 2017 by Fruhbeck et al. a strong negative correlation was found between c-reactive protein level, serum amyloid A and A/L ratio. These findings may indicate that the A/L ratio reflects adipose tissue dysfunction-induced systemic inflammation (10). Work on the anti-inflammatory adiponectin suggests that it is involved in the activation of nuclear factor κB (NF-κB) transcription. NF-κB is a protein kinase that regulates the immune system through the activity of T cells and plays an important role in acute rejection of a vascularized organ or in the etiopathogenesis of several autoimmune diseases. The authors of Vu et al. evaluated the association between NF-κB gene polymorphisms and the outcome of the transplant itself in a sample of 607 Hispanics after KT. Recipients with the NF-κB1 polymorphism had significantly fewer biopsy-verified acute graft rejections (17). Alam et al. in 2013 investigated graft survival in 987 patients after KT based on the adiponectin level. However,

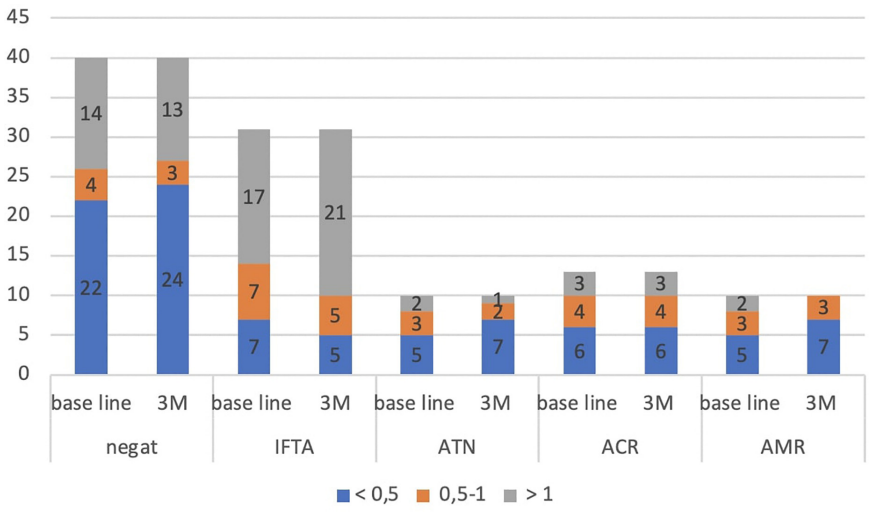

FIGURE 1
Development of the A/L ratio during 3 months of follow-up in all subgroups (according to histological findings in protocol graft biopsy). IFTA, interstitial fibrosis and tubular atrophy; ATN, acute tubular necrosis; ACR, acute cellular rejection; AMR, antibody-mediated rejection; M, month.

an increased level of the protective acting adiponectin was not associated with better graft survival (18).

Fonseca et al. in 2015 were the first to investigate the clinical significance of adipokines in the context of graft dysfunction in patients after KT and not in the context of cardiometabolic complications. In a sample of 40 adult patients who underwent KT, the relationship of leptin, adiponectin levels with DGF and acute rejection were evaluated. Serum levels of adipokines were measured before transplantation and subsequently in the first 7 days after KT. Leptin level was significantly higher in the group of patients who developed DGF compared to those who had prompt onset of graft function. Even serum leptin on the first day after KT predicted DGF slightly better than serum creatinine. Conversely, adiponectinemia was not significantly higher in graft dysfunction and was not a predictor of DGF. In the mentioned study, the authors also monitored the possible prediction of the development of acute graft rejection and the formation of anti-human leukocyte antigen (HLA) antibodies based on leptinemia, but no significant predictive value was found. A possible reason was also the minimal number of patients with acute rejection in the study (19). The importance of adiponectinemia in predicting the development of graft function after KT was presented in the study published by Roos et al. In 206 patients, they examined the level of total adiponectin and the high molecular weight multimer, as its main active form, before transplantation. At a 36-month follow-up, both forms were significantly associated with markers of endothelial dysfunction, arteriosclerosis, and at the same time significantly predicted graft survival. This inverse association between adiponectin and graft survival may be explained, at least in part, by its protective effects on endothelial cells and vascular inflammation (20).

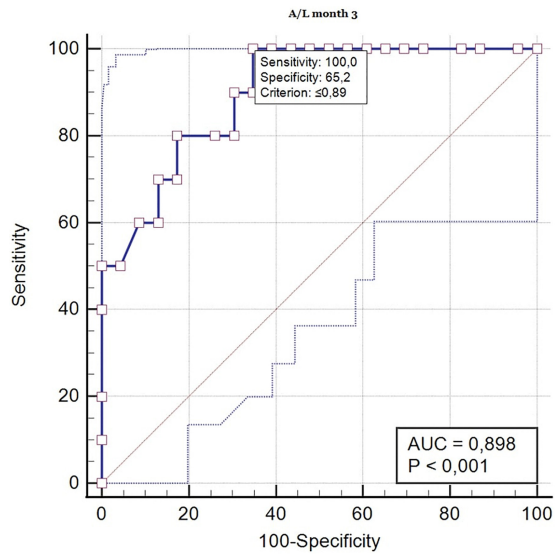

FIGURE 2
ROC curve analysis (A/L ratio in month 3 as a predictor for AMR 3 months after kidney transplantation). ROC, receiver operating characteristic; AUC, area under the curve.

A secondary finding in our study was that recipients who showed a pre-transplant high-risk A/L ratio < 0.5 spent a significantly longer time in a chronic hemodialysis program. The cause is probably chronic inflammation with oxidative stress, which are one of the basic determinants of cardiovascular morbidity and mortality in long-term dialysis patients (21). An unsurprising finding was a significantly higher representation of obese patients (BMI > 30 kg/m^2) in the group with A/L ratio < 0.5 three months after KT.

Based on our findings, we assume the importance of the level of adipokines in transplant patients, not only for the occurrence of already known cardiometabolic complications, but especially in the context of the development of rejection changes and DSA production. The results of our study indicate that the evaluation of the ratio of these hormones with the opposite effect has a greater clinical significance than monitoring them separately. In clinical practice, the monitoring of the A/L ratio can be an important

early predictor of risk groups of recipients for the development of rejection changes, DSA production and, probably, resulting worse function or survival of the graft. However, these claims will require further studies with longer follow-up and a larger sample of patients.

The limitation of this study is the low number of patients included in the individual monitored subgroups. On the other hand, this is the first study that deals with this issue and therefore we consider the sample size for this pilot project to be acceptable. Another limitation may be the absence of previous works on a transplanted sample of patients, so we designed our study based on the findings in the general population cohorts.

Conclusion

This is the first study to investigate the relationship between A/L ratio and immunological risk in terms of the development of rejection changes in patients after KT. In our study, we found that A/L ratio < 0.5 is an independent risk factor for the development of AMR and *de novo* DSA production in the third month after KT. Based on our findings, we attribute an importance to adipokines not only in the occurrence of metabolic, but especially immunological complications with a possible impact on the survival of grafts. A/L ratio can be an important early indicator of risk groups of patients undergoing KT. Further studies in a larger sample of patients will be needed to confirm our findings.

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

Informed consent for included participants was checked and approved by University Hospital's and Jessenius Faculty of Medicine's in Martin, Slovakia, Ethical Committees (EK 33/2018). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KG and ID participated in writing the manuscript, performing of the research, and data analysis. MV, MB, PK, and MM participated in data collection. MP participated in performing the research. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Project of Research Agency of Slovak Ministry of Education, Science and Sports (VEGA) 1/0238/21 and by Research and Development Project ITMS2014 +: 313011V344.

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. Kershaw E, Flier J. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab.* (2004) 89:2548–56.

2. Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat Rev Endocrinol.* (2019) 15:507–24.

3. Wensveen F, Valentiæ S, Šestan M, Wensveen T, Poliæ B. Interactions between adipose tissue and the immune system in health and malnutrition. *Semin Immunol.* (2015) 27:322–33. doi: 10.1016/j.smim.2015.10.006

4. Trzeciak-Ryczek A, Tokarz-Deptula B, Deptula W. Adipocytokines affecting the immune system – selected data. *Centr Eur J Immunol.* (2011) 36:92–4.

5. Song J, Deng T. The adipocyte and adaptive immunity. *Front. Immunol.* (2020) 11:593058. doi: 10.3389/fimmu.2020.593058

6. Fujita Y, Yanagida H, Mimori T, Jin Z, Sakai T, Kawanami T, et al. Prevention of fasting-mediated bone marrow atrophy by leptin administration. *Cell Immunol.* (2012) 273:52–8. doi: 10.1016/j.cellimm.2011.11.007

7. Barnes M, Carson M, Nair M. Non-traditional cytokines: how catecholamines and adipokines influence macrophages in immunity, metabolism and the central nervous system. *Cytokine.* (2015) 72:210–9. doi: 10.1016/j.cyto.2015.01.008

8. Zhang K, Guo Y, Ge Z, Zhang Z, Da Y, Li W, et al. Adiponectin suppresses T helper 17 cell differentiation and limits autoimmune CNS inflammation via the SIRT1 / PPARgamma / RORgammat pathway. *Mol Neurobiol.* (2017) 54:4908–20. doi: 10.1007/s12035-016-0036-7

9. Chimen M, McGettrick H, Apta B, Kuravi S, Yates C, Kennedy A, et al. Homeostatic regulation of T cell trafficking by a B cell-derived peptide is impaired in autoimmune and chronic inflammatory disease. *Nat Med.* (2015) 21:467–75. doi: 10.1038/nm.3842

10. Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J, et al. Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. *Sci Rep.* (2017) 7:6619. doi: 10.1038/s41598-017-06997-0

11. Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte.* (2018) 7:57–62. doi: 10.1080/21623945.2017.1402151

12. Joffre O, Santolaria T, Calise D, Al Saati T, Hudrisier D, Romagnoli P, et al. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. *Nat Med.* (2008) 14:88–92.

13. Graòák K, Vnuèák M, Belianèinová M, Kleinová P, Pytliaková M, Miklušica J, et al. Adiponectin/Leptin ratio as an index to determine metabolic risk in patients after kidney transplantation. *Medicina.* (2022) 58:1656. doi: 10.3390/medicina58111656

14. De Rosa V, Procaccini C, Calì G, Pirozzi G, Fontana S, Zappacosta S, et al. A key role of leptin in the control of regulatory T cell proliferation. *Immunity.* (2007) 26:241–55. doi: 10.1016/j.immuni.2007.01.011

15. Moraes-Vieira P, Bassi E, Larocca R, Castoldi A, Burghos M, Lepique A, et al. Leptin deficiency modulates allograft survival by favoring a Th2 and a regulatory immune profile. [corrected]. *Am J Transplant.* (2013) 13:36–44.

16. Nagy K, Nagaraju S, Rhee C, Mathe Z, Molnar M. Adipocytokines in renal transplant recipients. *Clin Kidney J.* (2016) 9:359–73.

17. Vu D, Tellez-Corrales E, Sakharkar P, Kissen M, Shah T, Hutchinson I, et al. Impact of NF-κB gene polymorphism on allograft outcome in Hispanic renal transplant recipients. *Transpl Immunol.* (2013) 28:18–23. doi: 10.1016/j.trim.2012.11.001

18. Alam A, Molnar M, Czira M, Rudas A, Ujszaszi A, Kalantar-Zadeh K, et al. Serum adiponectin levels and mortality after kidney transplantation. *Clin J Am Soc Nephrol.* (2013) 8:460–7.

19. Fonseca I, Oliveira J, Santos J, Malheiro J, Martins L, Almeida M, et al. Leptin and adiponectin during the first week after kidney transplantation: biomarkers of graft dysfunction? *Metabolism.* (2015) 64:202–7. doi: 10.1016/j.metabol.2014.10.003

20. Roos M, Baumann M, Liu D, Heinemann F, Lindemann M, Horn P, et al. Low pre-transplant adiponectin multimers are associated with adverse allograft outcomes in kidney transplant recipients a 3-year prospective study. *Regul Pept.* (2012) 178:11–5. doi: 10.1016/j.regpep.2012.06.001

21. Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. *Nephrol Dial Transplant.* (2018) 33(Suppl. 3):iii35–40.

frontiers | Frontiers in Medicine

TYPE Case Report
PUBLISHED 14 March 2023
DOI 10.3389/fmed.2023.1090659

OPEN ACCESS

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

RECEIVED 05 November 2022
ACCEPTED 24 February 2023
PUBLISHED 14 March 2023

CITATION
Zhang C, Qian C, Wang W, Chen Z, Lin Y and Sun M (2023) AIDS with obesity, hypothyroidism and elevated serum creatinine: A case report.
Front. Med. 10:1090659.
doi: 10.3389/fmed.2023.1090659

AIDS with obesity, hypothyroidism and elevated serum creatinine: A case report

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Hypothyroidism is a prevalent endocrine illness with a variety of clinical symptoms, but among which elevated serum creatinine is uncommon. Hypothyroidism is also common in acquired immunodeficiency syndrome (AIDS) patients, especially those receiving highly active antiretroviral treatment (HAART). Here we present a case of a young AIDS patient with hypothyroidism, increased serum creatinine, and obesity. Despite the lack of a kidney biopsy, following levothyroxine (LT4) therapy, his serum creatinine recovered to normal levels, and weight loss, edema, weakness, rough skin and other clinical symptoms obtained notable improvement. This highlights the need of clinicians paying attention to whether thyroid function is aberrant in human immunodeficiency virus (HIV) patients with increased creatinine, edema and significant weight gain since prompt thyroid hormone therapy can restore the alterations in renal function and avoid invasive renal biopsy.

KEYWORDS

hypothyroidism, elevated serum creatinine, obesity, levothyroxine, HIV, HAART

Introduction

Hypothyroidism is a prevalent systemic metabolic condition marked by inadequate thyroid hormones secretion, and common symptoms include weakness, weight gain, chills, constipation, edema and others, but renal involvement is rare (1), which affects the kidney by decreasing renin release, increasing vascular resistance, lowering renal plasma flow, and causing renal tubular dysfunction (2). Moreover, hypothyroidism is also common in HIV patients, especially those with HAART (3). Here we present a case of an AIDS patient with hypothyroidism, elevated serum creatinine and obesity who gradually recovered to energy, physical strength and normal renal function after receiving LT4 treatment.

Case report

A 30-year-old Chinese male was admitted to the hospital in June 2021 for weakness with edema of both lower limbs for 1 year and elevated serum creatinine for 1 week. 1 year before to admission, the patient began to experience intermittent edema in both lower limbs for no apparent reason. Because there were no noticeable abnormalities in the urine routine, renal function, or liver function throughout this time, no further examinations were conducted. 1 week ago, the patient noticed that his edema in both lower limbs had worsened, and his renal function revealed an increase in serum creatinine. Prior medical history comprised diagnosed

with hypothyroidism 10 months earlier but untreated, AIDS 8 months earlier and had been receiving HAART (Tenofovir disoproxil fumarate 300 mg, Lamivudine 300 mg and efavirenz 600 mg). The most recent $CD4^+$ T lymphocyte count was 414.1 /μL (normal range: 346.4–985.1 /μL), while HIV-RNA was negative.

The main clinical manifestations included weakness, fatigue, hypomnesis, slow movement, chills, thirst, poor appetite, drowsiness with greater snoring, and constipation. Physical examination showed dull complexion, lags in response, slow speech, rough skin, and edema in both lower limbs. His heart rate and blood pressure and body mass index (BMI) were 75 bpm, 135/86 mm/Hg and 33.3 kg/m^2 (body weight: 102 kg; body length: 1.75 m), respectively. Laboratory tests revealed that serum creatinine 1.43 mg/dL (normal: 0.64–1.10), proteinuria 0.00 g/24 h (normal: <0.15), creatine kinase (CK) 289 U/L (normal: 25–200 U/L), aspartate aminotransferase (AST) 83.1 U/L (normal range 15.0–40.0) and alanine aminotransferase (ALT) 64.8 U/L (normal range 9.0–50.0), serum albumin 47.0 g/L (normal: 40.0–55.0), and hemoglobin 108 g/L (normal: 130–175). The thyroid function test marked severe hypothyroidism: thyroid-stimulating hormone (TSH) 71.36 μIU/mL (normal values: 0.35–4.94), free triiodothyronine (FT3) < 1.64 pmol/L (normal: 2.43–6.01), free thyroxine (FT4) <5.15 pmol/L (normal: 9.01–19.05), anti-thyroglobulin antibodies (TG-Ab) >1000.00 IU/mL (normal: 0–4.11), anti-thyroid peroxidase antibodies (TPO-Ab) >1000.00 IU/mL (normal: 0–5.61). Additionally, routine urine examination, serological tests for antinuclear antibodies, anti-double-strand DNA antibodies, anti-neutrophil cytoplasmic antibodies and anti-phospholipase A2 receptor antibodies were all negative. Meanwhile, imageological examination detected left atrial and left ventricular enlargement, pericardial effusion, bilateral pleural effusion and diffuse ultrasonographic changes in thyroid parenchyma with a high possibility of hypothyroidism. The results of the abdominal color doppler ultrasonography were normal, and the size of the left kidney was 100*54 mm as well as the right kidney was 105*48 mm. We recommended a renal biopsy to determine the cause of the patient's illness, but he ultimately declined due to the associated risks and concerning about how much impact kidney biopsy made in therapy.

Combined with the patient's main symptoms and thyroid function tests, the diagnosis of hypothyroidism was confirmed. After admission, we administered LT4 50 μg orally once daily for 3 days, and then adjusted the dose to 75 μg. During the follow-up, the main abnormal laboratory parameters gradually improved (Table 1). 1 month later, the patient's body weight decreased to 85 kg (BMI 27.7 kg/m^2), edema was lightened, serum creatinine returned to normal (1.09 mg/dL). Then 2 months later, besides his response lags, slow speech, chills, weakness, fatigue, and roughness of the skin had significantly improved, edema disappeared and liver function normalized, he took on a completely new look compared to his admission. After 5 months of treatment, his weight dropped to 80 kg (BMI 26.1 kg/m^2); (Figure 1), hemoglobin returned to normal, and the LT4 dose increased from 75 μg to 125 μg. The follow-up conducted until now, his serum creatinine levels had been in the normal range and thyroid function tests were nearly normal. Meanwhile he was also in stable condition with AIDS with no opportunistic infections.

Discussion

For one thing, thyroid hormones influence kidney development and function in both direct and indirect ways, including influencing the size and structure of the fetal kidney, directly affecting renal blood flow (RBF), glomerular filtration rate (GFR), and renal tubular secretion and reabsorption function, and indirectly influencing kidney function by regulating systemic vascular resistance and cardiac output (4). Previous studies have shown that hypothyroidism can cause elevated CK and serum creatinine, proteinuria, even acute kidney injury (AKI), and the underlying mechanisms of associated renal injury include reduced myocardial contractility, increased peripheral vascular resistance, decreased cardiac output, decreased renal vasoconstriction and vasodilator synthesis, and structural pathological changes such as glomerular basement membrane thickening and mesangial matrix increasing, leading to decreased RBF and GFR (5–9). Pathological categories of hypothyroid-related renal damage shown by renal biopsy included membranous glomerulonephritis, focal segmental glomerulosclerosis, minimal change disease, membranoproliferative glomerulonephritis and so on (10).

In rare cases, severe hypothyroidism can induce rhabdomyolysis leading to AKI (7, 11, 12). But rhabdomyolysis is ruled out in our instance due to the absence of muscular discomfort and tenderness, a normal urine test, and, most critically, a CK value less than 5 times the upper limit of the normal range. In addition, the main renal manifestations of this patient were elevated serum creatinine without hematuria or proteinuria, color Doppler ultrasound revealed that both kidneys were normal in size, and serum creatinine declined to the normal range after 1 month, which was not consistent with typical nephritis or chronic kidney disease (CKD). It was surprising that this patient experienced reversible alterations in renal function following LT4 treatment. And Gou et al. also obtained comparable results to those indicated above (6, 7, 13). In a prospective severe hypothyroidism research, thyroid hormone replacement treatment could lower urine protein excretion while improving estimated GFR (14).

Hypothyroidism is also a risk factor for the occurrence and progression of CKD (8), increasing the hazard of mortality in the dialysis population (15). Although it is debatable whether subclinical hypothyroidism (SCH) necessitates thyroid hormone therapy, previous research has indicated that LT4 therapy helps protect renal function in CKD with SCH (16).

For another thing, HIV is a critical component that must not be overlooked. Hypothyroidism is common in the AIDS community, nevertheless, the pathophysiology is still unknown at present, which seems to be related to immune activation triggered by opportunistic infections (17). Ji et al. discovered that FT3 and FT4 levels were adversely connected to HIV duration and favorably related to CD4 cell counts (18). In addition, Patients on HAART had a higher prevalence of hypothyroidism and a lower level of FT4, with the likely cause being that HAART that HAART induced autoimmunity in the process of immunological reconstitution by suppressing HIV replication and increasing the number of CD4 positive memory and naive cells (3, 18). And TSH levels increased with the duration of HAART (19). This patient was in the asymptomatic stage of HIV infection with no other opportunistic infections. He had been on HAART and evaluated on a regular basis prior to admission. Even though the diagnosis of hypothyroidism came before the diagnosis of HIV and the initiation of HAART, the role of HIV and HAART in the development of hypothyroidism cannot be ruled out. As a result, clinicians should be on the high alert for thyroid dysfunction in AIDS patients with unexplained increased serum creatinine. We urge that HIV patients, particularly

TABLE 1 Main laboratory tests during admission and follow-up.

Time since levothyroxine					
laboratory tests	0month	1month	2months	5months	7months
creatinine (mg/dL)	1.43	1.09	0.93	0.73	0.73
proteinuria (g/24 h)	0.00	–	0.05	0.10	–
HGB (g/L)	108	110	120	135	141
AST (U/L)	83.1	41.7	25.5	23.6	27.7
ALT (U/L)	64.8	44.7	34.4	26.0	32.3
TSH (μIU/mL)	71.36	49.53	–	19.09	–
FT3 (pmol/L)	< 1.64	2.15	–	3.49	–
FT4 (pmol/L)	< 5.15	5.18	–	9.71	–
TPO-Ab (IU/mL)	>1000.00	–	–	–	–
TG-Ab (IU/mL)	>1000.00	–	–	–	–

Creatinine (0.64–1.10 mg/dL); proteinuria (<0.15 g/24 h); HGB, hemoglobin (130–175 g/L); AST, aspartate aminotransferase (15.0–40.0 U/L); ALT, alanine aminotransferase (9.0–50.0 U/L); TSH, thyroid-stimulating hormone (0.35–4.94μIU/mL); FT3, free triiodothyronine (2.43–6.01 pmol/L); FT4, free thyroxine (9.01–19.05 pmol/L); TPO-Ab, anti-thyroid peroxidase antibodies (0–4.11 IU/mL); TG-Ab, anti-thyroglobulin antibodies (0–5.61 IU/mL).

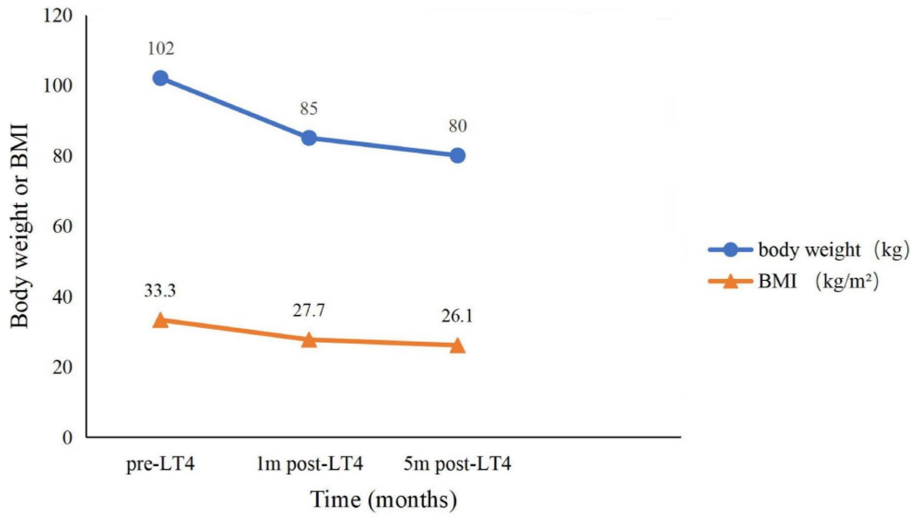

FIGURE 1
Trends in body weight and BMI over time. Pre-LT4, before levothyroxine treatment; 1m post-LT4, 1month after levothyroxine treatment; 5m post-LT4, 5months after levothyroxine treatment; BMI, body mass index.

those on HAART, have their thyroid function checked on a frequent basis.

Furthermore, the patient in question is an obese guy with a BMI of 33.3 kg/m^2. TSH levels that are consistently high may cause chronic low-grade inflammation, increasing leptin and inflammatory factor release, resulting in adipose tissue malfunction and weight gain (20). Obesity can affect thyroid function as well, the mechanisms of which are uncertain and may be linked to inflammatory factors reducing the iodine uptake activity of thyroid cells by inhibiting the mRNA expression of symporter sodium/iodide, regulating the expression of deiodinase and adipokines such as leptin inhibiting thyroglobulin expression to affect thyroid function according to researches (20, 21). As a result, we also counseled the patient to lose weight throughout LT4 treatment by food and lifestyle adjustments in order to attain a better prognosis, and the patient's weight steadily reduced during follow-up.

Conclusion

Hypothyroidism can cause serious weakness, increase in serum creatinine, and considerable weight gain. In severe situations, it can render patients unable to carry out everyday tasks and drastically degrade their quality of life, resulting in a devastating physiological and psychological blow to patients. We suggest that clinicians should be alert to abnormal thyroid function when they encounter elevated serum creatinine. Because if hypothyroidism is corrected, blood creatinine levels can return to normal in some individuals, and weight loss, energy, and physical strength can all be greatly improved, allowing patients to not only return to society but also avoid invasive kidney biopsy to achieve a good prognosis.

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

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

CZ, CQ, and YL gathered clinical data. CZ and CQ analyzed the data and wrote the manuscript. MS, WW, and ZC revised the content. All authors contributed to the article and approved the submitted version.

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.

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References

1. Chaker L, Razvi S, Bensenor IM, Azizi F, Pearce EN, Peeters RP. Hypothyroidism. *Nat Rev Dis Primers*. (2022) 8:1–17. doi: 10.1038/s41572-022-00357-7

2. Iglesias P, Bajo MA, Selgas R, Díez JJ. Thyroid dysfunction and kidney disease: an update. *Rev Endocr Metab Disord*. (2017) 18:131–44. doi: 10.1007/s11154-016-9395-7

3. Parsa AA, Bhangoo A. Hiv and thyroid dysfunction. *Rev Endocr Metab Disord*. (2013) 14:127–31. doi: 10.1007/s11154-013-9248-6

4. Mariani LH, Berns JS. The renal manifestations of thyroid disease. *J Am Soc Nephrol*. (2012) 23:22–6. doi: 10.1681/asn.2010070766

5. Weerakkody RM, Lokuliyana PN. Severe hypothyroidism presenting as reversible proteinuria: two case reports. *J Med Case Rep*. (2019) 13:270. doi: 10.1186/s13256-019-2216-3

6. Gou YL, Huang Y, Li JD, Chen H. Acute kidney injury induced by hypothyroidism: case reports and literature review. *Asian J Surg*. (2022) 45:2347–8. doi: 10.1016/j.asjsur.2022.05.040

7. Baghi MA, Sirajudeen J, Naushad VA, Alarbi KS, Benshaban N. Severe hypothyroidism-induced rhabdomyolysis: a case report. *Clin Case Rep*. (2021) 9:e05107. doi: 10.1002/ccr3.5107

8. Rhee CM. The interaction between thyroid and kidney disease: an overview of the evidence. *Curr Opin Endocrinol Diabetes Obes*. (2016) 23:407–15. doi: 10.1097/med.0000000000000275

9. Salomon MI, Di Scala V, Grishman E, Brener J, Churg J. Renal lesions in hypothyroidism: a study based on kidney biopsies. *Metab Clin Exp*. (1967) 16:846–52. doi: 10.1016/0026-0495(67)90186-2

10. Singh U, Rai V, Singh R, Santosh D, Parkash J, Singh RG, et al. Renal biopsy findings in patients with hypothyroidism: report of 16 cases. *J Clin Diagn Res*. (2016) 10:Ec27–9. doi: 10.7860/jcdr/2016/19362.8356

11. Alshamam MS, Gurung DO, Nso N, Saliaj M, Seitaj A. Rhabdomyolysis secondary to hypothyroidism: report of a case. *Cureus*. (2021) 13:e12746. doi: 10.7759/cureus.12746

12. Naz A, Issa M. Rhabdomyolysis and acute renal impairment in a patient with hypothyroidism: a case report. *Case Rep Med*. (2014) 2014:139170. doi: 10.1155/2014/139170

13. Vikrant S, Chander S, Kumar S, Gupta D. Hypothyroidism presenting as reversible renal impairment: an interesting case report. *Ren Fail*. (2013) 35:1292–4. doi: 10.3109/0886022x.2013.824381

14. Gondil VS, Chandrasekaran A, Rastogi A, Yadav AK, Sood A, Ramachandran R, et al. Proteinuria in severe hypothyroidism: a prospective study. *J Clin Endocrinol Metab*. (2021) 106:e749–56. doi: 10.1210/clinem/dgaa871

15. Rhee CM, Alexander EK, Bhan I, Brunelli SM. Hypothyroidism and mortality among dialysis patients. *Clin J Am Soc Nephrol*. (2013) 8:593–601. doi: 10.2215/cjn.06920712

16. Shin DH, Lee MJ, Kim SJ, Oh HJ, Kim HR, Han JH, et al. Preservation of renal function by thyroid hormone replacement therapy in chronic kidney disease patients with subclinical hypothyroidism. *J Clin Endocrinol Metab*. (2012) 97:2732–40. doi: 10.1210/jc.2012-1663

17. Micali C, Russotto Y, Celesia BM, Santoro L, Marino A, Pellicanò GF, et al. Thyroid diseases and thyroid asymptomatic dysfunction in people living with Hiv. *Infect Dis Rep*. (2022) 14:655–67. doi: 10.3390/idr14050071

18. Ji S, Jin C, Höxtermann S, Fuchs W, Xie T, Lu X, et al. Prevalence and influencing factors of thyroid dysfunction in Hiv-infected patients. *Biomed Res Int*. (2016) 2016:3874257. doi: 10.1155/2016/3874257

19. Madeddu G, Spanu A, Chessa F, Calia GM, Lovigu C, Solinas P, et al. Thyroid function in human immunodeficiency virus patients treated with highly active antiretroviral therapy (Haart): a longitudinal study. *Clin Endocrinol*. (2006) 64:375–83. doi: 10.1111/j.1365-2265.2006.02472.x

20. Walczak K, Sieminska L. Obesity and thyroid Axis. *Int J Environ Res Public Health*. (2021) 18:9434. doi: 10.3390/ijerph18189434

21. Song RH, Wang B, Yao QM, Li Q, Jia X, Zhang JA. The impact of obesity on thyroid autoimmunity and dysfunction: a systematic review and meta-analysis. *Front Immunol*. (2019) 10:2349. doi: 10.3389/fimmu.2019.02349

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TYPE Original Research
PUBLISHED 27 March 2023
DOI 10.3389/fendo.2023.1081543

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 27 October 2022
ACCEPTED 01 March 2023
PUBLISHED 27 March 2023

CITATION
Zhao Z, Yan Q, Li D, Li G, Cai J, Pan S, Duan J, Liu D and Liu Z (2023) Relationship between serum iPTH and peritonitis episodes in patients undergoing continuous ambulatory peritoneal dialysis. *Front. Endocrinol.* 14:1081543. doi: 10.3389/fendo.2023.1081543

Relationship between serum iPTH and peritonitis episodes in patients undergoing continuous ambulatory peritoneal dialysis

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Background: Peritonitis is considered as one of the most serious complications that cause hospitalization in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). There is limited evidence on the impact of the parathyroid hormone (PTH) on the first peritoneal dialysis (PD)-associated peritonitis episode. We aimed to investigate the influence of serum intact parathyroid hormone (iPTH) on peritonitis in patients undergoing PD.

Methods: This was a retrospective cohort study. Patients undergoing initial CAPD from a single center in China were enrolled. The baseline characteristics and clinical information were recorded. The primary outcome of interest was the occurrence of the first PD-associated peritonitis episode. Five Cox proportional hazard models were constructed in each group set. In group set 1, all participants were divided into three subgroups by tertiles of the serum concentration of iPTH; in group set 2, all participants were divided into three subgroups based on the serum concentration of iPTH with 150 pg/ml interval (<150, 150–300, and >300 pg/ml). Hazard ratios and 95% confidence intervals (CIs) were calculated for each model. The multivariate linear regression analysis elimination procedure assessed the association between the clinical characteristics at baseline and the iPTH levels. Restricted cubic spline models were constructed, and stratified analyses were also conducted.

Results: A total of 582 patients undergoing initial PD (40% women; mean age, 45.1 ± 11.5 years) from a single center in China were recruited. The median follow-up duration was 25.3 months. Multivariate Cox regression analysis showed that, in the fully adjusted model, a higher serum iPTH level (tertile 3, iPTH >300 pg/ml) was significantly associated with a higher risk of PD-associated peritonitis at 3 years [tertile 3: hazard ratio (HR) = 1.53, 95%CI = 1.03–2.55, p = 0.03; iPTH > 300 pg/ml: HR = 1.57, 95%CI = 1.08–2.27, p = 0.02]. The hazard

ratio for every 100 pg/ml increase in serum iPTH level was 1.12 (95%CI = 1.05–1.20, $p < 0.01$) in the total cohort when treating iPTH as a continuous variable.

Conclusions: An elevated iPTH level was significantly associated with an increased risk of peritonitis in patients undergoing CAPD.

