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Hypertensive disorders of pregnancy and the cardiovascular system: Causes, consequences, prevention and therapy

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Hypertensive disorders of pregnancy and the cardiovascular system: Causes, consequences, prevention and therapy

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Table of contents

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Editorial: Hypertensive disorders of pregnancy and the cardiovascular system: Causes, consequences, prevention and treatment

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Editorial on the Research Topic
Editorial: Hypertensive disorders of pregnancy and the cardiovascular system: Causes, consequences, prevention and therapy

Introduction

Hypertension occurs in one in ten pregnancies and may progress into preeclampsia when signs of organ damage emerge, and eclampsia when seizures ensue (1). Unsurprisingly, hypertension puts the pregnant mother and her child at risk of severe morbidity and death. Over the past years incidence rates of gestational hypertensive disorders have risen despite strenuous efforts of researchers and medical professionals (1). After gestational hypertension, women are at risk of developing cardiovascular and cardiometabolic disease, including diabetes, atherosclerosis, and chronic hypertension later in life. Proper insights into its causes, consequences, prevention and treatment are imperative in our efforts to improve future women's health.

This special issue of "*Hypertensive disorders of pregnancy and the cardiovascular system*" presents substantial scientific work aimed at underlying mechanisms, risks factors, and sequelae of preeclampsia. Important steps towards a better understanding of gestational hypertension and identifying pregnancies at risk of severe morbidity are discussed and a new study protocol is introduced.

Causes

Preeclampsia is a multifaceted syndrome with numerous hypotheses regarding its aetiology and pathophysiology. A generally accepted consensus is yet to be obtained and the studies presented here aim to gain a better understanding of its causes from genetic, metabolic, and hemodynamic perspectives.

Disturbances of lipid metabolism precede the onset of preeclampsia. Both overweight and underweight link to poor maternal and neonatal outcomes. Hu et al. observed lowest risks of disease when the BMI is between 19 and 23 preconceptionally, which is a narrower range than the common notion of healthy BMI between 18.5 and 25. Interestingly, risks of gestational hypertension already increase from BMI values 20 and up.

As preeclampsia has the tendency to run in families, a genetic origin of disturbed lipid metabolism was studied by Liu et al. A specific enzyme involved in lipid metabolism, APOBEC3A, is overexpressed in preeclampsia. Its hypothetical contribution to preeclampsia is further substantiated by the observation of disturbed lipid metabolism and a preeclampsia-like phenotype in an animal model of pregnant APOBEC3A-overexpressing mice. Complications associated with preeclampsia, such as renal impediments, may also have genetic origins. The Chinese Han population, for instance, has a relatively high incidence of hypertensive renal damage and impaired renal function during preeclampsia. In this population, Yun et al. discovered that the C677T gene mutation of the MTHFR-gene that affects methionine metabolism constitutes an independent risk factor for impaired renal function during preeclampsia.

Adequate maternal cardiovascular adaptation is key to uncomplicated course of pregnancy. Cardiovascular maladaptation can result in preeclampsia and may originate from a maternal prothrombotic state, increased vascular tone, or reduced volume circulation as is discussed in the works of Godtfredsen et al., Dierickx et al. and Gyselaers and Lees Components of the contact activation system are not only involved with coagulation and inflammation processes, but also with the regulation of placental function. Godtfredsen et al. used new methods focusing on the dynamic interactions and total capacity of the contact activating system in blood. They found that several components, specifically kallikrein generating capacity, prekallikrein and cleaved H-kininogen, are reduced in women with preeclampsia.

Findings supporting vascular maladaptation during preeclampsia are presented by Dierickx et al. Their combined Doppler-ECG assessment of the internal jugular veins demonstrated a lower venous pulse transit time at internal jugular veins in early-onset preeclampsia, suggesting that there is an increased venous vascular tone at supracardial level.

Gyselaers and Lees review existing evidence on the association between maternal intravascular filling state and fetal growth (Gyselaers and Lees). Previous studies in pregnant rodents showed that low plasma volume predisposes to poor fetal growth and beneficial effects are seen after enhancing plasma volume. A low intravascular volume combined with a high vascular resistance is suggested to cause hypertension in women with preeclampsia and fetal growth restriction. This review is an invitation to further investigate whether elevating the maternal intravascular volume in pregnant women with fetal growth retardation and preeclampsia may be a potential target for prevention and management.

Microvasculature changes also occur during pregnancy as is demonstrated with nailfold video capillaroscopy by Thevissen et al. The capillary bed of women with a low-risk cardiovascular profile differs in density and diameters compared to women with a high-risk cardiovascular profile. Furthermore, the capillary bed remains unchanged during gestation in low-risk women, whereas distinct differences exist in women with a high-risk cardiovascular profile and women with preeclampsia separately. Future studies should investigate whether these differences can be used to predict onset of preeclampsia.

Consequences

Pregnancy may be viewed upon as cardiometabolic stress test. As supported by the meta-analysis of Xu et al. pregnancy-induced hypertension and preeclampsia may be the first early signs flagging women who are especially prone to develop chronic hypertension later in life. These risks differ based on ethnic and racial origins as is clearly reviewed by Burger et al. Hypertensive diseases of pregnancy are associated consistently with increased cardiovascular disease risk across racial and ethnic groups. However, it is remarkable that the risk of gestational hypertension in non-White women is lower or similar compared to that found in non-Hispanic White women, while chronic hypertension, (superimposed) preeclampsia, and eclampsia risks are increased among most non-White populations.

From a logical perspective, strict blood pressure control seems especially important in women with a history of gestational hypertension to prevent chronic hypertension and its associated complications later in life. Increasing blood pressure, for example, is associated with a higher risk of mortality in patients with aortic dissection as shown by Wu et al. Interestingly, blood pressure is best interpreted dynamically over time because decelerating drops in pressure are associated with better outcomes while accelerating rises had poor prognosis. Nonetheless, low blood pressure can be harmful as well. In an intensive care unit setting, Shao et al. demonstrate that the mortality risk of patients with atrial fibrillation increases with hypertension, but also increase with pressures below 110 mmHg systolic blood pressure, below 55 mmHg diastolic blood pressure, and below 70 mmHg mean arterial pressure.

One interesting hypothesis is that women after preeclampsia are older in a vascular sense compared to women of equal biological age with a history of normotensive pregnancies. From a functional perspective, this seems not the case as Jansen et al. show that brachial artery flow-mediated dilation and sublingually administered nitroglycerine-mediated dilation decrease with age at comparable rates in young- to middle-aged women with a history of preeclampsia versus a history of normotensive pregnancy. These findings suggest that an increased

cardiovascular risk in the first decades after preeclampsia does not originate from an accelerated decline in endothelial function. Perhaps a different site, the microcirculation, ages more rapidly or another mode of endothelial action is involved, such as hemostatic and inflammatory related integrity. These hypotheses remain subjects for further investigation.

Prevention and treatment

Once preeclampsia develops, it progressively worsens. There is currently no treatment other than terminating the pregnancy once maternal or fetal life is in serious danger (2). Therefore, many researchers try to find ways to prevent the onset of preeclampsia. As described above, pregestational low intravascular volume and preeclampsia associate with fetal growth restriction. Gyselaers and Lees propose physical exercise before pregnancy as an attempt to increase maternal intravascular volume and reduce the risks of fetal growth restriction and early-onset preeclampsia. The beneficial effects of an increased filling state have been shown previously in pregnant rodents although its effectiveness is yet to be demonstrated in humans.

To reduce the risk of preeclampsia onset, administration of low-dose aspirin is commonly advised for women at risk (3). Because this recommendation is based on studies with singleton pregnancies, its effectiveness in twin pregnancies was studied by Zhou et al. Perhaps due to poor compliance, no risk reduction is observed. Their findings highlight the difficulties faced when assessing risk reducing regimens.

Means of preventing disease progression are still highly desired when preeclampsia strikes. In early onset preeclampsia expectant management is usually attempted to decrease the likelihood of fetal morbidity. Women with mild hypertension should be induced to deliver after 37 weeks of gestation (4). However, the timing of hospitalization, corticosteroids, and delivery remain a clinical challenge in case of severe hypertension. Villalain et al. developed two models with promising clinical potential using clinical characteristics such as BMI, gestational age, and fetal weight, and biomarkers placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). Their first model predicts the need to deliver within 7 days in women with early-onset preeclampsia and their second model the development of HELLP or placental abruption with 76% and 90% predictive values, respectively. These models potentially aid decision making regarding corticosteroid administration and expectant management.

Tanaka et al. further explored the use of biomarkers in timing delivery of women with early onset preeclampsia. Delivery within one week was predicted using sFlt-1 with a 67% positive predictive value. However, the study population was small and larger cohorts are necessary to determine the clinical feasibility of sFlt-1 in predicting time of delivery. Ratnik et al. also studied the use of biomarkers. They discovered that blood samples should be obtained between 10 and 12 weeks of gestation to predict preeclampsia with 80% specificity and 88% accuracy using a panel of biomarkers (PTX3, sFlt-1, and ADAM12) in combination with the subject's parity and gestational age at blood sampling. In future studies, their model as a first trimester screening tool for hypertensive gestational disease should be validated to allow careful early intervention and monitoring of individuals at-risk.

The use of biomarkers is costly which might prevent their feasibility as a screening instrument. To overcome this hurdle, Trilla et al. present a prospective, multicentric, cohort study protocol that aims to screen for preeclampsia in a two-step sequential screening model. The first step aims to distinguish women with low-risk from those with an intermediate or high risk of preeclampsia based on their medical history, mean blood pressure, Pregnancy-Associated Plasma Protein A (PAPP-A), and pulsatility index of the uterine arteries. In the second step, PlGF and sFLT-1 values are determined in women with an intermediate and high risk to allow reclassification into low-risk and high-risk groups. Predictive and preventive capacity of this two-step sequential model and the financial cost will be compared to the universal screening system.

All together, we hope you will enjoy reading these articles in this issue of Frontiers in Cardiovascular Medicine.

Author contributions

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

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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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ORIGINAL RESEARCH
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Copy Number Analyses Identified a Novel Gene: *APOBEC3A* Related to Lipid Metabolism in the Pathogenesis of Preeclampsia

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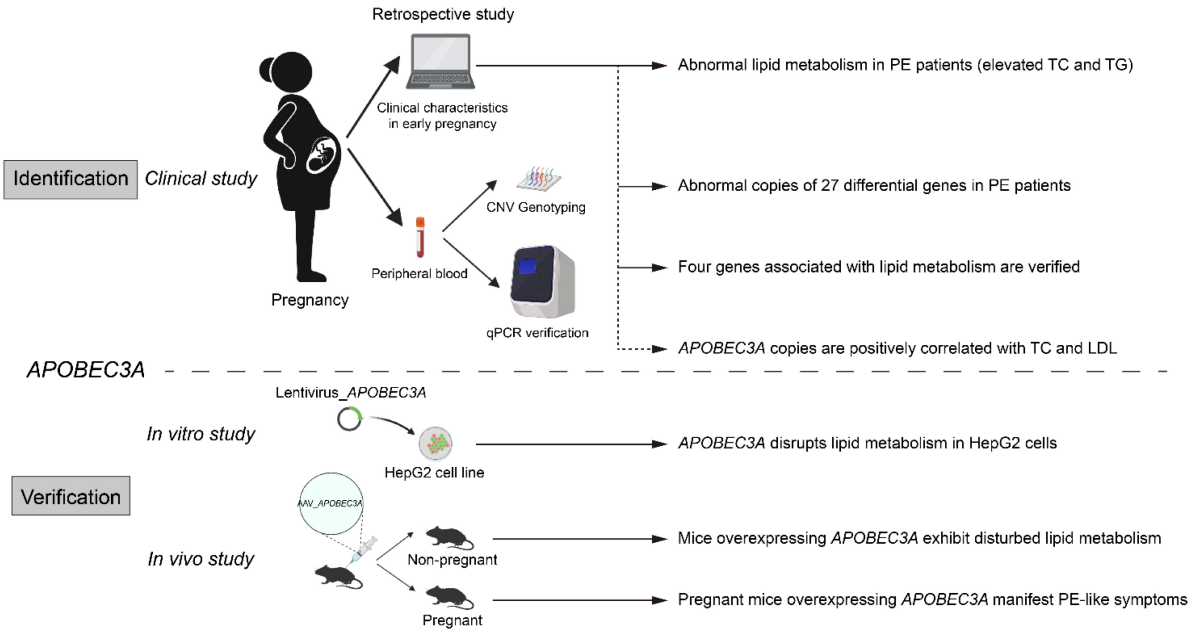
Background: Preeclampsia is a heterogeneous and complex disease with its pathogenesis mechanism not fully elucidated. A certain subset of patients with preeclampsia exhibit disturbances in lipid metabolism before clinical symptoms. Moreover, there is a tendency for preeclampsia to run in families. Whether genetic factors play a role in abnormal lipid metabolism during the incidence of preeclampsia has not been well investigated.

Methods: Preeclampsia patients (n = 110) and healthy age- and gravidity-matched pregnant women (n = 110) were enrolled in this study. Peripheral blood specimens were used for genomic analysis (n = 10/group) or laboratory validation (n = 100/group). We retrospectively obtained the baseline clinical characteristics of 68 preeclampsia patients and 107 controls in early pregnancy (12–14 gestational weeks). Correlation analyses between differential genes and baseline lipid profiles were performed to identify candidate genes. *In vitro* and *in vivo* gain-of-function models were constructed with lentivirus and adeno-associated virus systems, respectively, to investigate the role of candidate genes in regulating lipid metabolism and the development of preeclampsia.

Results: We observed that preeclampsia patients exhibited significantly elevated plasma TC (P = 0.037) and TG (P < 0.001) levels and increased body mass index (P = 0.006) before the disease onset. Within the region of 27 differential copy number variations, six genes potentially connected with lipid metabolism were identified. The aberrant copies of *APOBEC3A*, *APOBEC3A_B*, *BTNL3*, and *LMF1* between preeclampsia patients and controls were verified by quantitative polymerase chain reaction. Especially, *APOBEC3A* showed a significant positive correlation with TC (P < 0.001) and LDL (P = 0.048) in early pregnancy. Then, our *in vitro* data revealed that overexpression of *APOBEC3A* disrupted lipid metabolism in HepG2 cells and affected



GRAPHICAL ABSTRACT | Identification and validation of APOBEC3A'srole in regulating lipid metabolism in PE.

both cholesterol and fatty acid metabolisms. Finally, *in vivo* study in a hepatic-specific overexpressed *APOBEC3A* mouse model revealed abnormal parameters related to lipid metabolism. Pregnant mice of the same model at the end of pregnancy showed changes related to preeclampsia-like symptoms, such as increases in sFlt-1 levels and sFlt-1/PLGF ratios in the placenta and decreases in fetal weight.

Conclusion: Our findings established a new link between genetics and lipid metabolism in the pathogenesis of preeclampsia and could contribute to a better understanding of the molecular mechanisms of preeclampsia.

Keywords: preeclampsia, *APOBEC3A*, lipid metabolism, copy number variation, genetics

INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific complication mainly characterized by hypertension and proteinuria and remains one of the leading causes of morbidity and mortality in pregnant women and fetuses (1). Currently, the incidence of PE in developed countries is about 1.3–6%, which is even higher in developing countries (2). PE is a heterogeneous and complex disease caused by multiple factors, including heredity and environment (3). However, the underlying mechanism remains elusive due to diverse races, geography, and other factors (4).

An emerging body of research has demonstrated that abnormalities in lipid metabolism may be connected with the etiology of PE, with certain patients manifesting lipid metabolism disturbances before the onset of clinical symptoms (5–7). The lipid profiles are clinically accessible parameters. We have previously reported that triglycerides (TG) and low-density lipoprotein cholesterol (LDL) are independent risk factors for PE, while high-density lipoprotein cholesterol (HDL) is an independent protective factor (8). Therefore, elucidating the relationship between abnormal lipid metabolism and the pathogenesis of PE can guide the early identification of high-risk individuals for PE.

Previous findings have revealed that PE is relatively common among daughters and sisters of women with PE (9, 10), indicating genetic factors play an essential role in the development of PE. Most studies on genetic factors in PE are based on expression profiles, including mRNA and protein levels, making it challenging to clarify the causal relationship between these factors and the onset of PE.

To elucidate the mechanistic link between abnormal lipid metabolism and genetics in the pathogenesis of PE, we analyzed

Abbreviations: AAV, Adeno-associated Virus; CNVs, copy number variations; FPKM, fragment of the exon model per million mapped reads; GO, Gene Ontology; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PE, preeclampsia; PLGF, placental growth factor; qPCR, quantitative polymerase chain reaction; sFlt-1, soluble fms-like tyrosine kinase-1; SNVs, single nucleotide variants; TC, total cholesterol; TG, triglyceride.

the genomes of clinical specimens with DNA microarrays and performed laboratory validation. Additionally, we retrospectively collected clinical characteristics of pregnant women in early pregnancy (12–14 gestational weeks) and performed the correlation analysis of lipid profiles at baseline with the candidate genes. Furthermore, we constructed *in vitro* and *in vivo* gain-of-function models to explore the role of candidate genes in regulating lipid metabolism and participating in the development of PE.

MATERIALS AND METHODS

Clinical Study Design and Oversight

From October 2013 to December 2017, we recruited 220 participants, including 110 PE patients and 110 controls, from two institutes in Shanghai, China (Department of Obstetrics, International Peace Maternity and Child Health Hospital, and Department of Obstetrics, Renji Hospital, Shanghai Jiao Tong University School of Medicine). The case-control study flow chart is displayed in **Figure 1**. Fasting peripheral blood specimens were collected before delivery. The inclusion criteria for the PE group were pregnant women with hypertension ($\geq$ 140 mmHg systolic and $\geq$ 90 mmHg diastolic blood pressure), proteinuria higher than 0.3 g/d or qualitatively + above. In contrast, healthy age- and gravidity-matched pregnant women with normotensive pre-pregnancy and pregnancy and without obstetric complications as the control group.

Table 1 lists clinical features at the patients' initial diagnosis of PE. We retrospectively collected clinical characteristics of subjects at their initial routine prenatal examination in early pregnancy (12–14 gestational weeks), which is listed in **Table 2**. Since the Department of Obstetrics at Renji Hospital is a consultation and resuscitation center for maternal near-miss in Shanghai, some PE patients in this hospital were referred from other institutions, so the baseline data of some subjects in early pregnancy (12–14 weeks of gestation) are missing. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (the Ethics Committee Approval Protocol No. 5 of 28 February 2014). All subjects provided broad informed consent for the research use of their biological samples.

Copy Number Variation Genotyping and Data Analysis

Genomic DNA was extracted from the peripheral blood (n = 10/group) of pregnant women and detected using Affymetrix CytoScan HD Cytogenetics Chips according to the manufacturer's recommended protocol. Quality control analysis indicated that all microarrays were qualified, except one was judged as male by the Chromosome Analysis Suite software for unclear reasons (**Supplementary Table 1**). Therefore, we excluded this sample from the analysis of the microarray data.

The raw data was processed by Chromosome Analysis Suite analysis software from Affymetrix to obtain all the CNVs, including duplications and deletions. Given the small sample size for microarray analysis, we opted for CNVs that occurred with a frequency difference of more than 30% between the two groups. These CNVs did not overlap with the high-frequency CNV regions of ordinary individuals in the Database of Genomic Variants[1] as differential CNVs. The differential genes within the region of differential CNVs were given gene annotation by the Gene Ontology (GO) database. The GO function enrichment analysis was employed to obtain the special functions for these genes using Fisher's exact test and $\chi 2$ test with the threshold of $P < 0.05$ and FDR < 0.25.

Verification of Gene Copy Numbers and Clinical Correlation Analysis

Total genomic DNA was extracted from peripheral blood specimens (n = 100/group) of pregnant women using the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Scientific) following the manufacturer's instructions. The genes linked to lipid metabolism were verified by quantitative polymerase chain reaction (qPCR), and *GAPDH* was used as an endogenous control at the DNA level. Details of the primer sequences are shown in **Supplementary Table 2**. In this study, correlations between candidate genes and clinical lipid profiles were analyzed by SPSS Statistics 25, using Pearson's correlation test.

Establishment of Lipid Accumulation Model *in vitro*

HepG2 (ATCC) cell line was cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% Penicillin-Streptomycin (Thermo Fisher Scientific) at 37°C in a 5% CO2 atmosphere. We infected cells with lentivirus carrying *APOBEC3A* _Flag or negative control and screened the cells using puromycin (Thermo Fisher Scientific) to obtain a pool of cells overexpressing the *APOBEC3A* gene (or negative control). DMEM medium with 1 mM oleic acid (Sigma) served as the fat accumulation inducer for the cells. Cell cultures were collected after being starved overnight and treated with the fat accumulation inducer for 24 h. The intracellular lipid content was determined by a biochemical analyzer (Roche). The results were normalized to the total protein concentration.

Adeno-Associated Virus-Mediated Overexpression of *APOBEC3A* in Mice

The coding sequence of human *APOBEC3A*, the 3 × flag tag, and a green fluorescent protein were simultaneously subcloned into the AAV2/8 plasmid (AAV_*APOBEC3A*) under the control of the thyroxine-binding globulin promoter, a hepatic-specific promoter. This serotype is known to have a stable expression in the liver after injection (11). A virus containing green fluorescent protein alone under control of the thyroxine-binding globulin promoter was used as a control virus (AAV_Mock).

Separate groups of 6- to 8-week-old wild-type, C57BL/6J female mice (n = 14/group) were injected intraperitoneally with 1.5×10^{10} vector genomes/mouse of the relevant vectors. Mice

[1] http://dgv.tcag.ca/dgv/app/home

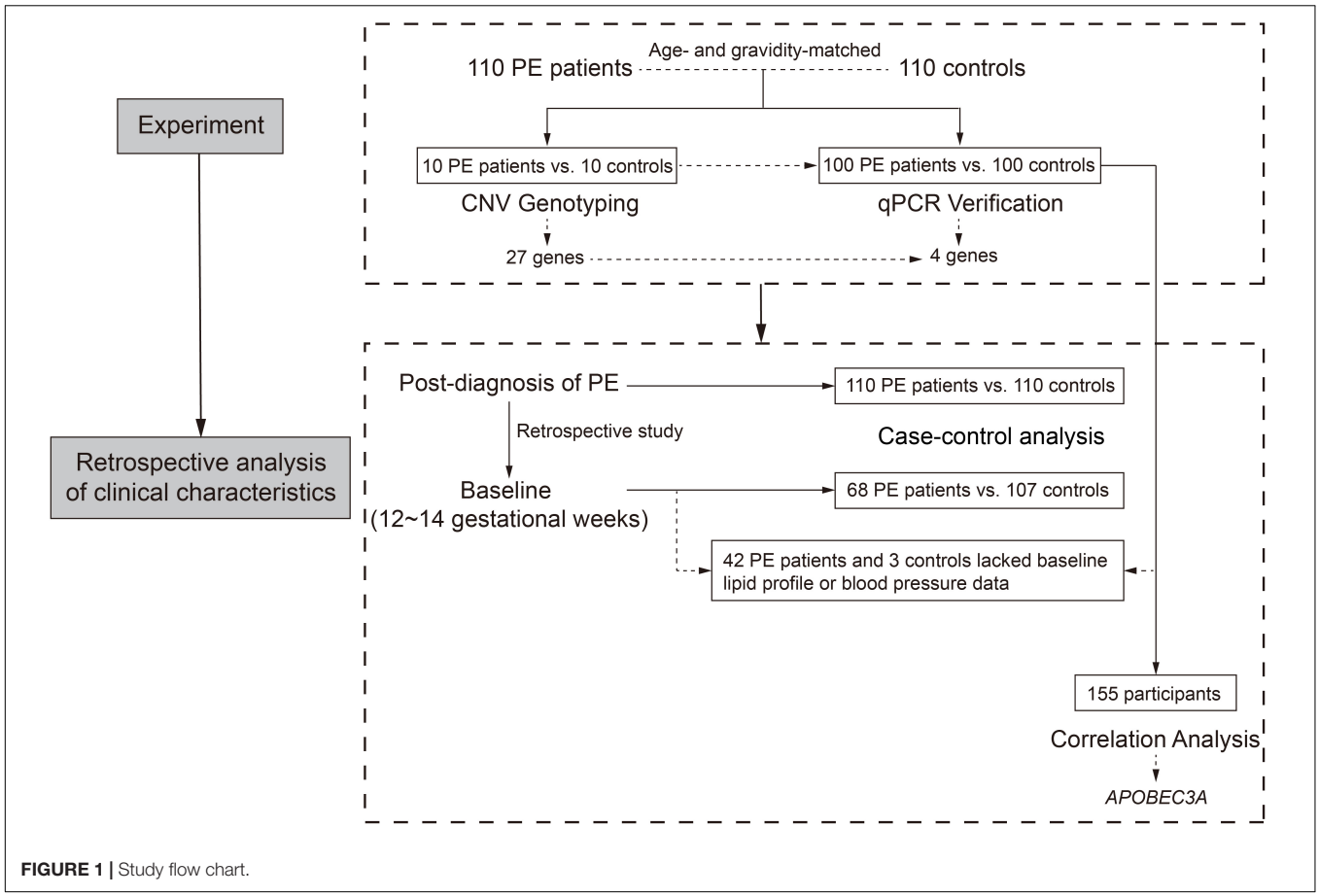


FIGURE 1 | Study flow chart.

were fed conventional chow throughout the study. They were sacrificed to investigate the impact of *APOBEC3A* on lipid metabolism (n = 6/group) or mated to explore the effect of the *APOBEC3A* gene on pregnant mice (n = 8/group, a positive vaginal plug was marked as gestation day 0, pregnant mice were sacrificed on gestation day 18) at 5 weeks (35 days) after AAV administration. Mice were fasted for 4 h, anesthetized with isoflurane, and whole blood was collected by cardiac puncture into BD Microtainer EDTA collection tubes. Plasma samples were used for lipid analysis and assayed using a biochemical analyzer (Roche). Liver and placenta specimens were obtained and immediately fast-frozen in nitrogen and stored at −80°C for subsequent testing. Fetuses were delivered by cesarean section, and their weights were measured. We performed all animal procedures according to protocols approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

RNA-Seq and Cluster Analysis

Total RNA was extracted from HepG2 cells (n = 3/group) and used for RNA-seq analysis. cDNA library construction and sequencing were performed by the Shanghai Majorbio Bio-Pharm Technology Co., Ltd. using the majorbio cloud platform. High-quality reads were aligned to the human reference genome (GRCh38) using Bowtie2. We normalized the expression level of each gene to the fragment of the exon model per million mapped reads (FPKM) based on the expectation-maximization method. NOISeq method was used to screen out differentially expressed genes between the two groups with a fold change $\geq$ of 1.5. Function and pathway annotation and enrichment analysis were based on the GO database and the Kyoto Encyclopedia of Genes and Genomes database.

We obtained the list of genes connected with cholesterol homeostasis and fatty acid metabolism from the Gene Set Enrichment Analysis website. The FPKM of these genes were logarithmically (fold-change) converted, and we visualized the differences between the two groups through heatmap, which was performed by GraphPad Prism V.8 software.

Reverse Transcription-Polymerase Chain Reaction and Immunoblot Analysis

Cell cultures (n = 3/group) or liver tissues (n = 6/group) were lysed in TRIzol (Invitrogen), and total RNA was extracted according to the manufacturer's instructions. cDNA was synthesized using PrimeScript^{TM} RT Master Mix (Takara). A reverse transcription-polymerase chain reaction was performed using PowerUp^{TM} SYBR^{TM} Green Master Mix (Thermo Fisher Scientific) on an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific). Primer sequences for human cells are listed in **Supplementary Table 3**

TABLE 1 | Clinical characteristics of participants at diagnosis.

Variables	PE patients (*n* = 110)	Controls (*n* = 110)	*P*-value
Age, years	31.00 (8.00)	31.00 (5.25)	0.205
SBP, mmHg	147.00 (16.00)	119.00 (11.00)	< 0.001[a]
DBP, mmHg	94.00 (12.00)	75.00 (9.00)	< 0.001[a]
Gestational age at delivery, week	37.00 (3.20)[b]	38.80 (1.30)	< 0.001[a]
Proteinuria, g/24 h	0.42 (0.95)	0.06 (0.05)	< 0.001[a]
Fetal weight, g	2830.00 (1170.00)[b]	3307.50 (428.75)	< 0.001[a]

PE, preeclampsia; SBP, systolic blood pressure; DBP, diastolic blood pressure. Variables were expressed as median (IQR). The p-values were calculated by the unpaired two-tailed Mann-Whitney U-test.
[a]$P \leq 0.05$; [b]Excluding 7 stillbirths due to early termination of pregnancy in patients with severe PE.

TABLE 2 | Clinical characteristics of participants in early pregnancy (12–14 gestational weeks).

Variables	PE patients (n = 68)	Controls (*n* = 107)	*P*-value
Body mass index, kg/m^2	23.00 (4.00)	21.40 (3.10)	0.006[a]
SBP, mmHg	110.00 (14.00)	110.00 (13.00)	0.102
DBP, mmHg	70.00 (11.00)	70.00 (4.50)	0.240
TC, mmol/L	4.62 (0.93)	4.46 (0.70)	0.037[a]
TG, mmol/L	1.61 (1.13)	1.27 (0.64)	< 0.001[a]
HDL, mmol/L	1.82 (0.69)	1.81 (0.38)	0.839
LDL, mmol/L	2.71 (0.85)	2.48 (0.64)	0.056

PE, preeclampsia; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Variables were expressed as median (IQR). The p-values were calculated by the unpaired two-tailed Mann-Whitney U-test. [a]$P \leq 0.05$.

(**Supplementary Table 4** is for mouse tissues). All results were normalized to *GAPDH* expression and calculated using the comparative CT ($\Delta\Delta$ CT) method.

Cell cultures (n = 3/group) were lysed with RIPA lysis buffer (Thermo Fisher Scientific). Protein lysates were separated on 10% SDS-PAGE gels and electrophoretically transferred to polyvinylidene fluoride membranes (Millipore). The membranes were blocked with 5% skim milk and incubated overnight at 4°C with mouse monoclonal anti-FLAG® M2 (Sigma) as primary antibodies. Then, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Goat anti-rabbit, or -mouse IgG, Jackson ImmunoResearch). Protein band chemiluminescence was detected using an ECL Plus immunoblot detection system (Millipore). We performed densitometric analysis on low-exposure images with ImageJ (National Institutes of Health). Rabbit polyclonal anti-β-Tubulin antibody (Proteintech) was used as a loading control to confirm equivalent protein loading on the same membrane.

Immunohistochemistry Analysis

Fresh liver tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin. We then sectioned them into 4-μm-thick sections for immunohistochemical staining. The primary antibodies used in this assay were rabbit monoclonal IgG [EPR25A]-isotype control (Abcam) and rabbit polyclonal to APOBEC3A (Abcam). Immunostaining was performed according to the standard protocol. Then, labeling was visualized using the diaminobenzidine method. Counterstaining was performed using Mayer's hematoxylin.

Enzyme-Linked Immunosorbent Assay

Approximately 25 mg of the liver (n = 6/group) or placental tissue (n = 8/group) was weighed and made into a 10% saline tissue homogenate, centrifuged at 4°C for 10 min at 3,000 g to obtain the supernatant for subsequent assays. TG or total cholesterol (TC) levels in the liver were measured by a biochemical analyzer (Roche). Placental growth factor (PLGF) or soluble fms-like tyrosine kinase-1 (sFlt-1) concentrations in plasma or placenta were measured by mouse PLGF or sFlt-1 Enzyme-linked immunosorbent assay kits (R&D Systems). The results were normalized by tissue mass.

Untargeted Relative Quantitative Lipidomics

Lipids from liver specimens (n = 6/group) were extracted according to the methyl tert-butyl ether method (12). Samples were separated and analyzed using an ultra-performance liquid chromatography system (Waters) equipped with a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Lipid lists were obtained by annotation using Lipid Search software, and differential lipids were identified by t-test and orthogonal partial least squares discrimination analysis test. The Beijing Genomics Institute performed this assay.

Statistical Analysis

Data were expressed as mean (*SD*) or median (IQR) when appropriate. Comparisons of continuous variables between two groups were made by unpaired two-tailed Student's t-test or Mann-Whitney U-test when variables were normally or non-normally distributed, respectively. Statistical tests were performed by GraphPad Prism V.8. Correlation analysis was conducted by SPSS Statistics 25. $P \leq 0.05$ (*) was considered statistically significant. Other statistical analysis methods used have been described separately in the respective methods section.

Graphical abstract was created using graphics from www.Biorender.com.

RESULTS

Preeclampsia Patients Showed a Trend of Abnormal Lipid Metabolism Before the Onset of the Disease

Compared with the control group, patients with PE presented with elevated blood pressure, increased proteinuria, decreased gestational age at delivery, and fetal growth restriction (**Table 1**), consistent with previous reports (13). The clinical characteristics of 68 PE patients and 107 controls in early pregnancy (12–14

gestational weeks) were retrospectively obtained, as shown in **Figure 1** and **Table 2**. The findings showed that those pregnant women who subsequently developed PE exhibited distinctly elevated plasma TC (P = 0.037) and TG (P < 0.001) levels before the disease onset and had significantly increased body mass index (P = 0.006). However, their blood pressure (systolic blood pressure, P = 0.102; diastolic blood pressure, P = 0.240) did not differ substantially in early pregnancy compared to controls. These findings indicate that PE patients tend to have abnormal lipid metabolism before the onset of the disease, but the causative mechanisms have not been elucidated.

Differential Genes Between Two Groups Were Enriched in Functions Related to Metabolic Processes

From the genomic analysis of 10 PE patients and 10 controls (clinical characteristics are shown in **Supplementary Table 5**), we obtained 27 differential CNVs, including 22 deletions and 5 duplications (**Figure 2A**). The identified variants ranged in size from 1,409 base pairs to nearly 151.7 kb (median = 10.8 kb), and these CNV regions contained 27 differential genes (**Figure 2B** and **Supplementary Table 6**). GO functional enrichment analysis revealed that the biological functions of these differential genes were enriched in response to nutrient levels, protein hydrolysis, developmentally programmed cell death, and regulation of triglyceride/cholesterol/lipoprotein metabolism (**Figure 2C**).

Aberrant Copies of Genes Linked to Lipid Metabolism Were Verified in 100 Preeclampsia Patients and 100 Controls

We identified six genes in the differential CNV regions that may be connected with lipid metabolism. The *LPA* gene located in the 6q26 region from 161.032 to 161.047 Mb was reported in previous research on PE (14–17), and we detected this duplication in one case (10%) and four controls (44.4%). *APOBEC3A*, *APOBEC3A_B*, and *APOBEC3B* in 22q13.1 from 39.350 to 39.390 Mb were detected in one case (10%) and four controls (44.4%). The *BTNL3* gene in the 5q35.3 region from 180.379 to 180.431 Mb, with this deletion detected in one case (10%) and four controls (44. 4%). The *LMF1* gene in the 16p13.3 region from 1.002 to 1.003 Mb, with this duplication detected in no one case and three controls (33.3%). These four regions of differential CNVs were confirmed by qPCR with significantly increased copy numbers of *APOBEC3A*, *APOBEC3A_B*, *BTNL3*, and *LMF1* in PE patients compared with controls (**Figures 3A–D**). In contrast, the decrease in copies of *LPA* and the increase in copies of *APOBEC3B* were not statistically significant (**Figures 3E,F**).

APOBEC3A Showed a Significant Positive Correlation With Total Cholesterol and Low-Density Lipoprotein Cholesterol in Human Subjects

To identify the predisposing genes causing lipid metabolism disturbance in PE patients, we collected clinical lipid profiles from these participants' initial prenatal examination (12–14 gestational weeks) and analyzed the correlation between lipid profiles and the copy number of these candidate genes. The results indicated significant positive correlations between the copy number of *APOBEC3A* and both TC (P < 0.001) and LDL (P = 0.048) levels in serum (**Figure 3G**). In contrast, we did not find significant correlations between other candidate genes and clinical lipid profiles here, except for copies of both *BTNL3* (P = 0.012) and *LPA* (P = 0.006), which were significantly positively correlated with HDL levels (**Supplementary Figures 1A–E**). For the first time, we report an interesting finding that the *APOBEC3A* gene may play a role in regulating lipid metabolism and be related to the pathogenesis of PE. Subsequently, we investigated how the novel gene *APOBEC3A* participates in regulating lipid metabolism and the pathogenesis of PE.

APOBEC3A Disrupted Lipid Metabolism in HepG2 Cells *in vitro*

The liver is recognized as an essential metabolic organ in humans (18). To explore the underlying mechanism of *APOBEC3A* in lipid metabolism regulation, we constructed an *in vitro* model of HepG2 (HepG2^{APOBEC3AOE}), a human hepatocellular carcinoma cell line overexpressing *APOBEC3A* (**Figure 4A**). Both TC and TG levels were distinctly upregulated in HepG2^{APOBEC3AOE} cells compared to mock cells treated with or without fat accumulation inducer (**Figure 4B**). RNA-seq analysis revealed that the differentially expressed genes in HepG2^{APOBEC3AOE} were enriched in the biological function of response to lipid (**Figure 4C**) and the pathway of regulation of lipolysis in the adipocytes (**Figure 4D**). Furthermore, hallmark gene sets related to cholesterol homeostasis and metabolism of fatty acids in HepG2^{APOBEC3AOE} (**Figure 4E**) showed distinct differences compared to the mock cells. The genes linked to cholesterol biosynthesis (*FDPS*, *DGAT2*) and fatty acid biosynthesis (*SCD*, *FASN*), as well as fatty acid absorption (*CD36*), were upregulated in HepG2^{APOBEC3AOE}. On the other hand, the genes associated with cholesterol catabolism (*GPD1*) and fatty acid β-oxidation (*CPT1A*) were downregulated (**Figure 4F**). These findings indicate that *APOBEC3A* may be involved in both cholesterol and fatty acid metabolic processes.

Mice With Hepatic-Specific Overexpression of *APOBEC3A* Exhibit Disturbed Lipid Metabolism

To explore the potential roles of the *APOBEC3A* gene in regulating lipid metabolisms and participation in the pathogenesis of PE *in vivo*, we injected AAV_*APOBEC3A* and AAV_Mock intraperitoneally in adult C57BL/6J female mice (**Figures 5A,B** and **Supplementary Figure 2**). The first important finding was that TC and TG levels were elevated in both plasma and liver of *APOBEC3A* over-expressing mice (**Figure 5C**). Moreover, the expression of genes related to cholesterol biosynthesis (*Hmgcr*, *Hmgcs2*, *Fdps*) and fatty acid biosynthesis (*Srebp1*, *Fasn*, *Acc1*) were

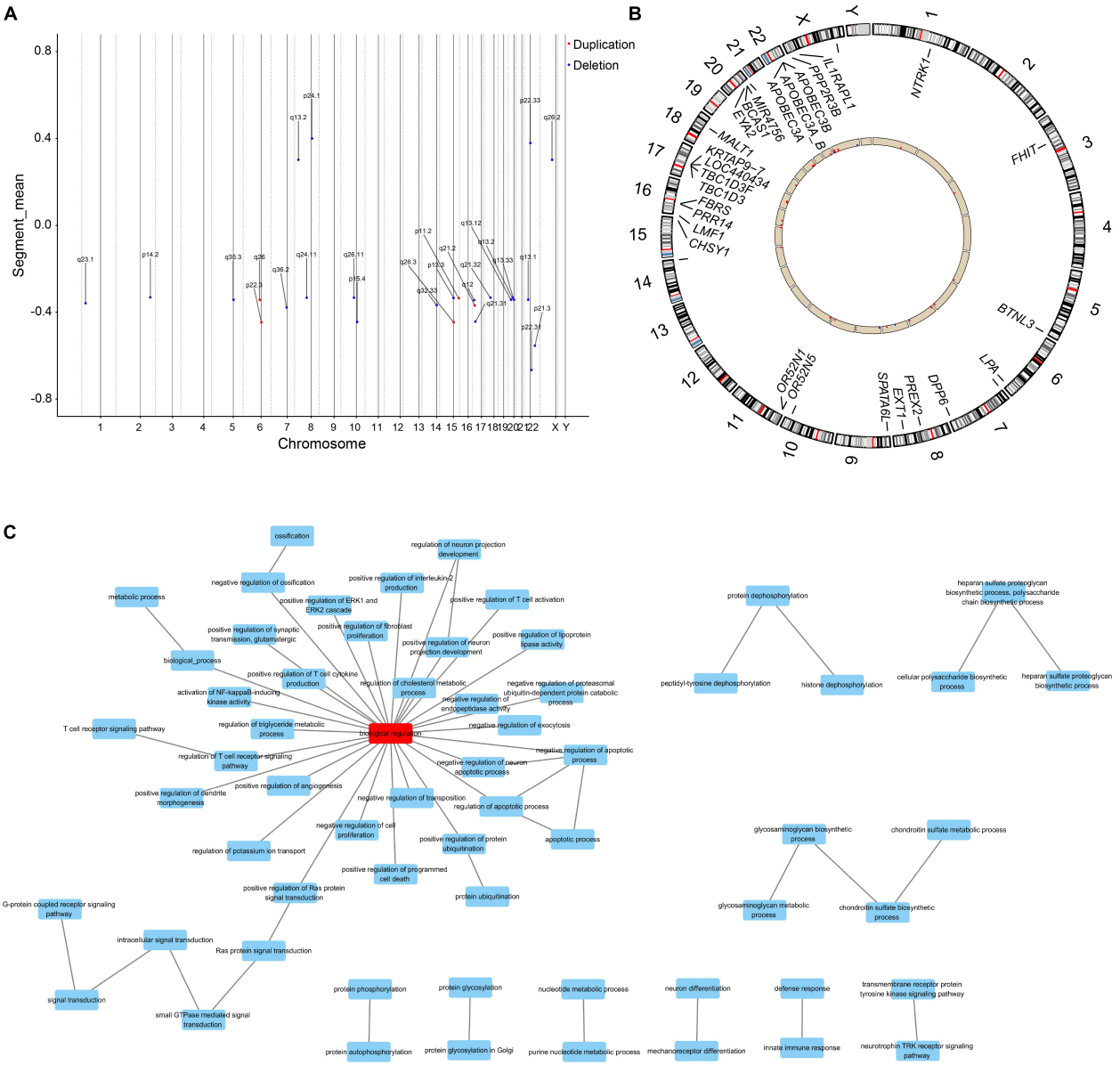


FIGURE 2 | The differential genes linked to aberrant CNVs are enriched in metabolism-related biological functions. **(A)** Distributions of aberrant CNVs on each chromosome. Red and blue dots indicate whether the type of the CNV is duplication or deletion, respectively. **(B)** Distribution of differential genes within the region of differential CNVs in the genome. Red columns indicate that the copy number of this gene is increased in PE patients compared to controls, and blue columns indicate a decrease. **(C)** Differential genes were enriched in functions associated with lipid and cholesterol metabolism, immunity, and development.

increased. In contrast, the expression of genes linked to fatty acid β-oxidation (*Adiporq*, *Acoxl*) was decreased in the liver of *APOBEC3A* over-expressing mice (**Figure 5D**). Lipidomics indicated that fatty acids, (O-acyl)-1-hydroxy fatty acids, triglycerides, and diacylglycerol were elevated in mice over-expressing *APOBEC3A* (**Figure 5E** and **Supplementary Figure 3A**). Moreover, pathway enrichment analysis indicated that differential lipids were enriched in glycerophospholipid metabolism, adipocytokine signaling pathway, fat digestion and absorption, regulation of lipolysis in adipocytes, and cholesterol metabolism (**Supplementary Figure 3B**). These results imply that *APOBEC3A* may play a significant role in the biosynthesis of fatty acids and triglycerides.

Pregnant Mice With Hepatic-Specific Overexpression of *APOBEC3A* Exhibit Preeclampsia-Like Symptoms

Fetuses in the AAV_*APOBEC3A* group had decreased fetuses/placenta ratios, which appeared to be driven by a significant reduction in fetal weight (**Figure 5F**), showing intrauterine growth restriction, a common clinical manifestation

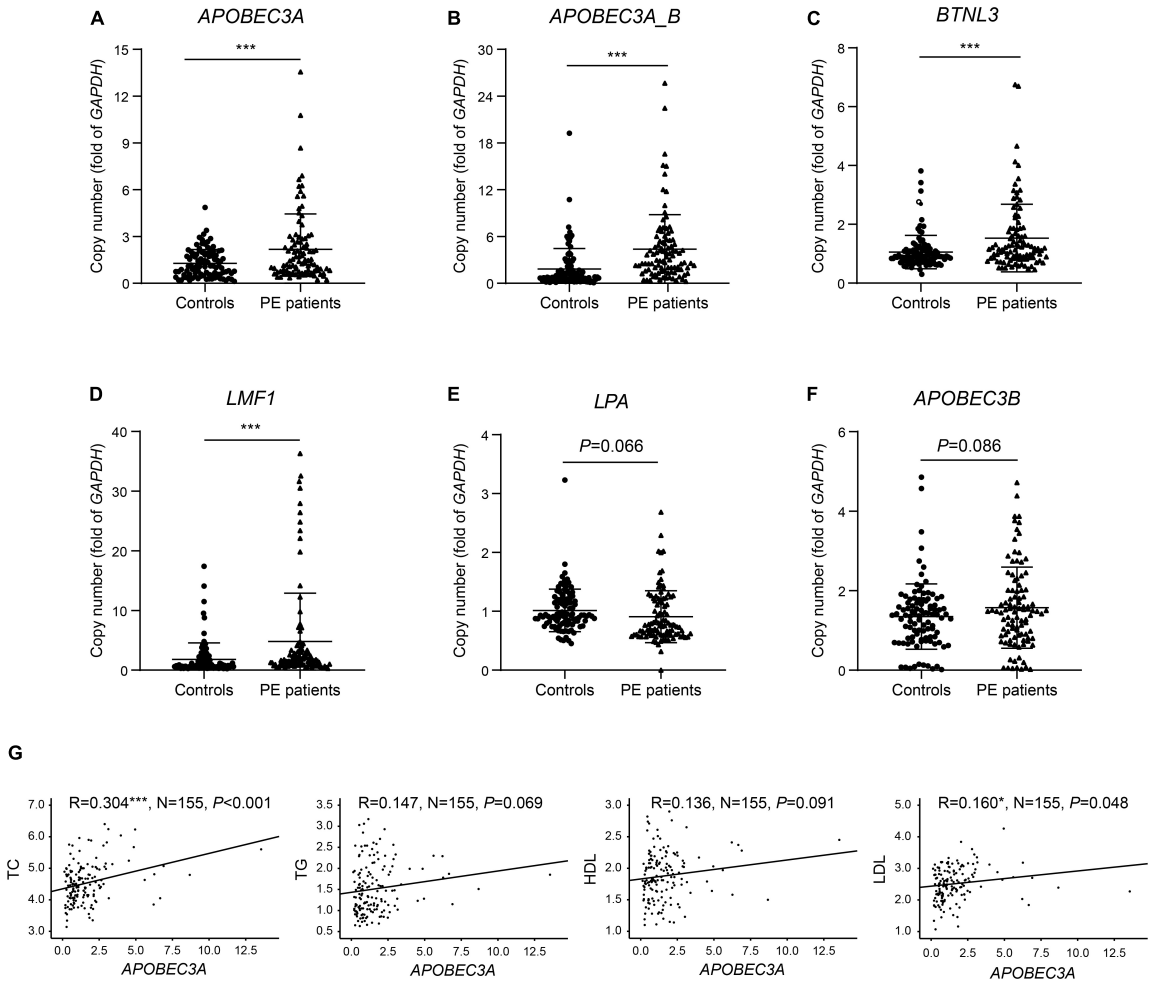


FIGURE 3 | Verification of copies for lipid metabolism-related genes and correlation of copies of *APOBEC3A* with lipid profiles. The copy numbers of **(A)** *APOBEC3A*, **(B)** *APOBEC3A_B*, **(C)** *BTNL3*, **(D)** *LMF1*, **(E)** *LPA*, **(F)** *APOBEC3B* in PE patients and controls (n = 100/group). **(G)** Correlation of plasma lipid profiles with *APOBEC3A* copies (n = 155). The unpaired two-tailed Student's t-test determined statistical significances between two groups. The Pearson correlation coefficient analyzed the correlations. Data were represented as the mean ± SEM. ***$P < 0.001$, *$P < 0.05$. PE, preeclampsia.

of PE. The sFlt-1/PLGF ratio is a well-known marker for the short-term prediction of PE (19, 20). We measured the levels of PLGF and sFlt-1 in mouse placenta homogenates and serum by ELISA. The protein and mRNA levels of sFlt-1 and the sFlt-1/PLGF ratio were significantly increased in the placenta of the AAV_*APOBEC3A* group compared with the AAV_Mock group (**Figure 5G**). In contrast, there was no substantial change in both protein and mRNA levels of PLGF in placental tissues (**Supplementary Figure 4A**). Besides, these changes mentioned above were not observed in serum samples (**Supplementary Figure 4B**). These findings indicate that *APOBEC3A* may be involved in the pathogenesis of PE.

DISCUSSION

Overall, our study identified 27 aberrant CNVs related to PE, including 22 deletions and 5 duplications. The differential genes in these CNV regions were enriched mainly in metabolism-related biological functions. Moreover, aberrant copies of *APOBEC3A*, *APOBEC3A_B*, *BTNL3*, and *LMF1* were validated in our cohort, especially the copy number of *APOBEC3A* was markedly correlated with clinical lipid parameters of human subjects in early pregnancy. To our best knowledge, we are the first to report that *APOBEC3A* plays a role in regulating lipid metabolism and participating in the pathogenesis of PE.

Despite the growing attention to the pathogenesis of PE, there are still few reports investigating its pathogenesis related to CNV, i.e., duplications or deletions of genomic segments greater than 1 kb in length (21). Although most genome studies have focused on single nucleotide variants (SNVs), CNVs cover more bases than SNVs and may have more potent effects on gene expression and structure (22, 23). Zhao et al. (24) analyzed CNVs from a US cohort and identified two relevant genes, *PDXDC1* and *PSG11*. However, the hypothesis

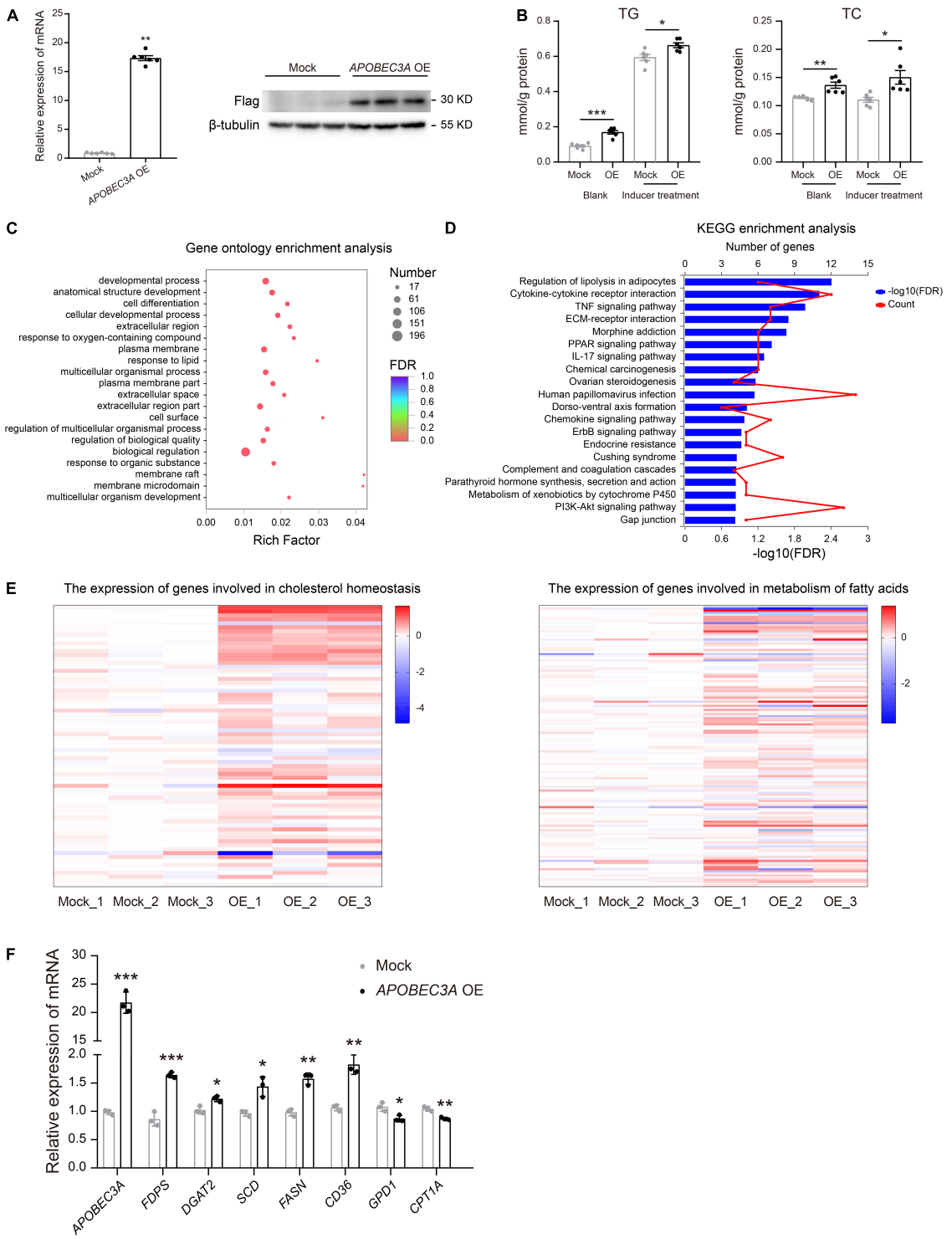


FIGURE 4 | HepG2^{APOBEC3AOE} exhibited perturbed cholesterol and fatty acid metabolism. **(A)** HepG2 cell lines over-expressing *APOBEC3A* were constructed. **(B)** TG and TC levels in the lysates of HepG2^{APOBEC3AOE} and mock cells (n = 6/group). **(C)** Differential genes between HepG2^{APOBEC3AOE} and mock cells were enriched in functions of respond to lipid. **(D)** Differential genes between HepG2^{APOBEC3AOE} and mock cells were enriched in pathways of the regulation of lipolysis in adipocytes. **(E)** The expression of genes connected with cholesterol homeostasis and metabolism of fatty acids in HepG2^{APOBEC3AOE} and mock cells (n = 3/group). **(F)** The mRNA expression of multiple genes related to cholesterol and fatty acid metabolism in HepG2^{APOBEC3AOE} and mock cells (n = 3/group). The unpaired two-tailed Student's t-test determined statistical significances between two groups. Data were represented as the mean ± SEM. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. OE, HepG2^{APOBEC3AOE} cells; Mock, HepG2 mock cells.

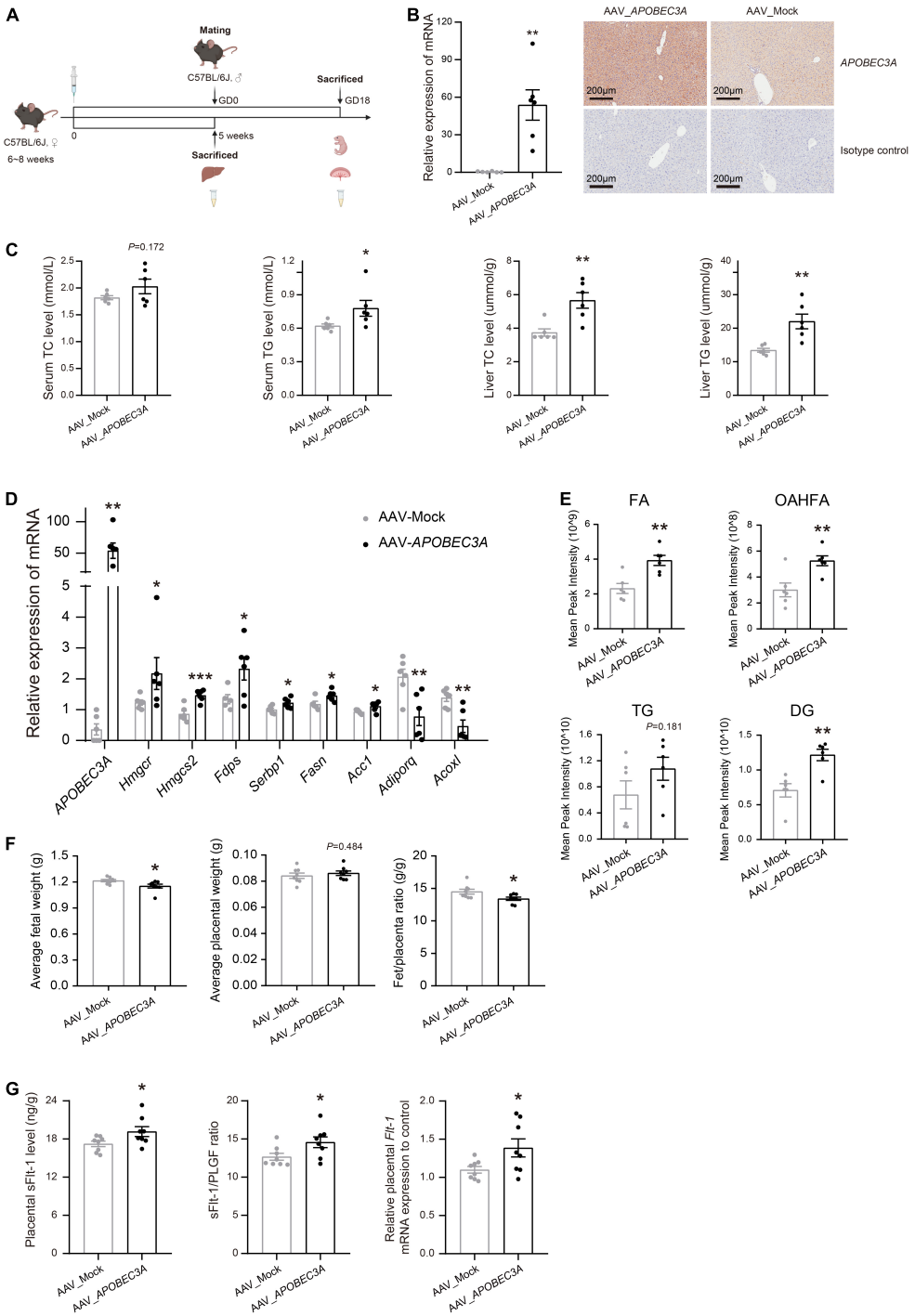


FIGURE 5 | Hepatic-specific overexpression of *APOBEC3A* in mice manifested abnormal lipid metabolism and PE-like symptoms. **(A)** Experimental design and timeline for the *in vivo* experiment in mice. **(B)** The mRNA and protein expression of *APOBEC3A* gene in the liver of non-pregnant mice injected with AAV_*APOBEC3A* or AAV_Mock (n = 6/group). **(C)** TC and TG levels in plasma and liver of mice injected with AAV_*APOBEC3A* or AAV_Mock at 5 weeks after AAV administration (n = 6/group). **(D)** The mRNA expression of multiple genes related to cholesterol and fatty acid metabolism in mice injected with AAV_*APOBEC3A* or AAV_Mock (n = 3–6/group). **(E)** Levels of FA, OAHFA, TG, and DG metabolites in mice injected with AAV_*APOBEC3A* or AAV_Mock (n = 6/group). **(F)** The average weight of fetuses and placentas in pregnant mice injected with AAV_*APOBEC3A* or AAV_Mock (n = 8/group). **(G)** The protein and mRNA levels of sFlt-1 in the placenta of pregnant mice injected with AAV_*APOBEC3A* or AAV_Mock (n = 8/group). The unpaired two-tailed Student's t-test determined statistical significances between two groups. Data were represented as the mean ± SEM. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. FA, fatty acids; OAHFA, (O-acyl)-1-hydroxy fatty acids; TG, triglycerides; DG, diacylglycerol. GD, day of gestation.

of them being involved in the pathogenesis of PE was not fully supported by further laboratory studies. The above two genes have no overlap with our findings, which may be interpreted as a difference in the genetic background of the two study cohorts.

Abnormal lipid metabolism in pregnant women has been reported to be possibly connected with the pathogenesis of PE (6). PE-related endothelial dysfunction may be associated with changes in lipid structure and oxidative stress caused by abnormal lipid metabolism (25, 26). Physiological hyperlipidemia, which occurs during normal pregnancy, increases the energy supply to both mothers and fetuses. Once the concentrations of TG and free fatty acid in pregnant women gradually increase beyond the physiological tolerance ranges of normal pregnancy, lipid molecules could accumulate and cause extensive vascular endothelial damage and systemic inflammation, eventually leading to PE (25). Consistent with previous reports, we found a significant increase of TC and TG in PE patients in early pregnancy compared with controls, which provides further evidence for abnormal lipid metabolism as a susceptibility factor for PE.

APOBEC3A belongs to the apolipoprotein B mRNA editing enzyme and catalytic polypeptide-like protein family, catalyzing cytidine deamination in single-stranded DNA/RNA to uridine (27). It plays an essential role in the host's innate immune by inducing viral mutations to protect cells from viral infection (28). Apart from this, *APOBEC3A*-induced genomic mutations may drive the progression of multiple cancers (29). In this study, we identified a deletion CNV including *APOBEC3A*, *APOBEC3A_B*, and *APOBEC3B* in the genomes of controls (44%). In contrast, the incidence of this CNV is lower in PE patients (10%), which may lead to elevated levels of both *APOBEC3A* and *APOBEC3B* in PE patients. *APOBEC3A_B* has the promoter and coding region of *APOBEC3A* and the 3'UTR of *APOBEC3B*. The resulting chimeric *APOBEC3A* transcription product would lead to a further increase in intracellular *APOBEC3A* levels (30). Subsequently, we also confirmed by qPCR that the copy numbers of *APOBEC3A* and *APOBEC3A_B* genes were markedly higher in PE patients than in controls. Notably, our findings showed that the copy number of *APOBEC3A* was statistically positively correlated with serum concentrations of TC and LDL. *LPA* is known to be associated with metabolic diseases (31) and may be involved in the pathogenesis of PE (14–17). *BTNL3* has also been reported to have a possible role in intestinal metabolism and inflammation in neonates with intrauterine growth restriction (32). Our study found that copies of both *LPA* and *BTNL3* were significantly and positively associated with HDL levels. Moreover, *in vivo* and *in vitro* studies suggested that *APOBEC3A* may regulate cholesterol and fatty acid metabolisms. These findings filled the gap in the role of *APOBEC3A* in regulating lipid metabolisms. Of note, we found that *APOBEC3A* expression was also increased in patients with non-alcoholic steatohepatitis (NASH), especially in NASH in combination with fibrosis (unpublished data), suggesting that *APOBEC3A* may play a role in other metabolic diseases as well. Overall, elevated *APOBEC3A* copies may lead to increased cholesterol and fatty acid biosynthesis and decreased catabolism, which increases the risks of various metabolic diseases. These findings may guide the dietary management and clinical use of drugs in such high-risk groups and have good clinical application prospects, although the specific mechanism by which *APOBEC3A* regulates lipid metabolism needs in-depth study.

PE is a gestational disease, and the main manifestations in animal models include elevated blood pressure and urinary protein, restricted fetal growth, elevated sFlt-1, decreased PLGF, and increased sFlt-1/PLGF ratio (33). In the present study, we found that the average fetal weight per pregnant mice overexpressing *APOBEC3A* was distinctly reduced (**Figure 5F**), a manifestation of growth restriction. Both sFlt-1 levels and sFlt-1/PLGF ratios were distinctly increased in the placenta (**Figure 5G**). However, changes in plasma parameters were not evident (**Supplementary Figure 4**), which led us to speculate that to be due to vascular endothelial damages may be only related to local (placenta) changes in PE rather than systemic ones. On the other hand, PE is a complex disease induced by multiple factors where a single element may be difficult to interpret the pathogenesis of PE. Our molecular and clinical data suggest that aberrant *APOBEC3A* copies could at least partly explain the pathogenesis of PE.

In terms of limitations, we did not conduct a subgroup analysis of the collected cases as early-onset and late-onset PE due to limited recruitment, despite the pathogenesis of PE may differ among patients in those two subtypes. Further, our recruited participants were all from one city in China, as PE patients from other regions were not available at the time of this study. This study retrospectively collected information on the lipid profile of pregnant women in early gestation, which resulted in a certain percentage of missing data. We did not account for the effect of possible confounding factors, such as familial dyslipidemia and the use of lipid-lowering drugs, on this study. In addition, as the blood pressure of mice may be affected by various conditions such as sounds, light, and temperature, we did not measure the blood pressure of mice in this study. Finally, *in vivo* and *in vitro* knockout models need to be constructed to explore further the more detailed mechanisms that *APOBEC3A* may modulate lipid metabolism and participate in the pathogenesis of PE.

Collectively, this study established a novel link between genetics and lipid metabolism in the development of PE. That is, aberrant copies of *APOBEC3A* may be involved in the pathogenesis of PE by regulating lipid metabolism. This critical finding will likely facilitate a better understanding of the molecular mechanisms of PE. It is also our understanding that *APOBEC3A* copies and lipid profiles may potentially have clinical applications to benefit the early identification of pregnant women at high risk of PE, leading to early clinical intervention to reduce the risk of developing PE.

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 Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by The Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

AUTHOR CONTRIBUTIONS

NL, L-KG, and B-SW conceived and designed the experiments. NL, ML, and J-HS performed the experiments. Y-NG, X-JW, JM, XZ, YC, and J-HL provided clinical materials. NL, Y-TH, FZ, HH, and J-LX collected and analyzed the data. NL wrote the manuscript. L-KG and B-SW reviewed and edited the manuscript. All authors read and approved the submitted version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.841249/full#supplementary-material

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C677T Gene Polymorphism of MTHFR Is a Risk Factor for Impaired Renal Function in Pregnant Women With Preeclampsia in the Chinese Han Population

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Impaired renal function in pregnant women with preeclampsia is particularly common, yet there is no consensus about implementation. This lack of consensus is due in part to uncertainty about risks for disease progression. Limited evidence suggests that C677T gene polymorphism of 5, 10-methylenetetrahydrofolate reductase (*MTHFR* C677T) may affect impaired renal function in pregnant women with preeclampsia in Chinese Han population. To investigate the association between *MTHFR* C677T and impaired renal function in pregnant women with preeclampsia, a total of 327 pregnant women diagnosed with gestational hypertension (GH) or preeclampsia-eclampsia (PE) from January 2016 to December 2021 were selected as the study subjects. The personal information, gestational information, clinical indicators, and the C677T gene polymorphism of *MTHFR* were tested. Compared with the GH group, the PE renal function impairment group had increased in blood pressure, homocysteine level, liver and kidney function indicators (creatinine, uric acid, urea nitrogen, cystatin C, alanine aminotransferase, aspartate aminotransferase, cholyglycine), and blood lipids (total cholesterol, triglycerides and low density lipoprotein) but had reductions in plasma protein (total protein, albumin, globulin, prealbumin), trace elements (calcium and zinc), prothrombin time and fibrinogen. The homocysteine level in the TT genotype was higher than that in the CC and CT genotypes. Binary logistic regression analysis showed that the *MTHFR* C677T gene polymorphism was associated with PE renal function impairment in the recessive model (OR: 1.620, 95% CI: 1.033–2.541, $P < 0.05$). These findings show that the C677T gene polymorphism of *MTHFR* is an independent risk factor for impaired renal function in pregnant Chinese Han women with PE.

Keywords: pregnancy hypertension, preeclampsia, impaired renal function, *MTHFR*, risk factor

INTRODUCTION

Hypertensive disorders of pregnancy (HDP), including gestational hypertension (GH), preeclampsia-eclampsia (PE), pregnancy-associated chronic hypertension and chronic hypertension with superimposed preeclampsia (1), are idiopathic diseases of pregnancy, posing serious threats to the health of mothers and infants. The incidence of PE in developing countries such as China is higher than that in developed countries (2). Since pregnancy is a special physiological process, hypertension and other diseases with specific occurrences in pregnancy deserve special attention. Different from primary hypertension, the basic pathological changes of HDP include systemic arteriolospasm, which leads to poor blood flow in the organs throughout the body, insufficient blood supply in the microcirculation, and damage of tissues and organs due to ischemia and hypoxia. Among these factors, impaired renal function is particularly common. Impaired renal function is very important in the assessment and treatment of HDP because its occurrence mechanism in HDP is not exactly equal to the renal damage caused by chronic hypertension. Therefore, the pathogenesis of impaired renal function in HDP has gradually gained attention from the research field.

Genetic factors play an important role in the occurrence and development of HDP. For example, *CCR5* gene polymorphism is associated with PE in Brazilian women (3); endothelial nitric oxide synthase (*eNOS*) gene polymorphism is associated with PE in Egyptian women (4); and *CDH13* gene polymorphism is associated with PE in Han women (5). However, based on the current literature in China and abroad, there are no studies identifying the susceptibility genes for impaired renal function in HDP or PE, and there are few studies conducting comprehensive analyses of the pathogenesis of impaired renal function in HDP or PE based on genetic and environmental factors.

Combined plasma homocysteine elevation is a special feature of the hypertensive population in China. Due to genetic and environmental factors, the average plasma homocysteine level in Chinese adults with primary hypertension is 15 μmol/L, and approximately 75% of patients have elevated plasma homocysteine levels (6). Elevated plasma homocysteine is associated with the C677T gene polymorphism of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) (7), which is the key enzyme in the metabolism of homocysteine. The frequency of the mutant T allele of the *MTHFR* gene polymorphism C677T is high, present in 41% of the Chinese population (8). Our previous study confirmed that plasma homocysteine was associated with early renal impairment in Chinese Han patients with primary hypertension (9). It is possible that the C677T gene polymorphism of *MTHFR* may also affect the occurrence of impaired renal function among Chinese Han pregnant women with HDP, which has not been investigated previously.

Due to the different pathogenic mechanisms of HDP and primary hypertension, this study focused on pregnant women with GH and PE and did not include pregnant women with chronic hypertension. Urinary protein is recognized as an important indicator of impaired renal function. Therefore, this study used urinary protein as a marker of impaired renal function in PE patients. We used the case-control method and detected plasma homocysteine and the C677T gene polymorphism in its key metabolic enzyme *MTHFR*, urinary albumin, and multiple clinical indicators possibly associated with HDP to analyze the risk factors of impaired renal function in pregnant Chinese Han women with PE. This study intended to explain the reasons for the high incidence of renal function impairment in pregnant Chinese Han women with PE from a new perspective and to provide a theoretical basis for the early prevention and treatment of renal function impairment in pregnant women with PE in clinical practice.

METHODS

Enrolled Populations

A total of 327 pregnant women diagnosed with GH or PE in our hospital from January 2016 to December 2021 were selected as the study subjects. We recorded the patients' age, height, weight, history of smoking, gestational week, gestational week upon the occurrence of elevated blood pressure, family history of hypertension, HDP history, and other related personal information. Among them, the gestational week was defined as the gestational week at the end of pregnancy; gestational week upon the occurrence of elevated blood pressure was defined as the gestational week at the time of the first diagnosis of HDP; a family history of hypertension was defined as the presence of immediate family members with a history of hypertension; and a history of HDP was defined as the diagnose of HDP during prior pregnancies.

Grouping Criteria

Inclusion criteria: GH was defined as the first occurrence of hypertension after 20 weeks of gestation, with a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, which returned to normal within 12 weeks postpartum, and with negative results in urinary protein tests. PE was defined as the first occurrence of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation, with random urinary protein ≥ (+) or any organ or systemic involvement. We used random urinary protein, which is commonly used clinically and is easy to detect, as a basis for grouping based on impaired renal function. Since random urinary protein ≥(2+) is considered to indicate the presence of impaired renal function (1), only PE patients with random urinary protein ≥(2+) were enrolled in this study.

Exclusion criteria: (1) secondary hypertension; (2) pregnancy complicated with chronic hypertension, or chronic hypertension with superimposed preeclampsia; (3) renal parenchymal or vascular lesions; (4) severe heart failure or liver and kidney failure; (5) obstetric and gynecological acute and critical illness (amniotic fluid embolism, etc.); (6) tumor; (7) recent serious infections; and (8) multiple organ dysfunction syndrome.

Grouping criteria: (1) GH group (159 cases): systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and negative random urine protein results; (2) PE renal function impairment group (168 cases): systolic blood pressure

≥140 mmHg and/or diastolic blood pressure >90 mmHg and prenatal random urine protein ≥(2+).

Laboratory Methods

Urine protein was measured using the Siemens BN ProSpec special protein analyzer. Plasma homocysteine was determined using the Oppland OP-162 micro-fluorescence detector. Liver and kidney function, blood glucose, and blood lipids were detected using the Hitachi MODULAR PP automated biochemical analyzer. Trace elements were measured using the Tiancheng TC-3010B trace element analyzer.

In total, 4 ml peripheral blood samples from subjects were collected and stored at 4°C. Genomic DNA was extracted from the samples using Blood DNA System (NOBELAB BIOTECHNOLOGIES CO, LTD, Beijing) and stored at −80°C for later use. Then, the *MTHFR* C677T (rs1801133) was genotyped with the direct DNA sequencing method. The primer sequences were as follows: 5′-CAA GCA ACG CTG TGC AAG TTC TGG-3′and 5′-TGT GCT GTG CTG TTG GAA GGT GCA-3′. PCR amplification was performed. DNA was denatured at 95°C for 5 min, amplified by 40 s cycles at 95°C for 30 s and cooled at 58°C for 30 s, 72°C for 1 min, and a final elongation at 72°C for 5 min. For SNP rs1801133, the PCR products were sequenced by DNA sequencing. The inner primers were used for the cycle-sequencing reaction, and genotyping was analyzed using an ABI3730XL DNA sequencer.

Statistical Methods

SPSS 17.0 software was used for statistical analysis. Measurement data are expressed as the means ± standard deviations ($\bar{x} \pm s$). Counting data are expressed as proportions (%). Comparisons between means of two groups were conducted using the t test. Comparisons between means of three groups were conducted using one-way analysis of variance. Pair-wise comparisons between two groups were conducted using the Bonferroni test. Count data were conducted using the chi-square (χ^2) test. Gene distribution was tested using the Hardy–Weinberg equilibrium test. The relationship between disease and gene polymorphism was analyzed by binary logistic regression. $P < 0.05$ indicated that the difference was statistically significant.