KEYWORDS

continuous ambulatory peritoneal dialysis (CAPD), parathyroid hormone, peritonitis, end-stage renal disease (ESRD), hazard ratio (HR)

Introduction

The high incidence of end-stage renal disease (ESRD) has become one of the major health problems worldwide (1, 2). Studies have estimated that 1.9 million people in Asia die prematurely every year due to lack of dialysis services (3, 4). Peritoneal dialysis is one of the renal replacement therapy methods for patients with ESRD (5). The semi-permeable membrane could transport uremic toxins from the bloodstream to the peritoneal dialysate, which is perfused into the abdominal cavity and refreshed routinely (6). One of the most important factors affecting the application of peritoneal dialysis (PD) is PD-associated peritonitis, which is a major infectious complication in patients undergoing PD and is usually accompanied by cloudy peritoneal effusion and abdominal pain (7, 8). Although a Peritoneal Dialysis Outcomes and Practice Patterns Study (PDOPPS) survey showed that the incidence of peritonitis varied among institutions within the same country (9), it was strongly associated with the risk of hospitalization, encapsulating peritoneal sclerosis, technical failure, and even death (10, 11). Poor patient compliance and a non-standard operation, which lead to bacteria entering through the air from the interface of the PD tube, are the suspected causal factors. However, previous studies have shown that a low immune status, such as hypoalbuminemia and vitamin D deficiency, and a self-inflammatory state are also closely related to PD-associated peritonitis (12–14).

The parathyroid hormone (PTH), a single-chain peptide hormone secreted by the main cells of the parathyroid gland, regulates the calcium levels in the body (15). It is composed of 84 amino acids, and the coding gene is located on the broken arm of chromosome 11. By stimulating the PTH receptor (PTHR), PTH controls the inorganic calcium matrix in the bone and plays a crucial role in the homeostasis of Ca^{2+} and phosphorus (16, 17). Serum PTH abnormalities are common in ESRD, particularly in patients undergoing dialysis. Previous studies have indicated that maintaining serum calcium, phosphorus, and intact PTH (iPTH) in healthy levels is beneficial for patients undergoing dialysis (18). However, from stage 3 chronic kidney disease (CKD) onwards, the renal excretion of phosphorus decreases; subsequently, the blood concentration of phosphorus and the PTH level increase from stage 3 of CKD (19, 20). Insufficient levels of 1,25(OH)2 vitamin D further decrease the absorption of intestinal calcium, elevate the PTH level, and result in secondary hyperparathyroidism (SHPT) (21, 22). The most obvious biochemical alteration of SHPT is the elevation of PTH, which becomes increasingly aggravated as CKD progresses (23). However, previous studies have rarely considered the iPTH level, an important factor in trial completion or termination.

To date, only a handful of studies have investigated the association between PTH and mortality in patients undergoing PD. Therefore, the purpose of the present study was to investigate the possible link between the baseline serum concentration of iPTH and new-onset peritonitis after adjusting for a variety of crucial variables and providing scientific evidence for iPTH treatment strategy in patients undergoing continuous ambulatory peritoneal dialysis (CAPD).

Materials and methods

Study population

This was a retrospective cohort study in a single center. Patients undergoing initial CAPD in the Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, between January 2017 and October 2018 were enrolled in the present study. The exclusion criteria were as follows: 1) age less than 18 years; 2) comorbid malignancy; 3) comorbid severe underlying diseases such as primary respiratory or digestive system disease; and 4) peritonitis occurring within 3 months of dialysis initiation. All patients and caregivers received PD training at the hospital after catheter placement. The occurrence of peritonitis within 3 months was excluded to reduce bias due to improper handling at the commencement of dialysis. Accordingly, a total of 582 patients undergoing CAPD were finally recruited. This study was approved by the Research and Clinical Trial Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All data were fully anonymized and all patient information collection procedures complied with the principle of confidentiality.

Outcomes and measurements

The included patients were followed up for 3 years from the date of commencing CAPD. The primary outcome of interest was the occurrence of the first PD-associated peritonitis episode. At the

end of follow-up, or death, transfer to hemodialysis and renal transplantation without previous peritonitis were censored. According to the International Society for Peritoneal Dialysis (ISPD) guidelines, PD peritonitis was diagnosed when at least two of the following were present: have clinical features such as abdominal pain and/or cloudy effluent; 2) dialysis effluent (intra-abdominal stay of at least 2 h) white blood cell (WBC) count >100 cells/μl, with >50% polymorphonuclear leukocytes; and 3) positive dialysate culture or Gram stain.

The baseline characteristics and clinical information of the whole cohort were retrieved from the Hospital Information System of The First Affiliated Hospital of Zhengzhou University. Patient characteristics were recorded at the initiation of PD, which included information on sex, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), the primary cause of ESRD, body mass index (BMI), medication use, comorbidities, and smoking and drinking status. Medication use included erythropoietin (EPO), iron supplements, phosphorus-lowering drugs, uric acid-lowering drugs (e.g., allopurinol and febuxostat), PTH-regulating drugs (e.g., osteotriol, calcimimetics, and vitamin D analogs), and statins. The criteria for comorbidities were as follows: hypertension, diabetes mellitus (DM), cardiocerebral vascular disease (CVD) and edema confirmed by a physician with prescribing authority and documented medical history, and chronic hepatitis B confirmed by a documented medical history. The clinical parameters, which were measured at the laboratory of The First Affiliated Hospital of Zhengzhou University, included the following: WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil, and red blood cell (RBC) counts; hematocrit (Hct), hemoglobin (Hb), and platelet (PLT) counts; fasting blood glucose (FPG); $CD4^+$, $CD8^+$, and $CD3^+$ counts; serum phosphorus (P), corrected calcium (cCa^{2+}), potassium (K), sodium (Na), chloride (Cl), and magnesium (Mg) levels; carbon dioxide binding capacity (CO_2); serum Fe level; unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC); C-reactive protein (CRP); erythrocyte sedimentation rate (ESR); and the levels of uric acid (UA), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (Tbil), direct bilirubin (Dbil), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), albumin (ALB), iPTH, blood urea nitrogen (BUN), and serum creatinine (Scr). Information on PD-related characteristics included laparoscopic insertion of PD catheter (yes or no), daily ultrafiltration, 24-h urine volume, total weekly *Kt*/*V*, and residual renal function (RRF). The parameters obtained in the echocardiography included right ventricular end dimension (RVD), interventricular septal thickness at diastole (IVSD), left ventricular end dimension (LVD), diameter of the left atrium (DLA), and ejection fraction (EF). Routine 12-lead electrocardiogram (ECG) included corrected QT (QTc), heart rate (HR), P–R interval (P.R), and ST- and T-wave (ST.T) changes.

The patients were divided into tertiles according to the serum iPTH levels in this cohort: tertile 1, ≤189 pg/ml; 189 pg/ml < tertile 2 < 340 pg/ml; and tertile 3, ≥340 pg/ml. Tertile 2 was used as a reference due to the mortality risk being lowest for this category. Moreover, the categories iPTH < 150 pg/ml, 150 pg/ml ≤ iPTH ≤ 300 pg/ml, and iPTH > 300 pg/ml, with iPTH 150–300 pg/ml as the reference, were also analyzed according to the recommendation of 2016 ISPD and the CORES study (24, 25). The baseline serum iPTH levels were treated as a categorical variable in the outcome analysis.

Statistical analysis

All statistical analyses were performed using R software (version 4.1.2; R Project, www.r-project.org) and RStudio (version 1.4.1). Parameter values were presented as the mean ± standard deviation (SD) or median with interquartile range (IQR) for continuous variables, or as number (*n*) and percentage for categorical variables. A *p*-value <0.05 was considered as statistically significant.

The modeling time to the occurrence of the first episode of PD-associated peritonitis was performed using Kaplan–Meier survival analysis, and differences were analyzed using the log-rank test. The association between the baseline serum iPTH levels and the first PD-associated peritonitis episode was analyzed using Cox proportional hazards models, which were constructed using the baseline variables that were thought to be related to the outcomes chosen for five levels of confounding factor adjustments: model 1, adjusted for age, sex, BMI, and smoking and drinking status; model 2, adjusted for model 1 covariates plus comorbidities [hypertension, DM, CVD history, hepatitis B and edema, and medication use (e.g., EPO, iron supplements, phosphorus- and uric acid-lowering drugs, PTH-regulating drugs, and statins)]; model 3, adjusted for model 2 covariates plus the dialysis-related parameters including ultrafiltration volume, 24-h urine output, laparoscopy, *Kt*/*V*, and RRF; model 4, adjusted for model 3 covariates plus the laboratory variables including WBC, RBC, Hb, PLT, GLU, CD4, CD8, CD3, ESR, CPR, Na, Cl, Ca, P, Mg, CO_2, BUN, K, Fe, UIBC, TIBC, Scr, UA, ALB, ALT, AST, ALP, TP, Tbil, Dbil, TC, TG, HDL, and LDL; and model 5, adjusted for model 4 covariates plus RVD, IVSD, LVD, DLA, EF, QTc, HR, P.R, and ST.T changes. The results were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariate linear regression analysis with a stepwise elimination procedure assessed the associations between the clinical characteristics at baseline and iPTH. To strengthen the findings in order to examine the relationship between iPTH level and peritonitis, analyses of the continuous variables were conducted, with HRs expressed per 100-pg/ml and per 1-SD higher iPTH level. Two sensitivity analyses were also conducted. All of the patients in the cohort were dichotomized into men or women and with or without PTH-regulating drugs for repeated Cox regression analysis. Furthermore, we examined iPTH as a continuous predictor using restricted cubic spline models (26) based on the fully adjusted Cox proportional hazards model. In addition, we also conducted stratified analyses based on potentially relevant markers, including BUN, Scr, ALB, phosphorus, cCa^{2+}, and TIBC, and plotted forest plots using the "forestplot" package in R.

Results

Patient characteristics

As shown in Figure 1, a total of 582 patients undergoing CAPD were enrolled in the present study. Table 1 and Supplementary Table S1 detail the baseline demographic and clinical characteristics of all patients and each subgroup, along with the whole cohort. The mean age of the patients was 45.1 ± 11.5 years, with 40% women (n = 233), 12.2% with diabetes (n = 71), and with a median follow-up period of 25.3 months. For all patients, the mean serum iPTH level was 310.0pg/ml (range, 8.2–2760 pg/ml). A total of 346 participants (59.5%) were taking PTH-regulating drugs at baseline. Compared to patients with iPTH levels in the lowest tertile, those with higher serum iPTH levels were more likely to have higher BMI and be men, as well as have a higher prevalence of hypertension and edema. The baseline serum creatinine and phosphorus levels increased, while the cCa^{2+} level and RBC and Hb counts decreased in the higher tertiles. The other characteristics were not significantly different between the three tertiles. There were 126 patients who had PD-associated peritonitis during the follow-up period, including 90 that occurred within 1 year of initiating PD.

iPTH and the occurrence of the first PD-associated peritonitis episode

The Kaplan–Meier cumulative incidence curves are shown in Figure 2, and the survival curves with a risk table are plotted in Supplementary Figure S1. The lower and higher iPTH level groups showed a significantly increased risk of occurrence of first peritonitis episode at 3 years in both classification categories (log-rank test: p = 0.02 and p = 0.004, respectively), while the highest iPTH level group showed a significantly increased 1-year cumulative risk of occurrence of first peritonitis episode.

The associations of the tertiles and different iPTH level groups with the risk of first peritonitis episode by Cox regression analysis are shown in Tables 2, 3. Compared with iPTH tertile 2, iPTH tertile 3 was associated with a higher cumulative risk of peritonitis episode in the fully adjusted Cox model [model 5: adjusted HR (aHR) = 1.90, 95%CI = 1.02–3.53, p = 0.04, at 1 year; aHR = 1.48, 95%CI = 1.01–2.17, p = 0.05, at 3 years] (Table 2). In the multivariate fully adjusted model, the iPTH > 300 pg/ml group *versus* the 150 pg/ml < iPTH < 300 pg/ml group showed significant association with the risk of first peritonitis episode at 1 year (aHR = 2.35, 95%CI = 1.30–4.26, p < 0.01) and at 3 years (aHR = 1.56, 95%CI = 1.08–2.26, p = 0.02) (Table 3).

We used Pearson's correlation analysis to confirm that there was no significant correlation ($|r|$ > 0.6) between every two baseline clinical data variables, except for the combination of hypertension and diabetes (Supplementary Figure S2). Moreover, as shown in Table 4, the baseline iPTH level was positively correlated with PTH-regulating drug use, edema, and the ALB, ALP, Na, P, and TIBC levels and negatively correlated with age, smoking status, Cl, Ca, Mg, UA, AST, and Dbil levels, and ST.T changes in the multivariate linear regression analysis.

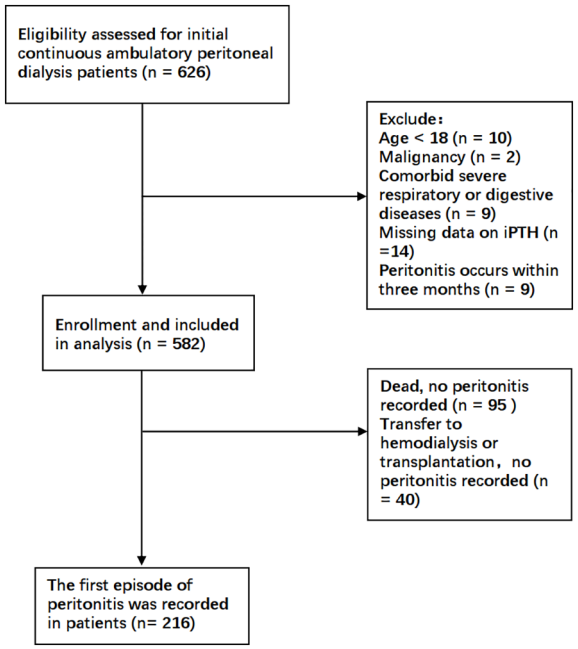

FIGURE 1
Flowchart for patient selection.

Sensitivity and stratified analysis

The association of the serum iPTH concentration with the first peritonitis episode, treating iPTH as a continuous variable, was examined using multivariate fully adjusted Cox models. As shown in Table 5, in the total cohort, the aHRs for every 100-pg/ml increase in iPTH level were 1.12 (95%CI = 1.05–1.20, p < 0.01, at 3 years) and 1.19 (95%CI = 1.10–1.29, p < 0.01, at 1 year), while those for every 1-SD increase were 1.34 (95%CI = 1.14–1.58, p < 0.01, at 3 years) and 1.56 (95%CI = 1.28–1.88, p < 0.01, at 1 year). With each 100-pg/ml and 1-SD increase in iPTH level, similar associations were found in men (aHR = 1.25, 95%CI = 1.13–1.39, p < 0.01, at 1 year; aHR = 1.22, 95%CI = 1.13–1.31, p < 0.01, at 3 years) and in patients taking PTH-regulating drugs (aHR = 1.29, 95%CI = 1.18–1.42, p < 0.01, at 1 year; aHR = 1.22, 95%CI = 1.13–1.31, p < 0.01, at 3 years).

Furthermore, these associations were validated in the restricted cubic spline model analyses. The results are summarized in Figure 3. In contrast to the peritonitis risk for lower iPTH levels not reaching statistical significance, an increase in the peritonitis risk was more distinguished in the higher iPTH levels with a narrow CI range. The results leaned toward a more J-shaped association between the levels of iPTH and the risk of first PD-related peritonitis episode after adjusting for multiple confounding factors in patients undergoing PD. The CI values in the cubic spline curves may indicate that an increase in the risk of peritonitis episodes at 1 and 3 years was more pronounced in the higher than about 600 and 400 pg/ml iPTH levels, respectively.

10.3389/fendo.2023.1081543

TABLE 1 Baseline demographic and clinical characteristic of the study population by serum intact parathyroid hormone (iPTH) tertile.

	Total (*N* = 582)	Tertile 1 (*N* = 194)	Tertile 2 (*N* = 194)	Tertile 3 (*N* = 194)	*p*-value
Sex, male	349 (60.0%)	109 (56.2%)	123 (63.4%)	117 (60.3%)	0.35
Age (years)	45.1 ± 11.5	46.4 ± 11.4	46.7 ± 11.1	42.1 ± 11.4	<0.01
SBP (mmHg)	140 ± 18.2	137 ± 18.6	140 ± 15.4	143 ± 19.9	0.01
DBP (mmHg)	87 ± 12.4	85.5 ± 12.2	86.8 ± 11.0	88.7 ± 13.7	0.04
Smoker, yes	47 (8.1%)	19 (9.8%)	14 (7.2%)	14 (7.2%)	0.56
Drinker, yes	32 (5.5%)	9 (4.6%)	13 (6.7%)	10 (5.2%)	0.65
BMI (kg/m^2)	23.4 ± 4.83	22.8 ± 4.81	23.2 ± 4.70	24.3 ± 4.87	<0.01
Comorbidities					
Hypertension, *n*(%)	507 (87.1%)	162 (83.5%)	179 (92.3%)	166 (85.6%)	0.03
Diabetes, *n*(%)	71 (12.2%)	29 (14.9%)	14 (7.2%)	28 (14.4%)	0.03
CVD, *n*(%)	97 (16.7%)	38 (19.6%)	27 (13.9%)	32 (16.5%)	0.32
Hepatitis B, *n*(%)	35 (6.0%)	11 (5.7%)	10 (5.2%)	14 (7.2%)	0.67
Edema, *n*(%)	278 (47.8%)	81 (41.8%)	91 (46.9%)	106 (54.6%)	0.04
Medication use					
EPO (%)	436 (74.9%)	142 (73.2%)	149 (76.8%)	145 (74.7%)	0.71
Iron supplements (%)	428 (73.5%)	138 (71.1%)	144 (74.2%)	146 (75.3%)	0.63
Phosphorus-lowering drugs (%)	374 (64.3%)	145 (74.7%)	115 (59.3%)	114 (58.8%)	<0.01
PTH-regulating drugs (%)	346 (59.5%)	95 (49.0%)	101 (52.1%)	150 (77.3%)	<0.01
UA-lowering drugs (%)	102 (17.5%)	42 (21.6%)	31 (16.0%)	29 (14.9%)	0.17
Statins (%)	56 (9.6%)	14 (7.2%)	25 (12.9%)	17 (8.8%)	0.15
Dialysis-related data					
Laparoscopy (%)	372 (63.9%)	113 (58.2%)	126 (64.9%)	133 (68.6%)	0.10
Ultrafiltration (ml/day)	400 (−1,500 to 2,500)	420 (−900 to 2,500)	400 (−800 to 2,000)	400 (−1,500 to 2,300)	0.86
24-h urine output (L)	200 (0–2,100)	200 (0–2,100)	200 (0–2,100)	200 (0–2,100)	0.71
Total weekly *Kt/V*	2.09 ± 0.31	2.11 ± 0.32	2.07 ± 0.29	2.09 ± 0.31	0.47
RRF (ml/min/1.73 m^2)	3.59 ± 1.57	3.5 ± 1.48	3.65 ± 1.68	3.62 ± 1.55	0.60
Laboratory variables					
WBC count ($\times10^9$/L)	6.14 ± 1.99	6.32 ± 2.32	6.12 ± 1.75	5.98 ± 1.84	0.25
RBC count ($\times10^{12}$/L)	3.33 ± 0.80	3.52 ± 0.82	3.25 ± 0.7	3.37 ± 0.78	<0.01
Hemoglobin (g/L)	102 ± 22.6	106 ± 22.7	101 ± 23.3	98.5 ± 21.1	<0.01
Platelet ($\times10^9$/L)	190 ± 69.3	195 ± 70.2	186 ± 71.3	189 ± 66.2	0.41
FPG (mmol/L)	4.94 ± 2.2	4.91 ± 2.07	4.93 ± 2.46	4.97 ± 2.04	0.96
Phosphorus (mmol/L)	1.76 ± 0.51	1.63 ± 0.49	1.82 ± 0.53	1.84 ± 0.47	<0.01
Corrected calcium (mmol/L)	2.15 ± 0.26	2.25 ± 0.28	2.1 ± 0.25	2.09 ± 0.23	<0.01
Potassium (mmol/L)	4.31 ± 0.74	4.30 ± 0.74	4.36 ± 0.79	4.28 ± 0.70	0.53
Serum Fe (μmol/L)	14.5 ± 6.68	14.2 ± 7.04	14.3 ± 5.9	14.9 ± 7.06	0.60
CRP (mg/L)	13.4 ± 31.4	14.5 ± 32.8	13 ± 31	12.7 ± 30.4	0.82
iPTH (pg/ml)	262 (8.20–2,760)	110 (8.20–189)	262 (189–339)	478 (340–2,760)	<0.01
BUN (mmol/L)	23.2 ± 8.6	21.9 ± 7.89	24.1 ± 9.63	23.7 ± 8.06	0.03

(Continued)

TABLE 1 Continued

	Total (*N* = 582)	Tertile 1 (*N* = 194)	Tertile 2 (*N* = 194)	Tertile 3 (*N* = 194)	*p*-value
Creatinine (mg/dl)	10.65 ± 4.32	836 ± 289	979 ± 478	999 ± 329	<0.01
Uric acid (mmol/L)	375 ± 95.9	368 ± 108	385 ± 94.8	372 ± 83.5	0.21
Albumin (g/L)	35.9 ± 6.25	34.8 ± 6.46	35.9 ± 6.16	36.9 ± 5.97	0.01
Cholesterol (mmol/L)	4.46 ± 1.74	4.56 ± 1.92	4.28 ± 1.04	4.54 ± 2.06	0.22
Triglycerides (mmol/L)	1.43 ± 0.84	1.44 ± 0.88	1.4 ± 0.79	1.44 ± 0.84	0.84
HDL (mmol/L)	1.13 ± 0.34	1.12 ± 0.32	1.11 ± 0.35	1.15 ± 0.36	0.52
LDL (mmol/L)	2.81 ± 0.92	2.85 ± 0.98	2.74 ± 0.86	2.84 ± 0.91	0.45
ECG and echocardiography					
EF (%)	61.1 ± 5.36	61.7 ± 4.57	61.4 ± 5.45	60.3 ± 5.92	0.03
QT interval (ms)	392 ± 57.8	393 ± 40.6	386 ± 72.9	396 ± 55.1	0.23
HR (bpm)	77.3 ± 12.4	76.4 ± 11.7	78.2 ± 13.1	77.5 ± 12.5	0.37
ST.T changes, yes (%)	210 (36.1%)	72 (37.1%)	63 (32.5%)	75 (38.7%)	0.42

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CVD, cardiocerebral vascular disease history; EPO, erythropoietin; UA, uric acid; RRF, residual renal function; WBC, white blood cell; RBC, red blood cell; FPG, fasting blood glucose; CRP, C-reactive protein; iPTH, intact parathyroid hormone; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; EF, ejection fraction; HR, heart rate.

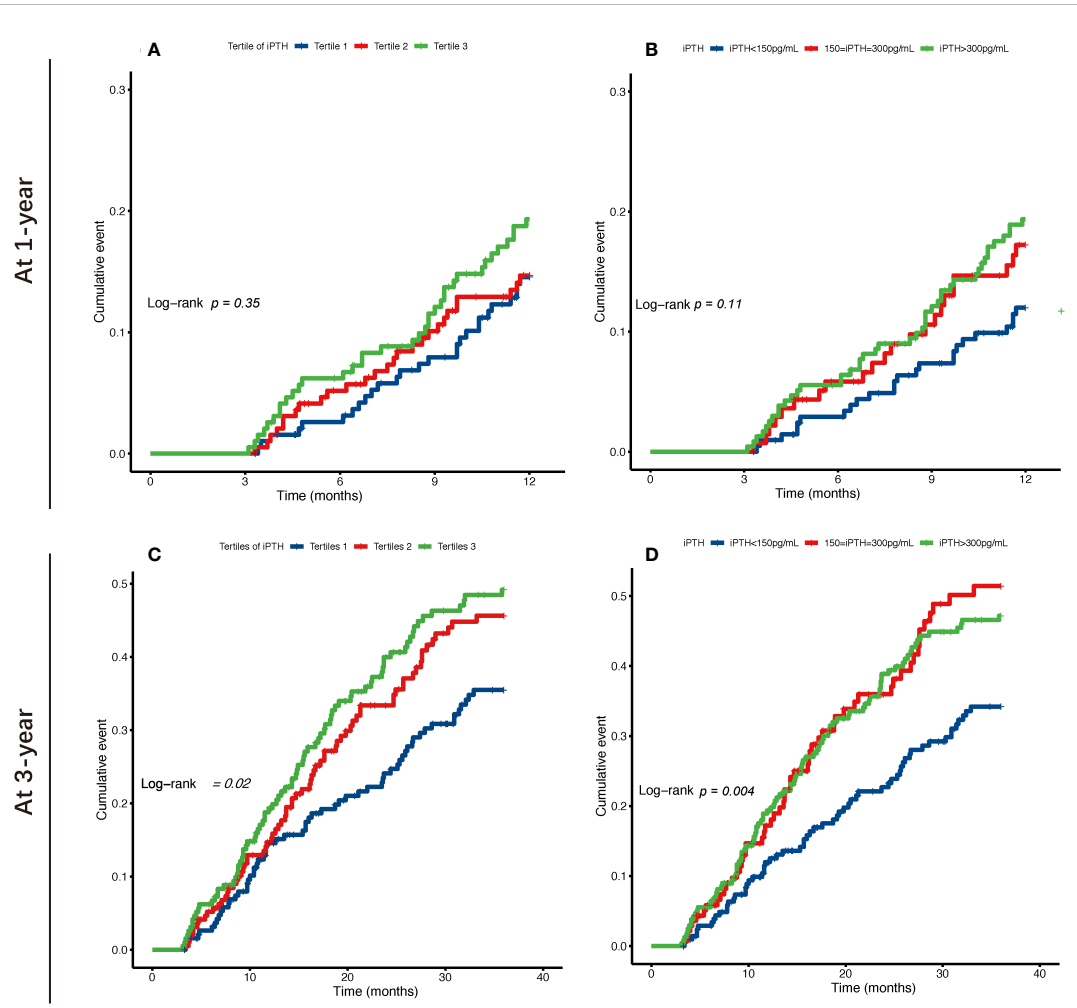

FIGURE 2
Kaplan–Meier survival curves of the risk of occurrence of first peritonitis episode at 1 and 3 years for tertiles **(A, C)** and categories by 150–300 pg/ml **(B, D)** of the serum intact parathyroid hormone (iPTH) levels in patients undergoing continuous ambulatory peritoneal dialysis.

10.3389/fendo.2023.1081543

TABLE 2 Cox regression models of the first peritonitis episode for serum intact parathyroid hormone (iPTH) level (tertiles as categories).

	iPTH tertiles	First peritonitis episode at 1 year		First peritonitis episode at 3 years	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Unadjusted	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	1.04 (0.61–1.77)	0.89	1.39 (0.99–1.96)	0.06
	Tertile 3	1.39 (0.84–2.29)	0.20	1.59 (1.14–2.21)	0.01
Model 1	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	1.03 (0.60–1.76)	0.92	1.40 (0.99–1.98)	0.05
	Tertile 3	1.29 (0.77–2.15)	0.33	1.56 (1.11–2.19)	0.01
Model 2	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	1.06 (0.61–1.82)	0.85	1.41 (0.99–2.00)	0.06
	Tertile 3	1.27 (0.75–2.14)	0.38	1.40 (0.99–2.00)	0.05
Model 3	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	1.04 (0.60–1.80)	0.89	1.39 (0.98–1.99)	0.07
	Tertile 3	1.30 (0.77–2.20)	0.33	1.44 (1.02–2.05)	0.04
Model 4	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	0.81 (0.44–1.48)	0.49	1.25 (0.85–1.85)	0.26
	Tertile 3	1.96 (1.07–3.56)	0.03	1.44 (0.98–2.10)	0.06
Model 5	Tertile 2	1 (as reference)		1 (as reference)	
	Tertile 1	0.80 (0.43–1.48)	0.48	1.30 (0.87–1.94)	0.21
	Tertile 3	1.94 (1.04–3.61)	0.04	1.53 (1.03–2.25)	0.03

Tertile 2 of iPTH was taken as the reference group. Model 1: adjusted for age, sex, BMI, and smoking and drinking status. Model 2: adjusted for model 1 covariates plus comorbidities (hypertension, DM, CVD history, hepatitis B and edema, and medication use (e.g., EPO, iron supplements, phosphorus- and uric acid-lowering drugs, PTH-regulating drugs, and statins). Model 3: adjusted for model 2 covariates plus ultrafiltration volume, 24-h urine output, laparoscopy, Kt/V, and RRF. Model 4: adjusted for model 3 covariates plus WBC, RBC, Hb, PLT, GLU, CD4, CD8, CD3, ESR, CPR, Na, Cl, cCa^{2+}, P, Mg, CO_2, BUN, K, Fe, UIBC, TIBC, Scr, UA, ALB, ALT, AST, ALP, TP, Tbil, Dbil, TC, TG, HDL, and LDL. Model 5: adjusted for model 4 covariates plus RVD, IVSD, LVD, DLA, EF, QTc, HR, P.R, and ST.T changes.
HR, hazard ratio; CI, confidence interval.

The results of the stratified analyses based on the BUN, Scr, phosphorus, cCa^{2+}, ALB, and TIBC levels are shown in Table 6 and Figure 4. The cohort was subdivided into two subgroups based on the median of these parameters. We found that Scr (<10.5 mg/dl) and cCa^{2+} (≤2.14 mmol/L) modified the association between iPTH and the risk of first peritonitis episode at 1 year (both $p < 0.01$ for interaction), while TIBC (≤40 μmol/L) modified the risk association at 3 years ($p < 0.02$ for interaction). The other *p*-values for subgroup interaction revealed non-significant results, implying that there were no significant interaction effects between iPTH and these clinical markers.

Discussion

In this retrospectively cohort study, we found that elevated serum iPTH levels were associated with a higher risk of the occurrence of first PD-associated peritonitis episode in patients undergoing initial CAPD, independent of multiple confounding factors such as demographic characteristics, comorbidities, and laboratory variables. Every 100-pg/ml elevation of the iPTH level at baseline was also significantly associated with a higher risk of the development of peritonitis at 1 and 3 years. The restricted cubic spline models further confirmed these results. A significant association remained particularly in men or after excluding the patients taking PTH-regulating agents.

The occurrence and consequences of PD-associated peritonitis, which is associated with a higher mortality risk and is the leading cause of technique failure among patients receiving PD, increased the cost of treatment and restricted the widespread utilization of PD (10). However, peritonitis is still a common and serious complication in patients undergoing PD (27, 28). A study involving multiple countries showed that the crude rate of peritonitis was 0.28 episode/patient-year; however, there is a low overall peritonitis cure rate, ranging between 54% and 68%, among all participating countries (29). Peritonitis frequently results in a decreased peritoneal ultrafiltration capacity and is the most common cause of transfer to long-term hemodialysis (30). Previous studies have identified common risk factors for peritonitis, such as exposure to dialysis fluid and catheters and touch contamination, and the preventive measures are mainly the use of prophylactic antibiotics before PD catheter insertion, daily

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TABLE 3 Cox regression models of the first peritonitis episode for serum intact parathyroid hormone (iPTH) level (150 and 300 pg/ml as categories).

	iPTH (pg/ml)	First peritonitis episode at 1 year		First peritonitis episode at 3 years	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Unadjusted	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.50 (0.84–2.67)	0.17	1.71 (1.19–2.46)	0.00
	iPTH > 300	1.70 (1.03–2.79)	0.04	1.60 (1.16–2.20)	0.00
Model 1	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.49 (0.83–2.66)	0.18	1.71 (1.19–2.47)	0.00
	iPTH > 300	1.58 (0.96–2.63)	0.07	1.54 (1.12–2.13)	0.01
Model 2	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.52 (0.84–2.74)	0.17	1.65 (1.13–2.41)	0.01
	iPTH > 300	1.55 (0.92–2.62)	0.10	1.39 (0.99–1.95)	0.01
Model 3	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.48 (0.81–2.70)	0.20	1.59 (1.09–2.33)	0.02
	iPTH > 300	1.59 (0.94–2.68)	0.09	1.41 (1.01–1.97)	0.05
Model 4	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.33 (0.68–2.61)	0.40	1.56 (1.03–2.38)	0.04
	iPTH > 300	2.51 (1.40–4.52)	0.00	1.54 (1.07–2.23)	0.02
Model 5	150 ≤ iPTH ≤ 300	1 (as reference)		1 (as reference)	
	iPTH < 150	1.26 (0.64–2.48)	0.50	1.57 (1.02–2.40)	0.04
	iPTH > 300	2.34 (1.29–4.24)	0.01	1.57 (1.08–2.27)	0.02

Tertile 2 of iPTH was taken as the as reference group. Model 1: adjusted for age, sex, BMI, and smoking and drinking status. Model 2: adjusted for model 1 covariates plus comorbidities (hypertension, DM, CVD history, hepatitis B and edema, and medication use (e.g., EPO, iron supplements, phosphorus- and uric acid-lowering drugs, PTH-regulating drugs, and statins). Model 3: adjusted for model 2 covariates plus ultrafiltration volume, 24-h urine output, laparoscopy, Kt/V, and RRF. Model 4: adjusted for model 3 covariates plus WBC, RBC, Hb, PLT, GLU, CD4, CD8, CD3, ESR, CPR, Na, Cl, cCa^{2+}, P, Mg, CO_2, BUN, K, Fe, UIBC, TIBC, Scr, UA, ALB, ALT, AST, ALP, TP, Tbil, Dbil, TC, TG, HDL, and LDL. Model 5: adjusted for model 4 covariates plus RVD, IVSD, LVD, DLA, EF, QTc, HR, P.R, and ST.T changes.
HR, hazard ratio; CI, confidence interval.

topical application of antibiotic cream to the catheter exit site, and training and nursing practice (31–35). Risk factors including demographic characteristics such as gender and age, comorbidities such as diabetes, and laboratory indicators such as albumin have also been pointed out and summarized in previous studies (36–39).