RESULTS

Comparison of Basic Information Between the Two Groups

There were 159 cases in the GH group, with a mean age of (31.46 ± 5.76) years. There were 168 patients with impaired renal function in the PE group, with a mean age of (31.78 ± 5.08) years. There were no significant differences in age, body mass index (BMI), history of smoking, family history of hypertension, history of HDP, infant gender, twin ratio, number of pregnancies, and number of births between the two groups ($P > 0.05$).

Compared with the GH group, the PE renal function impairment group had an earlier gestational week at the end of pregnancy (37.18 ± 2.59 weeks, 33.92 ± 3.64 weeks, $P < 0.01$) and an earlier gestational week upon the occurrence of elevated blood pressure (34.84 ± 4.32 weeks, 32.54 ± 4.42 weeks, $P < 0.01$) and had an increased proportion of cesarean sections for pregnancy termination (60.4, 92.9%, $P < 0.01$). In addition, the PE renal function impairment group had a lower neonatal body weight (3,023.27 ± 641.28 g, 2,340.06 ± 816.29 g, $P < 0.01$) and Apgar score (9.32 ± 0.57, 8.78 ± 1.14, $P < 0.01$) than those in the GH group. The details are shown in **Table 1**.

TABLE 1 | Comparison of basic information between the two groups.

Item	GH group ($n = 159$)	PE group ($n = 168$)	*P*-Value
Age (year)	31.46 ± 5.76	31.78 ± 5.08	0.595
Body mass index (kg/m²)	31.08 ± 5.14	31.39 ± 4.07	0.552
<24	7.5%	2.4%	0.080
24–27.9	18.2%	16.7%	
≥28	74.2%	81.0%	
History of smoking (%)	1.9%	1.8%	0.946
Gestational week (week)	37.18 ± 2.59	33.92 ± 3.64[a]	0.000
GWEBP (week)	34.84 ± 4.32	32.54 ± 4.42[a]	0.000
Family history of hypertension (%)	3.8%	4.8%	0.787
History of HDP (%)	3.8%	8.9%	0.071
Cesarean sections (%)	60.4%	92.9%[a]	0.000
Infant gender (male%)	42.8%	50.6%	0.183
Neonatal body weight (g)	3,023.27 ± 641.28	2,340.06 ± 816.29[a]	0.000
Apgar score (score)	9.32 ± 0.57	8.78 ± 1.14[a]	0.000
Twin ratio (%)	8.8%	3.6%	0.064
Number of pregnancies (%)			
1	37.1%	25.0%	0.125
2	28.3%	28.6%	
3	16.4%	20.8%	
>3	18.2%	25.6%	
Number of births (%)			
0	52.8%	45.2%	0.141
>1	47.2%	54.8%	

GH group, gestational hypertension group; PE group, preeclampsia-eclampsia renal function impairment Group; GWEBP, gestational week upon the occurrence of elevated blood pressure; HDP, hypertensive disorders of pregnancy.
[a]P < 0.01.

Comparison of Clinical Biochemical Indicators Between the Two Groups

Clinical biochemical indexes between the two groups were compared by t test. Compared with the GH group, the PE renal function impairment group showed increases in the systolic blood pressure (142.53 ± 16.18, 157.29 ± 18.84 mmHg, $P < 0.01$), diastolic blood pressure (96.26 ± 12.17, 100.57 ± 11.42 mmHg, $P < 0.01$), homocysteine (12.20 ± 6.84, 16.58 ± 9.88 μmol/L, $P < 0.01$), creatinine (46.56 ± 13.62, 54.18 ± 15.49 μmol/L, $P < 0.01$), uric acid (307.31 ± 92.35, 391.47 ± 89.37 μmol/L, $P < 0.01$), urea nitrogen (3.62 ± 1.10, 4.82 ± 1.79

TABLE 2 | Comparison of clinical biochemistry indicators between the two groups.

Item	GH group (n = 159)	PE group (n = 168)	P-Value
Systolic blood pressure (mm Hg)	142.53 ± 16.18	157.29 ± 18.84[a]	0.000
Diastolic blood pressure (mm Hg)	96.26 ± 12.17	100.57 ± 11.42[a]	0.000
Homocysteine (μmol/L)	12.20 ± 6.84	16.58 ± 9.88[a]	0.000
White blood cell (10^9/L)	11.78 ± 3.98	10.74 ± 2.78[a]	0.006
Hemoglobin (g/L)	109.45 ± 20.11	111.23 ± 16.36	0.382
Platelet (10^9/L)	215.66 ± 64.60	204.46 ± 63.96	0.117
Ferritin (ng/ml)	53.03 ± 27.28	48.32 ± 29.75	0.370
Serum creatinin (μmol/L)	46.56 ± 13.62	54.18 ± 15.49[a]	0.000
Uric acid (μmol/L)	307.31 ± 92.35	391.47 ± 89.37[a]	0.000
Blood urea nitrogen (mmol/L)	3.62 ± 1.10	4.82 ± 1.79[a]	0.000
Cystatin C (mg/L)	1.08 ± 0.33	1.41 ± 0.37[a]	0.000
Fasting plasma glucose (mmol/L)	5.22 ± 1.26	5.12 ± 1.43	0.533
Total protein (g/L)	59.77 ± 5.97	52.93 ± 5.97[a]	0.000
Albumin (g/L)	33.27 ± 3.99	28.78 ± 3.49[a]	0.000
Globulin (g/L)	26.29 ± 3.54	24.15 ± 3.45[a]	0.000
Prealbumin (mg/L)	181.05 ± 44.98	145.60 ± 38.78[a]	0.000
Alanine aminotransferase (U/L)	10.62 ± 5.83	15.26 ± 14.30[a]	0.001
Aspartate aminotransferase (U/L)	18.90 ± 8.07	23.32 ± 11.56[a]	0.000
Total bilirubin (μmol/L)	7.41 ± 5.39	5.62 ± 3.09[a]	0.002
Direct bilirubin (μmol/L)	2.82 ± 1.30	2.35 ± 0.99[a]	0.001
Indirect bilirubin (μmol/L)	4.59 ± 4.52	3.24 ± 2.36[a]	0.005
Glutamyltranspetidase (U/L)	14.39 ± 30.31	15.59 ± 21.73	0.723
Superoxide dismutase (U/L)	154.57 ± 25.62	133.70 ± 25.82[a]	0.000
Alkaline phosphatase (U/L)	166.14 ± 211.66	126.92 ± 54.19[b]	0.028
Creatine kinase MB (U/L)	8.72 ± 16.54	6.93 ± 9.19	0.349
Total bile acid (μmol/L)	3.35 ± 2.08	3.89 ± 4.05	0.196
Cholyglycine (mg/L)	1.64 ± 1.40	2.66 ± 3.16[a]	0.007
Serum calcium (mmol/L)	2.20 ± 0.17	2.05 ± 0.26[a]	0.000
Serum iron (μmol/L)	15.14 ± 9.86	14.31 ± 8.35	0.471
Serum zinc (μmol/L)	9.06 ± 2.60	7.29 ± 1.95[a]	0.000
Serum phosphorus (mmol/L)	1.19 ± 0.24	1.32 ± 0.18[a]	0.000
Serum magnesium (mmol/L)	0.83 ± 0.23	0.91 ± 0.32[b]	0.019
Total cholesterol (mmol/L)	6.01 ± 1.45	6.81 ± 1.63[a]	0.005
Triglycerides (mmol/L)	3.12 ± 1.40	4.05 ± 1.98[a]	0.005
Low-density lipoprotein (mmol/L)	3.07 ± 0.94	3.96 ± 1.21[a]	0.000
High-density lipoprotein (mmol/L)	1.62 ± 0.46	1.71 ± 0.45	0.263
Prothrombin time (s)	11.82 ± 0.69	11.50 ± 0.76[a]	0.001
Activated partial thromboplastin time (s)	25.76 ± 6.67	26.67 ± 4.02	0.201
Fibrinogen (g/L)	4.78 ± 2.16	3.98 ± 1.07[a]	0.000
d-Dimer (mg/L)	2.95 ± 4.56	4.56 ± 9.81	0.150

GH group, gestational hypertension group; PE group, preeclampsia-eclampsia renal function impairment group.
[a]$P < 0.01$.
[b]$P < 0.05$.

mmol/L, $P < 0.01$), cystatin C (1.08 ± 0.33, 1.41 ± 0.37 mg/L, $P < 0.01$), alanine aminotransferase (10.62 ± 5.83, 15.26 ± 14.30 U/L, $P < 0.05$), aspartate aminotransferase (18.90 ± 8.07, 23.32 ± 11.56 U/L, $P < 0.05$), cholyglycine (1.64 ± 1.40, 2.66 ± 3.16 mg/L, $P < 0.01$), total cholesterol (6.01 ± 1.45, 6.81 ± 1.63 mmol/L, $P < 0.01$), triglycerides (3.12 ± 1.40, 4.05 ± 1.98 mmol/L, $P < 0.01$), low density lipoprotein (3.07 ± 0.94, 3.96 ± 1.21 mmol/L, $P < 0.01$), serum magnesium (0.83 ± 0.23, 0.91 ± 0.32 mmol/L, $P < 0.05$), and serum phosphorus (1.19 ± 0.24, 1.32 ± 0.18 mmol/L, $P < 0.01$), but reductions in white blood cell (11.78 ± 3.98, 10.74 ± 2.78 10^9/L, $P < 0.01$), serum total protein (59.77 ± 5.97, 52.93 ± 5.97 g/L, $P < 0.01$), serum albumin (33.27 ± 3.99, 28.78 ± 3.49 g/L, $P < 0.01$), serum globulin (26.29 ± 3.54 g/L, 24.15 ± 3.45 g/L, $P < 0.01$), serum prealbumin (181.05 ± 44.98, 145.60 ± 38.78 mg/L, $P < 0.01$), calcium (2.20 ± 0.17, 2.05 ± 0.26 mmol/L, $P < 0.01$), zinc (9.06 ± 2.60, 7.29 ± 1.95 μmol/L, $P < 0.01$), total bilirubin (7.41 ± 5.39, 5.62 ± 3.09 μmol/L, $P < 0.01$), direct bilirubin (2.82 ± 1.30, 2.35 ± 0.99 μmol/L, $P < 0.01$), indirect bilirubin (4.59 ± 4.52, 3.24 ± 2.36 μmol/L, $P < 0.01$), superoxide dismutase (154.57 ± 25.62, 133.70 ± 25.82 U/L, $P < 0.01$), alkaline phosphatase (166.14 ± 211.66, 126.92 ± 54.19 U/L, $P < 0.05$), prothrombin time (11.82 ± 0.69, 11.50 ± 0.76 s, $P < 0.01$), and fibrinogen (4.78 ± 2.16, 3.98 ± 1.07 mg/L, $P < 0.01$). The remaining indicators showed no significant differences ($P > 0.05$). The details are shown in **Table 2**.

MTHFR C677T Genotyping

MTHFR C677T was divided into three genotypes (**Figures 1–3**), including the CC genotype in 79 cases, the CT genotype in 122 cases, and the TT genotype in 126 cases. There were no significant differences between the three genotypes in terms of age and BMI ($P > 0.05$). One-way ANOVA analysis of plasma homocysteine in the three genotypes showed that the homocysteine level in the TT genotype was higher than that in the CC and CT genotypes (11.77 ± 5.22 μmol/L, 10.67 ± 4.01 μmol/L, 19.79 ± 11.06 μmol/L, $P < 0.01$). There was no significant difference between the CC and CT genotype groups ($P > 0.05$). The details are shown in **Table 3**.

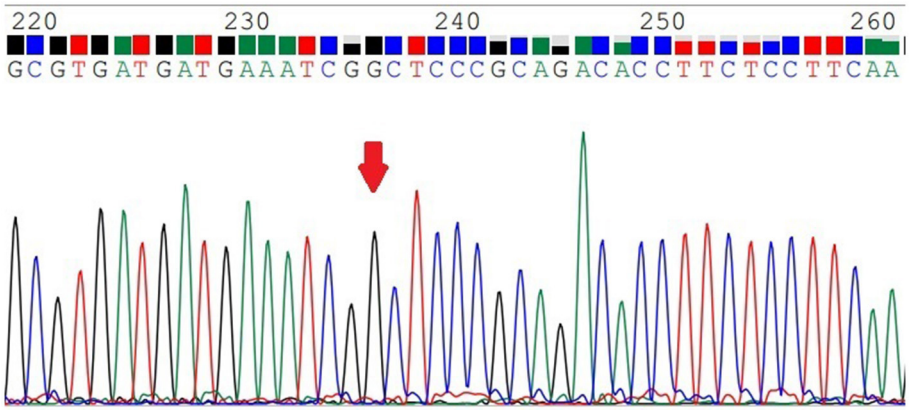

FIGURE 1 | The *MTHFR*-C677T CC genotype.

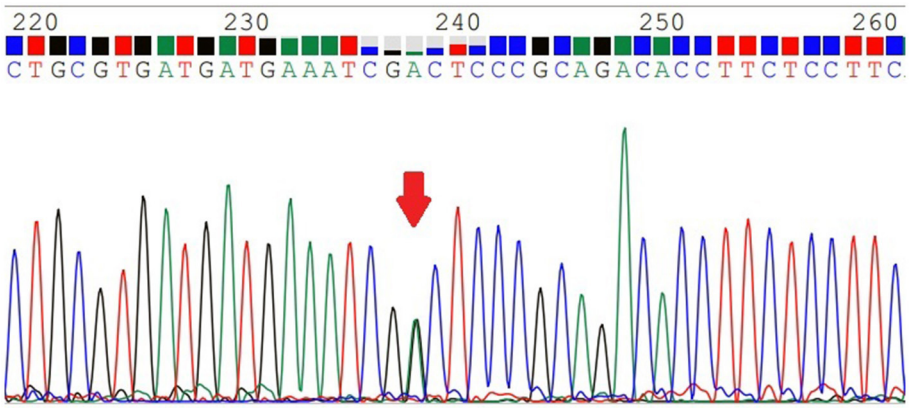

FIGURE 2 | The *MTHFR*-C677T CT genotype.

Analysis of the Relationships Between *MTHFR* C677T Gene Polymorphism and Impaired Renal Function in PE

The distributions of genes in the GH group and the PE renal function impairment group were in accordance with Hardy-Weinberg equilibrium ($P > 0.05$, **Table 4**).

Binary logistic regression analysis showed that the *MTHFR* C677T gene polymorphism was associated with PE renal function impairment in the recessive model [odds ratio (OR): 1.620, 95% confidence interval (CI): 1.033–2.541, $P < 0.05$]. In the dominant and additive models, *MTHFR* C677T gene polymorphism had no correlation with PE renal function impairment ($P > 0.05$). The details are shown in **Table 5**.

DISCUSSION

Hypertensive disorders of pregnancy is an idiopathic disease that occurs in mid-stage or late-stage pregnancy, seriously damaging maternal and child health. The Chinese Han population has a relatively high incidence of hypertensive renal damage and additional, more prominent issues with impaired renal function in pregnant women with HDP. Pregnant women with PE are often in critical condition upon admission and require emergency cesarean sections; in such cases, it is not suitable to conduct prenatal collection for 24-h urine protein determination. Many postpartum PE patients recover quickly, and postpartum random urine protein testing or 24-h urine protein determination cannot truly reflect the patient's prenatal condition. Therefore, we used prenatal random urinary protein as a reference standard to determine the presence of impaired renal function in pregnant women with PE. This grouping mode shifted the research focus from the previous grouping mode of "mild preeclampsia, severe preeclampsia and eclampsia" to the grouping mode of "whether or not renal function is impaired," with clearer aims and better research focus.

The age, BMI, history of smoking, family history of hypertension, history of HDP, sex of infants, proportion of twins,

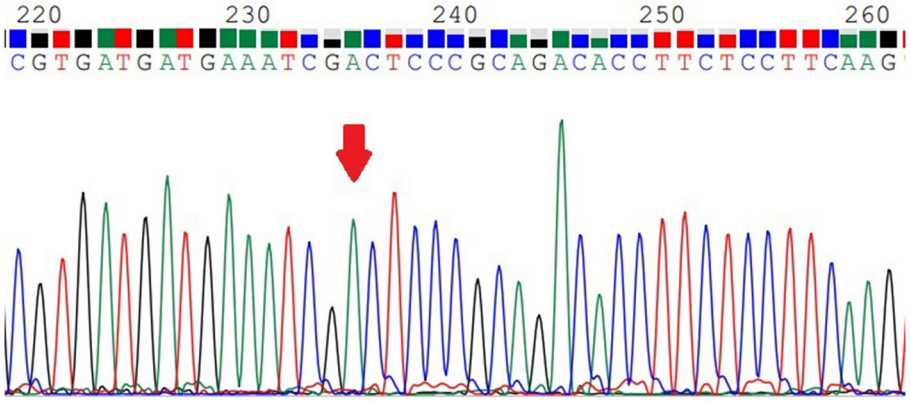

FIGURE 3 | The *MTHFR*-C677T TT genotype.

TABLE 3 | *MTHFR* C677T genotyping.

Item	CC group ($n = 79$)	CT group ($n = 122$)	TT group ($n = 126$)	*P*-Value
Age (year)	31.89 ± 5.72	31.75 ± 4.71	31.34 ± 5.86	0.746
Body Mass Index (kg/m^2)	31.79 ± 6.24	31.23 ± 3.44	30.90 ± 3.97	0.412
Homocysteine (μmol/L)	11.77 ± 5.22	10.67 ± 4.01	19.79 ± 11.06^{ab}	0.000

Compared with CC group, $^a P < 0.01$, Compared with CT group, $^b P < 0.01$.

TABLE 4 | Hardy-weinberg equilibrium testing.

Group (n)	Allele		*P*-Value
	C (%)	T (%)	
GH group ($n = 159$)	147 (46.2%)	171 (53.8%)	0.086
PE group ($n = 168$)	133 (39.6%)	203 (60.4%)	

GH group, gestational hypertension group; PE group, preeclampsia-eclampsia renal function impairment group.

number of pregnancies, and number of births matched between the two groups that we selected had no significant differences between the groups ($P > 0.05$), ensuring the reliability of this study. Compared with the GH group, the PE renal function impairment group had an earlier gestational week at the end of pregnancy and an earlier gestational week upon the occurrence of elevated blood pressure and had an increased proportion of cesarean sections ending pregnancy. In addition, neonatal body weight and Apgar scores in the PE renal function impairment group were lower than those in the GH group, indicating that the occurrence of PE renal function impairment increases risks in pregnancy. To ensure the safety of mothers and infants, the cesarean section rate was increased, as was the premature birth rate, but the preterm infants' body weights and Apgar scores were lower. Therefore, investigating the pathogenesis of PE renal function impairment, identifying the risk factors, and conducting early prevention and treatment are conducive to improving maternal and child health.

Differing from the pathogenesis of primary hypertension, the current recognized pathogenic mechanisms of HDP include immune theory, placental ischemia theory, and theory of heredity. We explored the pathogenesis of renal damage in PE from the perspective of genetics combined with environmental factors to more comprehensively and objectively evaluate the early prediction value of gene polymorphism, which can provide the theoretical basis for future early clinical diagnosis and treatment. As mentioned above, the investigation of the relationship between the C677T gene polymorphism of *MTHFR*, a key enzyme of plasma homocysteine metabolism, and PE renal function impairment is based on the high mutation rate of the *MTHFR* T allele and the high hyperhomocysteinemia incidence in the Chinese population. Our previous study also confirmed that the *MTHFR* C677T gene polymorphism was associated with impaired renal function in a hypertensive Chinese Han population (9). Does the correlation of this genetic polymorphism still exist in the special group of pregnant Han Chinese women? We performed *MTHFR* genotyping of the populations enrolled in this study and found that homocysteine expression in the TT genotype group was higher than in the CC and CT genotype groups ($P < 0.01$), with no significant difference between the CC genotype and CT genotype groups ($P > 0.05$). We also conducted a binary logistic regression analysis of the relationships between genetic polymorphisms and impaired renal function in PE and found that in the recessive model, the C677T gene polymorphism of *MTHFR* was associated with impaired renal function in PE.

The above results indicate that the mutation of the T allele may be an independent risk factor for impaired renal function in Chinese pregnant women with PE. The C677T mutation is the most common missense mutation in *MTHFR*; it can occur stably in populations around the globe and has a worldwide distribution of high frequency. In normal populations, the

TABLE 5 | Analysis of the relationships between *MTHFR* C677T gene polymorphism and impaired renal function in PE.

MTHFR genotype	GH group ($n = 159$)	PE group ($n = 168$)	Recessive		Dominant		Additive	
			OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value
CC	40 (25.2%)	39 (23.2%)	1.620 (1.033–2.541)	0.036[a]	1.112 (0.670–1.845)	0.682	1.246 (0.942–1.649)	0.124
CT	67 (42.1%)	55 (32.7%)						
TT	52 (32.7%)	74 (44.1%)						

GH group, gestational hypertension group; PE group, preeclampsia-eclampsia renal function impairment group; MTHFR, methylenetetrahydrofolate reductase; CI, confidenceinterval; OR, odd ratio.

[a] $P < 0.05$.

frequency of TT homozygotes is approximately 4% in Egyptian (10) and approximately 23% in Italians in Europe (11), while the T allele frequency is at a higher level of 41% in the Chinese population (8). According to Hou (12), the T allele frequency of healthy pregnant women is 27%. But our study showed that the frequency of the T allele in the GH group was 53.8%, and the frequency of the T allele in the PE renal function impairment group was 60.4%, both of which were higher than those reported above and similar to the findings of Ding et al. (13). As mentioned above, our previous study found that *MTHFR* C677T gene polymorphism is an important cause of renal damage in hypertensive Han Chinese patients.This feature still exists in Chinese Han pregnant women. This also proves that genetics plays an important role in this disease. The activity of normal *MTHFR* decreases by 60% after 5 min at 46°C, whereas the activity of the *MTHFR* mutant encoded by the C677T mutation decreases by 80%−90%. This change in thermolability leads to decreased *MTHFR* activity in the human body, which in turn results in an increase in plasma homocysteine concentration. A plasma homocysteine level above 10 μmol/L is known as hyperhomocysteinemia (14). Association with hyperhomocysteinemia is a special feature of Chinese hypertension populations. The patients included in this study had an average plasma homocysteine level above 10 μmol/L and a hyperhomocysteinemia rate of 75.2%, with rates of 73.0% in the GH group and 77.4% in the PE renal function impairment group. Hyperhomocysteinemia can damage glomerular capillary endothelial cells through a variety of mechanisms (15), affecting endothelial function, exacerbating urinary protein, and leading to impaired renal function, similar to the results of Li et al. (16). Therefore, we infer that the mutation rate of the T allele of *MTHFR* C677T is higher in Chinese Han PE patients with impaired renal function, affecting the metabolism of homocysteine and leading to an increase in the proportion of patients with hyperhomocysteinemia, in turn producing more urinary protein.

In addition to homocysteine expression and *MTHFR* C677T gene polymorphism, there are many factors affecting PE renal function impairment. This study also found that compared with the GH group, the PE renal function impairment group had increases in blood pressure (systolic blood pressure, diastolic blood pressure), liver and kidney function indicators (creatinine, uric acid, urea nitrogen, cystatin C, alanine aminotransferase, aspartate aminotransferase, cholyglycine), and blood lipids (total cholesterol, triglycerides and low-density lipoprotein) but had reductions in plasma protein (total protein, albumin, globulin, prealbumin), trace elements (calcium and zinc), prothrombin time and fibrinogen. These results are similar to the observations of Seremak-Mrozikiewicz et al. (17). Systemic arteriolospasm in PE patients leads to an increase in blood pressure, causing hyperperfusion, hyperfiltration, and high transmembrane pressure in the glomerulus, along with impaired glomerular endothelial cells and increased urinary protein secretion (18). Urine protein loss from the kidneys, coupled with insufficient liver synthesis of proteins, leads to impaired renal function in pregnant women with PE, often accompanied by hypoproteinemia and edema. Pregnant women with PE in severe conditions often experience ischemia and hypoxia in organs throughout the body on top of impaired liver and kidney functions. In our clinical practice, we found that the brain and myocardial cells were also damaged. Regular testing of liver and kidney function in patients with PE can lead to a dynamic understanding of the disease progress in patients. Trace elements such as calcium are involved in the regulation of a variety of physiological functions in the body. Hypocalcemia can lead to increased intracellular calcium concentrations and activation of myosin and myofibrillar proteins in vascular smooth muscle, thus causing arteriolar spasm, which is involved in the occurrence of HDP (19). Because of the demands of fetal growth and fat reserves, blood lipid levels are often elevated during pregnancy. However, a significant increase in blood lipid levels can inhibit anti-oxidation *in vivo* and can cause vasospastic contraction, thus affecting the development of HDP (20).

Many other diseases are also associated with C677T gene polymorphism of *MTHFR*. Such as cardiovascular diseases, diabetes, venous thrombosis (21) and breast cancer (22). Hyperhomocysteinemia is an emerging risk factor for various cardiovascular diseases. Folate and vitamin B_{12} are key elements of the one-carbon metabolism pathway in which *MTHFR* matters, supplementation of which may help reduce homocysteine levels (23). Different doses of folate have different efficacy of lowering homocysteine in hypertensive Chinese adults. One study showed that, in patients with hyperhomocysteinemia, 0.4 mg/day folate can significantly reduce the homocysteine level in CC genotype but at least 0.8 mg/day folate can reduced the

homocysteine level in TT genotype (24). Therefore, if pregnant women with a high homocysteine level, the *MTHFR* C677T genotypes should be tested and different doses of folate can be selected according to the different genotype. However, if homocysteine level is normal, screening for the *MTHFR* may be not necessary.

Although we comprehensively analyzed the relationships between the *MTHFR* gene polymorphism C677T, homocysteine, and various biochemical indicators of pregnancy and PE renal function impairment, we did not investigate whether the reduction in plasma homocysteine (such as with folic acid supplementation) can help to reduce urinary protein levels. We plan to investigate the pathways by which plasma homocysteine affects the impaired renal function from the perspective of molecular biology to provide a theoretical basis for the clinical prevention and treatment of renal function impairment in PE patients.

In summary, since the C677T polymorphism of the *MTHFR* gene, a key enzyme in plasma homocysteine metabolism, is an independent risk factor for impaired renal function in pregnant Chinese Han women with PE, we should conduct early detection of the *MTHFR* gene polymorphism C677T in this population and should control plasma homocysteine levels within the normal range. In addition, regular monitoring of blood pressure, liver and kidney functions, blood lipids, platelets, trace elements, and other indicators can lead to a dynamic understanding of PE renal impairment so that intervention can be conducted as early as possible to ensure the safety of mothers and infants.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

LY and MG acquired, analyzed and interpreted data, and wrote the manuscript. FZ and XZ reviewed and edited the manuscript. XL analyzed data. RX designed the study, acquired and analyzed, and interpreted the data. All authors read and approved the final manuscript.

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Changing Trends of Adverse Pregnancy Outcomes With Maternal Pre-pregnancy Body Mass Index: A Join-Point Analysis

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Objective: Adverse pregnancy outcomes have been related to obesity and thinness; however, the changing trends of the specific outcome with pre-pregnancy BMI remain unknown. The aim of this study was to investigate the change in risk trends of specific adverse outcomes for different pre-pregnancy BMI and analyze the recommended BMI range for pre-pregnancy counseling.

Methods: Data were extracted from the medical records of 39 public hospitals across 14 provinces in China from 2011 to 2012. The eligibility criteria were singleton birth with delivery week ≥28 weeks. Join-point analysis was adopted to explore changing trends with pre-pregnancy BMI and calculate slopes and join points of different pregnancy complications.

Results: A total of 65,188 women were eligible for analysis. There were three categories of trend style. Continuously increasing trends were linear for intrahepatic cholestasis of pregnancy, postpartum hemorrhage, and low 1-min Apgar score, and non-linear for cesarean delivery with one join point of BMI 23, hypertension disorder in pregnancy with two join points of BMI 20 and 28, gestational diabetes mellitus with one join point of BMI 22, and macrosomia with one join point of BMI 19. The trend was continuously and linearly decreasing for anemia. The bidirectional trends were downward and upward for premature rupture of the membrane with join BMI 22, preterm premature rupture of the membrane with join BMI 22, placenta abruption with join BMI 23, preterm birth with join BMI 19, and low birth weight with join BMI 19.

Conclusions: The changes in the trends of specific outcomes differed with pre-pregnancy BMI. Our results suggested that a pre-pregnancy BMI ranging between 19 and 23 may help reduce the risk of poor maternal and neonatal outcomes.

Keywords: pre-pregnancy body mass index, hypertension disorder in pregnancy, gestational diabetes mellitus, macrosomia, preterm birth, low birthweight

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INTRODUCTION

Numerous studies have reported that abnormal pre-pregnancy body mass index (BMI) could be associated with adverse maternal or neonatal outcomes. Existing evidence shows that women with overweight or obesity before pregnancy are at increased risk for gestational diabetes mellitus (GDM), hypertensive disorders in pregnancy (HDP), macrosomia, cesarean section, and neonatal

mortality (1–5). In contrast, low BMI before pregnancy may contribute to a higher risk of preterm birth, fetal growth restriction (FGR), and small for gestational age (SGA) status (6).

Most previous studies examined maternal and neonatal outcomes across different BMI groups based on the Institute of Medicine (IOM) classification. However, the influence of pre-pregnancy BMI on pregnancy outcomes may be accumulated from quantitative change to qualitative change, and the risk of adverse outcomes may accelerate after a certain point, which has not been previously reported. Besides, the pattern of effect of pre-pregnancy BMI on morbidity is diverse for each complication. Therefore, the objective of the current study was to investigate the change in risk trends of specific adverse outcomes for different pre-pregnancy BMI and to analyze the recommended BMI range for women before pregnancy.

MATERIALS AND METHODS

Data were extracted from the medical records of 39 public hospitals across 14 provinces in China between 2011 and 2012. The inclusion criteria encompassed women with singleton pregnancies and gestational week of birth ≥28 weeks. A total of 65,188 women were included in the final analysis (**Figure 1**).

All women were required to provide their medical records within the first 12 weeks of gestation and accept systematic antenatal care until delivery. Relevant data were collected from these medical records by trained hospital staff. Women's height was measured, and their pre-pregnancy weight was self-reported during the first antenatal visit. Gestational week of birth and pregnancy outcomes were extracted from the discharge records after delivery. The birthweight of the newborn was obtained within 1 h after delivery. BMI was calculated as weight (kg)/height^2 (m^2).

Adverse maternal outcomes included cesarean section, HDP, GDM, anemia, intrahepatic cholestasis of pregnancy (ICP), premature rupture of membrane (PROM), preterm premature rupture of membrane (PPROM), postpartum hemorrhage, and placental abruption. Adverse neonatal outcomes included preterm birth (28–37 weeks), macrosomia (>4,000 g), low birthweight (<2,500 g), and low 1-min Apgar score (≤7). Other adverse outcomes like birth defects or stillbirth were not analyzed for low morbidity. Diagnoses were recorded as International Classification of Diseases-10 codes by healthcare providers in the hospital. Women with pre-pregnancy BMI of 15 or lower as well as women with pre-pregnancy BMI of 34 or higher were combined into one separate group because of the small number of such cases. BMI-specific rates for each pregnancy outcome indicator were calculated. The Hospital Committee for Medical Research Ethics approved the study under the ethic approval code 2017–18. All of the methods used in the study were in accordance with the relevant guidelines, and informed consent to participate was obtained from all subjects.

Abbreviations: BMI, body mass index; HDP, hypertension disorder in pregnancy; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PROM, premature rupture of membrane; PPROM, preterm premature rupture of membrane; FGR, Fetal growth restriction; SGA, Small for gestational age.

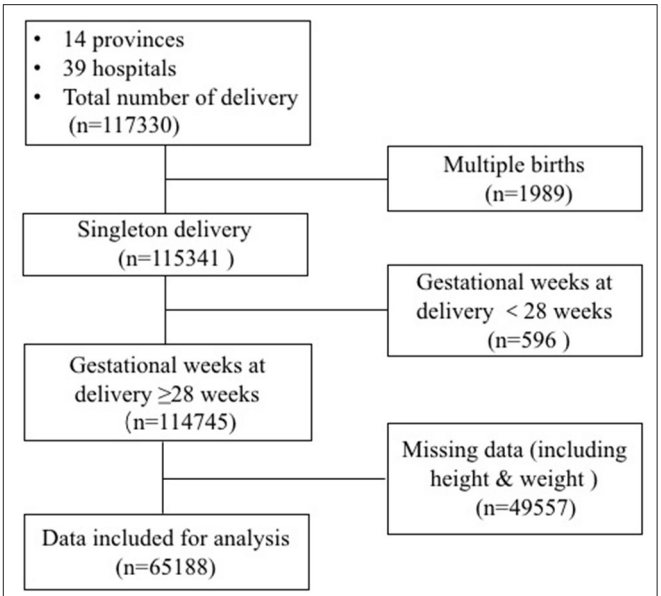

FIGURE 1 | Patient flow chart.

SPSS 21 was used for preparation and descriptive analyses of all data. Baseline characteristics were presented as numbers (percentage) for categorical variables. Join-point regression analysis was used to test whether an apparent change in temporal trend is statistically significant, where several linear segments are connected together at the "join points." It was applied in the analysis of cancer temporal trends previously and developed to be applied in other fields. In our study, join-point regression analysis (version 4.5.0.1) was adopted to establish the trend of pregnancy outcomes with maternal pre-pregnancy BMI. The crude rates of adverse pregnancy outcomes were used as dependent variables, and maternal pre-pregnancy BMI was used as the independent variable. Poisson variance was used to estimate the non-constant variance of segmental models by assuming that the dependent variable counts follow a Poisson distribution. The join-point analysis identified the best fitting piecewise continuous linear model by Bayesian information criterion. This approach allowed us to identify the specific BMI when there were significant changes in the trend and to estimate the magnitude of the increase or the decrease in each segment by estimating the slopes.

RESULTS

A total of 65,188 women were eligible for analysis. The baseline data of all enrolled women in different pre-pregnancy BMI groups are shown in **Table 1**. Results showed that there were no differences in demographic characteristics between the four groups.

Adverse maternal and neonatal outcomes are shown in **Table 2**. A total of < 5% of data for adverse outcomes was missing. Maternal complications included cesarean delivery in 53.76% (35046), HDP in 5.14% (3350), GDM in 4.71% (3072), anemia in 5.77% (3760), ICP in 0.54% (353), PROM in 15.60%

TABLE 1 | Demographics of women in different pre-pregnancy BMI groups.

Characteristic	Underweight (7,840)	Normal (49,214)	Overweight (7,131)	Obese (1,003)	*P*-value
Maternal age					0.06
≤34	702 0 (89.97%)	44,103 (89.91%)	6,304 (88.83%)	875 (87.94%)	
35∼39	630 (8.07%)	3,995 (8.14%)	641 (9.03%)	95 (9.55%)	
≥40	153 (1.96%)	952 (1.94%)	152 (2.14%)	25 (2.51%)	
Parity					0.10
Primipara	6,564 (83.72%)	40,732 (82.77%)	5,877 (82.41%)	842 (83.95%)	
Multipara	1,276 (16.28%)	8,482 (17.23%)	1,254 (17.59%)	161 (16.05%)	
Maternal educational level					0.51
High (universities and above)	3,676 (47.68%)	23,053 (47.81%)	3,390 (47.82%)	477 (48.77%)	
Middle (secondary schools)	2,445 (31.72%)	15,035 (31.18%)	2,275 (32.09%)	301 (30.78%)	
Low (primary schools and lower)	1,588 (20.60%)	10,134 (21.02%)	1,424 (20.09%)	200 (20.45%)	
Maternal self-reported smoking history					0.36
Yes	34 (0.43%)	190 (0.39%)	21 (0.29%)	6 (0.60%)	
No	7,806 (99.57%)	49,024 (99.61%)	7,110 (99.71%)	997 (99.40%)	
Maternal self-reported drinking history					0.37
Yes	139 (1.77%)	656 (1.33%)	86 (1.21%)	13 (1.30%)	
No	7,701 (98.23%)	48,558 (98.67%)	7,045 (98.79%)	990 (98.70%)	

BMI, body mass index.

(10169), PPROM in 2.05% (1335), placenta abruption in 0.49% (321), and postpartum hemorrhage in 3.61% (2355) women. Adverse neonatal outcomes included preterm birth in 4.94% (4571), macrosomia in 7.01% (4571), low birth weight in 4.94% (3223), and a low 1-minute Apgar score (≤7) in 2.35% (1532) women. Besides, result showed that there were differences between groups of different pre-pregnancy BMI in the aspects of cesarean section, HDP, GDM, anemia, ICP, PPROM, postpartum hemorrhage, preterm birth, macrosomia and low birthweight ($P < 0.05$).

The trends of adverse pregnancy outcomes with maternal pre-pregnancy BMI assessed by join-point analysis are shown in **Table 3**. Seven adverse outcomes revealed a continuously increasing trend with maternal pre-pregnancy BMI, one had a continuously decreasing trend, and five had a bidirectional trend, with a decreasing trend in thin women and an increasing trend in obese women at different nadir BMI (**Figures 2**–**4**).

The trends were continuously increasing for seven adverse pregnancy outcomes (**Figure 1**). The trend of ICP, postpartum hemorrhage, and low 1-min Apgar score increased linearly with pre-pregnancy BMI ranging from 15 to 34 without join-points (slope 0.01, 0.20, and 0.02%, respectively). The trends of the other four adverse pregnancy outcomes were continuous but non-linear with different join-points. The trend of cesarean delivery increased rapidly from BMI 15 to 23 (slope 2.63%) and then a little slowly from BMI 23 to 34 (slope 1.55%). The trend of HDP increased a little from BMI 15 to 20 (slope 0.08%), and then slightly increased from BMI 20 to 28 (slope 0.92%), followed by a significant increase from BMI 28 to 34 (slope 2.83%). The trend of GDM slightly increased from BMI 15 to 22 (slope 0.28%) and then increased rapidly from BMI 22 to 34 (slope 1.34%). The trend of macrosomia slightly increased from BMI 15 to 19 (slope 0.40%), while a rapid increase ensued from BMI 19 to 34 (slope 1.07%). The trend for anemia was continuously and linearly decreasing (slope -0.03%) (**Figure 2**).

Trends were bidirectional, downward-to-upward, with different nadir BMI for PROM, PPROM, placenta abruption, preterm birth, and low birthweight (**Figure 3**). The trend of PROM was decreasing before BMI 22 and increasing after that (slope−0.34% before BMI 22 and slope 0.10% after BMI 22). Similarly, the trend of PPROM was decreasing before BMI 22 and increasing after that (slope−0.16% before BMI 22 and slope 0.14% after BMI 22). The trend of placenta abruption was downward (slope−0.01%) before BMI 23 and upward (slope 0.05%) after it. The trend of low birthweight was downward before BMI 19 (slope−1.18%) and upward after it (slope 0.04%).

DISCUSSION

Our data revealed the trend changes of pre-pregnancy BMI in relation to different adverse outcomes. Seven adverse pregnancy outcomes including cesarean section, HDP, GDM, ICP, postparturm hemorrhage, macrosomia, and low 1-min Apgar score revealed a continuously increasing trend with maternal pre-pregnancy BMI; anemia had a continuously decreasing trend, while five other adverse outcomes, including PROM, PPROM, placenta abruption, preterm birth, and low birth weight had a bidirectional trend that was decreasing in thin women and increasing in obese women at different nadir BMI.

TABLE 2 | Incidence of adverse maternal and fetal outcomes of women in different pre-pregnancy BMI group.

Adverse outcomes	All women (65,188)	Underweight (7,840)	Normal (49,214)	Overweight (7,131)	Obese (1,003)	*P*-value
Maternal complications						
Cesarean section	35,046 (53.76%)	3,403 (43.41%)	26,397 (53.64%)	4,526 (63.47%)	720 (71.78%)	<0.01
HDP	3,350 (5.14%)	227 (2.90%)	2,188 (4.45%)	721 (10.11%)	214 (21.34%)	<0.01
GDM	3,072 (4.71%)	205 (2.61%)	1,974 (4.01%)	704 (9.87%)	189 (18.84%)	<0.01
Anemia	3,760 (5.77%)	446 (5.69%)	2,869 (5.83%)	394 (5.53%)	51 (5.08%)	0.56
ICP	353 (0.54%)	35 (0.45%)	267 (0.54%)	39 (0.55%)	12 (1.20%)	0.03
PROM	10,169 (15.60%)	1,300 (16.58%)	7,590 (15.42%)	1,115 (15.64%)	164 (16.35%)	0.06
PPROM	1,335 (2.05%)	201 (2.56%)	921 (1.87%)	181 (2.54%)	32 (3.19%)	<0.01
Postpartum hemorrhage	2,355 (3.61%)	240 (3.06%)	1,713 (3.48%)	348 (4.88%)	54 (5.38%)	<0.01
Placental abruption	321 (0.49%)	43 (0.55%)	239 (0.49%)	35 (0.49%)	4 (0.40%)	0.87
Adverse neonatal outcomes						
Preterm birth	3,768 (5.78%)	494 (6.30%)	2,679 (5.44%)	507 (7.11%)	88 (8.77%)	<0.01
Macrosomia	4,571 (7.01%)	299 (3.84%)	3,227 (6.59%)	883 (12.45%)	162 (16.25%)	<0.01
Low birthweight	3,223 (4.94%)	496 (6.38%)	2,298 (4.69%)	368 (5.19%)	61 (6.12%)	<0.01
Low 1-min Apgar score (≤7)	1,532 (2.35%)	177 (2.28%)	1,134 (2.32%)	191 (2.70%)	30 (3.01%)	0.12

HDP, Hypertension disorder in pregnancy;GDM:gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PROM: premature rupture of membrane; PPROM, preterm premature rupture of membrane.

TABLE 3 | Join-point analysis of changing trend of adverse pregnancy outcomes with pre-pregnancy BMI.

Complications	Trend 1			Trend 2			Trend 3		
	BMI	Slope	*P*-value	BMI	Slope	*P*-value	BMI	Slope	*P*-value
Cesarean section	15–23	2.63	<0.01*	23–34	1.55	<0.01*			
HDP	15–20	0.08	0.68	20–28	0.92	<0.01*	28–34	2.83	<0.01*
GDM	15–22	0.28	<0.01*	22–34	1.34	<0.01*			
Anemia	15–34	−0.03	0.27						
ICP	15–34	0.01	0.16						
PROM	15–22	−0.34	0.02*	22–34	0.10	0.33			
PPROM	15–22	−0.16	0.08	22–34	0.14	0.03*			
Postpartum hemorrhage	15–34	0.20	<0.01*						
Placenta abruption	15–23	−0.01	0.51	23–34	0.05	0.10			
Preterm birth	15–19	−0.87	0.05	19–34	0.24	<0.01*			
Low birthweight	15–19	−1.18	<0.01*	19–34	0.04	0.23			
Macrosomia	15–19	0.40	0.21	19–34	1.07	<0.01*			
Low 1-minApgar score (≤7)	15–34	0.02	0.45						

HDP, Hypertension disorder in pregnancy;GDM:gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PROM: premature rupture of membrane; PPROM: preterm premature rupture of membrane.

In most previous studies, adverse pregnancy outcomes related to different pre-pregnancy BMI were mostly analyzed across different BMI groups. In our study, join-point regression was adopted to investigate the continuous changing trend of adverse pregnancy outcomes in relation to pre-pregnancy BMI. As a result, the trend of risky pregnancy started to change at join BMI, meaning the impact of risk factors changed with pre-pregnancy BMI, even though the change could be insignificant. Join point is the theoretical point at which two adjacent trends cross. Our join BMI was mostly at the range of normal BMI of the IOM classification, but it was more specific, and the results were in line with those of previous studies on the effect of maternal pre-pregnancy BMI (analyzed by BMI groups) (1, 6, 7). Therefore, there is good reason to believe that most of our results on the association between abnormal pre-pregnancy BMI and adverse pregnancy outcomes are applicable to other reports.

Our results revealed a continuously increasing trend of HDP, GDM, ICP, macrosomia, cesarean section, postpartum hemorrhage, and low 1-min Apgar score, suggesting that only high pre-pregnancy BMI was a risk factor for these outcomes. The impact of high pre-pregnancy BMI on these outcomes was similar to previous reports (8–19). According to existing

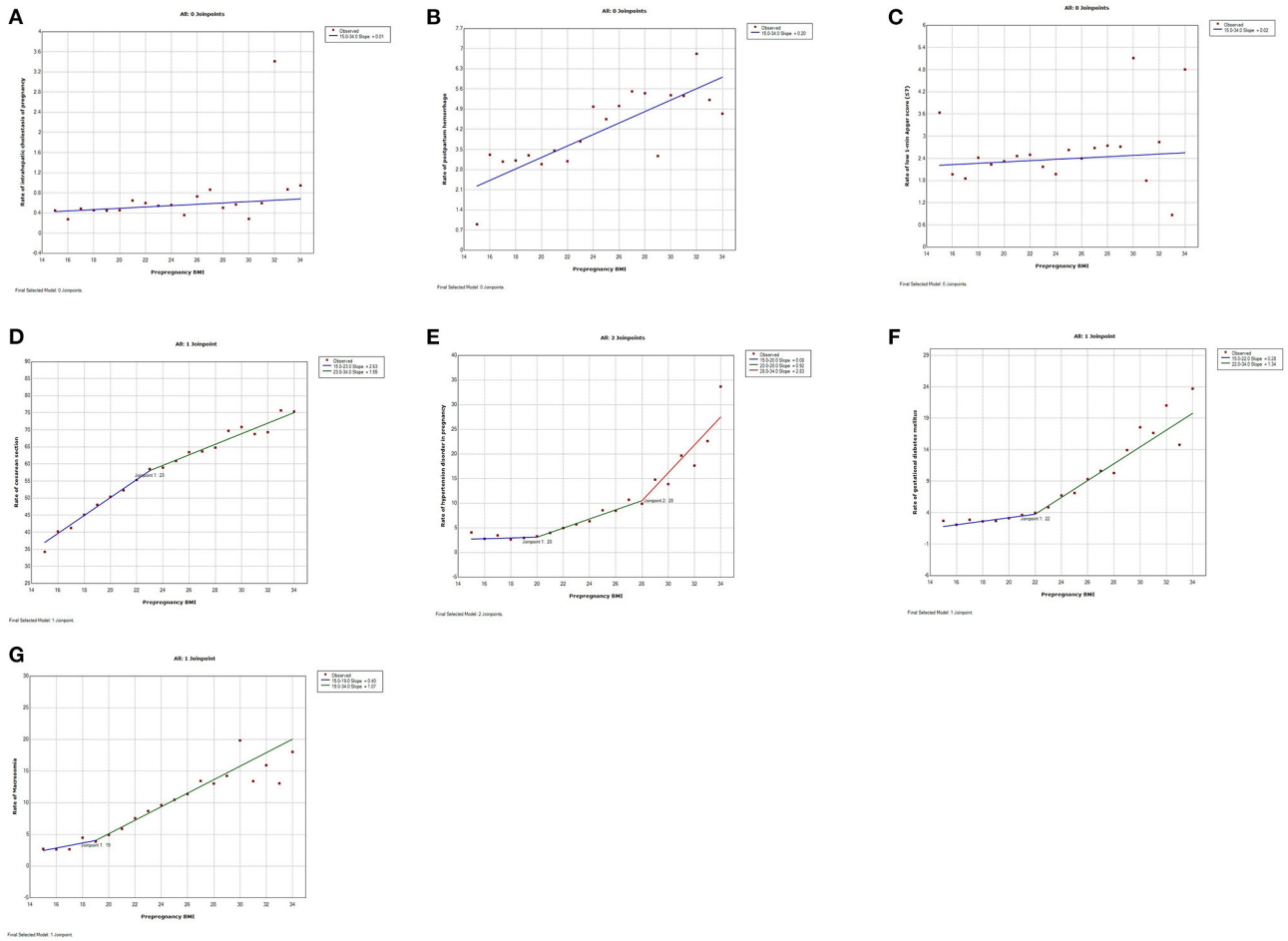

FIGURE 2 | Trends of adverse pregnancy outcomes in relation to maternal pre-pregnancy BMI by join-point analysis (increasing trends). **(A–C)** Continuous linear increase for intrahepatic cholestasis of pregnancy (ICP), postpartum hemorrhage, and low 1-min Apgar score (≤7). **(D–G)** Continuous non-linear increase for cesarean section, hypertension disorder in pregnancy (HDP), gestational diabetes mellitus (GDM), and macrosomia.

studies, obese (BMI 30 to 33.9) and morbidly obese (BMI > 40) primigravid women have 3 and 7 times higher risks of pre-eclampsia, respectively (8). Our study showed the incidence of HDP increased when pre-pregnancy BMI reached 20, which is traditionally considered normal BMI, and significantly increased when pre-pregnancy BMI reached 28. Similarly, the incidence slope of GDM increased when pre-pregnancy BMI reached 22 and significantly increased when pre-pregnancy BMI reached 27. While other studies have found similar results, they made no recommendations regarding BMI (13, 14). A previous study showed that compared with the normal group, the obese group was at 1.7 times higher risk of macrosomia, while the risk in the overweight group did not increase (15). Our results revealed that pre-pregnancy BMI affected the incidence of macrosomia in a continuous and linear way. Our results were also consistent with earlier reports, which have shown an association between increasing BMI and cesarean delivery and postpartum hemorrhage (16). This may be subsequent to induction, including altered uterine contractility combined with dysfunctional labor (17, 18). Besides, the link between high pre-pregnancy BMI and low 1 min Apgar score may be secondary to the result of increasing pregnancy complications. On the contrary, the negative association between low BMI and maternal anemia may be due to poor nutrition, including iron, folic acid, and other micronutrient deficiencies (19).

Low and high pre-pregnancy BMI affected some adverse outcomes, including PROM, PPROM, placenta abruption, preterm birth, and low birth weight. Previous studies showed that low maternal BMI was associated with more spontaneous preterm deliveries and low birth weight (20). Nevertheless, our results showed that trends of preterm birth, as well as low birth weight, were bidirectional, with a decrease in BMI lower than 19 and an increase higher than 19, suggesting that mainly being underweight (usually defined as BMI < 18.5) was a risk factor for preterm birth and low birthweight. Among other populations, including those with normal weight, overweight and obese, the risk slightly went up as BMI increased, which may be due to other increasing pregnancy complications. Similar trends were

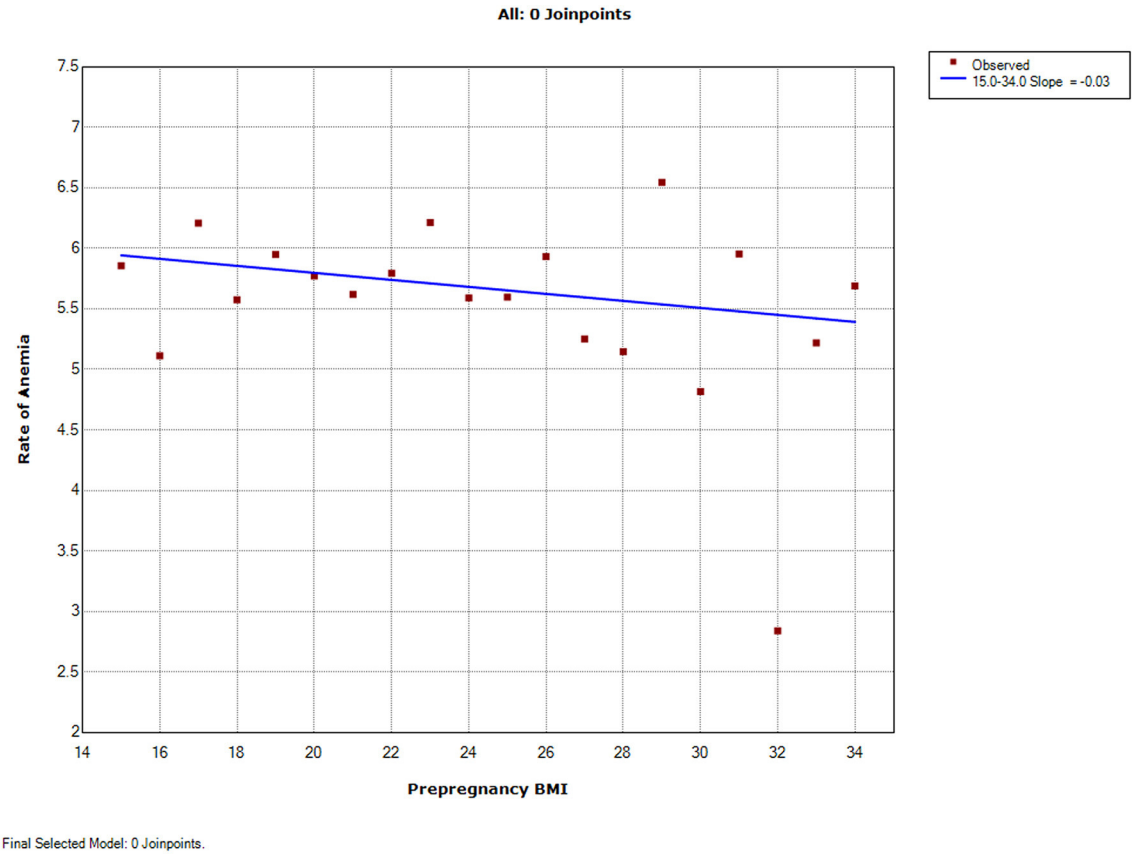

FIGURE 3 | Trends of adverse pregnancy outcomes with maternal pre-pregnancy BMI by join-point analysis (decreasing trends). Continuous linear decrease for anemia.

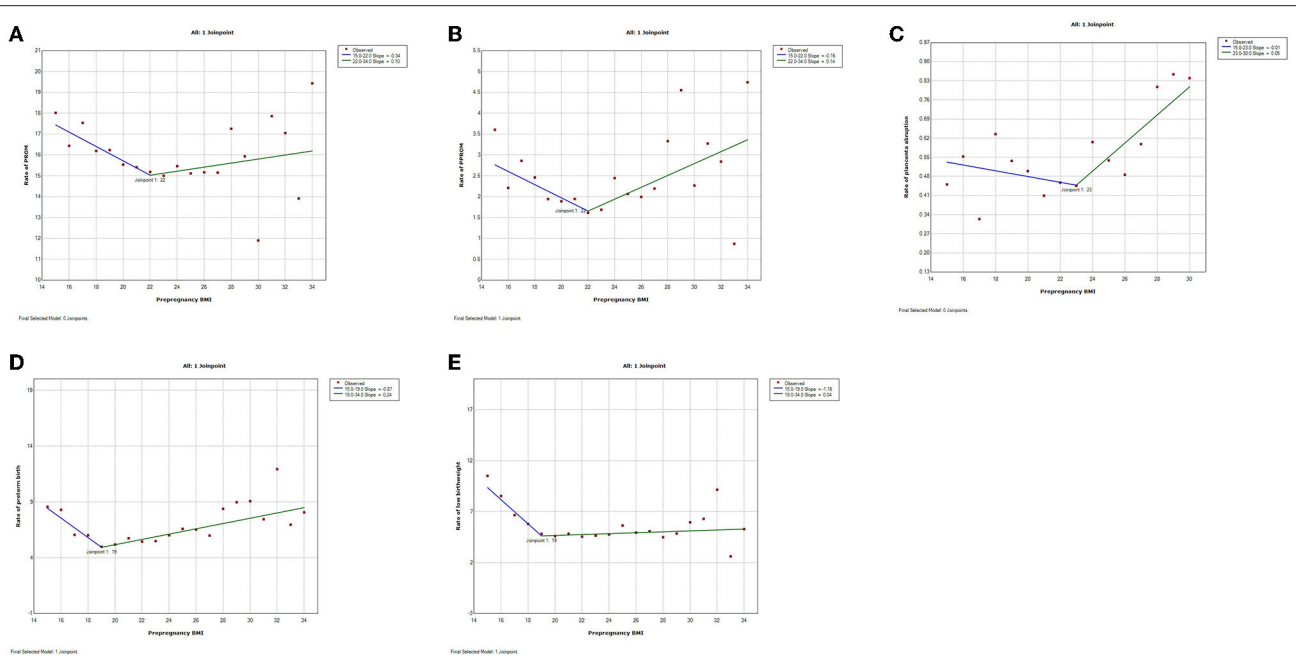

FIGURE 4 | Trends of adverse pregnancy outcomes with maternal pre-pregnancy BMI by join-point analysis (Bidirectional trends). **(A–E)** Bidirectional changes with down-and-upward trends for premature rupture of membrane (PROM), preterm premature rupture of membrane (PPROM), placenta abruption, preterm birth, and low birthweight.

also found in PROM and placenta abruption with join BMI of 22 and 23, respectively, which have not been previously reported in other researches.

The strengths of this study included large, retrospective, and continuous data of pregnant women collected from 39 hospitals across 14 provinces in a multi-center and cross-sectional way, including rural and urban populations, thus making the sample quite representative. The large sample size also made it possible to calculate the rate of different pre-pregnancy BMI and describe the incidence trend with BMI by join-point regression analysis. While most of the previous studies had grouped comparison design according to IOM guidelines, join-point analysis added priority to continuously investigate BMI.

However, there are some limitations in the present study that should be considered. First, pre-pregnancy BMI was determined by self-reported weight at their first antenatal visit, and there may be a possibility of confounding bias given the retrospective study design. Second, factors including gravity and parity, occupation, education, and unhealthy habits like smoking or drinking were not adjusted, which may cause bias. Large-scale prospective studies may be needed to investigate these problems further.

CONCLUSION

Maternal HDP, GDM, ICP, macrosomia, cesarean section, postpartum hemorrhage, and low 1-min Apgar score were only affected by high pre-pregnancy BMI, whereas maternal anemia was only affected by low pre-pregnancy BMI. Low and high pre-pregnancy BMI affected the risk of PROM, PPROM, placenta abruption, preterm birth, and low birth weight in different modes, and the satisfactory BMI before pregnancy appeared to be between 19 and 23. The estimated pre-pregnancy BMI could be helpful in identifying targeted BMI and providing pre-pregnancy counseling for reducing the risk of poor maternal and neonatal outcomes. Besides, according to our results, clinicians could particularly pay attention to specific pregnancy complications of high risks during pregnancy for women with different pre-pregnancy BMI.

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 Hospital Committee for Medical Research Ethics approved the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RH contributed to writing of the manuscript. HY contributed to data collection and data analysis. XL contributed to the planning of the project and revising of the manuscript. All authors have read and approved the manuscript.

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Prognostic Impact of Blood Pressure Change Patterns on Patients With Aortic Dissection After Admission

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Objectives: Hypertension is a predominant risk factor for aortic dissection (AD), and blood pressure (BP) control plays a vital role in the management of AD. However, the correlation between BP change and the prognosis for AD remains unclear. This study aims to demonstrate the impact of BP change patterns on AD prognosis.

Methods: This retrospective study included AD patients at two institutions (Shanghai Ninth People's Hospital Affiliated with Shanghai Jiao Tong University School of Medicine and the Vascular Department of the First Affiliated Hospital of Anhui Medical University) between 2004 and 2018. The systolic BP (SBP) change patterns of these patients were analyzed by functional data analysis (FDA). The relationship between BP change patterns and the risk of adverse events (AEs) was assessed using survival analysis.

Results: A total of 458 patients with AD were eligible for analysis. The logistic regression analysis indicated that compared with that in patients with low SBP variation (SBPV), the incidence of AEs in patients with high SBPV was significantly higher (35.84 vs. 20.35%, OR 2.19, $P < 0.001$). The patients were divided into four categories (accelerating rise, accelerating drop, decelerating rise, and decelerating drop) based on their SBP patterns after FDA fitting. The results of Kaplan–Meier analysis showed that at the 15- and 20-min time points, the incidence of AEs in the decelerating-drop group was significantly lower than that in the accelerating-rise group (OR 0.19, $P = 0.031$ and OR 0.23, $P = 0.050$). However, at the 25- and 30-min time points, the difference between these four groups was not significant (OR 0.26, $P = 0.08$ and OR 0.29, $P = 0.10$).

Conclusions: This study classified AD patients into four groups according to the SBP change patterns the first 30 min following admission, of which those with accelerating rises in SBP are at the highest risk of AEs, while those with decelerating drops have the best prognosis in the first 24 h after admission. Clinical practitioners may benefit from analyzing patterns of in-hospital SBP.

Keywords: aortic dissection, blood pressure, classification, functional data analysis, adverse events

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INTRODUCTION

Aortic dissection (AD) is a catastrophic aortic disease with an in-hospital mortality rate of 10–18% and a death rate of approximately 20–30% before admission to the hospital (1, 2). Hypertension is a predominant risk factor for AD because the increasing shear stress resulting from high blood pressure (BP) can lead to the initial tear in the aortic intima and the subsequent progression of AD (3), and 72.1% of AD patients have a history of hypertension (4). Thus, in the clinical management of AD, BP control has played a vital role, as hypertension (systolic BP, SBP >150 mm Hg) is associated with a higher incidence of vascular complications, and hypotension (SBP ≤80 mm Hg) is associated with a higher incidence of malperfusion syndromes in patients with acute AD (5, 6).

The latest clinical guidelines recommend a rapid SBP reduction to the target value of 100–120 mm Hg (7–10). However, this antihypertensive therapy has not been effective, and the mortality rate has not decreased over the last 20 years (1). There are two potential reasons for this inefficiency. First, it is unclear to what extent practitioners should control BP to optimize the survival rates of AD patients (11, 12). Several scholars found that this population-based BP control strategy was not suitable for all AD patients, where certain individuals may develop severe malperfusion syndromes due to intensive BP control (5, 11). On the other hand, a recent randomized trial revealed that intensive BP control (SBP <120 mm Hg) could significantly reduce the incidence of cardiovascular complications in hypertensive patients (12). Moreover, researchers found a negative correlation between the SBP at admission and the in-hospital mortality rate for patients with Stanford type A AD (13). Second, the fixed threshold fails to take into account the dynamic nature of BP, which is critical in the development of cardiovascular events. For instance, earlier studies have suggested that the circadian rhythm of BP fluctuation has an impact on the occurrence of AD (14–17) and that BP variations have strong predictive power for adverse events (AEs) in AD (18, 19). There is, however, no clear correlation between the pattern of change in BP and the prognosis for AD. Considering the urgency of the need to control BP in AD patients after admission, we aimed to demonstrate the impact of BP change patterns on AD prognosis and provide guidelines for the management of BP in AD patients.

METHODS

This was a retrospective, multicenter study of AD. We collected the clinical information of consecutive AD patients at two institutions (Shanghai Ninth People's Hospital Affiliated with Shanghai Jiao Tong University School of Medicine and the Vascular Department of the First Affiliated Hospital of Anhui Medical University) between January 2004 and December 2018. The need for written patient consent was waived because of the observational nature of this study. This study was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR1900025818). The inclusion criteria were hospital admission for AD patients diagnosed by computed tomographic angiography (CTA). The following were used as exclusion criteria: age < 18 years, pregnancy, lack of BP record, patients with traumatic, inflammatory or iatrogenic dissections, patients with a previous aortic surgery, or incomplete medical history.

All AD patients received either conservative treatment or emergency surgery after admission, and each sample included BP data from up to 14 days. The patients' BPs were measured approximately every 15– 30 min by automated noninvasive BP monitors, and the demographic information and medical history of each patient was collected from his or her medical records. In this analysis, the presence of another acute AD in the past is considered as having an AD history, and complicated AD is defined as persistent pain, uncontrolled hypertension, early aortic expansion, malperfusion, and signs of rupture (18).

To examine the relationship between the patient's history of hypertension, in-hospital BP variation, and progression of AD, we calculated the standard deviation (SD) of SBP in each patient during his or her hospitalization and stratified them into high and low SBP variation (SBPV) groups according to the average SBPV, which is the sample mean of all patients' SBPVs. A high SBPV group consists of patients whose SBPV is greater than the average, and a low SBPV group consists of patients whose SBPV is lower than the average (20). The incidence of AE was compared between the two groups through the *t* test. The first logistic regression model considers the impact of having a history of hypertension on the patient's SBPV, and the second model determines the impact of SBPV on the incidence of AE after admission. In this paper, AEs included fatal or nonfatal aortic rupture, organ or limb ischemia, and death.

In addition, our study analyzes the relationship between the fluctuation in SBP during the patients' first 30 min at the hospital and the incidence of AEs on the first day after admission. We should note before moving forward that each patient's SBP was discretely recorded at different time points, while it indeed exits at any point in time over a continuous period of time. Thus, the underlying SBP process is a function over time intervals, and it was necessary to conduct functional data analysis (FDA) to first estimate the process before analysis (21, 22). FDA is a nonparametric and continuous analysis technique proposed by Ramsay for functional data and has been shown to be an accurate estimation tool that can automatically adapt to the correct limit and recover the true underlying structure from discretely observed data in a wide variety of fields such as biomedical science, medicine, economics, finance, linguistics, psychology and sports (18, 23–28). The complete estimation process is illustrated in the **Supplementary File**. With the estimated underlying process, we can then determine the patients' SBP at particular time points after admission and monitor their temporary changes. We tracked the patient's condition at four different time points: 15, 20, 25, and 30 min following admission. Finally, based on the fitted curves for SBP, we computed the first and second derivatives and classified patients into mutually exclusive groups based on their signs. The first derivative of a curve indicates the slope of the SBP curve, with positive values indicating an increasing SBP and negative values indicating a decreasing SBP. The second derivative corresponds to the curvature, with a positive value

representing accelerating changes in SBP and a negative value representing decelerating changes in SBP. Taking the sign of each derivative, we categorized the patients into four groups at each predetermined time point: accelerating rise, accelerating drop, decelerating rise, and decelerating drop (24). The relationship between each BP classification and the outcomes was assessed using survival curves and the logistic regression for each of the four time points. Kaplan-Meier is a non-parametric statistic that is often used to estimate the survival function from lifetime data, and it has been used extensively in a variety of disciplines including but not limited to medical, economics, and engineering (24, 29). Survival curves were constructed using Kaplan–Meier analysis and parallels with the log-rank test. R software (http://www.r-project.org) was used for statistical analyses. Continuous variables are expressed as the mean ± SD, and categorical variables are shown as percentages. A two-tailed $p < 0.05$ implies that the statistics are significantly different.

RESULTS

A total of 458 patients were enrolled in the current study, and **Table 1** summarizes the patients' demographical and clinical characteristics. The average age of the patients in the sample was 57.1 years; the initial presentation with chest or abdominal pain occurred in 81.2% of patients; 35% of the patients were diagnosed with Stanford type A dissection, and the rest were diagnosed with type B.

The logistic regression analysis indicated that a history of hypertension was associated with a high SBPV (OR 1.56, $P < 0.05$). The average SBPV of all patients was 13.19. According to our SBPV classification, 173 patients were classified as having high SBPV, and 285 patients were classified as having low SBPV. Compared with that in the low SBPV group, the incidence of AEs in the high SBPV group was significantly higher (35.84 vs. 20.35%, $P < 0.001$; **Table 2**). Moreover, logistic regression analysis further confirmed the strong association between high SBPV and AE (OR 2.19, $P < 0.001$).

Figure 1 shows the SBP curves for nine randomly selected individuals and the average curve for all enrolled patients during their first 24-h hospitalization. The average curve presented the BP pattern with a drop during the first 5 h, followed by a leveling off. However, the BP pattern was not consistent across all patients. The rate and degree of BP reduction after admission varied among AD patients, and some patients had progressively elevated BP after their BP dropped in the first few hours after admission.

As all patients had their BP measurements within the first 30 min after admission, this analysis included all 458 patients. Based on the SBP pattern during hospitalization after FDA fitting, we divided patients into four categories (**Supplementary Figure 1**). Among all patients, when the patients were classified at 15 min, 80.57% of them had SBP drops: 19.65% with decelerating drops and 60.92% with accelerating drops (**Table 3**). The proportion of patients in the accelerating-drop group increased, while the proportion of patients in the decelerating-drop group decreased with the extension of admission time. More Stanford type B patients were found in the Accelerating-drop and Decelerating-drop groups, and there were equal numbers of Stanford type A and type B patients in the Accelerating-rise and Decelerating-rise groups (**Supplementary Table 1**).

Kaplan–Meier analysis was performed to determine the association between BP pattern at 15, 20, 25, and 30 min after admission and the prognosis of AD during the first 24 h of hospitalization (**Figure 2**). The results showed that patients with an accelerating rise at these four time points had the highest risk of AEs. In contrast, patients in the decelerating-drop group had the lowest incidence of AEs. At the 15- and 20-min time points, logistic regression analysis revealed that the incidence of AEs in the decelerating-drop group was significantly lower than that in the accelerating-rise group (**Table 4**). However, at the 25- and 30-min time points, the difference between these four groups was not significant. The subgroup analyses among patients with acute AD were conducted as well, and the results were consistent

TABLE 1 | Physical and clinical characteristics of the included patients.

	***n* = 458**
Age, years mean (±SD)	57.1 ± 13.4
Male	363 (79.3%)
Symptom	
0-None	42 (9.2%)
1-Pain	372 (81.2%)
2-Shock	8 (1.7%)
3-Others	36 (7.9%)
ODT, days mean (±SD)	24.7 ± 111.1
Marfan syndrome	22 (4.8%)
COPD	29 (6.3%)
Hypertension	315 (68.8%)
Diabetes mellitus	28 (6.1%)
History of AD	24 (5.2%)
Cardiac diseases	85 (18.6%)
Renal insufficiency	34 (7.4%)
PAD	15 (3.3%)
Maximum aortic diameter ≥ 5.5 cm	98 (21.4%)
Type of AD	
0-Stanford type A	161 (35.2%)
1-Stanford type B	297 (64.8%)
Complicated AD	89 (19.4%)
Pericardial effusion	43 (9.4%)
Pleural effusion	105 (22.9%)

ODT, onset to door time; COPD, chronic obstructive pulmonary disease; AD, aortic dissection; PAD, peripheral arterial disease.

TABLE 2 | The incidence of AEs in the high SBPV group vs. the low SBPV group.

	Overall (*n* = 458)	**High SBPV (*n* = 173)**	**Low SBPV (*n* = 285)**	***P* value**
AE (%)	120 (26.20)	62 (35.84)	58 (20.35)	< 0.001

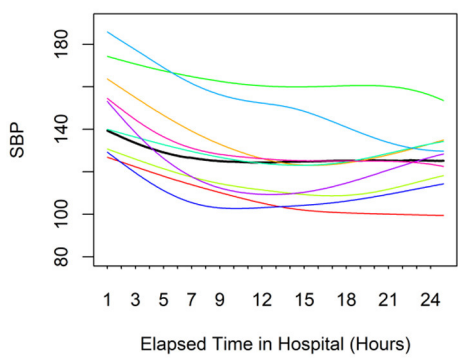

FIGURE 1 | Individual SBP curves for nine random individuals. The black line represents the average curve for the entire sample. There is considerable variation among individuals.

TABLE 3 | Distribution of AD patients after SBP classification.

	Accelerating rise	Accelerating drop	Decelerating rise	Decelerating drop
15 min	7.21%	60.92%	12.23%	19.65%
20 min	7.64%	62.45%	11.79%	18.12%
25 min	7.64%	65.07%	11.57%	15.72%
30 min	7.86%	65.94%	11.35%	14.85%

with that of the overall population (**Supplementary Figure 2** and **Supplementary Table 2**).

DISCUSSIONS

Summary of Main Results

The current study demonstrates that when AD patients are classified based on their in-hospital SBPV, patients with high SBPVs are at higher risk of AEs during hospitalization. Furthermore, when the patients were classified into four groups according to their SBP patterns during the 30-min period after admission, the results of the Kaplan–Meier analysis revealed that patients with an accelerating rise had the highest risk of AEs, while patients in the decelerating-drop group had the best prognosis during the first 24 h after admission.

The Review in the Context of Other Literature

Hypertension poses a substantial risk for AD, as shear stress associated with high BP can cause a tear in the aortic intima (30). Earlier studies indicated that the incidence of AD varied within a day in that the highest incidence occurred between 6:00 am and 12:00 am (31). The chronobiological patterns of AD onset were consistent with the circadian variation in BP (14), and patients who lack a nocturnal BP fall may have a higher risk of AD (17). These results indicated that the circadian rhythm of BP fluctuations had impacts on the occurrence of AD. In their study, Mehta et al. demonstrated that the incidence of AEs and in-hospital mortality of acute AD patients was not different among four different time periods within 1 day (0:00 am−6:00 am, 6:00 am−12:00 pm, 12:00 pm−6:00 pm, 6:00 pm−12:00 am) (32). Since circadian changes in BP reflect short-term fluctuations in BP, subsequent researchers began examining the relationship between BP variation and the development and progression of AD. Karatas et al. demonstrated that compared with hypertensive patients without AD, AD patients had significantly higher variations in BP, although the 24-h mean BP was similar between the two groups (33). Zhang et al. analyzed BP variation before endovascular therapy in AD patients and found that patients with high BP variation had an apparently higher incidence of aorta-related mortality (28.4 vs. 9.1%) (19). In addition, the thrombosis ratio of the false lumen was significantly lower in the high BPV group at the 6-month follow-up (86.4 vs. 69.7%) (19). Qiu et al. showed the association between unit increases in BP and the incidence of in-hospital AEs in AD patients by FDA (18). In the current study, based on our multicenter data, we found that patients with a history of hypertension were more likely to have high SBPV, which was further associated with an increased incidence of AEs. These findings could be explained by the abnormal BP rhythm and poor BP control and were in line with the results of previous studies (17, 19, 33). There is a challenge, however, in estimating the average SBPV and classifying the patients into high and low SBPV groups given different target populations in clinical practice; additionally, when using only the SD of the SBP as a summary measure, there is a loss of information. Otherwise, once these high SBPV patients are identified, it is unclear what the appropriate BP control strategy should be. For these reasons, a more specific and detailed classification of BP fluctuation patterns in AD patients is of great significance for guiding clinical practice.