PTH consists of 84 amino acids that are secreted after it is cleaved from the preproparathyroid hormone (115 amino acids) to the proparathyroid hormone (90 amino acids). The active biological form is the intact PTH (1–84), which plays a major role in regulating calcium metabolism (40). In a physiological state, PTH generates a high bone turnover state and the release of calcium from the skeleton, upregulating the *CYP27B1* (1α-hydroxylase) gene and inhibiting phosphate reabsorption in the proximal tubules of the kidney, as well as converting the circulating form of vitamin D, 25-hydroxy vitamin D [25(OH)D], into the active form, 1,25(OH)2D3 (40, 41). In CKD, and especially in patients on dialysis, SHPT manifested by an elevated serum PTH is a particularly common complication (23). With the reduction in kidney function, there is a preferential increase in serum PTH, leading to the increased expression of fibroblast growth factor 23 (FGF-23). On the one hand, FGF-23, which has been shown to have intact activity in patients undergoing PD, can increase the expression and secretion of inflammatory factors (42, 43) and has been reported to activate local inflammation in organs *via* nuclear factor of activated T cells, FGF receptor 4 (FGFR4)/phospholipase C gamma (PLCγ), and other pathways (44), all of which increase its potential association with peritonitis. On the other hand, increased skeletal resistance to PTH results in osteodystrophy in CKD, and continued development of SHPT leads to hyperphosphatemia, vascular and organ calcification, and an increased risk of all-cause mortality (45–47). Interestingly, the latest research found that the all-cause mortality with low PTH is similar to that of patients with SHPT undergoing hemodialysis (17), that a low serum iPTH level is an independent predictor of infection-related mortality in incident dialysis patients (48), and that even combinations of low iPTH with other specific indicators are independently associated with increased all-cause and cardiovascular mortality in patients undergoing PD (49, 50). For dialysis patients, the phase changes in the levels of serum PTH, calcium, and phosphorus are more complex and are closely related to the emergence of CKD–mineral and bone disorder and adjustments in the treatment regimens (47, 51). Treatment with

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TABLE 4 Multivariate linear regression of the intact parathyroid hormone (iPTH) levels with clinical parameters.

	Estimate	SE	*t*	*p*-value
Age	−2.087	0.827	−2.524	0.012
Smoker (yes *vs.* no)	−71.970	34.306	−2.098	0.036
Use of PTH drug (yes *vs.* no)	67.072	19.325	3.471	0.001
Combined edema (yes *vs.* no)	39.220	18.793	2.087	0.037
Albumin	5.993	1.936	3.096	0.002
Alkaline phosphate	0.755	0.140	5.390	0.000
Serum Na	7.647	2.801	2.730	0.007
Serum Cl	−7.013	2.262	−3.101	0.002
cCa^{2+}	−277.438	40.655	−6.824	0.000
Serum phosphorus	58.024	20.245	2.866	0.004
Serum Mg	−162.446	59.497	−2.730	0.007
Uric acid	−0.211	0.104	−2.036	0.042
AST	−2.188	0.895	−2.444	0.015
Dbil	−13.304	4.772	−2.788	0.005
TIBC	3.282	1.296	2.532	0.012
ST.T changes (yes or no)	−47.030	19.487	−2.413	0.016

SE, standard error; cCa^{2+}, corrected calcium; AST, aspartate aminotransferase; Dbil, direct bilirubin; TIBC, total iron binding capacity.

oral PTH-lowering vitamin D or its analogs has been shown to protect against peritoneal remodeling on PD and even reduce the risk of peritonitis (12, 52). Sevelamer, which belongs to another class of drugs, may be beneficial in reducing the endotoxin levels in dialysis patients and in improving the endothelial function and inflammatory response in patients undergoing PD, despite studies showing that it is not associated with a higher risk of peritonitis (53, 54). However, there are still no definitive accepted explanations on whether increased or decreased PTH levels in patients undergoing PD increase the occurrence of peritonitis risk, and the underlying possible pathophysiological mechanisms are still unclear. Thus far, to the best of our knowledge, there are only a few existing cohort studies that attempted to understand the associations between serum PTH and peritonitis in patients undergoing PD. A cohort study that included 270 patients who had PD revealed that, after adjusting for limited confounders, the multivariate analysis showed

TABLE 5 First peritonitis episode for each 100-pg/ml and 1-SD increase in the serum intact parathyroid hormone (iPTH) level.

	Outcome	For each 100-pg/ml increase in iPTH level		For each 1-SD increase in iPTH level	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Total cohort (*n* = 582)	1 year	1.19 (1.10–1.29)	<0.01	1.56 (1.28–1.88)	<0.01
	3 years	1.12 (1.05–1.20)	<0.01	1.34 (1.14–1.58)	<0.01
Men (*n* = 349)	1 year	1.25 (1.13–1.39)	<0.01	1.76 (1.36–2.27)	<0.01
	3 years	1.22 (1.13–1.31)	<0.01	1.64 (1.36–1.98)	<0.01
Women (*n* = 233)	1 year	1.13 (0.90–1.43)	0.29	1.37 (0.76–2.44)	0.29
	3 years	1.07 (0.94–1.21)	0.30	1.18 (0.87–1.61)	0.30
Excluding taking PTH drug (*n* = 236)	1 year	1.40 (0.86–2.28)	0.17	2.33 (0.69–7.81)	0.17
	3 years	1.07 (0.89–1.28)	0.47	1.13 (0.72–1.78)	0.60
Patients taking PTH drug (*n* = 346)	1 year	1.29 (1.18–1.42)	<0.01	1.88 (1.50–2.37)	<0.01
	3 years	1.22 (1.13–1.31)	<0.01	1.64 (1.36–1.98)	<0.01

Adjusted for the model 5 covariates in Table 2.
SD, standard deviation; HR, hazard ratio; 95%CI, 95% confidence interval.

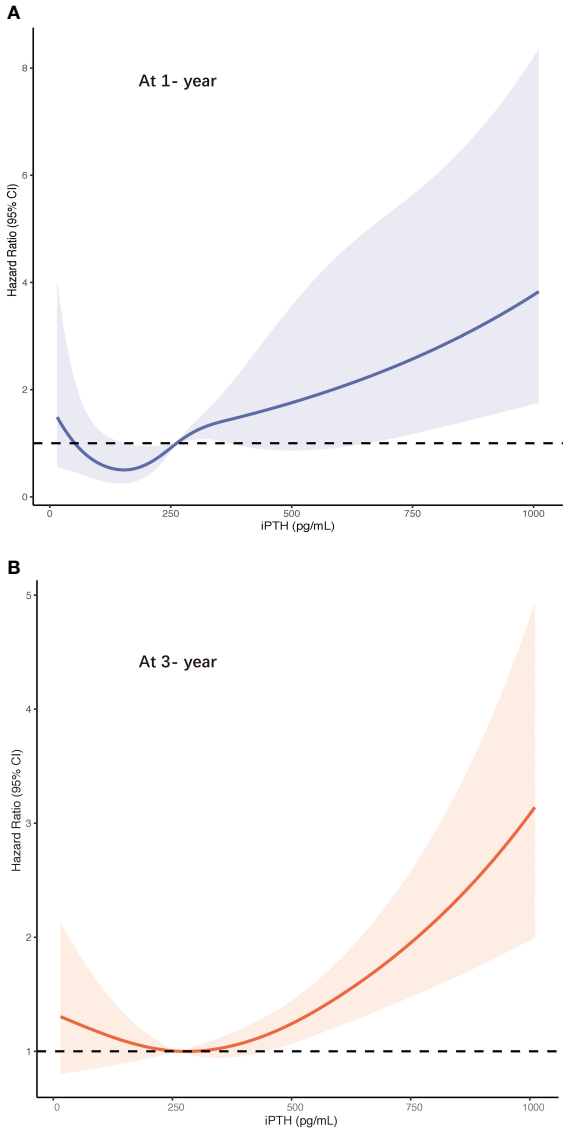

FIGURE 3
Risk of first peritonitis episode according to serum intact parathyroid hormone ((iPTH) levels with the fully adjusted (model 5) restricted cubic spline models at 1 year **(A)** and at 3 years **(B)**. *CI*, confidence interval.

that lower serum PTH levels (with 150 pg/ml as a category) were independently associated with peritonitis in incident PD patients (55). Another retrospective, observational study showned that the unioncombinations of low PTH levels with either high Ca levels or with low/normal P levels wereas a considerable risk factors of for the occurrence of first episode of peritonitis in patients with undergoing PD (56).

In this study, we found that there was no statistical significance in the association between the lower PTH (tertile 1 or <150 pg/ml) group and peritonitis episodes after multivariate adjustment, although the lower iPTH tertile (tertile 1) was associated with 3-year peritonitis risk in the Kaplan–Meier survival analysis. The sensitivity analysis further showed the J-shaped association between serum iPTH and risk of first episode of peritonitis. Determining the target range for iPTH control in patients on PD remains difficult, and the underlying mechanisms by which elevated PTH leads to an increased risk of peritonitis are still poorly understood. However, there are some possible explanations for this phenomenon. Firstly, excess PTH may disturb the ability of the beta cells to improve insulin secretion appropriately, causing insulin resistance through a calcium-dependent mechanism (57, 58). It has also been demonstrated that the pancreatic β-cell function is relatively more impaired in patients with severe hyperparathyroidism on hemodialysis (59). Alterations, including glucose disorders, cause a state of metabolic syndrome in the body, which can cause susceptibility to peritonitis (60–63). Secondly, hyperparathyroidism aggravates hematopoietic dysfunction, inhibits erythropoiesis, and increases erythrocyte osmotic vulnerability, while muscular toxic effects lead to dysfunction and increased energy expenditure (64, 65), which worsen the fragile nutritional status of patients undergoing PD. Thirdly, increased PTH levels can lead to myelofibrosis and cardiac fibrosis in ESRD and may mediate cardiovascular fibrosis and apoptosis through the TGF-β signaling pathway, resulting in a hyperinflammatory state (66–68). Last but not least, as mentioned above, some of the different drugs used to treat SHPT, such as calcimimetics and vitamin D analogs, may have potential interactions between PTH and peritonitis. Moreover, PTH itself is considered a uremic toxin, and previous studies have confirmed that chronic exposure to higher PTH levels is associated with reduced T-lymphocyte proliferation, impaired immunoglobulin production, and immune dysfunction, while the individual immune system is strongly associated with the development of peritonitis in patients on PD (69–71). Further research is required for a better understanding of the mechanisms involved in this association.

There are some limitations in the present study. Firstly, this was a single-center, retrospective study. Ideally, a prospective study should be conducted for the purpose of averting the analysis bias associated with retrospective studies. Secondly, despite extensive adjustments for confounding factors, some factors that may affect the occurrence of peritonitis have not been taken into account, such as seasonality, training, and socioeconomic status, among others (27, 72, 73). Lastly, the clinical data, including the serum PTH levels, were baseline values for one measurement only. The lack of data from multiple measurements may lead to missing dynamic change factors. It should also be ensured that unnecessary bias is avoided.

In conclusion, higher serum iPTH levels are associated with an increased risk of peritonitis episodes in patients treated with CAPD. According to our findings, the deleterious effects associated with PTH appear to transcend its beneficial effects. Our results provide helpful data regarding the control of the serum PTH levels in patients on CAPD. The underlying mechanisms remain unclear, and further prospective studies with larger sample sizes are needed to confirm this relationship.

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TABLE 6 Stratified analyses based on various clinical markers.

Parameter	iPTH tertiles	1-year cumulative peritonitis risk			3-year cumulative peritonitis risk		
		HR (95% CI)	*p*-value	*p* interaction	HR (95% CI)	*p*-value	*p* interaction
BUN ≤ 22.6	Tertile 1	0.76 (0.32–1.78)	0.52		1.32 (0.74–2.35)	*0.35*	
	Tertile 3	1.62 (0.60–4.37)	0.34	0.28	1.63 (0.92–2.89)	*0.10*	0.24
BUN > 22.6	Tertile 1	0.65 (0.14–3.11)	0.59		1.00 (0.48–2.08)	*0.99*	
	Tertile 3	7.20 (1.76–29.5)	0.01	0.24	2.54 (1.28–5.00)	*0.01*	0.35
Scr < 10.5	Tertile 1	0.96 (0.53–1.74)	0.88		2.33 (1.16–4.65)	*0.02*	
	Tertile 3	2.28 (1.21–4.29)	0.01	0.00	2.37 (1.09–5.17)	*0.03*	0.09
Scr ≥ 10.5	Tertile 1	0.37 (0.11–1.26)	0.11		0.82 (0.43–1.54)	*0.53*	
	Tertile 3	2.41 (0.91–6.42)	0.08	0.38	2.16 (1.21–3.84)	*0.01*	0.41
Phosphorus ≤ 1.68	Tertile 1	1.57 (0.59–4.17)	0.37		1.96 (1.06–3.60)	*0.03*	
	Tertile 3	5.71 (1.69–0.74)	0.01	0.74	2.02 (1.05–3.87)	*0.04*	0.46
Phosphorus > 1.68	Tertile 1	0.21 (0.05–0.85)	0.03		0.99 (0.49–1.99)	*0.98*	
	Tertile 3	2.91 (1.00–8.46)	0.05	0.99	3.03 (1.63–5.62)	*0.00*	0.65
cCa^{2+} ≤ 2.14	Tertile 1	0.77 (0.26–2.33)	0.65		1.94 (0.98–3.83)	*0.06*	
	Tertile 3	7.83 (2.18–28.13)	0.00	0.00	3.26 (1.64–6.48)	*0.00*	0.09
cCa^{2+} > 2.14	Tertile 1	1.32 (0.42–4.15)	0.63		1.09 (0.57–2.08)	*0.79*	
	Tertile 3	2.55 (0.75–8.68)	0.13	0.40	1.59 (0.82–3.08)	*0.17*	0.39
ALB ≤ 36	Tertile 1	1.28 (0.52–3.15)	0.60		1.74 (0.98–3.07)	*0.06*	
	Tertile 3	2.97 (1.01–8.73)	0.05	0.14	3.44 (1.88–6.26)	*0.00*	0.82
ALB > 36	Tertile 1	0.60 (0.16–2.25)	0.45		1.08 (0.52–2.25)	*0.83*	
	Tertile 3	0.63 (0.18–2.21)	0.47	0.71	0.96 (0.51–1.80)	*0.89*	0.29
TIBC < 40	Tertile 1	0.77 (0.27–2.23)	0.64		1.53 (0.81–2.92)	*0.19*	
	Tertile 3	2.41 (0.77–7.53)	0.13	0.12	2.03 (1.08–3.82)	*0.03*	0.08
TIBC ≥ 40	Tertile 1	1.73 (0.42–7.11)	0.45		1.77 (0.89–3.55)	*0.11*	
	Tertile 3	9.20 (2.14–39.65)	0.00	0.24	3.10 (1.50–6.42)	*0.00*	0.02

Tertile 2 of iPTH was taken as the reference group.

HR, hazard ratio; 95% CI, 95% confidence interval; p interaction: p value for the interaction analysis; BUN, blood urea nitrogen; Scr, serum creatinine; cCa^{2+}, corrected calcium; ALB, albumin; TIBC, total iron binding capacity.

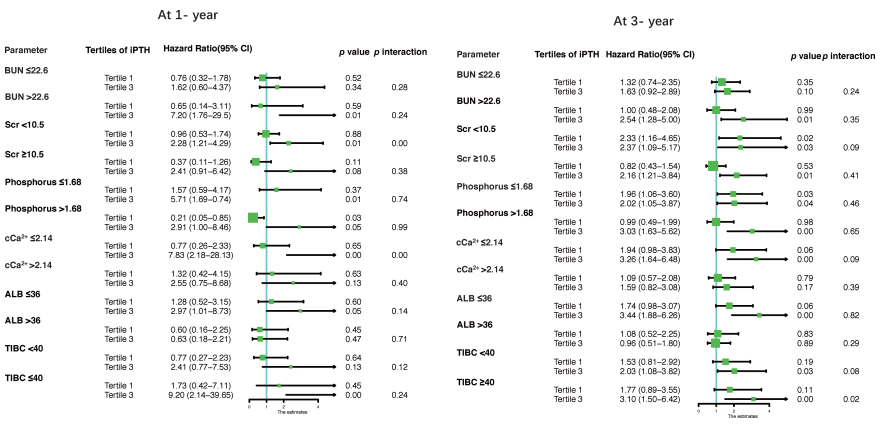

FIGURE 4
Forest plots for stratified analyses. *BUN*, blood urea nitrogen; *Scr*, serum creatinine; *cCa^{2+}*, corrected calcium; *ALB*, albumin; *TIBC*, total iron binding capacity.

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 conformed to the ethical standards of the Research and Clinical Trial Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZZ and ZL designed the study, ZZ, DuL, GL, JC and SP collected and analyzed the data, ZZ and QY wrote the manuscript. WDL, JD, and ZL reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (grant no. 82103916) and the General Program of the National Science Foundation of China General Project (no.81970633).

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/fendo.2023.1081543/full#supplementary-material

References

1. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: A systematic review. *Lancet (London England)* (2015) 385(9981):1975–82. doi: 10.1016/s0140-6736(14)61601-9

2. Johansen KL, Chertow GM, Gilbertson DT, Herzog CA, Ishani A, Israni AK, et al. Us renal data system 2021 annual data report: Epidemiology of kidney disease in the united states. *Am J Kidney Dis* (2022) 79(4 Suppl 1):A8–a12. doi: 10.1053/j.ajkd.2022.02.001

3. Thurlow JS, Joshi M, Yan G, Norris KC, Agodoa LY, Yuan CM, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement therapy. *Am J Nephrol* (2021) 52(2):98–107. doi: 10.1159/000514550

4. Saxena AB. Recent advances in the management of peritoneal dialysis patients. *F1000Prime Rep* (2015) 7:57. doi: 10.12703/p7-57

5. Maxwell MH, Rockney RE, Kleeman CR, Twiss MR. Peritoneal dialysis. 1. technique and applications. *J Am Med Assoc* (1959) 170(8):917–24. doi: 10.1001/jama.1959.03010080025004

6. Mehrotra R, Devuyst O, Davies SJ, Johnson DW. The current state of peritoneal dialysis. *J Am Soc Nephrol* (2016) 27(11):3238–52. doi: 10.1681/ASN.2016010112

7. Li PK, Chow KM, Cho Y, Fan S, Figueiredo AE, Harris T, et al. Ispd peritonitis guideline recommendations: 2022 update on prevention and treatment. *Peritoneal Dialysis Int* (2022) 42(2):110–53. doi: 10.1177/08968608221080586

8. Szeto CC, Li PK. Peritoneal dialysis-associated peritonitis. *Clin J Am Soc Nephrol CJASN* (2019) 14(7):1100–5. doi: 10.2215/cjn.14631218

9. Boudville N, Johnson DW, Zhao J, Bieber BA, Pisoni RL, Piraino B, et al. Regional variation in the treatment and prevention of peritoneal dialysis-related infections in the peritoneal dialysis outcomes and practice patterns study. *Nephrology dialysis Transplant* (2019) 34(12):2118–26. doi: 10.1093/ndt/gfy204

10. Boudville N, Kemp A, Clayton P, Lim W, Badve SV, Hawley CM, et al. Recent peritonitis associates with mortality among patients treated with peritoneal dialysis. *J Am Soc Nephrol* (2012) 23(8):1398–405. doi: 10.1681/asn.2011121135

11. Cho Y, Badve SV, Hawley CM, McDonald SP, Brown FG, Boudville N, et al. Peritoneal dialysis outcomes after temporary haemodialysis transfer for peritonitis. *Nephrology Dialysis Transplant* (2014) 29(10):1940–7. doi: 10.1093/ndt/gfu050

12. Rudnicki M, Kerschbaum J, Hausdorfer J, Mayer G, König P. Risk factors for peritoneal dialysis-associated peritonitis: The role of oral active vitamin d. *Peritoneal Dialysis Int* (2010) 30(5):541–8. doi: 10.3747/pdi.2009.00108

13. Sutherland TE, Shaw TN, Lennon R, Herrick SE, Rückerl D. Ongoing exposure to peritoneal dialysis fluid alters resident peritoneal macrophage phenotype and activation propensity. *Front Immunol* (2021) 12:715209. doi: 10.3389/fimmu.2021.715209

14. Chow KM, Szeto CC, Leung CB, Kwan BC, Law MC, Li PK. A risk analysis of continuous ambulatory peritoneal dialysis-related peritonitis. *Peritoneal Dialysis Int* (2005) 25(4):374–9.

15. Boonstra CE, Jackson CE. Serum calcium survey for hyperparathyroidism: Results in 50,000 clinic patients. *Am J Clin Pathol* (1971) 55(5):523–6. doi: 10.1093/ajcp/55.5.523

16. Izquierdo-Lahuerta A. The parathyroid hormone-related Protein/Parathyroid hormone 1 receptor axis in adipose tissue. *Biomolecules* (2021) 11(11):1570. doi: 10.3390/biom11111570

17. Guo W, Zhang H, Zhang Y, Huang H, Liu W, Diao Z. Low parathyroid hormone versus secondary hyperparathyroidism and survival in patients undergoing hemodialysis: A propensity-matched analysis. *Front Endocrinol (Lausanne)* (2022) 13:869330. doi: 10.3389/fendo.2022.869330

18. Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, et al. Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and pth: The dialysis outcomes and practice patterns study (Dopps). *Am J Kidney Dis* (2008) 52(3):519–30. doi: 10.1053/j.ajkd.2008.03.020

19. Evenepoel P, Bover J, Ureña Torres P. Parathyroid hormone metabolism and signaling in health and chronic kidney disease. *Kidney Int* (2016) 90(6):1184–90. doi: 10.1016/j.kint.2016.06.041

20. Sprague SM, Bellorin-Font E, Jorgetti V, Carvalho AB, Malluche HH, Ferreira A, et al. Diagnostic accuracy of bone turnover markers and bone histology in patients with ckd treated by dialysis. *Am J Kidney Dis* (2016) 67(4):559–66. doi: 10.1053/j.ajkd.2015.06.023

21. Pazianas M, Miller PD. The ckd-mbd syndrome: Hysteresis in pth involvement and pth administration for its management. *J Bone Mineral Res* (2020) 35(12):2313–7. doi: 10.1002/jbmr.4155

22. Al-Aly Z, Qazi RA, González EA, Zeringue A, Martin KJ. Changes in serum 25-hydroxyvitamin d and plasma intact pth levels following treatment with ergocalciferol in patients with ckd. *Am J Kidney Dis* (2007) 50(1):59–68. doi: 10.1053/j.ajkd.2007.04.010

23. Rodríguez-Ortiz ME, Rodríguez M. Recent advances in understanding and managing secondary hyperparathyroidism in chronic kidney disease. *F1000Research* (2020) 9:F1000 Faculty Rev-1077. doi: 10.12688/f1000research.22636.1

24. Li PK, Szeto CC, Piraino B, de Arteaga J, Fan S, Figueiredo AE, et al. Ispd peritonitis recommendations: 2016 update on prevention and treatment. *Peritoneal Dialysis Int* (2016) 36(5):481–508. doi: 10.3747/pdi.2016.00078

25. Naves-Díaz M, Passlick-Deetjen J, Guinsburg A, Marelli C, Fernández-Martín JL, Rodríguez-Puyol D, et al. Calcium, phosphorus, pth and death rates in a Large sample of dialysis patients from Latin america. the cores study. *Nephrology Dialysis Transplant* (2011) 26(6):1938–47. doi: 10.1093/ndt/gfq304

26. Johannesen CDL, Langsted A, Mortensen MB, Nordestgaard BG. Association between low density lipoprotein and all cause and cause specific mortality in Denmark: Prospective cohort study. *BMJ (Clinical Res ed)* (2020) 371:m4266. doi: 10.1136/bmj.m4266

27. Al Sahlawi M, Zhao J, McCullough K, Fuller DS, Boudville N, Ito Y, et al. Variation in peritoneal dialysis-related peritonitis outcomes in the peritoneal dialysis outcomes and practice patterns study (Pdopps). *Am J Kidney Dis* (2022) 79(1):45–55.e1. doi: 10.1053/j.ajkd.2021.03.022

28. Teitelbaum I. Peritoneal dialysis. *New Engl J Med* (2021) 385(19):1786–95. doi: 10.1056/NEJMra2100152

29. Perl J, Fuller DS, Bieber BA, Boudville N, Kanjanabuch T, Ito Y, et al. Peritoneal dialysis-related infection rates and outcomes: Results from the peritoneal dialysis outcomes and practice patterns study (Pdopps). *Am J Kidney Dis* (2020) 76(1):42–53. doi: 10.1053/j.ajkd.2019.09.016

30. Htay H, Cho Y, Pascoe EM, Darssan D, Nadeau-Fredette AC, Hawley C, et al. Multicenter registry analysis of center characteristics associated with technique failure in patients on incident peritoneal dialysis. *Clin J Am Soc Nephrol CJASN* (2017) 12(7):1090–9. doi: 10.2215/cjn.12321216

31. Witowski J, López-Cabrera M. Peritoneal dialysis and its local and systemic complications: From the bench to the clinic. *Front Physiol* (2020) 11:188. doi: 10.3389/fphys.2020.00188

32. Raby AC, González-Mateo GT, Williams A, Topley N, Fraser D, López-Cabrera M, et al. Targeting toll-like receptors with soluble toll-like receptor 2 prevents peritoneal dialysis solution-induced fibrosis. *Kidney Int* (2018) 94(2):346–62. doi: 10.1016/j.kint.2018.03.014

33. Strippoli GF, Tong A, Johnson D, Schena FP, Craig JC. Antimicrobial agents to prevent peritonitis in peritoneal dialysis: A systematic review of randomized controlled trials. *Am J Kidney Dis* (2004) 44(4):591–603.

34. Wang J, Zhang H, Liu J, Zhang K, Yi B, Liu Y, et al. Implementation of a continuous quality improvement program reduces the occurrence of peritonitis in pd. *Renal failure* (2014) 36(7):1029–32. doi: 10.3109/0886022x.2014.927771

35. McQuillan RF, Chiu E, Nessim S, Lok CE, Roscoe JM, Tam P, et al. A randomized controlled trial comparing mupirocin and polysporin triple ointments in peritoneal dialysis patients: The Mp3 study. *Clin J Am Soc Nephrol CJASN* (2012) 7(2):297–303. doi: 10.2215/cjn.07970811

36. Han SH, Lee SC, Ahn SV, Lee JE, Kim DK, Lee TH, et al. Reduced residual renal function is a risk of peritonitis in continuous ambulatory peritoneal dialysis patients. *Nephrology dialysis Transplant* (2007) 22(9):2653–8. doi: 10.1093/ndt/gfm242

37. Oo TN, Roberts TL, Collins AJ. A comparison of peritonitis rates from the united states renal data system database: Capd versus continuous cycling peritoneal dialysis patients. *Am J Kidney Dis* (2005) 45(2):372–80. doi: 10.1053/j.ajkd.2004.10.008

38. Okayama M, Inoue T, Nodaira Y, Kimura Y, Nobe K, Seto T, et al. Aging is an important risk factor for peritoneal dialysis-associated peritonitis. *Adv peritoneal Dialysis Conf Peritoneal Dialysis* (2012) 28:50–4.

39. Kerschbaum J, König P, Rudnicki M. Risk factors associated with peritoneal-Dialysis-Related peritonitis. *Int J Nephrol* (2012) 2012:483250. doi: 10.1155/2012/483250

40. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol CJASN* (2015) 10(7):1257–72. doi: 10.2215/cjn.09750913

41. Conigrave AD. The calcium-sensing receptor and the parathyroid: Past, present, future. *Front Physiol* (2016) 7:563. doi: 10.3389/fphys.2016.00563

42. 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(2):578–85. doi: 10.1210/jc.2009-1603

43. 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(5):985–96. doi: 10.1016/j.kint.2016.05.019

44. Krick S, Helton ES, Hutcheson SB, Blumhof S, Garth JM, Denson RS, et al. Fgf23 induction of O-linked n-acetylglucosamine regulates il-6 secretion in human bronchial epithelial cells. *Front In Endocrinol* (2018) 9:708. doi: 10.3389/fendo.2018.00708

45. Silver J, Naveh-Many T. Fgf-23 and secondary hyperparathyroidism in chronic kidney disease. *Nat Rev Nephrol* (2013) 9(11):641–9. doi: 10.1038/nrneph.2013.147

46. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* (2004) 15(8):2208–18. doi: 10.1097/01.asn.0000133041.27682.a2

47. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 kdigo chronic kidney disease-mineral and bone disorder (Ckd-mbd) guideline update: What's changed and why it matters. *Kidney Int* (2017) 92(1):26–36. doi: 10.1016/j.kint.2017.04.006

48. Hong YA, Kim JH, Kim YK, Chang YK, Park CW, Kim SY, et al. Low parathyroid hormone level predicts infection-related mortality in incident dialysis patients: A prospective cohort study. *Korean J Internal Med* (2020) 35(1):160–70. doi: 10.3904/kjim.2018.264

49. Chen Z, Zhang X, Han F, Xie X, Hua Z, Huang X, et al. High alkaline phosphatase and low intact parathyroid hormone associate with worse clinical outcome in peritoneal dialysis patients. *Peritoneal Dialysis Int* (2021) 41(2):236–43. doi: 10.1177/0896860820918131

50. Rhee CM, Molnar MZ, Lau WL, Ravel V, Kovesdy CP, Mehrotra R, et al. Comparative mortality-predictability using alkaline phosphatase and parathyroid hormone in patients on peritoneal dialysis and hemodialysis. *Peritoneal Dialysis Int* (2014) 34(7):732–48. doi: 10.3747/pdi.2013.00110

51. Isakova T, Nickolas TL, Denburg M, Yarlagadda S, Weiner DE, Gutiérrez OM, et al. Kdoqi us commentary on the 2017 kdigo clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (Ckd-mbd). *Am J Kidney Dis* (2017) 70(6):737–51. doi: 10.1053/j.ajkd.2017.07.019

52. Stavenuiter AWD, Farhat K, Vila Cuenca M, Schilte MN, Keuning ED, Paauw NJ, et al. Protective effects of paricalcitol on peritoneal remodeling during peritoneal dialysis. *BioMed Res Int* (2015) 2015:468574. doi: 10.1155/2015/468574

53. Chennasamudram SP, Noor T, Vasylyeva TL. Comparison of sevelamer and calcium carbonate on endothelial function and inflammation in patients on peritoneal dialysis. *J Ren Care* (2013) 39(2):82–9. doi: 10.1111/j.1755-6686.2013.12009.x

54. Kerschbaum J, König P, Hausdorfer J, Mayer G, Rudnicki M. Sevelamer use and incidence of peritonitis in peritoneal dialysis. *Wien Klin Wochenschr* (2011) 123(7-8):204–8. doi: 10.1007/s00508-011-1551-1

55. Yang Y, Da J, Jiang Y, Yuan J, Zha Y. Low serum parathyroid hormone is a risk factor for peritonitis episodes in incident peritoneal dialysis patients: A retrospective study. *BMC Nephrol* (2021) 22(1):44. doi: 10.1186/s12882-021-02241-0

56. Liao CT, Zheng CM, Lin YC, Wu MY, Lin YF, Hsu YH, et al. Aberrant serum parathyroid hormone, calcium, and phosphorus as risk factors for peritonitis in peritoneal dialysis patients. *Sci Rep* (2021) 11(1):1171. doi: 10.1038/s41598-020-80938-2

57. Fortes PC, de Moraes TP, Mendes JG, Stinghen AE, Ribeiro SC, Pecoits-Filho R. Insulin resistance and glucose homeostasis in peritoneal dialysis. *Peritoneal Dialysis Int* (2009) 29 Suppl 2:S145–8. doi: 10.1177/089686080902902S28

58. Dong J, Wang Q, Chen MH, Zhao HP, Zhu TY, Tian N, et al. Associations between serum-intact parathyroid hormone, serum 25-hydroxyvitamin d, oral vitamin d analogs and metabolic syndrome in peritoneal dialysis patients: A multi-center cross-sectional study. *Peritoneal Dialysis Int* (2014) 34(4):447–55. doi: 10.3747/pdi.2013.00001

59. Ahamed NAS, Abdul-Aziz MY, El-Bauomy AA, Salem TS. Parathyroid hormone: Effects on glucose homeostasis and insulin sensitivity in chronic renal failure patients on regular hemodialysis. *J Taibah Univ Med Sci* (2008) 3(1):44–54. doi: 10.1016/S1658-3612(08)70052-0

60. Ueda R, Nakao M, Maruyama Y, Nakashima A, Yamamoto I, Matsuo N, et al. Effect of diabetes on incidence of peritoneal dialysis-associated peritonitis. *PLoS One* (2019) 14(12):e0225316. doi: 10.1371/journal.pone.0225316

61. Szeto CC, Kwan BC, Chow KM, Leung CB, Cheng MS, Law MC, et al. Metabolic syndrome in peritoneal dialysis patients: Choice of diagnostic criteria and prognostic implications. *Clin J Am Soc Nephrol CJASN* (2014) 9(4):779–87. doi: 10.2215/cjn.06620613

62. Yuan F, Zhang Q, Dong H, Xiang X, Zhang W, Zhang Y, et al. Effects of des-acyl ghrelin on insulin sensitivity and macrophage polarization in adipose tissue. *J Trans Internal Med* (2021) 9(2):84–97. doi: 10.2478/jtim-2021-0025

63. McDonald SP, Collins JF, Rumpsfeld M, Johnson DW. Obesity is a risk factor for peritonitis in the Australian and new Zealand peritoneal dialysis patient populations. *Peritoneal Dialysis Int* (2004) 24(4):340–6. doi: 10.1177/089686080402400408

64. Swaminathan S, Shah SV. New insights into nephrogenic systemic fibrosis. *J Am Soc Nephrol* (2007) 18(10):2636–43. doi: 10.1681/asn.2007060645

65. Kir S, Komaba H, Garcia AP, Economopoulos KP, Liu W, Lanske B, et al. Pth/Pthrp receptor mediates cachexia in models of kidney failure and cancer. *Cell Metab* (2016) 23(2):315–23. doi: 10.1016/j.cmet.2015.11.003

66. Amann K, Ritz E, Wiest G, Klaus G, Mall G. A role of parathyroid hormone for the activation of cardiac fibroblasts in uremia. *J Am Soc Nephrol* (1994) 4(10):1814–9. doi: 10.1681/asn.v4101814

67. Duque EJ, Elias RM, Moysés RMA. Parathyroid hormone: A uremic toxin. *Toxins (Basel)* (2020) 12(3):139. doi: 10.3390/toxins12030189

68. Helmke A, Nordlohne J, Balzer MS, Dong L, Rong S, Hiss M, et al. Cx3cl1-Cx3cr1 interaction mediates macrophage-mesothelial cross talk and promotes peritoneal fibrosis. *Kidney Int* (2019) 95(6):1405–17. doi: 10.1016/j.kint.2018.12.030

69. Yasunaga C, Nakamoto M, Matsuo K, Nishihara G, Yoshida T, Goya T. Effects of a parathyroidectomy on the immune system and nutritional condition in chronic dialysis patients with secondary hyperparathyroidism. *Am J Surg* (1999) 178(4):332–6. doi: 10.1016/s0002-9610(99)00194-4

70. Klinger M, Alexiewicz JM, Linker-Israeli M, Pitts TO, Gaciong Z, Fadda GZ, et al. Effect of parathyroid hormone on human T cell activation. *Kidney Int* (1990) 37 (6):1543–51. doi: 10.1038/ki.1990.147

71. Zhang J, Friberg IM, Kift-Morgan A, Parekh G, Morgan MP, Liuzzi AR, et al. Machine-learning algorithms define pathogen-specific local immune fingerprints in peritoneal dialysis patients with bacterial infections. *Kidney Int* (2017) 92(1):179–91. doi: 10.1016/j.kint.2017.01.017

72. Wang Q, Hu KJ, Ren YP, Dong J, Han QF, Zhu TY, et al. The association of individual and regional socioeconomic status on initial peritonitis and outcomes in peritoneal dialysis patients: A propensity score-matched cohort study. *Peritoneal Dialysis Int* (2016) 36(4):395–401. doi: 10.3747/pdi.2015.00100

73. Zeng Y, Jiang X, Feng S, Jiang L, Wang Z, Shen H, et al. The influence of seasonal factors on the incidence of peritoneal dialysis-associated peritonitis. *Renal failure* (2020) 42(1):807–17. doi: 10.1080/0886022x.2020.1804401

frontiers | Frontiers in Endocrinology

TYPE Correction
PUBLISHED 10 May 2023
DOI 10.3389/fendo.2023.1208563

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

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RECEIVED 19 April 2023
ACCEPTED 01 May 2023
PUBLISHED 10 May 2023

CITATION
Zhao Z, Yan Q, Li D, Li G, Cai J, Pan S, Duan J, Liu D and Liu Z (2023) Corrigendum: Relationship between serum iPTH and peritonitis episodes in patients undergoing continuous ambulatory peritoneal dialysis. *Front. Endocrinol.* 14:1208563. doi: 10.3389/fendo.2023.1208563

Corrigendum: Relationship between serum iPTH and peritonitis episodes in patients undergoing continuous ambulatory peritoneal dialysis

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KEYWORDS

continuous ambulatory peritoneal dialysis (CAPD), parathyroid hormone, peritonitis, end-stage renal disease (ESRD), hazard ratio (HR)

A corrigendum on

Relationship between serum iPTH and peritonitis episodes in patients undergoing continuous ambulatory peritoneal dialysis

by Zhao Z, Yan Q, Li D, Li G, Cai J, Pan S, Duan J, Liu D and Liu Z (2023) *Front. Endocrinol.* 14:1081543. doi: 10.3389/fendo.2023.1081543

In the published article, an author name was incorrectly written as Zhangzuo Liu. The correct spelling is Zhangsuo Liu.