Presently, the antihypertensive therapy of AD is controversial in that the current clinical guidelines of BP control lack high-level clinical evidence (7–10), and clinical data have indicated no significant improvement in the efficacy of antihypertensive therapy for AD over the last two decades (1). Several studies found that the target BP level of 100–120 mm Hg was not suitable for all AD patients because rapid intensive BP lowering may lead to organ ischemia for patients with high basal BP (11, 34, 35). Therefore, individualized antihypertensive therapy for AD patients is essential. However, there is a lack of screening methods for high-risk patients that may be useful in developing a sufficient BP control strategy. In this paper, we classified AD patients into four groups based on their SBP patterns at four time points (15, 20, 25, and 30 min) after admission and conducted a predictive analysis for these patients. Patients with SBP increases, especially accelerating rises at the four time points, exhibited the highest incidence of AEs during the first 24 h after admission, suggesting that the analysis of BP patterns during the first 30 min after admission is helpful in identifying high-risk AD patients. Moreover, the risk of AEs was comparable between the decelerating-rise and accelerating-drop groups, which might result from the differences in the pathogenesis of aortic rupture and ischemia complications. Specifically, a continuous increase in SBP may lead to AD progression, while a rapid reduction in

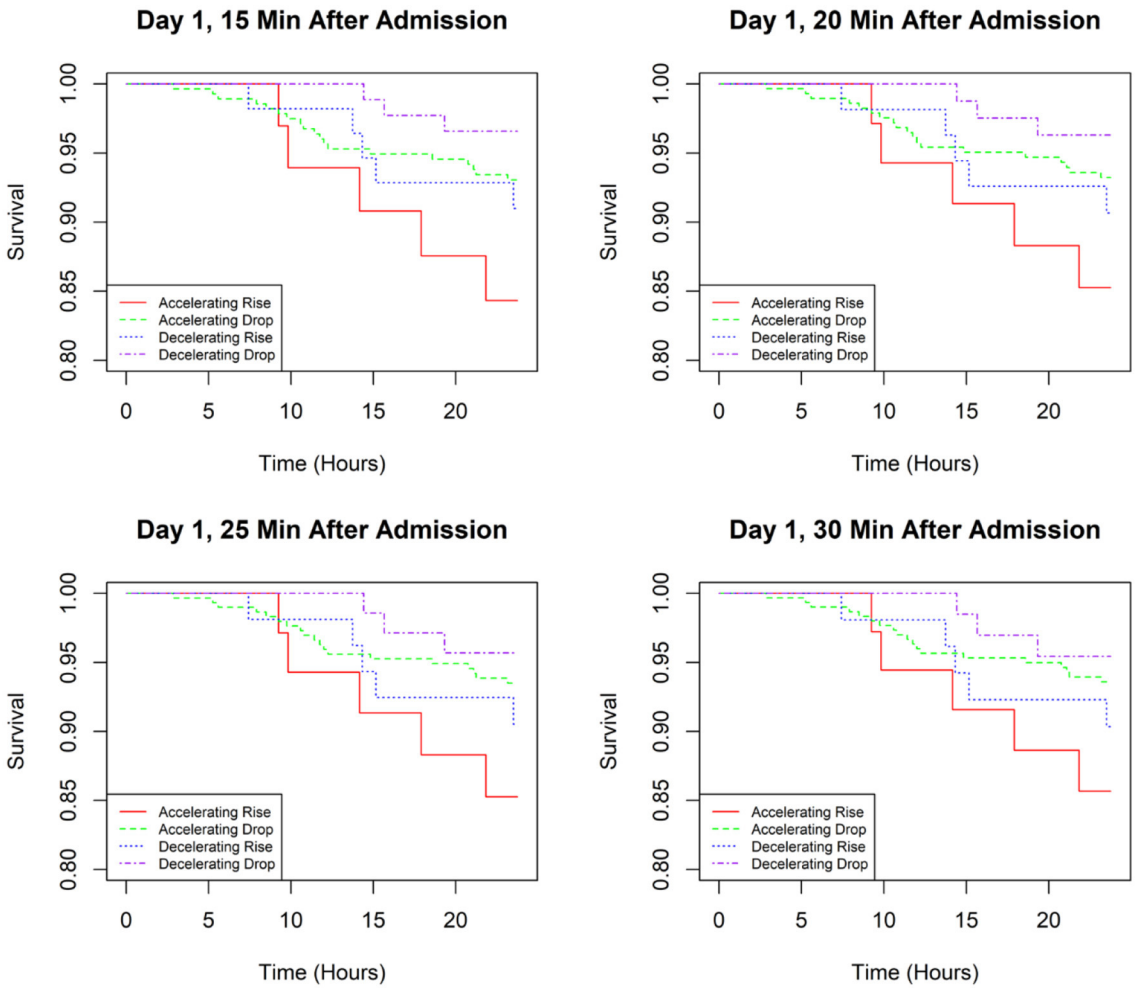

FIGURE 2 | Kaplan–Meier curves for time to an adverse event in the first 24 h of admission based on SBP classifications at different time points.

TABLE 4 | The risk of AEs compared with the accelerating-rise group.

	15 min	20 min	25 min	30 min
Accelerating rise	/	/	/	/
Accelerating drop	0.41 (0.10)	0.43 (0.11)	0.41 (0.10)	0.42 (0.10)
Decelerating rise	0.53 (0.35)	0.59 (0.43)	0.60 (0.45)	0.64 (0.51)
Decelerating drop	0.19 (0.03)	0.21 (0.04)	0.25 (0.07)	0.27 (0.09)

The values indicate the odds ratio (p value). The results were adjusted for Marfan syndrome and cardiac diseases.

BP may result in organ hypoperfusion. It was also noteworthy that patients in the decelerating-drop group exhibited the best prognosis at 24 h after admission, which implied that steady and stable BP reduction may be applicable in clinical settings.

Based on the results of the present study, we can prescreen patients at high risk for vascular and organ complications with their SBP patterns during the first 30 min after admission. Moreover, close monitoring and appropriate SBP control strategies can be applied to these patients. For patients with persistently elevated SBP after admission, we can adjust the type and dose of antihypertensive drugs to gradually lower their SBP. In addition, resistant hypertension, defined as SBP $\geqq$ 135/80 mm Hg despite the prescription of at least three antihypertensives, is sometimes used as an indication for surgery (36, 37). Nevertheless, the current definition of resistant hypertension does not reflect the dynamic process of BP. Based on our classifications, the pattern of BP changes that are characterized as accelerated rises or decelerating rises after medical treatment may be more consistent with the nature of resistant hypertension.

Limitations

There are several limitations to this work. First, there is the possibility of bias due to the observational nature of this study, and the causal relationship between changes in BP and the occurrence of AEs is unclear. To establish causation between the two variables, a prospective database with randomized controlled experiments should be utilized. Second, the sample size was relatively small, primarily due to the low incidence and high prehospital mortality rate of AD. Further studies

with larger sample sizes are warranted to replicate these preliminary findings. Third, most patients included in this study were Chinese; therefore, caution should be exercised in extrapolating our findings to other ethnic groups. Forth, continuous monitoring of BP is imperative for analyzing the changing pattern of BP after admission, which is not taken sporadically as laboratory tests. This may be difficult to implement in some clinical situations. Finally, the effect of the BP fluctuation pattern on the mid- and long-term prognosis of AD patients remains unclear and requires further investigation.

CONCLUSIONS

The current study confirms that SBPV is associated with the prognosis of AD. In addition, AD patients can be classified into four groups according to the patterns of changes in SBP exhibited during the first 30 min following admission, of which those with accelerating rises in SBP are at the highest risk of AE, while those with decelerating drops have the best prognosis in the first 24 h after admission. In light of these results, it appears that clinical practitioners may benefit from analyzing patterns of in-hospital SBP at different time points. These results need to be verified in a large-sample prospective AD database.

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 Institutional Review Board of Shanghai Ninth People's Hospital. The Ethics Committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

WL, RW, TC, and XL led in the conception and design of the study, revised the manuscript, supervised, validated the clinical work, and results. ZW, YL, and PQ collected research data, performed the statistical analysis, and drafted the manuscript. KL and HL revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.832770/full#supplementary-material

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ORIGINAL RESEARCH
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Depressed Kallikrein Generation in Women With Preeclampsia: A Matched Cross-Sectional Study

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Objective: The pathophysiology of preeclampsia is not fully understood. Disturbances in the contact system are associated with preeclampsia. Few studies have investigated the association between preeclampsia and alterations in the contact system in plasma. This study aims to elucidate whether this basic biological system is affected in preeclampsia using new methods focusing on the dynamic interactions and total capacity of the contact system in blood.

Design: Cross-sectional study matching women with preeclampsia and controls without preeclampsia regarding age, pregestational body mass index, and gestational age at onset of the disease.

Setting: Two Danish University hospitals.

Sample: A cohort of 117 women with preeclampsia and 117 controls.

Methods: The turnover and capacity of the contact system were determined with new methods. Paired *t*-test, Wilcoxon signed-pairs signed rank test, Mann-Whitney or Chi^2-test were applied, as appropriate.

Main Outcome Measurements: Kallikrein generation (peak kallikrein concentration and endogenous kallikrein potential), coagulation factor XII, prekallikrein, H-kininogen, cleaved H-kininogen, and complement C1 esterase inhibitor.

Results: The endogenous kallikrein potential, peak kallikrein concentration, prekallikrein and cleaved H-kininogen were significantly lower in women with preeclampsia compared to the controls, $p \leq 0.005$, whereas the concentration of coagulation factor XII, H-kininogen and complement C1 esterase inhibitor was not significantly different, $p > 0.05$.

Conclusion: This study demonstrates significant reduction in kallikrein generating capacity, prekallikrein and cleaved H-kininogen indicating that the contact system is affected in preeclampsia suggesting a link to the pathophysiology of the disease.

Keywords: preeclampsia, disease severity, contact activation, kallikrein generation, cleaved H-kininogen

INTRODUCTION

Worldwide, preeclampsia (PE) is a major contributor to maternal morbidity and mortality and can cause severe perinatal complications such as prematurity and intrauterine growth retardation (IUGR). Approximately 5% of all pregnancies are affected by PE (1), which occurs only during pregnancy or early post-partum. The incidence of preterm PE (before 37 weeks of gestation) has remained unchanged during the last decade (2) despite innumerable attempts of predicting the disease and providing prophylactic care. Still, the pathophysiology of PE is only partly understood, and the root cause is not clear. However, placenta dysfunction or reduced maternal cardiovascular adaption or a combination of these conditions may contribute to the pathophysiology of PE.

It has been reported, that proteins of the contact system (CAS) regulate the placental function, and demonstrated that components of CAS are present in human placenta, and that the system may contribute to the placental function by regulating blood flow and transplacental transport of metabolites (3). These observations prompted us to speculate whether CAS in blood plays a role in the development of PE. The plasma proteins coagulation factor XII (FXII), prekallikrein (PK) and H-kininogen (HK) are the constituents of CAS. The activity of the system is regulated by protease inhibitors, of which C1-esterase inhibitor (C1-inh) is of particular importance. CAS is initiated by the interaction between FXII, PK, and an activating substance. The interaction determines the further propagation of CAS (4) which may lead to activation of coagulation, inflammation or fibrinolysis. By these actions, CAS may contribute to the maternal pro-thrombotic state, the fibrinolytic changes and the increased inflammatory response characterizing PE (5, 6).

Conversely, only few studies have investigated the association between PE and alterations in the activation and regulation of CAS in plasma despite the possibility that disturbance in CAS may be associated with early gestational loss (7, 8) and adverse pregnancy outcomes like preterm birth (9) and PE (10–12). Few prospective and cross-sectional studies demonstrate that the plasma levels of CAS proteins are affected during pregnancies complicated by PE (10, 13–18). However, most of these studies are more than 30 years old, and the complex interactions characterizing the initiation and propagation of CAS cannot be deduced by the methods used previously. We have developed new methods focusing on the dynamic interactions of the CAS proteins and the total capacity of CAS in blood (19–21), thereby giving us the opportunity comprehensively to study whether this basic biological system is associated with the pathophysiology of PE.

Abbreviations: BMI, Body mass index; CAS, contact activation system; cHK, cleaved H-kininogen; CLSI, Clinical and Laboratory Standards Institute; C1-inh, complement 1-esterase inhibitor; EDTA, dipotassium-ethylene-diamine-tetra-acetate; EKP, endogenous kallikrein potential; ELISA, enzyme-linked immunosorbent assay; FGR, fetal growth restriction; FXII, coagulation factor XII; GA, gestational age; HK, H-kininogen; HRP, Horseradish Peroxidase; IUGR, intrauterine growth retardation; Mab, monoclonal antibody; N, antiserum; PE, preeclampsia; Pka, kallikrein; PK, prekallikrein; PreCAS, The Preeclampsia and ContAct System study.

Thus, the aim of the present cross-sectional study is to compare the plasma concentration of CAS proteins and the turnover and capacity of CAS in pregnant women suffering from PE and matched healthy pregnant women, in particular concerning the inflammatory pathway of CAS.

MATERIALS AND METHODS

Patient Cohort

The Preeclampsia and ContAct System study (PreCAS) is a matched cross-sectional trial conducted from January 2020 to October 2021. Patients were included at Departments of Obstetrics and Gynecology at University Hospital of Southern Denmark, Esbjerg and Odense. The hospitals take care of ∼6,000 births annually.

PE is defined by a national guideline from 2018 (22) as a combination of hypertension and proteinuria after 20 weeks of gestation or hypertension accompanied by one of the following: hematological or neurological complications, liver dysfunction, renal failure, pulmonary edema or utero placental insufficiency. The National Guideline is generally in accordance with the International Society for the Study of Hypertension in pregnancy guideline from 2018. Severe PE was defined as blood pressure > 160/100 mmHg and/or severe subjective symptoms or severity of the definition criteria (22). Preterm PE is defined as delivery before 37 weeks of gestation (2). Fetal growth restriction (FGR) is defined as fetal weight below −15% of expected (22). Pregnant women above the age of 18 fulfilling the diagnostic criteria of PE were enrolled in the study either in the outpatient clinic or when admitted to the hospital. One-hundred and seventeen women were asked to participate and all accepted.

For each women with PE, a healthy pregnant woman was included. The control subjects were matched with cases with respect to gestational week (GA) (±1 week), age (±1 year) and pregestational body mass index (BMI) (±1 kg/m^2).

During the period of inclusion, 117 women with PE were enrolled. The control subjects were recruited and included in the study in relation to an antenatal care session and 1,044 pregnant women consented to be possible controls. Data on the matching criteria was obtained in a secure database. Controls with the best possible match on all three criteria were contacted by telephone and invited to participate. Women suffering from PE in a former pregnancy, receiving cortisone in current pregnancy or developed PE were excluded as controls.

Of these 129 women were matched and invited to participate in the project. Twelve control subjects were excluded (**Figure 1**). Three women developed PE after blood sampling. One woman received systemic treatment with adrenal cortex hormone due to chronic inflammatory bowel disease. One woman had a severe medical condition and had prenatal care and delivery elsewhere. One woman suffered from late miscarriage and was not pregnant when contacted. Five women gave birth before blood sampling. One woman declined participation.

Details of Ethics Approval

Informed written consent was obtained from the participants before inclusion. The study was conducted according to the

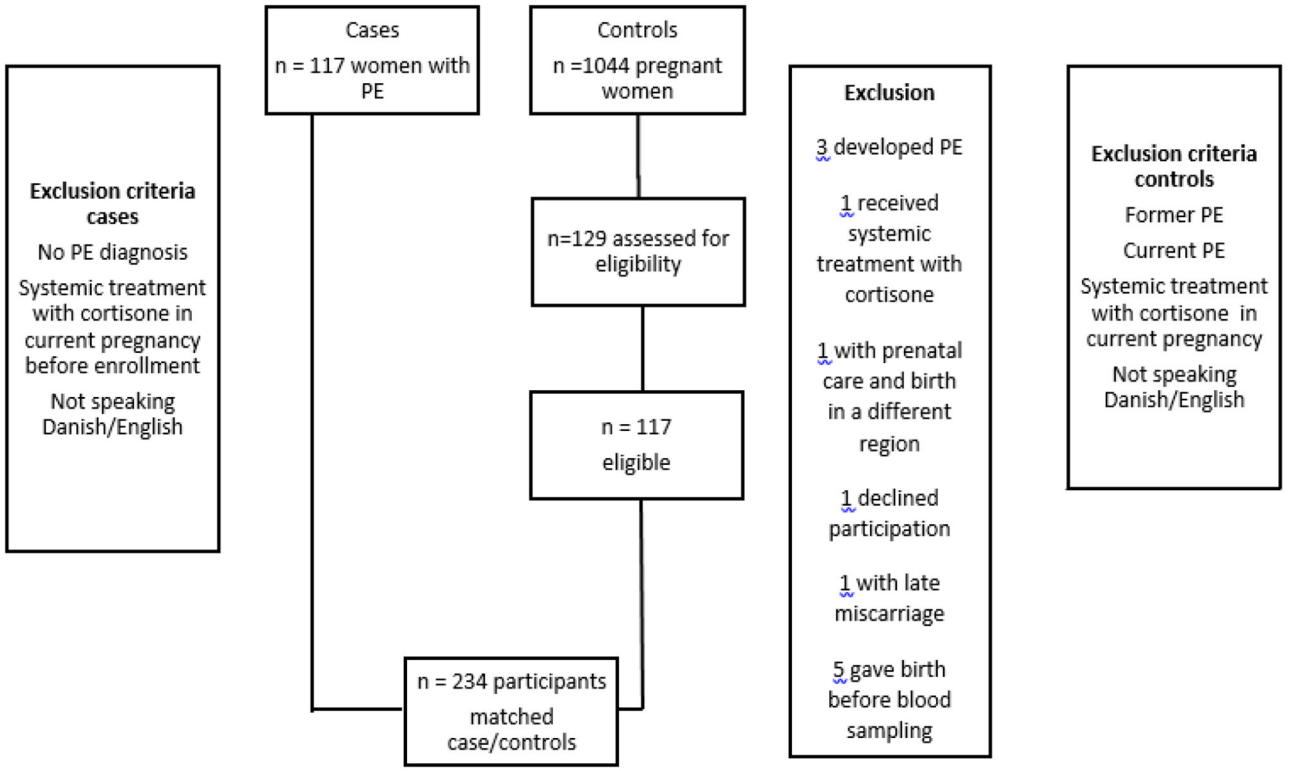

FIGURE 1 | Patients with preeclampsia (PE) and controls enrolled in the PreCas-study. All invited PE-patients (117) accepted to participate. The control group consisted of 1,044 patients and 129 were assessed as eligible. Twelve controls were excluded from the study based on the exclusion criteria.

Helsinki declaration including study approval by the Danish Data Protection Agency. Data from study participants are protected according to the Act on Processing of Personal Data. The Regional Ethics Committee in the Region of Southern Denmark approved the project (project ID S-20190142, 10 December 2019). The project was registered at www.clinicaltrials.gov as NCT04825145.

Blood Collection and Handling

Blood samples were collected from the patients upon inclusion when they fulfilled the above mentioned criteria for being diagnosed with PE and from the controls. The collection and handling of blood samples followed the G41 guideline from Clinical and Laboratory Standards Institute (CLSI) (23) with specific focus on the recommendations for collection, transport and processing of blood specimens for testing plasma-based coagulation assays as detailed in the H21-A5 guideline from CLSI (24).

In brief, 16.8 ml blood was collected from an antecubital vein in four evacuated 2.7 ml tubes containing 0.105 mol/L sodium citrate (Vacutainer 9NC, Becton Dickinson, Plymouth, UK) and two evacuated 3 ml tubes containing 5.4 mg dipotassium-ethylene-diamine-tetra-acetate (EDTA) (Vacutainer K2E, Becton Dickinson). Platelet poor plasma was collected after centrifugation for 20 min at 2,000 × g. The citrate and EDTA-stabilized plasma samples, respectively, were subsequently stored at −80°C in tightly capped cryotubes (Sarstedt, VWR-Bie & Berntsen, Søborg, Denmark). Before analysis, the samples were thawed for 5 min at 37°C, kept at room temperature, and analyzed within 1 h.

Laboratory Assays

Measures of kallikrein (PKa) generation, i.e., the lag time, time to peak, peak PKa concentration, and endogenous PKa potential (EKP) were recorded by an automated PKa generation assay as previously reported (19).

An in-house enzyme-linked immunosorbent assay (ELISA) that specifically recognizes cleaved HK (cHK), was used to measure the plasma concentration of cHK. Briefly, 96-well polystyrene flat bottom MicroWell™ MaxiSorp™ plates (Thermo Fisher Scientific, Lillerød, Denmark) were coated with 2 μg cHK mAb 19-20-8 per ml. The citrate plasma samples were analyzed at a 1:100 dilution and calibrated against kininogen depleted plasma (Affinity Biologicals, Ancaster, Ontario, Canada) spiked with 10 mg/ml purified cHK purchased from Enzyme Research Laboratories, Inc. South Bend, IN, USA. Biotinylated cHK mAb 19-31-18 (0.14 μg/ml) was used as detection antibody, and plates were visualized using HRP-conjugated streptavidin and ultra TMB ELISA substrate (Kementek, Taastrup, Denmark). The plates were read at 450 nm using a Tecan Sunrise ELISA reader (Tecan, Männedorf, Switzerland).

TABLE 1 | Matching criteria.

	Women with preeclampsia $n = 117$	Controls $n = 117$
Age (years)	29 (20–42)	29 (20–42)
Pregestational BMI (kg/m^2)	27.5 (16.5–50.0)	27.4 (16.0–48.2)
Gestational age for PE (weeks + days)	37+3 (28+3–41+6)	37+3 (29+0–40+6)

The results are presented as median (min-max).
BMI, Body mass index; PE, preeclampsia.

The protein concentration of HK was assessed by in-house prepared ELISA employing specific monoclonal antibodies. Briefly, 96-well polystyrene flat-button MicroWell MaxiSorp plates were coated with 1.0 μg Mab HK-6 per ml. Plasma samples were analyzed at a 1:4.000 dilution and a citrate plasma pool from 30 healthy volunteers was used as a calibrator. Biotinylated Mab HK 19-31-18 (0.14 μg/ml) was used as a detection antibody and plates were visualized using horseradish peroxidase-conjugated streptavidin and the TMB One-substrate (Kementek). The plates were read at 450 nm with 650 nm as reference using the Tecan Sunrise ELISA reader.

The plasma protein concentration of FXII and PK was determined with ELISA as described previously (20, 21).

The protein concentration of C1 inh was determined using N antiserum against human C1 inh, buffers, and reagents, employing the BN II analyser (all from Siemens Healthcare Diagnostics, Marburg, Germany).

Statistics

The power calculation was based on the EKP, i.e., the total amount of PKa that can be generated in plasma after contact activation (19). The mean EKP determined in a reference population ($n = 85$) is 2,172 mIU/ml × min with a standard deviation of 515 mIU/ml × min. Aiming at a change in EKP of 10%, and using an alpha-value of 0.05 and a beta-value of 80% revealed that 2 × 90 women should be included in the study. The maximal dropout frequency was stipulated to 25% and consequently 2 × 113 women had to be included in the study.

Statistical calculations were performed by GraphPad Prism version 9.1.2 (GraphPad Software, San Diego, CA, USA). The distribution of the results were verified by a QQ plot and the Kolmogorov-Smirnov test. Paired *t*-test, Wilcoxon matched-pairs signed rank test, unpaired *t*-test, Mann-Whitney or Chi^2-test were applied when appropriate. Results are presented as mean ± standard deviation or median and 25–75 percentile range, as appropriate. A p-value < 0.05 was considered statistical significant.

RESULTS

Women with PE were comparable with controls with respect to the matching criteria, maternal age, pregestational BMI and gestational age (**Table 1**).

The characteristics of the study participants are shown in **Table 2**.

TABLE 2 | Characteristics of the study participants.

	Women with preeclampsia $n = 117$	Controls $n = 117$	p-value
Pregnancy (number)	2 (1–9)	2 (1–11)	0.15
Nullipara	56.4%	44.4%	0.07
Ethnicity	94.9% Caucasian	97.4% Caucasian	0.40
	3.4% Afro-Caribbean	0.9% Afro-Caribbean	
	1.7% East-Asian	1.7% East-Asian	
Smoking status	81.2% non-smokers	91.5% non-smokers	0.028
	12.8% former smokers	3.4% former smokers	
	6.0% current smokers	5.1% current smokers	
Current pregnancy singleton/multifetal	93.2% singleton	96.6% singleton	0.24
	6.8% gemelli	3.4% gemelli	
Blood pressure in early pregnancy (mmHg)	Systolic: 124 ± 12	Systolic: 117 ± 12	<0.0001
	Diastolic: 81 ± 10	Diastolic: 75 (48–96)	
Baby birth-weight (g)	2,992 ± 688	3,612 ± 549	<0.0001
Gestational age at delivery	38+2 (30+1–42+0)	40+3 (35+5–42+3)	<0.0001
Assisted reproductive treatment	12.0%	9.5%	0.54

The results are presented as mean ± SD or median (min-max), as appropriate.

No significant differences were seen in the number of nulliparous women in women with PE and controls ($p = 0.07$).

In early pregnancy the blood pressure was higher in the women developing PE, than in the control women ($p < 0.0001$). The weight of the newborn was lower ($p < 0.0001$) and the duration of pregnancy was shorter ($p < 0.0001$) in women with PE compared to their controls. No difference was seen in in the number of pregnant women who had received assisted reproductive treatment ($p = 0.54$).

Thirty-four of the women with PE 34 had severe disease and 29 had preterm PE. Fifteen of the women had both severe disease and preterm PE.

The peak PKa concentration was significantly lower in the PE-women compared with controls; 1,413 nmol/l ± 287 vs. 1,653 nmol/l ± 362, $p < 0.0001$. The EKP was significantly lower in the PE-women compared to the controls; 1,956 ± nmol/l × min ± 447 and 2,195 nmol/l × min ± 471, respectively, $p = 0.0001$ (**Figure 2**). In addition, the lag time and the time to peak of PKa generation were significantly longer in PE-women than in controls, $p = 0.03$ and $p = 0.02$, respectively (data not shown).

The women in the PE group had significantly lower PK concentration than the controls; 27.5 μg/ml ± 6.9 and 29.4 μg/ml ± 7.0 respectively, $p = 0.005$ and the plasma concentration of cHK was also significantly lower in the PE group with a median of 3.8 μg/ml (2.9–5.4) than the controls; 4.9 μg/ml (3.5–6.0), $p = 0.002$ (**Figure 2**).

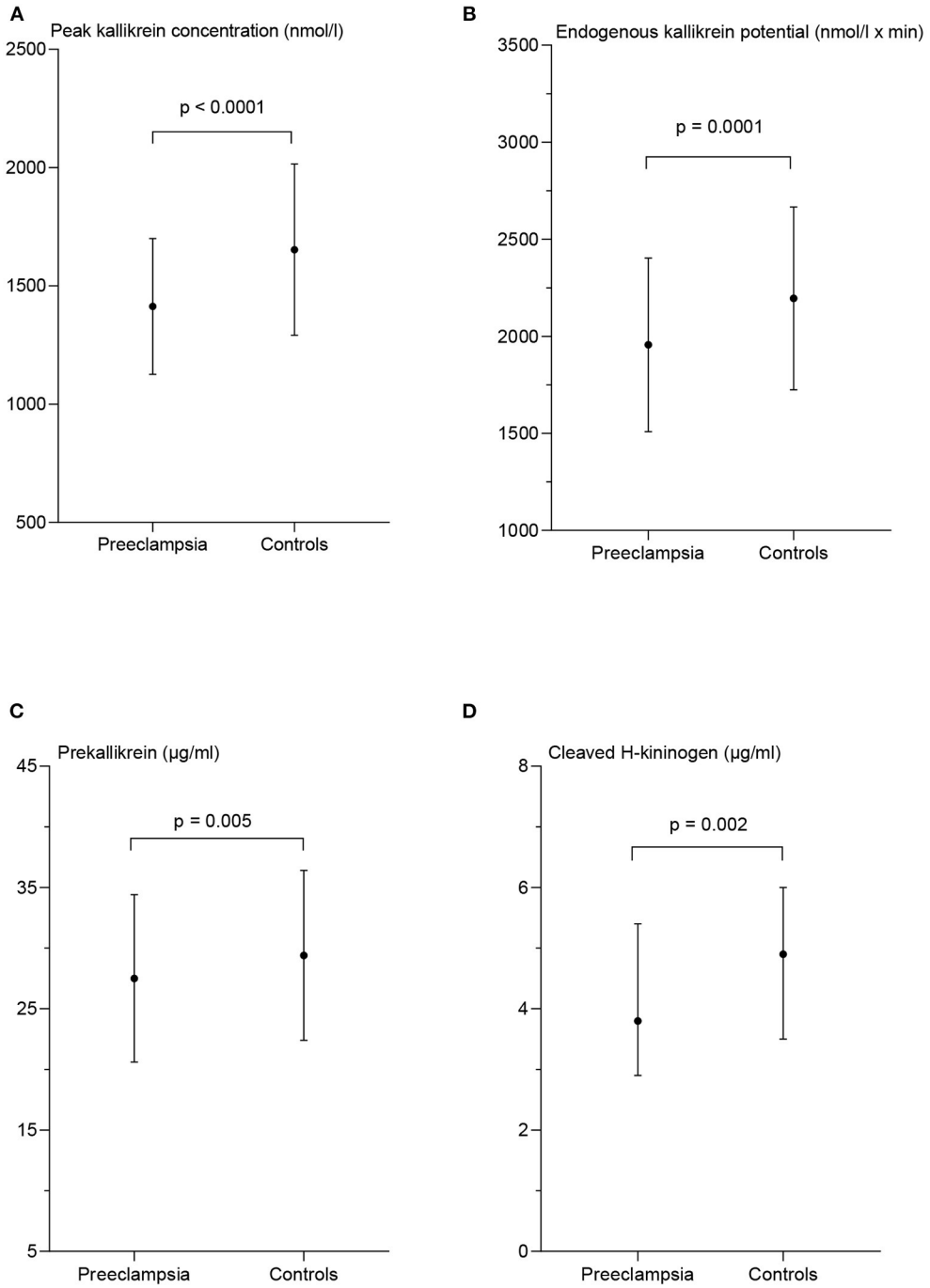

FIGURE 2 | Measures of contact activation in patients with preeclampsia and matched controls. Peak kallikrein concentration **(A)**, endogenous kallikrein potential **(B)**, and prekallikrein concentration **(C)** are presented as mean ± standard deviation. The concentration of cleaved H-kininogen **(D)** is presented as median and interquartile range. *p*-values from the paired *t*-test **(A–C)** and the Wilcoxon matched-pairs signed rank test **(D)** between the two patient groups.

The plasma concentrations of FXII, HK and C1-inh in women with PE were 35.8 mg/l ± 8.1, 65.5% ± 23.6, and 0.17 g/l (0.15–0.19), respectively, and not significantly different from control subjects where the corresponding concentrations were 35.8 mg/l ± 8.5, 63.7 ± 20.1, and 0.17 g/l (0.16–0.19), $p = 0.98$, $p = 0.48$, and $p = 0.14$, respectively (**Figure 3**).

Sub analyses of the PE group were performed, and peak PKa, EKP, the plasma concentration of PK, and cHK

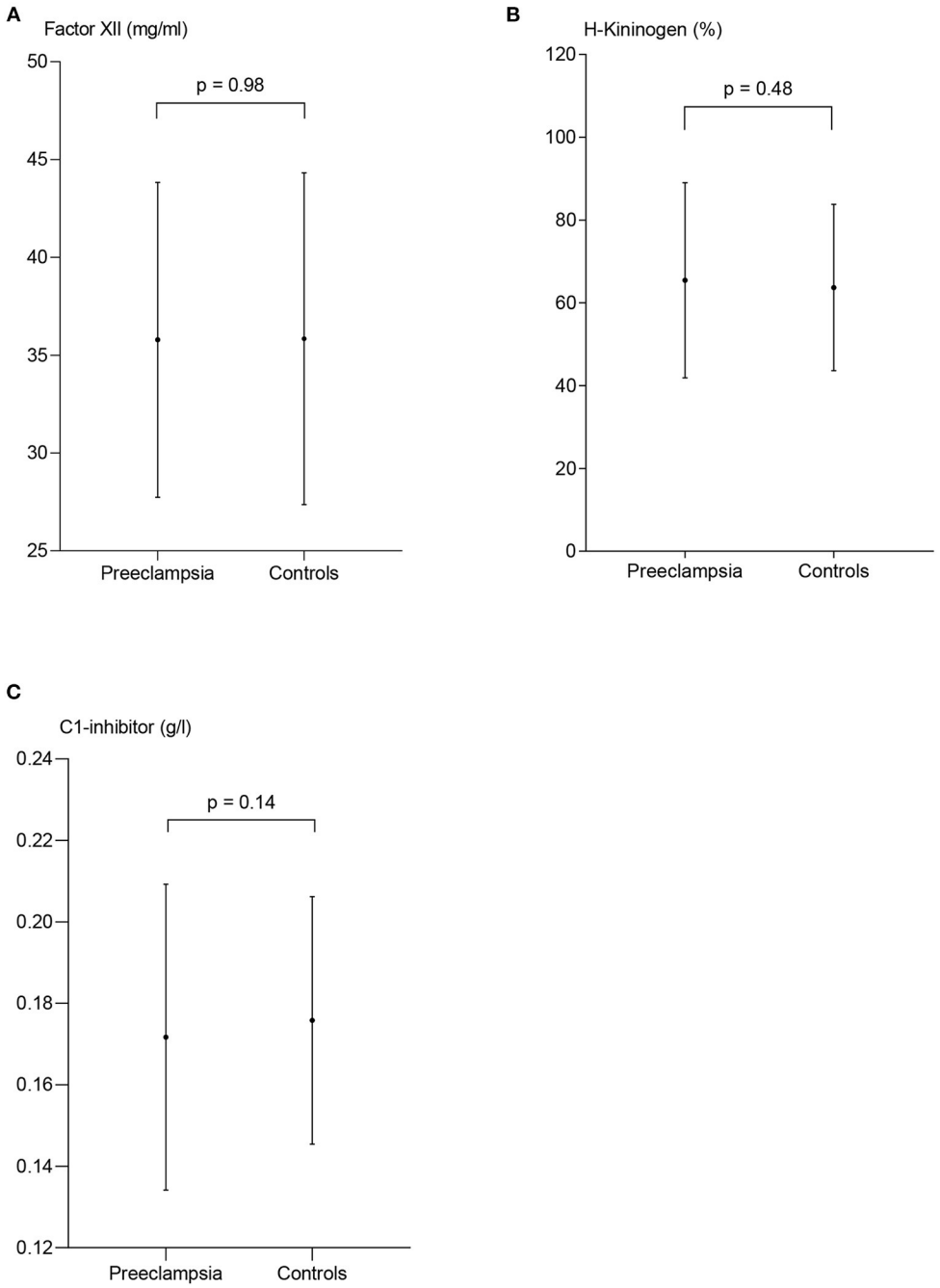

FIGURE 3 | Measures of contact activation in patients with preeclampsia and controls. The plasma concentration of coagulation factor XII (FXII) **(A)** and H-kininogen **(B)** are presented as mean ± standard deviation. C1-esterase inhibitor **(C)** is presented as median and interquartile range, p-values from the paired t-test **(A,B)** and the Wilcoxon matched-pairs signed rank test **(C)** between the two patient groups.

in women with severe and preterm PE are shown in **Figures 4**, **5**.

The peak PKa concentration in women with severe PE, 1,358 nmol/l ± 320 did not deviate significantly from the women with mild/moderate PE, 1,435 nmol/l ± 272 ($p = 0.19$). Also EKP was comparable in women with severe PE, 1,840 nmol/l × min ± 445 and women with mild/moderate PE, 2,002 nmol/l × min ± 442, $p = 0.08$ (**Figure 4**).

The concentration of cHK was significantly reduced in the women with severe PE, 3.37 μg/ml (2.47–5.22) compared to the women with mild/moderate PE, 4.94 μg/ml (3.66–5.95), $p = 0.002$. The PK concentration was not significantly different

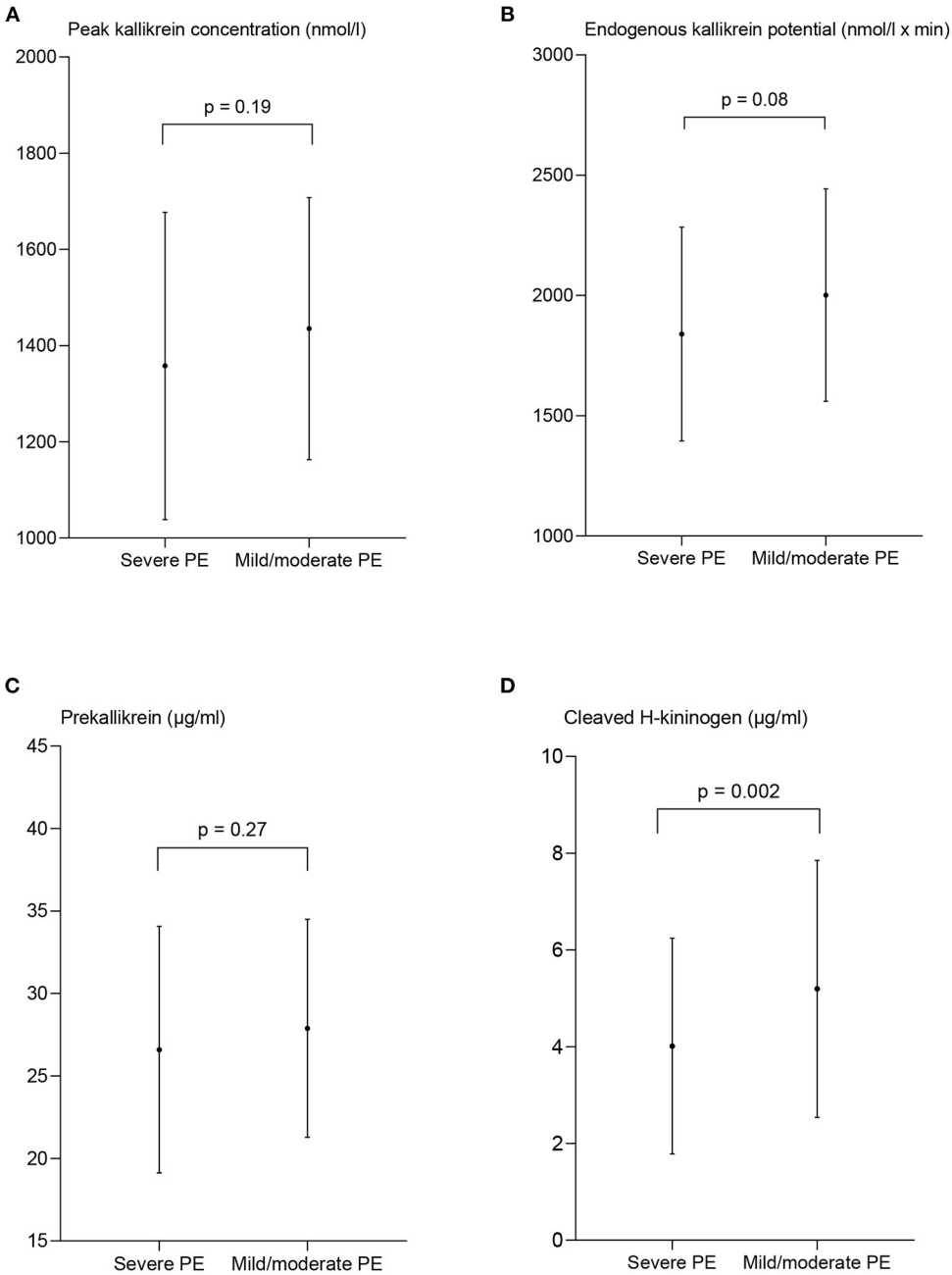

FIGURE 4 | Measures of contact activation in women with severe and mild/moderate preeclampsia (PE). The peak kallikrein concentration **(A)**, endogenous kallikrein potential **(B)**, and prekallikrein concentration **(C)** are presented as mean ± standard deviation. The concentration of cleaved H-kininogen **(D)** is presented as median and interquartile range. *p*-values from the paired *t*-test **(A–C)** and the Wilcoxon matched-pairs signed rank test **(D)** between the two patient groups.

among women with severe PE, 26.6 µg/ml ±7.5, and women with mild/moderate PE, 27.9 µg/ml ± 6.6, $p = 0.27$.

The peak PKa concentration was lower in women with preterm PE 1,327 nmol/l ± 329 than term PE, 1,438 nmol/l ± 273, although not significant ($p = 0.08$). EKP was significantly lower in women with preterm PE than in women with term PE, 1,804 nmol/l ± 485 vs. 1,999 ± 432, $p = 0.04$.

The concentration of cHK was significantly reduced in the women with preterm PE compared to the women with term PE ($p = 0.03$) with a concentration of 3.6 µg/ml ± 1.8 compared to 5.1 µg/ml ± 3.2, $p = 0.03$.The PK concentration was not significantly different between the groups; 26.4 µg/ml ± 6.7 in the preterm group and 27.8 µg/ml ± 6.9 in the term group, $p = 0.38$ (**Figure 5**).

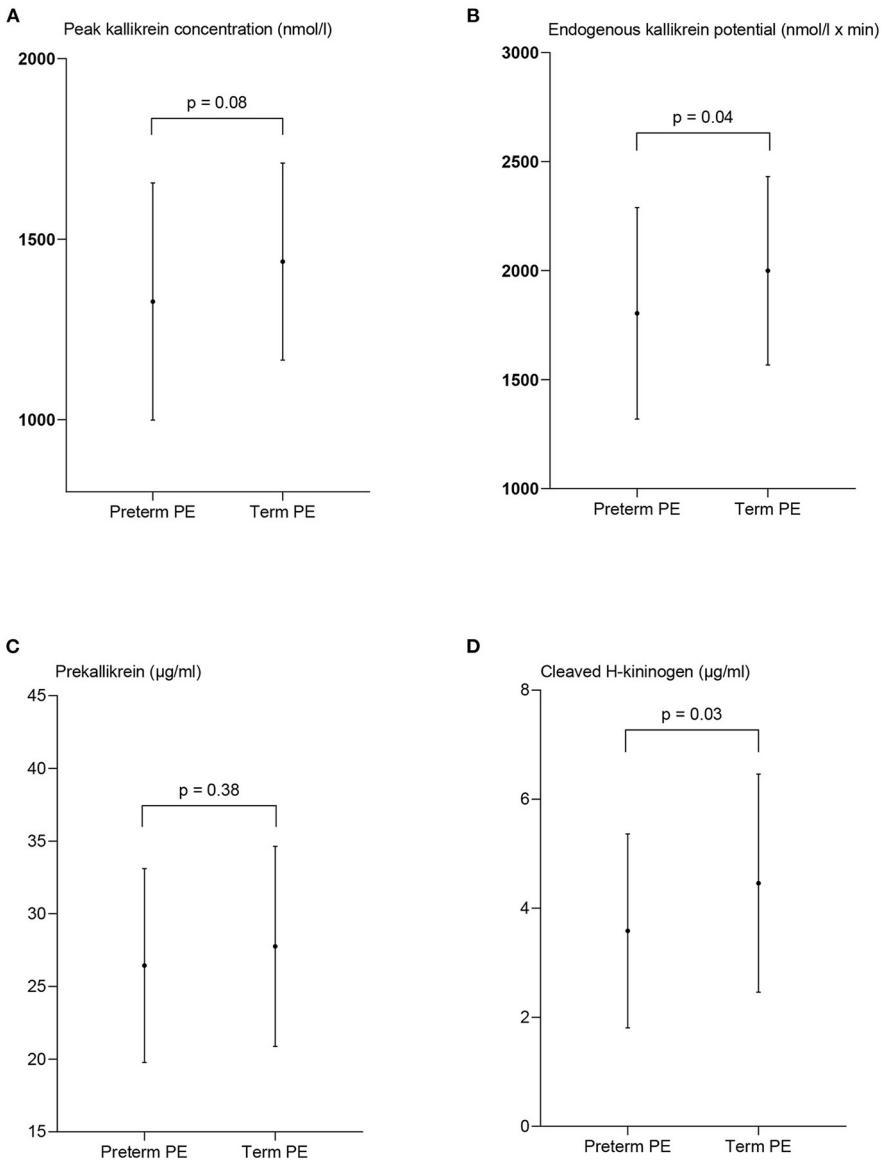

FIGURE 5 | Measures of contact activation in women with preterm preeclampsia and term preeclampsia (PE). The peak kallikrein concentration **(A)**, endogenous kallikrein potential **(B)**, prekallikrein concentration **(C)**, and the concentration of cleaved H-kininogen **(D)** are presented as mean ± standard deviation. *p*-values from the paired *t*-test between the two patient groups.

Of the 117 women with PE included in the study, 22 had FGR. Sub analysis showed no significant difference in measures of CAS between women with PE and FGR and women with PE and without FGR.

DISCUSSION

Main Findings

The present trial is the first matched cross-sectional study addressing the potential association between CAS and PE. Only few studies have investigated the association between PE and alterations in the activation and regulation of CAS in plasma (10, 13–18). Most studies are more than 30 years old, and it is not possible to reveal the complex protein interactions, including regulation and propagation of CAS with the methods used previously. Using a matched design and a more advanced analytic setup, we presently demonstrate that CAS is affected in pregnant women suffering from PE.

The key findings are that both the peak PKa concentration and the PKa generating capacity are reduced in women with PE compared to their controls, suggesting that CAS capacity is significantly depressed in PE patients. Accordingly, we observe that cHK is decreased in women with PE,

and additionally decreased in women with severe disease or preterm PE.

Strengths and Limitations

The strength of this study is the advanced analytical setup and the matched design. The PKa generating capacity and the concentration of CAS proteins might be dependent on the maternal and gestational age and the maternal BMI. Conversely, our design prevents the potential bias caused by these factors, and in addition the number of participants in our study are considerably higher than in former studies. On the other hand, the cross sectional design is a limitation. It would have been profitable to use a prospective study design to reveal the activation of CAS even before onset of PE, but this would require a much larger number of participants. Moreover, the observed effects on CAS in PE women could be related to the disease or a genuine condition in these women. This might contribute to the explanation of the increased risk of cardiovascular disease later in life in this group of patients (25). A follow up period could therefore be beneficial to shed light on the behavior of CAS beyond pregnancy.

We separated the PE patients into sub-groups with respect to severity of the disease and gestational age at delivery. The sizes of these groups were rather small, and the statistical power is not sufficient to allow firm conclusions. It has, however, been suggested that the pathophysiology of PE might be different in the groups regarding placental dysfunction and reduced maternal cardiovascular adaption to pregnancy (25, 26). Nonetheless, subgroup analysis regarding severity of PE and gestational age at delivery revealed trends in the key findings toward more extensive changes of CAS.

Interpretation

PKa is formed through activation of PK and PKa cleaves HK thereby releasing bradykinin and the two-chain molecule cHK that is a specific marker for CAS activation. Accordingly, depressed cHK corresponds conceptually with the finding of reduced PKa generating capacity in the PE group as demonstrated with the PKa generation assay. Even further reduced PKa generating capacity was noticed in women with preterm PE.

The present study also demonstrates significantly lower plasma concentration of PK in patients with PE compared to the control group. This is in line with former studies (10, 14, 15). A small cross-sectional study (14) revealed lower levels of PK in women with PE than in late term pregnant women. Another cross-sectional study (10) compared normal pregnant women with women developing PE and subdivided this group with respect to the severity of the disease. A significantly lower PK was detected in women with PE, and the reduction in PK correlated to the severity of the disease. Therefore, a link between the level of PK and the pathophysiology of PE was proposed. One small cross-sectional study (15) showed no difference in the concentration of PK in women with PE compared to pregnant women without PE. The different result might be due to small sample size. These studies suggest that both the plasma concentration and the activity of PK are reduced in women with PE. The concentration of PK is of importance for PKa generation thereby helping to explain the observed depression in CAS capacity in pregnant women with PE compared to healthy pregnant women.

In contrast to a previous study (13), reporting increased levels of FXII in women with PE, we observe no significant difference in the plasma concentration of FXII between our two groups. However, in the previous study, the women with PE were not comparable to control subjects regarding age, BMI, and GA for PE as the patients included in our study. Moreover, the study employed a functional method for determination of FXII, whereas in our study the protein concentration of FXII was determined.

The potential relation between PE and HK has previously been addressed in only one small study (16) using a semi quantitative immunological method for detection of HK. Reduced levels of HK were observed in women with PE complicated with babies who were small for gestational age. In contrast, we quantified the plasma protein concentration of HK and observed no difference in the concentration of native HK between women with PE and controls.

The plasma concentration of C1-inh was not different in the two groups. Similarly, two small cross-sectional studies (17, 18) have investigated C1-inh in women with PE. Halbmayer et al. revealed no difference in PE women compared to pregnant women without PE, whereas Hsieh et al. found a decrease in women with PE. Both studies included only few participants, and the method used for determination of the C1-inh, was different than the method employed in the present study.

The reason for a depressed CAS capacity in women with PE is unclear. It could be speculated whether women with a genuine low CAS capacity have an increased risk of PE. In order to answer this question sufficiently there is a need for a large prospective study. Another explanation could be a consumption of CAS proteins in early pregnancy in the individuals later suffering from PE. Bryant and Shariat-Madar (27) considered the theory of consumption. Their study described a possible activation of CAS due to reduced levels of total kininogen (high- and low molecular weight HK) in the placentas of PE women (28), indicating consumption of the proteins. Interestingly, and in this context, it has been reported that women developing PE produce misfolded proteins (29, 30) and that misfolded proteins *per se* have the capability to activate CAS (31). A third tentative explanation could be that an affected liver function could lower the concentration of CAS proteins in plasma. However, among the CAS proteins, only the concentration of PK was reduced and lower PK levels were not observed in patients with PE complicated by liver affection (ALAT $>$ 70 U/l).

The reduced capacity of CAS reported in the present study may reduce the fibrinolytic potential in women with PE compared to controls and by this action contribute to the increased thrombotic risk observed in women suffering from PE. The potential association between CAS and the fibrinolytic system will be addressed in future studies.

Conclusion

The current study addresses changes in CAS proteins in women with PE compared to matched pregnant women without PE. The study demonstrates that the peak PKa concentration, the PKa generating capacity, and the concentration of cHK are significantly reduced in women with PE. The results suggest that CAS is affected in PE suggesting a contribution to the pathophysiology of the disease.

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 Regional Ethics Committee in the Region of Southern Denmark (project ID S-20190142, 10 December 2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JBG, JJS, ACG, JSJ, and YP designed and directed the research. ACG collected and managed data in collaboration with BBD. ACG conducted the statistical analyses. ACG and JJS wrote the initial draft. JJS, JBG, STDP, and YP developed methods for the study. All authors contributed to the article and approved the submitted version.

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REVIEW
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Maternal Low Volume Circulation Relates to Normotensive and Preeclamptic Fetal Growth Restriction

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This narrative review summarizes current evidence on the association between maternal low volume circulation and poor fetal growth. Though much work has been devoted to the study of cardiac output and peripheral vascular resistance, a low intravascular volume may explain why high vascular resistance causes hypertension in women with preeclampsia (PE) that is associated with fetal growth restriction (FGR) and, at the same time, presents with normotension in FGR itself. Normotensive women with small for gestational age babies show normal gestational blood volume expansion superimposed upon a constitutionally low intravascular volume. Early onset preeclampsia (EPE; occurring before 32 weeks) is commonly associated with FGR, and poor plasma volume expandability may already be present before conception, thus preceding gestational volume expansion. Experimentally induced low plasma volume in rodents predisposes to poor fetal growth and interventions that enhance plasma volume expansion in FGR have shown beneficial effects on intrauterine fetal condition, prolongation of gestation and birth weight. This review makes the case for elevating the maternal intravascular volume with physical exercise with or without Nitric Oxide Donors in FGR and EPE, and evaluating its role as a potential target for prevention and/or management of these conditions.

Keywords: maternal hemodynamic changes in pregnancy, fetal growth, intravascular volume, cardiac output, venous hemodynamics, vascular resistance, body water volume

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INTRODUCTION

For the past 50 years, defective placentation and inadequate adaptation of uterine spiral arteries have been considered the key role players in the so-called obstetrical syndromes: preeclampsia (PE), fetal growth restriction (FGR), preterm labor, preterm premature rupture of the membranes, late spontaneous abortion, and abruptio placentae (1). Placental malperfusion and subsequent oxidative stress are associated with the impairment of both maternal and fetal systemic physiologic functions and, eventually, maternal disease, and/or fetal distress (2). In recent years, however, the focus of

research into the origins of gestational hypertensive disorders with or without FGR has shifted from the placenta to maternal cardiovascular dysfunction, present before conception or developing during the earliest stages of placentation (3, 4). The interplay between maternal hemodynamics and the placentation process explains the two hemodynamic phenotypes of PE – one with high cardiac output (CO) and low vascular resistance and another with low CO and high vascular resistance – (5, 6), as well as FGR (7), and also links maternal low CO to fetal increased umbilical and reduced cerebral Doppler impedance (8). Next to this, suboptimal plasma volume expansion is considered an intrinsic pathophysiologic feature of PE with FGR (9), and maternal normotensive low volume circulation has been associated with neonatal birthweight of <10th percentile (10). The feasibility and clinical relevance of non-invasive assessment of maternal body water volumes, in association with cardiovascular assessments in both latent and symptomatic stages of PE and/or FGR, have been reported (11, 12), offering an opportunity for volume expansion strategies as potential management options in the prevention and treatment of poor fetal growth.

This narrative literature review aimed to summarize reported evidence on the association between poor fetal growth and low maternal circulating volume as a constitutional predisposing condition in normotensive FGR or as a consequence of suboptimal volume expansion in PE with FGR. The methodology was performed according to Sandra's principles (13) using the following keywords (alone or in combination): FGR, intrauterine growth restriction, poor fetal growth, early onset preeclampsia (EPE), gestational hypertensive disorders, maternal hemodynamics, maternal cardiovascular function, CO, total vascular resistance (TVR), plasma volume, body water volume, maternal venous Doppler, fetal Doppler, venous hemodynamics, and volume regulation.

MATERNAL HEMODYNAMICS AND FETAL GROWTH

Birth weight relates to many variables, such as parental anthropometrics (height, weight, BMI), race, gender, gestational age, diet, drinking habits and substance (ab)use, and medical and obstetric history (14). Poor fetal growth and, in particular, FGR may relate to a chronic state of intrauterine hypoxia resulting from preplacental, placental (e.g., abruption), and postplacental causes (e.g., cord insertion, fetal genetics; 15). Known causes of preplacental hypoxia are not only pathologic conditions, such as chronic maternal cardiovascular, pulmonary or systemic disease, anemia, and infections, but also physiological determinants, such as high altitude and maternal CO.

Abbreviations: CO, cardiac output; DV, ductus venosus; EPE, early onset preeclampsia; FGR, fetal growth restriction; HR, heart rate; HV, hepatic veins; IL6, interleukin 6; MAP, mean arterial pressure; PAPP-A, pregnancy associated placental protein A; PE, preeclampsia; PlGF, placental growth factor; RV, right ventricle; S-FLT1, soluble FMS-like tyrosine kinase 1; SGA, small for gestational age; SV, stroke volume; SV (**Figure 3**), splenic vein; TGFβ, transforming growth factor β; TVR, total vascular resistance; UV, umbilical vein; VEGF, vascular endothelial growth factor.

Cardiac output is linked mathematically to mean arterial blood pressure (MAP) and TVR according to the hemodynamics' variant of Ohm's law

$$CO = MAP/TVR,$$

where CO is the product of stroke volume (SV) and heart rate (HR; 16). In the preconception period, low maternal CO, mostly in combination with increased TVR, predisposes pregnant women to gestational complications, such as PE with or without FGR (3), and the lowest CO values are observed in normotensive FGR (17). In an uncomplicated pregnancy, there is a positive correlation between CO change from preconception to mid-gestation and neonatal birth weight (18). During pregnancy from the first trimester onward, maternal CO is directly related to singleton birth weight (19–21), particularly at advanced maternal age (22), with parity (23), and multiple pregnancies (24), whereas there is an inverse relation with altitude (25) and gestational hypertensive disorders (26, 27). **Figure 1** shows the gestational trends of CO and TVR as measured by the bioimpedance technology: contrary to pregnancies eventually developing PE, the evolution is similar in FGR and uncomplicated pregnancy, however, at lower CO and higher TVR (10, 11, 28). This observation has also been reported by others (29) and linked to a condition of low CO that is already present before conception (17). Low CO in FGR pregnancies mainly results from low SV (30, 31) and to a lesser degree from low HR (32, 33). Throughout an uncomplicated pregnancy, the fraction of CO deviated to the uterus doubles from 6 to 12% (34) and is achieved by an increase of (distal) internal iliac artery impedance in concert with a reduction of uterine artery impedance (35). Uterine artery blood volume flow positively correlates with birth weight (36, 37), reduces from maternal upright to the supine position with poor response to supine exercise (38), and is lower in FGR than in normal fetal growth (39). These observations all indicate that maternal cardiovascular function and uterine arterial blood supply strongly contribute to fetal growth and neonatal birth weight.

Recently, it was shown that the venous compartment and body water volume load are also involved in the regulation of fetal growth (40). Next to its metabolic functions, the liver serves as a hemodynamic organ, where a large fraction of the unstressed blood volume is stored, as a reserve volume available for an instant increase of CO by sympathetic nervous-stimulated drainage of hepatic veins in the inferior vena cava. Inverse correlations have been reported between maternal CO and birth weight, on the one hand, and hepatic venous Doppler impedance index, on the other hand (40). Next to this, in normotensive women giving birth to neonates small for gestational age (SGA), low CO was associated with low body water volume and high Doppler impedance of uterine artery and hepatic veins, all of which are indicators of a low volume circulation (10; **Figure 1**). These observations are in line with reported impaired expansion of maternal plasma volume in FGR pregnancies in normal (41), small, and lean (42) women. Here, it is important to mention that the

reduced plasma volume, as compared to normal pregnancy, precedes a suboptimal rise of the volume regulating hormones aldosterone, progesterone, and estrogens (43). The presence of a low intravascular volume before or during pregnancy can explain why low CO and high TVR can present with normotension and why a normal gestational plasma volume expansion fails to achieve normal values of maternal CO. Contrary to normotensive FGR, EPE – which mostly is associated with poor fetal growth – low CO and high TVR present with high body water volumes already from the first trimester onwards, suggesting a different pathophysiologic background mechanism (11; **Figure 1**).

PATHWAYS OF MATERNAL LOW VOLUME CIRCULATION IN EARLY ONSET PREECLAMPSIA AND FETAL GROWTH RESTRICTION

In an uncomplicated pregnancy, maternal plasma volume expansion is triggered by a primary fall in systemic vascular resistance (44). The subsequent state of intravascular underfilling causes a reduction of cardiac afterload, which, in turn, is responsible for a rise in SV (45). Together with a rising HR, increased SV induces a 25% increase in CO at 6 weeks of gestation (17). Due to anatomic and physiologic properties of the high volume/low resistance of pulmonary circulation, increased CO results in a reduction of pulmonary vascular resistance *via* capillary recruitment and distention (46). In pregnant women, increased CO and the associated enlarged pulmonary capillary bed are responsible for a rise of intrathoracic water as measured by bioimpedance technology as early as 6–7 weeks of gestation (47, 48).

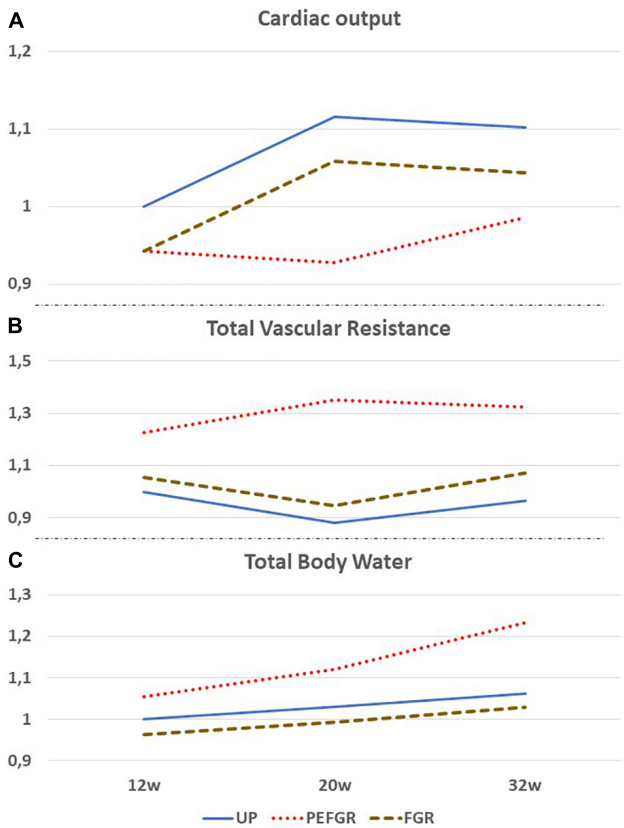

FIGURE 1 | Comparative gestational evolution of maternal cardiac output **(A)**, total vascular resistance **(B)**, and total body water volume **(C)**, as measured by the bioimpedance technology between uncomplicated pregnancies (UP), normotensive fetal growth restriction (FGR), and preeclampsia with FGR (PEFGR). Values are expressed as multiples of mean first trimester values in UP. Figures adapted from 10, 11, and 28.

Meanwhile, SV and left atrial dimensions continue to rise in normal pregnancies; this is not true for pregnancies destined to develop severely impaired fetal growth (45) and/or EPE (33). As shown in **Figure 1**, CO is persistently lower than normal in normotensive FGR despite a normal gestational rise. In EPE, however, CO fails to rise after 8 weeks of gestation (17), and this is associated with the echocardiographic reduced left atrial area and fractional area change (33), as well as increased left ventricular end-systolic and end-diastolic volumes (49). This pathophysiologic condition is responsible for the increase of left atrial filling pressure, with the subsequent retrograde rise of pulmonary venous pressure and capillary hydrostatic pressure, resulting in exudation of intravascular fluids similar to the mechanisms observed in heart failure (50). A rise of pulmonary interstitial fluids before the clinical presentation of symptomatic pulmonary edema is a known phenomenon in chronic heart failure, and the detection of this condition by bioimpedance technology is useful to predict and timely counteract the severity of pulmonary edema (50, 51). As shown in **Figure 1**, the same mechanism is likely to occur in early preeclampsia (EPE): an asymptomatic exudation of intravascular fluids in the pulmonary interstitium can precede the development of edema elsewhere in the female body and explains the combination of increased total body water volume without the concomitant rise of CO in EPE. The constant exchange between intravascular and interstitial volumes is a normal physiologic function of the microcirculation and indicates that abnormal changes in plasma volume cannot be interpreted correctly without considering changes in other body volume compartments. This phenomenon is illustrated visually in **Figure 2**. The nature of this pathophysiologic pathway is in line with the reported increased serum concentrations of atrial natriuretic peptide (52) and copeptin/vasopressin in PE (53) and with the impaired expansion of maternal plasma volume in EPE (9). The early gestational onset of this phenomenon is also supported by the shallow, but significant, rise of serum hemoglobin concentrations and hematocrit in the first trimester of pregnancies destined to develop EPE (31, 54, 55) but not in those eventually leading to FGR (56). As explained above and illustrated in **Figure 1**, FGR pregnancies show a normal rise of plasma volume and total body water, superimposed upon constitutionally low body water already present before conception.

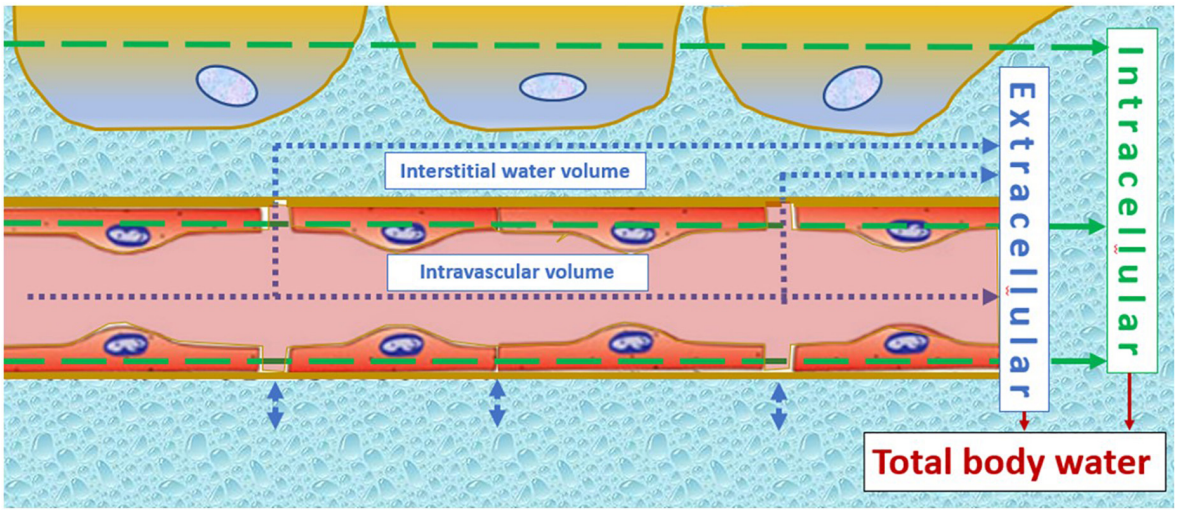

FIGURE 2 | Illustration of the constant fluid exchange between intravascular and interstitial volumes at the level of the microcirculation. This interconnectivity indicates that plasma volume changes cannot be interpreted without knowledge of interstitial water volume, or indirectly *via* measurement of total body water volume. Normotensive fetal growth restriction relates to a constitutionally low intravascular volume, remaining lower in uncomplicated pregnancies despite normal gestational volume expansion. Early onset preeclampsia, on the other hand, is a state of increased adrenergic activation (155), shifting blood from the venous capacitance bed into the circulation and despite this, cardiac output fails to rise. This can only be explained by a shift from the intravascular compartment to the interstitium, which is in line with clinical signs, such as malleolar or pulmonary edema, in early onset preeclampsia. The arrows indicate the principle of volume estimation by bioimpedance spectrum analysis of intracellular (green) and extracellular (blue) compartments, the latter being the sum of intravascular and interstitial volumes.

LABORATORY AND CLINICAL EXPERIMENTS SUPPORTING MATERNAL LOW VOLUME CIRCULATION IN FETAL GROWTH RESTRICTION

A low sodium diet for pregnant rats prevents normal maternal plasma volume expansion and induces FGR and low placental weight (57). This association presents with poor dilatation of the uterine and radial arteries, together with reduced uterine blood volume flow and increased vascular resistance, and the activation of the renin-angiotensin-aldosterone system with reduced expression of placental Angiotensin II receptor subtype 1 (58) and with increased concentrations of placental markers of hypoxia (57, 58). Murine FGR presents with a reduced placental blood flow rate, demonstrable by both contrast-enhanced sonography and magnetic resonance imaging (MRI; 59).

Different types of MRI technology have been proven useful for non-invasive studies of placental perfusion in animals and human (60), allowing for the quantification of blood flow volume (61), and mapping and fractional differentiation of perfused and non-perfused areas (62, 63). As compared to normal pregnancies, FGR presents with a one-third decrease of placental perfusion fraction (64), strongly correlating with increased Doppler impedance measurements of uterine and umbilical arteries, particularly of the ductus venosus (DV; 64, 65). Blood flow velocity in the FGR placenta is reduced, is non-homogenous, and shows intermittent stops in severe cases with increased DV pulsatility index (65). Taken together, all MRI placenta perfusion studies show evidence for underfilling of the intervillous space, which is a trigger for both maternal and fetal reflex responses. Laboratory and animal models show that incomplete spiral artery trophoblast invasion results in an increase of oxygen tension (66) and perfusion pressure (67) in the intervillous space, with constriction of maternal chorionic plate venules (68), enhanced myogenic activity of uterine and radial arteries (69) despite an activated pathway of nitric oxide (NO)-dependent vasodilation (70), and altered placental cell populations and trophoblast differentiation (71).

Poor placenta perfusion in both PE and FGR is supported by histologic signs, such as accelerated villus branching, large and numerous syncytial knots, and small sclerotic villi, suggestive of placental hypoxia and/or oxidative stress (72).

EPIDEMIOLOGIC DATA SUPPORTING MATERNAL LOW VOLUME CIRCULATION IN FETAL GROWTH RESTRICTION

Epidemiologic studies have shown an intergenerational association of FGR: women who, themselves, were born with low birth weight are more likely to reproduce low birth weight offspring (73–75). Apart from genetic, familial, and socioeconomic predispositions, complex molecular processes, such as genetic imprinting, microchimerism, and epigenetic modifications, are involved (76, 77), and this results from early neonatal life onward in the disruption of endocrine and metabolic systems (78, 79) together with permanent dysfunctions of vital organ systems (80, 81), such as the kidneys (82, 83), the

heart and blood circulation (76, 84, 85), the endothelium (86), and the immune system (80). In the long run, these systemic dysfunctions predispose to early onset adult disease (87). Body water volume homeostasis is another system that is dysfunctional in FGR, involving the renin-angiotensin system (88) and natriuretic peptides (89, 90). A particularly interesting observation is that FGR predisposes to low plasma volume in adult life (91). In former preeclamptic women, it was shown that preconceptional low plasma volume predisposes to recurrence of PE (92). Even more important is that, in nulliparous women, preconceptional vascular dysfunction (93) and angiotensinogen phenotype-dependent low plasma volume (94) predispose to abnormal perinatal outcomes (95). Low plasma volume coexists with poor venous reserves, resulting from abnormal venous capacitance and vascular compliance together with autonomic nervous dysfunction (96), and is associated with recurrent first-trimester pregnancy loss (97). As explained in the section, combined venous hemodynamic dysfunction in both mother and fetus in PE with FGR has also been observed in studies using Doppler-ECG ultrasonography.

INTERACTIVE MATERNAL-FETAL HEMODYNAMICS IN FETAL GROWTH RESTRICTION

Mother-to-Fetus Circulatory Interactions in Fetal Growth Restriction

Placental angiogenesis and vasculogenesis involve cell-communicating factors including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and oxygen (98). Placental histology studies have shown that FGR is characterized by decreased branching angiogenesis with increased apoptosis, resulting in a reduced number of terminal villi and stromal capillaries, poor villi vascular density, fewer intervillous pores, increase of intervillous thrombi, villous infarctions, villitis, and thickening of the basal membrane (99, 100), finally resulting in overall reduced exchange surface area (101–103). This is associated with a lower and higher release of VEGF-A and PlGF, respectively, an unbalanced production of their cell receptors, and the release of anti-angiogenic factors, mechanisms supported by reduced oxygen tension and volume flow (39, 98). As a result, endothelial cells from the FGR placenta show dysregulated biochemical signaling with failed compensatory response to resist high blood flow (104), demonstrable by high Doppler impedance measurements at the level of uterine and umbilical arteries (15). Doppler sonography is a useful method to assess uterine and umbilical artery flow impedance. Increased uterine artery Doppler pulsatility index and resistivity index have long been considered a result of abnormal spiral artery adaptation at implantation, but, currently, increasing number of experimental, clinical, and epidemiological data are in favor of the opposite pathway, suggesting abnormal placentation merely as the result rather than the cause of abnormal uterine perfusion (105, 106). In this context, the inverse correlation between preconceptional uterine artery Doppler impedance measurements and subsequent birthweight in formerly preeclamptic women is illustrative (107).

With advancing gestation of FGR, the intrauterine environment becomes more and more hypoxic, to which the fetus responds by redirecting the blood supply, preferably to vital organs like the heart and the brain, at the expense of subdiaphragmatic organ perfusion (15). Fetal brain sparing can be documented by Doppler flow measurements at the level of umbilical and cerebral arteries with the calculation of relative impedance ratios (108–110). Associations have been reported between abnormal uterine-fetal Doppler measurements and maternal hemodynamic dysfunction (8) and between fetal cerebral Doppler changes and adverse outcomes (111).

On top of fetal arterial Doppler flow changes, DV Doppler flow patterns, shifting from biphasic to triphasic, offer additional information on deteriorating fetal condition (112–114; **Figure 3**). This evolution is very similar to the change of Doppler flow patterns in the maternal hepatic vein from early pregnancy to the clinical stage of EPE (115; **Figure 3**). The sequence of venous Doppler waveform changes, however, is different between the FGR fetus and the woman with EPE. In FGR, DV Doppler flow is secondary to altered cardiac function due to increased afterload for the right ventricle (RV) but not the left one, with subsequently reduced RV compliance and increased right atrium filling pressure, which reflects in the reversed DV Doppler A-wave (116, 117) and reduced CO to the placenta (118). In EPE, triphasic HV Doppler flow patterns are already present weeks before the clinical onset of disease (119), indicating the involvement of venous vascular wall activity (120, 121). Further research should elucidate whether this difference relates to a different intravascular filling state, which is low for the woman with PEFGR (**Figure 1**) and is linearly related to birth weight, irrespective of preceding intrauterine fetal condition (122, 123).

Conceptus-to-Mother Circulatory Interactions in Fetal Growth Restriction

An important fetal-maternal communication system is the intravascular shedding of placental particles, varying in size and shape between multinucleated syncytial aggregates and subcellular nanovesicles, originating from not only apoptosis in a normal pregnancy but also necrosis in PE (124), and is associated with increased serum total cell-free DNA (125). These particles act *via* intravesical molecules and micro-RNA or circular RNA that, after phagocytosis by endothelium and immune cells, is capable of inducing sterile inflammation *via* increased surface expression of monocyte adhesion receptors, such as E-selectin, secretion of pro-inflammatory cytokines, namely interleukin 6 and transforming growth factor β (124). Downregulation of specific micro-RNAs has been reported in FGR, some of which are shared with PE (126). Next to this, the production of mediators of angiogenesis and vasoactivity is stimulated in FGR, such as soluble FMS-like tyrosine kinase and VEGF (127), whereas pregnancy-associated placental protein A (PAPP-A) is reduced (128). Many other vasoactive and immunologic mediators have also been studied in maternal serum concerning diagnosis or prediction of PE and/or FGR.