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.

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 03 April 2023
DOI 10.3389/fendo.2023.1085133

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 31 October 2022
ACCEPTED 03 March 2023
PUBLISHED 03 April 2023

CITATION
Gu D, Ding Y, Jiang X, Shen B, Musante L, Holthofer H and Zou H (2023) Diabetes with kidney injury may change the abundance and cargo of urinary extracellular vesicles.
Front. Endocrinol. 14:1085133.
doi: 10.3389/fendo.2023.1085133

Diabetes with kidney injury may change the abundance and cargo of urinary extracellular vesicles

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Background: Urinary extracellular vesicles (uEVs) are derived from epithelia facing the renal tubule lumen in the kidney and urogenital tract; they may carry protein biomarkers of renal dysfunction and structural injury. However, there are scarce studies focusing on uEVs in diabetes with kidney injury.

Materials and methods: A community-based epidemiological survey was performed, and the participants were randomly selected for our study. uEVs were enriched by dehydrated dialysis method, quantified by Coomassie Bradford protein assay, and adjusted by urinary creatinine (UCr). Then, they identified by transmission electron microscopy (TEM), nanoparticle track analysis (NTA), and western blot of tumor susceptibility gene 101.

Results: Decent uEVs with a homogeneous distribution were finally obtained, presenting a membrane-encapsulated structure like cup-shaped or roundish under TEM, having active Brownian motion, and presenting the main peak between 55 and 110 nm under NTA. The Bradford protein assay showed that the protein concentrations of uEVs were 0.02 ± 0.02, 0.04 ± 0.05, 0.05 ± 0.04, 0.07 ± 0.08, and 0.11 ± 0.15 μg/mg UCr, respectively, in normal controls and in prediabetes, diabetes with normal proteinuria, diabetes with microalbuminuria, and diabetes with macroproteinuria groups after adjusting the protein concentration with UCr by calculating the vesicles-to-creatinine ratio.

Conclusion: The protein concentration of uEVs in diabetes with kidney injury increased significantly than the normal controls before and after adjusting the UCr. Therefore, diabetes with kidney injury may change the abundance and cargo of uEVs, which may be involved in the physiological and pathological changes of diabetes.

KEYWORDS

urinary extracellular vesicles, diabetes, diabetic nephropathy, diabetes with microalbuminuria, diabetes with macroproteinuria

Introduction

Over the past three decades, diabetes and prediabetes are significantly increased nationwide among children, adolescents, and younger adults (1). The estimated overall prevalence of prediabetes and diabetes were 10.9% and 35.7%, respectively, in Chinese adults (2). Most importantly, half of diabetic patients do not know that they have diabetes. Adults and children with impaired fasting glucose and/or impaired glucose tolerance were considered as prediabetes cases since they have not met the criteria of a diabetes diagnosis. Before presenting with obvious clinical symptoms, prediabetes may have complications, even if these were not observed. According to the survey of 1999–2016 in America, youth with prediabetic levels of HbA1c or fasting glucose were a high-risk population prone to developing cardiometabolic diseases (3). Hyperglycemia and diabetes are rising globally, and they are the most common cause of chronic kidney disease, and diabetic nephropathy is the major cause of end-stage renal disease (4). Therefore, more and more attention should be drawn to diabetes and its complications.

Extracellular vesicles (EVs) are secreted by cells of all tissues and organs under normal and disease conditions, ranging in size from approximately 30 to 10,000 nm in diameter and containing surface receptors, membrane and soluble proteins, lipids, ribonucleic acids, and genomic and mitochondrial DNAs (5–7). Urinary extracellular vesicles (uEVs) are derived from epithelia throughout the urogenital tract, podocyte, and transitional epithelia from the urinary collection system and released into the urinary lumen. Glomerular, tubular, prostate, and bladder cells are the most common sources of these vesicles (8). The uEV excretion is related to estimated glomerular filtration rate, creatinine clearance, total kidney volume, and kidney weight; one document revealed that nephrectomy may reduce uEV excretion, but depending on the loss of nephron mass (9). Therefore, 99.96% of the proteins presenting in uEVs may be the characteristic of cells under normal and disease conditions as assessed by proteomic analysis (8), and uEVs are being explored for non-invasive biomarkers (10, 11) of kidney function, kidney disease, and urological disease by proteomic and transcriptomic analyses, such as acute kidney injury (12), chronic kidney disease (13), polycystic kidney disease (14), prostate cancer (15), and renal allograft rejection (16, 17).

uEVs may be involved in the progress of diabetic nephropathy because they carry valuable sources for disease-stage-specific information and have the natural quality of fingerprints in disease progression (18). However, there are scarce data reported about the application of uEV research in different stages of diabetes (19). Therefore, we perform this study in order to research the physiological and pathological secretion of uEVs in healthy controls and diabetes patients with and without kidney injury.

Materials and methods

Participants

A schematic representation of the methodology is shown in Figure 1. A total of 75 participants coming from a community-based screening in Zhuhai, Southern China, were randomly selected in our study (20, 21). The selected participants were separated into five groups: healthy controls (n = 15), prediabetes (n = 15), diabetes with normal proteinuria (n = 15), diabetes with microalbuminuria (n = 15), and diabetes with macroproteinuria (n = 15). Written informed consent prior to data collection was given to all participants. Leaflets were read to the illiterate participants, followed by a thumb impression. This study was approved by the Human Ethics Committee of the Third Affiliated Hospital of Southern Medical University, Guangzhou, China (20, 21).

Demographic characteristics, anthropometric measurements, and urine and blood collection

All participants successfully filled in the questionnaire, which included data on age, sex, personal and family history of disease, waist circumference, height, and three readings of blood pressure taken at 1-min intervals. The first morning midstream urine without proteases inhibitor was collected from all the participants. Women who had their menstrual period must be excluded, but they are included once their menstruation is over. Blood was drawn on an empty stomach at local community clinics or health stations. The blood samples were disposed as soon as possible or, if there was a delay, stored at 4°C for less than 2 days (22, 23). Immunoturbidimetric test

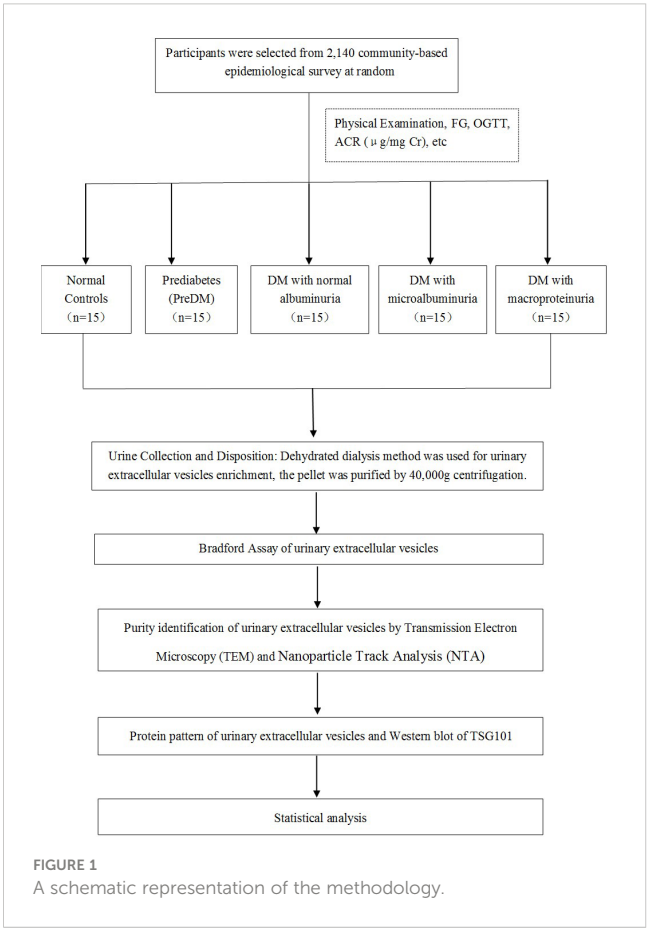

FIGURE 1
A schematic representation of the methodology.

was carried for albuminuria examination. Jaffe's kinetic method was used for UCr examination. Examinations on urinary albumin-to-creatinine ratio (ACR; μg/mg creatinine), fasting serum insulin concentration, serum creatinine, serum total cholesterol, high-density lipoprotein cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol were performed simultaneously. Because the urine samples must be disposed in the central laboratory, they were transported with dry ice and then stored at -80°C (24).

Evaluation criteria

Normal controls were selected from healthy participants, who should be non-smokers and non-drinkers and had no history of hypertension, dyslipidemia, central obesity, cardiovascular disease, stroke and tumor, infectious disease, and other kinds of disease. Prediabetes was defined as fasting plasma glucose (FPG) in the range 5.6–6.9 mmol/L (100–125 mg/dl) or OGTT 2 h after eating in the range 7.8–11.1 mmol/L (140–200 mg/dl). An empty stomach was defined as 8 h without eating. FPG ≥7.0 mmol/L (≥126 mg/dl) or OGTT 2 h after eating greater than 11.1 mmol/L (200 mg/dl) was defined as diabetes (25). ACR lower than 30 μg/mg in the spot urine of diabetes was defined as diabetes with normal proteinuria, ACR in the range of 30–299 μg/mg in the spot urine of diabetes was defined as diabetes with microalbuminuria, and ACR greater than 300 μg/mg in the spot urine of diabetes was defined as diabetes with macroproteinuria (25, 26).

uEV enrichment

A hydrostatic filtration dialysis method was used for uEV enrichment, which was established based on our previous method (27). First, fresh urine or thawed urine from -80°C was centrifuged at 2,000 *g* of relative centrifugal force for 30 min, and then the supernatant was collected. Second, a 1,000-kDa nanomembrane (Spectrum Laboratories, Inc., CA, USA) was used for dehydrated dialysis in order to discard the soluble proteins in the supernatant at 2,000 *g*. The dehydrated dialysis was stopped when the remaining urine volume in the tube was approximately 3–5 ml, and then the urine in the tube was collected. Dialyzed urine was experienced at 40,000 *g* centrifugation (Beckman JA-25.15; Beckman Coulter, CA, USA) for 1 h, and the pellet at 40,000 *g* was collected and finally suspended in ultra-pure water.

Bradford assay of uEVs

Coomassie Protein Assay Kit (Thermo Scientific, IL, USA) was used for the Bradford assay (28) according to the manufacturer's instructions. The content of one ampule of bovine serum albumin (BSA) was diluted into six clean vials with ultra-pure water by twofold serial dilutions; thus, we got the standard concentrations of 25, 50, 100, 200, 400, and 800 μg/ml. Coomassie Reagent Solution was mixed immediately by gently inverting the bottle a couple of times. An equilibrated amount of reagent was removed at room temperature before use. Next, a 20-μl standard, unknown samples, and ultra-pure water as blank were pipetted into a 96-well plate; this was repeated three times each. Each well was added with 200 μl Coomassie Reagent and then mixed with a plate shaker for 30 s. Incubation for 10 min at room temperature carried out after this progression. The absorbance was detected at 595 nm by SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices LLC, CA, USA).

Identification of uEVs by TEM

uEVs were identified by TEM. The Formvar-coated electron microscopy grid was held with forceps and washed gently with a drop of (10 μl) 0.01% BSA. After a while, a filter paper was used to suck from the edge of the grid. Then, a 2-μl sample was drawn immediately onto the grid and left on for 5 min, and a filter paper was used to suck from the edge of the grid. The grid was stained with 10 μl of 3% phosphotungstic acid (w/v) for 1 min, and then a filter paper was used again to suck from the edge of the grid. The grid was put directly into the grid box and then air-dried for half an hour before observation. After that, uEVs were identified by TEM (Hitachi H-7650; Hitachi, Tokyo, Japan).

Identification of uEVs by NTA

NanoSight NS300 equipped with sCMOS camera was used for uEV analysis (NanoSight Ltd., Salisbury, UK). NTA Version 2.3 Build 0033 was used throughout. All experiments were carried out at 1:200 dilution factor, except for diabetes with macroproteinuria group with 1:1,600, and 0.25 ml was loaded for each sample. An aliquot of 20 μl of each sample of the same group was mixed together as a one-go experiment, and five group experiments were performed.

Western blot of TSG101

Western blot of tumor susceptibility gene 101 (TSG101) was conducted according to our previous report (29–31). Specifically, 5-μg protein samples were loaded on resolving gels. After sodium dodecyl sulfate polyacrylamide gel electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane and then saturated with 5% BSA–phosphate-buffered saline (PBS) solution. The membrane was incubated with rabbit anti-human TSG101 antibody (Sigma Aldrich, Dorset, UK), followed by 6 times of washing with PBS-0.1% Tween, and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Dako, Ely, UK). After another round of six times of washing, the membrane was incubated with a detection reagent for 30 s and then visualized by Kodak IS 4000R image station (Kodak, USA).

Statistical analysis

A one-way ANOVA with *post hoc* analysis was used for multiple comparisons of the basic characteristics and protein

concentration of uEVs among five groups by SPSS 16.0. A *P*-value less than 0.05 was considered as significant difference. Graphs were created by GraphPad Prism 5.0.

Results

Baseline information of normal controls and diabetes patients

The characteristics of normal controls and diabetes patients are shown in Table 1. One-way ANOVO revealed that there was a significant difference in between group variation of blood glucose, albumin-to-creatinine ratio, systolic blood pressure, heart rate, serum creatinine, and triglycerides. There was no significant difference in between group variation of sex, age, diastolic blood pressure, urine creatinine, blood urea nitrogen, waist circumference, and total cholesterol.

Purity of uEVs by transmission electron microscopy and nanoparticle track analysis

The morphology of uEVs from the five groups as observed by TEM is shown in Figure 2. Our results indicate that uEVs have a homogeneous distribution, presenting a membrane-encapsulated structure like cup-shaped (Figure 2B) or roundish with a diameter of 30–100 nm (Figures 2A, C, D, E). A more comprehensive size analysis of uEVs was carried out by NTA on the pool made for each group. The profile showed that the main peak was between 55 and 110 nm, with a predominance of small vesicle in the healthy control (60–80 nm) DM and DM-macro (Figures 3A, C, E) and a shift in abundance (100 nm) for PreDM and DM-micro (Figures 3B, D). Moreover, some more peaks at a higher diameter were recorded with a characteristic trend for each group (Figure 3). All these uEVs under the under the magnification have active Brownian motion and present a membrane-encapsulated structure. In this analysis, all the samples were dialyzed first, and the pellet was then re-suspended in the same buffer eliminating differences in the viscosity of the liquid which can affect the rate of movement.

Protein concentration of uEVs

Data on protein concentration as determined by the Coomassie Bradford assay of uEVs in normal controls and diabetes patients are shown in Table 2. The Bradford assay showed that the protein concentration of the five groups differ from each other. The protein concentration of uEVs was 0.16 ± 0.07, 0.28 ± 0.23, 0.33 ± 0.19, 0.36 ± 0.22, and 0.60 ± 0.73 μg/ml, respectively, in normal controls and in prediabetes, diabetes with normal proteinuria, diabetes with microalbuminuria, and diabetes with macroproteinuria groups (Figure 4A). There was a higher protein concentration of uEVs in diabetes patients and a higher number of complications than normal controls; however, the protein concentration of uEVs in normal controls and prediabetes did not show a significant difference.

After adjusting the protein concentration with urinary creatinine by calculating the EVs-to-creatinine ratio, the protein concentration of uEVs was 0.02 ± 0.02, 0.04 ± 0.05, 0.05 ± 0.04, 0.07 ± 0.08, and 0.11 ± 0.15 μg/mg UCr, respectively, in normal controls and in prediabetes, diabetes with normal proteinuria, diabetes with microalbuminuria, and diabetes with macroproteinuria groups (Figure 4B). We found that protein concentration presents the same trend as unadjusted.

According to the NTA report, the size distribution was 162 ± 124, 138 ± 84, 90 ± 7, 163 ± 89, and 123 ± 76 nm in normal controls

TABLE 1 Characteristics of participants in different groups.

	NC	Pre-DM	DM	DM- micro	DM-macro	P value
Sex -male%	37.5%	43.7%	37.5%	50%	45.5%	P>0.05
Age -Year	40.75±11.1	61.75±1.31	57.62±7.89	60.81±12.05	65.63±12.59	P>0.05
Blood Glucose -mmol/l	4.64±0.36	6.00±0.43	6.23±1.49	8.59±3.27	6.78±2.18	P<0.001
ACR -μg/mg	6.70±2.71	10.67±5.64	11.39±6.42	71.26±55.64	380.57±61.74	P<0.001
SBP -mmHg	114.53±12.93	131.72±13.19	132.00±14.89	140.77±20.43	154.61±25.82	P<0.001
DBP-mmHg	74.22±10.33	77.09±9.62	82.03±9.06	77.73±6.53	79.55±12.44	P>0.05
HR - beats/min	74.62±7.99	78.40±6029	79.84±6.59	74.25±9.92	88.62±20.50	P<0.001
SCr –μmol/l	68.43±11.67	78.68±15.53	70.56±13.18	80.33±15.69	87.09±35.31	P<0.05
UCr –μmol/l	12.74±6.99	11.19±5.40	9.01±3.65	9.08±4.34	10.22±4.68	P>0.05
BUN –mmol/l	4.48±0.88	5.04±0.98	5.52±1.32	6.09±2.02	6.42±2.38	P>0.05
Waist -cm	78.53±9.90	90.81±7.62	87.00±10.05	90.83±10.01	85.33±12.74	P>0.05
TG –mmol/l	1.32±0.73	2.21±1.39	1.86±1.09	1.91±1.03	2.44±3.92	P<0.05
TC –mmol/l	5.23±0.80	6.29±2.06	5.29±0.99	5.83±0.98	5.05±1.34	P>0.05

NC, normal controls; PreDM, Prediabetes; DM, diabetes with normal proteinuria; DM-micro, diabetes with microalbuminuria; DM-macro, diabetes with macroproteinuria.
ACR, ablumin-to-creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SCr, serum creatinine; Ucr, urine creatinine; BUN, blood urea nitrogen; TG, triglyceride; TC, cholesterol.

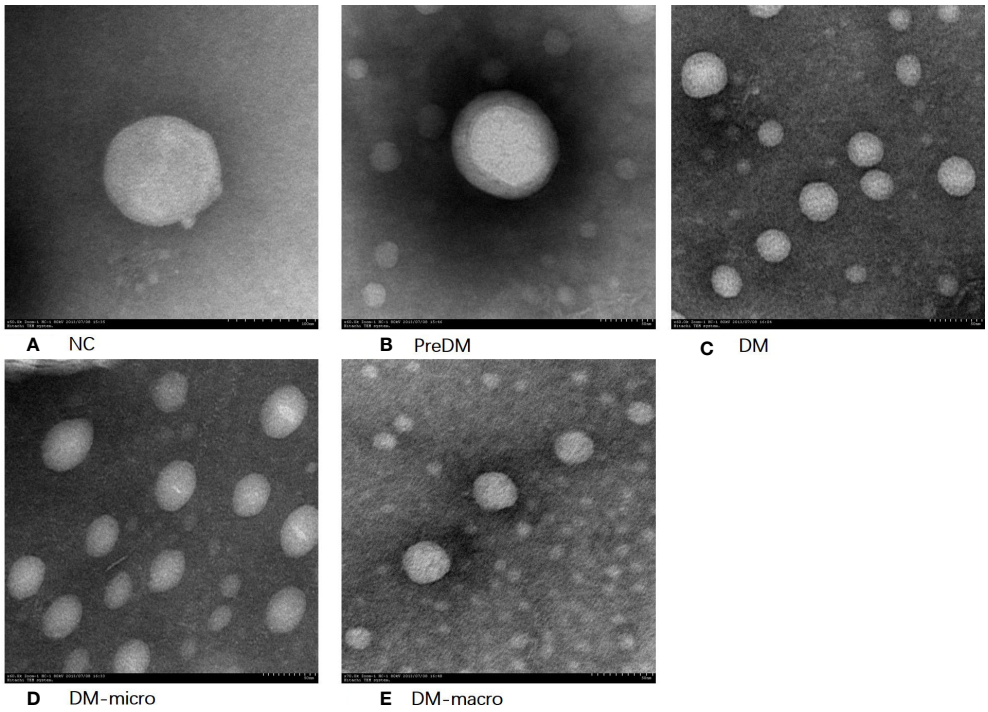

FIGURE 2
Morphology of uEVs observed by TEM. **(A)**. Morphology of uEVs under TEM in NC. **(B)**. Morphology of uEVs TEM in Prediabetes. **(C)**. Morphology of uEVs under TEM in Diabetes with normal proteinuria. **(D)**. Morphology of uEVs under TEM in Diabetes with microalbuminuria. **(E)**. Morphology of uEVs under TEM in Diabetes with macroproteinuria.

and in prediabetes, diabetes with normal proteinuria, diabetes with microalbuminuria, and diabetes with macroproteinuria groups. There was significant difference among the five groups ($p < 0.05$). According to the NTA report, the total concentration was 4.0 × E10, 7.16 × E10, 11.6 × E10, 11.8 × E8, and 17.44 × E8 particles/ml (total concentration equals detected concentration multiplied by the dilution factor) in normal controls and in prediabetes, diabetes with normal proteinuria, diabetes with microalbuminuria, and diabetes with macroproteinuria groups. It was significantly increased from prediabetes to diabetes with kidney injury groups ($p < 0.05$).

Protein pattern and western blot of TSG101 on uEVs

In total, 4 μg of exosome protein for each group was loaded on the colloidal gel. The protein pattern of Coomassie brilliant blue G-250 staining revealed that the protein band varied in the different groups (Figure 5A). TSG101 is the biogenesis biomarker of eUVs, and the western blot of TSG101 showed that TSG101 was present in all these five groups and exhibit a bit of difference (Figure 5B).

Discussion

Our study was established on the epidemiological survey, and all of the participants were selected from our community-based research (20). The baseline information of normal controls and diabetes patients is listed in Table 1, showing that the between-group variations of blood glucose, ACR, SBP, HR, SCr, and TG were significant by one-way ANOVO analysis. Diabetes increased the risk of developing a number of complications, such as neuropathy, nephropathy, retinopathy, and micro- and macro-vascular diseases. Our research focuses on kidney injury; however, there are still some unavoidable confounding factors such as SBP, HR, and TG.

In this study, we introduce a simple method of uEV recovery by hydrostatic filtration dialysis which employs a dry membrane with a molecular weight cutoff of 1,000 kDa to efficiently enrich the uEVs (27). Traditionally, uEVs have been separated by ultracentrifugation. Luca Musante found that the ultracentrifugation method is expensive and has poor efficiency because there are at least 40% of small uEVs that cannot be fully isolated from the supernatant after 200,0000 *g* ultracentrifugation (32). Our hydrostatic filtration dialysis method has the advantage of pre-processing and concentration of samples, the same as the conventional differential centrifugation method and density gradient ultracentrifugation method (27). It is super cost-efficient to enrich the uEVs from urine for clinical application. Since the uEV separation method shows specific advantages and disadvantages, the selected isolation method may play an important role in reflecting the characteristics of isolated EVs and contaminants, and there was not yet a standard operation procedure for their isolation; therefore, we should focus on the purity and yield of uEVs (8).

Decent uEVs were finally obtained in our present study. It has a homogeneous distribution, presents a membrane-encapsulated structure that was cup-shaped or roundish under TEM, shows active

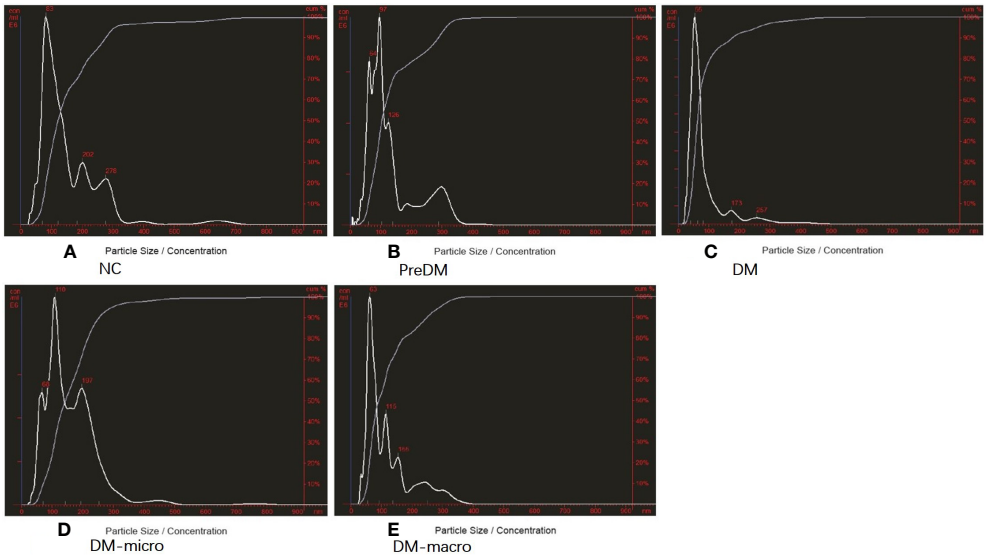

FIGURE 3
Purity of uEVs by NTA. **(A)**. Purity of uEVs under NTA in NC. **(B)**. Morphology of uEVs under TEM in Prediabetes. **(C)**. Morphology of uEVs under TEM in Diabetes with normal proteinuria. **(D)**. Morphology of uEVs under TEM in Diabetes with microalbuminuria. **(E)**. Morphology of uEVs under TEM in Diabetes with macroproteinuria. NC, normal controls; PreDM, Prediabetes; DM, diabetes with normal proteinuria; DM-micro, diabetes with microalbuminuria; DM-macro, diabetes with macroproteinuria.

Brownian motion, and presents the main peak between 55 and 110 nm under NTA. All these uEVs under the magnification field show active Brownian motion and present a membrane-encapsulated structure by NTA analysis. The roundish morphology observed under TEM may be caused by our phosphotungstic acid negative staining method instead of the more common negative staining based on uranyl acetate. The diameter of uEVs under TEM and NTA has some difference, which may be due to the fact that NTA observes the hydrodynamic particle diameter of the uEVs through a liquid medium; another is that larger particles contribute more strongly to dynamic light scattering than the smaller ones (33). According to the NTA report, the size distribution was significantly different among the five groups. This difference may be caused by aggregation of the microspheres, optical alignment, polydisperse in preparation procedure, *etc.* However, detailed calibration—different dilution factors of 1:1,600 in diabetes with macroproteinuria group and 1:200 in the rest of the groups—was carried out during the identification procedure in order to avoid such confounders. Another question is that there was no standard for uEV measurement. Furthermore, the size of our vesicles ranged from 30 to 500 nm according to the characteristic of EVs, and the total concentration was significantly increased from prediabetes to diabetes with kidney injury groups.

There were three centrifugation methods popular in EV extraction, such as low-force centrifugation at less than 10,000 *g*, ultracentrifugation that varies from 100,000 to 200,000 *g*, and differential centrifugation steps including both of those previously mentioned. With the progress in technology of EV preparation, P21 (pellet after 21,130 *g* centrifugation) was proved to have the rigorous characteristics of uEVs by Luca Musante and his team (31). After hydrostatic filtration dialysis and a relatively low-force centrifugation of 40,000 *g* in our study, P40 presents the biogenic characteristics of uEVs under TEM and NTA. There are couples of biomarkers of EVs. TSG101 was the biogenesis biomarker of uEVs (34) and expressed throughout the urogenital tract, having the nature for uEV identification. The western blot of TSG101 confirmed our urinary uEVs. Therefore, P40 is suitable for clinical application.

Our protein assay result suggests that there is a significant difference between normal controls and diabetes. After adjustment by UCr, the adjusted uEV concentrations still vary a lot between the disease cases and the normal controls. Therefore, uEVs may vary in

TABLE 2 Protein concentrations of uEVs in normal controls and diabetes (Mean±SD).

	NC	Pre-DM	DM	DM-micro	DM-macro	P value
P40 (μg/ml)	0.16±0.07	0.28±0.23	0.33±0.19	0.36±0.22	0.60±0.73	P<0.05
P40 (μg/mg UCr)	0.02±0.02	0.04±0.05	0.05±0.04	0.07±0.08	0.11±0.15	P<0.05
SN40	0	0	0	0	0	——

Protein concentration = Protein amount (μg) / Original urine volume (ml).
Protein concentration adjusted by Ucr = Protein amount (μg) / urinary creatinine (mg).
P40, Pellet 40,000 after 40,000g centrifugation; SN40, Supernatant 40,000 after 40,000g centrifugation.

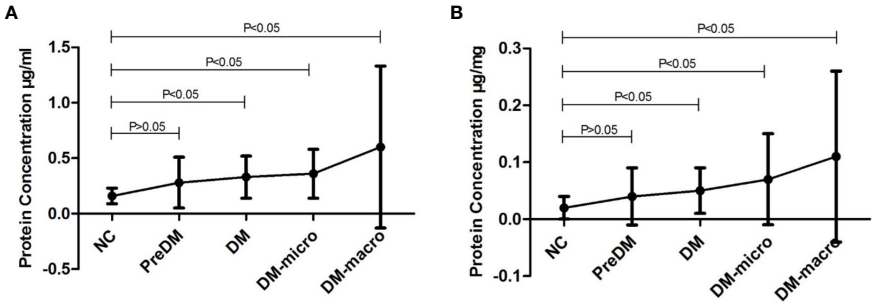

FIGURE 4
Protein concentration of uEVs in normal controls and diabetes. **(A)**. Protein concentration of uEVs in normal controls and diabetes (Mean±SD). **(B)**. Protein concentration of uEVs in normal controls and diabetes (Mean±SD) adjusted by Ucr.

disease cases to healthy individuals. Unfortunately, the lack of a housekeeping protein does not allow the precise normalization of the vesicle quantity as well as any other protein target carried by uEVs. Moreover, other than diabetes and its complication, several variables such as age, gender, diet, nutritional status, physical activity, collection time, volume status, environmental factors, *etc.*, might have impact on uEV secretion, thus resulting in a discrepancy between the two groups of participants (24, 35). The UCr concentration has the natural ability of a normalizer for uEV concentration because of its correlation with particle number and its excretion being not affected by water intake (36, 37). Concerning these confounding factors, UCr was used to adjust the protein concentration of uEVs as recommended (24) in order to have better quantification. However, the adjusted uEV protein concentration remains in the same trend. Therefore, the uEV-to-UCr ratio may be considered as a housekeeper indicating the stage of DN. uEVs may be non-invasive biomarkers in predicting and monitoring the progression of renal physiological and pathological conditions (38, 39).

More and more evidence have established a valuable role of uEVs in renal physiology and pathology (38, 40–42). A study focused on the interaction of the glomerular endothelial-derived EVs and podocytes established the central role of EV-mediated communication in playing a negative effect on podocyte function (40). Moreover, podocyte-derived EVs might establish a crosstalk between glomeruli and tubules and impair tubular epithelial cell by initializing an apoptosis program (41). That EVs could mediate long-distance cell-to-cell communication has already been proven. This might promote tubulointerstitial fibrosis and aggravate pathological progression, thus amplifying the damage of the kidney (43). However, whether the protein content in uEVs faithfully reflects the characteristics of renal cells and tissue or not is still a subject in debate. An animal study established a significant correlation between uEVs and renal protein abundance by proteomic analysis (34). A recent study revealed that the abundance of phosphorylated sodium chloride cotransporter in uEVs was significantly higher (1.89 folds) than that of the controls. Therefore, the protein biomarker of uEVs may be considered as an indicator of adrenal venous sampling (44). Our results also established the reliability of using uEV protein changes to monitor physiological and pathological responses in diabetic nephropathy.

In short, a simple method called hydrostatic filtration dialysis was used to enrich uEVs in our study, and it revealed that the abundance and cargo of uEVs vary in diabetes with or without kidney injury, which may be involved in the physiological and pathological changes of diabetes. However, the different techniques used to isolate EV subtypes and EVs from complicated components may result in preparations with different levels of abundance and purity of EVs

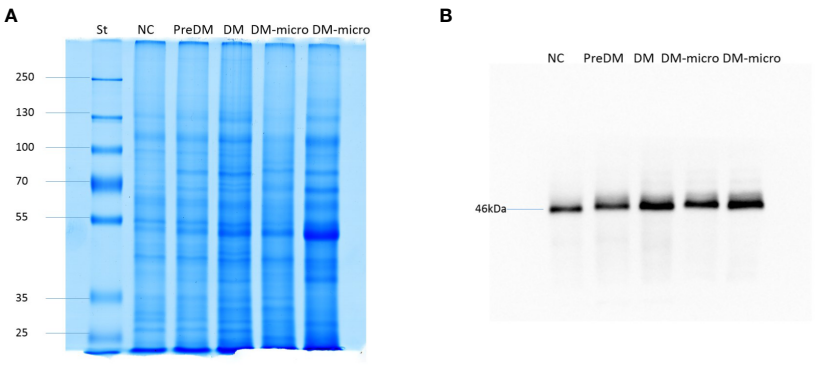

FIGURE 5
Protein pattern of uEVs and western blot of TSG101. **(A)**. Protein pattern of uEVs on the colloidal gel. **(B)**. Western blot of TSG101 as biogenesis biomarker of uEVs.

(45). Our study revealed that the uEV-to-UCr ratio may have advantage over urine protein–creatinine ratio or albumin–creatinine ratio, which may indicate different stages of DN. Furthermore, it has cost-effectiveness in terms of patient management. However, further research still needs to be developed to prove this.

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 Ethics Committee of the Third Affiliated Hospital of Southern Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DG performed the experiment and interpreted the data. DG and YD drafted the manuscript. XJ and BS collected the samples and epidemiological data. LM initiated the method of uEV enrichment. HZ and HH designed the study and reviewed the manuscript. All authors contributed to the manuscript and approved the submitted version.

Funding

This work was supported by the Medical Key Technologies R & D Program of Henan Province (LHGJ20200687 and 201802823) and EU FP7 Program, UroSense, 2011.

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. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. *Nat Rev Endocrinol* (2011) 8 (4):228–36. doi: 10.1038/nrendo.2011.183

2. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. *JAMA* (2017) 317 (24):2515–23. doi: 10.1001/jama.2017.7596

3. Wallace AS, Wang D, Shin JI, Selvin E. Screening and diagnosis of prediabetes and diabetes in US children and adolescents. *Pediatrics* (2020) 146(3). doi: 10.1542/peds.2020-0265

4. Sagoo MK, Gnudi L. Diabetic nephropathy: An overview. *Methods Mol Biol* (2020) 2067. doi: 10.1007/978-1-4939-9841-8_1

5. Abels ER, Breakefield XO. Introduction to extracellular vesicles: Biogenesis, RNA cargo selection, content, release, and uptake. *Cell Mol Neurobiol* (2016) 36(3):301–12. doi: 10.1007/s10571-016-0366-z

6. Iraci N, Leonardi T, Gessler F, Vega B, Pluchino S. Focus on extracellular vesicles: Physiological role and signalling properties of extracellular membrane vesicles. *Int J Mol Sci* (2016) 17(2):171. doi: 10.3390/ijms17020171

7. Zhu Q, Li H, Ao Z, Xu H, Luo J, Kaurich C, et al. Lipidomic identification of urinary extracellular vesicles for non-alcoholic steatohepatitis diagnosis. *J Nanobiotechnol* (2022) 20(1):349. doi: 10.1186/s12951-022-01540-4

8. Erdbrugger U, Blijdorp CJ, Bijnsdorp IV, Borras FE, Burger D, Bussolati B, et al. Urinary extracellular vesicles: A position paper by the urine task force of the international society for extracellular vesicles. *J Extracell Vesicles* (2021) 10(7): e12093. doi: 10.1002/jev2.12093

9. Blijdorp CJ, Hartjes TA, Wei KY, van Heugten MH, Bovee DM, Budde RPJ, et al. Nephron mass determines the excretion rate of urinary extracellular vesicles. *J Extracell Vesicles* (2022) 11(1):e12181. doi: 10.1002/jev2.12181

10. Raimondo F, Morosi L, Chinello C, Magni F, Pitto M. Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. *Proteomics* (2011) 11(4):709–20. doi: 10.1002/pmic.201000422

11. Zhu Q, Cheng L, Deng C, Huang L, Li J, Wang Y, et al. The genetic source tracking of human urinary exosomes. *Proc Natl Acad Sci USA* (2021) 118(43). doi: 10.1073/pnas.2108876118

12. Miller D, Eagle-Hemming B, Sheikh S, Joel-David L, Adebayo A, Lai FY, et al. Urinary extracellular vesicles and micro-RNA as markers of acute kidney injury after cardiac surgery. *Sci Rep* (2022) 12(1):10402. doi: 10.1038/s41598-022-13849-z

13. Oshikawa-Hori S, Yokota-Ikeda N, Sonoda H, Sonoda H, Sasaki Y, Ikeda M. Reduced urinary release of AQP1- and AQP2-bearing extracellular vesicles in patients with advanced chronic kidney disease. *Physiol Rep* (2021) 9(17):e15005. doi: 10.14814/phy2.15005

14. Ding H, Li LX, Harris PC, Yang J, Li X. Extracellular vesicles and exosomes generated from cystic renal epithelial cells promote cyst growth in autosomal dominant polycystic kidney disease. *Nat Commun* (2021) 12(1):4548. doi: 10.1038/s41467-021-24799-x

15. Almeida A, Gabriel M, Firlej V, Martin-Jaular L, Lejars M, Cipolla R, et al. Urinary extracellular vesicles contain mature transcriptome enriched in circular and long noncoding RNAs with functional significance in prostate cancer. *J Extracell Vesicles* (2022) 11(5):e12210. doi: 10.1002/jev2.12210

16. Golebiewska JE, Wardowska A, Pietrowska M, Pietrowska M, Wojakowska A, Debska-Slizien A. Small extracellular vesicles in transplant rejection. *Cells* (2021) 10 (11). doi: 10.3390/cells10112989

17. Rutman AK, Negi S, Saberi N, Khan K, Tchervenkov J, Paraskevas S. Extracellular vesicles from kidney allografts express miR-218-5p and alter Th17/Treg ratios. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.784374

18. Musante L, Tataruch DE, Holthofer H. Use and isolation of urinary exosomes as biomarkers for diabetic nephropathy. *Front Endocrinol* (2014) 5. doi: 10.3389/fendo.2014.00149

19. Ding X, Wang X, Du J, Han Q, Zhang D, Zhu H. A systematic review and meta-analysis of urinary extracellular vesicles proteome in diabetic nephropathy. *Front Endocrinol (Lausanne)* (2022) 13. doi: 10.3389/fendo.2022.866252

20. Gu DF, Shi YL, Chen YM, Liu HM, Ding YN, Liu XY, et al. Prevalence of chronic kidney disease and prediabetes and associated risk factors: A community-based screening in zhuhai, southern China. *Chin Med J* (2013) 126(7):1213–9. doi: 10.3760/cma.j.issn.0366-6999.20123504

21. Gu D, Chen Y, Masucci M, Xiong C, Zou H, Holthofer H. Potential urine biomarkers for the diagnosis of prediabetes and early diabetic nephropathy based on ISN CKHDP program. *Clin Nephrol* (2020) 93(1):129–33. doi: 10.5414/CNP92S123

22. Collins AC, Sethi M, Macdonald FA, Brown D, Viberti GC. Storage temperature and differing methods of sample preparation in the measurement of urinary albumin. *Diabetologia* (1993) 36(10):993–7. doi: 10.1007/BF02374489

23. Giampietro O, Penno G, Clerico A, Cruschelli L, Cecere M. How and how long to store urine samples before albumin radioimmunoassay: A practical response. *Clin Chem* (1993) 39(3):533–6. doi: 10.1093/clinchem/39.3.533

24. Zhou H, Yuen PS, Pisitkun T, Gonzales PA, Yasuda H, Dear JW, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. *Kidney Int* (2006) 69(8):1471–6. doi: 10.1038/sj.ki.5000273

25. American Diabetes Association. Standards of medical care in diabetes–2011. *Diabetes Care* (2011) 34Suppl 1. doi: 10.2337/dc11-S011

26. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Am J Kidney Dis* (2002) 39 (2 Suppl 1):S1–266. doi: 10.1053/j.ajkd.2020.05.006

27. Musante L, Tataruch D, Gu D, Benito-Martin A, Calzaferri G, Aherne S, et al. A simplified method to recover urinary vesicles for clinical applications, and sample banking. *Sci Rep* (2014) 4. doi: 10.1038/srep07532

28. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* (1976) 72. doi: 10.1006/abio.1976.9999

29. Musante L, Saraswat M, Duriez E, Byrne B, Ravida A, Domon B, et al. Biochemical and physical characterisation of urinary nanovesicles following CHAPS treatment. *PloS One* (2012) 7(7):e37279. doi: 10.1371/journal.pone.0037279

30. Musante L, Saraswat M, Ravida A, Byrne B, Holthofer H. Recovery of urinary nanovesicles from ultracentrifugation supernatants. *Nephrol Dial Transplant* (2012) 28 (6):1425–33. doi: 10.1093/ndt/gfs564

31. Musante L, Bontha SV, La Salvia S, Fernandez-Pineros A, Lannigan J, Le TH, et al. Rigorous characterization of urinary extracellular vesicles (uEVs) in the low centrifugation pellet - a neglected source for uEVs. *Sci Rep* (2020) 10(1):3701. doi: 10.1038/s41598-020-60619-w

32. Musante L, Saraswat M, Ravida A, Byrne B, Holthofer H. Recovery of urinary nanovesicles from ultracentrifugation supernatants. *Nephrol Dial Transplant* (2013) 28 (6):1425–33. doi: 10.1093/ndt/gfs564

33. Kestens V, Bozatzidis V, De Temmerman PJ, Ramaye Y, Roebben G. Validation of a particle tracking analysis method for the size determination of nano- and microparticles. *J Nanopart Res* (2017) 19(8):271. doi: 10.1007/s11051-017-3966-8

34. Wu Q, Poulsen SB, Murali SK, Grimm PR, Su XT, Delpire E, et al. Large-Scale proteomic assessment of urinary extracellular vesicles highlights their reliability in reflecting protein changes in the kidney. *J Am Soc Nephrol* (2021) 32(9):2195–209. doi: 10.1681/ASN.2020071035

35. Harpole M, Davis J, Espina V. Current state of the art for enhancing urine biomarker discovery. *Expert Rev Proteomics* (2016) 13(6):609–26. doi: 10.1080/14789450.2016.1190651

36. Hunter RW, Dear JW. Urinary vesicles: Are they ready for real-world use? *J Am Soc Nephrol* (2021) 32(5):1013–5. doi: 10.1681/ASN.2021030332

37. Blijdorp CJ, Tutakhel OAZ, Hartjes TA, van den Bosch TPP, van Heugten MH, Rigalli JP, et al. Comparing approaches to normalize, quantify, and characterize urinary extracellular vesicles. *J Am Soc Nephrol* (2021) 32(5):1210–26. doi: 10.1681/ASN.2020081142

38. Grange C, Bussolati B. Extracellular vesicles in kidney disease. *Nat Rev Nephrol* (2022) 18(8):499–513. doi: 10.1038/s41581-022-00586-9

39. Wu A, Wolley MJ, Fenton RA, Stowasser M. Using human urinary extracellular vesicles to study physiological and pathophysiological states and regulation of the sodium chloride cotransporter. *Front Endocrinol (Lausanne)* (2022) 13. doi: 10.3389/fendo.2022.981317

40. Hill N, Michell DL, Ramirez-Solano M, Sheng Q, Pusey C, Vickers KC, et al. Glomerular endothelial derived vesicles mediate podocyte dysfunction: A potential role for miRNA. *PloS One* (2020) 15(3):e0224852. doi: 10.1371/journal.pone.0224852

41. Jeon JS, Kim E, Bae YU, Yang WM, Lee H, Kim H, et al. microRNA in extracellular vesicles released by damaged podocytes promote apoptosis of renal tubular epithelial cells. *Cells* (2020) 9(6). doi: 10.3390/cells9061409

42. Braun F, Muller RU. Urinary extracellular vesicles as a source of biomarkers reflecting renal cellular biology in human disease. *Methods Cell Biol* (2019) 154. doi: 10.1016/bs.mcb.2019.04.014

43. Medeiros T, Myette RL, Almeida JR, Silva AA, Burger D. Extracellular vesicles: Cell-derived biomarkers of glomerular and tubular injury. *Cell Physiol Biochem* (2020) 54(1):88–109. doi: 10.33594/000000207

44. Kong L, Tang X, Kang Y, Dong L, Tong J, Xu J, et al. The role of urinary extracellular vesicles sodium chloride cotransporter in subtyping primary aldosteronism. *Front Endocrinol (Lausanne)* (2022) 13. doi: 10.3389/fendo.2022.834409

45. Cocozza F, Grisard E, Martin-Jaular L, Mathieu M, Thery C. SnapShot: Extracellular vesicles. *Cell* (2020) 182(1):262–e1. doi: 10.1016/j.cell.2020.04.054

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 12 April 2023
DOI 10.3389/fendo.2023.1083614

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Renal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 29 October 2022
ACCEPTED 27 March 2023
PUBLISHED 12 April 2023

CITATION
Xu Y, Liu B, Lin L, Lei F, Sun T, Zhang X, Song X, Huang X, Zeng Q, Cai J, Wang Z and Li H (2023) The association of apolipoprotein B with chronic kidney disease in the Chinese population. *Front. Endocrinol.* 14:1083614. doi: 10.3389/fendo.2023.1083614

The association of apolipoprotein B with chronic kidney disease in the Chinese population

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Background: Whether serum apolipoprotein B (ApoB) is a risk factor for the development of Chronic kidney disease (CKD) has not been fully established in the general population. Therefore, our study evaluated the correlation between serum ApoB level and CKD to look for an alternative approach for CKD prevention and treatment in the general population.

Methods: There were 146,533 participants in this cross-sectional study. 3,325 participants with more than 2 measurements were enrolled in the retrospective longitudinal study with at least a 3-year follow-up. ApoB was measured by the immunoturbidimetric method in 6 centers. Our study defined CKD as an estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2. The Spearman rank correlation analysis and the Random Forest algorithm were applied to rank the importance of variables determining the levels of eGFR. We used the logistic regression model to explain the correlation between serum ApoB and CKD. We used the Cox model to detect the correlation between baseline serum ApoB and the subsequent occurrence of CKD.

Results: Based on a cross-sectional study, 66.5% of the participants were males, with a median age of 49 (interquartile range [IQR] 43-55). Compared to the non-CKD group, the CKD group has higher levels of lipid profile and fasting glucose as well as the proportion of hypertension and hyperuricemia. The Spearman rank correlation analysis and the Random Forest algorithm revealed that ApoB has the highest correlation with eGFR decline among lipid profiles. The logistic regression analysis revealed that ApoB had a positive correlation with the prevalence of CKD after fully controlling confounders (odds ratio [OR], 1.07; 95% confidence interval [CI]: 1.02-1.11). Moreover, baseline ApoB level was correlated with a new-onset CKD in the longitudinal cohort after full

adjustment for confounding factors (hazard ratio [HR], 1.61; 95% CI: 1.02-2.54). The correlation between ApoB level and the new-onset CKD was consistently observed in all sensitivity analyses.

Conclusion: Serum ApoB had the strongest correlation with CKD among all lipid variables. Moreover, high serum ApoB levels might precede the occurrence of CKD, suggesting that monitoring and reducing serum ApoB levels may provide an alternative method to prevent and treat CKD.

KEYWORDS

apolipoprotein B, chronic kidney disease, estimated glomerular filtration rate, dyslipidemia, atherosclerosis

Background

As a major global public health problem, now chronic kidney disease (CKD), with a worldwide prevalence of 9.1% (1), is rising gradually and particularly in Asian countries with aging populations like China (2). CKD is associated with high morbidity and mortality, and is expected to become the fifth leading cause of death globally by 2040 (3). CKD has a serious impact on life quality and family economic status. The average per-patient to health care costs when the disease progresses to end-stage renal disease ranges from $20,110 to $100,593, without accounting for societal costs and productivity loss (4). Metabolic abnormalities are risk factors for CKD progression (5). Dyslipidemia is one of the most important components of metabolic abnormalities (6), which may contribute to renal lipid disturbances or aggravate glomerular and tubulointerstitial disease through the combination of inflammation and oxidative stress (7). Therefore, changes in lipid levels are important indicators reflecting the decline of renal function. Apolipoprotein B (ApoB) is a specific lipoprotein composition of serum lipids and has not received much attention in the previous studies on the relation of dyslipidemia and CKD compared with the traditional lipid parameters. Recent papers have found that serum ApoB level is a better biomarker for CVD of the risk diagnosis, prognosis (8, 9), and lipid-lowering therapy than traditional lipid factors. Whether ApoB plays a character in the development of CKD, previous studies are few and the results are inconclusive.

Abbreviations: CKD, chronic kidney disease; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate; ApoA-I, apolipoprotein A-I; Lp(a), lipoprotein (a); CI, confidence interval; OR, odds ratio; HR, hazard ratio; IQR, interquartile range; BMI, body mass index; WBC, white blood cell; HGB, hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; Scr, serum creatinine; SUA, serum uric acid; BUN, blood urea nitrogen; KDIGO, Kidney Disease Improving Global Outcomes; NHANES, National Health and Nutrition Examination Survey; Multivariable HR, Multivariable hazard ratio.

At present, lipid-lowering therapy by reducing the production of ApoB is mainly used in patients with atherosclerosis (10, 11). For example, mipomersen is the first Food and Drug Administration approved antisense ApoB synthesis inhibitor for patients who tolerate statin therapy and are at high CVD risk (12, 13). In the study of clinical trials, dose-dependent reductions were produced by mipomersen in different types of ApoB-containing lipoproteins, including lipoprotein (a) [Lp(a)] and low-density lipoprotein-cholesterol (LDL-C) (14). If ApoB proves to be a risk factor for CKD, mipomersen may be an alternative strategy for alleviating such metabolic risk-associated impairment in kidney function.

The current study aimed to examine the correlation between serum ApoB levels and CKD both on cross-sectional and longitudinal based on Chinese health check-up centers. If this hypothesis is established, it will provide an alternative approach to CKD prevention and treatment.

Methods

Study population

150,033 individuals had tested for serum ApoB and creatinine in six health management centers screened in this study from January 2009 to December 2017. All the participants who entered this study followed voluntary bases. Individuals who were less than 18 years old (n=208), used lipid-lowering agents (n=96), drugs that affect kidney function (n=387), and with a history of severe kidney disease (renal carcinoma, nephrectomy, kidney transplant, nephrotuberculosis) (n=419) were excluded. The severe kidney disease may be accompanied by changes in nutritional status, which may be a potential confounding factor affecting the measurement of serum creatinine and leading to inaccurate estimates of renal function. There were 146,533 participants eligible for the cross-sectional study (Figure 1A). Individuals with estimated glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m^2 at the initial test and had at least two examinations for serum ApoB and creatinine with an interval of over 3 years were selected for the retrospective longitudinal cohort. There were 3,331 participants

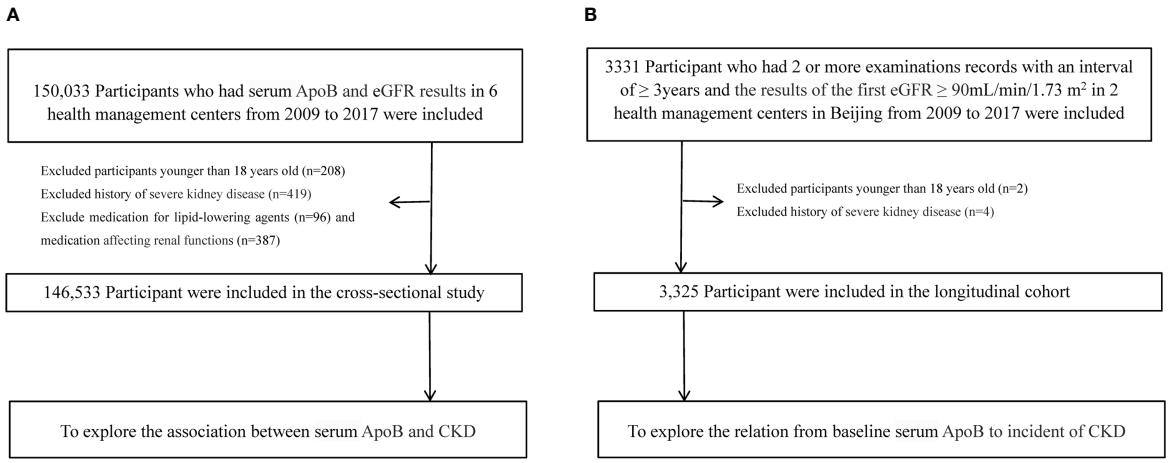

FIGURE 1
Flow chart of participants. **(A)** Flow chart of participants in the cross-sectional analysis. **(B)** Flow chart of participants in the longitudinal analysis.

from 2 health management centers in Beijing from 2009 to 2017. After excluding 2 participants younger than 18 years old and 4 participants with a history of severe kidney disease. Finally, 3,325 participants were included to calculate the correlation between serum ApoB level and the incidence of CKD (Figure 1B). The study was approved by the central ethics board of Renmin Hospital. Individual identification data was removed, and only anonymous information was kept during the study. The ethics committees from each hospital waived patient informed consent.

Anthropometric and laboratory data

All the individuals underwent clinical examinations and anthropometric measurements by experienced medical teams in the health check-up centers. The height (cm) was measured with shoes off (nearest 0.1 cm), and the weight (kg) was measured without a heavy coat (nearest 0.1 kg) (15). The automatic electronic sphygmomanometer was used for the blood pressure (mmHg) measurements after resting for 5-10 minutes (16). The blood sample was taken from the anterior axillary vein on an empty stomach. Biochemical tests and blood routine tests were detected by an automatic biochemical analyzer in a fasting condition. Two hours after the blood sample was collected. Test methods and procedures are unified laboratory standard protocols and guidelines (17). The concentrations of blood glucose, white blood cell (WBC), hemoglobin (HGB), alanine aminotransferase (ALT), triglyceride (TG), LDL-C, high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), serum creatinine (Scr), blood uric acid (SUA), and blood urea nitrogen (BUN) were detected by the automatic biochemical analyzer. The six health management centers included in this study used the same method, namely the Jaffe method, to detect Scr (18). Apolipoprotein A-I (ApoA-1) and ApoB were detected by polyethylene glycol-enhanced immunoturbidimetry (19). The medical history and medication were collected based on self-report records during the health exam. Body mass index (BMI) was calculated as weight divided by height square (kg/m^2). Past medical history of diseases and medications was obtained based on the self-reported history in the physical examination record.

Diagnostic criteria

The estimated GFR was calculated by the Modification of Diet in the Renal Disease equation for Chinese patients (2006) (20). The eGFR < 90 mL/min/1.73 m^2 (CKD stages 2-5) was defined as impaired kidney function based on the criteria in Kidney Disease Improving Global Outcomes (KDIGO) (21). Elevated ApoB level was described as a serum ApoB level above 1.1 g/L, according to the Chinese Guidelines for the Prevention and Treatment of Dyslipidemia in adults (19). Systolic blood pressure (SBP) ≥ 140 mmHg, or/and diastolic blood pressure (DBP) ≥ 90 mmHg, or a history of hypertension were identified as hypertension (22). Fasting plasma glucose ≥7.0 mmol/L, blood glucose 2 hours postprandial ≥ 11.1 mmol/L, history of hypoglycemic drugs, or history of diabetes were identified as diabetes (23). We defined hyperuricemia as an SUA > 360 μmol/L (6.0 mg/dL) and SUA > 420 μmol/L (7.0 mg/dL) for females and males, respectively (24). We defined hypohemia as an HGB < 110 g/L in females and < 120 g/L in males which is based on the diagnostics definitions (25).

Statistical analysis

Categorical variables were represented as frequencies and percentages. Continuous variables were expressed as the median and interquartile range (IQR). Student's t-test was used for testing the difference in the two groups for variables with normal distribution, and the Kruskal-Wallis test was applied to two groups with skewed distribution. Fisher's exact test or χ^2 test was used for comparisons in categorical variables. The variables with a miss data rate of < 30% were complemented using Miss Forest (26). We used the Spearman rank correlation analysis and the Random Forest algorithm to select the important variables to determine the eGFR levels. The common

method - logistic regression was selected for detecting the correlation between serum ApoB level and CKD prevalence. We determined the correlation between baseline ApoB level and the subsequent occurrence of CKD under the Cox proportional hazards regression model. The mixed-effects Cox regression models was applied in sensitivity analysis II (27).Two-sided $P < 0.05$ was considered statistically significant. We used SPSS Statistics (version 25.0, IBM, Armonk, NY, USA) and R-4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) for data analysis.

Sensitivity analysis

We further conducted two sensitivity analyses of the correlation between baseline ApoB level and the subsequent occurrence of CKD in the retrospective cohort for quality control. In sensitivity analysis I, we validated the results by increasing the duration of the follow-up. There were 1,896 participants with two or more health check-up records with an interval of ≥ 4 years between examinations. In sensitivity analysis II, we used the mixed-effects Cox regression models (medical center as a random effect) to assess the relationship between baseline ApoB level and the new-onset CKD. E-value analysis to address potential unmeasured confounding effects in the Cox model to assess the robustness of the association between ApoB and CKD occurrence (28).

Results

Participants' characteristics of the cross-sectional population

The study included data from 146,533 participants (median age, 49 years; IQR, 43-55 years), and 66.5% were males. The median level of ApoB was 0.91 (IQR, 0.77-1.08) g/L, the median Scr level was 69.00 (IQR, 59.00-78.20) μmol/L, the median SUA level was 336.00 (IQR, 274.00-398.00) μmol/L, and the median BUN level was 5.00 (IQR, 4.20-5.83) mmol/L. 22.5% had hypertension, 14.3% had hyperuricemia, and 13.3% suffered from diabetes. A comparison of participants across eGFR normal and abnormal groups revealed that those individuals with low levels of eGFR had generally higher levels of age (53 years [IQR, 47-62] versus 48 years [IQR, 42-54], $P < 0.001$), BMI (25.56 kg/m^2 [IQR, 23.65-27.53] versus 25.01 kg/m^2 [IQR, 22.79-27.29], $P < 0.001$), SBP (126 mmHg [IQR, 115-140] versus 121 mmHg [IQR, 110-134], $P < 0.001$) and ApoB (0.95 g/L [IQR, 0.80-1.12] versus 0.91 g/L [IQR, 0.76-1.07], $P < 0.001$). Detailed characteristics are provided in Table 1.

Association between lipid variables and CKD

Supplementary Table 1 shows the correlation matrix of serum lipid variables with CKD (eGFR < 90 ml/min/1.73 m^2). Spearman rank correlation analysis shows that ApoB was directly proportional to CKD (r = 0.063, $P < 0.001$). Besides ApoB, TG (r = 0.055, $p < 0.001$), TC (r = 0.042, $P < 0.001$), and LDL-C (r= 0.027, $P < 0.001$) were directly proportional to CKD, whereas HDL-C (r = −0.039, $P < 0.001$) and AopA-I (r = −0.037, $P < 0.001$) was negatively associated with CKD prevalence. In the screening of important variables of random forest, ApoB (Mean Decrease Accuracy, 118.455) was considered the most important indicator among all lipid variables.

Association between ApoB and CKD prevalence in the cross-sectional population

This study applied logistic regression analysis to identify the correlation between serum ApoB level and CKD prevalence. In the crude model, there is a strong connection between ApoB and CKD (odds ratio [OR], 1.37; 95% confidence interval [CI]: 1.32-1.42) ($P < 0.001$). After adjusting for sex, age, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use, and smoke status, serum ApoB level was still significantly positively correlated with CKD prevalence. The OR value was 1.07 (95% CI: 1.02-1.11) ($P = 0.006$) (Table 2).

Baseline characteristics of longitudinal cohort

Longitudinal analysis was further conducted to verify the correlation between baseline ApoB and CKD incidence. A strong association between serum ApoB levels and CKD prevalence was found in cross-sectional analysis. In a three-year follow-up cohort of 3325 individuals with eGFR ≥ 90 ml/min/1.73 m^2 at baseline, 185 individuals developed eGFR decline (eGFR < 90mL/min/1.73 m^2). The population's median age of the eGFR decline group was 50 (IQR, 45-55) years, older than those with the normal eGFR group (47 [IQR, 42-51] years). The participants that developed eGFR decline had larger proportions of males (87.60% versus 67.20%), hypertension (22.80% versus 18.20%), and hyperuricemia (19.50% versus 11.90%). Accordingly, the levels of BMI, SBP, DBP, WBC, ALT, LDL-C, TC, TG, Scr, SUA, and BUN were higher in the developed eGFR decline group. There is a higher proportion of participants with an elevated ApoB level developed eGFR decline. Detailed characteristics are provided in Table 3.

Association between the baseline ApoB and incident of CKD in the longitudinal cohort

Individuals with increased baseline serum ApoB showed a greater risk of developing incident CKD during the three-year follow-up in the crude analysis, with a hazard ratio (HR) of 1.50 (95% CI: 1.07-2.11) ($P = 0.019$). After adjusting for age, sex, BMI,

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TABLE 1 Baseline characteristics of the cross-sectional population.

Characteristic	Overall (N=146533)	eGFR ≥ 90 ml/min/1.73 m^2 (N=127447)	eGFR< 90 ml/min/1.73 m^2 (N=19086)	*P* value*
Sex, male, n(%)	97583 (66.5)	81893 (64.3)	15690 (82.2)	<0.001
Age, median[IQR]years	49 [43-55]	48 [42-54]	53 [47-62]	<0.001
BMI (kg/m^2, median[IQR])	25.09 [22.89-27.34]	25.01 [22.79-27.29]	25.56 [23.65-27.53]	<0.001
SBP (mmHg, median[IQR])	122 [110-135]	121 [110-134]	126 [115-140]	<0.001
DBP (mmHg, median[IQR])	78 [70-85]	78 [70-85]	80 [72-88]	<0.001
Serum glucose (mmol/L, median[IQR])	5.30 [4.92-5.84]	5.30 [4.91-5.84]	5.34 [4.96-5.83]	<0.001
TG (mmol/L, median[IQR])	1.43 [0.98-2.12]	1.41 [0.97-2.11]	1.55 [1.10-2.21]	<0.001
TC (mmol/L, median[IQR])	4.83 [4.23-5.48]	4.81 [4.22-5.46]	4.94 [4.32-5.61]	<0.001
LDL-C (mmol/L, median[IQR])	2.99 [2.46-3.53]	2.98 [2.46-3.52]	3.05 [2.52-3.59]	<0.001
HDL-C (mmol/L, median[IQR])	1.22 [1.02-1.47]	1.23 [1.03-1.48]	1.19 [1.01-1.42]	<0.001
ApoA-I (g/L, median[IQR])	1.30 [1.14-1.48]	1.30 [1.15-1.48]	1.27 [1.12-1.45]	<0.001
ApoB (g/L, median[IQR])	0.91 [0.77-1.08]	0.91 [0.76-1.07]	0.95 [0.80-1.12]	<0.001
HGB (g/L, median[IQR])	147.00 [135.00-157.00]	147.00 [134.00-157.00]	150.00 [139.80-159.00]	<0.001
WBC (10^9/L, median[IQR])	5.93 [5.03-7.00]	5.90 [5.00-7.00]	6.04 [5.16-7.10]	<0.001
ALT (U/L, median[IQR])	20.00 [14.30-29.60]	20.00 [14.10-29.70]	20.60 [15.00-29.00]	<0.001
Estimated GFR (ml/min/1.73 m^2, median[IQR])	112.06 [98.23-128.88]	115.88 [103.63-131.76]	82.75 [76.23-86.83]	<0.001
Scr (μmol/L, median[IQR])	69.00 [59.00-78.20]	67.00 [57.00-75.00]	90.50 [86.00-96.30]	<0.001
SUA (μmol/L, median[IQR])	336.00 [274.00-398.00]	329.00 [268.10-391.00]	380.30 [325.00-440.50]	<0.001
BUN (mmol/L, median[IQR])	5.00 [4.20-5.83]	4.90 [4.11-5.7]	5.70 [4.80-6.68]	<0.001
Hypertension, n(%)	33067 (22.5)	27441 (24.7)	5626 (34.8)	<0.001
Diabetes, n(%)	19512 (13.3)	16954 (13.4)	2558 (13.5)	0.600
Hyperuricemia, n(%)	21092 (14.3)	15864 (12.4)	5228 (27.4)	<0.001

IQR, interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HGB, hemoglobin; WBC, white blood cell; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; SUA, Serum uric acid; BUN, blood urea nitrogen.
*P values were calculated by the Kruskal-Wallis test for continuous variables, as well as the chi-square test or Fisher's exact test for categorical variables.

hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use, and smoke status, the baseline ApoB increase was still correlated with the occurrence of CKD (HR, 1.61; 95% CI: 1.02-2.54) (P = 0.042) (Table 4).

Subgroup analysis

The associations between baseline ApoB levels and the occurrence of CKD were analyzed in individuals with or without hypertension,

TABLE 2 Association between ApoB and the prevalence of CKD in the cross-section population.

Model	OR(95%CI)		*P* value*
	ApoB(g/L) ≤ 1.1	ApoB(g/L) > 1.1	
Crude	Ref	1.37 (1.32,1.42)	< 0.001
Model1	Ref	1.30 (1.26,1.35)	< 0.001
Model2	Ref	1.07 (1.02,1.11)	0.006

OR, odds ratio; CI, confidence interval; ApoB, apolipoprotein B; CKD, chronic kidney disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HGB, hemoglobin; WBC, white blood cell.
Model1, adjusted for age, sex and BMI.
Model2, adjusted for age, sex, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use and smoke status.
*P values were calculated based on logistic regression model.

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TABLE 3 Baseline characteristics of the longitudinal cohort.