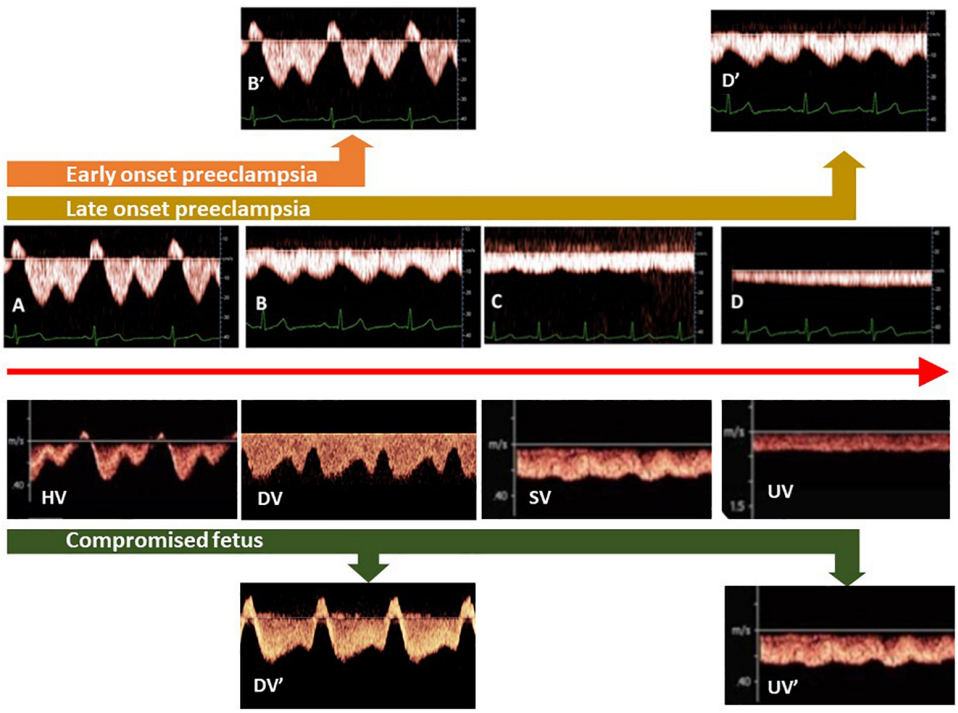

FIGURE 3 | Different types of venous Doppler waveforms in maternal and fetal circulations, varying between triphasic **(A)**, biphasic **(B)**, and monophasic **(C)** to flat **(D)**. In the fetus and non-pregnant adults, triphasic patterns are found close to the heart [hepatic veins (HV)], whereas flat patterns are present at distant locations [limbs, umbilical veins (UV)]. During uncomplicated pregnancy, HV patterns shift from **(A–D)**. In the clinical stage of preeclampsia with FGR, biphasic HV patterns become triphasic (orange arrow), whereas in term preeclampsia, monophasic patterns evolve to biphasic (brown arrow). In ductus venosus (DV) of FGR fetuses, a shift from biphasic to triphasic patterns occurs simultaneously with deteriorating fetal condition (green arrow). (SV: fetal splenic vein).

The most-reported biomarkers studied for this purpose are summarized in **Table 1**. It has generally been accepted that the placenta is the primary source of these factors and, as such, is the primary driver of the global functioning of the maternal circulation. It should be emphasized, however, that abnormal serum concentrations of many of these factors have also been documented in non-pregnant individuals with (pre-clinical) chronic cardiovascular and/or renal disease (**Table 1**). This indicates that, apart from some placenta-specific products, it cannot be concluded indisputably whether the origin of the vasoactive and/or immunomodulatory serum substances associated with PE ± FGR is placental, maternal, or combined.

CLINICAL IMPLICATIONS OF MATERNAL LOW VOLUME CIRCULATION IN FETAL GROWTH RESTRICTION

The association between low maternal volume circulation and FGR has important clinical implications. First, maternal hemodynamics can offer additional information in unexplained cases of FGR, where all other known etiologic factors have been excluded (129). In recent times, non-invasive assessment of CO and peripheral resistance is feasible by different types of technologies (130), which, when used with appropriate reference ranges under standardized conditions (8, 131), can easily identify those women with a low output/high resistance circulation who are particularly at risk for FGR. This information is not only useful for the diagnosis of FGR but can also be of value from the first trimester onward before poor fetal growth is evident (29, 45, 132). This opens perspectives toward the implementation of maternal hemodynamics parameters into current screening programs for FGR (7, 133). Preliminary, though promising, data on the reduction of FGR have been reported on the supplementation of the screen positive high-risk group with antiplatelet therapy (134, 135) and/or the antioxidants lycopene or L-Arginin (136).

Secondly, maternal low volume circulation can be a target for the prevention of FGR pregnancies. Physical exercise is a well-known useful intervention for the improvement of cardiovascular functions (137). In formerly preeclamptic women included in a program of controlled physical exercise, an increase in stroke and plasma volume was observed (138) together with the improvement of venous reserves up to the pretraining levels of controls (139). In overweight and obese pregnant women, physical exercise throughout gestation was associated with a lower incidence of gestational diabetes and reduced third-trimester systolic blood pressure (140). Lower systolic blood pressure was also observed in trained versus non-trained normotensive pregnant women (141). Importantly, maternal physical training was shown to influence fetal cardiovascular functions by the increase of left ventricular output and aortic peak

TABLE 1 | Serum markers of fetal growth restriction (FGR), preeclampsia (PET), and/or cardiovascular disease (CVD).

	Physiologic function	Pregnancy		Non-pregnant		References		
		FGR	PE		Type CVD	FGR	PE	CVD
CRP	Immunomodulation	↑	↑	↑	CHD, HF	(156)	(157)	(158)
VEGF	Pro-angiogenic Pro-vasculogenic	↓	↓	Polymorfisms	CHD	(98)	(159)	(160)
sFLT-1	Anti-angiogenic	↑	↑	↑	CHD, HF	(127)	(127)	(161)
sEng	Anti-angiogenic	↑	↑	↑	CHD	(162)	(159)	(163)
Activin A	Immunomodulation Apoptosis	↑	↑	↑	CHD, HT	(164)	(165)	(166)
Leptin	Immunomodulation Angiogenic	↑	↑	↑	CHD	(167)	(168)	(169)
sE-selectin	Immunomodulation	↑	↑	↑	HT	(170)	(171)	(172)
ADAM 12	Angiogenic Immunomodulation	↓	↓	↓	HF	(173)	(108)	(174)
ADMA	Vasodilatation	↑	↑	↑	CHD, HF, HT	(175)	(176)	(177)
PLGF	Pro-angiogenic	↓	↓	↑	CHD, HF	(127)	(159)	(161)
PAPP-A	Lysis IGF-BP	↓	↓↑↑	↑	CHD	(128)	(178)	(179)
ADM	Pro-angiogenic	↓	↓	↑	AMI	(179)	(180)	(181)

Abbreviations: FGR, fetal growth restriction; PE, preeclampsia; CVD, cardiovascular disease; ↑, high serum concentration; ↓, low serum concentration; ↓ ↑ ↑, cerum concentration changing from low to high; CHD, coronary heart disease; HT, hypertension; HF, heart failure; AMI, acute myocardial infarction; CRP, C-reactive protein; VEGF, vascular endothelial growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sEng, soluble endoglin; sE-selectin, soluble E-selectin; ADAM 12, A disintegrin and metalloproteinase 12; ADMA, asymmetric dimethylarginine; PLGF, placental Growth Factor; PAPP-A, pregnancy associated placental protein A; and ADM, adrenomedullin.

flow velocity (142) and by lower carotid artery wall thickness in offspring (143).

A third important implication for pregnancies complicated with both FGR and hypertension is the antihypertensive therapy of choice in addition to low dose aspirin (135) initiated before 16 weeks at ≥100 mg PD (134). Blood pressure-lowering pharmacologic mechanisms are different between beta-blockers, calcium blockers, and centrally active agents due to which the effects on neonatal birth weight are different. There is a growing body of evidence that adrenergic beta-blockers are associated with an increased birth rate of neonates SGA (144–148) with a mean effect estimated at ≤200 *g* at term (149). A similar but less pronounced effect has also been reported for Alfa-MethylDopa (145, 150) but not for calcium channel blockers (145). A possible explanation for this differential effect is that beta-blockers partially exert their effects *via* a reduction of CO (151), whereas calcium channel blockers mainly function *via* reduced peripheral resistance with a compensatory rise of CO (152). As such, from a theoretical perspective, calcium channel blockers might be a better choice than beta-blockers with respect to avoiding a negative pharmacologic impact on fetal growth. There, however, is an urgent need for more fundamental and clinical research into the differential mechanisms and outcomes of antihypertensive therapies in pregnant women.

An interesting clinical confirmation of the association between maternal intravascular volume and the gestational outcome has been reported in two studies by the Tor Vergata university of Rome's research team (153, 154) using the potently vasodilating NO donors. As compared to a historical control group, a cohort of 26 FGR pregnancies treated with NO donors and plasma volume expansion showed an improvement in maternal CO and TVR and in higher birth weight after 2 weeks (153). Similarly, in 32 women with hypertension and FGR with absent end-diastolic umbilical Doppler flow, randomized between conventional management with or without NO donors and plasma volume expansion, the reappearance of diastolic umbilical blood flow and the prolongation of gestation were observed in the treated group (154).

PERSPECTIVES

This review summarizes evidence from clinical, experimental, and laboratory observations on the association between low volume maternal circulation and poor fetal growth. Conditions of low maternal CO can present before conception or develop during the earliest stages of pregnancy, in conditions of both normotension or hypertension relative to the balance between flow volume and vascular resistance. Intravascular volume is intimately related to CO, renal function, and through aldosterone and the renin-angiotensin system to peripheral vascular resistance and blood pressure. Hence, acknowledging that the association between maternal intravascular filling state and fetal wellbeing opens perspectives toward prevention, management, and reduction of intergenerational transfer of poor fetal growth. However, more in-depth exploration is needed on the role of normal or abnormal maternal cardiovascular function in obstetric and neonatal outcomes.

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Predicting Preeclampsia Pregnancy Termination Time Using sFlt-1

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Background: The aim of this study was to determine the usefulness of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) in predicting the time for pregnancy termination in pregnant women with known preeclampsia (PE) onset.

Methods: Forty-four pregnant women diagnosed with PE (22 weeks 0 days to 33 weeks 6 days gestation) were included in this study. The levels of sFlt-1 and PlGF, and the sFlt-1/PlGF ratio were compared between the women that delivered in <24 h (T group) and those that delivered in more than 24 h (P group), and between women that delivered in <1 week (T group) and those that delivered in more than 1 week (P group). Cutoff values were calculated for the three markers that were the most significantly correlated with predicting pregnancy termination at <24 h and <1 week.

Results: Among sFlt-1, PlGF, and sFlt-1/PlGF, sFlt-1 was the most significantly associated with the timing of pregnancy termination. sFlt-1 cutoff values of 8682.1 pg/ml (AUC 0.71; 95%CI, 0.5191–0.9052) and 7,394.5 pg/ml (AUC 0.78; 0.78, 95%CI, 0.6394–0.9206) for delivery in <24 h and delivery within 1 week, respectively, were important predictive values. The positive predictive value for delivery within 24 h was 43.9%, with a sensitivity of 72.3% and specificity of 69.0%, when sFlt−1 was <8,682 pg/ml. A sFlt-1 level of 7,394 pg/ml or greater would result in delivery within 1 week, with a positive predictive value of 67.2%; the sensitivity was 79.0% and specificity was 72.0%.

Conclusion: This study showed that sFlt-1 may be effective in predicting the timing of pregnancy termination. However, the number of cases was small and, thus, the results were not definitive. This finding should be researched further in order to predict the optimal timing of pregnancy termination in PE to reduce severe maternal complications.

Keywords: preeclampsia, sFlt-1, PlGF, prediction, Angiogenetic factors

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INTRODUCTION

Preeclampsia (PE) is a disease with serious consequences for both the mother and fetus. Currently, deaths due to complications from PE are problematic in Japan (1–4). In particular, cerebral hemorrhage is more prevalent than cerebral arteriovenous malformation or Moyamoya disease in patients with PE (1–4). We have previously analyzed cases of PE-associated cerebral hemorrhage in Japan; the prognosis of PE-associated cerebral hemorrhage is determined by the degree of cerebral hemorrhage. Therefore, a backward analysis of those cases concluded that it was difficult to save a patient's life when cerebral hemorrhage was severe (5). Intervention before the onset of cerebral hemorrhage is a means of reducing maternal death from cerebral hemorrhage complicated by PE. Furthermore, Japanese people including to pregnant women are reported to be more prone to cerebral hemorrhage than cerebral infarction (4).

Once PE develops, it progressively worsens; there is currently no treatment other than terminating the pregnancy once PE becomes severe (6–8). Pregnancy cannot be easily terminated at <37 weeks due to fetal prematurity. Once PE develops, various clinical parameters (blood pressure, proteinuria, et al.) that indicate PE deterioration should be carefully monitored. The pregnancy is terminated when PE is determined to have become severe. However, this intervention method does not eliminate the mother's risk of developing cerebral hemorrhage. Furthermore, because the incidence of cerebral hemorrhage itself is very low, it is difficult to design studies to predict cerebral hemorrhage.

It is widely reported that placental growth factor (PlGF), an angiogenic factor involved in placentation, and its inhibitor, soluble fms-like tyrosine kinase-1 (sFlt-1), are involved in the pathogenesis of PE. The sFlt-1/PlGF ratio has attracted attention as an indicator to predict the onset of PE (9, 10). We hypothesized that PlGF and sFlt-1 could be used to predict the timing of PE pregnancy termination in patients who develop PE. Predicting the timing of PE pregnancy termination would allow for earlier intervention in PE cases. Therefore, the purpose of this study was to investigate the use of PlGF and sFlt-1 to predict the timing of pregnancy termination in women with known PE onset.

TABLE 1 | Maternal and neonatal background.

	***n* = 44**
Age (years)	34.3 ± 5.6
Hight (cm)	158.6 ± 5.4
Weight (g)	58.8 ± 12.9
Primipara	22 (50.0%)
Gestational age at onset preeclampsia (weeks)	30.5 ± 3.7
Gestational age at delivery (weeks)	32.9 ± 4.3
Delivery mode	
Caesarian delivery	36 (81.8%)
Vaginal delivery	8 (18.2%)
Obstetrics complication	
Fetal growth restriction	10 (22.7%)
Gestational diabetes mellitus	4 (9.0%)
Placental abruption	1 (2.3%)
Birth weight (g)	1759.6 ± 757.1
Z-score of birth weight	−1.1 ± 1.2
UA pH < 7.10	2 (4.5%)
Apgar score (5 mins) <7	5 (11.3%)

METHODS

Study Population

This study included pregnant women diagnosed with PE (22 weeks 0 days to 33 weeks 6 days gestation) at Mie University Hospital from 1 January 2016, to 30 October 2021, whose pregnancies were terminated for maternal indications (severe PE). Cases in which PlGF and sFlt-1 were measured at the time of diagnosis were enrolled.

Study Design

This was a single-center observational study. The study design was approved by the Ethics Committee of Mie University Hospital (approval No. H2022-013).

The levels of sFlt-1 and PlGF, and the sFlt-1/PlGF ratio were examined to determine which marker most significantly correlated with predicting the severity of PE after PE onset. If preterm delivery is anticipated in clinical practice, steroids should be administered to protect the fetus. Therefore, it is important to predict delivery within 24 h and 1 week in terms of onset and duration of steroid effects. The first group was defined as having delivered at less than 24 h (T group) and the second group as having delivered at more than 24 h (P group). Second, the time from measurement to pregnancy termination near to delivery was divided into two groups: <1 week (T group) and more than 1 week (P group). For the markers that were best correlated, cutoff values were calculated to predict pregnancy termination at <24 h and <1 week.

Diagnostic Criteria for PE

Diagnostic criteria for PE-related disorders were based on the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (11), which are as follows: after 20 weeks of gestation, hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or both) and proteinuria (>2+ protein on dipstick urine test, >300 mg protein per 24-h urine collection, >30 mg/dl protein on a spot urine sample, or a protein to creatinine ratio of >30 mg/mM), both of which are defined as new onset (11).

Criteria for Pregnancy Termination

After the diagnosis of PE, the decision to terminate the pregnancy was made if PE became severe. Severe disease was defined as meeting one of the following criteria: persistent hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >110 mmHg); Hemolysis, Elevated liver enzymes, and Low Platelets (HELLP) syndrome; renal dysfunction (serum creatinine >1.0 mg/dl or oliguria); thrombocytopenia (<100,000/μl); eclampsia; pulmonary edema; or stroke.

Pregnancies were also terminated in case of fetal growth retardation, even if the patient was judged to have a carefully controlled non-reassuring fetal status.

Evaluation of Serum Markers

Serum samples (≥2 ml) collected according to standard operating procedures were retrospectively analyzed in the Obstetrics and Gynecology Laboratory of Mie University. Concentrations of sFlt-1 and PlGF in maternal serum (both measured in pg/ml) were determined using an electrochemiluminescence immunoassay platform (Cobas E Analyzer, Roche Diagnostics) with a fully automated Elecsys assay for sFlt-1 and PlGF. The data obtained from the assay were then used to calculate the sFlt-1/PlGF ratio. The within-run coefficient of variation for control samples was <4% for both assays. Between-run coefficients of variation ranged from 2.3 to 5.6% for the Elecsys sFlt-1 assay and 2.4 to 4.6% for the Elecsys PlGF assay.

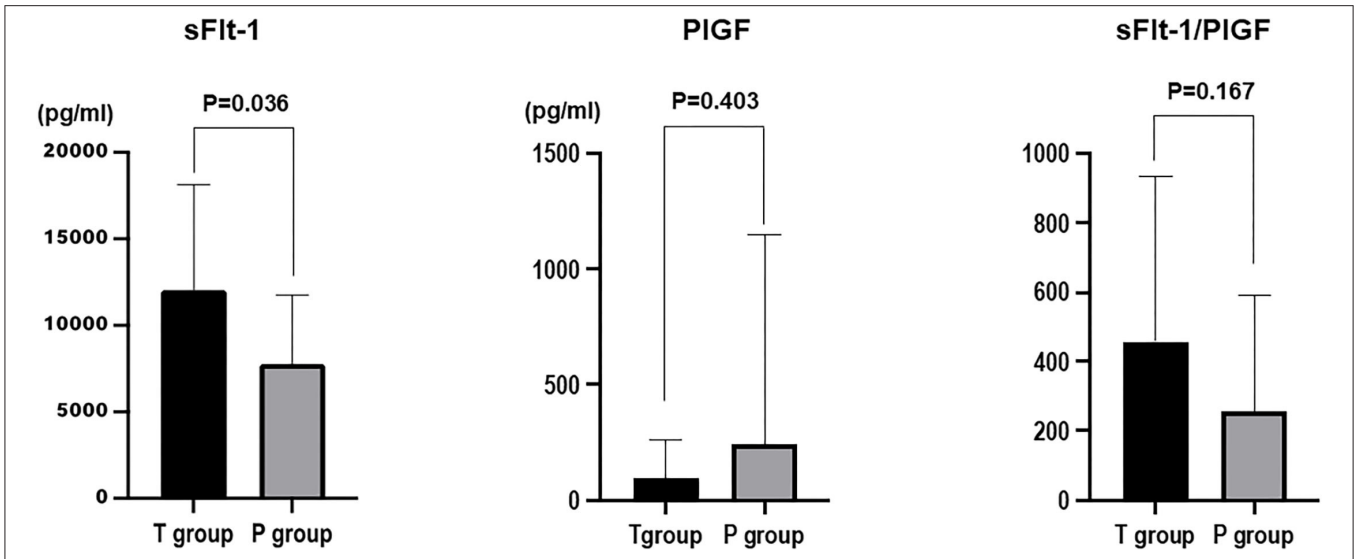

FIGURE 1 | Comparison of sFlt-1, PlGF, and sFlt-1/PlGF ratios between the group that delivered in <24 h (T group) and the group that delivered in more than 24 h (P group).

Statistical Analysis

Descriptive statistics (mean, standard deviation) were calculated for each group, and inter-group comparisons were made using the two-sided Student's *t*-test at a significance level of 5%. The cutoff value for predicting that a pregnancy will terminate within 24 h and <1 week was the value with the highest Youden's index.

RESULTS

Maternal Background

Forty-four patients were enrolled in the study. The patient background data is shown in **Table 1**. The mean age was 34.3 ± 5.6 years. The mean number of weeks for PE onset was 30.5 ± 3.7 weeks, and the mean number of weeks for pregnancy termination was 32.9 ± 4.3 weeks. The method of delivery was cesarean section in 81.1% of cases. The complication rate of fetal growth retardation was 22.7%, and the mean birth weight was 1759.6 ± 757.1 g.

Delivery in Less Than 24 h

The mean value of sFlt-1 was 12,034.3 ± 1,379.6 pg/ml in the 24 h T group and 7,779.2 ± 796.5 pg/ml in the 24 h P group ($P = 0.01$). The mean sFlt-1/PlGF ratio was 458.8 ± 112.8 in the 24 h T group and 265.12 ± 66.1 in the 24 h P group ($P = 0.12$). sFlt-1 was the most significantly associated factor with delivery in <24 h (**Figure 1**).

Delivery in Less Than 1 Week

The mean value of sFlt-1 was 1,1607.5 ± 984.2 pg/ml in the 1-week T group and 6,741.0 ± 858.0 pg/ml in the 1-week P group ($P = 0.0006$). The mean sFlt-1/PlGF ratio was 515.3 ± 76.7 in the 1-week T group and 149.6 ± 66.9 in the 1-week P group (P=0.0009). sFlt-1 was the most significantly associated factor with delivery in <1 week (**Figure 2**).

Cutoff Values for Delivery in <24 h and <1 Week

Among sFlt-1, PlGF, and sFlt-1/PlGF, sFlt-1 was the parameter that was the strongest statistical predictor. Therefore, we used sFlt-1 as the predictor. In this study, cutoff values of 8,682.1 pg/ml (AUC 0.71; 95%Cl, 0.5191–0.9052) and 7,394.5 pg/ml (AUC 0.78; 0.78, 95%Cl, 0.6394–0.9206) for sFlt-1 for delivery in <24 h and within 1 week, respectively, were identified as important predictive values (**Figures 3**, **4**). sFlt-1 below 8,682 pg/ml had a positive predictive value of 43.9% for delivery in <24 h, with a sensitivity of 72.3% and specificity of 69.0%. sFlt-1 >7,394 pg/ml would result in delivery within a week, with a positive predictive value of 67.2%, sensitivity of 79.0%, and specificity of 72.0%.

DISCUSSION

This study highlights two key novel findings. First, sFlt-1 may be more effective than PlGF or the sFlt-1/PlGF ratio in predicting the time of termination in pregnant women with known PE. Second, sFlt-1 cutoff values were calculated to predict the time of termination of pregnancy (<24 h and <1 week) after PE.

Although the etiology of PE remains controversial, the discovery of cardiovascular angiogenic factors has led to significant advances in both the diagnosis and prediction of prognosis. Of particular interest are the anti-angiogenic factor sFlt-1 and angiogenic factor PlGF. The sFlt-1 level increases in maternal blood in PE, whereas the PlGF level decreases as impaired spiral artery remodeling worsens placental return (12, 13). Therefore, various clinical studies have been conducted using the sFlt-1/PlGF ratio. The sFlt-1/PlGF ratio can be used to predict the development of PE in pregnant women who may be at risk of PE, and for the management planning and decision making

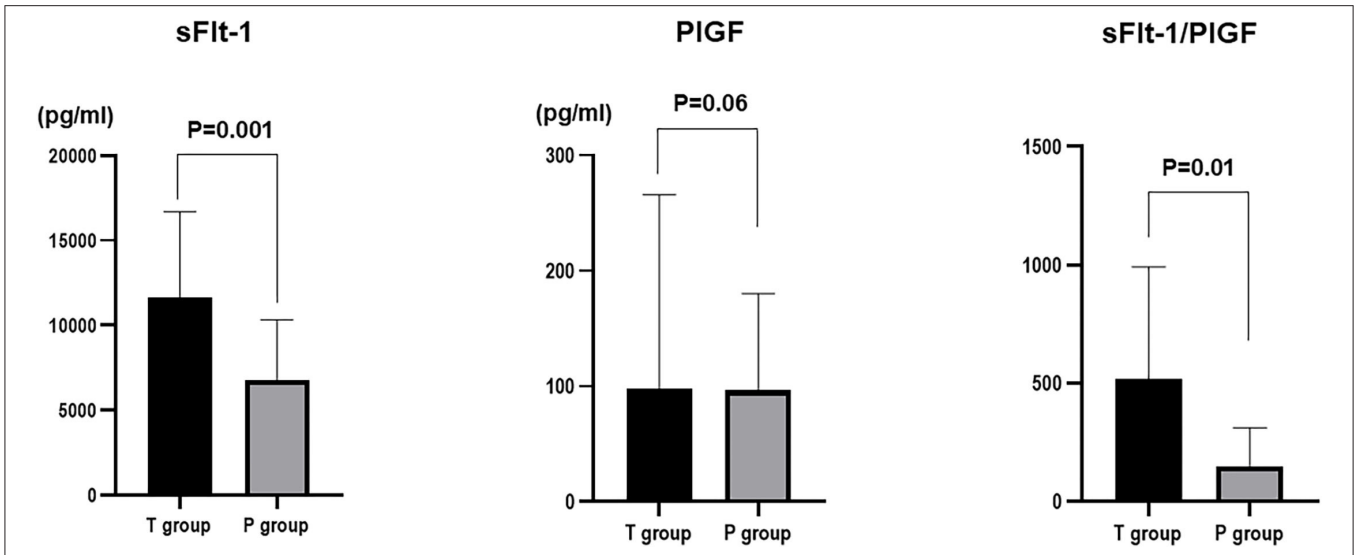

FIGURE 2 | Comparison of sFlt-1, PlGF, and sFlt-1/PlGF ratios in the group that delivered in <1 week (T group) and the group that delivered in more than 1 week (P group).

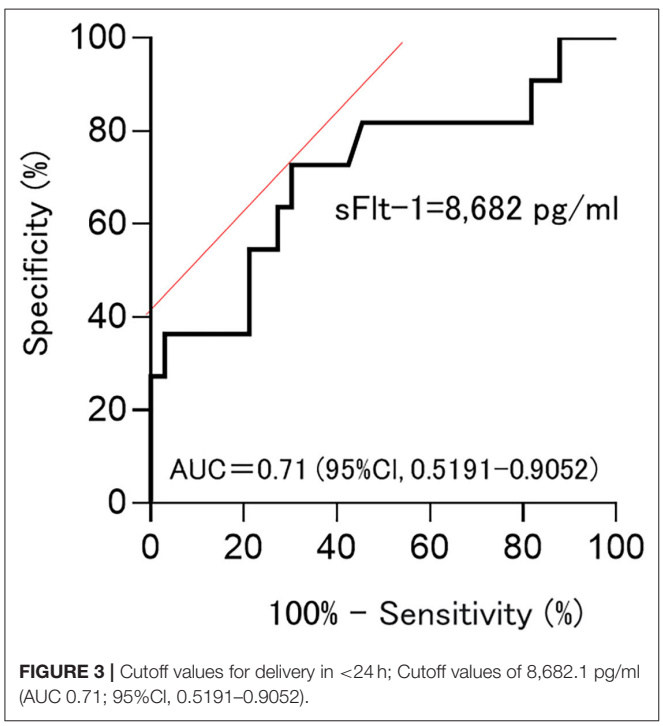

FIGURE 3 | Cutoff values for delivery in <24 h; Cutoff values of 8,682.1 pg/ml (AUC 0.71; 95%CI, 0.5191–0.9052).

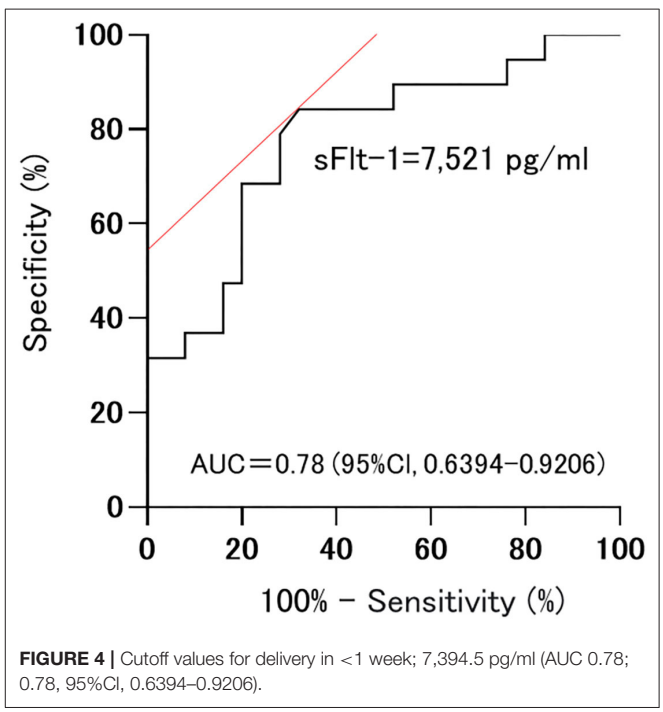

FIGURE 4 | Cutoff values for delivery in <1 week; 7,394.5 pg/ml (AUC 0.78; 0.78, 95%CI, 0.6394–0.9206).

in pregnant women with clinical features that are suspicious of PE or those diagnosed with PE. In particular, the sFlt-1/PlGF ratio is extremely useful in predicting the development of PE in pregnant women with suspected PE (9, 14, 15). In Japan, even standard low-risk pregnant women visit the clinic every 1–2 weeks for a checkup. Therefore, pregnant women with PE can be diagnosed at a relatively early stage. In Japan, after PE is diagnosed, the patient is generally hospitalized for observation. For these reasons, we are more interested in the application of the sFlt-1/PlGF ratio to the management and decision-making of pregnant women with known PE than in predicting the onset of PE. Specifically, we are interested in the possibility of terminating pregnancies after the onset of PE and before serious complications develop.

The association between angiogenic factors and adverse events in pregnant women with suspected PE was studied by Rana et al. (16). These studies could reduce unnecessary hospitalizations and inappropriate discharge of pregnant women with suspected PE and reduce the considerable morbidity associated with

unplanned preterm delivery. However, it is not currently possible to determine when a pregnancy should be terminated in pregnant women with known PE.

In the Hypertension and Preeclampsia Intervention Trial At near Term-I (HYPITAT I) study in the Netherlands, it was reported that women with gestational hypertension and mild hypertension should be induced to deliver after 37 weeks gestation, although certain angiogenic factors had not been investigated (17). In a study by Verlohren et al. it was possible to identify pregnant women at risk for impending delivery with different hypertension, chronic hypertension, and gestational hypertension (18). However, it was not possible to determine whether induction of labor should be recommended for these pregnant women.

The sFlt-1/PlGF ratio can be used to predict maternal adverse events in pregnant women with known PE. The higher the ratio, the higher the risk of maternal complications requiring hospital intervention, such as acute pulmonary edema, HELLP syndrome, placental abruption, renal failure, refractory hypertension, and eclampsia (10, 16, 18–22). However, these studies have not indicated when the pregnancy should be terminated.

Recent data has shown that the use of an algorithm based on PlGF levels in women with late preterm eclampsia results in a lower rate of progression to severe PE, fewer maternal complications, and no worsening of the neonatal outcome (23). Similarly, the use of the sFlt-1/PlGF ratio in standard practice has been reported to improve clinical accuracy and correctly identify both at-risk women and at-risk babies (24). In the future, it will be necessary to combine this with neonatal conversion to find the appropriate time to terminate the pregnancy.

Predicting the duration of pregnancy after the onset of gestational hypertension nephropathy would allow the obstetrician not to miss the timing of administering steroids before delivery, the obstetrician to observe the patient more intensively as the pregnancy is predicted to be nearing its end, and the neonatologist to be better prepared for the delivery. On the other hand, there is concern that setting a cutoff value may lead to an increase in excessive preterm births beyond the cutoff value. At this time, the indicator of pregnancy termination should be based on clinical findings.

This study had some potential limitations. Although the focus of this study was on the timing of pregnancy termination, it is necessary to examine this issue from the perspective of neonatal prognosis. However, this study was not conducted from the perspective of neonatal prognosis. Another limitation is the retrospective study design and the small number of cases, even though the cases were managed in a uniform manner. An important limitation of this study is the small number of cases. Though this study themselves show that there are different predictive values, the small group of cases considered may be the reason for this result. In the future, more cases should be added to validate the results of this study. sFlt-1 increases modestly with weeks. However, this study did not adjust for this increase, so caution should be exercised in interpreting the cutoff values in this study.

CONCLUSION

In summary, this study showed that sFlt-1 may be effective in predicting the timing of pregnancy termination. However, the number of cases was small and the results were therefore not definitive. In combination with previous reports, we would like to develop this study further to predict the optimal timing of pregnancy termination to prevent cerebral hemorrhage after PE.

AUTHOR'S NOTE

The authors of this article are board-certified Obstetrics and Gynecology physicians at Mie University Hospital, which is the largest Obstetrics and Gynecology resident training institution in Japan. The corresponding author is a member of the Japan Maternal Death Exploratory Committee.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mie University Hospital Medical Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HT conceived and designed the study, performed data acquisition, and conducted data analysis and interpretation. KT provided statistical expertise. HT wrote the manuscript draft. ST, NE, and TI made major revisions to the manuscript. All authors contributed to the article and approved the submitted version.

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U-Shaped Association Between Blood Pressure and Mortality Risk in ICU Patients With Atrial Fibrillation: The MIMIC-III Database

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Background: Existing evidence on the association between blood pressure (BP) and mortality risk in intensive care unit (ICU) patients with atrial fibrillation (AF) is scarce.

Aim: This study aimed to assess the associations between blood pressure (BP) and risks of in-hospital and all-cause mortality in ICU patients with AF.

Methods: A total of 2,345 records of patients with AF whose BP was monitored after admission to the ICU were obtained from the MIMIC-III database. Incidences were calculated for endpoints (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality). We performed smooth curve and logistic regression analyses to evaluate the association between BP and the risk of each endpoint.

Results: Smooth curve regression showed that systolic blood pressure (SBP), mean arterial pressure (MBP), and diastolic blood pressure (DBP) followed U-shaped curves with respect to endpoints (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality). The incidence of these endpoints was lowest at 110/70/55 mm Hg. There was an increased risk of 1-year mortality observed with BP > 110/70/55 mm Hg (SBP, odds ratio [*OR*] = 1.008, 95% *CI* 1.001–1.015, $p = 0.0022$; MBP, *OR* = 1.010, 95% *CI* 1.005–1.016, $p < 0.001$) after adjusting for age, sex, and medical history. In contrast, an inverse association between BP and the risk of 1-year mortality was observed with BP ≤ 110/70/55 mm Hg (SBP, *OR* = 0.981, 95% *CI* 0.974–0.988, $p < 0.001$; MBP *OR* = 0.959, 95% *CI* 0.939–0.979, $p < 0.001$; and DBP, *OR* = 0.970, 95% *CI* 0.957–0.983, $p < 0.001$).

Conclusions: We observed a U-shaped association between BP and in-hospital/all-cause mortality in ICU patients with AF. However, the underlying causes need to be investigated.

Keywords: blood pressure, atrial fibrillation, smooth curve, mortality, intensive care unit

INTRODUCTION

As a common cardiac arrhythmia, atrial fibrillation (AF) has increased considerably in prevalence in the general population aged ≥65 years (1). Evidence from previous studies has demonstrated that AF strongly contributes to an increased long-term risk of all-cause mortality. Existing studies suggest that demographic characteristics, such as advanced age and male sex; lifestyle factors, such as high body mass index (BMI) and low levels of physical exercise; and history of the disease, such as hypertension, myocardial infarction, valvular disease, heart failure, and diabetes mellitus, are all important factors contributing to AF (2–4). However, hypertension may be more important than other factors (2, 5) due to its high prevalence in the general population. Consequently, hypertension tends to be the most important target in the prevention of AF.

Blood pressure (BP) also has an important effect on mortality. Every 20/10 mm Hg increase in BP doubles cardiovascular risk in seniors with BP >115/75 mm Hg (6, 7). Previous studies have confirmed the strong association between BP and cardiovascular events. For instance, in certain individuals, such as patients with acute coronary syndrome or older adults, a J-shaped association between BP and adverse outcomes has been observed (8, 9). Low BP (<110/70 mm Hg) is related to increased incidence of negative outcomes, with mortality risk lowest at BP values ranging from (130 to 140)/(80 to 90) mm Hg (8). Similar results have also been demonstrated in individuals with stroke and chronic coronary artery disease (CAD) (10–13). However, few studies exist on the association between BP and mortality in specific individuals with AF. Only one study focused only on patients with AF, reporting a U-shaped association of BP with all-cause mortality. Their results showed that the incidence of all-cause mortality was lowest at 140/78 mm Hg (14). These correlative differences may have been due to differences among participants in various demographic characteristics, lifestyles, comorbidities, and different statistical methods.

Patients in the intensive care unit (ICU), as a special department, have high mortality risk, of which patients with AF account for a certain proportion. Reducing the mortality of ICU patients with AF has always been a major clinical objective. However, no study has focused on the association between BP and mortality risk and the optimal BP target in ICU patients with AF (14). Considering the loss of atrial contractility, the optimal value of BP in patients with AF may differ from that in the general population, which would be of great clinical significance for defining thresholds of BP below which adverse events may increase or decline in frequency. Therefore, by using records of patients obtained from the MIMIC-III database, we investigated whether a strong association exists between BP and mortality in ICU patients with AF. Our main objective was to investigate the nonlinear association between BP and mortality (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality) in a large cohort of patients with AF and determine the optimal BP at the lowest mortality. Furthermore, we attempted to evaluate the possible effects of age, sex, comorbidity, and medical treatment on the association of BP with mortality, and these confounding factors may be important moderators that few have previously taken into account.

MATERIALS AND METHODS

The data used in the present study were obtained from the MIMIC-III database (15). Briefly, the MIMIC-III database contains information on 46,520 patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) from 2001 to 2012 (15). The establishment of this freely available database was approved by the Institutional Review Boards (IRBs) of the Massachusetts Institute of Technology (MIT) and BIDMC. The database includes demographic data, laboratory tests, fluid balance data, vital status and blood gas analysis data, discharge summaries, electrocardiography, imaging examinations, and diagnostic information. We included ICU patients diagnosed with AF using diagnosis codes from the International Classification of Diseases, Ninth Revision (ICD-9), and a total of 2,345 patients were considered eligible for inclusion in this study after excluding patients with the absence of important variables. The study was conducted in accordance with the Declaration of Helsinki. This was consistent with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (16).

BP and Mortality

The BP was measured and recorded when entering the ICU, and the initial BP record values were further used for analysis in this study. The endpoints of the study were defined as hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality after the date of ICU admission. Hospital mortality was defined as death during hospitalization in the ICU. Furthermore, the 7-day mortality, 30-day mortality, and 1-year mortality were defined based on the time from the discharge date to the date of death.

Confounding Variables

A large amount of admission information was collected for each patient from MIMIC-III by the Structured Query Language, such as demographic data (age and sex), laboratory results [white blood cell count (WBC), red blood cell count (RBC), platelet count (PLC), hemoglobin, serum creatinine, and blood urea nitrogen], medication records [β receptor blockers (βRBs), statins, nitrates, warfarin, and heparin], and clinical comorbidities [hypertension, chronic heart failure (CHF), valvular disease, chronic kidney disease (CKD), stroke, diabetes, chronic bronchitis, depression, and malignancy].

Statistical Analysis

All statistical analyses in our study were conducted using SPSS 26.0 and EmpowerStats 3.0. Categorical data are presented as percentages, while continuous data are presented as the median (interquartile range, IQR). First, a smooth curve

TABLE 1 | Clinical characteristics of ICU patients with AF.

Variables	All $n = 2,345$
	Median (interquartile range) or n (%)
Age	73.60 (65.43–79.66)
Gender (male)	1,495 (63.75%)
DBP (mmHg)	58.00 (50.00–66.00)
Max	82.00 (72.00–97.00)
Min	38.00 (30.00–44.00)
MBP (mmHg)	77.00 (69.00-88.00)
Max	111.00 (98.00–139.00)
Min	52.00 (45.00–58.00)
SBP (mmHg)	114.00 (102.00–129.00)
Max	159.00 (144.00–179.00)
Min	77.00 (61.00–87.00)
Co-morbidity	
Hypertension	1,206 (51.43%)
CHF	874 (37.27%)
Valvular disease	904 (38.55%)
Stroke	61 (2.60%)
Diabetes	39 (1.66%)
CKD	126 (5.37%)
Chronic bronchitis	37 (1.58%)
Depression	42 (1.79%)
Malignant	119 (5.07%)
Medication	
βRBs	1,636 (69.77%)
Statins	878 (37.44%)
Nitrates	355 (15.14%)
Warfarin	108 (4.61%)
Heparin	695 (29.64%)
Blood biomarkers	
RBC (m/uL)	3.33 (2.92–3.76)
PLC (K/uL)	158.00 (118.00–210.00)
WBC (K/uL)	12.00 (9.00–15.70)
Hemoglobin (g/dL)	10.00 (8.70–11.60)
Creatinine (mg/dL)	0.90 (0.70–1.20)
Urea nitrogen (mg/dL)	18.00 (14.00–27.00)
ICU stay (hour)	86.00 (49.00–185.00)
Hospital mortality	294 (12.54%)
7-day mortality	326 (13.90%)
30-day mortality	381 (16.25%)
1-year mortality	610 (26.01%)

AF, atrial fibrillation; ICU, intensive care unit; DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure; CHF, chronic heart failure; CKD, chronic kidney disease; βRB, β receptor blockers; RBC, red blood cell; PLC, platelet count; WBC, white blood cell count.

analysis was performed to determine the relationships between BP (systolic blood pressure [SBP], diastolic blood pressure [DBP], and mean arterial pressure [MBP]) and endpoints (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality) and to further define the optimal value of BP with the lowest risk of mortality. According to the BP threshold, restrictive logistic regression models were then applied to determine whether BP was independently associated with endpoints (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality) after adjusting for potential confounders. The crude model had no adjustment. Model 1 was adjusted for age and gender. Model 2 was adjusted for Model 1 plus CHF, valvular disease, and stroke. Model 3 was adjusted for Model 2 plus CKD, chronic bronchitis, depression, diabetes, and malignancy. Furthermore, interaction analysis was conducted to determine the impacts of belonging in various subgroups, classified by age, sex, CHF, valvular disease, hypertension, and medication (βRBs, statins, nitrates, warfarin, and heparin).

RESULTS

Clinical Characteristics of Patients With AF in the ICU

The clinical characteristics of these included patients with AF are presented in **Table 1**. Their median age was 73.6 years, and the number of men was 1,497 (63.8%). The median levels of SBP, MBP, and DBP were 114, 77, and 58 mm Hg, respectively. The incidence of hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality was 294 (12.54%), 326 (13.90%), 381 (16.25%), and 610 (26.01%), respectively. Other clinical information, such as comorbidities, medication, and blood biomarkers in the ICU, is also described in **Table 1**. Importantly, smooth curve analysis showed approximate U-shaped relations of SBP, MBP, and DBP with mortality (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality), as shown in **Figure 1**. The BP levels with the lowest mortality risk, including those for SBP, MBP, and DBP, in these patients with AF were 110, 70, and 55 mm Hg, respectively.

Multivariable Analysis Suggested a Significant Association of DBP With Mortality Stratified by the DBP Value With the Lowest Mortality Risk (55 mm Hg)

Based on the lowest of the BP thresholds reported above, stratified analysis was performed to evaluate the associations of DBP with mortality in our study. As shown in **Table 2**, increased DBP levels were associated with reduced risks of hospital mortality (odds ration [*OR*] = 0.961, 95% *CI* 0.949–0.973, $p < 0.001$, crude model), 7-day mortality ($OR = 0.962$, 95% *CI* 0.950–0.974, $p < 0.001$, crude model), 30-day mortality ($OR = 0.963$, 95% *CI* 0.951–0.975, $p < 0.001$, crude model), and 1-year mortality ($OR = 0.965$, 95% *CI* 0.953–0.976, $p < 0.001$, crude model) in AF patients with DBP $\leq$ 55 mm Hg. However, in patients with DBP $>$ 55 mm Hg, increased DBP levels were only associated with the increased risk of 1-year mortality ($OR = 1.012$, 95% *CI* 1.001–1.023, $p = 0.037$, crude model) and not with those

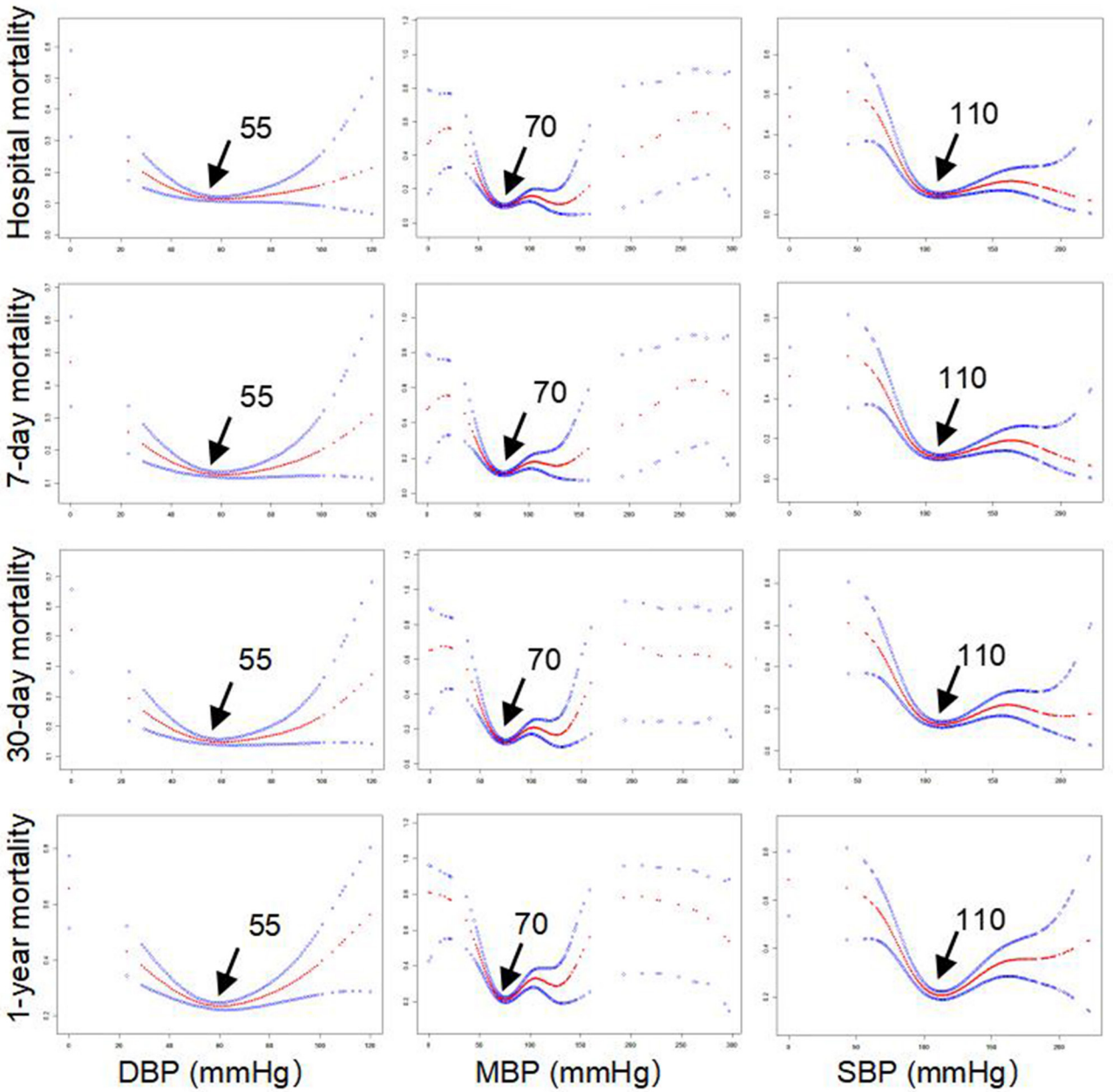

FIGURE 1 | Smooth curve analysis of the association between blood pressure (BP) and mortality.

of hospital mortality (*OR* = 1.006, 95% *CI* 0.993–1.019, $p = 0.376$, crude model), 7-day mortality (*OR* = 1.008, 95% *CI* 0.996–1.020, $p = 0.214$ crude model), or 30-day mortality (*OR* = 1.007, 95% *CI* 0.996–1.019, $p = 0.214$, crude model). Importantly, the interactions (all values of $p < 0.001$) of hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality with the BP threshold (separating patients into a group with DBP ≤ 55 mm Hg and a group with DBP > 55 mm Hg) were significant. Furthermore, after adjusting for confounding factors, such as age, sex, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes, and malignancy, these independent associations in Model 3 were only slightly changed, and significant interactions for hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality still existed.

Multivariable Analysis Suggested a Significant Association of MBP With Mortality Stratified by the MBP Value With the Lowest Mortality Risk (70 mm Hg)

As shown in **Table 3**, higher MBP levels were related to reduced risks of hospital mortality (*OR* = 0.955, 95% *CI* 0.936–0.973, $p < 0.001$, crude model), 7-day mortality (*OR* = 0.956, 95%

TABLE 2 | Multiple logistic regression analysis for relationship between DBP and mortality risk.

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#
Crude Model																
DBP ≤ 55 mmHg	0.961	0.949–0.973	<0.001	<0.001	0.962	0.950–0.974	<0.001	<0.001	0.963	0.951–0.974	<0.001	<0.001	0.965	0.953–0.976	<0.001	<0.001
DBP>55mmHg	1.006	0.993–1.019	0.376		1.008	0.996–1.020	0.214		1.007	0.996–1.019	0.214		1.012	1.001–1.023	0.037	
Model 1																
DBP≤55mmHg	0.960	0.948–0.972	<0.001	<0.001	0.962	0.950–0.974	<0.001	<0.001	0.962	0.950–0.974	<0.001	<0.001	0.963	0.951–0.975	<0.001	<0.001
DBP>55mmHg	1.006	0.994–1.019	0.347		1.008	0.996–1.020	0.199		1.008	0.996–1.020	0.199		1.014	1.002–1.026	0.026	
Model 2																
DBP≤55mmHg	0.963	0.951–0.975	<0.001	<0.001	0.964	0.952–0.977	<0.001	<0.001	0.965	0.953–0.978	<0.001	<0.001	0.967	0.955–0.979	<0.001	<0.001
DBP>55mmHg	1.000	0.984–1.016	0.969		1.002	0.988–1.017	0.755		1.002	0.988–1.016	0.800		1.008	0.997–1.019	0.168	
Model 3																
DBP≤55mmHg	0.965	0.953–0.978	<0.001	<0.001	0.967	0.955–0.980	<0.001	<0.001	0.968	0.955–0.980	<0.001	<0.001	0.970	0.957–0.983	<0.001	<0.001
DBP>55mmHg	1.001	0.986–1.017	0.869		1.004	0.990–1.018	0.587		1.003	0.989–1.017	0.660		1.009	0.998–1.020	0.113	

Crude Model: No adjustment.
Model 1:Adjusted for age and gender.
Model 2:Adjusted for age, gender, CHF, valvular disease and stroke.
Model 3:Adjusted for age, gender, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes and malignant.
P#: P-value for interaction.
DBP, diastolic blood pressure; CHF, chronic heart failure; CKD, chronic kidney disease.

CI 0.938–0.975, $p < 0.001$, crude model), 30-day mortality ($OR = 0.951$, 95% *CI* 0.932–0.970, $p < 0.001$, crude model), and 1-year mortality ($OR = 0.953$, 95% *CI* 0.934–0.972, $p < 0.001$, crude model) in AF patients with MBP ≤ 70 mm Hg. However, in patients with MBP > 70 mm Hg, higher MBP levels were associated with increased risks of hospital mortality ($OR = 1.012$, 95% *CI* 1.007–1.018, $p < 0.001$, crude model), 7-day mortality ($OR = 1.012$, 95% *CI* 1.007–1.018, $p < 0.001$, crude model), 30-day mortality ($OR = 1.013$, 95% *CI* 1.008–1.019, $p < 0.001$, crude model), and 1-year mortality ($OR = 1.013$, 95% *CI* 1.008–1.019, $p < 0.001$, crude model). Similarly, these independent associations in Model 3 were changed only slightly, and significant interactions with hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality still existed after adjusting for confounding factors, such as age, sex, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes, and malignancy.

Multivariable Analysis Suggested a Significant Association of MBP With Mortality Stratified by the SBP Value With the Lowest Mortality Risk (110 mm Hg)

As shown in **Table 4**, our results suggested that increased SBP levels contributed to lower risks of hospital mortality ($OR = 0.977$, 95% *CI* 0.971–0.983, $p < 0.001$, crude model), 7-day mortality ($OR = 0.978$, 95% *CI* 0.971–0.984, $p < 0.001$ crude model), 30-day mortality ($OR = 0.978$, 95% *CI* 0.972–0.984, $p < 0.001$, crude model), and 1-year mortality ($OR = 0.978$, 95% *CI* 0.971–0.984, $p < 0.001$, crude model) in AF patients with SBP ≤ 110 mm Hg. However, in patients with SBP > 110 mm Hg, increased SBP levels only contributed to increased risks of 30-day mortality ($OR = 1.009$, 95% *CI* 1.002–1.017, $p = 0.013$, crude model), and 1-year mortality ($OR = 1.013$, 95% *CI* 1.007–1.020, $p < 0.001$, crude model) but not that of hospital mortality ($OR = 1.008$, 95% *CI* 0.009–1.016, $p = 0.079$, crude model), and 7-day mortality ($OR = 1.008$, 95% *CI* 1.000–1.016, $p = 0.051$, crude model). Furthermore, after adjustment for confounding factors, such as age, sex, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes, and malignancy, the independent associations in Model 3 remained significant, and the values of *p* of the interactions with hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality were <0.001.

Analysis of Correlations Between BP and Mortality Stratified by Comorbidities and Medication

Interestingly, as shown in **Table 5**, in patients with MBP > 70 mm Hg, CHF ($p = 0.026$), nitrates ($p < 0.001$), and heparin ($p = 0.021$) modified the association between MBP and 1-year mortality. In patients with SBP > 110 mm Hg, nitrates modified the association between SBP and 1-year mortality ($p = 0.019$). Furthermore, hypertension ($p = 0.002$) and heparin ($p < 0.001$) modified the association between DBP and mortality in patients with DBP ≤ 55 mm Hg (**Table 6**). CHF ($p = 0.046$) and hypertension ($p = 0.025$) modified the association between MBP

TABLE 3 | Multiple logistic regression analysis for relationship between MBP and mortality risk.

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#
Crude Model																
MBP≤70mmHg	0.955	0.936–0.973	<0.001	<0.001	0.956	0.938–0.975	<0.001	<0.001	0.951	0.932–0.970	<0.001	<0.001	0.953	0.934–0.972	<0.001	<0.001
MBP>70mmHg	1.012	1.007–1.018	<0.001		1.012	1.007–1.018	<0.001		1.013	1.008–1.019	<0.001		1.013	1.008–1.019	<0.001	
Model 1																
MBP≤70mmHg	0.954	0.936–0.973	<0.001	<0.001	0.956	0.938–0.974	<0.001	<0.001	0.950	0.931–0.969	<0.001	<0.001	0.952	0.933–0.971	<0.001	<0.001
MBP>70mmHg	1.012	1.007–1.018	<0.001		1.013	1.007–1.018	<0.001		1.013	1.008–1.019	<0.001		1.014	1.008–1.019	<0.001	
Model 2																
MBP≤70mmHg	0.956	0.937–0.975	<0.001	<0.001	0.958	0.940–0.977	<0.001	<0.001	0.953	0.934–0.972	<0.001	<0.001	0.955	0.935–0.975	<0.001	<0.001
MBP>70mmHg	1.010	1.004–1.015	<0.001		1.010	1.004–1.015	<0.001		1.010	1.004–1.016	<0.001		1.010	1.005–1.016	<0.001	
Model 3																
MBP≤70mmHg	0.960	0.941–0.979	<0.001	<0.001	0.962	0.943–0.981	<0.001	<0.001	0.956	0.936–0.975	<0.001	<0.001	0.959	0.939–0.979	<0.001	<0.001
MBP>70mmHg	1.010	1.004–1.016	<0.001		1.010	1.004–1.016	<0.001		1.010	1.005–1.016	<0.001		1.010	1.005–1.016	<0.001	

Crude Model: No adjustment.
Model 1:Adjusted for age and gender.
Model 2:Adjusted for age, gender, CHF, valvular disease and stroke.
Model 3:Adjusted for age, gender, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes and malignant.
P#: P-value for interaction.
MBP, mean blood pressure; CHF, chronic heart failure; CKD, chronic kidney disease.

TABLE 4 | Multiple logistic regression analysis for relationship between SBP and mortality risk.

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P*	*P*#	OR	95% CI	*P*	*P*#	OR	95% CI	*P* Value	*P*#	OR	95% CI	*P* Value	*P*#
Crude Model																
SBP≤110mmHg	0.977	0.971–0.983	<0.001	<0.001	0.978	0.971–0.984	<0.001	<0.001	0.978	0.972–0.984	<0.001	<0.001	0.978	0.971–0.984	<0.001	<0.001
SBP>110mmHg	1.008	0.999–1.016	0.079		1.008	1.000–1.016	0.054		1.009	1.002–1.017	0.013		1.013	1.007–1.020	<0.001	
Model 1																
SBP≤110mmHg	0.977	0.971–0.983	<0.001	<0.001	0.977	0.971–0.983	<0.001	<0.001	0.978	0.971–0.984	<0.001	<0.001	0.977	0.970–0.983	<0.001	<0.001
SBP>110mmHg	1.006	0.998–1.015	0.163		1.006	0.998–1.015	0.124		1.008	1.000–1.016	0.040		1.012	1.006–1.019	<0.001	
Model 2																
SBP≤110mmHg	0.979	0.973–0.985	<0.001	<0.001	0.979	0.973–0.985	<0.001	<0.001	0.980	0.973–0.986	<0.001	<0.001	0.979	0.972–0.986	<0.001	<0.001
SBP>110mmHg	1.002	0.993–1.011	0.738		1.002	0.993–1.011	0.643		1.004	0.996–1.012	0.376		1.009	1.002–1.016	0.009	
Model 3																
SBP≤110mmHg	0.980	0.974–0.986	<0.001	<0.001	0.980	0.974–0.987	<0.001	<0.001	0.981	0.974–0.987	<0.001	<0.001	0.981	0.974–0.988	<0.001	<0.001
SBP>110mmHg	1.001	0.992–1.010	0.805		1.002	0.993–1.010	0.702		1.003	0.995–1.011	0.441		1.008	1.001–1.015	0.022	

Crude Model: *No adjustment.*
Model 1:*Adjusted for age and gender.*
Model 2:*Adjusted for age, gender, CHF, valvular disease and stroke.*
Model 3:*Adjusted for age, gender, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes and malignant.*
P#: P-value for interaction.
SBP, systolic blood pressure; CHF, chronic heart failure; CKD, chronic kidney disease.

and mortality in patients with MBP ≤ 70 mm Hg, respectively, as well as in patients with SBP ≤ 110 mm Hg.

DISCUSSION

We observed U-shaped relations between BP (SBP, MBP, and DBP) and mortality (hospital mortality, 7-day mortality, 30-day mortality, and 1-year mortality). The BP points for SBP, MBP, and DBP with the lowest mortality risk in patients with AF in our study were 110, 70, and 55 mm Hg, respectively (**Figure 1**). Studies on optimal BP in patients with AF have been few in the past and previous guidelines on hypertension therapy recommend tight control of BP (17–19). The clinical study including 3,947 patients with AF from the Atrial Fibrillation Follow-Up Investigation of Rhythm Management trial (AFFIRM) also suggested U-shaped curves between BP and all-cause mortality, and the risk of all-cause mortality was lowest at 140/78 mm Hg (14). In the AFFIRM study, patients with AF were either older adults or had at least one risk factor for cardiovascular events (20, 21). However, the authors further observed significantly greater mortality when patients with AF had an average BP (SBP/DBP) below 110/60 mm Hg, which is inconsistent with our finding that AF patients with SBP ≤ 110, MBP ≤ 70, or DBP ≤ 55 mm Hg tended to exhibit a reduced risk of mortality. This discrepancy may originate from different demographic characteristics and lifestyles, differences in comorbidities and treatment histories, different statistical methods, and different BP measurement methods. For example, the sample in the current study consists only of patients from the ICU. Their physiological status is worse, and there are more accompanying diseases than in ordinary patients. Previous studies have found that every 20/10 mm Hg increase in BP contributed to an increased risk of cardiovascular events in seniors with BP >115/75 mm Hg (6, 7). Roughly consistent with the findings of these previous studies, our results demonstrated that higher BP was associated with an increased risk of mortality in AF patients with SBP > 110, MBP > 70, or DBP > 55 mm Hg.

Although AF prevalence is affected by various factors, advanced age is the most important risk factor for AF (22). Existing epidemiological analyses have consistently confirmed a gradual increase in AF prevalence with advancing age (23–25). Thus, we also performed a stratified analysis by adding age as the stratification variable to evaluate the correlation between BP and mortality in patients with AF. However, our results showed that different age groups (age ≥ 65 and age < 65; age ≥ 73, and age < 73 years) have a little modifying effect on this relationship (data not shown). One possible explanation for these results is that the study sample consists of older adult ICU patients, and the influence of age on BP and mortality in patients with AF is disturbed by poor physiological state and accompanying diseases. Current epidemiological evidence also suggests a sex difference in the epidemiology of AF (26). A study of North American and European populations showed that the rate of AF was higher in men than women after adjustment for age. The results from the Framingham Heart Study (HFS) showed that the incidence of AF (per 1,000 person-years) was 1.6 in men and 3.8 in women (22). A significantly higher rate of AF in the male population is also observed in Asians, although there are few data (27, 28). Furthermore, one study showed that the AF prevalence was 7.4% in women and 10.3% in men among adults aged ≥65 years with Medicare beneficiaries (29). In our study, we still did not observe a modifying effect of sex, which suggests that there is no significant sex difference in this association between BP and mortality in patients with AF (data not shown). In addition to the explanation for the results described above, other potential factors need to be further studied in the future. Additionally, in AF patients with MBP >70 mm Hg or SBP >110 mm Hg, our results suggest that nitrates significantly modified the association between BP and 1-year mortality. Moreover, hypertension modified the association between DBP and mortality in patients with DBP ≤55 mm Hg. CHF and hypertension modified the association between MBP and mortality in AF patients with MBP ≤70 mm Hg and in patients with SBP ≤110 mm Hg, respectively. These significant results are also well explained by comorbidity and medication.

This study has several notable advantages. Our study data were obtained from the MIMIC-III database (15), which is a public critical care database that contains records from tens of thousands of ICU admissions to the Beth Israel Deaconess Medical Center from 2001 to 2012 and provides high-quality data. Professional researchers ensured the reliability and standardization of the data. Second, our study identified U-shaped associations of BP with risks of in-hospital mortality and post-hospital mortality in ICU patients with AF, providing the research evidence for controversial results on patients with AF. The SBP, MBP, and DBP levels with the lowest mortality risks in patients with AF in our study were 110, 70, and 50 mm Hg, respectively, which is inconsistent with the findings of previous relevant studies. Third, numerous disease histories and treatment histories were corrected for and stratified in our study, which improves the credibility of the conclusions of this study.

Of course, common defects in clinical research are also present in our study. Despite the MIMIC-III database prospectively providing high-quality data on ICU patients, the inevitable shortcomings of *post-hoc* analyses must be taken into consideration. Although meticulous adjustment for numerous potential confounding factors was made, regression analyses could not eliminate unknown or unmeasured variables. Overfitting models of regression analyses are likely to produce a bias toward the study hypothesis with the potential to conservatively underestimate the relationship between BP and mortality. In our results, baseline BP was recorded repeatedly, but we mainly used BP value from the first record after each patient entered the ICU ward. Therefore, the relationships of two BP measurements (maximum and minimum value BP during ICU) with mortality risk in these patients with AF were also analyzed (**Supplementary Materials**), suggesting a similarly U-shaped relationship, which suggested that our research results were reliable.

TABLE 5 | Multiple logistic regression analysis for relationship between BP (>55/70/110 mmHg) and mortality by stratified analysis.

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*
DBP																
CHF	0.983	0.958–1.009	0.208	0.225	0.992	0.968–1.017	0.547	0.449	0.994	0.972–1.017	0.624	0.331	1.002	0.982–1.023	0.835	0.570
NO CHF	1.008	0.993–1.024	0.306		1.008	0.993–1.024	0.314		1.008	0.993–1.023	0.322		1.011	0.997–1.024	0.124	
Valvular disease	0.992	0.947–1.039	0.742	0.615	0.995	0.954–1.040	0.807	0.496	0.992	0.957–1.029	0.678	0.350	1.000	0.980–1.021	0.998	0.122
NO valvular disease	1.003	0.984–1.022	0.769		1.007	0.989–1.026	0.421		1.008	0.990–1.025	0.392		1.017	1.001–1.034	0.033	
Hypertension	1.009	0.990–1.029	0.371	0.304	1.009	0.991–1.027	0.324	0.474	1.006	0.987–1.025	0.566	0.644	1.005	0.990–1.021	0.500	0.443
NO hypertension	0.991	0.970–1.013	0.438		0.996	0.975–1.018	0.708		0.998	0.978–1.019	0.868		1.016	0.996–1.035	0.115	
βRBs	0.991	0.966–1.016	0.470	0.441	1.000	0.978–1.022	0.984	0.777	0.999	0.980–1.019	0.946	0.785	1.010	0.998–1.023	0.111	0.684
NO βRBs	1.019	0.992–1.047	0.162		1.018	0.991–1.045	0.191		1.014	0.989–1.041	0.279		1.009	0.984–1.034	0.480	
Statins	0.998	0.952–1.048	0.949	0.756	0.992	0.947–1.039	0.734	0.834	0.985	0.945–1.028	0.491	0.717	1.007	0.989–1.026	0.432	0.930
NO Statins	0.997	0.978–1.016	0.772		1.004	0.986–1.022	0.690		1.003	0.985–1.021	0.737		1.010	0.994–1.026	0.243	
Nitrates	0.977	0.939–1.017	0.259	0.184	0.984	0.949–1.021	0.403	0.231	0.992	0.959–1.026	0.652	0.363	0.991	0.961–1.023	0.576	0.134
NO Nitrates	1.006	0.991–1.022	0.424		1.008	0.993–1.022	0.297		1.005	0.991–1.020	0.470		1.012	1.000–1.024	0.059	
Warfarin	–	–	–	–	1.103	0.995–1.223	0.063	0.060	1.076	0.981–1.180	0.118	0.125	1.031	0.964–1.103	0.372	0.521
NO Warfarin	1.001	0.986–1.017	0.868		1.002	0.987–1.017	0.764		1.002	0.987–1.016	0.822		1.008	0.997–1.020	0.148	
Heparin	0.984	0.956–1.013	0.268	0.179	0.980	0.953–1.008	0.166	0.062	0.983	0.957–1.009	0.203	0.077	1.012	0.989–1.036	0.318	0.847
NO Heparin	1.010	0.994–1.026	0.221		1.013	0.999–1.028	0.065		1.011	0.997–1.025	0.127		1.007	0.994–1.021	0.286	
MBP																
CHF	1.006	0.999–1.014	0.115	0.193	1.006	0.999–1.013	0.106	0.143	1.007	1.000–1.014	0.055	0.183	1.005	0.998–1.012	0.143	0.026
NO CHF	1.017	1.007–1.027	<0.001		1.017	1.008–1.027	<0.001		1.016	1.007–1.026	<0.001		1.019	1.009–1.029	<0.001	
Valvular disease	1.010	0.996–1.024	0.152	0.867	1.010	0.997–1.023	0.133	0.884	1.013	1.001–1.025	0.041	0.762	1.008	0.997–1.020	0.150	0.614
NO valvular disease	1.010	1.004–1.017	0.002		1.010	1.004–1.017	0.002		1.010	1.003–1.017	0.002		1.011	1.005–1.018	<0.001	
Hypertension	1.010	0.996–1.024	0.168	0.669	1.011	0.997–1.025	0.090	0.647	1.009	0.995–1.022	0.201	0.961	1.010	0.999–1.024	0.080	0.517
NO hypertension	1.008	1.002–1.014	0.016		1.008	1.001–1.014	0.016		1.009	1.002–1.015	0.006		1.008	1.002–1.015	0.011	
βRBs	1.005	0.994–1.015	0.391	0.472	1.006	0.996–1.016	0.227	0.637	1.009	1.000–1.018	0.039	0.931	1.010	1.003–1.018	0.007	0.591
NO βRB	1.013	1.004–1.021	0.004		1.012	1.004–1.021	0.005		1.011	1.002–1.019	0.011		1.010	1.002–1.018	0.019	
Statins	1.021	1.005–1.036	0.010	0.099	1.014	1.000–1.027	0.045	0.235	1.015	1.002–1.029	0.027	0.150	1.017	1.004–1.030	0.010	0.087
NO Statins	1.007	1.000–1.013	0.046		1.007	1.001–1.014	0.026		1.007	1.001–1.014	0.025		1.007	1.001–1.013	0.028	
Nitrates	0.991	0.970–1.011	0.421	0.014	0.993	0.975–1.013	0.484	0.013	0.995	0.978–1.013	0.609	0.007	0.984	0.967–1.003	0.093	<0.001
NO Nitrates	1.014	1.007–1.020	<0.001		1.014	1.007–1.020	<0.001		1.014	1.007–1.021	<0.001		1.018	1.010–1.025	<0.001	
Warfarin	1.015	0.973–1.058	0.499	0.843	1.005	0.973–1.038	0.779	0.694	1.003	0.972–1.036	0.831	0.726	0.984	0.956–1.014	0.292	0.775

(Continued)

TABLE 5 | Continued

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*	OR	95% CI	*P*	*P#*
NO Warfarin	1.010	1.004–1.016	<0.001		1.010	1.004–1.016	<0.001		1.010	1.004–1.016	<0.001		1.011	1.005–1.017	<0.001	
Heparin	0.997	0.987–1.008	0.627	0.003	0.998	0.988–1.008	0.681	0.003	1.000	0.990–1.009	0.927	0.004	1.001	0.993–1.010	0.739	0.021
NO Heparin	1.019	1.010–1.027	<0.001		1.018	1.010–1.027	<0.001		1.018	1.010–1.027	<0.001		1.016	1.008–1.024	<0.001	
SBP																
CHF	0.988	0.973–1.004	0.136	0.069	0.991	0.977–1.005	0.212	0.066	0.995	0.983–1.008	0.492	0.118	1.004	0.992–1.015	0.538	0.191
NO CHF	1.010	0.998–1.021	0.098		1.009	0.998–1.020	0.118		1.008	0.997–1.019	0.138		1.010	1.001–1.019	0.026	
Valvular disease	1.016	0.993–1.039	0.172	0.230	1.014	0.994–1.033	0.166	0.211	1.009	0.990–1.027	0.361	0.616	1.010	0.996–1.024	0.179	0.878
NO valvular disease	0.998	0.988–1.008	0.674		0.999	0.989–1.008	0.774		1.002	0.992–1.011	0.740		1.007	0.999–1.015	0.087	
Hypertension	1.006	0.991–1.022	0.402	0.125	1.007	0.993–1.022	0.307	0.215	1.005	0.992–1.019	0.429	0.371	1.010	0.999–1.021	0.085	0.277
NO hypertension	0.996	0.985–1.008	0.521		0.997	0.986–1.008	0.593		1.000	0.990–1.011	0.947		1.005	0.996–1.015	0.278	
βRBs	1.006	0.993–1.018	0.389	0.086	1.007	0.996–1.019	0.218	0.108	1.010	0.999–1.021	0.062	0.051	1.013	1.003–1.022	0.007	0.056
NO βRB	0.993	0.980–1.007	0.346		0.992	0.979–1.006	0.276		0.991	0.978–1.004	0.173		0.999	0.988–1.011	0.904	
Statins	1.008	0.981–1.035	0.571	0.401	1.002	0.978–1.026	0.883	0.613	1.001	0.980–1.023	0.914	0.788	1.015	1.000–1.031	0.049	0.180
NO Statins	0.998	0.988–1.008	0.683		0.999	0.990–1.009	0.895		1.001	0.992–1.010	0.847		1.004	0.996–1.012	0.325	
Nitrates	1.001	0.981–1.021	0.918	0.977	1.002	0.984–1.020	0.859	0.911	1.002	0.985–1.018	0.848	0.814	0.993	0.978–1.008	0.364	0.019
NO Nitrates	1.002	0.991–1.012	0.750		1.002	0.992–1.012	0.758		1.003	0.993–1.012	0.548		1.012	1.004–1.020	0.003	
Warfarin	0.954	0.827–1.099	0.513	0.474	1.026	0.963–1.094	0.424	0.468	1.029	0.970–1.091	0.339	0.405	1.016	0.972–1.063	0.484	0.731
NO Warfarin	1.001	0.992–1.010	0.782		1.002	0.993–1.010	0.713		1.003	0.995–1.011	0.472		1.008	1.001–1.015	0.023	
Heparin	0.997	0.984–1.011	0.689	0.519	0.998	0.985–1.011	0.738	0.613	0.999	0.987–1.011	0.880	0.343	1.002	0.991–1.012	0.770	0.180
NO Heparin	1.002	0.989–1.015	0.806		1.002	0.989–1.014	0.781		1.004	0.993–1.015	0.489		1.009	1.000–1.019	0.055	

Adjusted for age, gender, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes and malignant.

P#: P-value for interaction.

DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure; βRB, βreceptor blockers; CHF, chronic heart failure; CKD, chronic kidney disease.

TABLE 6 | Multiple logistic regression analysis for relationship between between BP (≤55/70/110 mmHg) and mortality by stratified analysis.