Characteristic	Overall (N=3325)	eGFR ≥ 90 ml/min/1.73 m^2 (N=3140)	eGFR < 90 ml/min/1.73 m^2 (N=185)	*P* value*
Sex, male, n(%)	2273 (68.30)	2111 (67.20)	162 (87.60)	<0.001
Age, median[IQR] years	47 [42-52]	47 [42-51]	50 [45-55]	<0.001
BMI (kg/m^2, median[IQR])	25.30 [23.23-27.42]	25.27 [23.18-27.42]	25.55 [23.88-27.42]	0.043
SBP (mmHg, median[IQR])	119 [109-130]	119 [109-130]	120 [112-133]	0.006
DBP (mmHg, median[IQR])	78.00 [71.00-85.00]	77.00 [71.00-85.00]	80.00 [74.00-85.25]	0.004
Serum glucose (mmol/L, median[IQR])	5.35 [4.97-5.89]	5.36 [4.97-5.90]	5.32 [4.95-5.79]	0.753
TG (mmol/L, median[IQR])	1.53 [1.05-2.32]	1.52 [1.04-2.30]	1.71 [1.20-2.43]	0.054
TC (mmol/L, median[IQR])	4.85 [4.26-5.50]	4.84 [4.26-5.49]	5.02 [4.29-5.64]	0.214
LDL-C (mmol/L, median[IQR])	3.06 [2.51-3.60]	3.05 [2.51-3.60]	3.15 [2.53-3.76]	0.209
HDL-C (mmol/L, median[IQR])	1.17 [0.99-1.40]	1.17 [0.99-1.40]	1.14 [0.99-1.31]	0.120
ApoA-I (g/L, median[IQR])	1.31 [1.17-1.48]	1.31 [1.17-1.48]	1.29 [1.16-1.43]	0.240
ApoB (g/L, median[IQR])	0.89 [0.74-1.05]	0.89 [0.74-1.05]	0.90 [0.75-1.10]	0.124
HGB (g/L, median[IQR])	149 [137-158]	149 [136-158]	154 [145-160]	<0.001
WBC (10^9/L, median[IQR])	5.80 [4.95-6.84]	5.79 [4.94-6.83]	6.16 [5.27-7.08]	0.004
ALT (U/L, median[IQR])	21.50 [14.7-32.20]	21.40 [14.60-32.20]	23.20 [16.65-31.73]	0.147
Estimated GFR (ml/min/1.73 m^2, median[IQR])	116.03 [104.69-130.82]	117.17 [106.27-131.89]	97.30 [93.30-103.71]	<0.001
Scr (μmol/L, median[IQR])	67.00 [58.00-75.00]	67.00 [57.10-74.60]	79.90 [73.00-83.10]	<0.001
SUA (μmol/L, median[IQR])	341.00 [277.30-400.50]	338.00 [275.00-399.00]	373.00 [339.00-430.00]	<0.001
BUN (mmol/L, median[IQR])	5.04 [4.30-5.90]	5.00 [4.30-5.90]	5.40 [4.60-6.18]	<0.001
Hypertension, n(%)	613 (18.40)	571 (18.20)	42 (22.80)	0.140
Diabetes, n(%)	439 (13.20)	417 (13.30)	22 (11.90)	0.670
Hyperuricemia, n(%)	410 (12.30)	374 (11.90)	36 (19.50)	<0.001

IQR, interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HGB, hemoglobin; WBC, white blood cell; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; SUA, Serum uric acid; BUN, blood urea nitrogen.
*P values were calculated by the Kruskal-Wallis test for continuous variables, as well as the chi-square test or Fisher's exact test for categorical variables.

TABLE 4 Association between the baseline ApoB and incident of CKD in the longitudinal cohort.

Model	HR(95%CI)		*P* value*
	ApoB(g/L) ≤ 1.1	ApoB(g/L) > 1.1	
Crude	Ref	1.50 (1.07,2.11)	0.019
Model1	Ref	1.48 (1.05,2.08)	0.024
Model2	Ref	1.61 (1.02,2.54)	0.042

HR, hazard ratio; CI, confidence interval; ApoB, apolipoprotein B; CKD, chronic kidney disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HGB, hemoglobin; WBC, white blood cell.
Model1, adjusted for age, sex and BMI.
Model2, adjusted for age, sex, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use and smoke status.
*P values were calculated based on Cox regression model.

diabetes, hyperuricemia, and hypohemia (Figure 2). In model 1, adjusting for confounders, the HRs were 1.71 (95% CI: 1.03-2.86) (P = 0.040) of the group without hypertension, 2.18 (95% CI: 1.24-3.82) (P = 0.007) of the group without hyperuricemia, 1.95 (95% CI: 1.14-3.32) (P = 0.015) of the group without hypohemia. However, in subgroups with hypertension, hyperuricemia and hypohemia, the associations between ApoB and CKD disappeared.

Sensitivity analysis

In sensitivity analysis I, we validated the results by increasing the duration of the follow-up to 4 years. When adjusted for sex, age, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use, and smoke status, ApoB at baseline remained significantly correlated with the CKD occurrence (HR, 1.76; 95% CI: 1.00-3.07) (P = 0.048) (Table 5). In sensitivity analysis II, the mixed-effects Cox regression model was applied. The association of the baseline ApoB and the development of CKD remained significant (HR, 1.62; 95% CI: 1.03-2.56) (P = 0.038) (Table 6). In the results of E-value analysis, we found that the estimated point of the primary endpoint in the Cox model was 2.60. Since this value is greater than the strong confounders, unmeasured confounders are unlikely to overcome the association between elevated ApoB and the occurrence of CKD

Discussion

Our study identified the positive association between serum ApoB level and CKD both in the cross-sectional study and the retrospective cohort. As far as we know, the current study is the first in China to elucidate the correlation based on a large data set of 146,533 individuals. We found that ApoB outperformed other lipid characteristics with the highest correlation coefficients with CKD, and the increase of ApoB has a positive correlation with CKD prevalence after fully adjusting for covariates in the cross-sectional population. We also observed a positive correlation between high serum ApoB levels and new-onset eGFR decline in the longitudinal cohort. In our subgroup analyses, higher ApoB levels were correlated with the incidence of CKD in people with non-hypertension, non-hyperuricemic, and non-anemic after adjustment for confounders.

Dyslipidemia is known to be an independent risk factor for CKD. ApoB is a special apolipoprotein of lipid profile (29, 30). In this study, we first found that the serum ApoB had a relatively stronger correlation with CKD compared with other variables in lipids. In the cross-sectional study, serum ApoB increases were notably correlated with the prevalence of CKD stages 2-5. Our results are consistent with previous findings based on the Chinese population, showing a great positive relationship between ApoB and the stages of CKD in cross-section (31). The other cross-section study by Mazidi et al. is main finding that individuals with high ApoB levels is more likely to develop CKD (eGFR < 60 ml/min/1.73 m^2) when LDL-C and ApoB are inconsistent, even after adjusting for a range of confounding variables (32). Ethnic differences may influence the association between ApoB and CKD. As shown in earlier cross-sectional research relied on the National Health and Nutrition Examination Survey (NHANES) III, ApoB was not associated with stages 3 to 5 of CKD (eGFR < 60 ml/min/1.73 m^2) after adjusting for confounders (30). The high ApoB level at baseline predicted subsequent CKD development in this longitudinal cohort study. Consistent with our findings, in a study of Korean men followed for 5 years, higher serum ApoB and the ratio of ApoB to ApoA-I values were associated with lower level eGFR at baseline and a higher risk of subsequent eGFR reduction (eGFR < 60 ml/min/1.73 m^2) (33). However, a retrospective longitudinal analysis of 10,288 subjects with a mean follow-up of 42.2-70.8 months showed that LDL-C/ApoB and HDL-C/ApoA-1 ratios, but not ApoB concentration, independently predicted an increased risk of developing CKD (eGFR < 60 ml/min/1.73 m^2). The differences in cohort results may be due to the differences in sample size and follow-up time (34). Different from conventional blood tests, nuclear magnetic resonance spectroscopy was used to quantify plasma levels of lipoprotein particles and their lipid components and low eGFR was also found to be significantly correlated with high levels of ApoB and the count of lipoprotein particles that contain most of the ApoB in a prospective study in Mexico (35).

A comprehensive assessment of the association between decreased eGFR and lipid variables in Kazakh hypertensives reported that serum ApoB level is significantly negatively

TABLE 5 Association between the baseline ApoB and incident of CKD in the 4 years longitudinal cohort.

Model	HR(95%CI)		*P* value*
	ApoB(g/L) ≤ 1.1	ApoB(g/L) >1.1	
Crude	Ref	1.83 (1.22, 2.75)	0.004
Model1	Ref	1.81 (1.20, 2.73)	0.004
Model2	Ref	1.76 (1.00, 3.07)	0.048

HR, hazard ratio; CI, confidence interval; ApoB, apolipoprotein B; CKD, chronic kidney disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HGB, hemoglobin; WBC, white blood cell.
Model1, adjusted for age, sex and BMI.
Model2, adjusted for age, sex, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use and smoke status.
*P values were calculated based on Cox regression model.

10.3389/fendo.2023.1083614

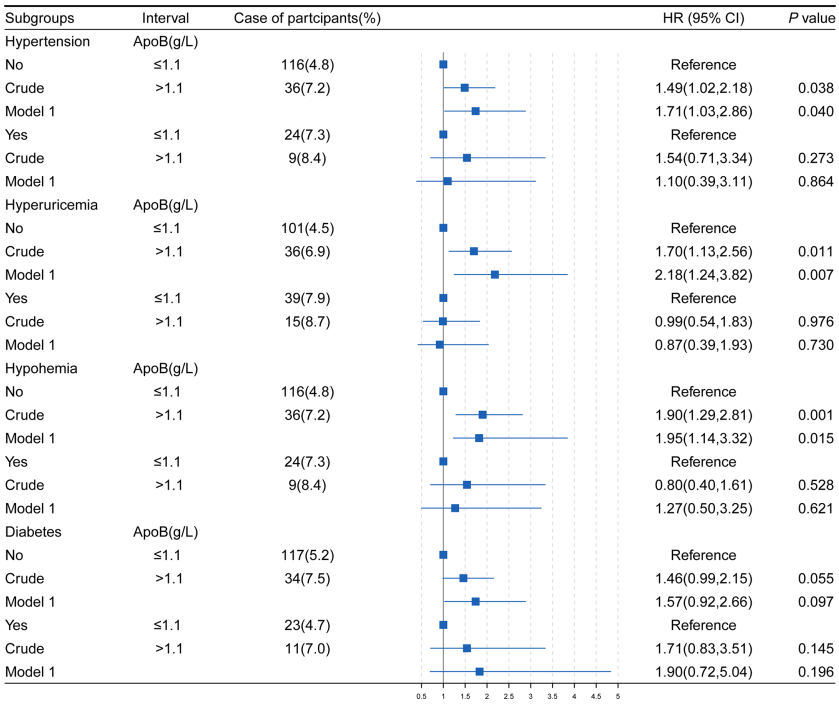

FIGURE 2
The association of ApoB and the development of CKD in study subjects stratifled by hypertension, hyperuricemia, hypohemia, diabetes. HR, hazard ratio; CI, confidence interval; BMI, body mass index; HGB, hemoglobin; ApoB, apolipoprotein B; TG, triglyceride; TC, total cholesterol; WBC, white blood cell. Model1, adjusted for age, sex, BMI, hypertension, diabetes, hyperuricemia, HGB, TG, TC, WBC, alcohol use and smoke status. *P values were calculated based on Cox regression.

associated with the early pre-CKD condition, and the final model was still significantly fitted even after adjustment of confounding factors, including diet, age, quality score and income (36). Hence, for patients with different comorbidities, the associations of increased serum ApoB level transitions with the development of CKD, and whether comorbidities can modify the effect are worth exploring. Therefore, we analyze the relationship between ApoB and eGFR decline in participants with diabetes, hypertension, dyslipidemia, and hypohemia. We have noticed the weakened association between ApoB and CKD in patients with hypertensive, hyperuricemia and hypohemia. It is known that hypertensive, hyperuricemia, hypohemia, and diabetes are strong risk factors for the initiation and development of CKD (37–40). The association between ApoB and CKD may be masked in patients with such diseases. Our study provided a hypothesis that ApoB may be an independent risk factor for CKD, especially in populations with hypertensive, hyperuricemia and hypohemia. A perspective study is warranted to confirm the causal relationship between ApoB and CKD in the general population and in patients with comorbidities.

CKD is a slowly progressing disease exposed to multiple risk factors. Dyslipidemia is one of the important propathogenic factors. ApoB, as an important component of the lipid profile, may affect the development and progression of CKD through the following mechanisms. High levels of ApoB-containing lipoproteins may initiate and promote atherosclerosis. When the plasma LDL-C

TABLE 6 Association between baseline ApoB and incident CKD by Mixed-effects Cox regression in the longitudinal cohort.

Model	HR (95%CI)		*P* value*
	ApoB(g/L) ≤ 1.1	ApoB(g/L) > 1.1	
Crude	Ref	1.51 (1.07,2.12)	0.018
Model1	Ref	1.49 (1.06,2.09)	0.022
Model2	Ref	1.62 (1.03,2.56)	0.038

Multivariable HR, Multivariable hazard ratio; CI, confidence interval; ApoB, apolipoprotein B; CKD, chronic kidney disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HGB, hemoglobin; WBC, white blood cell.
Model1, adjusted for age, sex and BMI.
Model2, adjusted for age, sex, BMI, hypertension, diabetes, hyperuricemia, TG, TC, WBC, HGB, alcohol use, smoke status and medical center as random effect.
*P values were calculated based on Mixed-effects Cox regression model.

and very low-density lipoprotein increase, the proportion of lipoprotein particles that contain most of the ApoB entering the arterial wall increases, and the fraction that cannot diffuse back into the circulation binds to the arterial wall proteoglycans (41, 42). The interaction between proteoglycans and ApoB motivates the accumulation of lipid particles in the subendothelium, which can accelerate oxidation and inflammation in the vascular wall (43, 44). With high concentrations of ApoB, glomerular endothelial cells and renal vessels may undergo a high level of oxidation stress and inflammation, and arteriosclerosis occurs in small and medium vessels, leading to decreased eGFR and CKD progression (45, 46). The mechanisms underlying the interaction between apolipoproteins, dyslipidemia, and CKD are complex.

Currently, the reagent inhibits the production of ApoB and is mainly used for treating atherosclerosis. It was found that the ApoB-100 peptide P210 vaccine significantly attenuated aortic atherosclerosis in a humanized mouse model (47). The decrease of dose-dependent in apolipoproteins, such as Lp(a), and LDL-C, were observed after the administration of mipomersen in clinical trials (13). If ApoB proves to be a risk factor for CKD, mipomersen may be an alternative strategy for alleviating such metabolic risk-associated impairment in kidney function.

Limitations

Some limitations exist in our study. First, due to the inherent bias of retrospective design, even if we use several statistical models to adjust for potential bias and perform sensitivity analyses to show that overall unmeasured confounders are unlikely to undermine our main conclusion, there will still be unforeseen confounders that could potentially alter the extent to which ApoB affects the occurrence of CKD. The relationship between serum ApoB and the incidence of CKD needs to be prospectively validated. Second, the population of this study was based on some community health examinations in China rather than random sampling, and there may be participant selection bias in this study. Third, using serum creatinine to estimate GFR, rather than direct measurement of kidney function, may overestimate or underestimate the actual GFR.

Conclusion

In summary, serum ApoB level has the strongest correlation with CKD among all lipid variables in the Chinese population. Moreover, the increase of serum ApoB level might precede the occurrence of CKD, suggesting that monitoring and reducing serum ApoB levels may provide an alternative approach for the prevention and treatment of CKD.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to HL, lihl@whu.edu.cn.

Ethics statement

The studies involving human participants were reviewed and approved by the central ethics board of Renmin Hospital.

Author contributions

YX and BL designed the study, collected and analyzed data, and drafted the manuscript. LL, FL, and TS performed the statistical analysis and interpreted data. XZ, XS, XH, and QZ assisted in data collection. JC performed critical revision of the manuscript for important intellectual content. ZW and HL conceived and supervised the study and made critical revisions to the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from the Medical flight plan of Wuhan University [TFJH2018006], the Hubei Province Innovation Platform Construction Project [2020420111730307223 8], and Wuhan Science and Technology Planning Project [2020021105012439].

Acknowledgments

We would like to thank the Supercomputer Center of Wuhan University for its support and help in this research.

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/fendo.2023.1083614/full#supplementary-material

References

1. 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

2. Liyanage T, Toyama T, Hockham C, Ninomiya T, Perkovic V, Woodward M, et al. Prevalence of chronic kidney disease in Asia: a systematic review and analysis. *BMJ Glob Health* (2022) 7:e007525. doi: 10.1136/bmjgh-2021-007525

3. Elshahat S, Cockwell P, Maxwell AP, Maxwell AP, Griffin M, O'Brien T, et al. The impact of chronic kidney disease on developed countries from a health economics perspective: A systematic scoping review. *PloS One* (2020) 15(3):e0230512. doi: 10.1371/journal.pone.0230512

4. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet* (2018) 392(10159):2052–90. doi: 10.1016/S0140-6736(18)31694-5

5. Huh JH, Yadav D, Kim JS, Son JW, Choi E, Kim SH, et al. An association of metabolic syndrome and chronic kidney disease from a 10-year prospective cohort study. *Metabolism* (2017) 67:54–61. doi: 10.1016/j.metabol.2016.11.003

6. Fox CS, Larson MG, Leip EP, Culleton B, Wilson PW, Levy D. Predictors of new-onset kidney disease in a community-based population. *JAMA* (2004) 291:844–50. doi: 10.1001/jama.291.7.844

7. Ruan XZ, Varghese Z, Moorhead JF. An update on the lipid nephrotoxicity hypothesis. *Nat Rev Nephrol* (2009) 5:713–21. doi: 10.1038/nrneph.2009.184

8. Ference BA, Kastelein JJP, Catapano AL. Lipids and lipoproteins in 2020. *JAMA* (2020) 324:595–6. doi: 10.1001/jama.2020.5685

9. Sniderman AD. ApoB vs non-HDL-C vs LDL-c as markers of cardiovascular disease. *Clin Chem* (2021) 67:1440–2. doi: 10.1093/clinchem/hvab140

10. Ference BA, Kastelein JJP, Ginsberg HN, Chapman MJ, Nicholls SJ, Ray KK, et al. Association of genetic variants related to CETP inhibitors and statins with lipoprotein levels and cardiovascular risk. *JAMA* (2017) 318:947–56. doi: 10.1001/jama.2017.11467

11. Sniderman AD, Navar AM, Thanassoulis G. Apolipoprotein b vs low-density lipoprotein cholesterol and non-High-Density lipoprotein cholesterol as the primary measure of apolipoprotein b lipoprotein-related risk: The debate is over. *JAMA Cardiol* (2022) 7:257–8. doi: 10.1001/jamacardio

12. Nurmohamed NS, Navar AM, Kastelein JJP. New and emerging therapies for reduction of LDL-cholesterol and apolipoprotein b: JACC focus seminar 1/4. *J Am Coll Cardiol* (2021) 77:1564–75. doi: 10.1016/j.jacc.2020.11.079

13. Lu Y, Cui X, Zhang L, Xu Y, Qin Z, Liu G, et al. The functional role of lipoproteins in atherosclerosis: Novel directions for diagnosis and targeting therapy. *Aging Dis* (2022) 13:491–520. doi: 10.14336/AD.2021.0929

14. Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, et al. Mipomersen, an apolipoprotein b synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet* (2010) 375:998–1006. doi: 10.1016/S0140-6736(10)60284-X

15. General Administration of Sport. *National physical fitness standard manual (Adult part)*. Beijing: Beijing People's Sports Publishing House (2003).

16. Wang Z, Chen Z, Zhang L, Wang X, Hao G, Zhang Z, et al. Status of hypertension in China: Results from the China hypertension survey, 2012-2015. *Circulation* (2018) 137:2344–56. doi: 10.1161/CIRCULATIONAHA.117.032380

17. Clinical Laboratory Centre, Ministry of Health. *National clinical laboratory procedures*. Nanjing: Southeast University Press (2006).

18. Guo W, Pan BS. Updates for clinical laboratories from K/DOQI 2002 to KDIGO 2012 clinical practice guidelines for chronic kidney disease. *J Lab Med* (2015) 7:663–7. doi: 10.3969/j.issn.1673-8640.2015.07.001

19. Joint committee issued Chinese guideline for the management of dyslipidemia in adults. *Chinese Guideline for the management of dyslipidemia in adults*. Zhonghua Xin Xue Guan Bing Za Zhi. 44(10):833–53. doi: 10.3760/cma.j.issn.0253-3758.2016.10.005.

20. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol* (2006) 17:2937–44. doi: 10.1681/ASN.2006040368

21. Stevens PE, Levin A. Kidney disease: Improving global outcomes chronic kidney disease guideline development work group members. 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

22. Joint Committee For Guideline Revision. Chinese Guidelines for prevention and treatment of hypertension-a report of the revision committee of Chinese guidelines for prevention and treatment of hypertension. *J Geriatr Cardiol* (2019) 16:182–241. doi: 10.11909/j.issn.1671-5411.2019.03.014

23. Chinese Diabetes Society. Guideline for the prevention and treatment of type 2 diabetes mellitus in China. *Chin J Endocrinol Metab* (2021) 37:311–98. doi: 10.3760/cma.j.cn311282-20210304-00142

24. Kidney Internist Branch of Chinese Medical Doctor Association. Chinese Practice guidelines for diagnosis and treatment of hyperuricemia in renal disease. *Chin Med J* (2017) 97:1927–36. doi: 10.3760/cma.j.issn.0376-2491.2017.25.003

25. Wan XH, Lu XF. Diagnostics. In: *People's medical publishing house*. Beijing: People's Medical Publishing House. (2018). p. 237

26. Waljee AK, Mukherjee A, Singal AG, Zhang Y, Warren J, Balis U, et al. Comparison of imputation methods for missing laboratory data in medicine. *BMJ Open* (2013) 3:e002847. doi: 10.1136/bmjopen-2013-002847

27. Alarcón-Soto Y, Langohr K, Fehér C, García F, Gómez G. Multiple imputation approach for interval-censored time to HIV RNA viral rebound within a mixed effects cox model. *Biom J* (2019) 61(2):299–318. doi: 10.1002/bimj.201700291

28. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: Introducing the e-value. *Ann Intern Med* (2017) 167(4):268–74. doi: 10.7326/M16-2607

29. Kwon S, Kim DK, Oh KH, Joo KW, Lim CS, Kim YS, et al. Apolipoprotein b is a risk factor for end-stage renal disease. *Clin Kidney J* (2021) 14:617–23. doi: 10.1093/ckj/sfz186

30. Goek ON, Köttgen A, Hoogeveen RC, Ballantyne CM, Coresh J, Astor BC. Association of apolipoprotein A1 and b with kidney function and chronic kidney disease in two multiethnic population samples. *Nephrol Dial Transplant* (2012) 27:2839–47. doi: 10.1093/ndt/gfr795

31. Zhao W, Li J, Zhang X, Zhou X, Xu J, Liu X, et al. Apolipoprotein b and renal function: across-sectional study from the China health and nutrition survey. *Lipids Health Dis* (2020) 19:110. doi: 10.1186/s12944-020-01241-7

32. Mazidi M, Webb RJ, Lip GYH, Kengne AP, Banach M, Davies IG. Discordance between LDL-c and apolipoprotein b levels and its association with renal dysfunction: Insights from a population-based study. *J Clin Med* (2022) 11(2):313. doi: 10.3390/jcm11020313

33. Ryoo JH. Association of apolipoproteins with incidence of decreased estimated GFR in Korean men. *Am J Kidney Dis* (2013) 61:564–70. doi: 10.1053/j.ajkd.2012.11.039

34. Bae JC, Han JM, Kwon S, Jee JH, Yu TY, Lee MK, et al. LDL-C/apoB and HDL-C/apoA-1 ratios predict incident chronic kidney disease in a large apparently healthy cohort. *Atherosclerosis* (2016) 251:170–6. doi: 10.1016/j.atherosclerosis.2016.06.029

35. Aguilar-Ramirez D, Alegre-Díaz J, Herrington WG, taplin N, Ramirez-Reyes R, Gnatiuc L, et al. Association of kidney function with NMR-quantified lipids, lipoproteins, and metabolic measures in Mexican adults. *J Clin Endocrinol Metab* (2021) 106:2828–39. doi: 10.1210/clinem/dgab497

36. Nurtazina A, Kozhakhmetova D, Dautov D, Khaidarova N, Chattu VK. Association of early renal dysfunction with lipid profile parameters among hypertensives in Kazakhstan. *Diagnostics (Basel)* (2021) 11:871. doi: 10.3390/diagnostics11050871

37. Zhu Q, Chen Y, Cai X, Cai L, Hong J, Luo Q, et al. The non-linear relationship between triglyceride-glucose index and risk of chronic kidney disease in hypertensive patients with abnormal glucose metabolism: A cohort study. *Front Med (Lausanne)* (2022) 9:1018083. doi: 10.3389/fmed.2022.1018083

38. Hamrahian SM, Falkner B. Hypertension in chronic kidney disease. *Adv Exp Med Biol* (2017) 956:307–25. doi: 10.1007/5584_2016_84

39. Johnson RJ, Bakris GL, Borghi C, Chonchol MB, Feldman D, Lanaspa MA, et al. Hyperuricemia, acute and chronic kidney disease, hypertension, and cardiovascular disease: Report of a scientific workshop organized by the national kidney foundation. *Am J Kidney Dis* (2018) 71(6):851–65. doi: 10.1053/j.ajkd.2017.12.009

40. Lankhorst CE, Wish JB. Anemia in renal disease: diagnosis and management. *Blood Rev* (2010) 24(1):39–47. doi: 10.1016/j.blre.2009.09.001

41. Hurt-Camejo E, Olsson U, Wiklund O, Bondjers G, Camejo G. Cellular consequences of the association of apoB lipoproteins with proteoglycans. potential contribution to atherogenesis. *Arterioscler Thromb Vasc Biol* (1997) 17:1011–17. doi: 10.1161/01.atv.17.6.1011

42. Nordestgaard BG, Nielsen LB. Atherosclerosis and arterial influx of lipoproteins. *Curr Opin Lipidol* (1994) 5:252–7. doi: 10.1097/00041433-199408000-00002

43. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* (2011) 473:317–25. doi: 10.1038/nature10146

44. Bae YS, Lee JH, Choi SH, Kim S, Almazan F, Witztum JL, et al. Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4- and spleen tyrosine kinase-dependent activation of NADPH oxidase 2. *Circ Res* (2009) 104:210–8. doi: 10.1161/CIRCRESAHA.108.181040

45. Scheuer H, Gwinner W, Hohbach J, Gröne EF, Brandes RP, Malle E, et al. Oxidant stress in hyperlipidemia-induced renal damage. *Am J Physiol Renal Physiol* (2000) 278:F63–74. doi: 10.1152/ajprenal.2000.278.1.F63

46. Trevisan R, Dodesini AR, Lepore G. Lipids and renal disease. *J Am Soc Nephrol* (2006) 17:S145–7. doi: 10.1681/ASN.2005121320

47. Chyu KY, Zhao X, Zhou J, Dimayuga PC, Lio NW, Cercek B, et al. Immunization using ApoB-100 peptide-linked nanoparticles reduces atherosclerosis. *JCI Insight* (2022) 7:e149741. doi: 10.1172/jci.insight.149741

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 19 May 2023
DOI 10.3389/fmed.2023.1086359

OPEN ACCESS

EDITED BY
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RECEIVED 01 November 2022
ACCEPTED 11 April 2023
PUBLISHED 19 May 2023

CITATION
Farah Yusuf Mohamud M, Nur Adan F, Osman Omar Jeele M and Ahmed MAM (2023) Major cardiovascular events and associated factors among routine hemodialysis patients with end-stage renal disease at tertiary care hospital in Somalia. *Front. Med.* 10:1086359. doi: 10.3389/fmed.2023.1086359

Major cardiovascular events and associated factors among routine hemodialysis patients with end-stage renal disease at tertiary care hospital in Somalia

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Introduction: Cardiovascular complications are the most significant cause of death in patients undergoing routine hemodialysi (HD) with end-stage renal disease (ESRD). The main objective of this study is to determine the significant cardiac events and risk factors in patients undergoing routine hemodialysis in Somalia.

Methods: We carried out a cross-sectional retrospective study in a single dialysis center in Somalia. Two hundred out of 224 were included. All of them had ESRD and were on hemodialysis during the study period between May and October 2021. The records of all patients were reviewed, and the following parameters were analyzed socio-demographic factors, risk factors for cardiovascular disease, and the presence of cardiovascular diseases.

Results: The mean age was 54 ± 17.5 years (range 18–88 years), and 106 (53%) patients were males. The prevalence of a cardiovascular disease among hemodialysis patients was 29.5%. Moreover, the distribution of cardiovascular diseases was different; heart failure was the most common, about 27.1%, followed by coronary artery disease (17%), pericarditis and pericardial-effusion (13.6%), dysrhythmia (10.2%), cerebrovascular-accident (8.5%), and peripheral vascular disease (3.4%). About 176 (88%) participants had at least one modifiable cardiovascular risk factor. The most common modifiable cardiovascular risk factor was hypertension ($n = 45$, 25.1%), followed by anemia ($n = 28$, 15.6%) and diabetes ($n = 26$, 14.5%). Younger (18–30) participants were six times less likely to have cardiovascular events among hemodialysis than older age 0.4 (0.11–1.12).

Conclusion: Low prevalence rate of cardiovascular complications was confirmed in ESRD patients receiving hemodialysis in the main HD center in Somalia. Diabetes, anemia, and hypertension were the highest significant risk factors for CVD in HD patients with ESRD in Somalia.

KEYWORDS

hemodialysis, end-stage renal disease, cardiovascular disease, heart failure, diabetic, hypertension

Introduction

CKD is becoming more common in Sub-Saharan Africa (SSA), primarily affecting young individuals in their prime for economic productivity. Additionally, many patients receive nephrologist referrals too late, are vulnerable to acute complications from dialysis, and struggle with infrastructure and financial issues that make it challenging to provide adequate dialysis to those with end-stage renal disease (1, 2). Although the prevalence of chronic kidney disease (CKD) in Somalia has not previously been studied, Muiru et al. reported that CKD in sub-Saharan Africa was determined to be 8% in 2020 (3).

Renal replacement therapy (RRT) as a whole and hemodialysis (HD) in particular remains a lifesaving intervention for a lot of patients whose kidneys do not work correctly (4). Nevertheless, in patients undergoing routine hemodialysis (HD), there is an increased risk for cardiovascular morbidity and mortality (5).

It's thought that cardiovascular diseases account for more than 50% of deaths among HD patients (6). Cardiovascular deaths among HD patients are also believed to be 10–20 times greater than in general (7). Sudden cardiac death is the leading cardiovascular death among HD patients, with 25% of all cardiovascular deaths (8).

Some researchers suggested that the HD process can activate the complement system, induce prothrombotic and proinflammatory responses in patients and thus predispose cardiovascular events to the development (9). Some researchers suggest that cardiovascular lesions may even appear before the initiation of the HD process in chronic renal failure patients, as chronic renal failure or end-stage renal disease (ESRD) is an independent risk factor for cardiovascular diseases (10). In addition, HD can lead to anemia and cause alteration in calcium and phosphate metabolism, which could play a significant risk factor for developing cardiovascular events (11).

To the best of our knowledge, the prevalence and risk factors of major cardiac events among patients undergoing HD in Somalia remain unknown. The main objective of this study is to determine the significant cardiac events and risk factors among patients undergoing routine hemodialysis in Somalia.

Methods

This retrospective study included all patients who have received the diagnostic code of ESRD in accordance with the International Classification of Diseases (ICD-10) system and underwent routine hemodialysis between May 2021 and October 2021 using the electronic hospital information system (HIS). Two hundred out of 224 patients who had ESRD and underwent routine hemodialysis (HD) were included in our study. Patients with renal transplantation, peritoneal dialysis, and those with incomplete data were excluded from the study.

The following parameters were analyzed: Socio-demographic and clinical parameters included age and gender, risk factors for cardiovascular disease (family history of coronary artery disease, diabetes mellitus, arterial hypertension, and dyslipidemia), anemia, duration on HD, and time of hemodialysis per week. The presence of cardiovascular diseases such as heart failure, coronary artery disease, dysrhythmia, pericarditis, pericardial effusion, cerebrovascular disease, and peripheral vascular disease was looked for in the hospital information system (FONET) record by using an electrocardiogram, two-dimensional and Doppler echocardiography, Doppler ultrasound, brain CT scan, and brain MRI records.

The Echocardiography was licensed in Turkey using a Toshiba Aplio^{TM} ultrasound system (TUS-A500, Shimoishigami, Japan) in accordance with the American Society of Echocardiography guidelines.

The presence of any of the following was considered a cardiovascular disease (CVD):

- Coronary heart disease: myocardial infarction or stable angina or unstable angina by assessing normal values for cardiac dimensions and EKG diagnostic criteria were obtained from standard references, or coronary artery bypasses graft or percutaneous coronary intervention (12).
- Heart failure: is defined as an aberrant left ventricular filling pattern and/or a mitral E/A ratio on echocardiography that is out of the range of 0.7–3.1 if under 64 years old or 0.5–1.7 if over 64 years old. b) Systolic dysfunction was defined as an ejection fraction of <50%.
- Cerebrovascular disease includes atherothrombotic cerebral infarction and transient ischemic attack with brain CT scan or MRI Confirmation.

- Peripheral vascular disease: is a chronic progressive atherosclerotic disease leading to partial or total peripheral vascular occlusion. PAD typically affects the abdominal aorta, iliac arteries, lower limbs, and occasionally the upper extremities.
- Dysrhythmias: evidence of ventricular tachycardia, fibrillation, or any other type of dysrhythmia on electrocardiographic criteria.
- Pericarditis and Pericardial effusion: diffuse ST-segment elevation on ECG, stiff or thick pericardium constricting the heart's normal movement or free fluid around the heart by echocardiography.

The study was carried out after receiving ethical approval and being granted permission by the research and ethical committee of the Mogadishu Somali Turkish Training and Research Hospital (Ref: MSTH/6384). This study was carried out in accordance with the Helsinki Declaration's contents. The information obtained from the medical records was kept strictly confidential and utilized only for research purposes. Furthermore, study participants are not recognized by name to ensure confidentiality.