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	95% CI	*P* Value	*P#*	OR	95% CI	*P* Value	*P#*	OR	95% CI	*P* Value	*P#*	OR	95% CI	*P* Value	*P#*
DBP																
CHF	0.963	0.947–0.980	<0.001	0.746	0.969	0.953–0.985	<0.001	0.755	0.969	0.953–0.985	<0.001	0.807	0.976	0.960–0.992	0.003	0.204
NO CHF	0.966	0.946–0.987	0.002		0.964	0.944–0.985	<0.001		0.965	0.946–0.986	<0.001		0.960	0.940–0.981	<0.001	
Valvular disease	0.957	0.929–0.986	0.004	0.550	0.964	0.937–0.991	0.010	0.824	0.969	0.943–0.996	0.023	0.980	0.980	0.956–1.005	0.103	0.342
NO valvular disease	0.967	0.953–0.982	<0.001		0.967	0.953–0.982	<0.001		0.967	0.953–0.981	<0.001		0.966	0.951–0.981	<0.001	
Hypertension	0.952	0.930–0.974	<0.001	0.080	0.951	0.929–0.973	<0.001	0.039	0.951	0.929–0.973	<0.001	0.026	0.943	0.920–0.968	<0.001	0.002
NO hypertension	0.972	0.956–0.989	<0.001		0.977	0.961–0.993	0.005		0.977	0.962–0.993	0.005		0.986	0.970–1.002	0.077	
βRBs	0.982	0.961–1.003	0.087	0.095	0.982	0.962–1.001	0.068	0.116	0.981	0.963–1.000	0.044	0.130	0.981	0.964–0.998	0.026	0.158
NO βRB	0.951	0.930–0.972	<0.001		0.954	0.933–0.975	<0.001		0.955	0.934–0.976	<0.001		0.961	0.940–0.983	<0.001	
Statins	0.948	0.914–0.983	0.004	0.086	0.954	0.922–0.987	0.007	0.226	0.949	0.919–0.979	<0.001	0.062	0.971	0.945–0.999	0.040	0.764
NO Statins	0.971	0.957–0.985	<0.001		0.971	0.957–0.986	<0.001		0.974	0.960–0.988	<0.001		0.974	0.960–0.989	<0.001	
Nitrates	0.989	0.945–1.035	0.625	0.102	0.992	0.949–1.037	0.726	0.075	0.987	0.948–1.028	0.532	0.103	0.977	0.945–1.009	0.161	0.233
NO Nitrates	0.961	0.947–0.975	<0.001		0.963	0.949–0.977	<0.001		0.964	0.950–0.977	<0.001		0.967	0.953–0.981	<0.001	
Warfarin	–	–	–	–	1.025	0.887–1.183	0.740	0.283	1.026	0.899–1.172	0.702	0.300	0.971	0.913–1.033	0.354	0.966
NO Warfarin	0.965	0.952–0.978	<0.001		0.966	0.953–0.978	<0.001		0.966	0.954–0.979	<0.001		0.970	0.957–0.982	<0.001	
Heparin	0.995	0.973–1.017	0.653	<0.001	0.993	0.973–1.014	0.531	<0.001	0.994	0.974–1.014	0.564	<0.001	0.997	0.977–1.016	0.731	<0.001
NO Heparin	0.947	0.931–0.965	<0.001		0.950	0.934–0.967	<0.001		0.950	0.934–0.967	<0.001		0.952	0.934–0.970	<0.001	
MBP																
CHF	0.985	0.961–1.009	0.227	<0.001	0.989	0.965–1.013	0.357	<0.001	0.979	0.956–1.002	0.070	0.001	0.976	0.952–1.000	0.051	0.046
NO CHF	0.913	0.877–0.950	<0.001		0.910	0.874–0.948	<0.001		0.910	0.875–0.947	<0.001		0.935	0.904–0.967	<0.001	
Valvular disease	0.984	0.944–1.025	0.440	0.176	0.987	0.948–1.027	0.513	0.134	0.985	0.949–1.022	0.417	0.051	0.988	0.955–1.022	0.487	0.052
NO valvular disease	0.951	0.928–0.975	<0.001		0.952	0.929–0.976	<0.001		0.942	0.917–0.968	<0.001		0.946	0.920–0.972	<0.001	
Hypertension	0.941	0.910–0.973	<0.001	0.079	0.941	0.910–0.974	<0.001	0.064	0.948	0.918–0.979	0.001	0.524	0.933	0.900–0.966	<0.001	0.025
NO hypertension	0.966	0.940–0.992	0.011		0.974	0.951–0.999	0.039		0.959	0.933–0.985	0.003		0.979	0.954–1.005	0.110	
βRBs	0.973	0.947–1.000	0.047	0.358	0.974	0.948–1.000	0.050	0.431	0.963	0.938–0.989	0.005	0.754	0.963	0.937–0.989	0.005	0.914
NO βRB	0.947	0.914–0.981	0.003		0.951	0.919–0.985	0.005		0.953	0.921–0.986	0.006		0.967	0.936–0.999	0.042	
Statins	0.993	0.906–1.090	0.890	0.758	0.987	0.908–1.074	0.769	0.866	0.987	0.908–1.074	0.769	0.555	0.987	0.940–1.037	0.602	0.665
NO Statins	0.965	0.945–0.985	<0.001		0.967	0.947–0.987	0.002		0.965	0.945–0.985	<0.001		0.968	0.947–0.990	0.004	
Nitrates	0.985	0.937–1.037	0.567	0.143	0.985	0.937–1.037	0.567	0.167	0.959	0.918–1.003	0.068	0.610	0.881	0.802–0.968	0.008	0.092
NO Nitrates	0.949	0.927–0.972	<0.001		0.953	0.930–0.975	<0.001		0.952	0.930–0.975	<0.001		0.966	0.944–0.988	0.003	

(Continued)

TABLE 6 | Continued

Variables	Hospital mortality				7-day mortality				30-day mortality				1-year mortality			
	OR	**95% CI**	***P* Value**	***P#***	**OR**	**95% CI**	***P* Value**	***P#***	**OR**	**95% CI**	***P* Value**	***P#***	**OR**	**95% CI**	***P* Value**	***P#***
Warfarin	–	–	–	–	1.051	0.838–1.316	0.668	0.254	1.058	0.838–1.336	0.635	0.177	0.958	0.876–1.048	0.351	0.986
NO Warfarin	0.959	0.939–0.979	<0.001		0.960	0.940–0.979	<0.001		0.952	0.932–0.973	<0.001		0.958	0.938–0.979	<0.001	
Heparin	0.973	0.942–1.005	0.095	0.057	0.983	0.955–1.013	0.266	0.057	0.961	0.932–0.992	0.014	0.540	0.970	0.940–1.001	0.059	0.403
NO Heparin	0.944	0.918–0.971	<0.001		0.945	0.919–0.972	<0.001		0.951	0.925–0.976	<0.001		0.952	0.926–0.979	<0.001	
SBP																
CHF	0.982	0.974–0.990	<0.001	0.493	0.984	0.976–0.992	<0.001	0.218	0.984	0.976–0.993	<0.001	0.243	0.988	0.980–0.996	0.003	0.013
NO CHF	0.976	0.965–0.987	<0.001		0.973	0.963–0.984	<0.001		0.974	0.964–0.985	<0.001		0.967	0.955–0.980	<0.001	
Valvular disease	0.973	0.959–0.988	<0.001	0.240	0.975	0.962–0.989	<0.001	0.368	0.979	0.965–0.993	0.001	0.661	0.984	0.971–0.997	0.006	0.809
NO valvular disease	0.982	0.975–0.989	<0.001		0.982	0.975–0.989	<0.001		0.982	0.974–0.989	<0.001		0.980	0.972–0.988	<0.001	
Hypertension	0.976	0.965–0.988	<0.001	0.384	0.973	0.962–0.985	<0.001	0.161	0.973	0.962–0.985	<0.001	0.093	0.964	0.950–0.979	<0.001	0.003
NO hypertension	0.981	0.973–0.989	<0.001		0.984	0.976–0.992	<0.001		0.985	0.977–0.993	<0.001		0.989	0.981–0.998	0.012	
βRBs	0.989	0.979–1.000	0.040	0.095	0.988	0.978–0.998	0.016	0.086	0.988	0.978–0.997	0.009	0.170	0.987	0.979–0.996	0.005	0.089
NO βRB	0.969	0.957–0.982	<0.001		0.971	0.959–0.984	<0.001		0.973	0.961–0.986	<0.001		0.975	0.963–0.988	<0.001	
Statins	0.974	0.957–0.991	0.003	0.208	0.974	0.959–0.989	0.004	0.387	0.974	0.959–0.989	<0.001	0.137	0.982	0.968–0.996	0.011	0.978
NO Statins	0.982	0.975–0.989	<0.001		0.982	0.974–0.989	<0.001		0.984	0.976–0.991	<0.001		0.982	0.974–0.990	<0.001	
Nitrates	0.986	0.966–1.006	0.168	0.172	0.987	0.967–1.008	0.232	0.140	0.984	0.966–1.002	0.090	0.309	0.984	0.967–1.001	0.063	0.422
NO Nitrates	0.978	0.971–0.985	<0.001		0.978	0.971–0.985	<0.001		0.979	0.972–0.986	<0.001		0.979	0.972–0.987	<0.001	
Warfarin	–	–	–	–	1.020	0.902–1.152	0.756	0.293	1.022	0.903–1.156	0.731	0.273	0.972	0.940–1.005	0.105	0.590
NO Warfarin	0.980	0.974–0.987	<0.001		0.980	0.973–0.987	<0.001		0.981	0.974–0.987	<0.001		0.981	0.974–0.988	<0.001	
Heparin	0.991	0.981–1.001	0.093	0.301	0.991	0.981–1.001	0.083	0.349	0.992	0.982–1.002	0.100	0.121	0.994	0.984–1.004	0.211	0.600
NO Heparin	0.972	0.963–0.981	<0.001		0.973	0.964–0.982	<0.001		0.973	0.965–0.982	<0.001		0.971	0.960–0.981	<0.001	

Adjusted for age, gender, CHF, valvular disease, stroke, CKD, chronic bronchitis, depression, diabetes and malignant.

P#: P-value for interaction.

DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, arterial systolic blood pressure; βRB, βreceptor blockers; CHF, chronic heart failure; CKD, chronic kidney disease.

CONCLUSIONS

We identified U-shaped associations between BP and in-hospital/all-cause mortality in ICU patients with AF. The BP levels with the lowest mortality risks were 110, 70, and 55 for SBP, MBP, and DBP, respectively. This study demonstrated that increased BP values when SBP >110, MBP >70, or DBP >55 mmHg are associated with a higher risk of all-cause mortality. In contrast, mortality risk declines with increasing BP when SBP ≤110, MBP ≤70, or DBP ≤55.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Boards (IRB) of BIDMC and MIT approved the project and informed consents were exempted due to all patients' data were anonymized before the data were obtained.

AUTHOR CONTRIBUTIONS

YS is responsible for the data analysis and writing. JH is responsible for the supervision and revision. Both authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.866260/full#supplementary-material

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REVIEW
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Hypertensive Disorders of Pregnancy and Cardiovascular Disease Risk Across Races and Ethnicities: A Review

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Pregnancy is often considered to be a "cardiometabolic stress-test" and pregnancy complications including hypertensive disorders of pregnancy can be the first indicator of increased risk of future cardiovascular disease. Over the last two decades, more evidence on the association between hypertensive disorders of pregnancy and cardiovascular disease has become available. However, despite the importance of addressing existing racial and ethnic differences in the incidence of cardiovascular disease, most research on the role of hypertensive disorders of pregnancy is conducted in white majority populations. The fragmented knowledge prohibits evidence-based targeted prevention and intervention strategies in multi-ethnic populations and maintains the gap in health outcomes. In this review, we present an overview of the evidence on racial and ethnic differences in the occurrence of hypertensive disorders of pregnancy, as well as evidence on the association of hypertensive disorders of pregnancy with cardiovascular risk factors and cardiovascular disease across different non-White populations, aiming to advance equity in medicine.

Keywords: hypertensive disorders of pregnancy, preeclampsia, cardiovascular disease, hypertension, diabetes, ethnicity, chronic kidney disease, dyslipidemia

INTRODUCTION

Cardiovascular diseases (CVD) are the number one cause of death globally, with 17.9 million deaths in 2016, representing 31% of all global deaths (1). There are significant differences between women and men in terms of prevalence, presentation, treatment, effects, and prognosis of CVD (2). CVD are diagnosed less often and treated less aggressively in women than in men, likely in

part due to practitioners missing the knowledge on the specific risks of women (3, 4). An important non-traditional risk factor for CVD, unique to women, is a history of pregnancy complications, especially hypertensive disorders of pregnancy (HDP) (5).

HDP complicate up to 6–8% of all pregnancies and are a leading cause of maternal and perinatal mortality and morbidity worldwide (6). Registration studies and systematic reviews have consistently shown that women with a history of HDP are at increased risk of subsequent CVD (7–15). HDP are often part of a placental syndrome that is associated with endothelial dysfunction, insulin resistance, oxidative stress, inflammatory activation, and dyslipidemia, all of which may remain in the postpartum period and contribute to an increase in CVD risk (16). Alternatively, it is hypothesized that HDP and future CVD risk are caused by common underlying factors and pregnancy can be seen as a cardiometabolic stress test, potentially identifying those at high CVD risk later in life (16). At highest risk of future CVD are those after the early onset of HDP, with severe and/or recurrent disease (16). The increased CVD risk may be present immediately after pregnancy and persist for more than 20 years (17).

There is substantial heterogeneity in the burden of HDP and CVD across different racial and ethnic (sub) populations, with some disproportionally affected compared to others (18–20). Yet, by far most of the research on the association between HDP and CVD has been conducted in white majority populations (18). A recent review identified that similar to the male-female disparity in research, a disparity exists in the attention to ethnicity: White women are heavily overrepresented in current studies, while there is limited and heterogeneous reporting of race and ethnicity information. Additionally, the potential interaction between race and ethnicity and other sociodemographic variables is not investigated in most studies (18). The few studies that were conducted in multi-ethnic populations and investigated how race and ethnicity interact with HDP on the CVD risk after pregnancy showed contradictory results (21–24).

The lack of research on and understanding of the role of race and ethnicity in HDP-related CVD risk prohibits evidence-based targeted prevention, monitoring, and intervention strategies in multi-ethnic populations and maintains the gap in health outcomes. The aim of this review is to present an overview of the evidence on racial and ethnic differences in the occurrence of HDP, as well as evidence on the association between HDP, cardiovascular risk factors, and CVD later in life in different racial and ethnic (sub) population, aiming to advance equity in medicine.

RACE AND ETHNICITY: DEFINITIONS AND LIMITATIONS

Important sensitivities and controversies related to use of the terms race, ethnicity and associated nomenclature exist in medical and health research, clinical practice, and society. We agree with Flanagin et al. that "terminology, usage, and word choice are critically important, especially when describing people and when discussing race and ethnicity" (25). In this review, we follow the JAMA guidance for Reporting Race and Ethnicity in Research Articles (26). We chose to use the aggregated "race and ethnicity," acknowledging that there are numerous subcategories within race and ethnicity (26). When addressing race and ethnicity, we refer to it as a social construct, that is applied to compare different groups based on a given socio-cultural or physical characteristic. When describing and comparing the results of included original studies, we use racial and ethnic categories as they have been applied in the original articles.

SEARCH STRATEGY, SELECTION CRITERIA, AND DATA EXTRACTION

An extensive systematic literature review was conducted to identify all relevant studies reporting on HDP and CVD risk following HDP in non-White subgroups and populations. We systematically searched PubMed and Embase from inception to February 2022. The full search strategy is available in **Supplementary Tables 1, 2**. Reference tracing was performed to identify additional studies of interest. Titles and abstracts of all identified studies were screened, after which potentially useful records were reviewed in full. Studies were included if they met the following inclusion criteria: (i) original research, (ii-a) reporting on the incidence, prevalence, or risk of HDP, HDP severity, or HDP-related complications, or (ii-b) reporting on the incidence, prevalence, or risk of CVD and CVD risk factors at least 6 weeks after a pregnancy complicated by HDP, (iii-a) in at least two different racial or ethnic groups, or (iii-b) in non-White (sub) populations. Data on study characteristics and outcomes were extracted from the included studies. **Supplementary Tables 3, 4** provide an overview of all included studies and relevant characteristics. **Figures 1–3** provide visual representations of point estimates for relative risk (RR, OR, or HR) of different HDP, CVD risk factors and CVD reported in the included studies among different racial and ethnic groups. Studies that did not report a measure of relative risk are not included in the figures. Study quality and precision of the estimates were not accounted for in the figure, and it should thus be interpreted as an overview of the available evidence, not as a formal statistical summary.

DEFINITIONS OF HYPERTENSIVE DISEASES IN PREGNANCY

Over the years and across countries, many different definitions and criteria have been used for HDP, although the cutoff for blood pressure to classify hypertension has been consistent. For this review, we followed the ISSHP 2018 classification (27). Where possible, we converted terminology used by original authors to fit the ISSHP classification. Hypertension is defined as a systolic blood pressure (BP) $\geq$140 mmHg and/or diastolic BP $\geq$ 90 mmHg. Chronic hypertension refers to high BP predating the index pregnancy or recognized before 20 weeks of gestation. Transient gestational hypertension is *de novo*

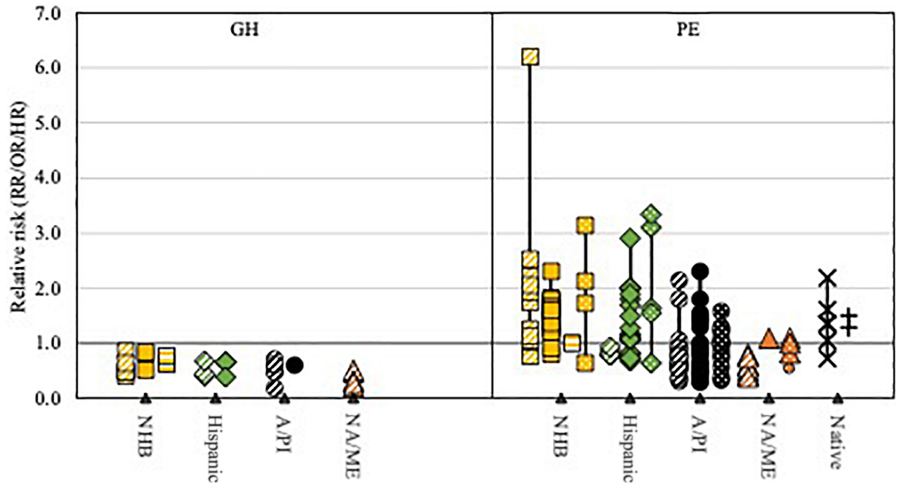

FIGURE 1 | Visual representation of point estimates for relative GH and PE risk reported in the included studies among different racial and ethnic groups compared to non-Hispanic White women. ▫ Non-Hispanic Black, African American or Black women; ◇ Latina or Hispanic women; ● Asian or Pacific Islander women; △ North African or Middle Eastern women (NA/ME); × American Indian/Alaska Native women; + Aboriginal/Torres Strait Islander or Maori women; ▨ living in Europe; ▪ living in the US; ⊟ living in South Africa; ▩ living in another predominantly White country. Study quality and precision of the estimates were not accounted for in the figure, and it should thus be interpreted as an overview of the available evidence, not as a formal statistical summary.

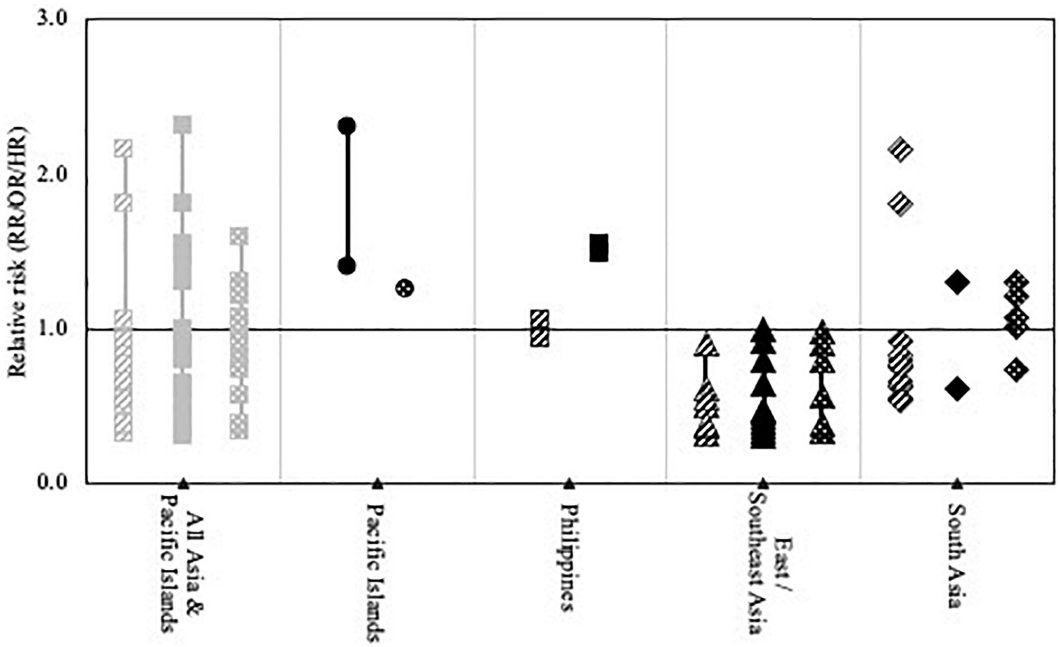

FIGURE 2 | Visual representation of point estimates for relative PE risk reported in the included studies among women of Asian and Pacific Islander origin compared to non-Hispanic White women. ▨ Asian or Pacific Islander women living in Europe; ▪ living in the US; ⊟ living in South Africa; ▩ living in another predominantly White country. Study quality and precision of the estimates were not accounted for in the figure, and it should thus be interpreted as an overview of the available evidence, not as a formal statistical summary.

hypertension that develops at any gestation that resolves without treatment during pregnancy. Gestational hypertension (GH) is persistent *de novo* hypertension that develops at or after 20 weeks' gestation in the absence of features of preeclampsia. Preeclampsia (PE) is GH accompanied by ≥1 of the following new-onset conditions at or after 20 weeks' gestation: (i) abnormal proteinuria (urine protein/creatinine ratio ≥30 mg/mmol), (ii) maternal organ dysfunction, or (iii) uteroplacental dysfunction (e.g., fetal growth restriction). PE superimposed on chronic hypertension (superimposed PE) is diagnosed if a woman with chronic hypertension develops *de novo* proteinuria or organ dysfunction consistent with

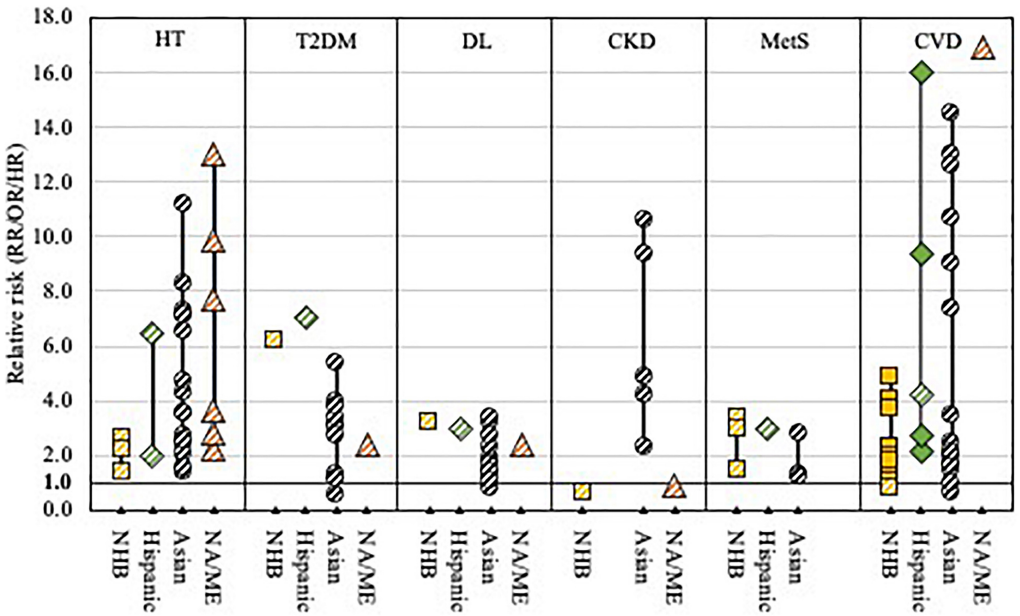

FIGURE 3 | Visual representation of point estimates for CVD risk and CVD risk factors reported in the included studies across races and ethnicities. ■ Non-Hispanic Black, African American or Black women; ◆ Latina or Hispanic women; ○ Asian women; ▲ North African or Middle Eastern women (NA/ME); ▨ living in country of origin; ■ living in the US. Note: study quality and precision of the estimates were not accounted for in the figure, and it should thus be interpreted as an overview of the available evidence, not as a formal statistical summary. HT, hypertension; T2DM, type 2 diabetes mellitus; DL, dyslipidemia; CKD, chronic kidney disease; MetS, metabolic syndrome; CVD, cardiovascular disease.

preeclampsia. Eclampsia and HELLP syndrome are considered part of the preeclampsia spectrum and not a separate disorder. In this review, we use HDP as an umbrella term for all the above-defined disorders.

HYPERTENSIVE DISORDERS OF PREGNANCY ACROSS RACIAL AND ETHNIC GROUPS

We identified n = 53 studies that reported on the prevalence of HDP, or on HDP severity and HDP-related complications across different racial and ethnic groups (**Supplementary Figure 1** and **Supplementary Table 3**) (28–80). Different classifications were used to describe race and ethnicity. To demonstrate this, for black women in America, classifications used the term non-Hispanic Black, African American, and Black. Race and ethnicity were determined in 28% by self-report, in 4% by researcher, in 39% based on database/electronic health reports, and in 30% based on maternal country of birth. Definitions of different types of HDP were not always provided and varied across studies (**Supplementary Table 3**).

Hypertensive Disorders of Pregnancy

Six studies reported on the overall prevalence of HDP (**Supplementary Table 5**) (40, 45, 54, 69, 72, 76). HDP prevalence among non-Hispanic Black, African American, and Black women in the United States of America (US) varied from 3.3 to 15.8% (40, 45, 54, 72). Two studies reported significantly increased HDP prevalence among non-Hispanic Black women compared to non-Hispanic White women (OR 1.3, OR other study NR), while two other studies reported no significant difference compared to White women and women of other race or ethnicity. The prevalence of HDP was higher among US-born non-Hispanic Black women (10.3%) than among foreign-born non-Hispanic Black women (7.1%) (40). Among women with Sub-Saharan African origin in Finland, HDP prevalence was 4.2%, which was significantly lower than among Finish women (4.6%; adjusted RR 0.84) (76).

Among Hispanic women in the US HDP prevalence was 4.5–9.1%, which was lower than among US non-Hispanic White women in both studies (40, 54). The prevalence of HDP was higher among US-born Hispanic women (5.3–6.2%) than among foreign-born Hispanic women (4.4–5.9%), except in Puerto Rican women (40). Similarly, HDP prevalence among Latin American women in Finland (2.2%) was significantly lower than among Finnish women (4.6%; adjusted RR 0.52) (76).

Among Chinese, Japanese, Korean, Vietnamese, and Asian-Indian women in the US and/or Australia, significantly lower HDP prevalence was found compared to non-Hispanic White US or Australian-born women (40, 69). Prevalence was higher in those born in the US compared to foreign-born women living in the US (40). Similarly, women from South and East Asian in Finland had significantly lower HDP prevalence (adjusted RR 0.33–0.63) (76). Among Filipino, Samoan and American Indian/Alaska Native (AI/AN) women in the US HDP prevalence was significantly higher than among non-Hispanic White US women (40, 69).

Prevalence of HDP was significantly lower among women from the Middle East and North African in Finland compared to Finish women (76).

Chronic Hypertension Before or in Pregnancy

Sixteen studies reported on the prevalence of chronic hypertension (**Supplementary Table 5**) (36, 38, 40, 42, 43, 45, 47–49, 51, 61, 67, 71, 72, 75, 78). Eleven of these studies reported on the prevalence of chronic hypertension among non-Hispanic Black, Black, or African American women in the US (36, 40, 42, 43, 45, 49, 67, 71, 72, 75, 78). In most studies, the prevalence of chronic hypertension in these women ranged between 0.8 and 3.3% and was 1.4–2.3-fold higher than among non-Hispanic White women (36, 40, 43, 45, 49, 67, 71, 72, 78). In one high-risk cohort that was oversampled with women who delivered preterm, a higher chronic hypertension prevalence was found among non-Hispanic Black women (7.8%). However, chronic hypertension prevalence among non-Hispanic White women was similarly increased, resulting in a relative risk for non-Hispanic Black women that was comparable to the other studies (75). The prevalence of chronic hypertension was higher among US-born non-Hispanic Black women than among foreign-born non-Hispanic Black women (43, 75). Among African Caribbean women living in the United Kingdom the incidence of chronic hypertension was 3.3%, significantly higher than among Caucasian women in the United Kingdom (adjusted OR 3.1) (48). In South Africa, the prevalence of chronic hypertension was higher in Black (1.1%) and Colored (1.7%) women compared to White women (0.6%) (61).

Six studies reported on the prevalence of chronic hypertension among Latina or Hispanic women in the US (36, 40, 43, 67, 71, 75). The chronic hypertension prevalence ranged from 0.7 to 1.6%; in a high-risk cohort, oversampled with women who delivered preterm, CH prevalence was 2.5–2.8% (36, 40, 43, 67, 71, 75). The prevalence of chronic hypertension in most Latina/Hispanic groups was similar to, or lower than in non-Hispanic Black and non-Hispanic White women. One study reported lower rates of chronic hypertension among foreign-born compared to US-born Latina or Hispanic women in the US, except in Puerto Rican women; a second study reported no significant difference (43, 75).

Five studies reported the prevalence of chronic hypertension among women from Asian and Pacific Islander (A/PI) origin living in the US (40, 43, 47, 67, 71). Chronic hypertension prevalence ranged from 0.1 to 2.3%. Rates were generally lower than among non-Hispanic White women, except among Filipino and Samoan women, who had increased rates of chronic hypertension. Lowest rates were described among Chinese, Korean, and Asian Indian women. Rates were lower among A/PI women who were born outside of the US compared to US-born A/PI women (43). Lower chronic hypertension rates were reported among Vietnam-born women living in Australia (38). One study found increased rates of chronic hypertension among South Asian women in the United Kingdom compared to Caucasian women (OR 1.9) (48). Rates among A/PI, Native Hawaiian, and White women living in Hawaii were low and similar across groups (0.1–0.3%) (51).

Among AI/AN women in the US, chronic hypertension prevalence was 1.4–1.5-fold increased in two studies compared to non-Hispanic White women (40, 71).

Gestational Hypertension and/or Preeclampsia

Eleven studies reported on the combined prevalence of GH and PE (**Supplementary Table 5**) (31, 32, 40, 43, 45, 46, 53, 59, 62, 66, 71). Among US non-Hispanic Black or African American women, GH/PE prevalence ranged from 2.9 to 10.5% (40, 43, 45, 53, 59, 62, 66, 71). In most studies, GH/PE prevalence was slightly higher among these women than among non-Hispanic White women (2.9–9.2%). Both studies that statistically tested the difference found a significantly higher rate of GH/PE among non-Hispanic Black or African American women (adjusted RR/OR 1.3), although in one study no difference was found when the analysis was limited to overweight and obese women (59, 62). Similar to all HDP, the prevalence of GH/PE was higher among US-born non-Hispanic Black women (10.5%) than among foreign-born non-Hispanic Black women (7.1%) (43).

GH/PE prevalence was lower among Hispanic women (1.3–7.8%) than among non-Hispanic White women in the US (31, 40, 43, 53, 59, 66, 71). The difference was statistically significant in two studies, although in one study only in overweight or obese women (59, 66). The rate of GH/PE was lower among foreign-born than among US-born Hispanic or Latina women, expect in Puerto Rican women, where high rates of GH/PE were found in both foreign and US-born women (43).

The three studies comparing GH/PE prevalence between A/PI women and non-Hispanic White women in the US showed lower rates among A/PI women (2.4–6.0%), except in Filipino (5.9–8.1%) and Samoan women (6.8%) (40, 43, 71). Foreign-born A/PI women had lower GH/PE rates than US-born A/PI women (43). Two other studies compared rates of GH/PE among different Asian subgroups (32, 46). They reported the lowest GH/PE rates among East Asian and Southeast Asian women (1.1–1.8%), and higher rates among Filipino (2.9–6.3%), South Asian (1.8–3.3%), and Pacific Island women (2.3–4.8%) (32, 46).

One study reported higher rates of GH/PE among non-Hispanic American Indian women, compared to non-Hispanic White women in the US (5.3% vs. 4.5%) (71).

Gestational Hypertension

Nine studies reported on the prevalence of GH (**Supplementary Table 5**) (33, 36, 39, 45, 61, 67, 72, 74, 76). **Figure 1** provides a visual overview of the GH risk among different non-White populations compared to non-Hispanic White women reported in these studies. In three US studies, lower rates of GH were reported in non-Hispanic Black or Black women (1.3–3.6%) compared to non-Hispanic White or White women (2.0–4.5%) (36, 67, 72). Among women from Sub-Saharan origin in Finland and Norway, GH rate was significantly lower than among Finish or Norwegian women (ORs 0.5) (39, 76). Women from Surinamese-Creoles (3.2%), Cape Verdean (3.2%), and Antillean

origin (2.9%) in the Netherlands had lower rates of GH than Dutch women (5.2%), but the difference was not statistically significant (74). In South Africa, the overall prevalence of GH was high, with highest rates among White women (14.1%) and lowest rates among Black women (8.8%) (61).

The prevalence of GH varied from 1.2 to 2.4% among Hispanics in the US, significantly lower than among non-Hispanic White or Caucasian women in two studies (adjusted OR 0.6, adjusted RR 0.4) (33, 36, 67). GH prevalence was also significantly lower among Latin American/Caribbean women in Finland and Norway compared to Finish and Norwegian women (adjusted RR 0.4, adjusted OR 0.5–0.7) (39, 76).

The prevalence of GH among A/PI women was 1.7% (one study), significantly lower than among non-Hispanic White women (adjusted OR 0.6) (20, 67). Prevalence of GH was also significantly lower among in South Asian (1.1–1.6%, adjusted OR 0.6–0.7) and East Asians (0.3–1.2%, adjusted OR 0.5–0.6) compared to Finish (2.3%) and Norwegian women (1.5–2.4%) (39, 76). Women of Oceanian origin in Norway had similar GH rates as Norwegian women (39). Among Surinamese-Hindustani women in the Netherlands, GH prevalence was 3.4%, compared to 5.2% in Dutch women (not significant) (74).

Women from Middle Eastern or North African origin in Finland (0.6%) and Norway (0.8–1.1%) had significantly lower GH rates compared to Finish (adjusted RR 0.2) and Norwegian women (adjusted OR 0.5) (39, 76). Turkish (1.7%) and Moroccan (1.5%) women in the Netherlands had significantly lower GH rates than Dutch women (adjusted ORs 0.3) (74).

Preeclampsia

Thirty-four studies reported on the prevalence of PE (**Supplementary Table 5**) (29, 30, 33–39, 41, 42, 44, 45, 47, 49–52, 55, 56, 58, 60, 61, 63, 65, 67, 70, 72–77, 80). **Figure 1** provides a visual overview of the PE risk among different non-White populations compared to non-Hispanic White women reported in these studies. The prevalence of PE was significantly higher among non-Hispanic Black, Black, and African American women compared to non-Hispanic White or White women in the US in most studies (adjusted OR 1.2–2.3), ranging from 2.5 to 8.3% (29, 36, 42, 45, 49, 55, 56, 60, 63, 65, 67, 72, 73, 75). In one high-risk cohort, oversampled with women who delivered preterm, a higher PE prevalence was found among non-Hispanic Black women (9.2–12.2%), but PE prevalence among non-Hispanic White women was similarly increased, so that the relative risk for non-Hispanic Black women was comparable to the other studies (75). One study reported higher PE prevalence among US-born non-Hispanic Black women than among foreign-born non-Hispanic Black women, although after 10 years of residence in the US, the difference was no longer statistically significant (75). Another study found no difference between US-born and foreign-born non-Hispanic Black women (65). Among women from Sub-Saharan Africa in Canada, risk of severe PE was significantly increased (0.7%, adjusted OR 3.1) compared to women from industrialized countries (34). In Israel, Ethiopian women had significantly higher rates of mild and severe PE compared to Israeli women (44). Prevalence of PE was significantly increased among women of Sub-Saharan African origin in Finland (3.0%, adjusted RR 1.8), in France (severe PE 1.6%, adjusted OR 2.5) and Australia, Canada, Spain, US, Denmark, and Sweden (2.8%, adjusted OR 1.7) compared to White native populations (35, 41, 76). Three Norwegian studies showed no significant difference in PE risk among women of Sub-Saharan African origin compared to Norwegian women, except in women from Burundi (5.9%, adjusted OR 1.8), Congo (5.9%, adjusted OR 1.9), Tanzania (7.4%, adjusted OR 2.2) and Somalia (4.0%, adjusted OR 1.3) (39, 50, 52). Among Cape Verdean women in the Netherlands, PE rate was significantly increased (4.2%, adjusted OR 2.1), while no significant difference was seen between Surinamese Creole (2.4%) and Dutch women (1.9%) (74). Among Sub-Saharan African women in Australia, prevalence of PE was significantly lower than among Australian or New Zealand born women (3.5% vs. 4.8%, adjusted OR 0.6) (80). In South Africa, no difference was seen in PE prevalence among White, Black or Colored women (2.9%) (61).

The prevalence of PE among Hispanic and Haitian women in the US ranged from 2.6 to 5.9%; in one high-risk cohort oversampled with women who delivered preterm PE prevalence was 7.9–9.1% (33, 36, 42, 49, 56, 60, 63, 65, 67, 75). In most US studies, PE rates were higher in Hispanic women than in non-Hispanic White women (adjusted OR/HR 1.1–2.9), but lower than in non-Hispanic Black women (33, 36, 56, 60, 63, 65, 67, 75). No difference was reported in PE rate among US-born and foreign-born Hispanic women in the US (65, 75). Severe PE was more prevalent among Hispanic and Caribbean women in Canada (0.6 and 0.7%; adjusted OR 2.0 and 3.3) (35). No significant difference was found in PE rate among Latin American and Caribbean women in Finland and Norway compared to Finish and Norwegian women, except for multiparous women in Norway (adjusted OR 0.8) (39, 50, 76). PE prevalence among Antillean women in the Netherlands was 3.7% compared to 1.9%, but the difference was not statistically significant (74). Prevalence of PE was significantly increased among women of Latin American and Caribbean origin in Australia, Canada, Spain, US, Denmark and Sweden (2.8% vs. 1.8%, adjusted OR 1.6) compared to White native populations (35). Another study reported 3.4% PE among Latin American and Caribbean women in Australia, which was significantly lower than among Australian or New Zealand born women (4.8%; adjusted OR 0.6) (80).

The prevalence of PE among A/PI women in the US and Hawaii ranged from 1.5 to 6.8% (47, 51, 56, 63, 65, 67, 70). **Figure 2** provides a visual overview of the PE risk among different A/PI populations compared to non-Hispanic White women reported in these studies. PE risk (1.4–3.7%) was lowest among East Asian women and significantly lower than among non-Hispanic White women in most studies (adjusted OR 0.6–0.9) (47, 51, 65, 67, 70). Among women from South Central Asia, prevalence of PE was increased (2.2%, adjusted OR 1.3) compared to non-Hispanic White women (65). Similarly, Philippine women in the US and Hawaii had significantly higher PE rates (4.0–6.8%; adjusted OR 1.6–2.8) (47, 51, 65). Among other Southeast Asian women in the US prevalence of PE (1.7–2.8%) was not significantly different from non-Hispanic White women (70). Foreign-born Southeast Asian and Pacific Island women had higher PE risks compared to US-born Southeast Asian and

Pacific Island women (65). In Canada, the risk of severe PE was significantly increased in one study among women of East Asian and Pacific origin (adjusted OR 1.6), but not in South Asian women (34). Among East Asian and Southeast Asian women in Finland, Norway, New Zealand, Australia, Canada, Spain, US, Denmark, and Sweden, PE prevalence was significantly lower than among the White populations in most studies (adjusted OR/RRs 0.3–0.9) (35, 39, 50, 52, 76, 77, 80). No significant difference was found in PE prevalence among Filipino, Indian, Myanmarese, or Oceanian women in Norway, Indian women in New Zealand, and South Asian women in Finland, Australia, Canada, Spain, US, Denmark, and Sweden, compared with White populations (35, 50, 52, 76, 77). Two other studies found significantly decreased PE rates among South Asian women in Norway and Australia (adjusted OR 0.6-0.8) (39, 80). PE prevalence was 3.8% among Surinamese-Hindustani women in the Netherlands compared to 1.9% among Dutch women, but the difference was not statistically significant (74). In Singapore, women from Malay origin had significantly higher risk of PE (4.2%) and severe PE (0.4%) than Chinese (3.5 and 0.3%) and Indian women (2.6 and 0.2%) (37, 58).

Among women of North African and Middle Eastern origin in Finland, Norway, the Netherlands, Australia, Canada, Spain, US, Denmark, and Sweden the prevalence of PE (0.6–2.7%) was similar to or lower (adjusted OR 0.3–0.6) than the PE prevalence among non-Hispanic White women (34, 35, 39, 41, 50, 52, 65, 74, 76, 80).

Significantly increased risk of PE was found among AI/AN, Native American, and Native Hawaiian women in most studies (4.0–8.9%, adjusted OR 1.1–1.4) (30, 51, 60). Among Maori women in New Zealand PE rate was significantly increased (4.7%, adjusted OR 1.5), while among Aboriginal and Torres Strait Islanders women, no difference in PE risk was found (77, 80).

Eclampsia

Nine studies reported on eclampsia prevalence separately (**Supplementary Table 5**) (28, 29, 32, 35, 40, 66–68, 71). Eclampsia occurred in 0.1–0.7% of non-Hispanic Black, Black, or African American women in the US, compared to 0.1–0.3% among non-Hispanic White women (29, 40, 66–68, 71). In two studies the risk of eclampsia was significantly higher in non-Hispanic Black women than in non-Hispanic White women; one study showed no significant difference (66–68). Among Sub-Saharan women living in the Netherlands (RR 6.2) and Australia, Canada, Spain, US, Denmark, or Sweden (0.1%, adjusted OR 2.1), risk of eclampsia was significantly elevated compared to White populations (28, 35).

Eclampsia was observed in 0.1–0.4% of Hispanic women in the US (40, 66–68, 71). Results were mixed: one study reported significantly lower rates of eclampsia among Hispanic women compared to non-Hispanic White women; one study reported higher rates of eclampsia (adjusted OR 1.3); one study reported no significant difference (66–68). A significantly higher risk of eclampsia was also described in women with Surinamese or Antillean origin in the Netherlands (RR 2.5) and in Latin American or Caribbean women (adjusted OR 1.6) in Australia, Canada, Spain, US, Denmark or Sweden (28, 35).

The prevalence of eclampsia among AP/I women in the US ranged from $<$ 0.1 to 0.5% (32, 40, 67, 68, 71). The risk of eclampsia among A/PI women did not differ significantly from non-Hispanic White women (67, 68). Among A/PI women, lowest eclampsia prevalence was seen in East Asian (0.1–0.2%), Southeast Asian (0.1%) and Asian Indian women (0.1%), while Filipino (0.2–0.3%, adjusted OR 3.0) and Pacific Island women (0.3–0.5%, adjusted OR 4.2–6.1) had significantly higher eclampsia risk compared to Chinese women (33, 40). No difference was found in eclampsia prevalence among South Asian and Southeast women in Australia, Canada, Spain, US, Denmark, or Sweden compared to White populations ($\leq$ 0.1%) (35).

Among AI/AN or Native American women, eclampsia prevalence was 0.1–0.6%, not significantly different from non-Hispanic White women in one study (40, 68, 71).

Among Moroccan and Turkish women in the Netherlands, and among North African and Middle Eastern women in Australia, Canada, Spain, US, Denmark, or Sweden, eclampsia prevalence (1.0%) was comparable to the White populations (28, 35).

Superimposed Preeclampsia

The prevalence of PE superimposed on chronic hypertension was reported in five US studies (**Supplementary Table 5**) (29, 36, 56, 67, 72). Among non-Hispanic Black, non-Hispanic African American, and Black women, superimposed PE prevalence ranged from 0.4 to 1.0% compared to 0.1–0.3% among non-Hispanic White and White women (29, 36, 56, 67, 72). One study reported a statistically significant difference (OR 2.0) (67). Among Hispanic (0.3–0.4%) and A/PI women (0.2–0.4%) in the US, superimposed PE prevalence was not significantly different from non-Hispanic White women (36, 56, 67). Non-Hispanic Black women with chronic hypertension in the UK were less likely to develop superimposed PE compared to White women with chronic hypertension in one study (13% vs. 17%) (57). Indo-Asian women with chronic hypertension were at a similar risk of developing superimposed PE as White women in the UK (19% vs. 17%) (57).

Severity and Hypertensive Disorders of Pregnancy-Related Complications

Among hypertensive women, pregnancy outcomes differed by race, with non-Hispanic Black women having the poorest outcome (**Supplementary Table 5**) (29, 42, 45, 52, 53, 55, 57, 61, 64, 70, 72, 79). Non-Hispanic Black women with PE were significantly more likely to suffer severe maternal morbidity (9.8%, adjusted OR 1.4, definition study-specific) and eclampsia (1.7%) (64). Non-Hispanic Black women with HDP or PE had 3–5-fold increased risk of maternal mortality compared to non-Hispanic White women with HDP or PE (42, 64, 79). Also, African American, Black of African Caribbean women with HDP were at significantly higher risk for intrauterine fetal death (IUFD; adjusted OR 2.5), perinatal mortality (3.8% vs. 1.6%), and neonatal morbidity (adjusted OR 1.1) (42, 55, 57). Preterm birth (PTB), low birthweight (LBW), and delivery of an infant small for gestational age (SGA) were more prevalence among

non-Hispanic Black, African American, or African Caribbean women in the US and Europe compared to White women with HDP (29, 52, 53, 57).

The risk of severe maternal morbidity (7.7% vs. 6.1%) or eclampsia (1.6% vs. 1.3%) was slightly higher in Hispanic women with PE than in non-Hispanic White women with PE, but lower than among non-Hispanic Black women (64). No differences were found in HDP-related mortality, perinatal mortality, and PTB risk in Hispanic women with HDP compared to non-Hispanic White women with GH or PE (42, 53). Hispanic women with GH or PE significantly more often had an LBW infant (adjusted OR 1.5) (53).

Severe maternal morbidity occurred in 7.5% of A/PI with PE in the US, significant more often than among non-Hispanic White women (adjusted OR 1.2) (64). One study among Indo-Asian women with chronic hypertension in the UK reported a very high perinatal mortality risk compared to White women (10% vs. 2%) (57). Two studies reported higher PTB rates among Southeast and South Asian women with PE and chronic hypertension (32.45%) compared to White women with PE or chronic hypertension; another study found no significant difference (52, 57, 70). Delivery of an LBW or SGA infant and neonatal admission >72 h was more prevalent among Southeast Asian women with PE or chronic hypertension compared to White women with PE or chronic hypertension (57, 70).

No significant difference in severe maternal morbidity was found between Native American women and non-Hispanic White women with PE (64). Prevalence of PTB among women with PE was increased in women from Afghanistan and Iraq in Norway compared to Norwegian women (52).

CARDIOVASCULAR DISEASE RISK FOLLOWING HYPERTENSIVE DISORDERS OF PREGNANCY ACROSS RACIAL AND ETHNIC (SUB) POPULATIONS

We identified n = 62 studies that reported on the incidence, prevalence, or risk of CVD and CVD risk factors after HDP in non-White subgroups and populations (**Supplementary Figure 2** and **Supplementary Table 4**) (22–24, 81–139). Most of the studies came from the Asian continent (n = 30), followed by Sub-Saharan Africa (n = 13), North Africa and the Middle East (n = 9), and South and Middle America (n = 4). One European study and five North American studies reported on Black, Non-Hispanic Black, Hispanic, and African American women. **Figure 3** provides a visual overview of the different CVD risk factors and CVD risk among different non-White populations compared to non-Hispanic White women reported in these studies.

Hypertension

The majority of the studies reported on the risk of hypertension after a pregnancy complicated by HDP (n = 42; **Supplementary Table 6A**) (81–83, 86, 87, 89–94, 97, 98, 101–106, 109–120, 122–124, 128, 132–134, 138, 139). All but one of the comparative studies showed a significantly increased risk of hypertension after pregnancy complicated by HDP, although follow-up time, absolute prevalence/incidence, and risk ratios differed substantially across different studies. Five studies among Chinese, Sudanese, Nigerian, South African, and Ugandan women reported a substantial prevalence of hypertension 6 weeks after pregnancy complicated by PE (28–36%) and GH/PE (26%) (90, 109, 113, 117, 119). Three months to 1 year after pregnancy, the prevalence of hypertension among Indian, Cameroonian, Ugandan, Cuban, and Black Dutch women with PE was 15–38%; among Kenyan women with GH/PE prevalence of hypertension was 24% (91, 101, 102, 112, 114, 120). Prevalence of hypertension was 22% 1 year after pregnancy in Nigerian women with GH, and 61% in women with PE (98).

Studies with longer follow-up times (mean 5–35 years) among Japanese (adjusted OR 2.6–7.1), Korean (RR 2.1, adjusted OR 1.53), Singaporean (adjusted RR 3.6), and Taiwanese women (adjusted HR 8.3–11.2) reported a significantly increased risk of hypertension after pregnancy complicated by GH/PE or HDP compared to women without a history of GH/PE or HDP (86, 97, 104, 106, 110, 118, 128, 133, 139). Risk of hypertension was similarly increased after GH and PE in two Taiwanese studies (97, 139). Among Brazilian women, risk of hypertension was 2–6-fold increased on average 13–15 years after GH/PE or HDP (89, 94). Significantly higher rates of hypertension (mean follow-up time 6–10 years) were also reported among Iranian women with a history of PE (adjusted HR 3.6) or GH/PE (adjusted RR 2.8), Jordanian women with history of PE (RR 13.0) or GH (RR 7.7), Pakistani women with a history of HDP (adjusted OR 2.2) and Turkish women with a history of PE (RR NR) (81, 83, 90, 93, 122–124). Among Tanzanian women, prevalence of hypertension was increased (29% vs. 13%) 5–7 years after pregnancy complicated by PE (111). Among US women, higher rates of hypertension after PE were reported in non-Hispanic Black women (21%) compared to Hispanic and Non-Hispanic White women on average 3 years postpartum (105).

Type 2 Diabetes Mellitus and Prediabetes Mellitus

Fourteen studies reported on type 2 diabetes mellitus (T2DM), four on T2DM or prediabetes (**Supplementary Table 6B**) (83, 86, 88, 92–94, 97, 103, 104, 110, 118, 120, 126, 129, 131, 133, 138, 139). Mean length of follow-up varied from 2.6 to 30.7 years. Most studies (n = 11) showed significantly increased rates of T2DM or prediabetes after HDP. Five studies from Taiwan reported significantly higher incidence rates of T2DM after GH/PE (adjusted HR 2.7–3.4), after PE (adjusted HR 3.1–5.4), and after GH (adjusted HR 3.3) compared to normotensive pregnancies (97, 103, 129, 131, 139). Studies from India (33%) and Indonesia (16% after early onset PE; 23.5% after late-onset PE) showed high rates of T2DM 5–10 years after PE, but no comparison group was available (88, 92). Studies from Brazil (RR 7.1) and Iran (adjusted RR 2.4) showed significantly higher T2DM rates after GH/PE (93, 94). Among Japanese (4 studies)

and Turkish women (1 study), both with and without HDP, the reported rates of T2DM were substantially lower and no significant association between HDP and T2DM was reported (83, 104, 110, 118, 133). Four studies among Korean (adjusted HR 1.1), Thai (RR 4.0), and Kenyan (adjusted RR 6.2) women reported significantly increased rates of prediabetes after PE/GH (86, 120, 126, 138).

Dyslipidemia

Fourteen studies reported on dyslipidemia (**Supplementary Table 6C**) (86, 88, 92–94, 103, 104, 110, 118, 120, 129, 133, 138, 139). Overall, the prevalence of dyslipidemia differed largely over the different study population. High rates of dyslipidemia were reported in Indian (33%) and Indonesian women (58% high triglycerides after early-onset PE, 40% after late-onset PE) 5–10 years after PE (88, 92). Even higher rates (87% after GH/PE; 66% after normotensive pregnancy; $p = 0.01$) were reported in a population-based cohort of Irani women 10 years after pregnancy (93). Four studies were conducted among Japanese women with (dyslipidemia 9.9–42.4%) and without a history of HDP (dyslipidemia 2.6–14.2%); two reported significantly higher rates of dyslipidemia after GH/PE (adjusted OR 3.2 and 1.4) (104, 110, 118, 133). Prevalence (1.5% vs. 0.5% and 4.5% vs. 2.8%) and incidence rates (15.0 vs. 4.4 per 1,000 person-years) in Taiwanese women were overall low, but significantly higher after a pregnancy complicated by PE (HR 3.4), HDP (RR 1.6) and GH/PE (adjusted OR 2.29) compared to women without a history of HDP (103, 129, 139). Among Korean women, dyslipidemia was significantly more prevalent on average 10 years after HDP (RR 1.3) than among women without HDP, while no association between PE and dyslipidemia 23 years after pregnancy was noted (86, 138). Among Kenyan women, dyslipidemia was significantly more prevalent among women with a history of GH/PE (adjusted RR 3.25) than among women without a history of GH/PE (120). No significant association was found between HDP and dyslipidemia 10–20 years after pregnancy in Brazilian women (20% vs. 6.7% in women with and without a history of HDP) (94).

Chronic Kidney Disease

Ten studies reported on the association between HDP and chronic kidney disease (CKD; **Supplementary Table 6D**) (84, 97, 100, 101, 110, 118, 123, 130, 136, 137). Among Japanese women, no association was found between GH/PE and CKD 5 years after pregnancy, while significantly higher rates of CKD were reported in women with HDP compared to normotensive pregnancies on average 31 years after pregnancy (adjusted OR 4.85) (110, 118). Three of four studies in Taiwanese women reported significantly higher incidence rates of CKD after GH/PE (adjusted HR 4.3), GH (adjusted HR 5.8), PE (adjusted HR 9.5), chronic hypertension (adjusted HR 16.0), and superimposed PE (adjusted HR 44.7), and increased rates of ESRD after GH (adjusted HR 12.4) and PE (adjusted HR14.0), 6–9 years after pregnancy compared to women without a history of HDP (97, 130, 136, 137). In Iranian women, one study found higher rates of proteinuria after PE compared to normotensive pregnancy (20% vs. 0%) on average 6 years after pregnancy; another study did not find an association between PE and CKD (84, 123). In women from Cameroon, proteinuria was reported in 1.8% of women with severe PE 6 months after pregnancy (101). In Nigerian women, 3.5% of women had CKD at 1 year after pregnancy complicated by HDP (100).

Metabolic Syndrome

The association between HDP and metabolic syndrome was reported in 11 studies, all using slightly different definitions of metabolic syndrome (**Supplementary Table 6E**) (86, 88, 89, 92, 99, 106, 108, 110, 117, 120, 138). Studies among Korean (adjusted OR 1.2 and 1.3), Brazilian (RR 2.9), and Kenyan women (adjusted RR 3.0) showed significantly increased risks of metabolic syndrome after HDP (86, 89, 120, 138). Among Singaporean and South African women, rates of metabolic syndrome were increased after GH/PE, but the difference did not reach statistical significance (106, 117). In Japanese women, no difference in metabolic syndrome prevalence was reported (110). Increased rates of metabolic syndrome 1 year after HDP compared to normotensive pregnancy were reported among Nigerian women (99).

Cardiovascular Diseases

CVD risk after a pregnancy complicated by HDP was investigated in 17 studies (**Supplementary Tables 6F–H**) (22–24, 85, 95–97, 103, 107, 121, 124, 125, 127, 129, 135, 136, 139). Three Taiwanese studies reported significantly increased incidence rates of combined CVD after GH/PE (adjusted HR 2.0), GH (HR 2.0), and PE (HR 3.0 and adjusted HR 6.4) (107, 137, 139). The incidence rate of congestive heart failure (HF) was also significantly increased after PE (HR 7.4) among Taiwanese women (103). Among Brazilian women, prevalence of CVD was increased fourfold ($p = 0.002$) (125). Among non-Hispanic Black women in the US, the incidence rate of HF was significantly increased after GH/PE (adjusted HR 3.74) and superimposed PE (adjusted HR 4.88), but not after chronic hypertension (22). Although overall rates of HF were higher among non-Hispanic Black women with a history of PE/GH or superimposed PE than among non-Hispanic White women with a history of GH/PE (2.28 vs. 0.96 per 1,000 persons-years) or superimposed PE (4.30 vs. 1.22 per 1,000 person-years), the hazard ratios for HF were similar in both groups, and no significant interaction between HDP and race for incident HF was found (22). One study among Cameroonian women showed a significantly decreased risk of CVD after PE, but the authors conclude that this unexpected result was potentially attributable due to selection bias among the control group (127).

The incidence rate of stroke was significantly higher among Korean women with a history of PE (adjusted OR 1.6) and non-Hispanic Black women in the US with a history of GH/PE (adjusted HR 1.7) or superimposed PE (adjusted HR 4.0) (23, 121). Although overall rates of stroke were higher among non-Hispanic Black women in the US with a history of PE/GH than among non-Hispanic White and Hispanic women in the US with a history of GH/PE (0.32 vs. 0.20 vs. 0.15 per 1,000 persons-years), no significant interaction between HDP and race for incident stroke was found (23). In non-Hispanic Black women stroke risk was significantly increased in women with history of

superimposed PE (adjusted HR 4.0), while among non-Hispanic White women the difference was not statistically significant (adjusted HR 1.9). However, no significant interaction between superimposed PE and race for incident stroke was found (23). Among Taiwanese women, a significantly increased risk of stroke after pregnancy complicated by HDP (adjusted HR 1.7–2.1), GH/PE (adjusted HR 2.0), GH (adjusted HR 1.7), PE (adjusted OR 1.6–2.1, HR 2.0–3.5), and superimposed PE (adjusted HR 3.1–3.9) were noted (95, 96, 103, 107, 129). Two Taiwanese studies found no significant association of GH and GH/PE with stroke (95, 97).

Ischemic heart disease (IHD) was significantly increased among Taiwanese women with a history of (superimposed) PE (adjusted HR 13.0), Iranian women with a history of PE (adjusted HR 16.9), and non-Hispanic Black and Hispanic women in the US with a history of GH/PE (adjusted HR 2.3 and 2.7, respectively) and superimposed PE (adjusted HR 4.0 and 9.4, respectively) (23, 85, 107). Although overall rates of IHD were higher among non-Hispanic Black women in the US with a history of PE/GH or superimposed PE than among non-Hispanic White and Hispanic women in the US with a history of GH/PE (1.52 vs. 0.88 vs. 0.34 per 1,000 persons-years) or superimposed PE (3.51 vs. 1.18 vs. 1.57 per 1,000 person-years), no significant interaction between GH/PE or superimposed PE and race for IHD was found (23). Additionally, the same study also did not find evidence for interaction between chronic hypertension and race for incident IHD (23). Two other studies among Taiwanese women, and one study among Black women in the US did not find a significant association between GH/PE and IHD (97, 129, 135).

CVD-related mortality was significantly increased among Taiwanese women with a history of PE/GE (adjusted HR 2.0) and (superimposed) PE (adjusted HR 6.4) (107, 137). Among US women a significant interaction between race and GH was found: African American women with a history of GH had an increased CVD mortality risk (adjusted HR 1.8), while among non-African Americans with a history of GH, no significant increase in CVD mortality risk was found (adjusted HR 0.9) (24).

DISCUSSION

Our review identified evidence on the risk of HDP and on the risk of CVD and CVD risk factors after a pregnancy complicated by HDP in non-White populations. It serves as an overview of the current evidence, and of gaps in the literature that need additional attention.

Compared to non-Hispanic White women in the US, prevalence of chronic hypertension, (superimposed) PE and eclampsia, but not GH, seemed increased among non-Hispanic Black women. Women from Sub-Saharan African origin in Europe mostly had lower rates of HDP, but higher rates of PE in part of the studies than White women. Combined HDP prevalence was lower among Hispanic US women than among non-Hispanic White women, but PE prevalence was increased. Women from East Asian and Southeast Asian origin both in the US and in Europe, and North African or Middle Eastern women in Europe seemed at decreased risk for HDP compared to non-Hispanic White women. In most studies, rates of HDP were lower among those born in their country of origin compared to women of the same origin born in the host country, and risks converged toward that of the host population with increasing duration of residence. While most studies accounted in their analyses for common confounders (i.e., maternal age, parity, socio-economic status, education level, BMI), understanding of sociodemographic, economic, or health behavioral factors underlying these differences is limited. It has to be noted that a fairly large part of the studies we identified used maternal country of birth as proxy for race and ethnicity, potentially misclassifying part of their population.

The results from the included articles on CVD risk after HDP among different racial and ethnic groups presented in this review are generally in line with results of in the previously published, systematic reviews and meta-analyses that have included a predominantly White population. The current review was designed to extend this work with an overview of the evidence on CVD risk after HDP in different racial and ethnic groups. The articles on CVD risk after HDP included in this review are almost exclusively published in the last decade. For that reason, most of these results are not included in the large meta-analyses that were published on this topic and suffer from overrepresentation of White women (8, 12–14, 16). It is important to ensure a racially and ethnically diverse study population in individual studies and systematic reviews, representative of the real-word diversity, to improve generalizability of outcomes and clinical recommendations. Therefore, updates of these systematic reviews and meta-analysis, and subsequently the guidelines based on this evidence, is needed in the future. Moreover, the studies identified in this current topical review are primarily from the Asian continent, and African women are still underrepresented. Better studies, especially on long-term CVD risk, are needed among these women.

We find contradicting evidence on differential CVD risk after HDP across racial and ethnic groups. Only five of the 62 identified studies reported on CVD risk after a pregnancy complicated by HDP in more than one racial or ethnic group (22–24, 105, 114). Three of these studies formally tested for interaction between the exposure variable and race for the studied CVD outcome. Two studies found no evidence of interaction between HDP (GH/PE, chronic hypertension, or superimposed PE) and race for incident HF, IHD, and stroke (22, 23). A third study did find a significant interaction between GH and race, with GH being a significant marker for CVD risk only for African American women (24). Further studies in multi-ethnic populations are needed to study the potential influence of race and ethnicity on the association between HDP and CVD risk in more detail, taking into account other relevant socio-economic parameters.

Another topic of interest, largely outside the scope of is this review, that needs to be taken into account is the role of migration on the risk of HDP and CVD after HDP among different racial and ethnic groups. Women with a migration history form a distinct group because their health is influenced both by the situation and presence of risk factors in the homeland and in the host country. This review showed higher rates of HDP among

non-White women born in the host country compared to women born in their country of origin who migrated to the host country. A better understanding of factors underlying these differences and targets for prevention of this increase in HDP risk could improve the overall health of non-White women. This is of particular interest as migration is expected to rise further in most contexts over the next years (39, 52).

It is remarkable that the risk of GH in non-White women is lower or similar compared to that found in non-Hispanic White women, while chronic hypertension, (superimposed) PE, and eclampsia risk in increased among most non-White populations. Further research on mechanisms underlying the racial and ethnic differences, including pre-existing cardiovascular risk profile, access to health care, interventions (e.g., iatrogenic delivery), and other obstetric characteristics, is needed to provide an explanation for this observation, and other differences identified in this review.

In conclusion, this review highlights that there are racial and ethnic differences in the prevalence of all types of HDP but that the body of literature is yet insufficient to draw firm conclusions. HDP is associated consistently with increased CVD risk across racial and ethnic groups, but further studies on potential differences and their etiology are required. Meta-analyses and guidelines should be updated and based on evidence from more racially and ethnically diverse study populations. This may contribute to a better understanding of the pathogenesis of HDP and subsequent CVD risk, improve monitoring strategies and allow timely interventions to reduce the unequal burden of HDP and CVD across races and ethnicities.

AUTHOR CONTRIBUTIONS

WG conceptualized the review. RB and HD performed the literature search and data extraction and wrote the manuscript. All authors critically reviewed the manuscript and gave final approval for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.933822/full#supplementary-material

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Prediction of Delivery Within 7 Days After Diagnosis of Early Onset Preeclampsia Using Machine-Learning Models

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Background: Early onset preeclampsia (eoPE) is a hypertensive disorder of pregnancy with endothelial dysfunction manifested before 34 weeks where expectant management is usually attempted. However, the timing of hospitalization, corticosteroids, and delivery remain a challenge. We aim to develop a prediction model using machine-learning tools for the need for delivery within 7 days of diagnosis (model D) and the risk of developing hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome or *abruptio placentae* (model HA).

Materials and Methods: A retrospective cohort of singleton pregnancies with eoPE and attempted expectant management between 2014 and 2020. A Mono-objective Genetic Algorithm based on supervised classification models was implemented to develop D and HA models. Maternal basal characteristics and data gathered during eoPE diagnosis: gestational age, blood pressure, platelets, creatinine, transaminases, angiogenesis biomarkers (soluble fms-like tyrosine kinase-1, placental growth factor), and ultrasound data were pooled for analysis. The most relevant variables were selected by bio-inspired algorithms. We developed basal models that solely included demographic characteristics of the patient (D1, HA1), and advanced models adding information available at diagnosis of eoPE (D2, HA2).

Results: We evaluated 215 eoPE cases and 47.9% required delivery within 7 days. The median time-to-delivery was 8 days. Basal models were better predicted by K-nearest-neighbor in D1, which had a diagnostic precision of 0.68 ± 0.09, with 63.6% sensitivity (Sn), 71.4% specificity (Sp), 70% positive predictive value (PPV), and 65.2% negative predictive value (NPV) using 13 variables and HA1 of 0.77 ± 0.09, 60.4% Sn, 80% Sp, 50% PPV, and 87.9% NPV. Models at diagnosis were better developed by support vector machine (SVM) using 18 variables, where D2's precision improved to 0.79 ± 0.05

with 77.3% Sn, 80.1% Sp, 81.5% PPV, and 76.2% NPV, and HA2 had a precision of 0.79 ± 0.08 with 66.7% Sn, 82.8% Sp, 51.6% PPV, and 90.3% NPV.

Conclusion: At the time of diagnosis of eoPE, SVM with evolutionary feature selection process provides good predictive information of the need for delivery within 7 days and development of HELLP/*abruptio placentae*, using maternal characteristics and markers that can be obtained routinely. This information could be of value when assessing hospitalization and timing of antenatal corticosteroid administration.

Keywords: preeclampsia, prediction, machine-learning, HELLP syndrome, placental abruption

INTRODUCTION

Preeclampsia (PE) is a multisystem disorder of pregnancy defined as *de novo* or worsening hypertension from 20 weeks of gestation with endothelial dysfunction manifested as proteinuria or end-organ damage (1). Its associated complications include refractory hypertension, renal failure, eclampsia, stroke, pulmonary edema, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome or *abruptio placentae*, making PE a leading cause of maternal morbidity and mortality, with 50,000 maternal deaths yearly worldwide, most of them in developing countries (2). Unfortunately, there is still no treatment for the disease beyond timely delivery (3).

The early onset PE (eoPE) subtype, defined as that diagnosed before 34 + 0 weeks, is a critical challenge since prompt delivery exposes the fetus to the consequences of prematurity. Therefore, expectant management is usually attempted at the expense of exposing the mother to the risk of developing complications (4). In eoPE, abnormal trophoblastic invasion in early pregnancy causes placental hypoperfusion and hypoxia, which compromise maternal–fetal exchange of nutrients and oxygen. As a response, an excess of placental antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1), are released into the maternal circulation. This reduces the bioavailability of proangiogenic factors, such as the placental growth factor (PlGF). The angiogenic imbalance induces maternal endothelial dysfunction, being responsible for hypertension, proteinuria, and end-organ disease (5). The increase of the sFlt-1/PlGF ratio is related to eoPE (6), being detectable up to 5 weeks before clinical symptoms (7). Moreover, there is an inverse relationship between the value of the sFlt-1/PlGF ratio and the time lapse until it is necessary to deliver due to disease progression. In particular, extremely high values of the ratio have been related to complications, such as HELLP syndrome and *abruptio placentae* (8), which are otherwise difficult to anticipate. Therefore, the integration of these biomarkers with other clinical, analytical, and ultrasound tools could be of use in the prediction of eoPE progression after diagnosis.

Current predictive models of PE have several limitations such as their short-term (within 48 h) predictive capability, the prediction of maternal but not fetal complications, the inclusion of solely severe cases from onset (9), or the focus on the development of PE but not its complications (10). Others have only included data obtained in the first trimester, and most of them have not used angiogenesis biomarkers (9, 11). Approaching such complex optimization problems can be challenging and supervised machine-learning techniques, which generate classification models that analyze patterns and trends in the variables of a large volume of data to ultimately predict the course and progression of the disease, can be of use (12). Our aim is to develop two predictive models with available data at the time of diagnosis of eoPE: (1) need to deliver within 7 days and (2) risk of developing HELLP syndrome or *abruptio placentae*.

MATERIALS AND METHODS

Our retrospective cohort study was on singleton women with a diagnosis of eoPE between January 2014 and December 2020. Inclusion criteria were singleton pregnancies with a diagnosis of PE before 34 + 0 weeks and attempted expectant management. Cases with congenital anomalies, lack of angiogenesis biomarkers determination at diagnosis (±48 h), or loss to follow-up were excluded. The study was approved by the local Ethics Committee (n 21/113). Due to its retrospective, non-interventional nature with the use of de-identified information, the requirement of informed consent was waived.

Data Collection, Follow-Up, and Outcome Measures

Baseline Characteristics

Maternal characteristics include age, height, weight, smoking status, race, method of conception, low-dose aspirin intake, heparin prophylaxis, and risk factors for PE and other PD-related disorders according to the National Institute for Health and Care Excellence (NICE) guidelines (13) were collected from the medical records. During the study period, PE was screened according to the NICE risk-factor guidelines criteria. Those women with $\geq$ 1 high risk factor or $\geq$ 2 moderate ones were considered at high risk of PE, and a recommendation of prophylaxis with aspirin was made. However, not all women were evaluated at first in our center so the screening protocol may have differed. Furthermore, there were some women with low molecular heparin treatment, either in the context of thrombophilia, systemic erythematosus lupus, or assisted reproduction techniques. Uterine artery evaluation at 20 weeks was recorded and centiles were calculated (14). Gestational age (GA) was estimated according to the American College of Obstetricians and Gynecologists, that is, reliable last menstrual

period was corrected by the crown-rump length before 14 + 0 weeks or biparietal diameter from 14 + 0 weeks to 21 + 6 weeks, when a significant discrepancy of more than 7 days or more than 10 days was found, respectively (15).

Preeclampsia Diagnosis and Management

Preeclampsia was defined as the presence of both hypertension and proteinuria, according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (16). In the clinical setting, patients were managed as having PE even in the absence of proteinuria when other severity criteria were met and indications for delivery followed current recommendations (17).

At diagnosis, a complete blood count, biochemistry including angiogenesis biomarkers, and spot protein/creatinine ratio were measured. The sFlt-1 and PlGF concentrations (picograms per milliliter) were determined using an automated assay system (Cobas® 6000 e701 module, Roche Diagnostics, Penzberg, Germany). The sFlt-1/PlGF ratio was expressed in absolute values, and the obstetricians involved were aware of the results of the sFlt-1/PlGF ratio. Values $\leq$ 38 were considered to rule out PE $\geq$ 85 as "aid in diagnosis," and $>$ 655 as a high risk of the need to deliver within 48 h (18). This information was not intently used to indicate delivery, although its knowledge could have influenced the clinicians in the interpretation of the severity of PE-related symptoms that leads to decisions about the continuation of the pregnancy.

Corticoids for fetal maturity (betamethasone 12 mg/day for 2 days) were administered before 34 + 6 weeks if fetal viability was reached [provided that the GA was $\geq$ 24 + 0 weeks in normally grown fetuses or 26 + 0 weeks and estimated fetal weight (EFW) $\geq$ 500 g in growth restricted fetuses]. A repeated cycle of corticoids was considered after 1 week of the administration of the first cycle if the GA remained below 34 + 6 weeks. Magnesium sulfate was indicated in the presence of severity features or regardless of maternal status when there was a risk of imminent delivery $<$ 32 weeks.

Expectant management was initially attempted for fetal interest whenever the GA was $\geq$ 24 weeks and was discussed with the parents at earlier weeks. Severe complications that indicated immediate delivery irrespectively of GA (19) were pulmonary edema, refractory hypertension (uncontrolled blood pressure despite two antihypertensive medications at maximum doses), HELLP syndrome, *abruptio placentae* [defined as placental detachment prior to delivery of the fetus, including the identification of a retroplacental hematoma or evidence of blood clot in at least 20% of its surface based on clinical data provided by the attending obstetrician at delivery (20)], renal failure (oliguria $<$ 500 mL/24 h, creatinine $>$ 1.2 mg/dl), and neurological deficit (persisting visual alterations, stupor, clonus, or eclampsia). Delivery was also recommended after 34 + 0 weeks in the presence of severe features of PE and in any PE case after 37 + 0 weeks.

Fetal Assessment

Fetal assessment including a detailed anatomical scan and growth evaluation was undertaken at our placental dysfunction consult within 48 h of eoPE diagnosis. Fetal weight was estimated (21) and centiles customized (22) to maternal and fetal characteristics. Fetal growth restriction (FGR) was diagnosed according to a stage-based classification (23): stage I was considered in cases with normal fetal Doppler or abnormal but with antegrade umbilical artery (UA) flow; stage II was those with absent-end diastolic UA flow; stage III those with reversed end-diastolic UA flow or ductus venosus PI $>$ 95th centile, and finally stage IV was limited to cases with a reversed a-wave on the ductus venosus or spontaneous decelerations on the CTG. Whenever anterograde flow in UA was present, biweekly monitoring (fetal Doppler including interrogation of the ductus venosus plus conventional cardiotocography) was planned, and vaginal delivery (in the absence of other contraindications) was recommended after 37 weeks. If the absent end-diastolic UA flow was detected, subsequent follow-up controls were performed every 48–72 h, and elective cesarean section was indicated at 34 weeks. When reverse end-diastolic UA flow or ductus venosus PI $>$ 95th centile was found, hospitalization and daily monitoring were carried out until elective cesarean section at 30 weeks. Whenever a reverse a-wave flow in the ductus venosus or spontaneous decelerations in the cardiotocography were noted, elective cesarean section was indicated.

Perinatal Data

Perinatal data included date and reason for delivery and perinatal mortality.

All data were recorded in a database created on the Research Electronic Data Capture (REDCap) tool (24) hosted by the "imas12" research institute.

Statistical Analysis

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was followed for reporting the results (25).

Descriptive Statistics

Continuous variables were expressed in mean (SD) or median (interquartile range) when non-normally distributed. Categorical variables were expressed in percentage (%). Univariate comparisons between the cases in which delivery occurred within 7 days after eoPE diagnosis and those that did not were performed using the *t*-test or Mann–Whitney *U*-test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Two-sided $p < 0.05$ was considered statistically significant. Statistical package STATA, version 14.1 (TX, United States: StataCorp LP) was used for this analysis.

Machine-Learning Model Development

First, we developed a predictive model of the need for delivery within 7 days of diagnosis (model D), considering this as the window of the effect of antenatal corticosteroids for fetal maturation (26). Second, we created a model to calculate the risk of developing HELLP syndrome or *abruptio placentae* at any point after eoPE diagnosis (model HA), as these are the most acute and harder to predict complications.

In order to cover different resource availability scenarios, we developed a reduced version of these models using only demographic characteristics of the patient (D1, HA1) and an extended version adding information available at diagnosis of eoPE (D2, HA2). Accordingly, variables were structured into two datasets: those prior to the onset of PE (baseline) and those evaluated at the moment of diagnosis (diagnosis).

Three preprocessing steps were performed. There were very few missing data in the baseline and "at diagnosis" variables and given the pattern they were considered missing at random. However, there were some variables and some patients with a high number of missing values, especially in the follow-up variables, since this depends on the time a patient participates in the clinical study. First, missing values were imputed using the MissForest imputation technique. This is an imputation method suitable for both categorical and numerical data. It performs an iterative imputation by training a Random Forest model followed by a prediction of the missing values in an iterative process (27). This technique was applied for variables with less than 20% missing data and patients with less than 27% missing data in order to avoid synthetic deviation of the statistical distribution. The remaining variables and patients were directly removed from the dataset. To apply the technique, the Iterative Imputer tool of scikit-learn was used with the Random Forest classifier (28). Treatment of missing values is shown as **Supplementary Material 1**. Subsequently, nominal variables were categorized and represented by one hot vector. Finally, numerical and ordinal nominal variables were normalized using the Min–MaxScaler tool of the scikit-learn library, which scales the data within a 0–1 range.

The available data present a high dimensionality, and some of them may be redundant or uninformative, which can negatively affect the performance of the classification models. Variable selection (feature selection) methods allow us to obtain the most relevant sets of variables, optimizing the development of machine learning models. We have used a genetic algorithm, a heuristic optimization technique that simulates the natural process of evolution and performs a bio-inspired exploration of a large space of solutions to find the best combination of features, something unfeasible with traditional feature selection techniques in high-dimensionality problems. A mono-objective genetic algorithm (PyWinEA python library)[1] was used since the main interest was minimizing the number of characteristics. During the process, variables are selected based on a series of previously fixed parameters and a fitness function that needs to be optimized. This function is based on a supervised classification model, using a specific evaluation metric (29). In our case, we tried support vector machine (SVM), K-nearest neighbor (KNN) algorithm, Gaussian Naïve Bayes (GNB), and decision tree (DT) models and selected them relying on the F1-score metric.

Finally, once selected, the best advanced models provided by the genetic algorithm (D2, HA2), we created an interface using Streamlit open-source app framework in Python language where we exported the models to generate a calculator that evaluates the risks as a function of the input variables.

[1]https://github.com/FernandoGaGu/pywinEA

RESULTS

There were 227 women with eoPE of which 7 were excluded for coexistence with congenital anomalies and only 5 due to lack of determination of angiogenesis biomarkers at diagnosis ($n = 4$), or loss of follow-up ($n = 1$). A total of 215 patients were included, among them, 103 (47.9%) required delivery within 7 days of diagnosis. Baseline characteristics of the study population stratified by the need for delivery within 7 days are depicted in **Table 1**. There were no statistically significant differences among groups, except for a lower percentage of women with an identifiable *a priori* risk of PE in those with early delivery, mostly at the expense of lower pregestational body mass index and maternal age. This resulted in a lower number of women taking low-dose aspirin before 16 weeks in this subgroup (15.5% vs. 33.9%, $p < 0.01$).

As shown in **Table 2**, regarding eoPE diagnosis, the mean (SD) GA at diagnosis was 29.6 (3.1) weeks. In cases that required delivery within 7 days, GA at diagnosis was significantly lower (29.0 vs. 31.0 weeks, $p < 0.01$), and angiogenesis biomarkers were significantly more altered in the overall ratio and its components, evaluated as absolute numbers, MoM values, or with standardized centile cut-offs. Of note, up to 32% of women who required delivery within 7 days had an sFlt-1/PlGF > 655 at diagnosis. Considering the rest of the blood work, platelets were lower and transaminases higher in those with the need to deliver within 7 days, but these differences were not seen when these parameters were dichotomized according to clinically relevant developed cut-offs. There was a higher rate of growth restricted fetuses among women with prompt delivery after eoPE diagnosis (65% vs. 40.2%, $p = 0.001$), with lower EFW, lower middle cerebral artery PI, and higher PI in the umbilical artery, ductus venosus, and maternal uterine arteries.

Considering outcomes (**Table 3**), the median GA at delivery was 32.1 weeks, with a median latency time from diagnosis of 8 days. Those cases that gave birth within 7 days mainly had a maternal indication for delivery in comparison to those with longer latency time (78.4% vs. 69.6%, $p < 0.01$). In the latter group, up to 8.9% delivered for causes unrelated to PE or FGR (intrahepatic cholestasis, premature rupture of membranes, and spontaneous onset of labor). There were no statistically significant differences in terms of the development of severe PE between groups. However, those with earlier delivery after eoPE diagnosis had higher rates of intrauterine demise (8.7% vs. 0%) and suffered from more maternal complications (53.4% vs. 23.2%, $p < 0.001$), especially so due to higher rates of HELLP syndrome (21.4% vs. 3.6%, $p < 0.001$) and *abruptio placentae* (17.5% vs. 4.5%, $p = 0.002$).

There were two models developed for both the primary (D) and the secondary (HA) outcomes. The best performance was achieved by the KNN model in time-to-delivery and the TD models for the prediction of HELLP-*abruptio placentae* for the basal approach, and the SVM model performed best in both cases when considering variables at diagnosis of eoPE. The resulting calculator for the prediction of D2 and HA2 can be found in **Supplementary Material 2**, and the selected variables for each one are in **Supplementary Material 3**. In the case of model D,

TABLE 1 | Baseline characteristics of the study population stratified by the presence of preeclampsia complications within 7 days of diagnosis.

Characteristics	Overall (*n* = 215)	Delivery within 7 days	
		No (*n* = 112)	Yes (*n* = 103)
Maternal age (years)	33.4 ± 6.4	33.9 ± 6.6	32.8 ± 6.1
Height (cm)	160 ± 9	159 ± 10	161 ± 6
Pre-pregnancy weight (kg)	68.7 ± 14.2	71.4 ± 15.3	65.5 ± 12.2
Pre-pregnancy BMI (kg/m^2)	26.7 ± 5.4	27.9 ± 5.9	26.2 ± 5.0
Smoking during pregnancy	8 (3.7)	4 (3.6)	4 (3.9)
Race or ethnic group†			
White or Caucasian	116 (54.0)	57 (50.9)	59 (57.3)
Hispanic	71 (33.0)	42 (37.5)	29 (28.2)
Asian	2 (0.9)	1 (0.9)	1 (1.0)
Black or African American	9 (4.2)	6 (5.4)	3 (2.9)
Arab/North African	17 (7.9)	6 (5.4)	11 (10.7)
Risk factors for preeclampsia			
High			
Previous preeclampsia	24 (11.2)	21 (18.8)	13 (12.6)
Chronic hypertension	35 (16.3)	22 (19.6)	13 (12.6)
Pre-pregnancy diabetes	8 (3.7)	4 (3.6)	4 (3.9)
Chronic kidney disease	5 (2.3)	3 (2.7)	2 (1.9)
Thrombophilia	5 (3.1)	4 (3.6)	1 (1.0)
Systemic lupus erythematosus	2 (0.9)	2 (1.8)	0 (0)
Moderate			
Nulliparity	161 (74.9)	83 (74.1)	78 (75.7)
Age ≥ 40 years	31 (14.4)	21 (18.8)	10 (9.7)
Pre-pregnancy BMI ≥ 35 kg/m^2	16 (7.4)	14 (12.5)	2 (1.9)
Family history of preeclampsia*	10 (4.7)	6 (5.1)	4 (3.8)
≥1 high-risk or 2 moderate-risk factors	80 (37.2)	42 (46.4)	28 (27.2)
Mode of conception			
Spontaneous	189 (87.9)	139 (87.4)	50 (89.3)
Assisted reproduction technique	24 (11.1)	14 (12.5)	10 (9.7)
Oocyte donation	12 (5.6)	10 (8.9)	2 (1.9)
Low-dose aspirin intake (100 mg/day)			
No	154 (71.6)	70 (62.5)	84 (81.6)
Starting at or before 16 weeks	54 (25.1)	38 (33.9)	16 (15.5)
Starting after 16 weeks	7 (3.3)	4 (3.6)	3 (2.9)
Low-dose heparin prophylaxis			
No	209 (97.2)	108 (96.4)	101 (98.1)
Starting at or before 16 weeks	6 (2.8)	4 (3.6)	2 (1.9)
Starting after 16 weeks	0 (0)	0 (0)	0 (0)
Uterine artery PI > 95th centile at 19–22 weeks γ	85/131 (63.1)	48/79 (60.8)	37/52 (71.2)

Data are mean ± standard deviation or n (%), unless otherwise stated.
**First-degree relative (mother or sister) with a history of PE.*
†Evaluated after the Bonferroni adjustment.
γ Measured in cases from our center.
BMI, body mass index.

TABLE 2 | Diagnosis characteristics of the study population stratified by the presence of preeclampsia complications within 7 days of diagnosis.

Diagnosis	Overall (*n* = 215)	Delivery within 7 days	
		No (*n* = 112)	Yes (*n* = 103)
GA at diagnosis, median (Q1–Q3)	30.0 (27.4–32.3)	31.01 (28.1–32.6)	29.0 (3.3)
sFlt-1/PlGF			
Median (Q1–Q3)	325 (140–550)	203 (97–380)	460 (285–828)
MoM	83 (33–160)	52 (23–121)	125 (64–228)
>655	41 (19.1)	8 (7.1)	33 (32.0)
sFlt-1			
Median (Q1–Q3)	10,939 (7,877–14,985)	9,958 (7,356–13,882)	13,116 (8,676–18,008)
MoM	7 (5–10)	5.6 (4.4–9.1)	8.1 (6.1–11.2)
>95th centile	197 (91.6)	97 (86.7)	100 (97.9)
PlGF			
Median (Q1–Q3)	42 (23–71)	55 (34–86)	29 (19–42)
MoM	0.9 (0.05–0.15)	0.12 (0.08–0.20)	0.06 (0.04–0.10)
<5th centile	191 (88.8)	91 (81.3)	100 (97.9)
Blood pressure (mmHg)			
Systolic	148 ± 14	146 ± 13	149 ± 15
Diastolic	93 ± 9	94 ± 9	93 ± 10
Mean	109 ± 11	109 ± 10	111 ± 11
Platelets			
Absolute (10ˆ3)	223 ± 86	234 ± 63	211 ± 105
<100.000	5 (2.3)	0 (0)	5 (4.9)
Creatinine			
Absolute (mg/dL)	0.65 ± 0.22	0.61 ± 0.12	0.69 ± 0.28
>1.1 mg/dL	3 (1.3)	0 (0)	3 (2.9)
AST	22 (17–30)	21 (16–24)	25 (20–36)
ALT	16 (12–28)	16 (12–24)	19 (13–40)
Estimated fetal weight			
median (Q1–Q3)	1,191 (816–1,741)	1,438 (973–103')	928 (720–1,272)
<10t^h centile	110 (51.2)	45 (40.2)	65 (63.1)
<3rd centile	77 (35.8)	29 (25.9)	48 (46.6)
Umbilical artery PI			
Mean	1.44 ± 0.75	1.27 ± 0.43	1.64 ± 0.96
>95th centile	17 (8.3)	9 (8.0)	8 (8.5)
Middle cerebral artery PI			
Mean	1.69 ± 0.40	1.79 ± 0.35	1.57 ± 0.42
<5th centile	53 (23.9)	16 (23.9)	37 (28.6)
Ductus venosus PI			
Mean	0.51 ± 0.20	0.49 ± 0.13	0.51 ± 0.24
>95th centile	9 (23.9)	0 (0)	9 (3.6)
Mean uterine artery PI			
Mean	1.63 ± 0.62	1.50 ± 0.58	1.80 ± 0.65
>95th centile	38 (23.9)	131 (82.4)	50 (89.3)
Fetal growth restriction			
No	103 (47.9)	67 (59.8)	36 (35.0)
Stage I	92 (42.8)	42 (37.5)	50 (48.5)
Stage II	9 (4.2)	3 (3.7)	6 (5.8)
Stage III	8 (3.7)	0 (0)	8 (7.8)
Stage IV	3 (1.4)	0 (0)	3 (2.9)

Data are mean ± standard deviation or n (%), unless otherwise stated.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GA, gestational age; MoM, multiples of the median; PI, pulsatility index; PlGF, placental growth factor; Q, quartile; sFlt-1, soluble fms-like tyrosine kinase 1.

the available result is the probability (%) of delivery within 7 days after diagnosis, whereas, in the case of HA, the unbalanced data only allowed for the dichotomic classification of HELLP/*abruptio* risk (yes/no). The performance of the resulting models is depicted in **Table 4**. Baseline algorithms (D1 and HA1) have an area under

TABLE 3 | Maternal and perinatal outcomes stratified by the presence of preeclampsia complications within 7 days of diagnosis.

Perinatal outcome	Overall (*n* = 215)	Delivery within 7 days		
		No (*n* = 112)	Yes (*n* = 103)	*p*
GA at delivery, median (Q1–Q3)	32.1 (29.0–34.1)	33.8 (31.6–35.4)	29.7 (27.4–32.1)	<0.001
Time to delivery in days, median (Q1–Q3)	8 (3–19)	18 (13–28)	3 (1–5)	<0.001
Reason for delivery				
Maternal, related to preeclampsia	158 (73.5)	78 (69.6)	80 (100)	0.004
Fetal, related to FGR	41 (19.1)	19 (17.0)	22 (21.6)	
Reached 37 weeks	5 (2.3)	5 (4.5)	0 (0)	
Other	11 (5.1)	10 (8.9)	1 (1.0)	
Intrauterine demise	9 (4.2)	0 (0)	9 (8.7)	0.001
Severity features				
Any	137 (63.7)	72 (64.3)	65 (63.1)	0.86
Severely elevated blood pressure	116 (54.0)	63 (56.3)	53 (51.5)	0.48
Elevated liver enzymes	40 (18.6)	16 (14.3)	24 (23.3)	0.09
Low platelets	22 (10.2)	3 (2.7)	19 (18.5)	<0.001
Elevated creatinine	9 (4.2)	4 (3.6)	5 (4.9)	0.64
Maternal complications				
Any	81 (37.7)	26 (23.2)	55 (53.4)	<0.001
Refractory hypertension	34 (15.8)	17 (15.2)	17 (16.5)	0.79
HELLP syndrome	26 (12.1)	4 (3.6)	22 (21.4)	<0.001
Abruptio placentae	23 (10.7)	5 (4.5)	18 (17.5)	0.002
Oliguria (<500 mL/24 h)	9 (4.2)	4 (3.6)	5 (4.9)	0.64
Pulmonary edema	6 (2.7)	2 (1.8)	4 (3.9)	0.35
Eclampsia	2 (0.9)	0 (0)	2 (1.9)	0.14

FGR, fetal growth restriction; GA, gestational age; HELLP, hemolysis, elevated liver enzymes, and low platelets.

TABLE 4 | Diagnostic performance of model D (need to deliver within 7 days of diagnosis) and model HA (occurrence of HELLP syndrome or *abruptio placentae*) in their basic (D1, HA1) and advanced (D2, HA2) versions.

	Area under ROC curve (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
D1	0.68 (0.53–0.82)	63.6 (40.7–82.8)	71.4 (47.8–88.7)	70.0 (45.7–88.1)	65.2 (42.7–83.6)
D2	0.79 (0.66–0.91)	77.3 (54.6–92.2)	80.1 (56.3–94.3)	81.5 (58.1–94.6)	76.2 (52.8–91.8)
HA1	0.77 (0.55–0.88)	60.4 (26.2–87.4)	80.8 (64.5–93.0)	50.0 (21.1–78.9)	87.9 (70.2–96.4)
HA2	0.79 (0.59–0.93)	66.7 (29.9–92.5)	82.8 (65.5–93.2)	51.6 (21.1–78.9)	90.3 (74.2–98.0)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

the curve (AUC) of 0.68 [95% confidence interval (CI) (0.53–0.82)] in the case of D1 (risk of delivery within 7 days) and of 0.79 (95% CI, 0.66–0.91) in the case of HA1 (risk of developing HELLP syndrome or *abruptio placentae*). These figures are 0.79 (95% CI, 0.66–0.91) and 0.79 (95% CI, 0.55–0.88) for D2 and HA2, respectively, that is, using the available information at diagnosis of eoPE. The AUC using 5 repeats of 10-fold cross-validation of the models is shown in **Figure 1**.

DISCUSSION

Main Findings

Our study provides two models based on machine-learning techniques to predict the need for delivery within 7 days (model D) and the future occurrence of HELLP syndrome or *abruptio placentae* (model HA). The advanced versions of such models (D2 and HA2), which include data obtained at diagnosis from angiogenic factors and ultrasonographic study of fetal biometry and Doppler parameters, reached the best performance. It is particularly remarkable their high negative predictive value (NPV) of 76.2% (95% CI, 52.8–91.8%) and 90.3% (95% CI, 74.2–98.0%), respectively.

Interpretation of the Results

The current evident-based management for eoPE is mainly guided by two clinical trials carried out in the 1990s (30, 31). According to them, expectant management should be pursued in the absence of an imminent threat of complications since it improves perinatal outcomes. Even in eoPE with severe features, this recommendation persists until 34 weeks of gestation. However, the natural course of eoPE in undelivered women tends to be a progressive end-organ dysfunction in which both its speed and type of manifestation have been considered virtually unpredictable. This may explain why it is not uncommon for some clinicians to deliver earlier than stated by current guidelines, aiming to reduce the risk of maternal adverse outcomes at the likely expense of incrementing neonatal morbidity. Therefore, choosing to prolong pregnancies in these circumstances requires meticulous maternal-fetal surveillance and the availability of the appropriate resources to resolve any of its associated complications (32). There have been several advances since those studies were carried out. In the last decade, angiogenic biomarkers (sFlt-1/PlGF ratio) have become available. Their relationship with the evolution of eoPE has allowed us to improve our anticipation of the diagnosis and complications (7, 18). The identification and evaluation of FGR, which is associated in more than half of the cases with eoPE, has also improved with the advances in ultrasound (33). Furthermore, the prognosis of preterm newborns under 34 weeks has improved dramatically, as a result of the continuous improvements in neonatal care (34). This has led to questioning whether the axiom of expectant management of eoPE is still valid and safe enough for any mother and fetus (4) or if there is room for an individualized assessment of risk.

There are paradigmatic examples of the use of clinical tools to predict adverse outcomes in other contexts, such as the scoring systems to estimate the mortality risk on admission to an intensive care unit or after the diagnosis of sepsis (35, 36). The main attempt to develop a tool to identify the risk of adverse maternal outcomes in PE was the full preeclampsia integrated estimate of risk (fullPIERS) model that was published in 2011

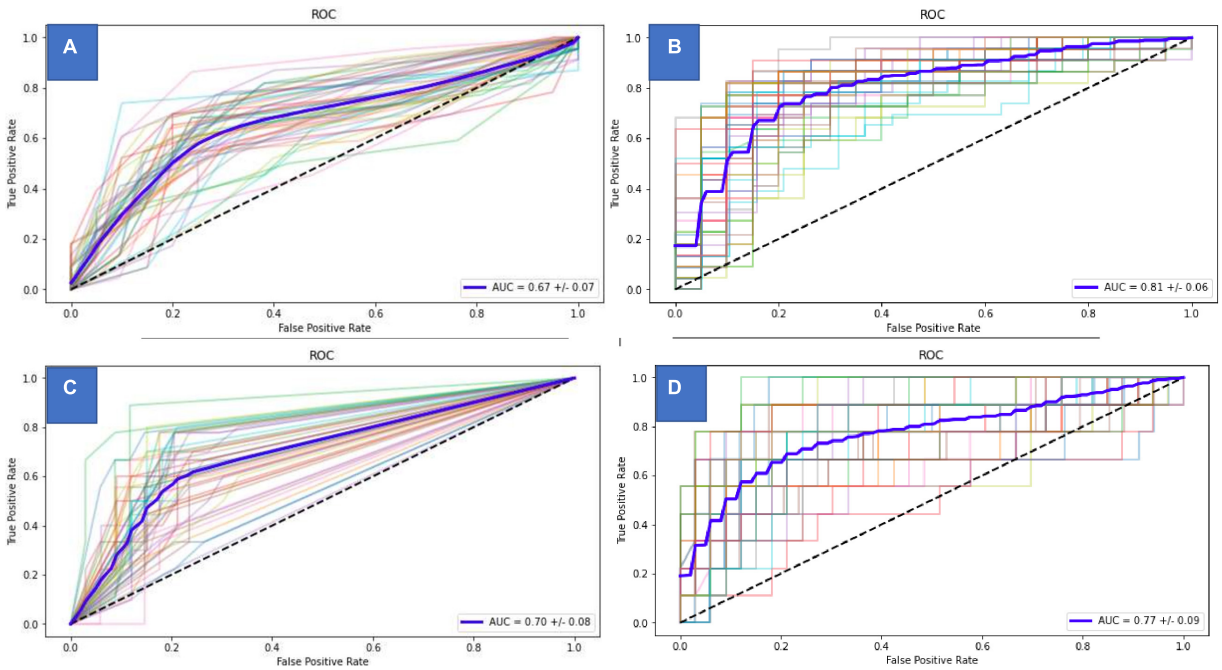

FIGURE 1 | Area under the curve of the different predictive models. **(A)** Delivery within one week predictive model (basal). **(B)** Delivery within one week predictive model (at diagnosis). **(C)** HELLP/Abruptio predictive model (basal). **(D)** HELLP/Abruptio predictive model (at diagnosis).

(8). It was subsequently externally validated in women with eoPE (37), showing good discrimination for the prediction of any adverse maternal outcome within 48 h of admission, with an area under the receiver operating characteristic curve of 0.80 (95% CI, 0.75–0.86). This model has been criticized for being dominated by variables that are themselves indicative of a complication, such as low platelets or elevated creatinine, and only provides a very short-term prediction. Furthermore, it neither incorporates information from angiogenic markers that have shown promising properties to predict complications, such as HELLP syndrome and *abruptio placentae* (7, 38), nor takes into account parameters of fetal wellbeing. On the contrary, our D2 model provides an expanded prediction of 7 days, which allows a greater margin for decision-making, including transfer to a tertiary center or the administration of corticosteroids. The HA2 model is useful for ruling out acute and highly feared complications, such as HELLP syndrome and *abruptio placentae*, which until now have been deemed completely unpredictable. Of note, the machine-learning methodology selected without any prior condition, both the angiogenic markers and feto-maternal Doppler study among the available parameters to compose the D2 and HA2 models.

The high NPV of the D2 and HA2 models is promising to help better select the appropriate candidates for expectant management among pregnant women with eoPE. These models could also help optimize the administration of antenatal corticosteroids and determine the need for hospitalization. The next steps should focus on the use of these models in a randomized trial to compare maternal and perinatal outcomes between a control group with standard care after eoPE diagnosis and an intervention group in which expectant management or planned early delivery is decided after the knowledge of the results of the D2 and HA2 predictive models.

Predicting maternal and perinatal outcomes in PE remains a challenge, and its unpredictability is a source of stress for patients, their families, and clinicians. Although it has been shown that adopting expectant management before 34 weeks is the best policy, it is not without risks, and this may trigger overattentive women may develop some subjective symptoms (headache and blurred vision), and clinicians prompt early delivery recommendations. On the other hand, not recognizing the onset of some severe complications, which may go unnoticed, such as HELLP syndrome and *abruptio placentae*, can be fatal for both the woman and the fetus. Improving the prediction of time-to-delivery and some complications can help reduce stress, optimize the administration of antenatal corticosteroids, and adjust better both the need for hospitalization and the clinical decision-making of when to deliver. The high NPV of the D2 and HA2 models is promising to help better select the appropriate candidates for expectant management among pregnant women with eoPE. The next steps should focus on the use of these models in a randomized trial to compare maternal and perinatal outcomes between a control group with standard care after eoPE diagnosis and an intervention group in which expectant management or planned early delivery is decided after the knowledge of the results of the D2 and HA2 predictive models.

Strengths and Limitations

The main limitations of our study come from the use of retrospective data, in which clinicians were not blinded to the knowledge of the sFlt-1/PlGF values or Doppler status. Although

they were not used to directly indicate delivery in any case, we cannot exclude that women with higher angiogenic imbalance or fetuses with poorer Doppler status were more closely observed and at higher risk of intervention. However, this represents a real-world evidence scenario in the era of angiogenic markers. In fact, their use has spread widely in recent years because among its strengths is that their simple knowledge is an aid to making better clinical decisions and avoiding thereby serious maternal complications (39, 40). The models have been developed from a relatively small sample size and lack external validation which makes us interpret results with caution. Furthermore, the values of the sFlt-1 and PlGF are not completely interchangeable between different laboratory platforms (17), and a sonographer trained in performing a feto-maternal Doppler study might not always be available in the emergency department where the initial care is given to women with eoPE. Finally, given the high proportion of Caucasian and Hispanic in our cohort, these results may not be applicable to different subsets of patients. Nevertheless, in the case of angiogenesis biomarkers, they have shown good validation in other ethnicities (41, 42).

However, several strengths must be noted as well, such as the use of machine-learning technology to develop the models, which limits pre conceptual bias when selecting the variables in the study. Given its single-center character, there was a great uniformity in management throughout the study period, using systematically the determination of angiogenic markers and ultrasound evaluation of fetal biometry and Doppler parameters at diagnosis.

CONCLUSION

We have developed, with the aid of machine-learning techniques and using commonly available clinical data, two models that are applicable at the time of eoPE diagnosis. The first model predicts the need to deliver within 7 days and the second one the future occurrence of HELLP syndrome or *abruptio placentae*. Their high NPV of 76 and 90%, respectively, seems promising for future clinical use as an aid to better select the appropriate candidates for expectant management after the diagnosis of eoPE.

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 Hospital Universitario 12 de Octubre Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

IH and AG contributed to the conception and design of the study. CV and IH collected the data and drafted the manuscript. PD, JA, PA, CV, IH, and AG analyzed and interpreted the data. JA and AG revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.910701/full#supplementary-material

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Nailfold Video Capillaroscopy in Pregnant Women With and Without Cardiovascular Risk Factors

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Objective: To evaluate microvasculature in pregnant women with and without cardiovascular risk factors.

Design: Cross-sectional, observational study.

Population: Women were recruited at the outpatient clinic for high risk prenatal care. Out of a total of 345 women assessed at first and/or second and/or third trimester, 169 women without and 176 with cardiovascular risk factors were included.

Methods: Nailfold video capillaroscopy (NVC) measurements were performed at magnification of 200x at all fingers except thumbs. Images were stored for offline measurement of capillary density (CDe) and capillary diameters (CDi). Maternal anthropometrics, obstetric, and medical history were used for categorization in low and high cardiovascular risk. Comparison between groups and trimesters, with respect to pregnancy outcome, was performed using linear mixed model analysis.

Results: Women with a high risk cardiovascular profile show higher CDe, regardless of pregnancy outcome. CDi drops during pregnancy, with lowest CDi in third trimester in patients with preeclampsia. Capillary bed (CB), as a composite of CDe and CDi, is stable during pregnancy in women with low risk cardiovascular profile. In women with high risk cardiovascular profile, CB drops from the first to the second trimester, regardless of pregnancy outcome. Only in women with pre-eclampsia, the CB is lower in the third trimester as compared to the first trimester.

There is an inverse association between CDe and mean arterial pressure (MAP) in women with high cardiovascular risk and pre-eclampsia.

Conclusion: Microcirculation is altered during the course of pregnancy and microcirculatory behavior is different in patients with low and high cardiovascular risk profile, as well as in patients with preeclampsia.

Keywords: Nailfold video capillaroscopy, microcirculation, pregnancy, pre-eclampsia, hypertension

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INTRODUCTION

Pregnancy requires profound adaptation in the circulatory system, in order to guarantee adequate blood supply to the mother and fetus (1). From the 5th week of gestation, peripheral vascular resistance (PVR) lowers and cardiac output rises by increasing heart rate and stroke volume. The state of an underfilled cardiovascular system urges a plasma volume expansion through hormonal changes and an increase in sodium and water reabsorption. Two weeks later, venous compliance begins to increase and this phenomenon persists throughout pregnancy in order to build up a buffer compartment in the splanchnic system. Between 8 and 12 weeks of gestation, morphological changes occur in the heart, which can possibly be explained by the continuous higher venous return and the improvement in cardiac performance. In the second half of the first trimester, retrograde trophoblast invasion into the spiral artery is one of the reasons for increase of uteroplacental vascular compliance (1, 2).

There are only sparse data on microcirculatory changes in pregnancy. The available data differ in used techniques and outcome measures (3). In a prospective study, Antonios et al., were able to show that quantifying structural rarefaction of skin capillaries in pregnancy is a potentially useful clinical marker for the prediction of preeclampsia (4). In most studies, microcirculation is studied in a dynamic way, with recruitment maneuvers (e.g., by applying pressure to congest vessels before measurement). Less is known about static features of microcirculation in pregnancy.

Nailfold video capillaroscopy (NVC) is a method for static evaluation of microcirculation at the nailfolds of the fingers at both hands. In daily practice, this technique is used to diagnose and evaluate systemic sclerosis. There, changing patterns can predict internal organ involvement (5). Only few papers exist on NVC in pregnancy (6, 7).

In this study, we aimed to evaluate structural changes in nailfold microcirculation in pregnancies of women with or without cardiovascular risk factors. Our hypothesis is that trimestral measurements of CDi and CDe are similar in uncomplicated pregnancies, regardless of cardiovascular risk.

PATIENTS AND METHODS

Study Design and Population

A cross-sectional, observational study is conducted in the Fetal Medicine Unit of Ziekenhuis Oost-Limburg (Genk, Belgium) to evaluate the microcirculatory adaptations during pregnancy. This hospital has a combined secondary and tertiary referral function. Approval of the local Committee for Medical Ethics of Ziekenhuis Oost-Limburg (Genk, Belgium) and Hasselt University (Hasselt, Belgium) was obtained before study onset (May 29, 2017—filenr. 17/019U). All participants gave written informed consent. Obstetric and clinical history, including age, length, weight before pregnancy, profession, nationality, marital status, number of previous pregnancies, medication use, smoking history, and history of Raynaud's phenomenon were recorded, as well as blood pressure (BP) and actual weight measurements.

A total of 345 women were assessed in first and/or second and/or third trimester, categorized in 3 groups: one group with low cardiovascular (CV) risk profile, one group with high CV profile but normal pregnancy outcome and one group with high CV profile who developed preeclampsia (PE). For this study our focus was mainly to describe the differences between low and high cardiovascular risk profile and a normal pregnancy outcome. We included also patients with preeclampsia as outcome, to show that our data in that patient group are in line with previous published data, to strengthen our findings in patients with normal pregnancy outcome. Cardiovascular risk profile was based on their anthropometrics, obstetric and medical history, as specified in **Table 1**. Low risk women were invited to participate during routine antenatal visits, whereas in the high risk group, NVC was part of the maternal cardiovascular assessment performed for clinical indications in the referral clinic for high risk prenatal care (8).

Methods

For capillary assessments, a hand-held video nailfold capillaroscope (Optilia Mediscope, Vällingby, Sweden) was used, equipped with a 200x magnification high-resolution lens.

To keep the core temperature of the patient in a normal range to exclude cold induced vasoconstriction, all capillaroscopy measurements were conducted after acclimatization in a temperature-controlled room (22°C), after the obstetric ultrasound scan. Additionally, the equipment and process of measurements were explained to the patient to avoid stress during the measurement (9). Before the NVC examination, patients were seated in a chair with their hands placed at the heart level resting on a table. Next, a small drop of oil was applied on the nailfold of all fingers, except thumbs, to maximize the amount of light and improve the visibility of capillaries. Subsequently, the nailfold of the second, third, fourth, and fifth fingers was examined bilaterally by one researcher (KT) using NVC. In order to reduce selection bias, four consecutive microscopic fields extending over 1 mm centered around the nailfold were studied per finger. The contact angle and the direction of the capillaroscope were manually adjusted to reduce light reflections (9) (see **Figure 1**). Finally, by manually adapting the focusing system and triggering the button on the device, a sharp image of the capillaries was taken (see **Figure 2**).

The microscopic images were obtained using the OptiPix system (OptiPix Capillary, version 1.7.7, Optilia, Sweden) and analyzed off-line from the stored data by one observer (KT), blinded to the pregnancy outcome and cardiovascular risk of the patient. The quantitative analysis of NVC changes is a validated technique in systemic sclerosis research, were images are more challenging to capture due to contractures of fingers and thicker nailfolds due to sclerosis (10–13). In earlier research there was a high reproducibility reported for NVC (14). More recent research also uses the technique of NVC in normal pregnancy (15).

CDe is defined as the number of capillaries that are perfused at the time of examination. The analysis of capillaries was performed within the region of interest (ROI), which is a

TABLE 1 | Baseline characteristics of study population.

	Low risk group Group 1	High risk group Normal outcome Group 2	High risk group Preeclampsia Group 3	Group 1 vs. 2	Group 2 vs. 3	Group 1 vs. 3
N patients	169	142	34	NA	NA	NA
Age (mean; SD)	30.26; 4.38	31.72; 4.26	30.91; 3.88	**0.004**	0.312	0.424
Smoking	21/169 (12.43%)	10/142 (7.04%)	3/34 (8.82%)	0.114	0.721	0.552
Raynaud	11/169 (6.51%)	10/142 (7.04%)	3/34 (8.82%)	0.852	0.721	0.627
BMI (mean; SD) *n* = 159 (normal risk)/139 (high risk)/34 (preeclampsia)	24.75; 5.08	27.18; 6.32	26.68; 5.91	**0.000**	0.674	0.052
Length (mean; SD) *n* = 159 (normal risk)/139 (high risk)/34 (preeclampsia)	165.2; 6.64	166.93; 6.38	166.18; 7.17	**0.022**	0.547	0.440
Weight (mean; SD) *n* = 169 (normal risk)/141 (high risk)/34 (preeclampsia)	68.03; 15.14	76.94; 18.06	75.70; 19.47	**0.000**	0.726	**0.011**
Mean Arterial Pressure (mean; SD) = 161 (normal risk)/142 (high risk)/34 (preeclampsia)	91.42; 8.95[£]	95.32; 11.44	104.68; 13.21	**0.001**	**0.000**	**0.000**
Parity				0.0000[¥]		
Nulliparous	61.54%	28.87%	59.38%	-	-	-
Primiparous/multiparous	38.46%	71.13%	40.63%	-	-	-
Gestational age at birth (mean; SD)	$39^{+1.6}$; 12.44 (days)	38^{+6}; 22.63 (days)	35; 22.63 (days)	0.058	**0.000**	**0.000**
Mode of delivery *n* = 169 (normal risk)/135 (high risk)/30 (preeclampsia)				0.247*		
Vaginal	81.55%	74.07%	73.33%	-	-	-
Section	18.45%	25.93%	26.67%	-	-	-
Birth Weight in grams (mean; SD) *n* = 168 (normal risk)/133 (high risk—normal outcome)/29 (high risk—preeclampsia)	3318.76; 537.87	3258.06; 502.37	2257.69; 934.91	0.318	**0.000**	**0.000**
Percentile (mean; SD) *n* = 168 (normal risk)/129 (high risk—normal outcome)/29 (high risk—preeclampsia)	52.12; 29.35	49.20; 26.76	35.64; 30.67	0.378	**0.018**	**0.006**
Medication (other than antihypertensive)	8.88%	17.04%	21.88%	**0.033**	0.522	**0.031**
Medication (antihypertensive)	0%	5.15%	37.50%	**0.003**	**0.000**	**0.000**
Reason for evaluation in high prenatal care outpatient clinic	0/169	142/142 (100%)	34/34 (100%)	NA		
History of abruption placentae		2/142		-		
History of eclampsia/pre-eclampsia		47/142	6/34	-		
Pre-eclampsia in current pregnancy		0/142	14/34	-		
Underlying systemic disease		10/142		-		
Newly discovered hypertension		27/142	9/34	-		
History of gestational hypertension		15/142	1/34	-		
History of HELLP		8/142	1/34	-		
Essential hypertension		4/142	1/34	-		
History of intra-uterine growth restriction		8/142		-		
Newly discovered intra-uterine growth restriction		2/142		-		
Age		1/142		-		
History of unexplained intrauterine demise		1/142		-		
Profound edema		1/142		-		
Thrombophilia		2/142	2/34	-		
History of premature rupture of membranes		3/142		-		
Screening of high uterine artery resistance		9/142		-		
History of premature birth		2/142		-		

**Vaginal vs. section.*
[¥] Nulliparous vs. primi-/multiparous.
[£] Based on 161 assessments.
NA, not applicable.
For continuous variables mean and standard deviation are given, together with a p-value obtained for a t-test. For categorical variables counts and % are given, with a p-value for a Chi-square test (or Fisher Exact test).
Bold values are the significant ones.

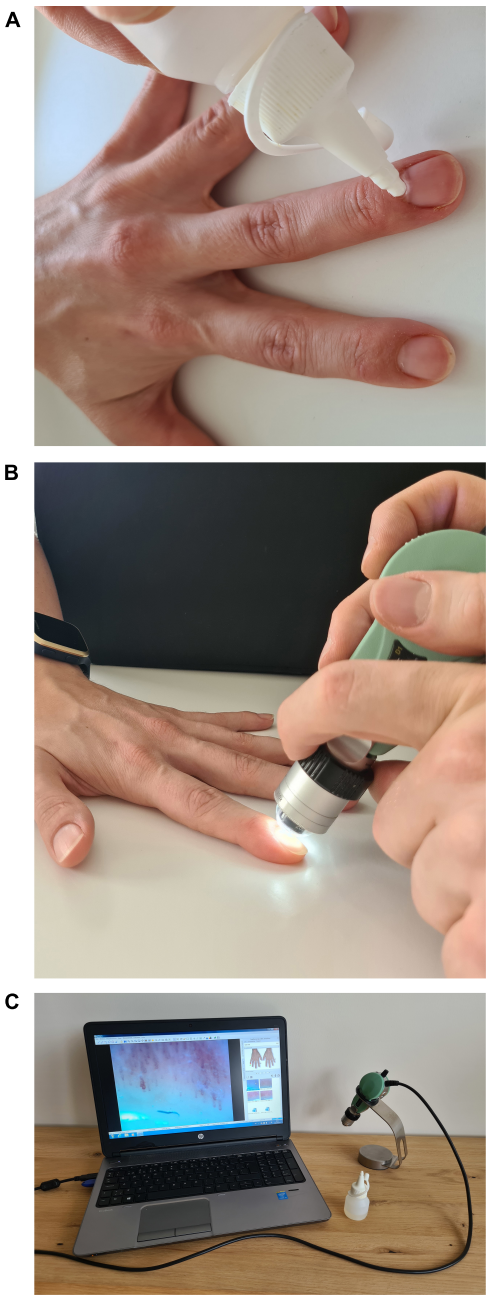

FIGURE 1 | Equipment and performance of NVC. **(A)** Oil application. **(B)** Adjusting. **(C)** Equipment to capture.

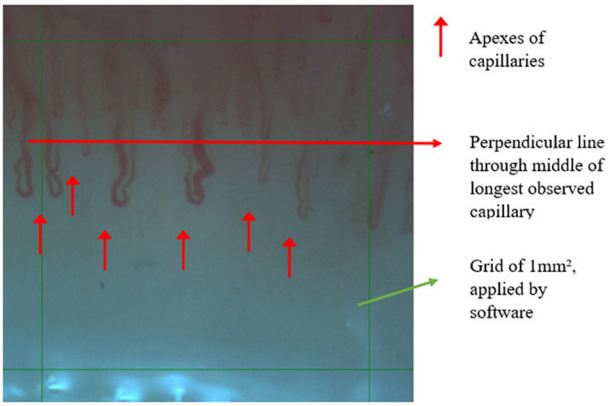

FIGURE 2 | Example of one measured field of 1 mm^2.

grid of 1 mm^2, containing the capillaries to be counted and measured. Therefore, every grid is positioned randomly before starting the analysis. All capillary apexes within the ROI were counted manually in two of the four microscopic fields chosen by the observer. The researcher directly observed the loops and indicated the loops that were considered to be distal, as per definition this is distal to a perpendicular line through the middle of longest observed capillary. If the common branch of the capillary positioned within the ROI, it was counted as being one capillary. Additionally, if only the apex of the capillary is present within the ROI, it was also counted as being one capillary.

The CDi was measured as the largest diameter of the erythrocyte column from the apex of the loop, which is also defined as the transitional diameter of a capillary. All quantifiable capillary loops were measured.

We estimated an individual's total capillary bed surface (CB) by multiplying for each finger the mean CDe with the mean CDi, followed by calculating the mean digital CB per patient by summation of all digital values divided by 8.

Besides nailfold capillaroscopy we also measured mean arterial pressure (MAP), body weight and height at each visit.

Statistical Analysis

For descriptive purposes, continuous data are shown as mean ± standard deviation (SD) and categorical data are presented as frequencies and percentages (%). At baseline, Student's *t*-test or the Mann-Whitney *U*-test (in case of a non-normally distributed data) was used to compare the normal and high-risk pregnancies for continuous variables, and a Chi-square test or Fisher's Exact test for categorical variables.

The following three capillaroscopic parameters, calculated for each patient at each trimester, are used in the statistical models: (1) Cde is the average value of the (at maximum) 16 counts (4 fingers per hand, 2 evaluations per finger. (2) Capillary diameter. A patient's value is obtained by first averaging the diameters of the (at max) four different fingers of each hand, then the average of these four values per hand and finally the average of the two hands. (3) Total capillary bed is a calculation whereby the average of eight fingers after summation of each digit's average diameter is multiplied by its average density.

For each capillaroscopic variable, a linear mixed model for repeated measures was employed to compare the evolution of the outcome over time for the two groups per trimester. Trimester, group (low risk vs. high risk) and their interaction were included as fixed explanatory effects in the model with the capillaroscopic variable as dependent variables. A non-linear evolution was allowed by including the trimester as a categorical variable. The linear mixed model also included a random patient effect to

take into account the correlation between the capillaroscopic measurements over the different trimesters of the same patient. Based on this model, changes within a group and differences between the groups at the three trimesters were investigated.

MAP and the capillaroscopic variables are associated by means of a linear mixed model. The measurements from all trimesters are used and models with MAP as dependent variable, the capillaroscopic variable as independent variable, the group and their interaction were fitted. Again, a random patient intercept was included. From this model, the change in MAP for a unit change in the capillaroscopic variable is estimated for each group, i.e., the regression slope.

No correction of multiple testing was used. All statistical analyses were performed in SAS v9.4.

RESULTS

Patient characteristics are depicted in **Table 1**. The differences between the high risk group vs. low risk group were found in age, parity, BMI, gestational age at birth, birth weight (but not percentile), MAP and medication use. Patients and assessment distributions per trimester are depicted in **Table 2**. In the low risk group we had 68, 35, and 28 women in each trimester, respectively. Thirty eight women had measurements in first and second trimester. In the high risk group we had 30, 47, and 62 women in each trimester, respectively. Six women had measurements in first and second trimester, 14 women in second and third trimester, 15 women in first and third trimester and two women in all three trimesters.

Capillary Density and Diameter

Table 3 shows the trimestral mean and 95% confidence intervals for CDe, CDi, and CB in and between the three groups.

Intertrimestral trends are similar, however, most pronounced in the low risk group where, CDe decreases from second to third trimester and CDi decreases from first to second trimester. We observed significant higher CDe in first and third trimester in women with a high risk cardiovascular profile and normal pregnancy outcome.

Capillary Bed

Women with high risk cardiovascular profile and normal pregnancy outcome had a significantly greater CB in the first trimester. In women who developed pre-eclampsia, we could observe a significant decrease of CB in the third trimester as compared to previous trimesters of pregnancy, but no differences along trimesters as compared to the other groups.

Association Between Parameters

An overview of MAP and the difference between groups is shown in **Table 4**.

Table 5 shows the association between NVC measurements and mean arterial pressure in women with pre-eclampsia. There is a statistical significant correlation between MAP and CDe. We could not observe clinically significant associations in the other groups between MAP, BMI, age, birth weight percentile and CDe/CDi.

Intra-Rater Variability

We did not perform an interrater variability measurement, since in earlier research this is well documented and NVC is found to be a reproducible technique in measurement of CDi and CDe. Although, we calculated an intra-rater variability by repeating the measurements of twenty randomly chosen patients in our cohort. We hereby report the results of intraclass correlation coefficient (ICC), (see **Table 6**).

Figure 3 is a graphical overview of the mean values of CDe, CDi and CB, as shown in **Table 3**. Per trimester and per risk group the mean values are given, which indicates the global evolution throughout pregnancy.

Figure 4 is a graphical overview of the mean values of MAP as shown in **Table 4**. Per trimester and per risk group the mean values are given, which indicates the global evolution throughout pregnancy.

DISCUSSION

Main Findings

1. Women with a high risk cardiovascular profile show higher CDe, regardless of pregnancy outcome. In women with high risk cardiovascular profile and with normal pregnancy outcome, the higher CDe is higher in first and third trimester as compared with women with low cardiovascular risk.
2. CDi drops in both the low risk and the group of women with preeclampsia from the first to the second trimester. In the low risk group, however, the difference is no longer observed in the third trimester, as is the case in the preeclampsia group.
3. CB, as a composite of CDe and CDi is stable during pregnancy in women with low risk cardiovascular profile. In women with high risk cardiovascular profile, CB drops from the first to the second trimester, regardless of pregnancy outcome. Only in women with pre-eclampsia, the CB is lower in the third trimester as compared to the 1st trimester.
4. There is an inverse correlation between CDe and MAP in women with high cardiovascular risk and pre-eclampsia.
5. In this study we were able to show the feasibility and potential of microvascular assessment using NVC in pregnant women. The easy accessibility makes it an attractive technique to use, since there is no need to a special environment to perform the examination. It is done by a handheld, USB coupled device, which can be used even bedside.

Interpretation

Microcirculatory assessment using NVC has previously been described in women with pregnancy induced hypertension (3, 7).

More recent research also investigated the role of NVC in normal pregnancy course (15). Assessment of the maternal

TABLE 2 | Distribution of patients and assessments.

Low risk			High risk		
1st trimester	**2nd trimester**	**3rd trimester**	**1st trimester**	**2nd trimester**	**3rd trimester**
68	35	28	30	47	62
38			6		
			14		
			----------> 15		
			2		
169 women in total			176 women in total (142 with normal outcome,		
207 measurements in total			34 with pre-eclampsia) 215 measurements in total		

TABLE 3 | Results.

Results		Trimester 1	Trimester 2	Trimester 3	Trimester 1 → 2	Trimester 2 → 3	Trimester 1 → 3
Capillary density	**Low risk group—normal outcome (= A)**	7.31 [7.16; 7.46]	7.38 [7.20; 7.56]	7.01 [6.7; 7.33]	$p = 0.47202$	**$p = 0.04552$**	$p = 0.09430$
	High risk group—normal outcome (= B)	7.64 [7.41; 7.86]	7.48 [7.27; 7.69]	7.39 [7.20; 7.57]	$p = 0.28476$	$p = 0.46680$	$p = 0.05786$
	High risk group—pre-eclampsia (= C)	7.49 [6.85; 8.13]	7.58 [7.15; 8.01]	7.13 [6.80; 7.46]	$p = 0.80606$	$p = 0.07041$	$p = 0.29217$
	B vs. A	**$p = 0.01823$**	$p = 0.48037$	**$p = 0.04488$**			
	C vs. A	$p = 0.59455$	$p = 0.40566$	$p = 0.61220$			
	C vs. B	$p = 0.67337$	$p = 0.68045$	$p = 0.18236$			
Capillary diameter	**Low risk group—normal outcome (= A)**	17.47 [16.89; 18.04]	16.54 [15.86; 17.22]	17.27 [16.08; 18.45]	**$p = 0.01773$**	$p = 0.29655$	$p = 0.76529$
	High risk group—normal outcome (= B)	17.96 [17.12; 18.79]	17.24 [16.43; 18.04]	17.54 [16.83; 18.24]	$p = 0.20303$	$p = 0.55957$	$p = 0.40655$
	High risk group—pre-eclampsia (= C)	19.61 [17.14; 22.06]	15.90 [14.19; 17.61]	16.68 [15.43; 17.93]	**$p = 0.01097$**	$p = 0.42563$	**$p = 0.02803$**
	B vs. A	$p = 0.34392$	$p = 0.19410$	$p = 0.70031$			
	C vs. A	$p = 0.09724$	$p = 0.49330$	$p = 0.50403$			
	C vs. B	$p = 0.21371$	$p = 0.16388$	$p = 0.24267$			
Capillary bed	**Low risk group—normal outcome (= A)**	127.12 [122.6; 131.65]	122.22 [116.88; 127.57]	120.59 [111.37; 129.82]	$p = 0.11171$	$p = 0.76384$	$p = 0.21216$
	High risk group—normal outcome (= B)	135.17 [128.6; 141.75]	126.12 [119.8; 132.45]	128.50 [122.95; 134.06]	**$p = 0.04196$**	$p = 0.55298$	$p = 0.09642$
	High risk group—pre-eclampsia (= C)	146.01 [126.63; 165; 38]	123.53 [110.11; 136.94]	119.79 [110; 129.57]	**$p = 0.04998$**	$p = 0.62926$	**$p = 0.01272$**
	B vs. A	**$p = 0.04801$**	$p = 0.35505$	$p = 0.14936$			
	C vs. A	$p = 0.06278$	$p = 0.85932$	$p = 0.90620$			
	C vs. B	$p = 0.29834$	$p = 0.73075$	$p = 0.12847$			

This table shows the results per parameter for each trimester and per risk group the mean value and the 95% confidence interval. Significance levels are specified by p-value.
Bold values are the significant ones.

microvascular system can offer insights in the maternal global cardiovascular and placental function and in the pathophysiological processes of hypertensive complications of pregnancy (16).

CDi seems to compensate to findings, seen in CDe in patients with low cardiovascular risk. CDi drops from the first to the second trimester, but in the third trimester there is no longer a significant lower CDi as compared to the first trimester. Meanwhile, CDe drops significant from the second to the third trimester. We can speculate that by lowering CDe, a compensatory vasodilatation occurs (CDi rises). This is different in patients with a high cardiovascular risk with evolution toward pre-eclampsia, there we do observe a lower CDi in the third trimester as compared to the first trimester, reflective of a vasoconstrictive status. CDe shows, however, no significant drop. Further research in conjunction with macrocirculatory assessment is needed to clarify these findings.

Our data on CDe are in line with earlier published research. Capillary rarefaction (defined as the reduction in the number

TABLE 4 | Overview of MAP in different groups and per trimester.

		Trimester 1	Trimester 2	Trimester 3	Trimester 1 → 2	Trimester 2 → 3	Trimester 1 → 3
MAP	**Low risk group—normal outcome (= A)**	92.38 [90.39; 94.36]	91.82 [89.53; 94.11]	87.85 [83.79; 91.91]	$p = 0.6659$	$p = 0.0943$	$p = 0.0495$
	High risk group—normal outcome (= B)	96 [93.22; 98.78]	93.38 [90.78; 95.98]	95.88 [93.53; 98.24]	$p = 0.1506$	$p = 0.1288$	$p = 0.9428$
	High risk group—pre-eclampsia (= C)	99.34 [91.05; 107.63]	102.29 [96.71; 107.86]	108.71 [104.55; 112.86]	$p = 0.5404$	**$p = 0.0460$**	**$p = 0.0353$**
	B vs. A	**$p = 0.0374$**	$p = 0.3772$	**$p = 0.0008$**			
	C vs. A	$p = 0.1086$	**$p = 0.0007$**	**$p = 0.0000$**			
	C vs. B	$p = 0.4526$	**$p = 0.0047$**	**$p = 0.0000$**			

Bold values are the significant ones.

TABLE 5 | Overview of MAP in different groups and per trimester.

	Dependent variable	Independent variable	Estimate	*P*-value
High risk group—pre-eclampsia	**Mean arterial pressure**	**Capillary density**	-5.7230	**0.0022**
	Mean arterial pressure	**Capillary diameter**	-0.1951	0.7458

This table depicts the association between CDe and CDi as dependent and independent variables and other variables. Based on the independent variables, we tried to predict the dependent variables. The models assumes a linear association between dependent and independent variables. The strength of association is reflected in the slope (estimate). The estimate depicts the change in dependent variable by increase of 1 unit of the independent variable. The P-value depicts whether there is a significant association or not.
Bold values are the significant ones.

of capillaries per visual field), described in normal pregnancy, is consistent with the significant drop in CDe in our study in the group of women with low cardiovascular risk (17). In earlier animal models, this was shown in multiple microvascular beds (18, 19). CDe shows no difference between the high risk group with preeclampsia and the other groups. This finding is also in line with earlier research in skin capillaroscopy (20). Our data show differences in CDe but in a selected group of women with high cardiovascular risk and normal pregnancy outcome. They have higher CDe in first and third trimester of pregnancy, which is surprising, since we hypothesized it would be similar regardless of cardiovascular risk. Whether this finding is linked to the eventual normal gestational outcome is an interesting topic for further research. Speculative is that the lack of rarefaction (higher CDe) is possibly protective against a raise in blood pressure and evolution toward pre-eclampsia, which makes that women with a high cardiovascular risk and a higher CDe (in comparison to women with normal cardiovascular risk) are more likely to have a normal pregnancy outcome. Our groups are too small to make distinction between early and late pre-eclampsia, which could be of interest. In further analysis, when also taking into account macrocirculatory parameters and/or serum biomarkers that reflect vascular remodeling. Of course, to further clarify this finding, we have to look at the macrocirculatory parameters in both groups, which is part of our future research. But, in this way, capillaroscopy can be a potentially new tool in addition to the other assessments, to make a distinction between women with high risk pregnancy who will have low or high risk to develop pre-eclampsia.

TABLE 6 | Intraclass correlation coefficient analysis (ICC).

	CDe	CDi
Mean value per patient	0.99	0.98

Although further research is needed, the process of capillary rarefaction seems to precede the onset of hypertension, suggesting that it is a primary phenomenon, rather than secondary (21). In high risk patients, capillary rarefaction can be a positive predictor for the development of pre-eclampsia, which could further increase sensitivity and specificity of uterine artery Doppler pulsatility index for the development of PE (4). In our preeclampsia group we confirmed the finding of capillary rarefaction, as CDe and MAP are inversely related with high statistical significance.

From the physiological point of view, microcirculatory function relates to both arterial and venous vascular activity. Arterial vasoconstriction reduces the capillary perfusion, whereas the opposite is true for venous congestion. Our data show that in early pregnancy of PE pregnancies, an expanded capillary bed is present, evolving to normal during the course of pregnancy in association with a rise of blood pressure. One conclusion might be that in these women, venous congestion is already present in the early stages of pregnancy, reducing to normal levels with increasing arteriolar constriction and blood pressure. The hypothesis of gestation induced hypertension as a protective mechanism against microcirculatory congestion is very interesting and invites for further assessment in future research (22).

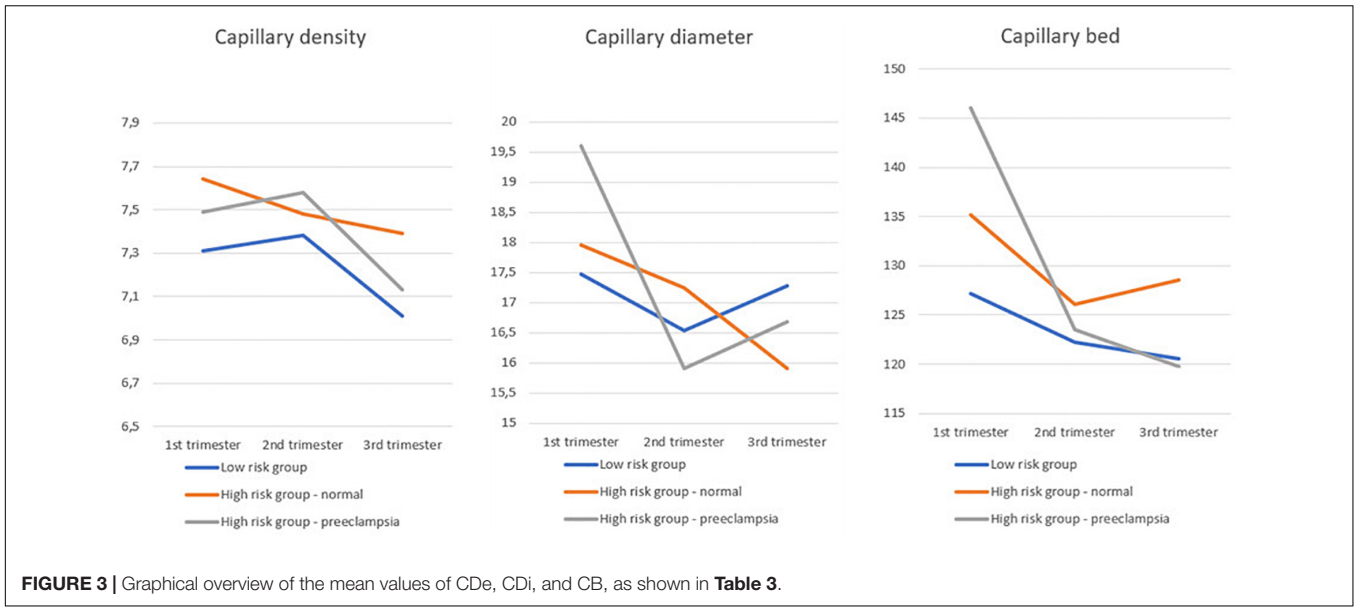

FIGURE 3 | Graphical overview of the mean values of CDe, CDi, and CB, as shown in **Table 3**.

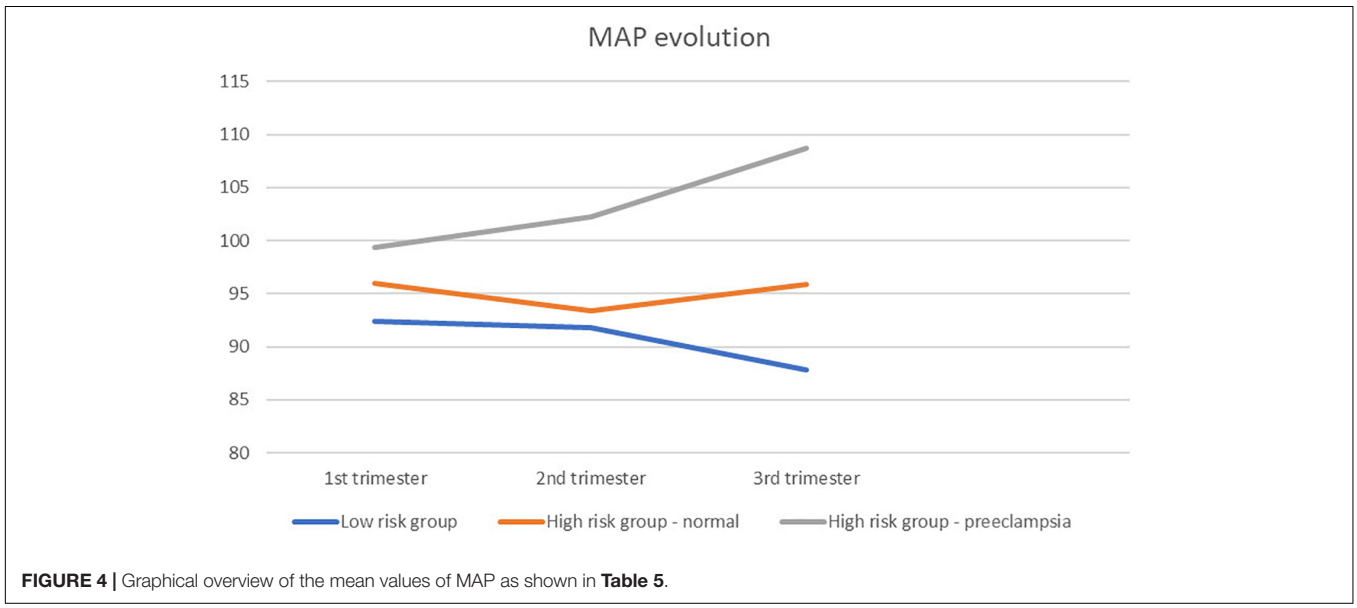

FIGURE 4 | Graphical overview of the mean values of MAP as shown in **Table 5**.

CDe and CDi measurement by NVC, are useful and feasible. They add information on the pathophysiology of hypertensive pregnancy disorders. As a standalone examination, they cannot discriminate, based on the current data, but together with macrocirculatory measurements, a more detailed profile of the total hemodynamic status in pregnancy can be generated. Based on our current study and on earlier published data, it is not yet possible to really define cut-off values. We can measure and see a trend, but variation throughout pregnancy is too small to make distinction between high and/or low and/or normal. Values are likely to be seen in relation to other measurements, but of course at this stage this is rather speculative. For further research, as microcirculation is influenced by pre- and postcapillary factors, it would be of uttermost interest to couple macrovascular observations to microvascular changes as they form a continuum and integral part of the cardiovascular system, to search for correlations, that can explain the microcirculatory observations. Despite normal pregnancy outcome, we observed differences based on cardiovascular risk profiles, which could be of value for early detection of cardiovascular events later on in life. Future research should also focus on microcirculatory changes in women with and without hypertensive and/or uteroplacental complications in pregnancy, taking the cardiovascular risk profile into account.

Strengths and Limitations

Our strength is the higher number of patients included. Most research on microcirculation in pregnancy is performed in small populations. We described the findings throughout pregnancy in both a large low risk, high risk with normal outcome and high risk

who developed PE. In this way, it is reassuring that our results in a larger population are in line with earlier published research in smaller populations.

Limitations are the lack of consistently, consecutive measurements in each subject. The population is a mix of longitudinal and cross-sectional measurements for each group, in each trimester. In the high risk group, this is due to the fact that patients were mostly seen once, as a tertiary advice for the peripheral colleague gynecologists. Patients are not routinely seen twice in our center. In the normal risk group, we did had the intention to see patients four times (during pregnancy and once post-partum) but due to COVID-19 pandemic, only necessary visits were allowed. Second limitation is that we don't have equal number of patients in every trimester. This last limitations is somewhat overcome by our higher number of patients in total.

CONCLUSION

With our observational study, we could confirm that microcirculation is altered during the course of pregnancy and that there is a difference in patients with low and with high cardiovascular risk profile, as well as in patients with high risk profile who do and do not develop preeclampsia. As capillary rarefaction seems to precede the onset of PE, capillaroscopy could be of diagnostic value in predicting pre-eclampsia in high risk 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 Ethical Committee ZOL Genk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TK and DM gathered data and analyzed the data. TK wrote the article. DM, CJ, and GW scrutinized the article to further optimization by providing insight and suggestions. CJ and GW were guiding TK in his Ph.D. trajectory. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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The Association Between Hypertensive Disorders in Pregnancy and the Risk of Developing Chronic Hypertension

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Objective: This meta-analysis comprehensively evaluated the association between hypertensive disorders in pregnancy (HDP) and the risk of developing chronic hypertension and the associations between specific types of HDP, including preeclampsia (PE) and gestational hypertension (GH), and the risk of developing chronic hypertension.

Design: Systematic review and meta-analysis.

Data Sources: The PubMed, Embase and Cochrane Library databases were searched from inception to August 20, 2021.

Methods: Depending on heterogeneity, the combined odds ratio (OR) of the 95% confidence interval (CI) was obtained with a random-effects or fixed-effects model. We used meta-regression analysis to explore the sources of heterogeneity. We analyzed the OR value after adjusting for age and BMI at recruitment, prepregnancy BMI, age at first delivery, and other factors. Additionally, we evaluated the results of the subgroup analysis by the year of publication (< 2016, ≥ 2016), study design, sample size (< 500, ≥ 500), region (North and South America, Europe, and other regions) and NOS score (< 7, ≥ 7).

Results: Our systematic review and meta-analysis comprehensively explored the relationships between HDP, GH, and PE and chronic hypertension. Twenty-one articles that included 634,293 patients were included. The results of this systematic review and meta-analysis suggested that women with a history of HDP are almost 3.6 times more likely to develop chronic hypertension than those without a history of HDP, women with a history of GH are almost 6.2 times more likely to develop chronic hypertension than those without a history of GH, and women with a history of PE are almost 3.2 times more likely to develop chronic hypertension than those without a history of PE. In addition, we further calculated the probability of developing chronic hypertension among patients with HDP or PE after adjusting for age and BMI at recruitment, prepregnancy BMI, age at first delivery, and other factors. The results suggested that women with a history of HDP are almost 2.47 times more likely to develop chronic hypertension than those

without a history of HDP and that women with a history of PE are almost 3.78 times more likely to develop chronic hypertension than those without a history of PE. People in Asian countries are more likely to develop chronic hypertension after HDP or PE, while American people are not at high relative risk.

Conclusion: These findings suggest that HDP, GH, and PE increase the likelihood of developing chronic hypertension. After adjustment for age and BMI at recruitment, prepregnancy BMI, age at first delivery, and other factors, patients with HDP or PE were still more likely to develop chronic hypertension. HDP may be a risk factor for chronic hypertension, independent of other risk factors. GH and PE, as types of HDP, may also be risk factors for chronic hypertension.

Systematic Review Registration: [www.ClinicalTrials.gov], identifier [CRD42021238599].

Keywords: hypertensive disorders in pregnancy (HDP), preeclampsia (PE), gestational hypertension (GH), hypertension, pooled odds ratios (ORs), confidence intervals (CIs), systematic review, meta-analysis

INTRODUCTION

Hypertension is one of the most common conditions that occur during pregnancy and the main cause of maternal death (1). Ten percent of pregnancies are affected by hypertension, especially those of primiparas. Hypertensive disorders in pregnancy (HDP) include a series of diseases classified as preeclampsia, eclampsia, gestational hypertension, pregnancy complicated with chronic hypertension and preeclampsia superimposed on chronic hypertension (2). Their definitions are shown in **Table 1**. HDP remains one of the leading causes of maternal and fetal disease incidence and mortality worldwide. Moreover, HDP is closely related to the patient's future health. A study found that women with prepregnancy hypertension and those with both HDP and prepregnancy hypertension had an increased risk of kidney disease 5 years after delivery (3). HDP increases the risk of future cardiovascular events and has been included in the guidelines for the risk assessment and prevention of stroke and cardiovascular disease (CVD) in women (4, 5). Recent evidence indicates that the incidence rate of HDP has increased over the past 30 years, suggesting that HDP, a sex-specific CVD risk factor, may become more important in the coming years (6, 7). A history of gestational hypertension/preeclampsia is related to subclinical atherosclerosis (increased carotid intima-media thickness (IMT) and plaque) (8). Pregnancy-induced hypertension is even hereditary, affecting the cardiovascular health of offspring (9).

Studies have shown that women with preeclampsia have a higher risk of developing chronic hypertension. Indeed, comprehensive data show that 20% of women with eclampsia develop hypertension within 15 years (10). However, the risk varies depending on the population studied and the criteria used for diagnosis. According to a study, the risk of hypertension in Sweden 5–12 years after pregnancy is approximately 40% (11, 12). Three other studies reached similar conclusions (13–15). The correlation between HDP and chronic hypertension fluctuates greatly. The results were different depending on the region and follow-up years. There are many other confounding factors, such as race or country; studies have shown that African women with a history of pregnancy-induced hypertension, followed by Hispanic and Asian women, have the highest risk of future high blood pressure. Moreover, individuals with normal blood pressure showed better health-related quality of life than patients with hypertension. Although systemic hypertension has almost always been considered a clinically asymptomatic disease, it can impair the quality of life of patients (16, 17). Therefore, the early prevention of hypertension is necessary. If the association between gestational hypertension and chronic hypertension can be identified, the early prevention and treatment of HDP will greatly benefit the long-term health of patients.

This systematic review and meta-analysis assessed recent reports to explore the association between HDP and chronic hypertension and evaluate the associations between specific types of HDP, including preeclampsia (PE), and gestational hypertension (GH), and the risk of developing chronic hypertension. We analyzed both crude and adjusted OR values to better determine the relationships between the variables and the stability of the results. We also conducted subgroup analysis by country and year to analyze the relationship between HDP and chronic hypertension.

METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (18).

Protocol, Eligibility Criteria, Information Sources, and Search Strategy

This review was based on a prior design recommended by a systematic review and meta-analysis. The PubMed, EMBASE, and Cochrane Library databases were searched electronically in August 2021 using a combination of terms, keywords and

TABLE 1 | Definition of HDPs.

Type of HDPs	Definition
Gestational hypertension	Hypertension occurring after 20 weeks of pregnancy, systolic blood pressure ≥ 140 mmHg and (or) diastolic blood pressure ≥ 90 mmHg, and a return to normal blood pressure within 12 weeks after delivery; urinary protein (–); the diagnosis can be made after delivery.
Preeclampsia	Systolic blood pressure ≥ 140 mmHg and (or) diastolic blood pressure ≥ 90 mmHg after 20 weeks of pregnancy, accompanied by urinary protein ≥ 0.3 g/24 h, or random urinary protein (+) Or without proteinuria, but combined with any of the following: • Thrombocytopenia (platelets < 100) × 10^{9}/L) • Liver function impairment (serum transaminase level is more than twice the normal value) • Renal function impairment (serum creatinine level > 1.1 mg/dl or more than twice the normal value) • Pulmonary edema • New central nervous system abnormalities or visual impairment
Eclampsia	Convulsions that cannot be explained by other reasons occurring on the basis of preeclampsia.
Preeclampsia superimposed on chronic hypertension	There was no proteinuria before pregnancy, and proteinuria was present after 20 weeks of pregnancy in women with chronic hypertension; or proteinuria was present before pregnancy, and proteinuria increased significantly after pregnancy; or blood pressure rises further; or thrombocytopenia < 100 × l0^{9}/L; or other serious manifestations such as liver and kidney function damage, pulmonary edema, nervous system abnormalities, or visual impairment.
Pregnancy complicated with chronic hypertension	Systolic blood pressure ≥ 140 mmHg and (or) diastolic blood pressure ≥ 90 mmHg before 20 weeks of pregnancy (excluding trophoblastic diseases), and there was no significant aggravation during pregnancy; or hypertension was first diagnosed after 20 weeks of pregnancy and continued beyond 12 weeks postpartum.

word variants related to the medical subject headings (MeSH) "hypertension, pregnancy," "preeclampsia," "eclampsia" and "hypertension." We used Endnote x9 to remove duplicate articles and then browsed the titles and summaries to exclude unrelated articles. Reviews, meta-analyses, articles lacking relevant data, letters and abstracts were excluded. There were no time or language restrictions. The reference lists of relevant articles and comments were manually searched for additional reports. The study was registered in the Prospero database (Registration number: CRD42021238599).

Study Selection, Data Collection, and Data Items

The main outcome was the incidence rate of chronic hypertension in patients with HDP or with the specific types PE and GH. We included case–control studies and cohort studies that provided data on how many patients developed hypertension several years after delivery. The research period of the different studies varied: the span was large, and the time period ranged from 1 to 30 years. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg occurring more than once in a clinical environment. The use of antihypertensive drugs and lower thresholds for defining hypertension were also included in the diagnostic criteria. When data were available, only patients affected by HDP, PE, and GH were considered in the analysis. We excluded studies in which chronic hypertension was present before pregnancy or before 20 weeks of gestation. If a study included patients with chronic hypertension, we considered only the articles that provided the number of patients with chronic hypertension. In addition, we did not include articles about the incidence rate of postpartum hypertension within 1 year of delivery.

Two researchers, Xu and Wang, independently performed all abstract screenings. The two researchers retrieved and independently evaluated the full texts of potentially eligible studies. Any inconsistencies or differences were discussed with a third reviewer, and a consensus was reached. Several articles were translated into languages other than English to determine whether they were suitable for inclusion. The reviewers extracted data on the study characteristics and results, especially the author, year, location, study type, population size, and reported results. If multiple studies with the same endpoint were published for the same cohort, the report containing the most comprehensive population information was used to avoid population overlap.

Risk of Bias and Study Quality

The quality of the included studies was assessed using the Newcastle–Ottawa Scale (NOS) for cohort and case–control studies, which was developed by Schokker et al. to assess the quality of non-randomized studies (19). With this protocol, the maximum score for each study was 9. Studies with a score ≥ 7 were considered high-quality articles. The two authors independently reviewed each study and determined whether it was eligible for inclusion in our meta-analysis. If there were any differences, the third author joined the discussion. Since the NOS could not be used to fully evaluate the potential confounding factors in the study analysis, information on which confounding factors were considered in each study was further extracted. Publication bias was assessed by a funnel plot using Begg's and Egger's tests (20). Subgroup analysis by publication year (< 2016, ≥ 2016), study design, location, sample size (< 500, ≥ 500) and NOS score (< 7, ≥ 7) was performed to further evaluate the associations between HDP, PE, and GH and chronic hypertension.

Statistical Analysis

We constructed forest plots to obtain pooled ORs and 95% CIs. We applied a fixed-effects model to calculate the combined effect estimate if $I_2 \geq 50\%$. Otherwise, we used a random-effects model.