Microsoft Excel and SPSS software version 23 were used to create the database. Continuous variables are presented as mean ± standard deviation, and categorical variables as the observed number of patients (percentage). Fisher's exact test was used for categorical variables to compare patient characteristics between groups (cardiac and non-cardiac events). For correlations, a correlation coefficient test was applied, binary logistic regression

was also used, and a p-value of <0.05 was considered statistically significant.

Results

In this retrospective observational study, 200 out of 224 routine hemodialysis patients at Mogadishu Somali Turkish Training and Research Hospital from May 1, 2021, to October 231, 2021, fulfilled the inclusion criteria and enrolled in the study.

Table 1 shows the socio-demographic characteristics of the 200 patients with HD with ESRD. The mean age was 54 ± 17.5 years (range 18–88 years), and 106 (53%) patients were males.

Based on the time of hemodialysis, most of the patients (78%) underwent hemodialysis twice a week. In comparison, 29 (14.5%) patients underwent one a week, and 15 (7.5%) patients underwent three times per week.

According to the hemodialysis duration, most patients (38.5%) experience hemodialysis for 1–5 years. Notably, the duration of hemodialysis < 1 year and >5 years were less, being experienced in 31 and 30.5%, respectively ($P = 0.222$) (Table 1).

This study revealed that about 176 (88%) of the study participants (hemodialysis patients with end-stage renal disease) had at least one modifiable cardiovascular risk factor. As shown in Figure 1, the most common modifiable cardiovascular risk factors among hemodialysis patients with end-stage renal disease were hypertension in 45 patients (25.1%), followed by anemia in 28 patients (15.6%), and diabetes mellitus in 26 patients (14.5%).

The prevalence of cardiovascular disease among hemodialysis patients with ESRD was 29.5%, as shown in Figure 2. About 27.1% of the hemodialysis patients with ESRD had heart failure, 17% had coronary artery disease, 13.6% had pericarditis and pericardial effusion, 10.2% had dysrhythmia, 8.5% had cerebrovascular accident, and 3.4% had peripheral vascular disease (Figure 3).

The data also showed among the 59 respondents that were diagnosed with at least one cardiovascular disease, near half of the respondents [30 out of 59 (50.8%)] were males. In comparison, females comprised the remaining 29 out of 59 respondents (49.2%) ($p = 0.757$).

Among the age subgroups, the prevalence of CVD was 16.9, 10.1, 44, and 28.8% in 18–30 years, 31–49 years, 51–69 years, and 70 years or older, respectively ($p = 0.086$).

Of the 16 respondents who were diagnosed with at least one cardiovascular disease, 23 (40%) had been on hemodialysis for 1 year or less, and 22 (37.3%) respondents have on hemodialysis for 2–5 years, while the remaining 14 respondents (23.7%) have been on hemodialysis for more than 5 years ($p = 0.222$).

Regarding the study population, 59 respondents were diagnosed with at least one cardiovascular disease. Most participants (84.7%) had undergone hemodialysis twice per week, while five (8.5%) had undergone hemodialysis once weekly. Only four (6.8%) participants had undergone hemodialysis three times or more per week ($p = 0.259$) (Table 2).

Table 2 shows younger (18–30) participants were six times less likely to have cardiovascular events among hemodialysis than older age 0.4 (0.11–1.12). Cardiovascular events were less in participants with previous risk factors than in those without.

Discussion

Chronic kidney disease (CKD) implies various degrees of declined renal function. The most severe and last stage of CKD is an end-stage renal disease (ESRD), which occurs when the kidneys

TABLE 1 Socio-demographic characteristics among hemodialysis patients with end-stage renal disease (*N*: 200).

Variables		All ($n = 2$) Freg (%)	Cardiac events		*P*-value
			Yes, $N = 59$	No, $N = 141$	
Age group	18–30 years	30 (15%)	10 (17%)	20 (14.2%)	0.086
	31–49 years	39 (19.5%)	6 (10.2%)	33 (23.4%)	
	50–69 years	89 (44.5%)	26 (44%)	63 (31.5%)	
	≥70 years	42 (21%)	17 (28.8%)	25 (12.5%)	
Sex	Male	106 (53%)	30 (50.8%)	76 (53.9%)	0.757
	Female	94 (47%)	29 (49.2%)	65 (46.1%)	
Time of hemodialysis	One a week	29 (14.5%)	5 (8.5%)	24 (17%)	0.259
	Twice a week	156 (78%)	50 (84.7%)	106 (75.2%)	
	Three times a week	15 (7.5%)	4 (6.8%)	11 (7.8%)	
Duration of hemodialysis	≤1 year	62 (31%)	23 (39%)	39 (27.6%)	0.222
	2–5 years	77 (38.5%)	22 (37.3%)	55 (39%)	
	>5 years	61 (30.5%)	14 (23.7%)	47 (33.3%)	
Risk factors	Yes	176 (88%)	56 (95%)	120 (85.1%)	0.135
	No	22 (12%)	3 (5%)	21 (14.9%)	

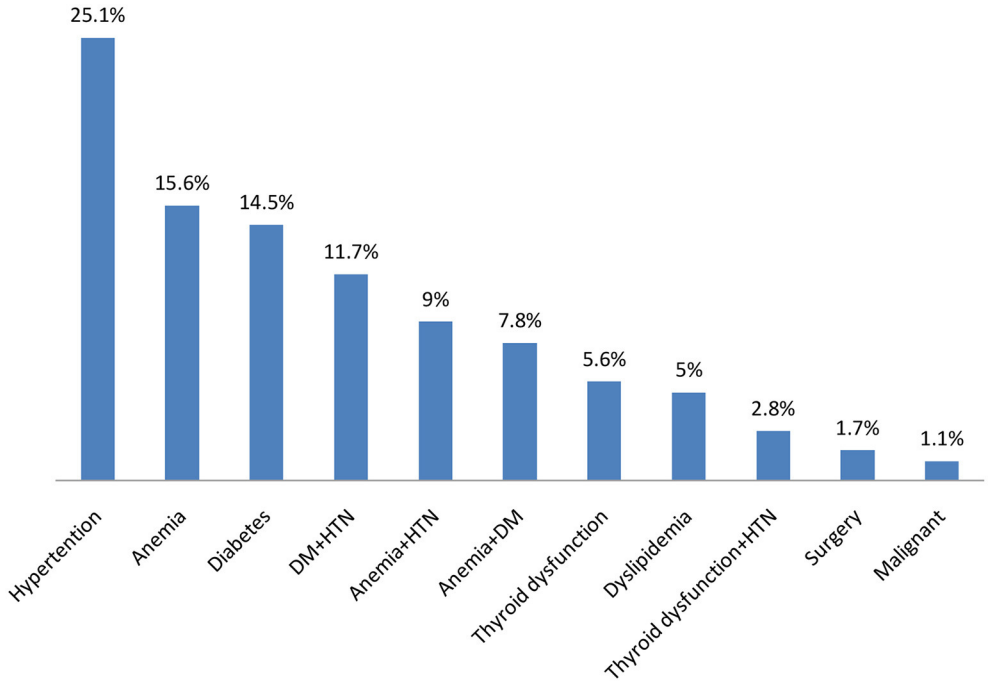

FIGURE 1
Cardiovascular risk factors among hemodialysis patients with ESRD in Somalia. DM, Diabetes; HTN, Hypertension.

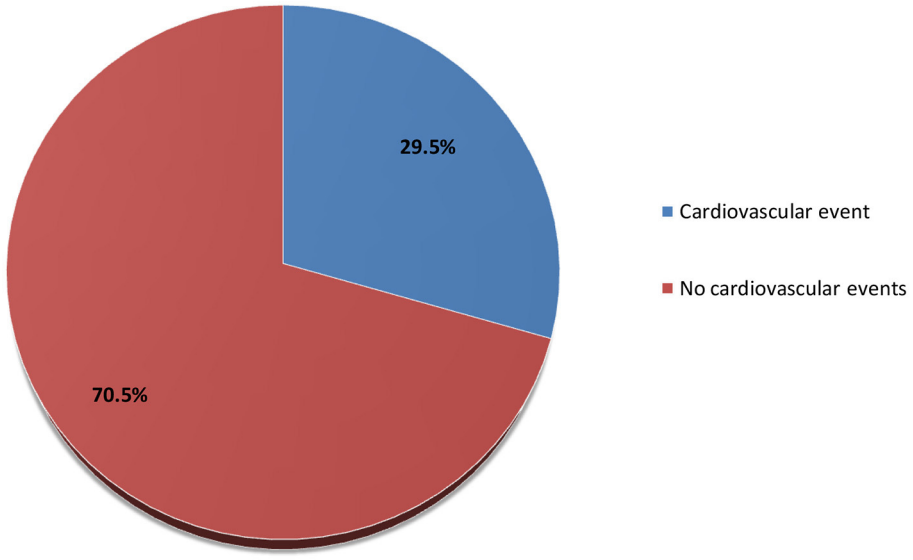

FIGURE 2
Prevalence of cardiovascular disease among hemodialysis patients with ESRD in Somalia.

cannot properly perform their essential functions. Finding regular hemodialysis or a kidney transplant is the only option available to individuals with ESRD to survive (13, 14).

Cardiovascular complications are the most significant cause of death in patients with end-stage renal disease (ESRD) on hemodialysis treatment (15). As early as 1836, Richard Bright suggested that the first cardiovascular disease (CVD) originated from renal disease (16).

The mechanism underlying the increased risk of cardiovascular events in patients with ESRD has not been well defined. In fact, a broad spectrum of risk factors influences cardiac function and structure in hemodialysis patients with ESRD.

Lindner et al. (17) discovered the significant burden of cardiovascular disease (CVD) in chronic renal disease (CRD) more than 40 years ago.

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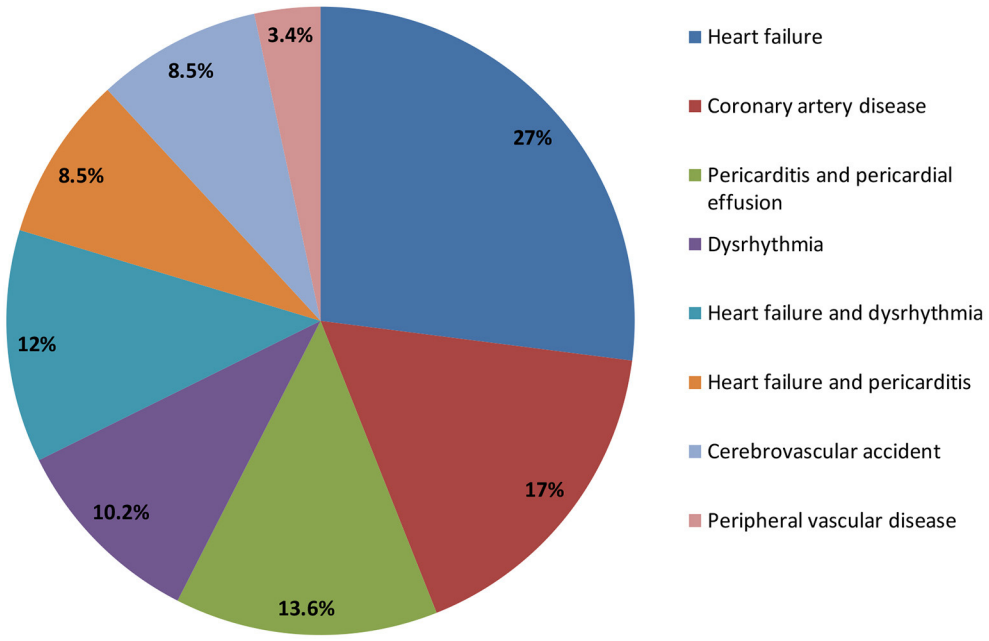

FIGURE 3
Distribution of cardiovascular events among hemodialysis patients with ESRD in Somalia.

TABLE 2 Age and risk factor differences in prevalence of cardiovascular events among routine hemodialysis patients with ESRD.

Variable	Odds ratio	95% CI	*p*-value
Age group			
18–30 years	1.0		
31–49 years	0.4	0.11–1.12	0.086
50–69 years	0.8	0.34–2.00	0.671
≥70 years	1.4	0.51–3.62	0.538
Risk factors			
Yes	1.0		
No	0.3	0.09–1.21	0.096

In general medical practice, patients in stages 3–4 CKD who have reduced renal function but are not in ESRD have a prevalence of ischemic heart disease of 25%, more than double the prevalence in patients without CKD, according to the NEORICA (New Opportunities for Early Renal Interventions by Computerized Assessment) study (18).

Cardiovascular disease is a common cause of death in hemodialysis patients, with a ratio of 10–20 times greater than in people with normal renal function (19).

Regarding the duration of HD, most patients (38.5%) had dialysis duration between 1 and 5 years, similar to study findings from Sudan (20).

Our findings showed that most of the participants (84.7%) had undergone hemodialysis twice per week, while five (8.5%) participants had undergone hemodialysis once per week. Only four (6.8%) participants had undergone hemodialysis three times or more weekly. In contrast to our report, a study from Ethiopia found that only 10.8% had undergone hemodialysis twice per week, while 89.2% had undergone hemodialysis Three times per week (21).

Our study found no association between cardiovascular disease and duration of HD and section of HD per week.

A hemodialysis study in the United States states that 40% of dialysis patients had cardiovascular disease at admission. Coronary artery disease was the cause of 63% of hospital admissions for cardiovascular causes (22).

In the present study, the prevalence of cardiovascular disease among hemodialysis patients with ESRD was 29.5% lower than that reported in previous studies (22, 23). Another study from Cameroon found that 84% of hemodialysis patients with ESRD had a cardiovascular illness, which is higher prevalent than this figure (2). The variation in cardiovascular events prevalence in our study compared with other reports could be due to several reasons: the diagnostic criteria for cardiovascular events in ESRD patients were not uniform, the different populations were genetically varied, or the inclusion criteria in the various studies may have been different.

The distribution of cardiovascular diseases among hemodialysis patients with ESRD was different. In our study, heart failure (27.1%) was the most common cardiovascular disease among hemodialysis patients with ESRD, followed by coronary artery disease (17%), pericarditis and pericardial effusion (13.6%), dysrhythmia (10.2%%), cerebrovascular accident (8.5%), and peripheral vascular disease (3.4%).

A randomized multi-center trial on 1,846 chronic hemodialysis patients at 15 clinical centers comprising 72 dialysis units, congestive heart failure (40%), ischemic heart disease (IHD) (39%), and arrhythmia (31%) were the most common prevalent cardiovascular disease (22).

Analysis of the Yaoundé General Hospital (YGH) hemodialysis center from December 2010 to February 2011 revealed that left ventricular hypertrophy (60%), valvular calcifications (38%), heart failure (36%), conduction disorders (33%), pericardial effusion (13%), valvular diseases (11%) and ischemic heart diseases (2%) were the highest distribution of cardiovascular diseases among hemodialysis patients with ESRD (2).

The increased prevalence of heart failure could be related to the higher prevalence of hypertension in this study sample which is the leading etiological factor of underlying renal disease. Another risk factor for heart failure is the high incidence of anemia caused by the low use of erythropoiesis-stimulating drugs. High cardiac output, a large stroke volume, increased heart rate, and deteriorating left ventricular dilatation are all associated with anemia (24).

A study from Spain on cardiovascular disease among hemodialysis patients reported that 16.7% had coronary disease, 13.9% had different degrees of heart failure, and 11.6% had arrhythmia (25). Rostand and his teammates reported that 73% of hemodialysis patients have coronary artery disease, representing the highest cardiovascular disease prevalence among ESRD patients (26).

In our study, 88% of dialysis patients had at least one pre-existing comorbidity before beginning dialysis therapy, significantly higher than the study published in Malaysia, which found that just 31.6% had such conditions (23). The most prevalent comorbidities in the current study were hypertension (21.5%), anemia (15.6%), and diabetes (14.4%). In bivariate or multivariate analyses, pre-existing comorbidities were also not statistically related to cardiovascular events. According to Lim et al. (23), hypertension (96.5% of all cases), diabetes (66.2%), and hyperlipidemia (58.1%) were the most prevalent comorbidities identified throughout their analysis.

Cardiovascular events were less in participants with previous risk factors than in those without. This may be due to progress in both prevention and treatment of CVD, including precipitous declines in cigarette smoking, improvements in hypertension and diabetic treatment and control, and widespread use of statins to lower circulating cholesterol levels.

Regarding the study population, most of the participants (84.7%) had undergone hemodialysis twice per week, while five (8.5%) participants had undergone hemodialysis once per week. Only four (6.8%) participants had undergone hemodialysis three times or more per week ($p = 0.259$). Inadequate or missed hemodialysis sessions also significantly affected cardiovascular disease among hemodialysis patients with ESRD. The overcrowding of our center for receiving many ESRD patients needing regular renal replacement therapy, lack of public awareness of the disease and the hemodialysis itself, discrimination, and social pressure on the patients were the leading factors of inadequate or missed hemodialysis sessions. In addition to this, insufficient skills of dialysis providers, higher costs belonging to each dialysis session that most of the patients are not affordable (low socioeconomic status), as well as; lack of access to the center because of rural and far distance distribution of the cases also played a role.

The limitations of our study included: a limited sample size, a retrospective study, and a single-center study that may not be representative of the country. Risk factors such as smoking, alcoholism, sedentary lifestyle, and obesity were not evaluated due to a retrospective study that cannot be obtained from the system. Several novel risk factors have yet to be explored due to technical drawbacks and the high cost of laboratory-based tests.

Despite the growing population of patients on maintenance hemodialysis in Somalia, there has been a relative lack of large clinical databases describing the specific cardiac diseases among HD patients with ESRD.

Although this study has several limitations, it is the first study to assess the prevalence, risk factors, and extent of cardiovascular disease in a significant condition in adult chronic hemodialysis patients in Somalia. This issue has been well addressed in adult ESRD patients.

Conclusion

Cardiovascular events are lower prevalent among hemodialysis patients with ESRD in Somalia when compared to other countries. The majority of the cardiovascular event confirmed in our HD patients were significantly higher in older patients and those with diabetes, anemia, and hypertension.

Data availability statement

We declared that we had full access to all of the data in this study and we take complete responsibility for the integrity of the data. All original data are available in the Mogadishu Somali Turkish Training and Research Hospital in Mogadishu, Somalia. Data used to support the findings of this study are available from the corresponding author upon request.

Ethics statement

The study was carried out after receiving ethical approval and granted permission from the Research and Ethical Committee of the Mogadishu Somali Turkish Training and Research Hospital (Ref: MSTH/6384). Written informed consent for participation was not required for this study in accordance with the National Legislation and the Institutional requirements.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, took part in drafting, revising or critically reviewing the article, gave final approval of the version to be published, have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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. Halle MP, Kengne AP, Ashuntantang G. Referral of patients with kidney impairment for specialist care in a developing country of sub-Saharan Africa. *Ren Fail.* (2009) 31:341–34. doi: 10.1080/08860220902882014

2. Kaze FF, Ashuntantang G, Kengne AP, Hassan A, Halle MP, Muna W. Acute hemodialysis complications in end-stage renal disease patients: the burden and implications for the underresourced Sub-Saharan African health systems. *Hemodial Int.* (2012) 16:526–31. doi: 10.1111/j.1542-4758.2012.00692.x

3. Muiru AN, Charlebois ED, Balzer LB, Kwarisiima D, Elly A, Black D, et al. The epidemiology of chronic kidney disease (CKD) in rural East Africa: a population-based study. *PLoS ONE.* (2020) 15:e0229649. doi: 10.1371/journal.pone.0229649

4. Robinson BM, Akizawa T, Jager KJ, Kerr PG, Saran R, Pisoni RL. Factors affecting outcomes in patients reaching end-stage kidney disease worldwide: differences in access to renal replacement therapy, modality use, and haemodialysis practices. *Lancet.* (2016) 388:294–306. doi: 10.1016/S0140-6736(16)30448-2

5. Li Z, Liu S, Liang X, Wang W, Fei H, Hu P, et al. Pulmonary hypertension as an independent predictor of cardiovascular mortality and events in hemodialysis patients. *Int Urol Nephrol.* (2014) 46:141–9. doi: 10.1007/s11255-013-0486-z

6. Shafi T, Powe NR, Meyer TW, Hwang S, Hai X, Melamed ML, et al. Trimethylamine N-oxide and cardiovascular events in hemodialysis patients. *J Am Soc Nephrol.* (2017) 28:321–31. doi: 10.1681/ASN.2016030374

7. Yotsueda R, Taniguchi M, Tanaka S, Eriguchi M, Fujisaki K, Torisu K, et al. Cardiothoracic ratio and all-cause mortality and cardiovascular disease events in hemodialysis patients: the Q-Cohort study. *Am J Kidney Dis.* (2017) 70:84–92. doi: 10.1053/j.ajkd.2016.11.026

8. Rutherford E, Ireland S, Mangion K, Stewart GA, MacGregor MS, Roditi G, et al. A randomized, controlled trial of the effect of allopurinol on left ventricular mass index in hemodialysis patients. *Kidney Int Rep.* (2021) 6:146–55. doi: 10.1016/j.ekir.2020.10.025

9. Poppelaars F, Gaya da Costa M, Faria B, Berger SP, Assa S, Daha MR, et al. Intradialytic complement activation precedes the development of cardiovascular events in hemodialysis patients. *Front Immunol.* (2018) 9:2070. doi: 10.3389/fimmu.2018.02070

10. Kobayashi S. Cardiovascular events in hemodialysis patients: challenging against vascular calcification. *Ann Vasc Dis.* (2017) 10:1–7. doi: 10.3400/avd.ra.17-00006

11. Arbiol-Roca A, Padró-Miquel A, Hueso M, Navarro E, Alía-Ramos P, González-Álvarez MT, et al. Association of ANRIL gene polymorphisms with major adverse cardiovascular events in hemodialysis patients. *Clin Chim Acta.* (2017) 466:61–7. doi: 10.1016/j.cca.2016.12.029

12. Ansari A, Kaupke CJ, Vaziri ND, Miller R, Barbari A. Cardiac pathology in patients with end-stage renal disease maintained on hemodialysis. *Int J Artif Organs.* (1993) 16:31–6. doi: 10.1177/039139889301600107

13. Muddu M, Mutebi E, Ssinabulya I, Kizito S, Mulindwa F, Kiiza CM. Utility of albumin to creatinine ratio in screening for microalbuminuria among newly diagnosed diabetic patients in Uganda: a cross sectional study. *Afr Health Sci.* (2019) 19:1607–16. doi: 10.4314/ahs.v19i1.36

14. Hodel NC, Hamad A, Praehauser C, Mwangoka G, Kasella IM, Reither K, et al. The epidemiology of chronic kidney disease and the association with non-communicable and communicable disorders in a population of sub-Saharan Africa. *PLoS ONE.* (2018) 13:e0205326. doi: 10.1371/journal.pone.0205326

15. Jardine AG, McLaughlin K. Cardiovascular complications of renal disease. *Heart.* (2001) 86:459–66. doi: 10.1136/heart.86.4.459

16. Subbiah AK, Chhabra YK, Mahajan S. Cardiovascular disease in patients with chronic kidney disease: a neglected subgroup. *Heart Asia.* (2016) 8:56–61. doi: 10.1136/heartasia-2016-010809

17. Lindner A, Charra B, Sherrard DJ, Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. *New Engl J Med.* (1974) 290:697–701. doi: 10.1056/NEJM197403282901301

18. de Lusignan S, Chan T, Stevens P, O'Donoghue D, Hague N, Dzregah B, et al. Identifying patients with chronic kidney disease from general practice computer records. *Family Pract.* (2005) 22:234–41. doi: 10.1093/fampra/cmi026

19. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis.* (1998) 32:S112–9. doi: 10.1053/ajkd.1998.v32.pm9820470

20. Dahnan M, Assabri AM, Khader YS. Risk factors for end-stage renal failure among patients on hemodialysis in aljomhory hospital, sa'adah governorate, yemen: hospital-based case-control study. *JMIR Public Health Surveillance.* (2019) 5:e14215. doi: 10.2196/14215

21. Derseh BT, Dadi AF. Mortality and predictors of Chronic Kidney Diseases (CKD) in selected dialysis centers in Addis Ababa, Ethiopia. *medRxiv*. (2022).

22. Cheung AK, Sarnak MJ, Yan G, Berkoben M, Heyka R, Kaufman A, et al. Cardiac diseases in maintenance hemodialysis patients: results of the HEMO Study. *Kidney Int.* (2004) 65:2380–9. doi: 10.1111/j.1523-1755.2004.00657.x

23. Lim CT, Yap XH, Chung KJ, Khalid MA, Yayha N, Latiff LA, et al. Predictor of cardiovascular risks in end stage renal failure patients on maintenance dialysis. *Pakistan J Med Sci.* (2015) 31:1300. doi: 10.12669/pjms.316.8039

24. Parfrey PS, Foley RN, Wittreich BH, Sullivan DJ, Zagari MJ, Frei D. Double-blind comparison of full and partial anemia correction in incident hemodialysis patients without symptomatic heart disease. *J Am Soc Nephrol.* (2005) 16:2180–9. doi: 10.1681/ASN.2004121039

25. Portolés J, Lopez-Gomez JM, Aljama P, AM Tato on behalf of MAR STUDY GROUP. Cardiovascular risk in hemodialysis in Spain: prevalence, management and targt results (MAR study). *Nefrologia.* (2005) 25:297–306. doi: 10.3389/fmed.2023.1086359

26. Rostand SG, Kirk KA, Rutsky EA. Dialysis-associated ischemic heart disease: insights from coronary angiography. *Kidney Int.* (1984) 25:653–9. doi: 10.1038/ki.1984.70

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 11 July 2023
DOI 10.3389/fendo.2023.1099975

OPEN ACCESS

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RECEIVED 16 November 2022
ACCEPTED 28 April 2023
PUBLISHED 11 July 2023

CITATION
Kee YK, Jeon HJ, Oh J, Cho A, Lee Y-K, Yoon J-W, Kim H, Yoo TH and Shin DH (2023) Fibroblast growth factor-23 and cardiovascular disease among prevalent hemodialysis patients focusing on residual kidney function.
Front. Endocrinol. 14:1099975.
doi: 10.3389/fendo.2023.1099975

Fibroblast growth factor-23 and cardiovascular disease among prevalent hemodialysis patients focusing on residual kidney function

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Background: In patients undergoing incident hemodialysis, increased fibroblast growth factor-23 (FGF-23) levels are associated with the development of cardiovascular disease (CVD), but the influence of residual kidney function (RFK) on this association is unclear. This study aimed to investigate the association between FGF-23 levels, RKF, and CVD in patients undergoing prevalent hemodialysis.

Methods: This cross-sectional and longitudinal observational study included 296 patients undergoing maintenance hemodialysis for at least three months who were followed up for a median of 44 months. RKF was defined as 24-h urine output >200 mL, left ventricular (LV) diastolic dysfunction as E/E' >15 on echocardiographic parameters. CVD was defined as hospitalization or emergency room visits due to cardiovascular causes, such as angina, myocardial infarction, or congestive heart failure.

Results: The median intact FGF-23 (iFGF-23) level was 423.8 pg/mL (interquartile range, 171–1,443). Patients with an FGF-23 level > 423.8 pg/mL significantly had a lower proportion of RKF (39.2% vs. 60.1%, P < 0.001) and a higher proportion of LV diastolic dysfunction (54. 1% vs. 29.1%, P < 0.001) than those with an iFGF-23 level ≤ 423.8 pg/mL. The odds ratio (OR) for LV diastolic dysfunction was significantly higher in patients with RFK (OR per one-unit increase in the natural log-transformed iFGF-23 levels, 1.80; 95% confidence interval [CI]: 1.11–2.93) than in patients without RKF (OR per one-unit increase in the natural log-transformed iFGF-23 levels: 1.42; 95% CI: 1.01–1.99) in multivariate analysis (p < 0.001). During the follow-up period, 55 patients experienced CVD. The hazard ratio (HR) for CVD development was also significantly higher in patients with RKF (HR per one-unit increase in the natural log-transformed iFGF-23 levels, 2.64; 95% CI: 1.29–5.40) than those without RKF (HR per one-unit

increase in the natural log-transformed iFGF-23 levels: 1.44; 95% CI: 1.04–1.99) in multivariate analysis (p = 0.05).

Conclusions: Increased iFGF-23 levels were associated with LV diastolic dysfunction and CVD development in patients undergoing prevalent hemodialysis; however, the loss of RKF attenuated the magnitude of these associations. Therefore, in these patients, RKF strongly influenced the detrimental role of iFGF-23 in the development of CVD.

KEYWORDS

fibroblast growth factor-23, cardiovascular disease, left ventricular diastolic dysfunction, hemodialysis patients, residual kidney function

1 Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with end-stage kidney disease (ESKD) undergoing hemodialysis (1, 2). Traditional risk factors for CVD, such as hypertension, diabetes, and dyslipidemia, do not fully account for the high prevalence of CVD in this population (3). Non-traditional risk factors for CVD, including vascular calcification, elevated asymmetrical dimethylarginine, anemia, volume overload, and others, contribute to the increased cardiovascular risk observed in patients with ESKD (4, 5). Fibroblast growth factor-23 (FGF-23), a hormone secreted by osteoblasts, plays an essential role in the development of CKD and MBD (6). In patients with CKD, serum FGF-23 levels gradually increase with decreasing kidney clearance. Increased FGF-23 levels help maintain a normal serum phosphate concentration range by enhancing urinary phosphate excretion and reducing phosphate absorption through decreased 1,25-dihydroxy vitamin D production (6). End-organ resistance to the phosphaturic action of FGF-23 due to a deficiency of Klotho, the required cofactor, may also increase FGF-23 levels (7). Usually, when these patients reach end-stage kidney disease (ESKD), FGE-23 levels can be up to 1,000-fold above the normal range (8).

Recent studies have found an association between elevated FGF-23 levels and CVD, including hypertension, LV hypertrophy, acute coronary syndrome, stroke, transient ischemic attack, and heart failure (9–13). In line with these findings, some observational studies on patients undergoing incident hemodialysis have shown that high FGF-23 levels are associated with a greater risk of CVD (14, 15). Interestingly, a few large-scale studies on patients undergoing prevalent hemodialysis also showed that high FGF-23 levels were associated with a greater risk of CVD (16, 17). However, the magnitude of this association was not as strong as that observed in patients undergoing incident hemodialysis. When hemodialysis is initiated, most patients with ESKD have substantial residual kidney function (RKF). Although these studies did not investigate RKF, the difference in the magnitude of this association might be related to RKF. Therefore, this study aimed to evaluate the association between increased FGF-23 levels and CVD development in patients undergoing prevalent hemodialysis, focusing on the differences in RKF.

2 Materials and methods

2.1 Ethics statement

The Institutional Review Boards of Kangam Sacred, Kangdong Sacred, and Chuncheon Sacred Heart Hospitals approved this study (Refs. 2018-03-19, 2018-07-01, 2018-96). This study was performed in accordance with the Declaration of Helsinki. All the patients provided written informed consent before enrolment in the study.

2.2 Patients

This study was conducted between January 2018 and January 2022 at three dialysis clinics: Kangam Sacred, Kangdong Sacred, and Chuncheon Sacred Heart Hospitals. Inclusion criterion was patients who had undergone hemodialysis for at least 3 months, three times a week for 4 h. Exclusion criteria were patients who were planning to transfer to other centers and those who did not undergo echocardiography.

2.3 Measurement of intact FGE-23 (iFGF-23)

Blood samples for iFGF-23, the full-length biological molecule, were collected in serum separator tubes, clotted at room temperature, centrifuged, frozen at -70 °C, and shipped on dry ice to Seoul Clinical Laboratories for measurement. The levels of iFGF-23 were measured using a commercial enzyme-linked immunosorbent assay kit (Kainos Laboratories Inc., Tokyo, Japan).

2.4 Data collection

Baseline characteristics, including demographic and clinical information, and biochemical parameters were collected from the medical records at the time of blood collection for iFGF-23 measurement. We conducted echocardiography within 1 week at baseline and calculated the mean interdialytic weight gain (IDWG) as an average of 10 values before baseline.

2.5 Echocardiographic measurements

This study used an ultrasound machine (Vivid 7; GE Vingmed Ultrasound AS, Horten, Norway) with a 2.5 MHz probe to perform comprehensive echocardiographic measurements based on the imaging protocols of the American Society of Echocardiography guidelines. This study calculated the LV ejection fraction and LV mass using modified biplane Simpson's and Devereus and Reichek's methods, respectively (18, 19). The LV mass index (LVMI) was calculated as follows: LVMI = LV mass/body surface area. This study assessed mitral inflow from an apical four-chamber view using Doppler echocardiography. Peak mitral in-flow velocities at early (E) and late (A) diastole and deceleration time were measured using mitral inflow profiles. The peak mitral annular velocities at early (E') and late (A') diastole were measured using Doppler tissue imaging.

2.6 Outcomes

Based on echocardiographic parameters, LV diastolic dysfunction was defined as E/E' >15. Additionally, we designated CVD as an event requiring hospitalization or emergency room visits due to cardiovascular causes, such as angina, myocardial infarction, or congestive heart failure.

2.7 Statical analyses

We used descriptive statistics to compare baseline characteristics according to iFGF-23. Normally distributed variables were described as mean ± standard deviation and compared using the t-test for two groups. Nonnormally distributed variables were presented as a median and interquartile range, and compared using Mann–Whitney U test for the two groups. Categorical variables were described as frequencies and percentages, and compared using a chi-square test or Fisher's exact test. Kaplan–Meier product estimation method was used to calculate the cumulative incidence of CVD according to the median iFGF-23 level. We used logistic regression to examine the association between iFGF-23 and LV diastolic dysfunction, and Cox proportional hazards analysis to investigate the association between iFGF-23 and CVD development. We used multivariate models to adjust for three models (1): demographic and clinical factors: age, sex, dialysis vintage, Kt/V, diabetes, and prior CVD (e.g., coronary artery disease and congestive heart failure); (2) markers of mineral metabolism: serum levels of calcium, phosphorus, and intact parathyroid hormone (iPTH); and (3) active vitamin D treatment: prescription of active or analog vitamin D. We analyzed the results of laboratory data on a continuous scale. Therefore, nonnormally distributed laboratory data (iPTH and iFGF-23 levels) were log-transformed. Hazard ratios (HR) or odds ratios (OR) and 95% confidence intervals (CI), corresponding to a one-unit increase in the natural log-transformed iFGF-23 levels, were provided. We also investigated the potential interactions of iFGF23 with RKF (defined as 24-h urine output >200 mL) for clinical outcomes. The correlation between mean IDWG and iFGF-23 was calculated using Pearson analysis. Statistical analyses were performed using SPSS 27.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Study population

In total, 123, 140, and 132 patients were receiving hemodialysis therapy in Kangnam Sacred, Kangdong Sacred, and Chuncheon Sacred Heart Hospitals, respectively, between January 2018 and January 2019. Of these, 311 patients were eligible for inclusion in the study, and 15 patients were excluded because of plans of transfer to other centers (n=5) or because they did not undergo echocardiography (n=10). Thus, 296 patients were included in the study (Figure 1).