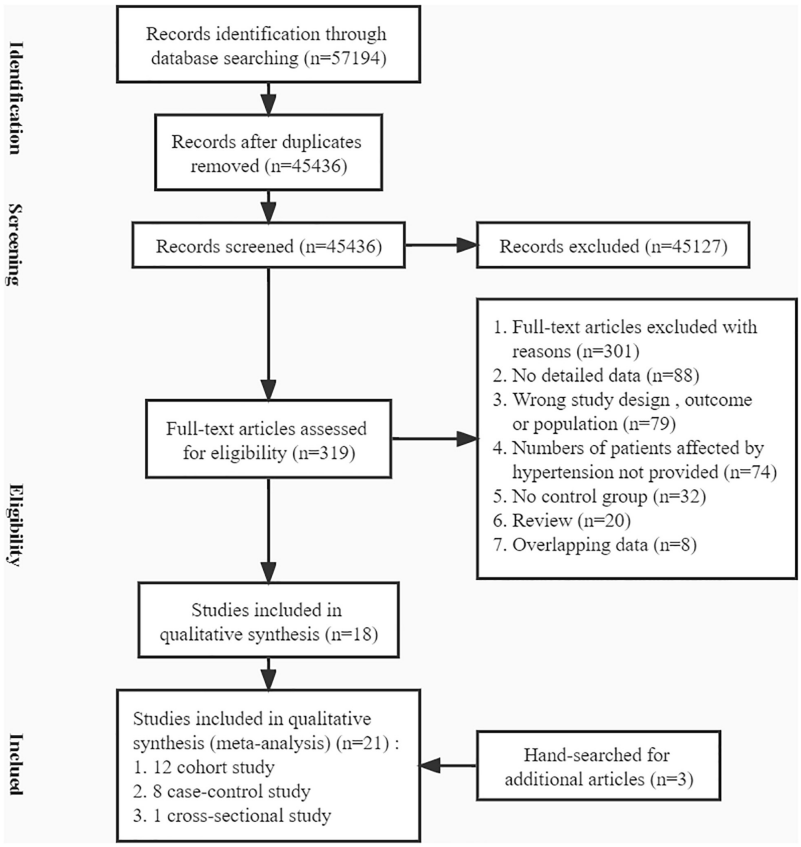

FIGURE 1 | A flow chart of the screening of studies on HDP/GH/PE and chronic hypertension.

TABLE 2 | Summary characteristics of the 21 studies included in the systematic review of pregnancy complications.

Articles	Publication years	Country	Study design	Included population	Sample size	NOS score
Garrido-Gimenez et al. (21)	2020	France	Cohort study	PE	79	6
Moreira et al. (22)	2009	Brazil	Cross-sectional study	HDP	1,141	7
Drost et al. (23)	2011	Netherlands	Cohort study	PE	874	7
Watanabe et al. (24)	2020	Japan	Case–control study	HDP/GH/PE/OTHER	245	7
Nordén Lindeberg and Hanson (25)	2000	Sweden	Cohort study	HDP	115	8
Shammas and Maayah (26)	2000	Jordan	Case–control study	GH/PE	180	6
Garovic et al. (27)	2010	America	Case–control study	HDP	5,796	6
Wilson et al. (28)	2003	Britain	Cohort study	GH/PE	1,865	8
Mito et al. (29)	2018	Japan	Cohort study	HDP	796	7
Garovic et al. (30)	2020	America	Cohort study	HDP/PE	3,283	7
Edlow et al. (31)	2009	America	Case–control study	PE	248	7
Honigberg et al. (32)	2019	Britain	Cohort study	HDP	277,011	7
Marín et al. (33)	2000	Spain	Cohort study	HDP	463	8
Kuo et al. (34)	2018	China	Cohort study	PE/Eclampsia	7,050	7
Gastrich et al. (35)	2020	America	Case–control study	PE	331,707	6
White et al. (36)	2016	America	Cohort study	PE	112	8
Qasim et al. (37)	2016	Pakistan	Case–control study	HDP	527	7
Ghossein-Doha et al. (38)	2014	Netherlands	Cohort study	PE	28	6
Ehrenthal et al. (39)	2014	America	Case–control study	HDP	82	6
Shahul et al. (40)	2018	America	Case–control study	HDP/PE	137	6
Martelly et al. (41)	2021	America	Cohort study	PE	55	7

Sensitivity analysis was used to explore the robustness of the included literature. Publication bias was assessed by funnel plots and linear regression equations. If the funnel plot was obviously asymmetric, we further used the trim-and-fill method to adjust the data. In addition, meta-regression analysis was performed based on the publication year, NOS score, status, sample size, and study design to explore the sources of heterogeneity. All analyses were conducted *via* R version 3.6. The critical value for statistical significance was set as $P < 0.05$.

RESULTS

Study Selection

To obtain relevant literature, we searched the PubMed, Embase and Cochrane Library databases from inception to August 20, 2021. A total of 57,194 studies were obtained (**Figure 1**). After removing duplicate articles, 45,436 articles remained. Then, we culled articles that were unrelated and lacked data by scanning the titles, abstracts, and full texts. In addition, three studies that were retrieved from the reference lists of previous relevant articles were included. Ultimately, 21 studies met all eligibility criteria (21–41).

Study Characteristics

The 21 studies included in this systematic review and meta-analysis varied in study design, year of publication, NOS score, country, and sample size. All studies were observational; 12 were described as cohort studies, eight as case–control studies and one as a cross-sectional study. The publication dates of these articles ranged from 2000 to 2021. Among these articles, the study areas included Europe for seven studies, North and South America for nine studies, and other regions for five studies. The

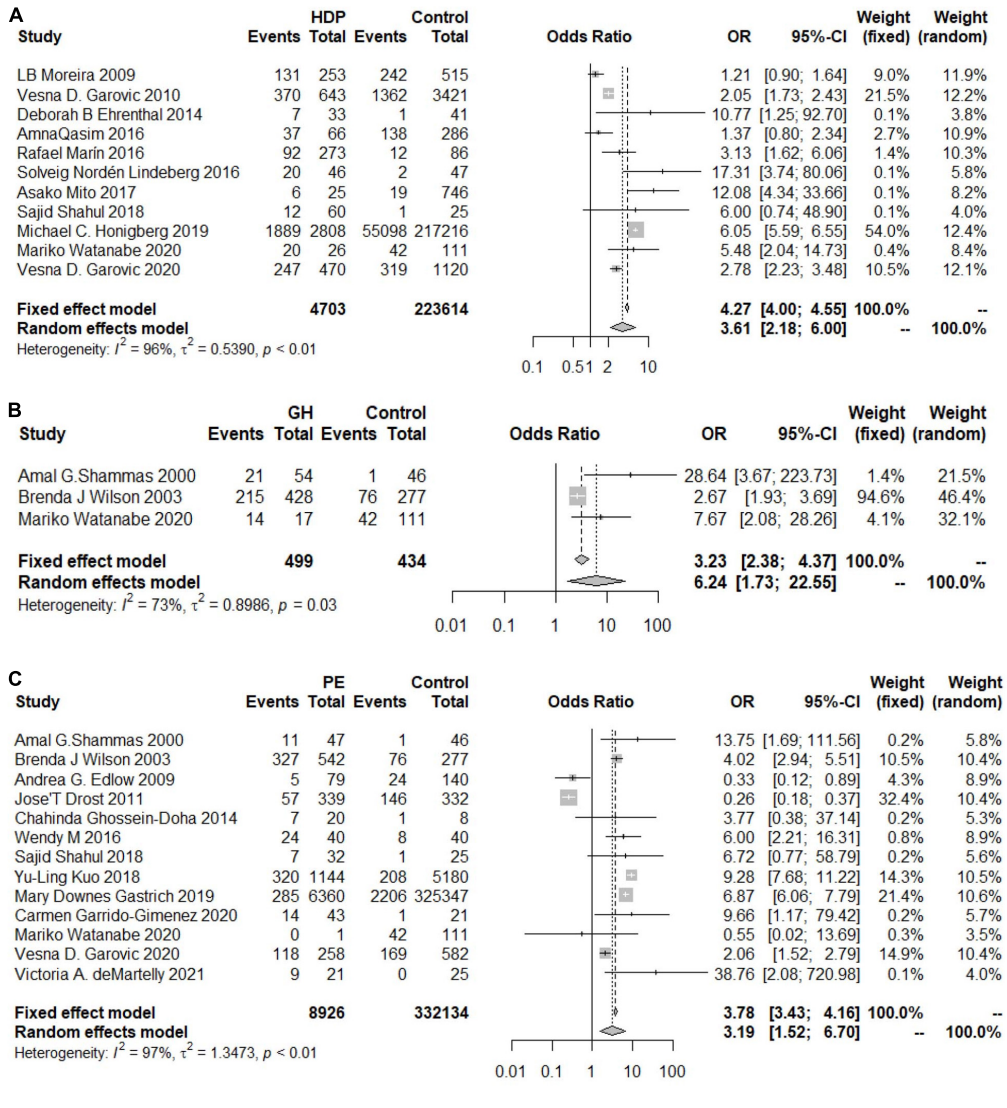

FIGURE 2 | Forest plots of the risk of developing chronic hypertension. **(A)** Forest plots of the risk of developing chronic hypertension in the HDP group; **(B)** forest plots of the risk of developing chronic hypertension in the GH group; **(C)** forest plots of the risk of developing chronic hypertension in the PE group.

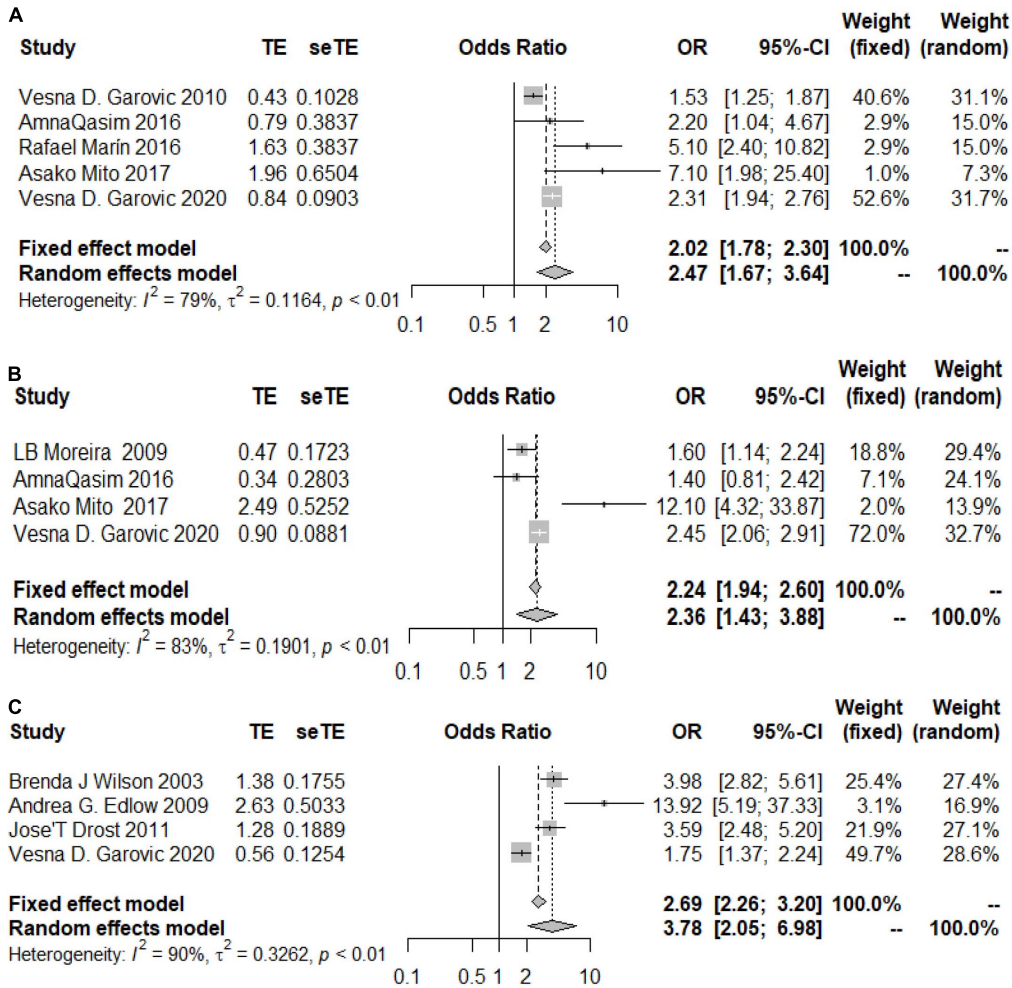

FIGURE 3 | Forest plots of the risk of developing chronic hypertension in the adjusted group. **(A)** Forest plots of the risk of developing chronic hypertension in the HDP-adjusted group; **(B)** forest plots of the risk of developing chronic hypertension in the HDP-unadjusted group; **(C)** forest plots of the risk of developing chronic hypertension in the PE-adjusted group.

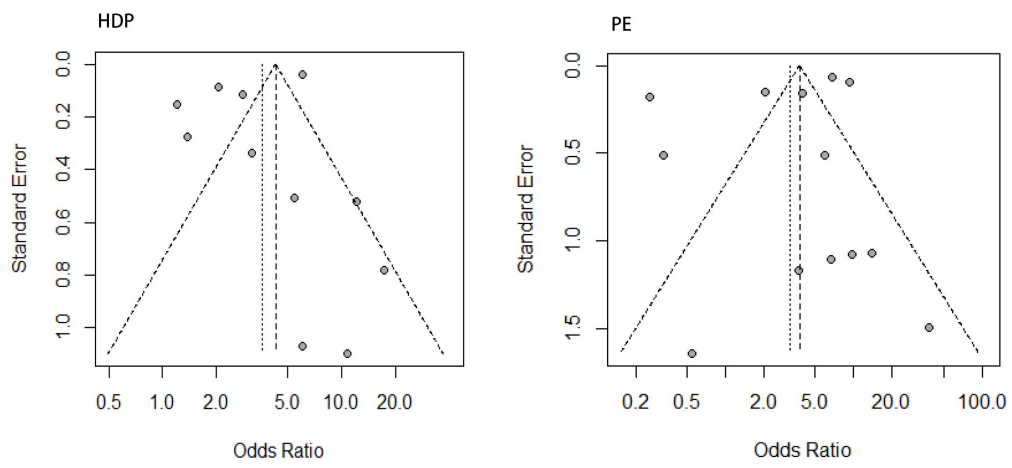

FIGURE 4 | Funnel plot of the included studies for the HDP and PE groups.

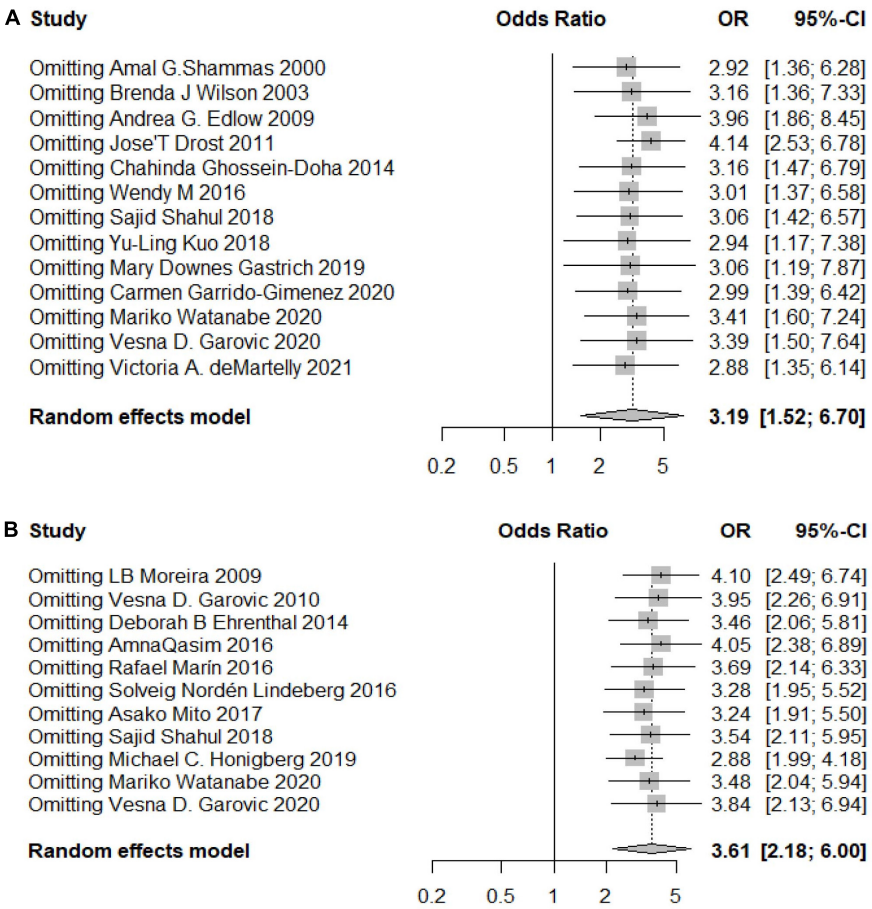

FIGURE 5 | Sensitivity analyses of the included studies. **(A)** Sensitivity analysis of the included studies for the HDP group; **(B)** sensitivity analysis of the included studies for the PE group.

smallest sample size was 28 (38), and the largest sample size was 331,707 (35). Eleven studies researched HDP, 3 researched GH, and 13 researched PE. There were five studies that included more than one disease. The research characteristics are summarized in **Table 2**.

Total Pooled Effect

As shown in **Figure 2A**, the heterogeneity among the eligible articles about HDP was I^2 = 96% ($P <$ 0.01), so we chose to use a random-effects model. The overall combined effect showed that HDP patients had a higher risk of developing chronic hypertension than healthy controls (OR 3.61, 95% CI 2.18–6.00). We also calculated the GH and PE results and chose to use random-effects models (I_{GH}^2 = 73%, P = *0.03*, I_{PE}^2 = 97%, $P <$ 0.01). Women with GH or PE were at higher risk of developing chronic hypertension than healthy controls (OR_{GH} 6.24, 95% CI 1.73–22.55, OR_{PE} 3.19, 95% CI 1.52–6.70) (**Figures 2B,C**).

Some articles reported adjusted OR values for age and BMI at recruitment, prepregnancy BMI, age at first delivery and other factors. We further evaluated the associations between HDP, GH, and PE and chronic hypertension based on the adjusted OR values.

The heterogeneity among the articles about HDP with adjusted OR values was 79% (OR 2.47, 95% CI 1.67–3.64) (**Figure 3A**), and the heterogeneity among those with unadjusted OR values was 83% (OR 2.36, 95% CI 1.43–3.88) (**Figure 3B**). The two results were similar, showing that patients with HDP are at higher risk of developing chronic hypertension than healthy controls. The same trend in the risk of chronic hypertension was observed in the PE group, and the OR

TABLE 3 | Results of the meta-regression analysis.

Study level variables	HDP Group		PE Group	
	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
Publication year	(0.0077, 0.2026)	0.03	(−0.07, 0.16)	0.41
NOS score	(−1.28, 1.35)	0.96	(−3.10, 0.61)	0.19
Region	(−0.22, 0.82)	0.26	(−1.03, 1.28)	0.83
Sample size	(−1.31, 0.76)	0.6	(−1.78, 1.09)	0.64
Study design	(0.24, 1.17)	0.003	(−1.55, 2.19)	0.74

values were adjusted (I^2 = 90%, OR = 3.78, 95% CI 2.05–6.98) (**Figure 3C**).

Publication Bias, Sensitivity Analysis and Risk Analysis

Through linear regression and funnel plots, we found that studies on HDP (P = 0.4639) and PE (P = 0.5380) had no publication bias (**Figure 4**). **Figure 5A** shows that when omitting one of these studies (22), the sensitivity analysis of the HDP group showed an OR of 4.10 (95% CI 2.49–6.74), which was nearly the same outcome as the total pooled effect (OR 3.61, 95% CI 2.18–6.00). Similarly, when omitting other studies, women with HDP were at higher risk for developing chronic hypertension than healthy controls. Sensitivity analysis of the PE group showed similar results after omitting other studies, and women with PE were at higher risk of developing chronic hypertension than those in the healthy control group (**Figure 5B**).

The quality assessment and risk of bias analysis of each included study are shown in **Table 2**.

Meta-Regression Analysis

In the total pooled effect, the heterogeneity of the HDP group was I^2 = 96%, and the heterogeneity of the PE group was I^2 = 97%. Thus, we conducted meta-regression analysis based on the publication year, NOS score, country, sample size and study design. The results confirmed that the publication year and study design had a significant effect on the heterogeneity in the HDP group ($P_{\text{publication year}}$ = 0.03, $P_{\text{study design}}$ = 0.003). Other factors showed no significant effect on the heterogeneity in the HDP group. The publication year and study design may be the sources of heterogeneity for the experimental results. None of the factors showed a significant effect on the heterogeneity in the PE group (**Table 3**).

Subgroup Analysis

We conducted subgroup analyses based on the year of publication (< 2016, ≥ 2016), study design, region (North America, South America, Europe, etc.), sample size (< 500, ≥ 500) and NOS score (< 7, ≥ 7) to further evaluate the correlations between HDP, GH, and PE and the risk of chronic hypertension. The subgroup analyses showed some inconsistencies; some of them seemed reasonable, while others did not.

An overall OR value of 5.75 (95% CI 3.92–8.44; I^2 = 49%) was found for the risk of developing postpartum hypertension among women with a history of HDP. According to the subgroup analysis, the risk of chronic hypertension in patients with HDP increased for different continents, but there were differences among the continents (P = 0.03). The increased risk in North and South America was the lowest (OR 2.11, 95% CI 1.42–3.14), and the risk in Europe was the highest (OR 5.52, 95% CI 3.01–10.14),

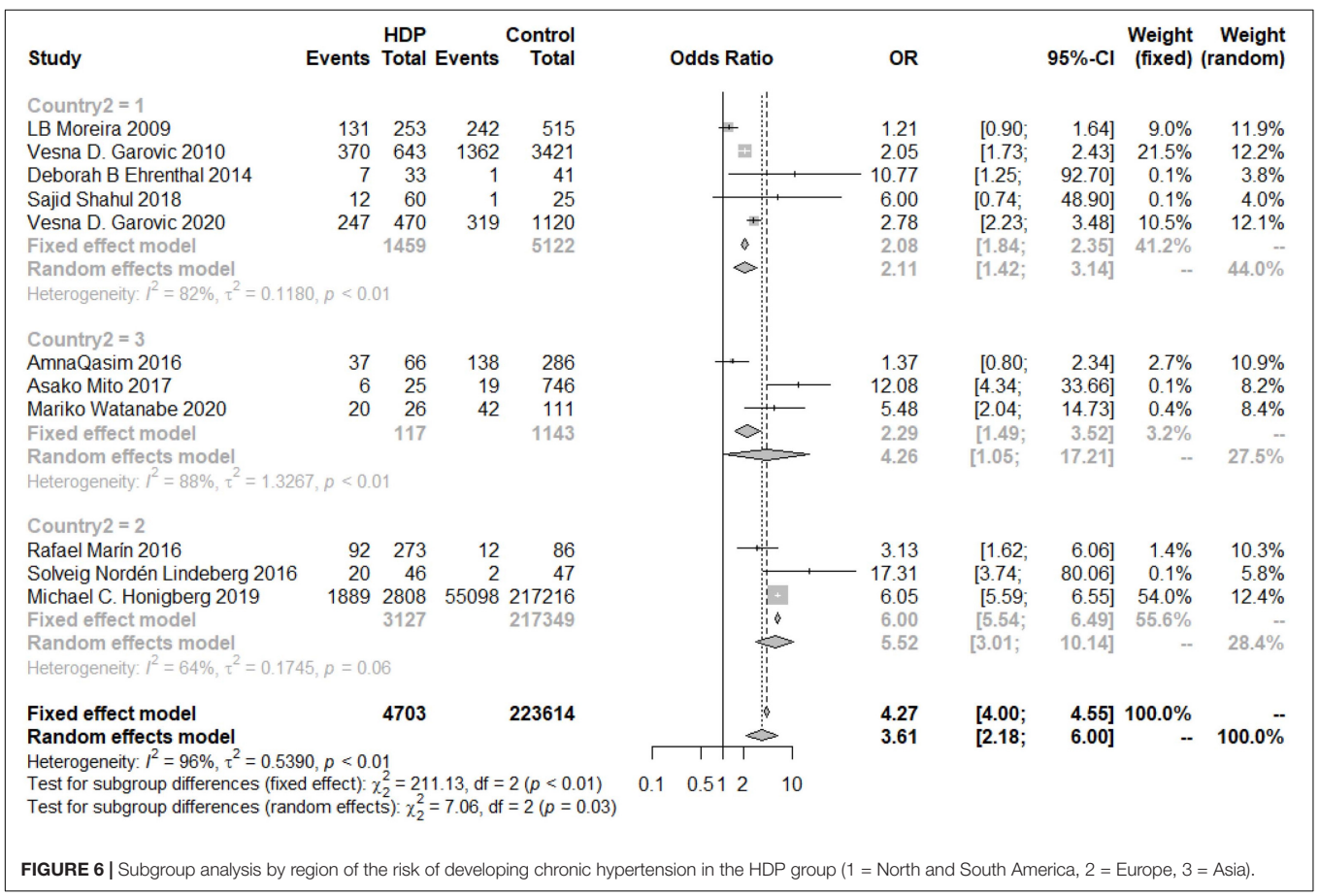

FIGURE 6 | Subgroup analysis by region of the risk of developing chronic hypertension in the HDP group (1 = North and South America, 2 = Europe, 3 = Asia).

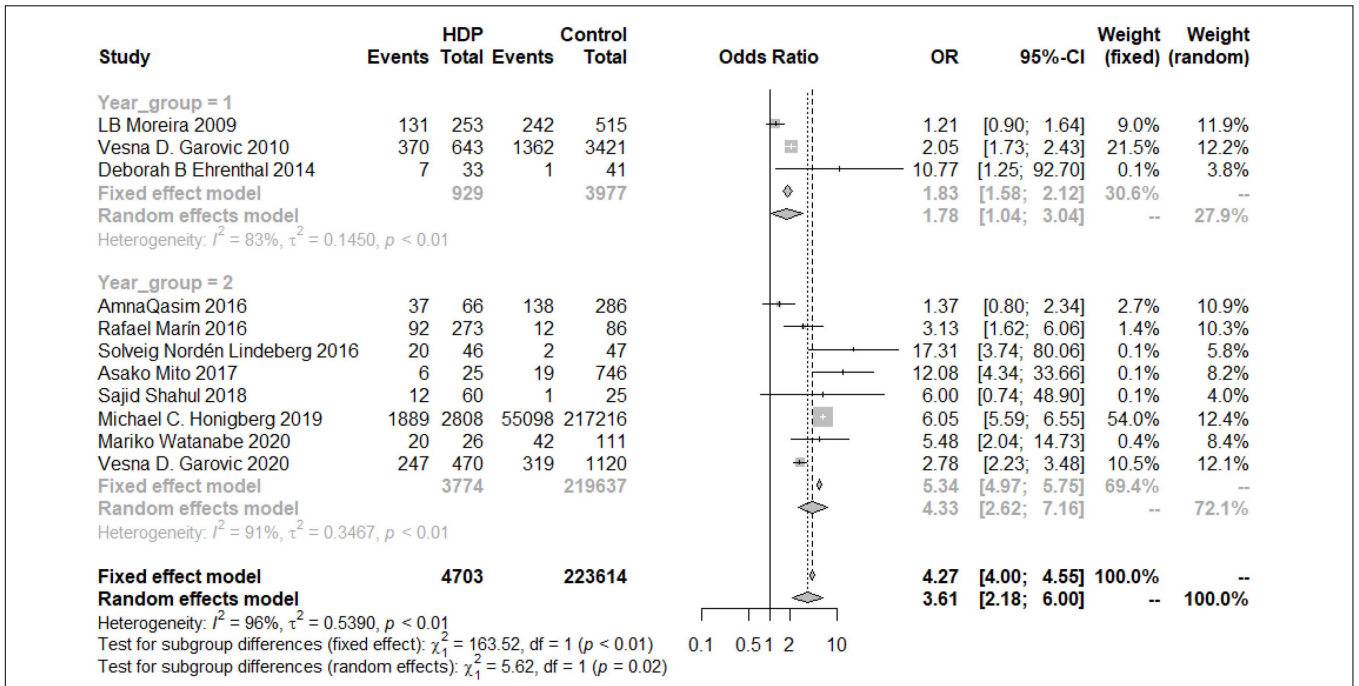

FIGURE 7 | Subgroup analysis by publication year of the risk of developing chronic hypertension in the HDP group (Group 1 = years < 2016, Group 2 = years ≥ 2016).

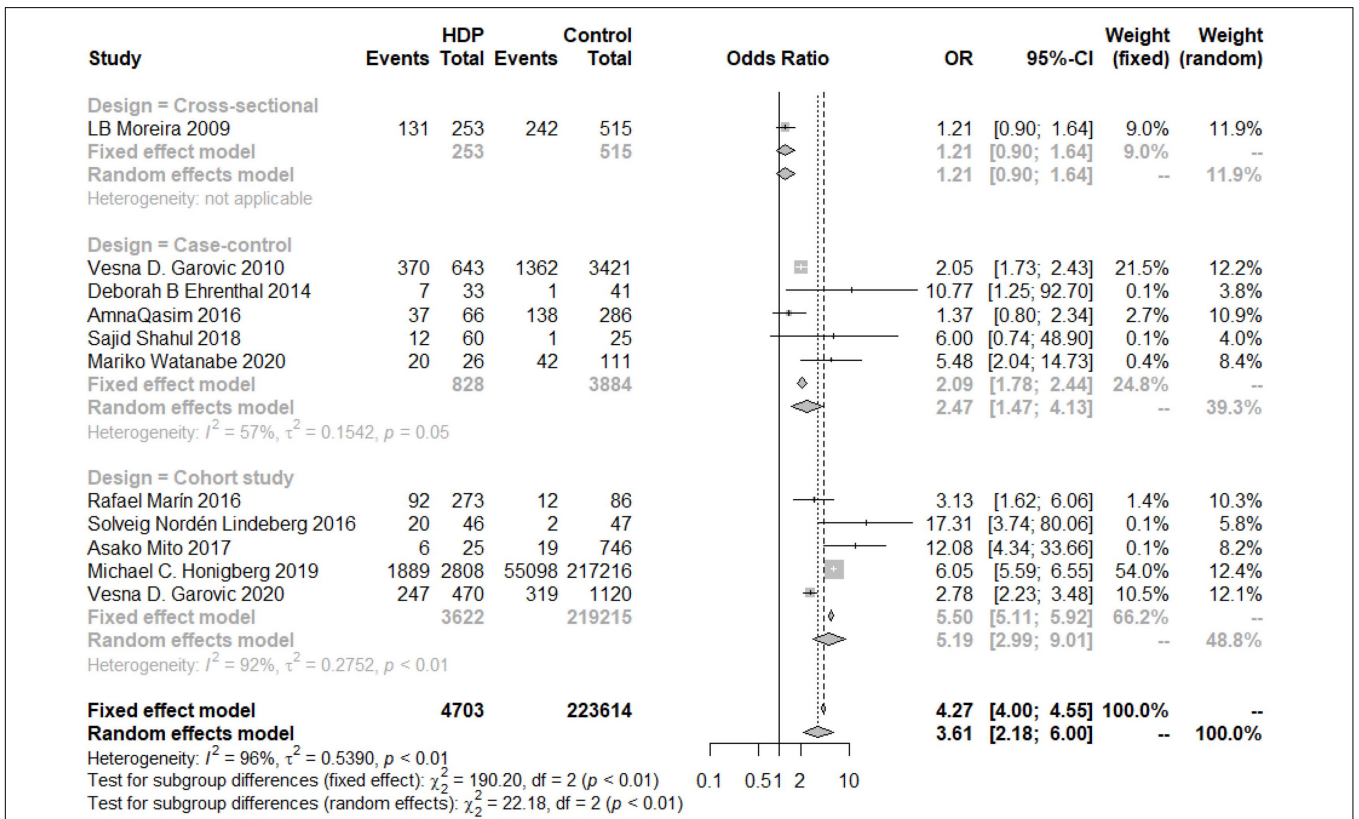

FIGURE 8 | Subgroup analysis by the study design of the risk of developing chronic hypertension in the HDP group.

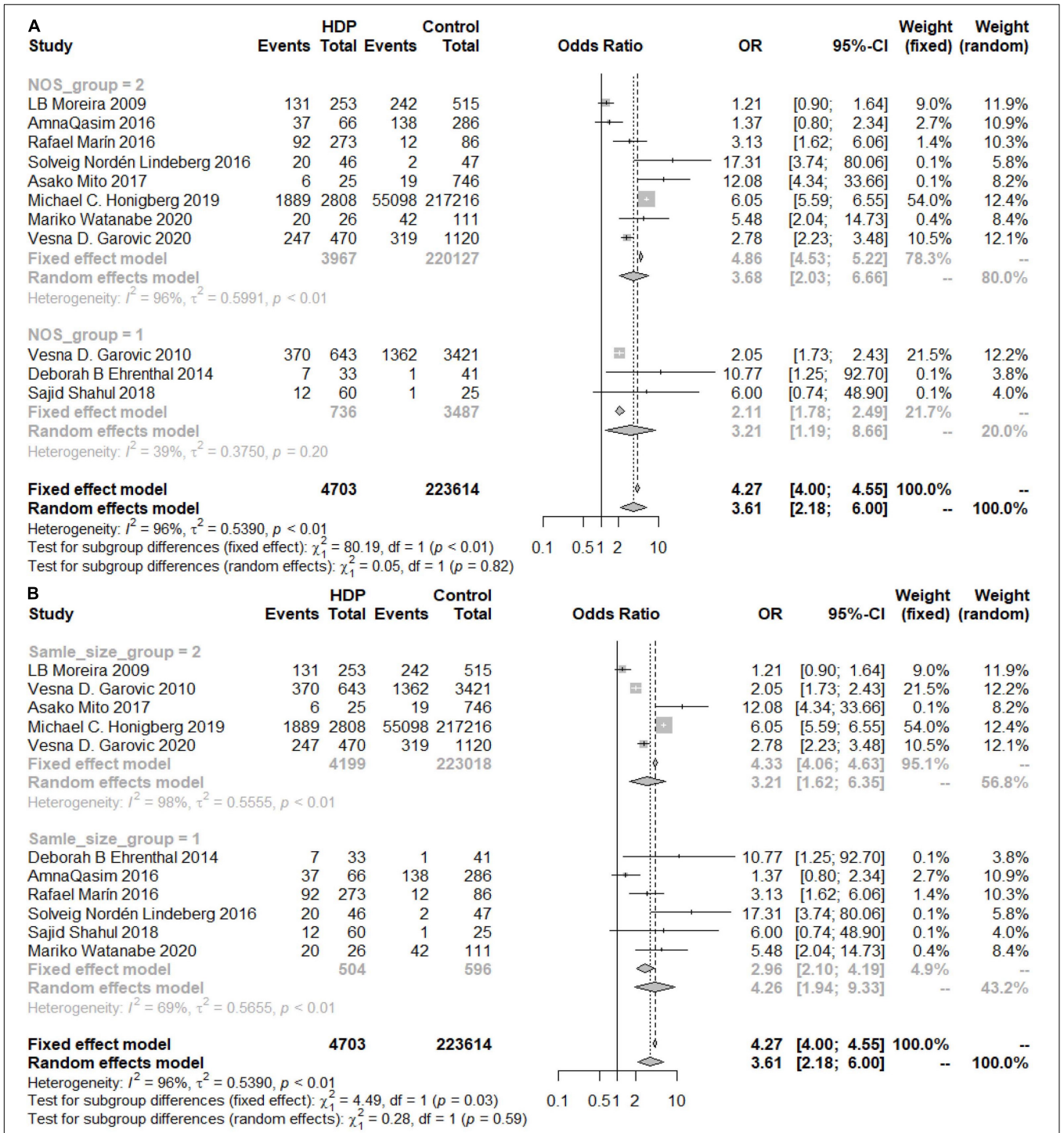

FIGURE 9 | (A) Subgroup analysis by NOS score of the risk of developing chronic hypertension in the HDP group (Group 1 = NOS < 7, Group 2 = NOS ≥ 7). **(B)** Subgroup analysis by the sample size of the risk of developing chronic hypertension in the HDP group (Group 1 = sample size < 500, Group 2 = sample size ≥ 500).

while the risk in Asia was similar to the overall assessment (OR 4.26, 95% CI 1.05–17.21) (**Figure 6**). According to the analysis of publication years, when the publication year was before 2016, the increase in the risk of developing chronic hypertension among patients with HDP was significantly lower than that among patients included in studies with a publication year of 2016 or later (P = 0.02, $OR_{<2016}$ 1.78, 95% $CI_{<2016}$ 1.04–3.04, $OR_{\geq 2016}$ 4.33, 95% $CI_{\geq 2016}$ 2.62–7.16) (**Figure 7**). Grouped by study

design, the OR value of the case–control group was 2.47 (95% CI 1.47–4.13), that of the cohort control group was 5.19 (95% CI 2.99–9.01), and that of the cross-sectional group was 1.21 (95% CI 0.90–1.64) (**Figure 8**). According to the NOS score and sample size, the increased risk of developing chronic hypertension among HDP patients was similar to that of the overall evaluation ($OR_{NOS\geq 7}$ 3.68, 95% $CI_{NOS\geq 7}$ 2.03–6.66; $OR_{NOS<7}$ 3.21, 95% $CINOS_{<7}$ 1.19–8.66; $OR_{>500}$ 3.21, 95% $CI_{>500}$ 1.62–6.35; $OR_{\leq 500}$ 4.26, 95% $CI_{\leq 500}$ 1.94–9.33) (**Figure 9**).

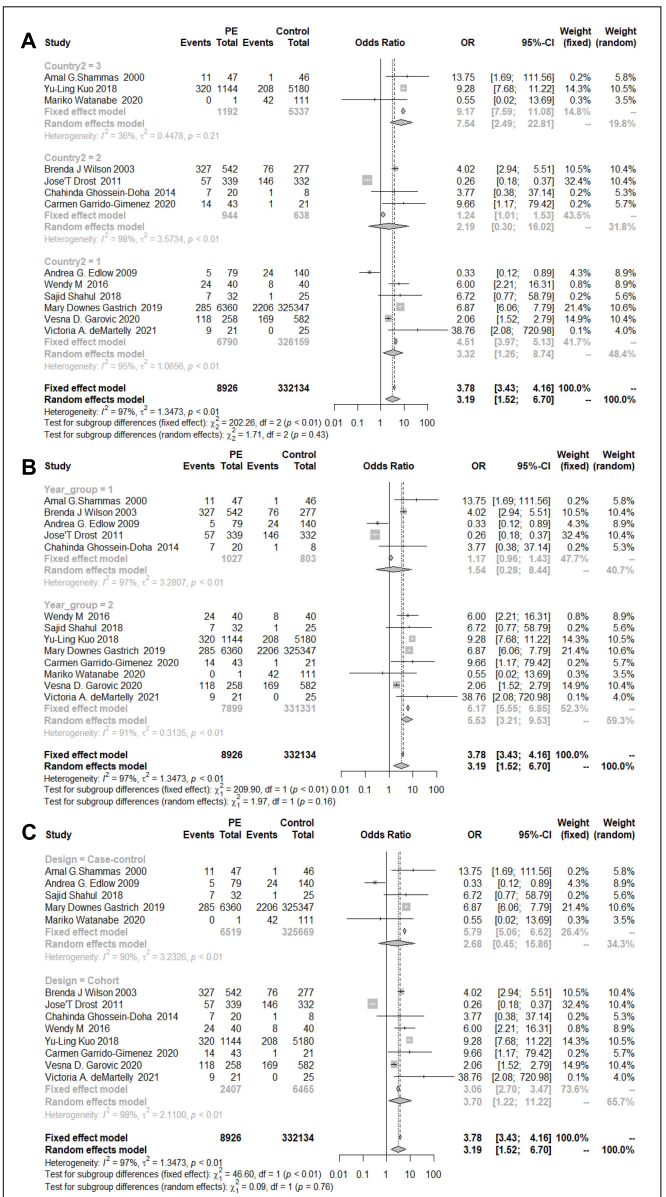

FIGURE 10 | (A) Subgroup analysis by region of the risk of developing chronic hypertension in the PE group (1 = North and South America, 2 = Europe, 3 = Asia). **(B)** Subgroup analysis by publication year of the risk of developing chronic hypertension in the PE group (Group 1 = years < 2016, Group 2 = years ≥ 2016). **(C)** Subgroup analysis by the study design of the risk of developing chronic hypertension in the PE group.

The overall OR was 3.19 (95% CI 1.52–6.70; I_2 = 97%), and women with a history of PE had a greater risk of developing postpartum hypertension than women without PE. The increased risks in the Americas and Europe were similar to the overall risk ($OR_{Americas}$ 3.32, 95% $CI_{America}$ 1.26–8.74; OR_{Europe} 2.19, 95% CI_{Europe} 0.3–16.02), while the risk of developing chronic hypertension in Asia was significantly increased (OR 7.54, 95% CI 2.49–22.81) (**Figure 10A**). According to the analysis of the publication years, when the publication year was before 2016, the increase in the risk of developing chronic hypertension among patients with PE was significantly lower than that among patients included in studies with a publication year of 2016 or later ($OR_{<2016}$ 1.54, 95% $CI_{<2016}$ 0.28–8.44, $OR_{\geq 2016}$ 5.53, 95% $CI_{\geq 2016}$ 3.21–9.53) (**Figure 10B**). Grouped by study design, the OR value of the case–control group was 2.68 (95% CI 0.45, 15.86) and that of the cohort control group was 2.70 (95% CI 1.22, 11.22) (**Figure 10C**). The OR value of the NOS score ≥ 7 group was 2.15 (95% CI 0.7–6.64), and the OR of the other group was 6.88 (95% CI 6.07–7.80) (**Figure 11A**). According to sample size, the increase in the risk of developing chronic hypertension among PE patients was similar to that of the overall evaluation ($OR_{<500}$ 4.05, 95% $CI_{<500}$ 1.12–14.69; $OR_{\geq 500}$ 2.69, 95%$CI_{\geq 500}$ 0.97, 7.45) (**Figure 11B**).

DISCUSSION

Principal Findings

Our systematic review and meta-analysis comprehensively explored the associations of HDP, GH, and PE with chronic hypertension. We included 21 articles with a total of 634,293 patients. The results of this systematic review and meta-analysis suggested that women with a history of HDP are almost 3.6 times more likely to develop chronic hypertension than those without a history of HDP, women with a history of GH are almost 6.2 times more likely to develop chronic hypertension than those without a history of GH, and women with a history of PE are almost 3.2 times more likely to develop chronic hypertension than those without a history of PE. In addition, we further calculated the probability of developing chronic hypertension among patients with HDP or PE after adjusting for age and BMI at recruitment, prepregnancy BMI, age at first delivery and other factors. The results suggested that women with a history of HDP were almost 2.47 times more likely to develop chronic hypertension than those without a history of HDP and that women with a history of PE were almost 3.78 times more likely to develop chronic hypertension than those without a history of PE (**Figure 12**). The above results show that women with HDP are more likely to develop chronic hypertension and that those with GH are more likely to have PE. Therefore, patients with HDP should monitor their blood pressure more actively in the future and choose a healthy lifestyle, such as a low-salt and low-fat diet, to reduce the possibility of hypertension. One meta-analysis showed that subclinical hypothyroidism during pregnancy is associated with an increased risk of developing HDP, and this association is present regardless of the gestational period (42). Some studies have shown that BMI or maternal prepregnancy obesity and

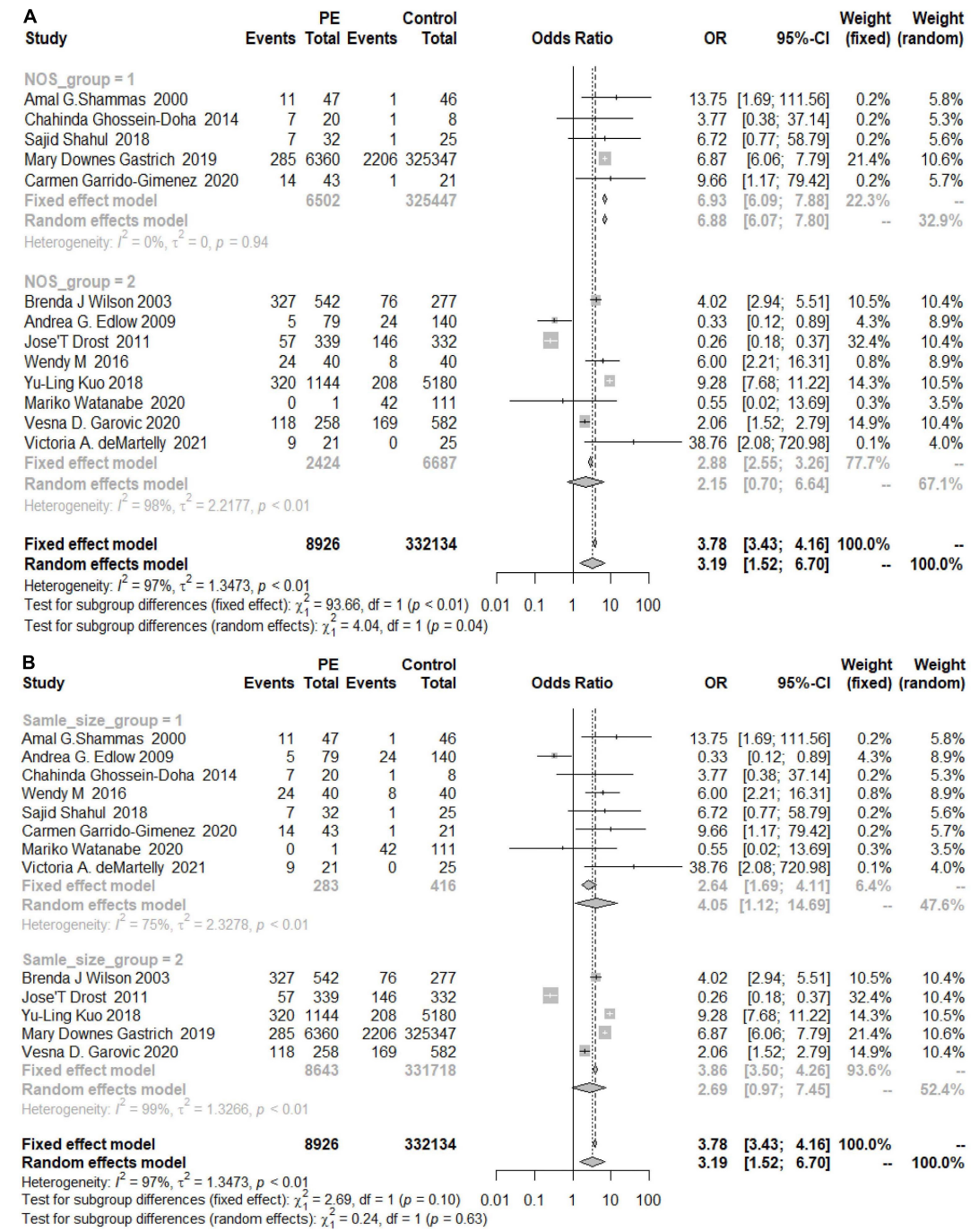

FIGURE 11 | (A) Subgroup analysis by NOS score of the risk of developing chronic hypertension in the PE group (Group 1 = NOS < 7, Group 2 = NOS ≥ 7). **(B)** Subgroup analysis by sample size of the risk of developing chronic hypertension in the PE group (Group 1 = sample size < 500, Group 2 = sample size ≥ 500).

abnormal gestational glucose metabolism are independently associated with an increased risk of HDP. Controlling these factors may reduce the occurrence of HDP (43, 44). Preventing or reducing the occurrence of HDP in pregnant women will inevitably reduce the probability of developing hypertension in the future. In terms of countries, women in Asian countries are more likely to develop chronic hypertension after HDP or PE, while the relative risk in the Americans is not high. This may be related to race, medical level and economic conditions. We look forward to future research.

Comparison With Other Studies

Our systematic review illustrates the risk of developing chronic hypertension among pregnant women with HDP, GH and PE.

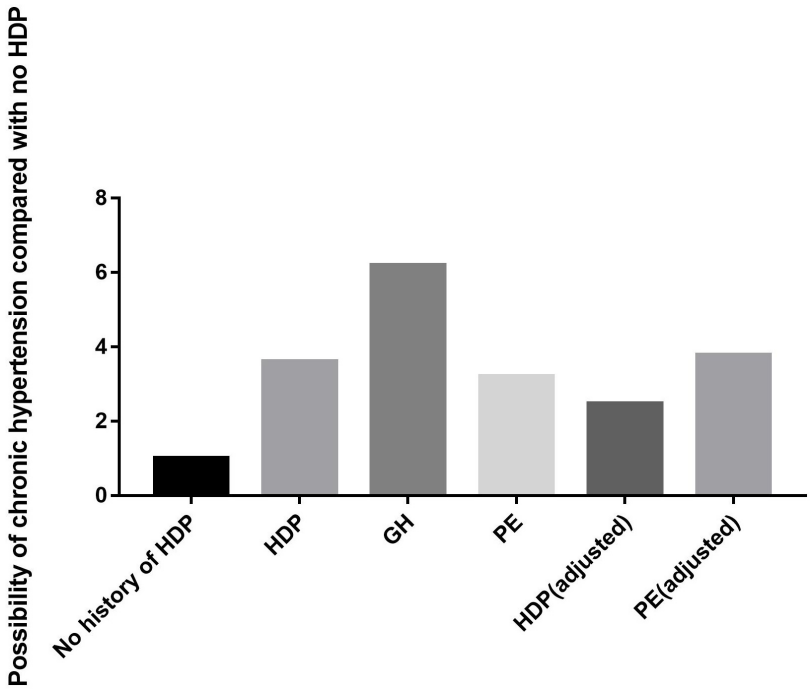

FIGURE 12 | Main findings of the study.

Although the evidence linking pregnancy-induced hypertension with the development of hypertension has been recognized, there are still many outstanding problems in a number of specific aspects (45).

In 2007, a systematic review and meta-analysis showed that preeclampsia patients had more than three times the risk of developing hypertension (OR 3.70, 95% CI 2.70–5.05) than those without preeclampsia; the follow-up time was adjusted to 14.1 years (46). Subsequent studies did not adjust the follow-up years. A systematic review and meta-analysis in 2013 showed that women with a history of preeclampsia or eclampsia had more than three times the risk of developing hypertension (RR 3.13, 95% CI 2.51, 3.89) (14) than those without a history of preeclampsia or eclampsia. In 2016, Mayri Sagady Leslie reviewed 48 unique studies from 20 countries that included a total of 3,598,601 women, and found similar results (47). This outcome was consistent with ours. In 2018, L Brouwers' team found that recurrent preeclampsia was consistently associated with an increased pooled risk ratio for hypertension (RR 2.3; 95% CI 1.9–2.9) (48). The above articles all studied the relationship between preeclampsia and chronic hypertension, and few meta-analyses have directly studied the relationship between HDP or GH and chronic hypertension.

The advantage of our study is that a large number of articles were selected, and the sample size was large. We not only studied the possibility of HDP leading to chronic hypertension but also accounted for the relevant data on various types of HDP and finally chose to analyze the large amount of relevant data for PE and GH. We also performed subgroup analysis (publication year, study design, country, sample size and NOS score) to analyze the sources of heterogeneity and the probability of developing chronic hypertension in each subgroup. In addition, we further calculated the probability of developing chronic hypertension for patients with HDP or PE after adjusting for age and BMI at recruitment, prepregnancy BMI, age at first delivery and other factors. In general, we carried out statistical analysis on all aspects of the obtained data that could be analyzed.

However, there are still some limitations of this study, which need further study. There are few studies with high scores. The ages of patients with HDP and chronic hypertension were not statistically analyzed because the data were seriously lacking, which may be the reason for the high heterogeneity. The published literature is insufficient to determine the best screening period for postpartum detection of hypertension. We could not determine an observation age or follow-up period to limit the screening of the articles. The heterogeneity of the population and hypertension definitions and the failure to obtain sufficient details make the results of the meta-analysis misleading, and they could not be adjusted using statistical tests.

CONCLUSION

HDP, GH, and PE increase the likelihood that patients will develop chronic hypertension. After adjustment for age and BMI at recruitment, prepregnancy BMI, age at first delivery and other factors, patients with HDP or PE were still more likely to develop chronic hypertension. HDP, GH, and PE may be risk factors for chronic hypertension, independent of other risk factors.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

JX, TL, and YW: study design, data extraction, statistical analysis, and manuscript writing. LX, ZM, WL, and KX: study design, data extraction, and verification. CH and HD: study design, statistical analysis, manuscript editing and reviewing, and funding. All authors contributed to the article and approved the submitted version.

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STUDY PROTOCOL
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First-Trimester Sequential Screening for Preeclampsia Using Angiogenic Factors: Study Protocol for a Prospective, Multicenter, Real Clinical Setting Study

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Introduction: The incidence of preeclampsia (PE) is about 2–8%, making it one of the leading causes of perinatal morbidity and maternal mortality in the world. Early prophylactic low dose administration (150 mg) of acetylsalicylic acid is associated with a significant reduction in the incidence of early-onset PE, intrauterine growth restriction (IUGR), and neonatal mean stay in the intensive care unit (ICU). Universal implementation of a first-trimester screening system including angiogenic and antiangiogenic markers [the Placental Growth Factor (PlGF) and/or soluble fms-like Tyrosine Kinase-1 (sFlt-1)] has shown a prediction rate of 90% for early-onset PE but entails a high financial cost. The aim of this study is to determine the predictive and preventive capacity of a universal PE first-trimester two-step sequential screening model, determining the PlGF only in patients previously classified as intermediate risk by means of a multivariate model based on resources already used in the standard pregnancy control, in a real clinical setting. We hypothesize that this screening model will achieve similar diagnostic performance as the universal determination of PlGF but at a lower economic cost.

Methods and Analysis: This is a prospective, multicentric, cohort study in a real-world clinical setting. Every singleton pregnancy will be recruited at the routine first pregnancy visit. In a first step, the first-trimester risk of PE will be calculated using a multivariate Gaussian distribution model, based on medical history, mean blood pressure, Pregnancy-Associated Plasma Protein A (PAPP-A), and Uterine Artery Doppler Pulsatility Index (UTPI). Patients will be classified into three risk groups for PE: (1) risk $\geq$ 1/50,

high-risk with no further testing (blinded PlGF); (2) risk between 1/51 and 1/500, medium-risk requiring further testing; and (3) risk $\leq$ 1/501, low-risk with no further testing. In a second step, the PlGF will only be determined in those patients classified as intermediate risk after this first step, and then reclassified into high- or low-risk groups. Prophylactic administration of aspirin (150 mg/day) will be prescribed only in high risk patients. As a secondary objective, sFlt-1 values will be blindly determined in patients with high and intermediate risk to assess its potential performance in the screening for PE.

Ethics and Dissemination: The study will be conducted in accordance with the principles of Good Clinical Practice. This study is approved by the Aragon Research Ethics Committee (CEICA) on 3 July 2020 (15/2020).

Clinical Trial Registration: ClinicalTrials.gov, identifier: NCT04767438.

Keywords: screening, first trimester, sequential, preeclampsia, growth restriction

INTRODUCTION

The incidence of preeclampsia (PE) is about 2–8%, making it one of the leading causes of perinatal morbidity, and responsible for 10–15% of the maternal deaths in the world (1, 2). Women with PE present a higher risk of cardiovascular morbidity, multisystemic complications, and a long-term increased risk for cardiovascular diseases (3) and Mortality (4). Early-onset PE (diagnosed before 32 weeks of gestation) is also frequently associated with intrauterine growth restriction (IUGR), and therefore affecting perinatal outcomes and long-term offspring development (5, 6). Late-onset PE, perhaps clinically less severe but much more common, is also an important cause of maternal and neonatal morbidity and mortality with long-term consequences (7).

Although a complete understanding of the pathogenesis of PE remains unclear, the current theory suggests a two-stage process. The first stage is caused by shallow invasion of the trophoblast, resulting in inadequate remodeling of the spiral arteries. This is presumed to lead to the second stage, which involves the maternal response to endothelial dysfunction and imbalance between angiogenic and antiangiogenic factors (8). In PE and IUGR, this vascular remodeling does not exist or it is incomplete, leading to placental hypoperfusion associated with endothelial cell dysfunction (9). For a correct placental development, a balance between the production of angiogenic (Placental Growth Factor, PlGF) and antiangiogenic factors (soluble Fms-like tyrosine kinase-1, sFlt-1) is required. PIGF and sFlt-1 are partially produced by the syncytiotrophoblast. Angiogenic biomarkers (PlGF and sFlt-1) have shown their ability to predict PE and its complications (10).

We have effective preventive strategies to reduce the incidence of PE. The nightly low dose (150 mg) administration of acetyl salicylic acid (ASA) before 16 weeks of gestational age until term is associated with a 90% reduction in the incidence of preterm-PE (11, 12) and IUGR (13, 14), as well as with a 70% reduction of the neonatal mean stay in intensive care units (ICUs) (15). Therefore, it is essential to develop universal PE screening strategies during the first trimester of pregnancy to determine the risk of each patient, selecting those high-risk candidates to start as soon as possible for the prophylactic treatment with low-dose ASA (16, 17).

Universal PE screening has been proven to be cost-effective (18). PE screening models based on maternal demographic characteristics and risk factors have been proposed (19), with a very low detection rate and an excess in false-positive rate (20). Hence, different multivariate models have been developed. A competitive risk approach at 11–13 weeks by means of a combination of maternal characteristics (age, parity, medical history as thrombophilia, nephrological diseases, and chronic hypertension), maternal biophysical variables [mean arterial pressure (MAP) and Doppler of Uterine Arteries (Uterine Artery Pulsatility Index, UTPI)], and biochemical variables (PlGF) has shown a prediction rate of 90% for early-PE and 75% for late-onset PE, with a 10% false-positive rate (21, 22). Additionally, observational retrospective studies have reported that including antiangiogenic biomarkers such as sFlt-1 could improve the first-trimester screening performance (23).

Unfortunately, universal implementation of any first-trimester screening system, such as angiogenic and antiangiogenic markers (PlGF and/or sFlt-1), entails a high financial cost, unaffordable for many healthcare systems. For that reason, more economical screening alternatives have been explored. In this line, taking advantage of the resources used on a regular basis for the screening of chromosomal abnormalities, an alternative multivariate model based on maternal characteristics, MAP, and Uterine Arteries Pulsatility Index combined with Pregnancy-Associated Plasma Protein A levels (PAPP-A), has shown a detection rate of early-onset PE of 80.8% and for late-onset PE of 39.6%, with a 10% false-positive rate (24).

Recent retrospective studies with large sample sizes (25, 26) suggest that a two-step screening protocol, performing only angiogenic markers in 30% of the population classified as moderate or high risk of PE after an economical first step (24), would correctly select high-risk patients for developing PE, but lower-economic cost. However, these results come from retrospective cohorts after universal screening models with PlGF, and therefore with potential intervention biases.

TABLE 1 | Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Singleton pregnancies	• Abnormal karyotype, structural abnormalities or congenital infections at inclusion
• Gestational age between 8 and 13+6 weeks according to first trimester crown-rump length	• Multiple pregnancies
• Could receive regular follow-up • Written informed consent	

TABLE 2 | Variables included in the first-step multivariate model for the estimation of the risk of preeclampsia (PE).

Maternal factors
• Age
• Ethnicity
• Weight
• Height
• Smoking status
• Parity
• History of preeclampsia
• Pre-existing diabetes
• Pre-existing hypertension
• Thrombophilia
• Renal diseases
• Autoimmune conditions
Biophysical markers
• Mean arterial pressure (MAP)
• Mean uterine arteries pulsatility index
Blood test samples
• Pregnancy-associated plasma protein A (PAPP-A) (MoMs)

The aim of this study is to prospectively determine, in a real clinical setting, the predictive and preventive capacity of a universal PE first trimester two-step sequential screening model, determining PlGF only in those patients previously classified as intermediate risk by means of a multivariate model based on medical history, MAP, PAPP-A, and UTPI. We hypothesize that this screening model will achieve similar diagnostic performance as the universal determination of PlGF but at a lower economic cost.

As a secondary objective, in every intermediate and high-risk patient, sFlT-1 values will be determined to assess their potential performance in the screening for PE. Even though this data will be blinded to the researchers and will not be considered for the clinical management. We also evaluate the predictive and preventive capacity of this first-trimester sequential screening model for the development of IUGR.

METHODS AND ANALYSIS

Study Design

This is a multicentric prospective cohort study in a real clinical setting. The study will be conducted in five tertiary Spanish hospitals: Hospital de la Santa Creu i Sant Pau (Barcelona), Complejo Hospitalario Universitario Insular Materno Infantil (Las Palmas), Hospital Universitario de Cruces (Bilbao), Hospital Son Llàtzer (Mallorca), and Hospital Clínico Universitario Lozano Blesa (Zaragoza).

Study Population and Groups

Participants will be recruited at the routine first pregnancy visit with an obstetrics specialist, always before the 14 weeks of gestation, based on the eligibility criteria presented in **Table 1**, and followed until delivery. Written informed consent will be obtained from all recruited patients. This study is approved by the Aragon Research Ethics Committee (CEICA) on 3 July 2020 (15/2020). Obstetricians will present the study to all the eligible patients, explaining the study, offering participation, and requesting written informed consent.

Intervention

We consider the first-trimester universal screening for PE as a routine clinical practice. Therefore, the first-trimester risk of PE will be calculated in every patient using a multivariate Gaussian distribution model validated in our population, based on maternal conditions, biophysical markers, and maternal serum PAPP-A (taken routinely for trisomy screening) (**Table 2**). No angiogenic factors will be used to determine the risk of PE at this first step.

All patients who meet the inclusion criteria will be offered the opportunity to participate in the study. If they do not wish to participate, in accordance with a previous pilot study, a risk cut-off of $\geq$1:250 for early PE will be considered high risk, and therefore prophylactic treatment with ASA 150 mg will be recommended (**Figure 1**).

In those patients who agree to participate in the study, a two-step contingent sequential screening model will be applied (**Figure 1**). According to the estimated risk of PE in the first screening step, three risk groups of early PE will be defined: (1) risk $\geq$ 1/50, high-risk with no further testing (blinded PlGF), (2) risk between 1/51 and 1/500, medium-risk requiring further testing, and (3) risk $\leq$ 1/501, low-risk with no further testing. According to a previous pilot study performed in the Hospital de la Santa Creu i Sant Pau and Hospital Universitario de Cruces, we expect to find 10% of high-risk patients and 20% of intermediate-risk after the first step of the screening. These results agree with those published by other authors (25).

For the second stage, serum concentrations of PlGF will be determined in the medium-risk group, giving a final risk at a cut-off value of 1/250. Prophylactic treatment with ASA (150 mg) up to 36 weeks of gestation will be recommended in patients classified as high risk of PE either in the first or second step of the model.

With the intention of performing secondary analysis at the end of the study but never to be considered for clinical decisions, the levels of maternal serum sFlT-1 will be determined and

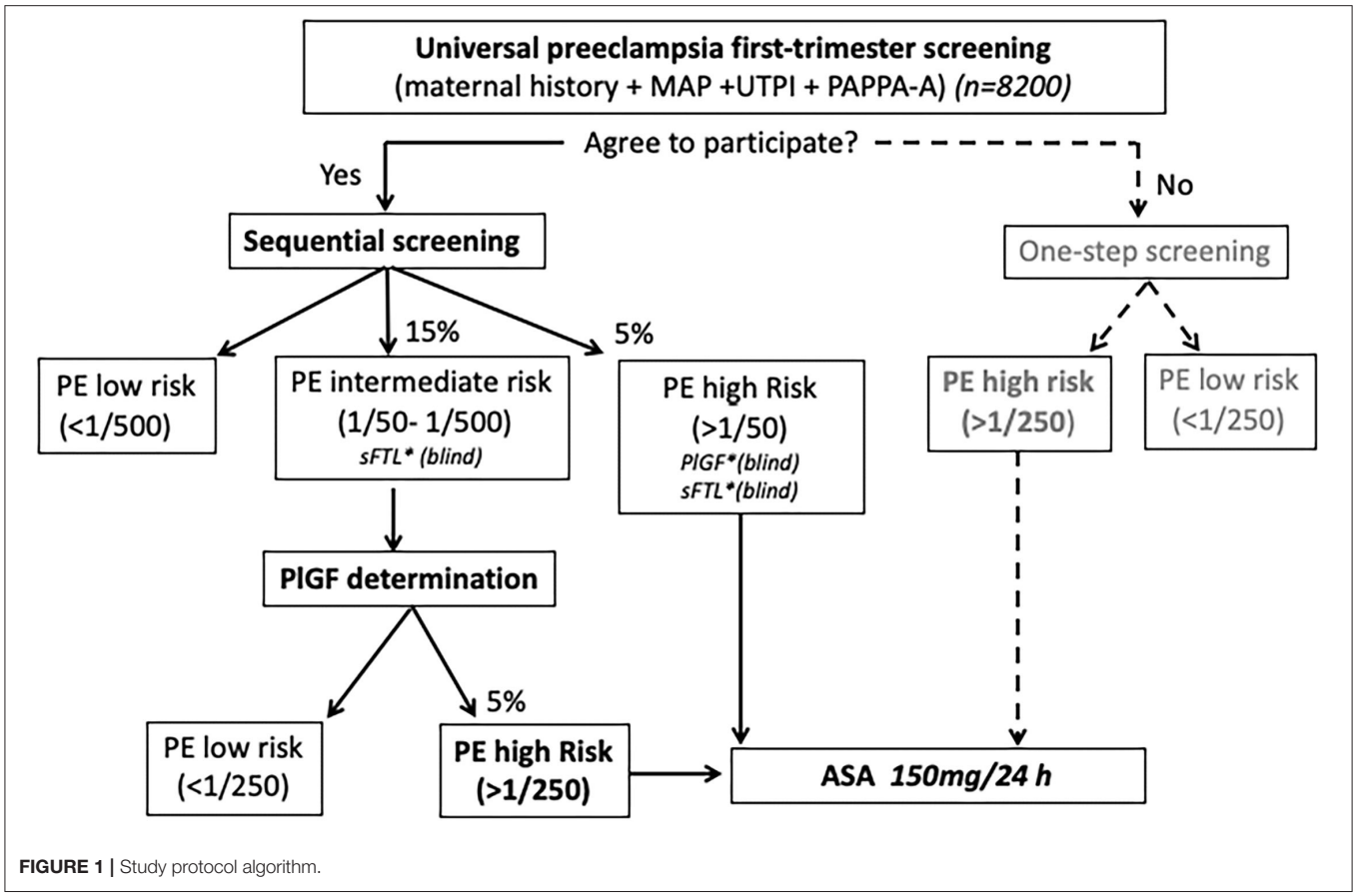

FIGURE 1 | Study protocol algorithm.

TABLE 3 | Study outcomes.

Primary outcomes

- Preeclampsia diagnoses during pregnancy (International Society for the Study of Hypertension in Pregnancy—ISSHP) (28)

Secondary outcomes

- Early-onset Preeclampsia: diagnosed before 32 weeks (28)
- Severe preeclampsia (ISSHP) (28)
- Pregnancy-induced hypertension (28)
- Birth weight below the 10th percentile (29)
- Intrauterine Growth Restriction (30)
- Perinatal mortality (>22 weeks of pregnancy - <28 days postpartum).
- Neonatal acidosis (arterial pH <7.10 + base excess >12 mEq/L)
- Neonatal Intensive Care Unit admission (days)
- Significant neonatal morbidity [convulsions, intraventricular hemorrhage > III grade, periventricular leukomalacia, hypoxic-ischemic encephalopathy, abnormal electroencephalogram, necrotizing enterocolitis, acute renal failure (serum creatinine >1.5 mg/dL) or heart failure (requiring inotropic agents)].
- Gestational age at birth
- Type of delivery (vaginal, instrumental, cesarean section)
- Economic cost the screening (euros)

blinded for clinicians in patients with an early PE risk ≥ 1/500 after the first step of screening (**Figure 1**).

In Spain, all pregnancies undergo routine ultrasound scans at ∼12, 20, and 36 weeks of gestation. All women with a high risk for preterm PE will be scheduled for an additional scan at approximately 28 weeks.

MEASUREMENTS AND OUTCOMES

The most important aim of this project is to develop an efficient first-trimester screening of PE using resources already used during pregnancy control. All the clinical data, sonography, and blood test results will be collected during the regular pregnancy control without the need for additional appointments, explorations, or extraordinary blood extractions.

Maternal characteristics will be prospectively recorded at the time of recruitment. Pregnancy outcomes will be registered during pregnancy and confirmed after delivery. Fetal crown-rump length (CRL) (27), and transabdominally uterine artery Doppler will be determined in all patients at the routine first-trimester ultrasound (11^0-13^6 weeks) by experienced fetal medicine specialists. Blood pressure (BP) will be measured once, after 5-min of rest with women seated at the time of inclusion. The MAP will be calculated as: diastolic BP + (systolic–diastolic BP)/3.

First-trimester routine blood samples will be performed between 10^0 and 13^6 weeks. PAPP-A (taken routinely for trisomy screening) will be initially determined in every patient. Serum remaining samples will be stored at −80°C, to be able to analyze PlGF and sFlT-1 after the first-trimester ultrasound in those patients with an estimated risk of PE risk ≥ 1/500 after the first step of screening. The serum will be separated by centrifugation at 1,500 g for 10 minutes at 4°C, and concentrations of PAPP-A, PlGF, and sFlT-1 will be determined by electrochemiluminescence immunoassays, fully automated on the Cobas e 601 analyzer, Roche Diagnostics. Multiples of the median (MoM) values for PAPP-A, PlGF, and sFlT-1, calculated from locally derived normal medians using the above-mentioned multivariate Gaussian distribution model, will be considered for analysis. To calculate the risk of PE, we will use the SsdwLab6 version 6.1 package (SBP Soft 2007 S.L.), previously developed in the pilot study.

The main outcome is the diagnosis of PE during pregnancy, according to the definition of the International Society for the Study of Hypertension in Pregnancy (ISSHP). Thus, the diagnosis will be based on systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg on repeated occasions after 20 weeks' gestation, and proteinuria [dipstick urinalysis ≥ 1+ or protein/creatinine ratio ≥ 30 mg/mmol (0.3 mg/mg)] or another maternal organ dysfunction. PE will be classified according to gestational age at delivery into early-onset (<34 weeks), preterm (<37 weeks), and term (>37 weeks). **Table 3** describes the secondary outcomes that will be assessed during the project.

Data Management

The processing of the data will be carried out in accordance with the current legislation, specifically article 28 of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 regarding the protection of natural persons regarding the processing of personal data and the free circulation of these data, and in accordance with the provisions of Organic Law 3/2018, of 5 December, on the Protection of Personal Data and guarantee of digital rights. The patient database will be anonymous and codified. Data checks will be regularly performed to ensure data quality. The number of eligible, included, and excluded of patients will be recorded. Withdrawals will also record as detailed as possible.

DATA ANALYSIS

Sample Size

According to the previously described inclusion criteria and considering the number of pregnancies controlled and delivered among all participating centers during the study period, we estimate that 8,200 patients will be potentially invited to participate in the study.

As the incidence of PE in Spain is ∼3% of pregnancies, approximately 246 patients of our sample will develop PE. We could assume that at least 80% of the patients will consent to participate in the study, meaning a final sample of 6,560 singleton pregnancies. Based on a pilot study previously performed, we estimate that 30% of the patients (which means approximately 1,968 pregnancies) will be classified as intermediate or high risk after the first-trimester screening for PE. This sample size assures enough statistic power, external validity, and extrapolation of the results to the clinical practice.

Statistical Analysis

Continuous variables will be expressed as means ± standard deviation (SD) or median [interquartile range (IQR)], where appropriate. Categorical variables will be expressed as counts (percentages). The variables in the study will be presented using descriptive statistics. Associations between variables were evaluated using Student's *t*-test, chi-square test, or Mann–Whitney's *U*-test where appropriate. A multivariate analysis will be performed using the binomial logistic regression. The diagnostic performance of the contingent screening model will be evaluated by determining the sensitivity, specificity, screen-positive rate, positive predictive value (PPV), and negative predictive value (NPV) for early PE. All reported *p*-values are 2-sided and unequal variances were assumed. A $p < 0.05$ will be used to define statistical significance. Statistical analyses will be performed with the IBMSPSS software program, v.26.0 (IBM-SPSS Inc., Chicago, IL; USA).

DISCUSSION

Since we have an effective preventive treatment with early administration of low dose ASA, universal screening for PE is cost effective (18), and a clinical and moral obligation. The objective of this project is to validate a first-trimester screening of PE protocol that guarantees access to all pregnant women, without sacrificing quality. For this reason, in the first step of the screening, we will calculate the risk of each patient according

to variables already included in the standard pregnancy control. As there is no economic cost involved, we ensure universal accessibility for all pregnant women to screening for PE. However, the estimation of angiogenic markers is the strategy with the best performance when it comes to screening the risk of PE (21, 22). By adding PlGF determination (and blinded sFlT-1) to only a reduced proportion of patients, we believe that we can offer an early PE screening with maximum quality, while marginally increasing the economic cost. This design has been previously proposed after retrospective analysis of randomized clinical trials based on the universal PE screening with PlGF (25, 26). Our objective is to validate these results in a multicenter, real-world clinical setting study. This sample size assures enough statistical power for the main outcomes. We consider that the design and methodology of the project guarantees the external validity of the results.

Some limitations of our study need to be mentioned. First, our design is not a randomized control trial, therefore it does not include a control group without intervention. Several randomized clinical trials have already shown the efficacy of the first-trimester PE screening for the early implementation of low-dose ASA prophylaxis. Our objective is to validate a two-step screening system previously proposed by retrospectively analyzing high methodological quality clinical trials in a real clinical scenario. We have not considered the creation of a non-intervention group in the design, as our intention is to compare our results with all the screening strategies published to date. However, we have quality information on historical patient cohorts prior to the implementation of PE screening in all centers. As a contingency plan, if we consider it necessary at the end of the study, we could also compare the results with the group of patients in whom we have not been able to screen for PE in the first trimester for other reasons. We assume that the study design allows us less control over the results than a clinical trial, as it is subjected to the potential biases of routine clinical practice. However, we consider that this is, on the other hand, one of the differentiating points of this project, since it offers us results with high external validity and transferability to clinical reality. Second, for clinical purposes to be able to publish our results as soon as possible, follow-up of the offspring is limited to the neonatal period. However, this is not an important limitation, since it is not the objective of the project, and since all the study patients are registered, a long-term cohort study with these neonates could be considered.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Aragon Research Ethics Committee (CEICA) on 3 July 2020 (15/2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EL, CT, MF, CL, and DO: conception and design of the research. DO: principal investigator. CT: co-investigator. SD, LR, AR-R, JM, TB, MF, AM, and SR-M: collaborators. CT, CL, and DO: wrote the first version of the manuscript, drafting, and revision of the manuscript. All authors revised it and contributed significantly in writing the final version that was accepted.