3.2 Baseline characteristics

Diabetes was the cause of ESKD in 173 (58.4%) patients. The median age was 63 years (interquartile range, 57–71 years), and 162 (54.7%) patients were men. The median iFGF-23 level was 423.8 pg/mL (interquartile range, 171.09–1,143.85 pg/mL) (Figure 2). Patients with iFGF-23 levels > 423.8 pg/mL had a longer hemodialysis vintage, lower proportion of RKF, greater mean IDWG, and were prescribed more noncalcium-based phosphate binders than those with iFGF-23 levels ≤423.8 pg/mL. Additionally, phosphate levels were higher in patients with iFGF-23 levels > 423.8 pg/mL (Table 1).

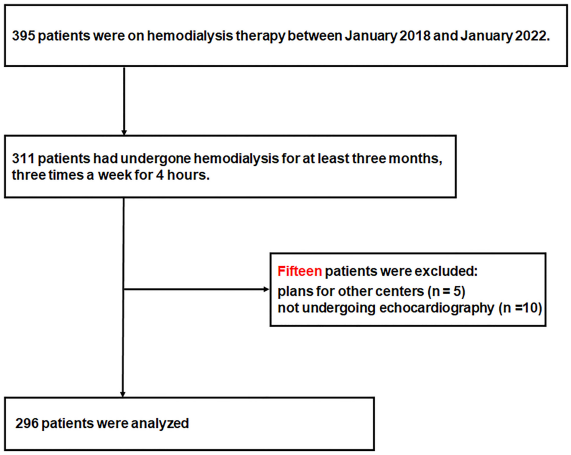

FIGURE 1
Flowchart of study population enrollment.

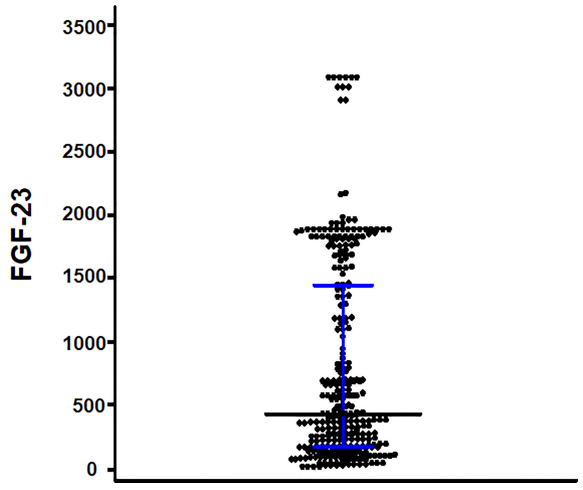

FIGURE 2
Scattered plots of the intact fibroblast growth factor-23 (iFGF-23) levels. The bar and error bars show the median and interquartile range.

3.3 Intact FGF-23 and LV diastolic dysfunction

Table 2 presents the echocardiographic parameters of the two groups: patients with iFGF-23 levels ≤423.8 pg/mL and those with iFGF-23 levels > 423.8 pg/mL. The proportion of LV diastolic dysfunction was significantly higher in patients with iFGF-23 levels > 423.8 pg/mL than in those with iFGF-23 levels ≤ 423.8 pg/mL (Table 2). In univariate logistic regression analysis, increased iFGF-23 levels were significantly associated with LV diastolic dysfunction (OR per unit increase in the natural log-transformed iFGF-23 levels, 1.60; 95% CI, 1.30–1.95). After adjusting for model 1 covariates, increased iFGF-23 levels were significantly associated with LV diastolic dysfunction. Even after adjusting for models 2 and 3, increased iFGF-23 levels were significantly associated with LV diastolic dysfunction (Table 3).

3.4 Intact FGF-23 and CVD

During a median follow-up of 44 months (interquartile range, 41.0–44.6 months), 55 patients experienced CVD, with 17 cases of angina, 15 cases of myocardial infarction, and 23 cases of congestive heart failure. Cardiovascular disease was significantly higher in patients with iFGF-23 levels > 423.8 pg/mL than in those with iFGF-23 levels ≤ 423.8 pg/mL (26.4% vs. 10.8%, P = 0.001) (Figure 2). The cumulative probability of incident CVD was also significantly higher in patients with iFGF-23 levels > 423.8 pg/mL than in those with iFGF-23 levels ≤ 423.8 pg/mL (Figure 3). In the univariate Cox regression analysis, increased iFGF-23 levels were significantly associated with the development of CVD. Additionally, increased iFGF-23 levels were significantly associated with the development of CVD, even when the analysis was adjusted for models 1, 2, and 3 (Table 4).

3.5 Interaction with RKF

Patients with RKF had lower iFGF-23 levels than those without RKF. However, the OR for LV diastolic dysfunction (OR per one-unit increase in the natural log-transformed iFGF-23 levels, 1.59; 95% CI: 1.13–2.52) in patients with RKF was more robust than that in patients without RKF (OR per one-unit increase in the natural log-transformed iFGF-23, 1.39; 95% CI: 1.07–1.79; $p < 0.001$). The higher magnitude of OR for LV diastolic dysfunction in patients with RKF than in patients without RKF was also maintained significantly by further adjustments for models 1, 2, and 3 (Table 3). Interestingly, the HR for developing CVD was significantly higher in patients with RKF than in patients without RKF when the analysis was adjusted for models 2 and 3.

3.6 Intact FGF-23, IDWG, and RKF

Although the mean IDWG was positively correlated with iFGF-23 levels in patients with RKF ($r = 0.24$, $p = 0.004$), there was no correlation between the mean IDWG and iFGF-23 levels in patients without RKF ($r = -0.27$, $p = 0.74$). However, in patients without RKF, the high mean IDWG was significantly associated with an increased risk of LV diastolic dysfunction and CVD development in the univariate and multivariate analyses (Supplemental Tables 1, 2).

4 Discussion

This cross-sectional and longitudinal observational study confirmed that increased iFGF-23 levels were significantly associated with LV diastolic dysfunction in patients undergoing prevalent hemodialysis. Additionally, increased iFGF-23 levels are significantly associated with the development of CVD. However, the loss of RKF attenuated the magnitude of these associations, even though the median iFGF-23 levels were higher in patients without RKF than in those with RKF.

Preservation of RKF has long been recognized as a significant beneficial factor in cardiovascular and overall mortality in hemodialysis patients. Even a minimal level of RKF has been associated with better fluid and electrolyte balance, enhanced clearance of middle molecules, lower inflammation levels, and improved quality of life (20, 21). Thus, understanding the role of RKF in mitigating the detrimental effects of FGF-23 on CVD is essential to developing potential therapeutic strategies for this patient population.

Observational cohort studies have shown a strong association between increased FGF-23 levels and the development of CVD in patients undergoing incident hemodialysis. These studies divided patients into three or four groups according to FGF-23 levels and demonstrated HRs of developing CVD for the highest FGF-23 level group versus the lowest FGF-23 level group, which ranged between 3–5 (14, 15). Meanwhile, in cohort studies on patients undergoing prevalent hemodialysis, HRs of developing CVD for the highest FGF-23 level group versus the lowest FGF-23 level group ranged

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TABLE 1 Baseline characteristics, overall and according to intact FGF-23.

Variables	Total (n = 296)	iFGF-23 of ≤423.8 pg/mL (n=148)	iFGF-23 of >423.8 pg/mL (n=148)	P-value
Demographic data				
Age (years)	63 (57.0–71.0)	67 (58.5–74.0)	62.0 (56.0–69.0)	0.001
Male, n (%)	162 (54.7)	71 (48.0)	91 (61.5)	0.03
Clinical data				
Dialysis vintage (months)	42.6 (17.9–68.5)	40.4 (15.1–59.1)	44.6 (25.4 – 80.4)	0.02
Initial end-stage kidney disease				
Diabetes, n (%)	173 (58.4)	89 (60.1)	84 (56.8)	0.64
Non-diabetes, n (%)	123 (41.6)	59 (39.9)	64 (43.2)	
Previous cardiovascular disease, n (%)	103 (34.8)	49 (33.1)	54 (36.5)	0.54
Urine output ≥200 mL/d, n	147 (49.7)	89 (60.1)	58 (39.2)	0.001
Interdialytic weight gain	2.6 ± 0.8	2.5 ± 0.9	2.9 ± 0.7	0.001
Medication				
Calcium-based phosphate binders, n (%)	81 (27.4)	52 (35.1)	29 (19.6)	0.004
Non-calcium-based phosphate binders, n (%)	153 (51.7)	49 (33.1)	104 (70.3)	<0.001
Vitamin D analogs, n (%)	165 (55.7)	82 (55.4)	83 (56.1)	0.99
Cinacalcet, n (%)	42 (14.2)	16 (10.8)	26 (17.6)	0.13
Laboratory data				
Hemoglobin (g/dL)	10.3 ± 1.2	10.1 ± 1.0	10.4 ± 1.3	0.03
Albumin (g/dL)	3.8 ± 0.4	3.8 ± 0.3	3.9 ± 0.4	0.01
Calcium (mg/dL)	8.5 ± 0.7	8.4 ± 0.6	8.6 ± 0.7	0.08
Phosphate (mg/dL)	4.8 (4.0–5.9)	4.3 (3.5–5.1)	5.4 (4.6–6.3)	<0.001
iPTH (pg/mL)	239.8 (147.8–417.0)	226.3 (153.5–383.0)	279.9 (143.5–438.8)	0.17
Kt/V	1.7 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	0.31

iFGF-23, intact fibroblast growth factor-23; iPTH, intact parathyroid hormone.
Values are expressed as median (interquartile range) or number (percentage).

between 1–1.2 (16, 17). Interestingly, a Japanese cohort study showed that long-term dialysis attenuated the association between increased FGF-23 levels and CVD development (22). Therefore, differences in the magnitude of this association might be related to differences in baseline characteristics between patients enrolled in the study, and RKF may be one of the differences. To understand these findings, we first evaluated the association between increased iFGF-23 levels and LV diastolic dysfunction in patients undergoing prevalent hemodialysis, focusing on differences in RFK.

Previous studies have indicated that LV diastolic dysfunction is associated with CVD, and is an independent predictor of CVD development in patients (23–25). Therefore, investigating LV diastolic dysfunction in these patients will help understand the association between increased FGF-23 levels and CVD development. Although several observational clinical studies showing that increased FGF-23 levels were associated with LV diastolic dysfunction cannot prove causality (26–29), *in vitro* and experimental animal studies have demonstrated that FGF-23 induced LV hypertrophy is related to LV diastolic dysfunction (11). FGF-23 causes pathological hypertrophy of isolated rat cardiomyocytes *via* FGF receptor-dependent activation of the calcineurin-nuclear factor of activated T cells signaling pathway, but this effect is independent of Klotho (11). These findings suggest that the pathogenesis of FGF-23-induced LV hypertrophy may be ongoing in patients undergoing hemodialysis, thereby promoting LV diastolic dysfunction. However, in this study, the magnitude of the association between increased iFGF-23 levels and LV diastolic dysfunction in patients without RKF was not as strong as that in those with RKF. Although iFGF-23 is associated with LV diastolic dysfunction, age, sex, diabetes, dialysis vintage, prior CVD, calcium,

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TABLE 2 Echocardiac parameters in prevalent hemodialysis patients according to intact FGF-23.

Variables	Total (n=296)	iFGF-23 of ≤423.8 pg/mL (n = 148)	iFGF-23 of >423.8 pg/mL (n=148)	P-value
Echocardiac parameters				
LVEF (%)	57.2 ± 10.7	56.1 ± 11.9	58.4 ± 9.1	0.29
LVMI (g/m)	139.8 ± 41.3	142 ± 45.8	132 ± 34.4	0.06
E (cm/s)	78.5 ± 24.0	73.5 ± 23.2	84.6 ± 68.4	0.09
A (cm/s)	91.4 ± 21.1	91.2 ± 24.1	92.2 ± 30.2	0.79
E/A ratio	0.9 ± 0.4	0.8 ± 0.3	0.8 ± 0.5	0.01
E'/A' ratio	0.6 ± 0.2	0.6 ± 0.4	0.8 ± 1.3	0.19
E/E' ratio	18.9 ± 9.1	15.2 ± 5.9	17.1 ± 8.6	0.02
Diastolic dysfunction, n (%)	123 (41.6)	43 (29.1)	80 (54.1)	<0.001
DT	216.3 ± 67.9	223.1 ± 72.9	213.7 ± 67.8	0.30

iFGF-23, intact fibroblast growth factor-23; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; DT, deceleration time.
Values are expressed as mean ± standard deviation.

TABLE 3 Associations of serum intact FGF-23 with left ventricular diastolic dysfunction and the interaction of intact FGF-23 with residual kidney function.

	Overall (n=296)	Urine output ≥200 mL/dL (n=147)	Urine output <200 mL/dL (n=149)	P for interaction
Median FGF-23 (IQR), pg/mL	423.8 (171.1–1443.9)	321.62 (135.41–681.11)	690.33 (257.08–1778.50)	
*OR (95% CI) for diastolic dysfunction				
No adjustment	1.60 (1.30–1.95)	1.59 (1.13–2.52)	1.39 (1.07–1.79)	<0.001
Model 1	1.54 (1.23–1.94)	1.99 (1.28–3.11)	1.38 (1.04–1.84)	<0.001
Model 2	1.51 (1.17–1.95)	1.51 (1.17–1.95)	1.41 (1.02–1.95)	<0.001
Model 3	1.50 (1.16–1.94)	1.87 (1.11–3.14)	1.41 (1.01–1.95)	<0.001

FGF-23, fibroblast growth factor-23; OR, odds ratio; CI, confidence interval; IQR, interquartile range; 1-SD, one-standard deviation
*ORs are per unit increase in natural log-transformed intact FGF-23 levels
Model 1 was adjusted for age, sex, diabetes, dialysis vintage, prior cardiovascular disease, interdialytic weight gain, and Kt/V.
Model 2 was adjusted for model 1 covariates plus calcium, phosphate, and natural log-transformed intact parathyroid hormone levels.
Model 3 adjusted for model 2 covariates plus vitamin D analogs treatment.

TABLE 4 Associations of serum intact FGF-23 with cardiovascular diseases and the interaction of intact FGF-23 with residual kidney function.

	Overall (n=296)	Urine output ≥200 mL/dL (n=147)	Urine output <200 mL/dL (n=149)	P for interaction
Median FGF-23 (IQR), pg/mL	423.8 (171.1–1443.9)	321.62 (135.41–681.11)	690.33 (257.08–1778.50)	
*HR (95% CI) for cardiovascular disease				
No adjustment	1.58 (1.24–2.02)	2.27 (1.36–3.80)	1.24 (0.95–1.61)	0.06
Model 1	1.52 (1.17–1.98)	2.84 (1.41–5.75)	1.33 (1.00–1.78)	0.06
Model 2	1.58 (1.17–2.13)	3.02 (1.37–6.67)	1.45 (1.05–2.02)	0.04
Model 3	1.58 (1.17–2.13)	3.17 (1.38–7.29)	1.45 (1.05–2.02)	0.04

FGF-23, fibroblast growth factor-23; HR, hazard ratio; CI, confidence interval; IQR, interquartile range.
*HRs are per unit increase in natural log-transformed iFGF-23.
Model 1 was adjusted for age, sex, diabetes, dialysis vintage, prior cardiovascular disease, interdialytic weight gain, and Kt/V.
Model 2 was adjusted for model 1 covariates and calcium, phosphate, and natural log-transformed parathyroid hormone levels.
Model 3 adjusted for model 2 covariates plus vitamin D analogs treatment.

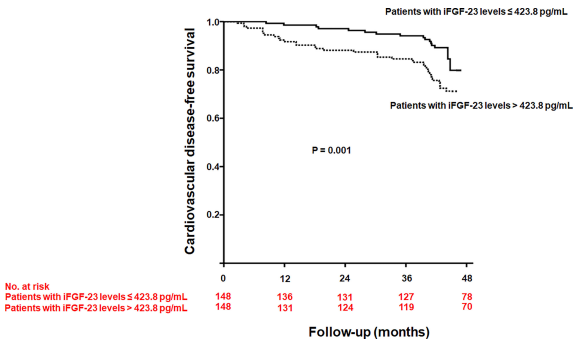

FIGURE 3
Kaplan-Meier curve of developing cardiovascular disease according to the median intact fibroblast growth factor-23 (iFGF-23) levels (P = 0.001).

phosphate, and iPTH play significant roles in inducing LV diastolic dysfunction (30–35). Especially mechanical stress, such as volume overload, causes LV hypertrophy as the primary maladaptive response (36). Persistent fluid overload is commonly observed in patients with ESKD without RKF. In our study, the high mean IDWG was significantly associated with the risk of LV diastolic dysfunction in patients without RKF. Therefore, in patients without RKF, IDWG may diminish the effect of increased iFGF-23 levels on LV diastolic dysfunction.

In recent years, it has become clear that FGF-23 directly influences calcium and sodium handling in the distal nephron of the kidney. In the distal tubular epithelium, FGF-23 regulates the apical membrane abundance of epithelial transient receptor potential vanilloid-5 and sodium-chloride cotransporter (9). In our study, iFGF 23 and mean IDWG were positively correlated in patients with RKF but not significantly in patients without RKF. This is probably because the direct effect of increased FGF-23 on the distal nephron of the kidney resulted in sodium retention and blood volume expansion in patients with RKF. Therefore, the absence of RKF would reduce the effect of increased FGF-23 levels on LV diastolic dysfunction. Another possible explanation for how the loss of RKF reduces the effect of elevated FGF-23 levels on left ventricular diastolic dysfunction is that it leads to decreased levels of certain hormones that contribute to the detrimental effects of FGF-23 on the cardiovascular system. For example, a study has suggested that the renin-angiotensin-aldosterone system (RAAS) may play a role in the adverse effects of FGF-23 on the heart and that the attenuation of these effects with the loss of RKF may be due to a decrease in RAAS activity (37). However, it is essential to remember that these are just hypotheses and that other mechanisms may also be involved. The exact mechanism of this attenuating effect has yet to be fully explored and requires further investigation.

Therefore, RKF plays a pivotal role in the detrimental effects of increased FGF-23 levels on the development of CVD. FGF-23 is an approximately 32-kDa glycoprotein with N- and C-terminal regions (38). There are two main types of assays for measuring FGF-23 levels in humans. The iFGF-23 assay binds two epitopes that flank the proteolytic cleavage site, presumably detecting only biologically active, full-length FEG-23 (~32 kDa) (39). In contrast, the C-terminal FGF-23 (cFGF-23) assay binds to epitopes within the C-terminal region of the FGF-23 protein, and detects both full-length and processed C-terminal fragments (~14kDa) (40). Based on the idea that the iFGF-23 assay might be superior because it detects the full-length FGF23 molecule and not a mixture of full-length FGF23 and degradation products (41), we used the iFGF-23 assay in this study. However, most prior studies have reported associations between FGF-23 and clinical outcomes using the cFGF-23 assay (14, 15, 42, 43). Additionally, a few studies have shown that the iFGF-23 assay showed much weaker associations with similar endpoints than the cFGF-23 assay (44, 45). Therefore, in our study, the association between increased FGF-23 levels and clinical outcomes may have been weakened because of the usage of an iFGF-23 assay. Nevertheless, we showed the importance of RKF by showing that the loss of RKF attenuated the magnitude of the associations between increased iFGF-23 and LV diastolic dysfunction and CVD development.

Our study had several limitations. First, this was a small-scale observational study. Second, we did not measure cFGF-23 levels, which might have provided additional information on the regulation of FGF-23. Third, we could not determine whether the association between increased iFGF-23 levels and LV diastolic dysfunction is causal. Lastly, there were numerous potential confounders that could affect the relationship between iFGF-23 levels, RKF, and CVD. Although we attempted to account for several of these factors in our multivariable model, there may be other unmeasured confounding factors that we could not adjust for.

In conclusion, we showed that increased iFGF-23 levels are associated with LV diastolic dysfunction and CVD development in patients undergoing prevalent hemodialysis. The loss of RKF attenuated the magnitude of these associations, highlighting the strong influence of RKF on the detrimental role of iFGF-23 in the development of CVD. Nonetheless, the observed associations between iFGF-23 levels, RKF, and CVD should be interpreted with caution, as there may be other unmeasured confounding factors. Further large-scale studies are needed to understand the complex relationship between these factors fully. If confirmed, FGF–receptor blocker treatment could be considered in patients undergoing hemodialysis with RKF to prevent CVD development.

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 Institutional Review Boards of Kangam Sacred,

Kangdong Sacred, and Chuncheon Sacred Heart Hospitals. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DHS conceived and designed the study. YKK, HJJ, YKL, AC., JWY, and HK acquired data. DHS, AC, and YKK analyzed and interpreted the data. DHS and YKK wrote the paper. DHS, YKL, and JWY reviewed the manuscript for important intellectual content and approved the final version. During the revision, THY provided invaluable advice and assistance in addressing the reviewers' comments and concerns.

Acknowledgments

The authors thank the dedicated volunteers who participated in this study, and the research nurse for collecting clinical data.

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/fendo.2023.1099975/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. *New Engl J Med* (2004) 351(13):1296–305. doi: 10.1056/NEJMoa041031

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(3 Suppl 1):A7–a8. doi: 10.1053/j.ajkd.2016.12.004

3. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American heart association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation* (2003) 108(17):2154–69. doi: 10.1161/01.Cir.0000095676.90936.80

4. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Salvatore Malatino L, et al. Asymmetric dimethylarginine, c-reactive protein, and carotid intima-media thickness in end-stage renal disease. *J Am Soc Nephrol JASN* (2002) 13(2):490–6. doi: 10.1681/asn.V132490

5. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol dialysis Transplant* (2003) 18(9):1731–40. doi: 10.1093/ndt/gfg414

6. Wolf M. Forging forward with 10 burning questions on Fgf23 in kidney disease. *J Am Soc Nephrol JASN* (2010) 21(9):1427–35. doi: 10.1681/asn.2009121293

7. Seiler S, Heine GH, Fliser D. Clinical relevance of fgf-23 in chronic kidney disease. *Kidney Int Supplement* (2009) 114):S34–42. doi: 10.1038/ki.2009.405

8. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, et al. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol JASN* (2005) 16(7):2205–15. doi: 10.1681/asn.2005010052

9. Andrukhova O, Slavic S, Smorodchenko A, Zeitz U, Shalhoub V, Lanske B, et al. Fgf23 regulates renal sodium handling and blood pressure. *EMBO Mol Med* (2014) 6 (6):744–59. doi: 10.1002/emmm.201303716

10. Fyfe-Johnson AL, Alonso A, Selvin E, Bower JK, Pankow JS, Agarwal SK, et al. Serum fibroblast growth factor-23 and incident hypertension: the atherosclerosis risk in communities (Aric) study. *J hyper* (2016) 34(7):1266–72. doi: 10.1097/hjh.0000000000000936

11. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. Fgf23 induces left ventricular hypertrophy. *J Clin Invest* (2011) 121(11):4393–408. doi: 10.1172/jci46122

12. 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(6):1020–32. doi: 10.1016/j.cmet.2015.09.002

13. Tuñón J, Fernández-Fernández B, Carda R, Pello AM, Cristóbal C, Tarín N, et al. Circulating fibroblast growth factor-23 plasma levels predict adverse cardiovascular outcomes in patients with diabetes mellitus with coronary artery disease. *Diabetes/metab Res Rev* (2016) 32(7):685–93. doi: 10.1002/dmrr.2787

14. Scialla JJ, Parekh RS, Eustace JA, Astor BC, Plantinga L, Jaar BG, et al. Race, mineral homeostasis and mortality in patients with end-stage renal disease on dialysis. *Am J Nephrol* (2015) 42(1):25–34. doi: 10.1159/000438999

15. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *New Engl J Med* (2008) 359(6):584–92. doi: 10.1056/NEJMoa0706130

16. Moe SM, Chertow GM, Parfrey PS, Kubo Y, Block GA, Correa-Rotter R, et al. Cinacalcet, fibroblast growth factor-23, and cardiovascular disease in hemodialysis: the evaluation of cinacalcet hcl therapy to lower cardiovascular events (Evolve) trial. *Circulation* (2015) 132(1):27–39. doi: 10.1161/circulationaha.114.013876

17. 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 JASN* (2016) 27(1):227–37. doi: 10.1681/asn.2014101009

18. Folland ED, Parisi AF, Moynihan PF, Jones DR, Feldman CL, Tow DE. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. a comparison of cineangiographic and radionuclide techniques. *Circulation* (1979) 60(4):760–6. doi: 10.1161/01.cir.60.4.760

19. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. anatomic validation of the method. *Circulation* (1977) 55(4):613–8. doi: 10.1161/01.cir.55.4.613

20. Obi Y, Rhee CM, Mathew AT, Shah G, Streja E, Brunelli SM, et al. Residual kidney function decline and mortality in incident hemodialysis patients. *J Am Soc Nephrol JASN* (2016) 27(12):3758–68. doi: 10.1681/asn.2015101142

21. Mathew AT, Fishbane S, Obi Y, Kalantar-Zadeh K. Preservation of residual kidney function in hemodialysis patients: reviving an old concept. *Kidney Int* (2016) 90 (2):262–71. doi: 10.1016/j.kint.2016.02.037

22. 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(11):1956–64. doi: 10.1016/j.ekir.2020.08.013

23. Han JH, Han JS, Kim EJ, Doh FM, Koo HM, Kim CH, et al. Diastolic dysfunction is an independent predictor of cardiovascular events in incident dialysis patients with preserved systolic function. *PloS One* (2015) 10(3):e0118694. doi: 10.1371/journal.pone.0118694

24. Yoon HE, Chung S, Whang HC, Shin YR, Hwang HS, Chung HW, et al. Abdominal aortic calcification is associated with diastolic dysfunction, mortality, and nonfatal cardiovascular events in maintenance hemodialysis patients. *J Korean Med Sci* (2012) 27(8):870–5. doi: 10.3346/jkms.2012.27.8.870

25. Iwabuchi Y, Ogawa T, Inoue T, Otsuka K, Nitta K. Elevated E/E' predicts cardiovascular events in hemodialysis patients with preserved systolic function. *Internal Med (Tokyo Japan)* (2012) 51(2):155–60. doi: 10.2169/internalmedicine.51.6250

26. Sharma S, Hanudel MR, Ix JH, Salusky IB, Ganz T, Nguyen KL. Elevated fibroblast growth factor 23 levels are associated with greater diastolic dysfunction in esrd. *Kidney Int Rep* (2019) 4(12):1748–51. doi: 10.1016/j.ekir.2019.07.022

27. Milovanova LY, Shvetsov MY, Milovanova SY, Lysenko Kozlovskaya LV, Kamyshova ES, Taranova MV, et al. Elevated fibroblast growth factor 23 and decreased klotho levels are associated with diastolic dysfunction in ckd G4-5d patients. *Kidney Int Rep* (2020) 5(7):1118. doi: 10.1016/j.ekir.2020.04.022

28. Negishi K, Kobayashi M, Ochiai I, Yamazaki Y, Hasegawa H, Yamashita T, et al. Association between fibroblast growth factor 23 and left ventricular hypertrophy in maintenance hemodialysis patients. comparison with b-type natriuretic peptide and cardiac troponin T. *Circ J* (2010) 74(12):2734–40. doi: 10.1253/circj.cj-10-0355

29. Jovanovich A, Ix JH, Gottdiener J, McFann K, Katz R, Kestenbaum B, et al. Fibroblast growth factor 23, left ventricular mass, and left ventricular hypertrophy in community-dwelling older adults. *Atherosclerosis* (2013) 231(1):114–9. doi: 10.1016/j.atherosclerosis.2013.09.002

30. Masugata H, Senda S, Goda F, Yoshihara Y, Yoshikawa K, Fujita N, et al. Left ventricular diastolic dysfunction in normotensive diabetic patients in various age strata. *Diabetes Res Clin Pract* (2008) 79(1):91–6. doi: 10.1016/j.diabres.2007.08.006

31. Redfield MM, Jacobsen SJ, Borlaug BA, Rodeheffer RJ, Kass DA. Age- and gender-related ventricular-vascular stiffening: a community-based study. *Circulation* (2005) 112(15):2254–62. doi: 10.1161/circulationaha.105.541078

32. Chatterjee K. Primary diastolic heart failure. *Am J geriatric Cardiol* (2002) 11 (3):178–87. doi: 10.1111/j.1076-7460.2002.00051.x

33. Utescu MS, Couture V, Mac-Way F, De Serres SA, Marquis K, Larivière R, et al. Determinants of progression of aortic stiffness in hemodialysis patients: a prospective longitudinal study. *Hyper (Dallas Tex 1979)* (2013) 62(1):154–60. doi: 10.1161/hypertensionaha.113.01200

34. Poudel K, Shah AM, Michos ED, Folsom AR, Konety S, Lutsey PL. Association of serum calcium and phosphorus with measures of left ventricular structure and function: the aric study. *Nutri metab Cardiovasc Dis NMCD* (2020) 30(5):758–67. doi: 10.1016/j.numecd.2020.01.003

35. Ohara N, Hiramatsu K, Shigematsu S, Hayashi Y, Ishihara F, Aizawa T, et al. Effect of parathyroid hormone on left ventricular diastolic function in patients with primary hyperparathyroidism. *Mineral electrol Metab* (1995) 21(1-3):63–6.

36. Carabello BA, Zile MR, Tanaka R, Cooper Gt. Left ventricular hypertrophy due to volume overload versus pressure overload. *Am J Physiol* (1992) 263(4 Pt 2):H1137–44. doi: 10.1152/ajpheart.1992.263.4.H1137

37. Leifheit-Nestler M, Kirchhoff F, Nespor J, Richter B, Soetje B, Klintschar M, et al. Fibroblast growth factor 23 is induced by an activated renin-Angiotensin-Aldosterone system in cardiac myocytes and promotes the pro-fibrotic crosstalk between cardiac myocytes and fibroblasts. *Nephrol Dialysis* (2018) 33(10):1722–34. doi: 10.1093/ndt/gfy006

38. Liu S, Quarles LD. How fibroblast growth factor 23 works. *J Am Soc Nephrol JASN* (2007) 18(6):1637–47. doi: 10.1681/asn.2007010068

39. Wolf M, White KE. Coupling fibroblast growth factor 23 production and cleavage: iron deficiency, rickets, and kidney disease. *Curr Opin Nephrol hyper* (2014) 23(4):411–9. doi: 10.1097/01.mnh.0000447020.74593.6f

40. Jonsson KB, Zahradnik R, Larsson T, White KE, Sugimoto T, Imanishi Y, et al. Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. *New Engl J Med* (2003) 348(17):1656–63. doi: 10.1056/NEJMoa020881

41. Bożentowicz-Wikarek M, Owczarek A, Kocełak P, Olszanecka-Glinianowicz M, Więcek A, Chudek J. C-terminal to intact fibroblast growth factor 23 ratio in relation to estimated glomerular filtration rate in elderly population. *Kidney Blood Pressure Res* (2016) 41(5):519–26. doi: 10.1159/000443452

42. Baia LC, Humalda JK, Vervloet MG, Navis G, Bakker SJ, de Borst MH. Fibroblast growth factor 23 and cardiovascular mortality after kidney transplantation. *Clin J Am Soc Nephrol CJASN* (2013) 8(11):1968–78. doi: 10.2215/cjn.01880213

43. Ix JH, Katz R, Kestenbaum BR, de Boer IH, Chonchol M, Mukamal KJ, et al. Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: chs (Cardiovascular health study). *J Am Coll Cardiol* (2012) 60(3):200–7. doi: 10.1016/j.jacc.2012.03.040

44. Kestenbaum B, Sachs MC, Hoofnagle AN, Siscovick DS, Ix JH, Robinson-Cohen C, et al. Fibroblast growth factor-23 and cardiovascular disease in the general population: the multi-ethnic study of atherosclerosis. *Circ Heart failure* (2014) 7 (3):409–17. doi: 10.1161/circheartfailure.113.000952

45. Ginsberg C, Craven TE, Chonchol MB, Cheung AK, Sarnak MJ, Ambrosius WT, et al. Pth, Fgf23, and intensive blood pressure lowering in chronic kidney disease participants in sprint. *Clin J Am Soc Nephrol CJASN* (2018) 13(12):1816–24. doi: 10.2215/cjn.05390518

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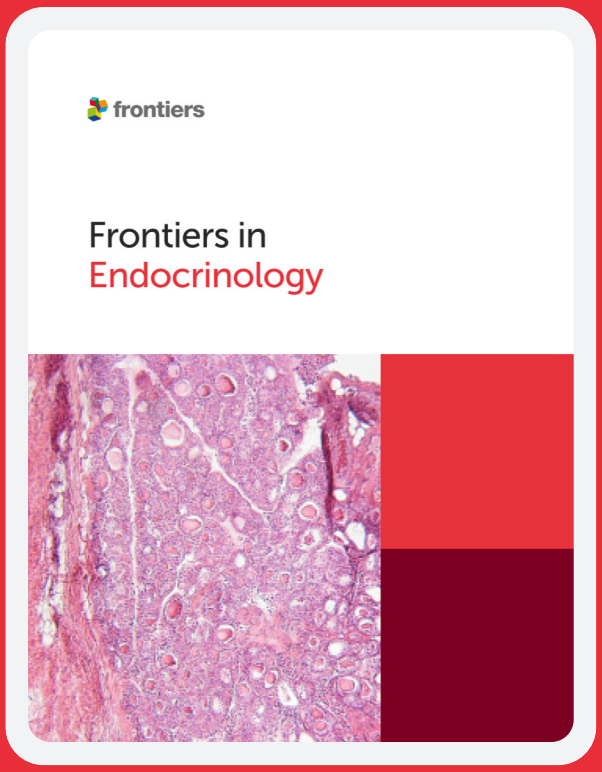

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