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Novel Early Pregnancy Multimarker Screening Test for Preeclampsia Risk Prediction

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Preeclampsia (PE) is a common pregnancy-linked disease, causing preterm births, complicated deliveries, and health consequences for mothers and offspring. We have previously developed 6PLEX, a multiplex assay that measures PE-related maternal serum biomarkers ADAM12, sENG, leptin, PlGF, sFlt-1, and PTX3 in a single test tube. This study investigated the potential of 6PLEX to develop novel PE prediction models for early pregnancy. We analyzed 132 serum samples drawn at 70–275 gestational days (g days) from 53 pregnant women (PE, $n = 22$; controls, $n = 31$). PE prediction models were developed using a machine learning strategy based on the stepwise selection of the most significant models and incorporating parameters with optimal resampling. Alternative models included also placental *FLT1* rs4769613 T/C genotypes, a high-confidence risk factor for PE. The best performing PE prediction model using samples collected at 70–98 g days comprised of PTX3, sFlt-1, and ADAM12, the subject's parity and gestational age at sampling (AUC 0.94 [95%CI 0.84–0.99]). All cases, that developed PE several months later (onset 257.4 ± 15.2 g days), were correctly identified. The model's specificity was 80% [95%CI 65–100] and the overall accuracy was 88% [95%CI 73–95]. Incorporating additionally the placental *FLT1* rs4769613 T/C genotype data increased the prediction accuracy to 93.5% [AUC = 0.97 (95%CI 0.89–1.00)]. However, 6PLEX measurements of samples collected at 100–182 g days were insufficiently informative to develop reliable PE prediction models for mid-pregnancy (accuracy <75%). In summary, the developed model opens new horizons for first-trimester PE screening, combining the easily standardizable 6PLEX assay with routinely collected antenatal care data and resulting in high sensitivity and specificity.

Keywords: Preeclampsia, early pregnancy, biomarkers, PTX3, multiplex immunoassay, risk prediction, statistical modeling, 6PLEX

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INTRODUCTION

Preeclampsia (PE) is considered a disease of the placenta, caused by impaired remodeling of spiral arteries during the first half of pregnancy and/or suboptimal placental capacity to support the maternal-fetal needs until natural delivery. As a result of insufficient modulation of the uterine vasculature in the beginning of the pregnancy, hypertension (HTN) and other signs of organ

dysfunction, the characteristics of PE, manifest during the second half of pregnancy (1, 2). This is mediated by hypoxic placenta releasing biomolecules into maternal circulation that causes endothelial damage and generalized inflammatory vascular stress. PE is a sudden and severe complication with a prevalence of 4.6% worldwide and in Europe alone affecting annually ∼400,000 cases. Currently, the most effective management of PE is delivery that can occur preterm. PE can also cause fetal growth restriction or even intrauterine or maternal death in extreme cases (3). In addition, hypertensive pregnancy complications result in a greater risk of developing cardiovascular and metabolic diseases, stroke, and end-stage renal disease later in life (4).

It has been recently shown that low-dose aspirin has a protective effect in the prevention of PE in high-risk cases (up to 82% for early PE, onset before 34 gestational weeks (g week)) if prophylaxis is started before g week 16 (5, 6). The development of effective screening tools for PE to be applied in the 1st trimester of pregnancy has been sought for and advised by professional organizations (4, 7–9). Traditionally, evaluation of PE risk is based on the assessment of maternal prepregnancy characteristics, such as maternal age >40 years, nulliparity, obesity, previous or family history of PE, diabetes mellitus, chronic HTN, and renal or autoimmune diseases. Although it is a maternal risk factor-based model that is easy to perform, it has a low detection rate (∼40%) for the subsequent manifestation of PE (10). Therefore, the application of other parameters to be measured during pregnancy has been proposed for the estimation of the PE risk. These include mean arterial pressure (MAP) and uterine arteries pulsatility index (UtA PI), a single or combination of biochemical serum markers (11). The predictive rate of these algorithms has been reported up to 96.3% [false-positive rate (FPR) 10%] for early PE with the onset before 34th g week, but remains lower (up to 76.6%) for late PE that represents the major fraction of PE cases (12, 13). The modest prediction rate of late PE has limited the investigation of its early preventive measures. For example, pravastatin, administered after 35–37 g week for high-risk women, did not prevent the manifestation of disease (14). It indicates either the ineffectiveness of pravastatin or suboptimal selection of high-risk patients destined to develop term PE.

We have recently developed an innovative, single-tube multimarker 6PLEX assay for the Luminex® xMAP platform to measure simultaneously six PE-related biomarkers in the sera of pregnant women, namely, soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF), soluble endoglin (sENG), leptin, disintegrin, and metalloproteinase domain-containing protein 12 (ADAM12), and Pentraxin 3 (PTX3) (15). Combining 6PLEX measurements of serum samples collected in the second half of pregnancy with gestational age and maternal weight at sampling generated a clinically applicable and effective prediction formula for PE development regardless of gestational age at its clinical manifestation. The new solution exhibited superior prognostic yield compared to the currently used sFlt-1/PlGF ratio in the diagnosis confirmation of symptomatic women in late pregnancy (96.5% vs. 73.7%) (15, 16).

This study aimed to investigate the potential of 6PLEX to develop novel PE prediction models for early pregnancy. We analyzed serum samples from pregnant women drawn during either 70–98 or 100–182 gestational days (g days), including cases who later experienced PE and controls with an uneventful pregnancy until delivery. Combining biomarker measurements with maternal data enabled the generation of new PE prediction models with high specificity and sensitivity to correctly identify PE onset many months later. Innovatively, the developed 1st trimester PE prediction models were further improved by incorporating the placental genotypes of the genetic variant rs4769613 T/C. This variant is localized upstream of the *FLT1* gene, and the carriership of the C-allele, especially CC-homozygosity, represents a high-confident genetic risk factor for late-onset PE (17, 18).

METHODS

Clinical Study Material

The study patients were recruited, and the clinical data and biomaterials were collected during a prospective observational "Happy Pregnancy" project (full name: "Development of novel non-invasive biomarkers for fertility and healthy pregnancy', PI: M.L.). The project has been approved by the Ethics Committee of Human Research of the University Clinic of Tartu, Estonia (permission no. 221/T-6, 17.12.2012, and 286/M-18, 15.10.2018) and was carried out in compliance with the Helsinki Declaration. Written informed consent to participate in the study was obtained from everyone prior to recruitment. All participants were of white European ancestry and lived in Estonia.

The pregnant women had been enrolled at their first antenatal visit at the Women's Clinic, Tartu University Hospital, Estonia in 2013–2015. The pregnancy follow-up was based on the national guidelines of the Estonian Gynaecologists' Society (19). The collected clinical and epidemiological data are specified in the **Supplementary Methods**. The diagnosis of PE followed the international guidelines at the time of recruitment and included co-occurrence of a new-onset HTN (blood pressure ≥140/ ≥90 mmHg) after 20 g week and proteinuria (PTN), or other signs of maternal organ dysfunction (3). Diagnosis of small-for-gestational-age (SGA) newborn was assigned at the delivery based on national guidelines (20).

For research purposes, serum samples were collected in parallel with blood samples for routine clinical tests. Each Happy Pregnancy study subject had been drawn a blood sample 2–5 times during the pregnancy. Serum was separated (centrifugation at 1,800 g for 10 min at room temperature, RT) within 2 h after sampling and kept at −80°C before further aliquoting and subsequent analysis.

Preeclampsia patients (n = 22, age 28.0 ± 5.2 years; prepregnancy body mass index (BMI) 26.8 ± 6.2 kg/m^2) and non-PE controls (n = 31; 28.5 ± 5.1 years; BMI 25.7 ± 4.8 kg/m^2) analyzed in this study were selected from the Happy Pregnancy biobank based on available gestational-age matched serum samples in both, PE case and control groups (**Supplementary Table S1**). The included pregnancies had been drawn from a total of 132 serum samples (**Table 1**). Early pregnancy was represented by 14 samples from women who later developed PE (drawn 88.8 ± 7.0; 70–98 g days)

and 20 samples from healthy gestations (88.2 ± 5.8; 76–96 g days). Within this time window, one PE pregnancy and two control cases were represented by two sera, drawn at 70th and 96th, 76th and 92nd, 90th, and 93rd g days, respectively. Mid-pregnancy sample set comprised 18 PE and 21 non-PE pregnancy sera, sampled between 100–180 (150.7 ± 25.7) and 109–182 (136.1 ± 24.1) g days, respectively. During mid-gestation, one woman in the PE and three in the control group had been sampled two times, at 117th and 180th, 111th and 182nd; 118th and 139th; and 121st and 147th g days, respectively. All 53 women, irrespective of the final pregnancy outcome (PE or non-PE), were normotensive at blood draw during early and mid-pregnancy blood sampling. The age at the onset of PE in cases with early- and mid-pregnancy serum samples was 257.4 ± 15.2 and 249.3 ± 25.3 g days, respectively.

Late pregnancy serum samples of these 53 cases (28 PE, 206–275 g days; 31 controls, 210–274 g days) have been utilized in our recent study reporting PE prediction models based on 6PLEX assay measurements of late pregnancy serum samples (15). Two serum samples (one PE and one control, 182 and 180 g days, respectively) from this seminal publication were reallocated in this study to the mid-pregnancy sample set, as more appropriate.

Biomarker Measurements Using the Luminex® 6PLEX Multiplex Assay

The Luminex® xMAP platform offers advanced immunoassay-based technology that allows rapid simultaneous analyses of a large number of biomarkers in a single test tube (21). Luminex® xMAP-based approach and development of the methodology for multiplex measurement of sFlt-1, PlGF, sENG, leptin, ADAM12, and PTX3 in a single test tube is detailed in a previously published study (15). Briefly, Luminex® magnetic microspheres (#MC100) and antibody coupling kit for covalent linking of antibodies and microspheres (Antibody Coupling Kit, #40–50016) were purchased directly from Luminex® Corporation (Austin TX, USA). Capture and detection antibodies and reference proteins were purchased from R&D Systems (Minneapolis, MN, USA) (**Supplementary Table S2**). The applied Luminex® sandwich immunoassay protocol followed the manufacturer's guidelines (The xMAP Cookbook, https://www.luminexcorp.com/). All serum samples were analyzed in one batch in duplicate using a 1:20 dilution factor. All dilutions of reference proteins and tested samples were prepared in General Assay Diluent (GAD; #620; ImmunoChemistry Technologies, LLC, Minnesota, USA). Details of Luminex® xMAP technology used equipment, reagents, and their dilutions are provided in

TABLE 1 | Maternal and pregnancy characteristics of the cases involved in PE prediction modeling.

	Early pregnancy samples		Mid-pregnancy samples	
	Control	**Later PE**	**Control**	**Later PE**
Sampling data				
Number of serum samples	20	14	21	18
Gestational age at sampling (g days)	88.2 ± 5.8	88.8 ± 7.0	136.1 ± 24.1	150.7 ± 25.7
Maternal weight at sampling (kg)	74.3 ± 15.5	70.2 ± 15.1	79.5 ± 15.4	77.1 ± 15.5
General data of the index pregnancy				
Number of cases	18	13	18	17
Maternal age (years)	26.8 ± 4.6	26.6 ± 3.7	29.3 ± 6.4	28.1 ± 5.5
Nulliparity (n, %)	8 (44.4%)*	12 (92.3%)	10 (55.5%)	13 (76.5%)
Gravidity (n)	1.8 ± 1.0	1.4 ± 0.9	2.2 ± 1.6	1.6 ± 1.2
Pre-pregnancy BMI (kg/m2)	25.3 ± 4.7	24.2 ± 4.4	26.1 ± 5.0	27.0 ± 7.0
Fetal sex (male/female)	10/ 8	7/ 6	9/ 9	11 / 6
Diagnosis of preeclampsia (g days)	n.a.	257.4 ± 15.2	n.a.	249.3 ± 25.3
Gestational diabetes (n, %)	2 (11.1%)	0 (0%)	2 (11.1%)	0 (0%)
Gestational age at delivery (g days)	278.6 ± 13.0	260.4 ± 16.2	278.4 ± 13.3	257.5 ± 22.2
Preterm delivery, <259 g days (*n*, %)	1 (5.5%) *	5 (38.4%)	1 (5.5%) *	9 (52.9%)
SGA[a] newborn (*n*, %)	1 (5.5%) *	6 (46.2%)	2 (11.31%) *	8 (47.1%)
Placental genotype for rs4769613 (CC/CT/TT) [b]	4/9/4	4/6/1	6/8/2	4/7/2

Data are given as either mean ± standard deviation or number (%) for the continuous or categorical variables, respectively.

[a] *Diagnosis of a small-for-gestational-age (SGA) newborn was assigned at the delivery based on national guidelines (20).*

[b] *Placental tissues were available for genotyping for 17 of 22 PE and 29 of 31 no-PE cases included in the study (in total 46 of 53 pregnancies); placental genotype of a single-nucleotide variant rs4769613 T/C represents a risk factor for late-onset PE as it is localized in an enhancer region near the FLT1 gene, modulating gene expression (17, 18).*

**P < 0.05 between PE and no-PE groups; categorical variables, chi-square test, non-categorical variables Wilcoxon rank-sum test.*

BMI, body mass index; g days, gestational days; gravidity, the total number of pregnancies including index pregnancy; n, number of subjects; nulliparity, no previous deliveries; PE, preeclampsia; n.a., not applicable.

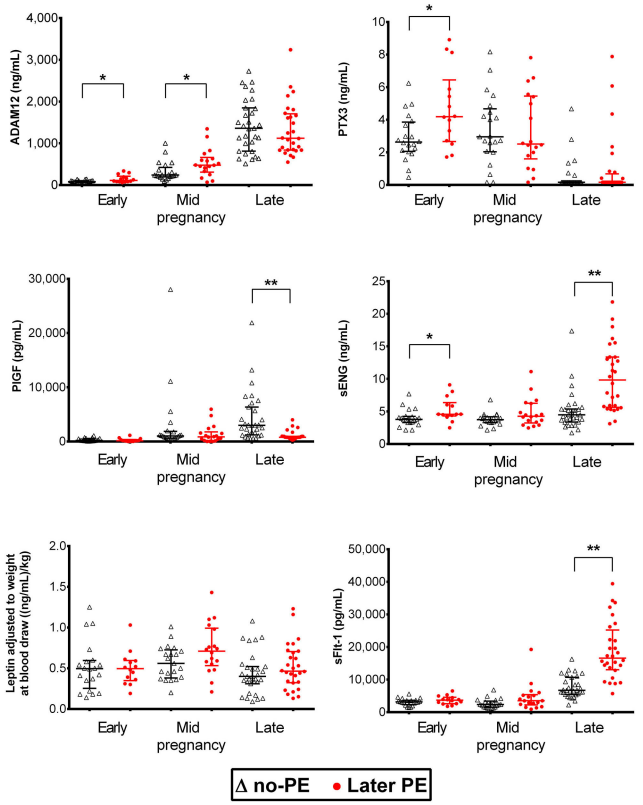


FIGURE 1 | Dynamics of preeclampsia-related biomarkers throughout gestation in healthy and preeclamptic pregnancies. Luminex® 6PLEX assay measurement data of concentrations of ADAM12, PTX3, PlGF, sENG, and sFlt-1 in 132 serum samples collected from pregnant women. The early pregnancy group was comprised of 14 samples drawn at 70–98 g days from women that later developed PE and 20 samples from controls collected at 76–96 g days. The mid-pregnancy sample set comprised 18 PE and 21 control pregnancy sera, sampled at 100–180 and 109–182 g days, respectively. Data of late pregnancy samples representing 28 PE (206–275 g days) and 31 control sera (210–274 g days) were obtained from our recent study (15). All women, irrespective of the pregnancy outcome (PE or no-PE), were normotensive at blood draw. The whiskers on the plot show the median with an interquartile range. The statistical difference in biomarker distributions between PE and control cases was compared using the Mann-Whitney U-test, * representing $P < 0.05$ and ** $P < 0.0001$. ADAM12, ADAM Metallopeptidase Domain 12; g days, gestational days; PlGF, placental growth factor; PTX3, Pentraxin3; sENG, endoglin; sFlt-1, soluble fms-like tyrosine kinase 1.

the **Supplementary Methods** and our seminal methodological publication (15).

Placental *FLT1* rs4769613 Genotyping

Placental tissues were collected after cesarean section or vaginal delivery, were washed with 1× PBS (phosphate-buffered saline, pH∼7.4) to remove maternal blood, and stored in a dry tube at −80°C until DNA extraction. DNA from the placental samples was extracted using the NucleoSpin Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. For genotyping of placental rs4769613 T/C, premade TaqMan Genotyping Assay was used according to the manufacturer's protocol (Applied Biosystems, Foster City; Assay ID: C__32231378_10). Placental tissue was available for 17 of 22 PE cases and 29 of 31 controls (in 46 of 53 pregnancies). Genotyping details have been described in the **Supplementary Methods** and a recent publication (18).

Statistical Analysis and PE Prediction Algorithm Development

Summary estimates of the data were calculated, and all statistical tests were implemented using the STATA software version 13.1 (StataCorp, TX, USA) or the R 3.3.3 language and environment (Free Software Foundation, Boston, MA, USA, http://www.r-project.org). To compare groups, the Mann-Whitney rank-sum test was used for continuous variables and the chi-square test for categorical variables. $P < 0.05$ was considered statistically significant.

Logistic regression models (GLM) were used to investigate associations between biomarker measurements and the clinical onset of PE during the index pregnancy. All biomarker values were centered and scaled before modeling for data normalization and standardization. First automated computational pre-filtration for the identification of the optimal prediction model has performed modeling with the stepAIC selection method (generalized linear model with stepwise feature selection) in package CARET (short for **C**lassification **A**nd **RE**gression **T**raining). This machine learning strategy, in combination with leave-one-out cross-validation (LOOCV), allows to select statistically most significant prediction model and to pick the complexity parameters that are associated with the optimal resampling statistics. Pre-filtration was carried out by using the following input variables: measured concentrations of six biomarkers (ADAM12, Leptin, Pentraxin3, sENG, sFlt-1, and PlGF) and maternal characteristics of blood sampling time in gestational days, maternal weight at blood draw, and parity as a binary variable (nulliparity defined as 0 and multiparity defined as 1). As an output of this procedure, all possible models generated from these input parameters were automatically ranked based on their area under the curve (AUC) estimates. The best-predicted model by the LOOCV + stepAIC approach was developed further by alternatively replacing and/or adding biomarker measurements to trial the model performance using the GLM package in R. Additionally, statistical models were built combining parameter combinations with the placental genotypes of the single-nucleotide polymorphism (SNP) rs4769613 T/C (in an additive manner, defined as variables 0, 1, or 2 according to genotypes TT, CT, and CC) either by replacing parity with the SNP data or considering them both.

The predictive power of the models was assessed using the ROC curve (receiver operating characteristic curve) and the area under the ROC curve (AUC). For every fitted model, model-based individual predictions were obtained, as estimated probabilities of PE (during the index pregnancy until term), denoted as p(i) with Epi::ROC. The p(i) represents the probability thresholds at the maximum Youden's J index on the curve. The p(i) equal or superior to a fitted model

TABLE 2 | Measured concentrations of biomarkers in maternal sera sampled in early, mid, and late pregnancy.

	Early pregnancy		Mid pregnancy		Late pregnancy[a]	
	Controls ***n*** **= 20**	**Later PE** ***n*** **= 14**	**Controls** ***n*** **= 21**	**Later PE** ***n*** **= 18**	**Controls** ***n*** **= 31**	**Later PE** ***n*** **= 28**
Gestational days at sampling (*n*)	88.2±5.8 89 (76–96)	88.8 ± 7.0 89 (70–98)	136.1 ± 24.1 137 (109–182)	150.7 ± 25.7 137 (100–180)	236.2 ± 17.2 230 (210–274)	236.3 ± 20.8 234 (206–275)
ADAM12 (ng/mL)	79.1 (69.1–98.5)	110.8* (97.8–202.3)	239.2 (196.6–421.0)	474.6* (249.3–632.3)	1362 (813.4–1847)	1121 (837.7–1705)
PTX3 (ng/mL)	2.63 (2.24–3.56)	4.19* (3.33–6.08)	2.95 (2.04–4.67)	2.55 (1.79–5.41)	0.16 (0.16–0.85)	0.16 (0.16–1.75)
PlGF (pg/mL)	196.4 (188.1–451.6)	136.10 (63.9–436.5)	1011 (611–1872)	884.1 (184.3–1559)	2974 (1197–6356)	780.1** (577–939)
sENG (ng/mL)	3.76 (3.35–4.59)	4.57* (4.18–6.30)	3.74 (3.31–4.17)	4.19 (2.90–6.22)	4.48 (3.37–5.38)	9.82** (5.62–13.36)
sFlt-1 (pg/mL)	3119 (2627–3638)	3660 (2895–4422)	2329 (1486–3394)	3472 (2384–5168)	6693 (5408–10686)	16543** (13012–25202)
Leptin adjusted to weight ((ng/mL)/kg)	0.49 (0.37–0.66)	0.49 (0.38–0.62)	0.56 (0.38–0.73)	0.69 (0.55–0.98)	0.40 (0.31–0.52)	0.46 (0.33–0.71)

All biomarkers were measured in a single test tube using the Luminex® 6PLEX assay. Biomarker data are presented as median with 95% CI.

*Gestational days are presented as mean ± standard deviation and median (range). The Mann–Whitney U–test for non–categorical variables was applied to compare biomarker levels between groups, * denotes $P < 0.05$ and ** $P < 0.0001$.*

[a]*Data from the study developing PE prognosis models based on 6PLEX assay measurements of late pregnancy serum samples (15).*

ADAM12, disintegrin and metalloproteinase domain–containing protein 12; PlGF, placental growth factor; PTX3, pentraxin-3; sENG, soluble endoglin; sFlt-1, soluble fms–like tyrosine kinase-1.

optimal cutoff point value indicates that the subject will develop PE or has PE, whereas the p(i) inferior to a fitted model optimal cutoff point value predicts that PE will not develop.

RESULTS

Gestational Dynamics of Maternal Serum ADAM12, PTX3, PlGF, sFlt-1, sENG, and Leptin Levels in Healthy and Preeclamptic Pregnancies

Luminex® 6PLEX multiplex assay measurements of 132 serum samples (drawn from 53 pregnant women between 70 and 275 g days; **Table 1**) revealed different gestational dynamics of the analyzed PE-linked biomarkers ADAM12, PTX3, PlGF, sFlt-1, sENG, and leptin (**Figure 1**, **Table 2**). In normal pregnancy, ADAM12 and PlGF levels gradually increase through all three trimesters. PTX3 serum concentrations maintained stable levels during early and mid-pregnancy with a significant decrease toward the term. In contrast, both sFlt-1 and sENG have an increasing trend specifically in late pregnancy. During early pregnancy, three biomarkers showed significantly ($P < 0.05$) increased serum levels in cases with a later onset of PE compared to controls: PTX3, ADAM12, and sENG, whereas only ADAM12 maintained higher concentration in the PE group also in mid-pregnancy (**Figures 1**, **2**). In late pregnancy serum samples from future PE cases, increased sENG and sFlt-1, and decreased PlGF were measured (PE vs. non-PE serum levels, $P < 0.001$).

Placental Rs4769613 T/C Variant Near *FLT1* Is Not Associated With sFlt-1 Serum Levels

Placental tissues from 46 women (17 PE and 29 non-PE) analyzed in this study were available for placental genotyping of *FLT1* rs4769613 T/C. No statistically significant differences were observed in serum sFlt-1 levels in early, mid, or late pregnancy between women with alternative genotypes CC, CT, and TT (**Table 3**).

Potential of 6PLEX Assay During Early Pregnancy in Predicting Risk for PE Development

Preeclampsia prediction models applicable in early pregnancy were developed by combining Luminex® 6PLEX multiplex-assay measurements of PE biomarkers in maternal serum (70-98 g days) with informative clinical data (**Figures 3A,B**; **Tables 4**, **5**; **Supplementary Table S3**). According to the machine-learning approach (LOOCV + stepAIC), the best performing PE prediction model comprised of PTX3, sFlt-1, and ADAM12 measurements, the subject's parity, and gestational age at sampling [model 1A: AUC 0.936 (95%CI 0.843–0.993)]. PTX3 concentration and parity information had a statistically significant contribution to the model ($P < 0.05$). This model correctly "ruled in" or "ruled out" the onset of PE for 30 of 34 analyzed samples (accuracy 88.2%; 95%CI 73.4–95.3). The PE prediction model 1A exhibited 100% sensitivity in identifying all 14 cases who developed PE several months later (onset 257.4 ± 15.2 g days). The specificity of this model was 80.0%. Four of 20 cases progressing healthy pregnancy until delivery received a false-positive outcome regarding the PE prediction.

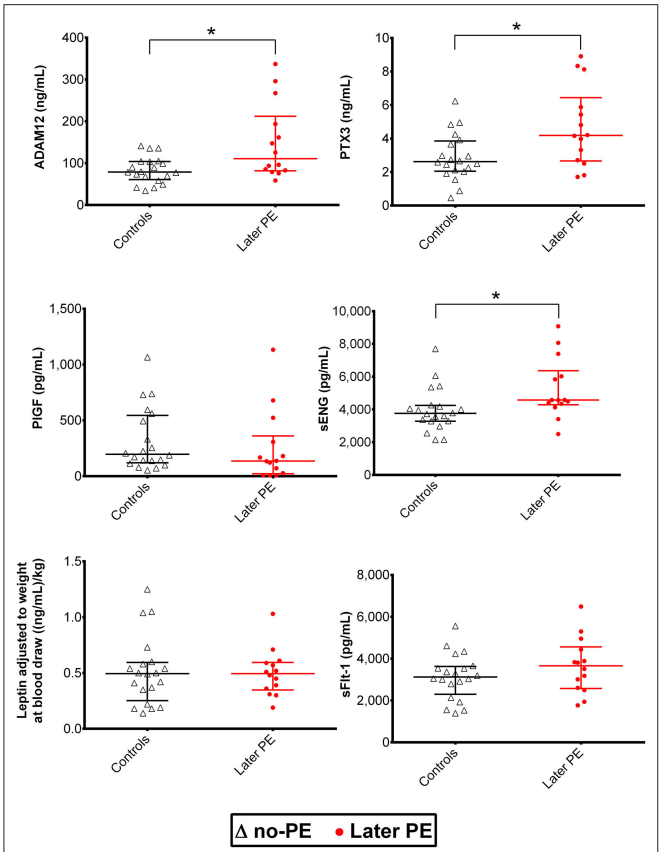


FIGURE 2 | Distribution of maternal serum biomarkers measured with the Luminex® 6PLEX assay during early pregnancy, stratified by the later onset of preeclampsia (PE). Samples from women that later developed PE ($n = 14$, drawn at 70–98 gestational days) were compared to samples representing uncomplicated gestations until term ($n = 20$, 76–96 gestational days; **Table 1**). The whiskers on the plot show the median with an interquartile range. The statistical difference in biomarker distributions between PE and control cases was compared using the Mann-Whitney U-test, * representing $P<0.05$. ADAM12, disintegrin and metalloproteinase domain-containing protein 12; PlGF, placental growth factor; PTX3, pentraxin-3; sENG, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1.

Early pregnancy sFlt-1 and sENG serum levels were highly correlated (**Figure 4**). When sFlt-1 was replaced with sENG (model 1B) or both biomarkers were incorporated (model 1C), these alternative formulas showed equivalent properties in predicting PE as the model 1A (**Tables 4**, **5**).

Placental *FLT1* Rs4769613 T/C Genotype Data Improve PE Prediction Models

We explored the added value of placental *FLT1* rs4769613 T/C, confidently associated with late-onset PE (17, 18), in the PE prediction models excluding (2A–2C) or including (3A−3C) the parity data (**Figures 3A,B**; **Tables 4**, **5**; **Supplementary Table S3**). The overall PE prediction accuracy in early pregnancy was the highest when combining gestational age-adjusted PTX3, sFlt-1, sENG, and ADAM12 measurements with the parity and placental *FLT1* rs4769613 additive genotype

TABLE 3 | Detailed data of sFlt-1 (pg/mL) measurements stratified into fetal *FLT1* rs4769613 T/C genotype of the 116 serum samples drawn during early, mid, and late pregnancy.

	Early pregnancy $n = 31$	Mid–pregnancy $n = 32$	Late–pregnancy $n = 53$
g days	90 (70–98)	144 (100–182)	236 (206–275)
CC	3531 (2131–6489)	2339 (1309–6489)	11862 (4782–39417)
CT	3191 (1531–5161)	2770 (813–5989)	9158 (2207–34221)
TT	3292 (1396–5298)	4427 (3491–6805)	8758 (4301–29884)

sFlt-1 (pg/mL) data are presented as medians with minimum and maximum values. Gestational age data in days are presented as median (minimum—maximum). sFlt-1, soluble fms-like tyrosine kinase-1; g days, gestational age in days.

data [model 3C: AUC 0.969 (95%CI 0.895–1.000)]. This model yielded a correct "rule in" or "rule out" PE in 29 out of 31 cases (93.5%; 95%CI 79.3–98.2%). In the series of models 3A–3C and model 2A, none of the true PE cases were missed. Models 3A–3C yielded only two false-positive PE predictions for pregnancies that remained normotensive. Taken together, including placental *FLT1* rs4769613 T/C genotype data further improved the specificity of PE prediction from 80% to 94.7% (model 1A vs. 3A, model 1C vs. 3C, **Table 5**).

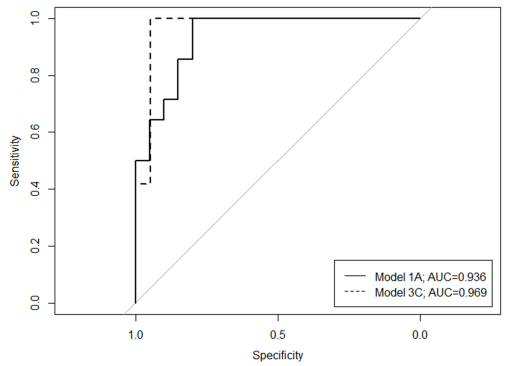
6PLEX Assay Data in Mid-pregnancy Are Moderately Informative for PE Prediction

The development of PE prediction models using 6PLEX assay measurement data in mid-pregnancy (100–182 g days) was less informative compared to early pregnancy (**Figure 3C**, **Tables 4**, **5**, **Supplementary Table S3**). The best performing model selected by the stepwise logistic regression model combined gestational age-adjusted PlGF measurements with parity data [Model 4: AUC 0.784 (95%CI 0.634–0.912)]. In total, 29 of 39 pregnancies [74.4% (95%CI 58.9–85.4)] received correctly "ruled in" or "ruled out" PE development. Including genotype data of the placental variant *FLT1* rs4769613 T/C (model 5) did not improve the prediction [accuracy 71.9% (95%CI 54.6–84.4)].

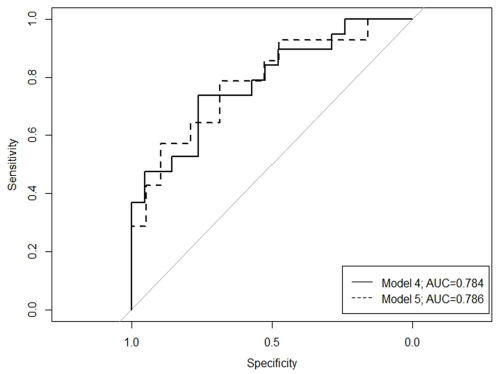
DISCUSSION

We have recently developed a maternal serum-based 6PLEX assay implemented on the Luminex® xMAP platform that measures six PE biomarkers in a single test tube and has shown its potential as an informative screening test (prediction accuracy of 96.5%) for PE prediction during the second half of pregnancy (15). This study demonstrated that 6PLEX assay measurements of serum samples collected in early pregnancy are also informative for developing sensitive and accurate PE prediction models applicable already during 70–98 g days (**Figure 3**, **Table 5**, **Supplementary Table S3**). Further innovative aspects in the study were the exploitation of an unbiased machine

A Early pregnancy best PE prediction models



C Mid-pregnancy best PE prediction models



B

Early pregnancy PE prediction formula 1A

$$p(i) = \frac{1}{1 + e^{(-15.9+1.8*log_2([sFlt-1])-1.7*log_2([ADAM12])-1.9*log_2([PTX3])-4.1*([parity])+0.15*([gest.days]))}}$$

Early pregnancy PE prediction formula 3C

$$p(i) = \frac{1}{1 + e^{(-8.7+3.9*log_2([sFlt-1])-2.7*log_2([ADAM12])-2.1*log_2([PTX3])-1.9*log_2([sENG])-20.8*([parity])+0.14*([gest.days])-2.5*\left(\left[rs4769613\frac{T}{C}\right]\right))}}$$

FIGURE 3 | ROC curves, AUC values, and the formulas of the best performing preeclampsia (PE) prediction models were developed based on the Luminex® 6PLEX assay data. The best PE prediction models are based on the analysis of serum samples collected during either **(A)** 70th-98th or **(C)** 100th−182nd g days**.** Early pregnancy model 1A incorporated data from three biomarkers (sFlt-1, ADAM12, and PTX3) and model 3C four markers (sFlt-1, sEng, ADAM12, and PTX3), whereas mid-pregnancy models 4 and 5 are based on only PlGF measurements. Model 3C and model 5 additionally included placental genotype data of the *FLT1* rs4769613 T/C variant. All models were adjusted for gestational days (gest. days) at sampling and maternal parity (nulliparity or multiparity). **(B)** PE prediction formulae for models 1A and 3C. Details are presented in **Tables 4**, **5** and **Supplementary Table S3**. AUC, the area under the curve; ROC, receiver operating characteristics.

learning approach to select statistically most significant PE prediction models (22–24), adjusting biomarker measurements for the gestational day at sampling, and incorporation of the placental genotypes of the *FLT1* rs4769613 T/C variant, confidently associated with PE susceptibility (17, 18). The prediction model combining gestational age-adjusted 6PLEX measurements of PTX3, sFlt-1, sENG, and ADAM12 with parity and placental *FLT1* rs4769613 T/C genotype data yielded a correct prediction of PE in 93.5% of analyzed cases with no false-negative predictions.

This is the first time a PE prediction model incorporated a genetic risk factor to be combined with maternal serum biomarker measurements and clinical data into a disease risk prediction algorithm. The proposed placental *FLT1* rs4769613 T/C genotyping that increased the predictive accuracy from 88.2% to 93.5% (**Figure 3**, **Table 5**) is a novel entry into the currently protein-based biomarker-ruled PE prediction landscape. The utility of the non-invasive prenatal screening (NIPS) approach that is based on cell-free fetal DNA (cffDNA) is rapidly developing, currently focusing on detecting large chromosomal aberrations in the fetus (25, 26). However, there are already available technological solutions that allow single gene variant detection using the cffDNA (27). Thus, using cffDNA to screen the placental *FLT1* rs4769613 T/C genotypes to be incorporated into the PE prediction models may soon be a feasible approach. In this perspective, the most rational solution would be the inclusion of this variant in gene panels developed for NIPS targeting fetal single gene defects. Simultaneous blood sampling and screening of pregnant women for fetal chromosomal and monogenic conditions, as well as for their risk to develop PE, is also a cost-effective, time-saving, and patient-friendly approach. Although currently available NIPS-based tests for fetal monogenic disorders cost hundreds of euros and are expensive for screening purposes, the prices are expected to drop in long run with possible new competitive technological and cost-effective solutions arriving on the market.

The developed 6PLEX assay-based PE prediction models performed at least equally or even better than most PE prediction algorithms that are currently implemented in early pregnancy (12, 13). One of the most widely used and validated algorithms for detecting PE is based on combining maternal factors, uterine arteries pulsatility index (UtA PI), mean arterial pressure (MAP), maternal serum PlGF, and/or PAPP-A (10). This algorithm can predict 90% of PE onset <32 g weeks, 75% of preterm PE (<37 g weeks), but only <50% for term PE cases. As three

TABLE 4 | Developed PE prognosis models and contributing variables.

Model acronym	Variables contributing to alternative models[a]						
Early pregnancy data modeling (70–98th gestational days)							
1A[b]	PTX3*	sFlt-1		ADAM12	parity*		g days
1B	PTX3*		sENG	ADAM12	parity*		g days
1C	PTX3*	sFlt-1	sENG	ADAM12	parity*		g days
2A	PTX3*	sFlt-1		ADAM12*		rs4769613 T/C	g days
2B	PTX3*		sENG	ADAM12*		rs4769613 T/C	g days
2C	PTX3*	sFlt-1	sENG	ADAM12*		rs4769613 T/C	g days
3A	PTX3	sFlt-1		ADAM12	parity	rs4769613 T/C	g days
3B	PTX3		sENG	ADAM12	parity	rs4769613 T/C	g days
3C	PTX3	sFlt-1	sENG	ADAM12	parity	rs4769613 T/C	g days
Mid–pregnancy data modeling (100–182th gestational days)							
4[b]			PlGF		parity		g days
5			PlGF		parity	rs4769613 T/C	g days

[a]Parity was treated as a binary variable; every woman was assigned as nulliparous referring to no previous deliveries, or multiparous indicating at least one childbirth before the index pregnancy; rs4769613 T/C refers to the placental genotype of a single–nucleotide variant near the FLT1 gene with T– and C–alleles.

[b]Selected as statistically most significant model using the automatic computational pre–filtration approach (LOOCV + stepAIC).

**p–value <0.05, showing a statistically significant contribution of this variable to the model.*

ADAM12, disintegrin and metalloproteinase domain–containing protein 12; g days, gestational age in days; PlGF, placental growth factor; PTX3, pentraxin−3; sENG, soluble endoglin; sFlt-1, soluble fms–like tyrosine kinase−1.

of four PE pregnancies develop ≥37th g week, this algorithm has its limitation. Further shortcomings of the currently used solution include a high false-positive rate (10, 28). In addition, the requirement of certified costly apparatus and trained personnel for the measurement of UtA PI is not a routine procedure in the management of pregnant women in many countries (11).

Combined data from this study and our previous report (15) provide strong evidence that placental biomarkers circulating in maternal serum have individual gestational dynamics that must be considered in PE prediction models. 6PLEX data are consistent with the published observations on the measurements of the same biomarkers using conventional single marker assays (29, 30). There is enough accumulated evidence that during each gestational period, the set of maternal serum metabolites reflecting placental and/or maternal pathology varies. For example, well-established PE biomarkers of late pregnancy sFlt-1 and PlGF (8, 11, 12, 16) show early pregnancy serum levels that are individually not equivocally discriminative for the PE onset during the 3rd trimester (**Figures 1**, **2**). In contrast, circulating levels of PTX3 in early pregnancy were shown as an informative biomarker for later PE development in this study and by others (31, 32).

The development of a potentially applicable predictive model using mid-pregnancy serum biomarker data has appeared to be a challenging task. In the current study, 6PLEX measurements of samples collected at 100–182 g days were insufficiently informative to develop reliable PE prediction models for mid-pregnancy (accuracy <75%). Further studies are needed to discover maternal serum biomarkers that are specific to the 2nd trimester of pregnancy.

Possible limitations of our study have to be acknowledged, including the moderate sample size that does not cover the real-life variability of pregnancy scenarios (e.g., twin pregnancies) and the narrow demographic origin of patients. Large-scale follow-up studies in independent pregnancy cohorts, both retrospective and prospective, are needed to validate the developed PE prediction models and evaluate their performance in clinical practice.

CONCLUSION

Despite all the research efforts and clinical advances, PE has remained a severe and rather common pregnancy complication with significant harm to maternal and perinatal morbidity. Timely surveillance and management of at-risk patients is the key approach to reducing its morbidities and most severe outcomes. Therefore, further early prediction tools, especially for late-onset PE, are needed to combine reliable screening performance with easily applicable protocols that do not need expensive infrastructure and special training and will be accessible to a large number of clinical centers. The developed model opens new horizons for first-trimester PE screening, combining the easily standardizable 6PLEX assay with routinely collected antenatal care data and resulting in high sensitivity and specificity of the test.

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.

TABLE 5 | Characteristics of PE prediction models.

Model[a]	Correct prognosis (accuracy%[95%CI)]	AUC [95%CI]	Sensitivity % [95%CI]	Specificity % [95%CI]
Early pregnancy data modeling (70–98th g days) [b]				
Models combining biomarkers, gestational age and parity				
1A[c]	30/34 (88.2% [73.4–95.3])	0.936 [0.843–0.993]	100.0 [92.9–100.0]	80.0 [65.0–100]
1B	29/34 (85.29% [69.9–93.6])	0.914 [0.804–0.989]	100.0 [78.6–100.0]	80.0 [60.0–100.0]
1C	30/34 (88.2% [73.4–95.2])	0.932 [0.839–0.993]	100.0 [92.9–100.0]	80.0 [65.0–100.0]
Models combining biomarkers, gestational age and rs4769613 T/C placental genotype				
2A	26/31 (83.9% [67.4–92.9])	0.934 [0.829–1.000]	100.0 [75.0–100.0]	78.9 [68.4–100.0]
2B	25/31 (80.7% [63.7–90.8])	0.930 [0.829–0.996]	91.7 [75.0–100.0]	78.9 [57.9–100.0]
2C	27/31 (87.1% [71.2–94.9])	0.934 [0.825–1.000]	91.7 [83.3–100.0]	89.5 [57.9–100.0]
Models combining biomarkers, gestational age, parity and rs4769613 T/C placental genotype				
3A	29/31 (93.5% [79.3–98.2])	0.969 [0.882–1.000]	100.0 [83.3–100.0]	94.7 [89.5–100.0]
3B	29/31 (93.5% [79.3–98.2])	0.947 [0.851–1.000]	100.0 [83.3–100.0]	89.5 [84.2–100.0]
3C	29/31 (93.5% [79.3–98.2])	0.969 [0.895–1.000]	100.0 [100.0–100.0]	94.7 [89.5–100.0]
Mid–pregnancy data modeling (100–182th g days)				
Model combining PlGF measurement, gestational age and parity				
4[c]	29/39 (74.4% [58.9–85.4])	0.784 [0.634–0.912]	73.7% [36.8–94.7]	76.2% [19.0–85.7]
Model combining PlGF measurement, gestational age, parity and rs4769613 T/C placental genotype				
5	23/32 (71.9% [54.6–84.4])	0.786 [0.613–0.932]	78.6% [28.6–92.9]	68.4% [36.8–100]

[a] *Detailed information is available in* ***Supplementary Table S3.***

[b] *Early pregnancy model A includes serum measurements of ADAM12, PTX3, and sFlt–1; model B measurements of ADAM12, PTX3, and sENG, and model C measurements of ADAM12, PTX3, sFlt–1, and sENG.*

[c] *Selected as statistically most significant model using the automatic computational pre–filtration strategy.*

AUC, the area under the curve; g days, gestational days.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Human Research of the University Clinic of Tartu, Estonia (permissions no. 221/T-6, 17.12.2012 and 286/M-18, 15.10.2018). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception: ML. Design: KRa, KRu, and ML. Provision of study materials: ML and KRu. Clinical data collection: KRu and EH. Experimental conduct: KRa and TK. Experimental guidance: KK and ML. Data analysis: KRa, OA, TK, and KF. Data interpretation: KRa, KRu, OA, TK, EH, KK, KF, and ML. Manuscript writing: KRa and ML. Critical reading and commenting on the article: KRu, EH, TK, KK, OA, and KF. All authors contributed to the article and approved the submitted version.

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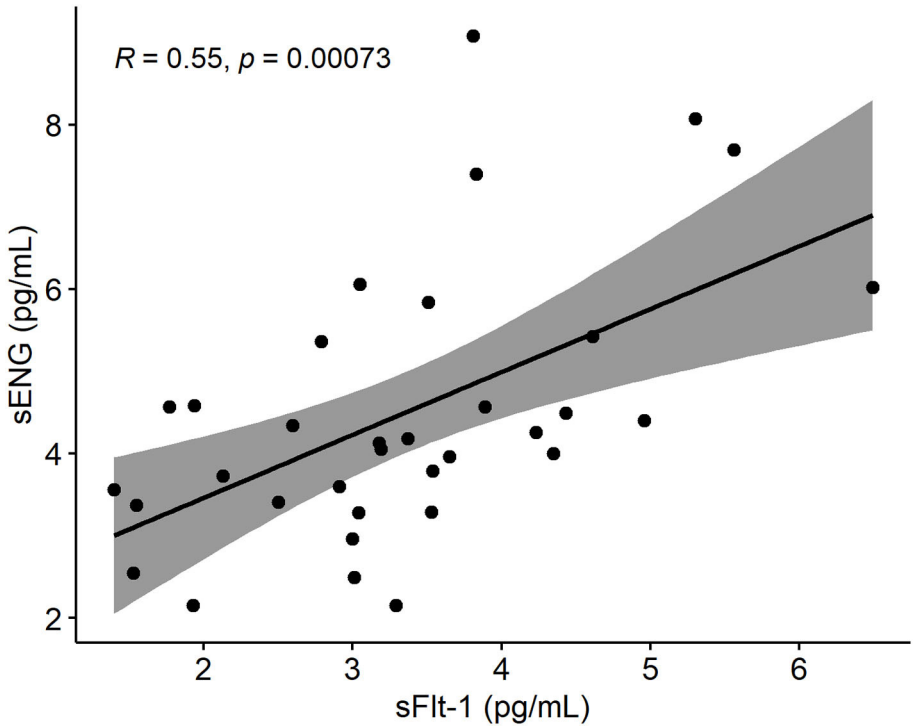

FIGURE 4 | Spearman's rank correlation plot between sENG and sFlt-1 measurements of sera drawn from pregnant women within 70 and 98 gestational days. Gray around the linear regression line (y = 0.7651x + 1,931.4) indicates the 95% confidence region. sENG, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.932480/full#supplementary-material

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No accelerated arterial aging in relatively young women after preeclampsia as compared to normotensive pregnancy

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Introduction: Preeclampsia, an endothelial disorder of pregnancy, predisposes to remote cardiovascular diseases (CVD). Whether there is an accelerated effect of aging on endothelial decline in former preeclamptic women is unknown. We investigated if the arterial aging regarding endothelial-dependent and -independent vascular function is more pronounced in women with a history of preeclampsia as compared to women with a history of solely normotensive gestation(s).

Methods: Data was used from the Queen of Hearts study (ClinicalTrials.gov Identifier NCT02347540); a large cross-sectional study on early detection of cardiovascular disease among young women (≥18 years) with a history of preeclampsia and a control group of low-risk healthy women with a history of uncomplicated pregnancies. Brachial artery flow-mediated dilation (FMD; absolute, relative and allometric) and sublingually administered nitroglycerine-mediated dilation (NGMD; absolute and relative) were measured using ultrasound. Cross-sectional associations of age with FMD and NGMD were investigated by linear regression. Models were adjusted for body mass index, smoking, antihypertensive drug use, mean arterial pressure, fasting glucose, menopausal state, family history of CVD and stress stimulus during measurement. Effect modification by preeclampsia was investigated by including an interaction term between preeclampsia and age in regression models.

Results: Of the 1,217 included women (age range 22–62 years), 66.0% had a history of preeclampsia and 34.0% of normotensive pregnancy. Advancing age was associated with a decrease in relative FMD and NGMD (unadjusted regression coefficient: FMD: −0.48%/10 years (95% CI:−0.65 to −0.30%/10 years), NGMD: −1.13%/10 years (−1.49 to −0.77%/10 years)) and increase in brachial artery diameter [regression coefficient = 0.16 mm/10 years (95% CI 0.13 to 0.19 mm/10 years)]. Similar results were found when evaluating FMD and NGMD as absolute increase or allometrically, and after confounder adjustments. These age-related change were comparable in former preeclamptic women and controls (*p*-values interaction ≥0.372). Preeclampsia itself was independently associated with consistently smaller brachial artery diameter, but not with FMD and NGMD.

Conclusion: In young- to middle-aged women, vascular aging in terms of FMD and NGMD was not accelerated in women after preeclampsia compared to normotensive pregnancies, even though former preeclamptic women consistently have smaller brachial arteries.

KEYWORDS

aging, preeclampsia, cardiovascular, flow-mediated dilation (FMD), arterial function, endothelial (dys)function, hypertensive pregnancy

Introduction

Arterial aging is a physiological process that develops gradually over time and increases the risk of cardiovascular diseases (CVD). With advancing age, several structural and functional arterial changes contribute to this increased cardiovascular (CV) risk (1–3). Age-related vascular dysfunction is characterized by a decline in endothelial function involving impaired vasodilatory capacity of the blood vessel.

Besides age, vascular dysfunction may be accelerated by both conventional and sex-specific CV risk factors (4, 5). Preeclampsia (PE), a hypertensive vascular complication of pregnancy, is associated with impaired endothelial function, both during pregnancy and in the first years after delivery (6, 7). Endothelial dysfunction during or after PE might contribute to the subsequently observed two- to seven-fold increased risk of CVD among these women (8, 9). On top of that, vascular aging might further be accelerated in former preeclamptic women since conventional risk factors, especially increased blood pressure, are highly prevalent after PE (10).

The major mediator of vasodilatory capacity of arteries is endogenous nitric oxide (NO) release by the endothelium, which relaxes vascular smooth muscles (11, 12) resulting in flow-mediated vasodilation in healthy conditions. This physiological response is favorable as it keeps local wall shear stress constant (13). Flow-mediated dilation (FMD) measurement of the brachial artery is a non-invasive method to assess endothelial dysfunction by high-resolution ultrasound imaging. Impaired FMD is used as surrogate measure for CV health as it is strongly associated with and predictive of CVD later in life (14–17).

Whether PE modifies the age-related decline in endothelial function is unknown. Therefore, we investigated whether the age-related decline in endothelial-dependent and –independent vasodilatory function is more pronounced in women with a history of PE as compared to women with normotensive pregnancies, independent of conventional CV risk factors.

Materials and methods

Study design and population

This study was part of a large cross-sectional study aimed at investigating subclinical CVD in women (Queen of Hearts study; ClinicalTrials.gov Identifier NCT02347540) and was approved by the Medical Ethics Committee of the Maastricht University Medical Center (METC azM/UM 14-2-20136 NL47252.068.14). All participating women provided written informed consent. Procedures were in conformity with institutional guidelines and adhered to the principles of the Declaration of Helsinki.

We included women aged ≥18 years with a history of PE and a control group of women who had normotensive pregnancies. Women were included within a postpartum interval of 0.5–30 years, which was based on delivery of their first (complicated) pregnancy. Women who participated between December 2014

and October 2019 were included in the current study. Women with a history of hypertension, autoimmune disease, or kidney disease prior to their first pregnancy were excluded.

PE was defined as new-onset hypertension (i.e. systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg) along with proteinuria (≥300 mg/24 h) after 20 weeks of gestation, or other maternal organ dysfunction (18). Diagnosis before 34 weeks was characterized as early onset PE. Uncomplicated pregnancy was defined as normotensive pregnancy in absence of any placenta-associated disease, including HELLP-syndrome, placental abruption, small for gestational age infancy and/or fetal demise.

Cardiovascular assessment

Postpartum cardiovascular assessment was performed following a standardized protocol during one morning study visit at the Maastricht University Medical Center (MUMC+), including vascular assessment, physical examination of weight and height, blood pressure measurements, fasting blood collection and (obstetrical) medical history taking. All women were instructed to fast for at least 10 h before the study visit.

Height and weight were measured to calculate body mass index (BMI). SBP, DBP, mean arterial pressure (MAP) and heart rate were measured for 30 min in sitting position by a semiautomatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL) with a three-minute measurement interval. The median value of these measurements was used for analyses. Hypertension was defined as antihypertensive drug use or SBP ≥140 and/or DBP ≥90 mmHg, as according to European guidelines (19). A positive family history of CVD was defined as a (grand)parent or sibling below the age of 65 years with CVD.

Measurements of endothelium-dependent and -independent vasodilation

Endothelium-dependent and -independent brachial artery dilation were evaluated by assessing FMD and the effect of a sublingual dose of nitroglycerine (i.e., nitroglycerine-mediated dilation [NGMD]), respectively. FMD and NGMD measurements were performed sequentially under standardized conditions in a temperature-controlled room (±22 °C). Before the measurements, participants rested in supine position on a comfortable bed for at least 15 min. The arm was in an extended position at ±80° from the torso. A rapid inflation and deflation cuff (Hokanson, Bellevue, VA 98005) was positioned around the forearm distal to the olecranon. A multi-frequency linear array probe attached to a high-resolution ultrasound machine (Voluson p6, GE Healthcare) with an operating frequency of five MHz was used to image the brachial artery in the distal third of the upper arm, two to five cm above the antecubital fossa. The probe was fixed during all measurements by a custom-made fixation device made by instrumental services.

For (endothelium-dependent) FMD evaluation, we acquired a 3-min baseline recording of the brachial artery diameter. Thereafter, the forearm cuff was inflated (200 mmHg) for 5 min followed by rapid deflation. The diameter and Doppler spectrum were assessed continuously from 3 min before inflation to 5 min after deflation, but interrupted from 30 s after inflation to 30 s before deflation.

For (endothelium-independent) NGMD evaluation, we also started with a 3-min baseline recording after which a dose of NG (0.4 mg/dose) was administered sublingually. The recording ended 10 min after sublingual administration of NG.

Image analysis of the brachial artery diameter was performed off-line with a custom designed edge-detection and wall-tracking software (13) in Matlab (Matlab R2013b, The Mathworks Inc. Natick, MA), which separates the measurements from the analyses and therefore reduced the risk of bias. Peak diameter was automatically detected, as described previously (13). In a previous pilot, two experienced sonographers performed repeated measurements of FMD in 15 volunteers, to quantify the inter-observer agreement. The corresponding inter-observer intraclass correlation coefficient (ICC) was 0.82, while the intra-observer ICC was 0.83.

FMD and NGMD outcome measures

FMD and NGMD were expressed both as an absolute and relative (i.e., percentage) increase in brachial artery diameter, which were based on the peak change in diameter with respect to baseline. Baseline diameter during FMD referred to the 3-min period before cuff-release. During NGMD, a single value for baseline was measured at the start of the response. Although the baseline brachial artery diameter was separately measured in FMD- and NGMD-assessment, the variability of these measurements was not statistically significant (paired sample t-test; $p = 0.659$).

FMD was also expressed with an allometric scaling to avoid baseline dependency as proposed by Atkinson et al. (20). It aims to compensate for potential differences in vessel diameter. For the allometric FMD, we calculated the regression slope between logarithmically transformed values of both baseline diameter and peak diameter and derived the correct scaling exponent for our dataset (20). A value of 1 is necessary for appropriate use of FMD% (21, 22). The regression slope between the logarithmically transformed values of both baseline diameter and peak diameter yielded a 1.014 scaling exponent, which was used to calculate the allometric scaled FMD.

Besides, we calculated the physiologic dilatory response to stress (i.e., FMD) as proportion of maximal dilatory capacity (i.e., NGMD) by (FMD%*100)/NGMD%.

Statistical analysis

Baseline data are presented as mean and standard deviation in case of normal distribution, otherwise as median and interquartile range [IQR]. Categorical variables are presented as number and percentage within group. To analyze between-group differences in baseline characteristics, we used the independent-samples *t*-test, Mann-Whitney U or Fisher's exact, as appropriate. To ensure no selection bias had occurred due to missing, incomplete or low-quality data on FMD and NGMD, we compared baseline characteristics of women who were excluded to those included in the analyses.

First, differences between age groups (20–29, 30–39, 40–49, ≥50 years) regarding brachial artery diameter, FMD, and NGMD were tested using one-way ANOVA and Kruskal Wallis tests, as appropriate. Subsequently, linear regression analysis was performed to evaluate the association of age with brachial artery diameter, FMD and NGMD, both unadjusted and fully-adjusted for BMI, smoking, antihypertensive drug use, MAP, fasting blood glucose level, menopausal state and a positive family history of CVD. When investigating absolute FMD and NGMD, we additionally adjusted for the baseline diameter. In all regression models on FMD and NGMD, we additionally adjusted for the potential effect of stress stimulus by adjusting for the velocity area under the curve (vAUC) as measured by Doppler during FMD and NGMD assessments, as described previously (13). Effect estimates (β) of the association between age and vascular function were presented per 10 years of advancing age.

Second, the potential interaction effect of age and history of PE was investigated by adding an interaction term between age and history of PE to the linear regression models described above. If the interaction term was not statistically significant (i.e. $p > 0.10$), the interaction was omitted from the model and we only evaluated the effect estimate of PE, while adjusting for age.

Finally, sensitivity analyses were performed to evaluate the association of postpartum interval instead of age and the robustness of our findings following the parameters we used to operationalize FMD and NGMD. We repeated the above analyses 1) by replacing age by postpartum interval, 2) by replacing leading baseline by baseline measured at a single point before response, 3) by replacing relative FMD by normalized relative FMD for stress stimulus (i.e., FMD%/stimulus instead of separate adjustment).

Statistical analyses were performed using the statistical software program IBM SPSS (version 24.0). *P*-values of main effects <0.05 and *p*-values of interactions <0.10 were considered statistically significant.

Results

Study population

Of the 1,465 participating women, 248 women were excluded from analysis due to (1) missing-, incomplete or low-quality FMD and NGMD measurements or (2) no or uncertain fasting state before the vascular evaluation (Figure 1). Baseline characteristics of in- and excluded women were comparable except for a higher BMI among those excluded (0.6 kg/m^2 higher, $p = 0.018$, Supplementary Table 1).

Of the 1,217 eligible participants, 803 (66.0%) had a history of PE and 414 (34.0%) women had a normotensive pregnancy. Baseline characteristics of the total study population and groups are presented in Table 1.

The age of the study population ranged between 22 and 62 years. Women with a history of PE were on average 6 years younger, less often in postmenopausal state, and at a shorter postpartum interval compared to women with normotensive pregnancy. Moreover, BMI, fasting glucose levels, SBP and MAP were higher and DBP level was lower in women with a history of PE.

Prevalence of hypertension (14.2 vs. 7.0%, $p < 0.001$), antihypertensive drug use (11.2 vs. 5.6%, $p = 0.001$), positive CVD family history (61.9 vs. 55.7%, $p = 0.041$) and smoking (5.7 vs. 9.4%, $p = 0.024$) were higher among women with a history of PE.

Baseline brachial artery diameter was lower in women with a history of PE than in those with normotensive pregnancies ($p \leq 0.008$). No statistically significant differences in FMD (absolute, relative and allometric), NGMD (absolute and relative) and physiologic response as proportion of maximal dilatory capacity were found between both groups (Table 1).

Association of age with FMD and NGMD

Across age deciles, a statistically significantly increasing trend was found for brachial artery diameter, whereas for all parameters of FMD and NGMD a decreasing trend was found (Supplementary Table 2). These trends were similar for women with and without a history of PE (Figures 2, 3, Table 2). The dilation in response to stress stimulus (FMD) as proportion of maximal dilatory capacity (NGMD) decreased with aging in former preeclamptic women, but did not in controls (Supplementary Table 2). Though this remained not statistically significant in fully-adjusted regression models, the trend of a decreased ability to maximally dilate among former PE remained (Table 3).

In line, unadjusted linear regression models revealed a statistically significant association of advancing age with brachial artery diameter (regression coefficient (95% CI) = 0.16 mm/10 years (0.13–0.19 mm/10 years), $p < 0.001$) and

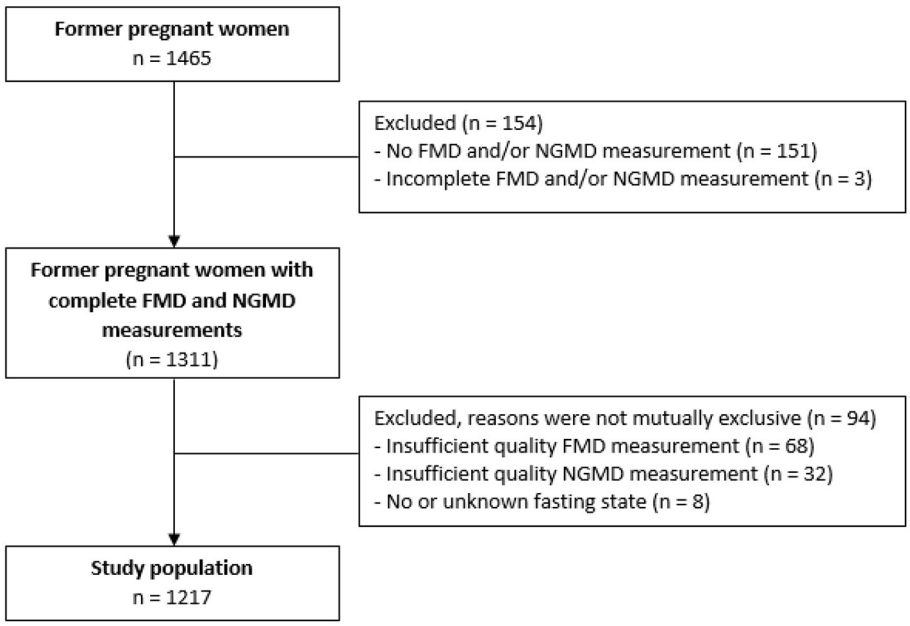

FIGURE 1
Flowchart of inclusion study population. FMD, flow-mediated-dilation; NGMD, nitroglycerine-mediated dilation.

all parameters of FMD (absolute: −0.001 mm/10 years (95 % CI −0.002-−0.002 mm/10 years), relative: −0.48 mm/10 years (95% CI−0.65-−0.30 mm/10 years), allometric: −0.49 mm/10 years (95 % CI −0.68-−0.31 mm/10 years); $p \leq 0.007$) and NGMD (absolute: −0.02 mm/10 years (95 % CI −0.03-−0.01), relative: −1.13 mm/10 years (95% CI −1.49-−0.77 mm/10 years); $p \leq 0.001$) (Supplementary Table 3). This result remained similar after adjustment for confounders ($p \leq 0.018$), with exception of the association of age with absolute FMD when adjusting for baseline diameter which became non-significant in multivariable analyses (regression coefficient = −0.01 mm/10 years (95% CI −0.01–0.003), p-value 0.060, Supplementary Table 3). If not adjusting for baseline, the effect of age on absolute FMD remained statistically significant in the multivariable analysis (regression coefficient = −0.01 mm/10 years (95 % CI −0.01-−0.001 mm/10 years), p-value 0.024).

No interaction effect between PE and age on FMD and NGMD

For baseline brachial artery diameter, the interaction term between age and history of PE was not statistically significant, both in unadjusted and fully-adjusted linear regression models (interaction term $p \geq 0.588$). Similar non-significant interactions were found for FMD (absolute, relative and allometric; p-values interaction ≥ 0.360), NGMD (absolute and relative; p-values interaction ≥ 0.443) and dilation as proportion of maximal dilatory capacity (≥ 0.224). These statistically non-significant interaction terms did not favor any stratification based on a history of PE.

Accordingly, we further investigated the independent effect of PE in all regression models. Following, for all measures of FMD and NGMD, no effect of a history of PE was found ($p \geq 0.170$) (Table 3). For brachial artery diameter, however, after adjustment for age and other confounders the effect of a history of PE on brachial artery diameter was −0.06 mm (95% CI −0.12-−0.10 mm, $p = 0.025$) as compared to history of normotensive pregnancy, indicating smaller brachial arteries in women with PE (Table 3). The results on the association with age remained similar in these models as compared with the regression analyses described above (Supplementary Table 3).

Sensitivity analyses

Sensitivity analyses on replacing age by postpartum interval did not provide different findings (data not shown), with exception of the confounder-adjusted association between postpartum interval and absolute NGMD which became non-significant (data not shown) in contrast to the reported result for age and absolute NGMD (Supplementary Table 3). Although the association between years postpartum and dilation as proportion of maximal dilation was not significant in univariable analyses, is was significant in multivariable analyses as in line with the analyses on age. Re-analyzing the data by replacing parameters

TABLE 1 Baseline characteristics of entire study population and stratified for history of preeclampsia.

	Total study population ($n = 1217$)	Women with history of PE ($n = 803$)	Women without history of PE ($n = 414$)	*p*-value
Age (years)	40.5 ± 8.6	38.5 ± 7.9	44.5 ± 8.4	*<0.001*
Parity	2 [1–2]	2 [1–2]	2 [2–3]	*<0.001*
Early onset PE[a]	411 (33.8%)	411 (51.2%)	N.A.	N.A.
HELLP-syndrome	567 (46.6%)	567 (70.6%)	N.A.	N.A.
Months postpartum	104 [28–198]	71 [17–155]	180 [91–272]	*<0.001*
Postmenopausal[a]	176 (14.6%)	78 (9.8%)	98 (23.9%)	*<0.001*
BMI (kg/m^2)	25.3 ± 4.6	25.7 ± 4.8	24.8 ± 4.1	*0.003*
Current smoking	85 (7.0%)	46 (5.7%)	39 (9.4%)	*0.024*
Positive CVD family history[a]	723 (59.8%)	493 (61.9%)	230 (55.7%)	*0.041*
Diabetes Mellitus	13 (1.1%)	12 (1.5%)	1 (0.2%)	0.072
Hypertension	143 (11.8%)	114 (14.2%)	29 (7.0%)	*<0.001*
Antihypertensive drugs use	113 (9.3%)	90 (11.2%)	23 (5.6%)	*0.001*
Multivitamin use	331 (27.2%)	205 (25.5%)	126 (30.4%)	0.077
Glucose level (mmol/L)[a]	5.1 ± 0.8	5.2 ± 0.9	5.0 ± 0.5	*0.001*
Systolic BP (mmHg)[a]	113 [107–122]	115 [108–123]	111 [105–118]	*<0.001*
Diastolic BP (mmHg)[a]	71 [66–77]	73 [67–79]	69 [64–74]	*<0.001*
MAP (mmHg)[a]	87 [82–94]	89 [83–96]	84 [79–90]	*<0.001*
Baseline brachial artery diameter (mm)	3.53 ± 0.44	3.50 ± 0.43	3.60 ± 0.46	*<0.001*
Absolute FMD (mm)	0.14 [0.09–0.21]	0.14 [0.09–0.20]	0.14 [0.09–0.21]	0.846
Relative FMD (%)	3.9 [2.5–5.9]	3.9 [2.5–5.9]	3.9 [2.4–5.9]	0.514
Allometric FMD (%)	4.0 [2.5–6.0]	4.0 [2.5–6.0]	4.0 [2.4–6.1]	0.514
Absolute NGMD (mm)	0.59 ± 0.18	0.59 ± 0.18	0.59 ± 0.19	0.921
Relative NGMD (%)	17.0 ± 5.5	17.1 ± 5.5	16.8 ± 5.5	0.260
Dilation (FMD) in proportion of maximal dilation (NGMD) (%)[a]	24.3 [14.5–35.8]	23.8 [14.4–35.8]	24.5 [14.6–35.9]	0.962

[a]Variable consisted few missing values (<2.0%), valid percentages are presented. Continuous variables are reported as mean ± standard deviation in case of normal distribution, otherwise as median [IQR]. Categorical variables are reported as number (%). Statistically significant p-values are presented in cursive.
PE, preeclampsia; BMI, body mass index; CVD, cardiovascular disease; BP, blood pressure; MAP, mean arterial pressure; FMD, flow-mediated-dilation; NGMD, nitroglycerine-mediated dilation.

for FMD and NGMD, as described in our methods section (i.e., replacing leading baseline by single-point baseline, and replacing relative FMD and relative NGMD by normalized relative FMD for stress stimulus instead of separate adjustment for stress stimulus), did also not alter the results (data not shown).

Discussion

In this cross-sectional study we show that the age-related decline in brachial artery vasodilation and increase in diameter was independent of obstetric history, suggesting no additional (accelerating) effect of PE on the decline in endothelial function with aging. The age-related decline in both endothelium-dependent and –independent brachial artery vasodilation itself was significant even after adjusting for important confounding factors. PE itself was consistently associated with a smaller brachial artery diameter across ages, but did not affect observed FMD and NGMD.

No accelerated age-effect on FMD and NGMD in former preeclamptic women

In agreement with previous studies, we show an independent decline in FMD and NGMD and increase in arterial diameter with advancing age (3, 23, 24). This may provide a valuable explanation for the increasing CVD risk with aging, especially as traditional risk factors do not completely explain the impact of age on CVD.

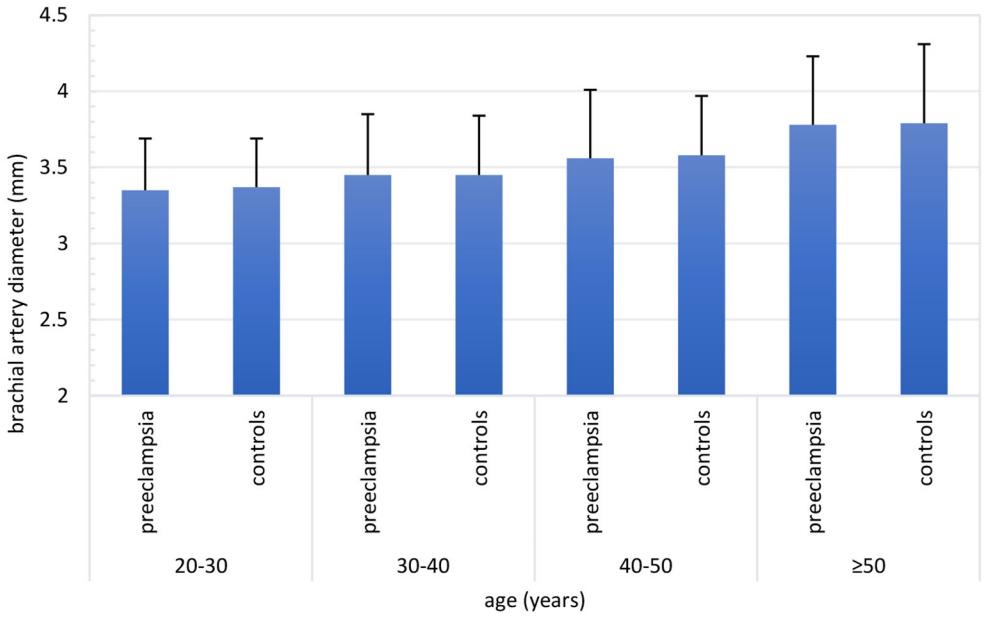

FIGURE 2
Brachial artery diameter (mm) across age categories stratified for women with a history of preeclampsia-complicated pregnancy (i.e. preeclampsia) and women with a history of normotensive pregnancy (i.e., controls). Both in women with and without a history of preeclampsia, brachial artery diameter increased with advancing age. Number of inclusions within groups: 20–30 years: preeclampsia $n = 103$, controls $n = 16$; 30–40 years: preeclampsia $n = 392$, controls $n = 111$; 40–50 years: preeclampsia $n = 238$, controls $n = 159$; ≥50 years: preeclampsia $n = 70$, controls $n = 128$.

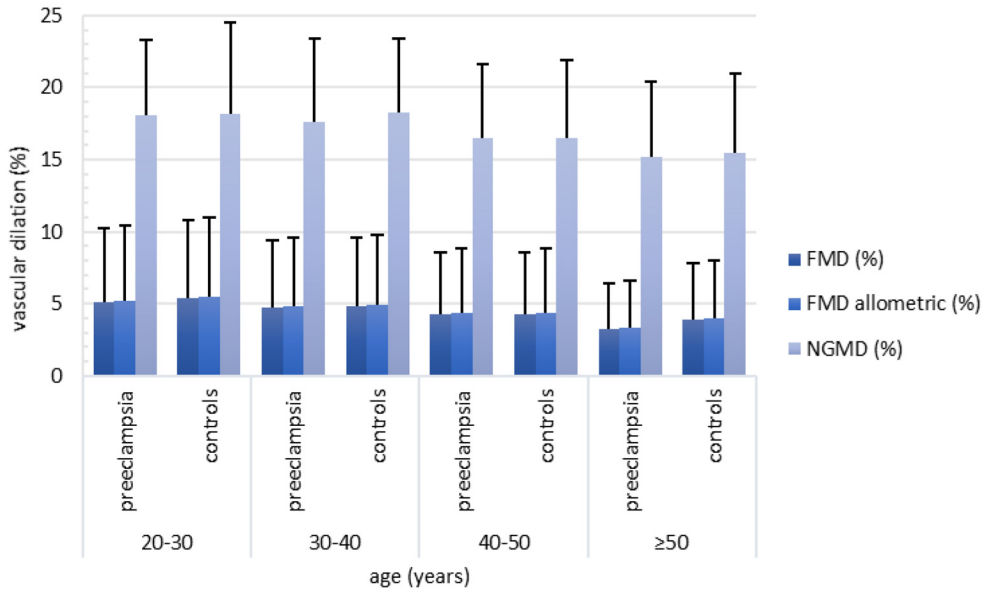

FIGURE 3
Relative FMD (%), allometric FMD (%) and NGMD (%) across age categories stratified for women with a history of preeclampsia-complicated pregnancy (i.e. preeclampsia) and women with a history of normotensive pregnancy (i.e. controls). Both in women with and without a history of preeclampsia, FMD (%), allometric FMD and NGMD (%) decreased with advancing age. Between women with preeclampsia and controls no clear differences were found with regard to FMD (%), allometric FMD (%) and NGMD (%). Number of inclusions within groups: 20–30 years: preeclampsia $n = 103$, controls $n = 16$; 30–40 years: preeclampsia $n = 392$, controls $n = 111$; 40–50 years: preeclampsia $n = 238$, controls $n = 159$, ≥50 years: preeclampsia $n = 70$, controls $n = 128$. FMD, flow-mediated-dilation; NGMD, nitroglycerine-mediated dilation.

TABLE 2 Trend effect of advancing age on FMD and NGMD.

Measurement	20–30 yr ($n = 119$)	30–40 yr ($n = 503$)	40–50 yr ($n = 397$)	≥50 yr ($n = 198$)	*p*-value of trend
Brachial artery diameter (mm)	3.36 ± 0.34	3.45 ± 0.40	3.57 ± 0.43	3.79 ± 0.50	*<0.001*
FMD					
Absolute FMD (mm)	0.16 [0.11–0.22]	0.15 [0.09–0.21]	0.13 [0.09–0.20]	0.12 [0.07–0.18]	*<0.001*
Relative FMD (%)	4.7 [3.3–6.7]	4.4 [2.6–6.4]	3.6 [2.4–5.7]	3.1 [2.0–5.1]	*<0.001*
Allometric FMD (%)	4.8 [3.3–6.9]	4.5 [2.6–6.6]	3.7 [2.4–5.8]	3.1 [2.0–5.2]	*<0.001*
NGMD					
Absolute NGMD (mm)	0.60 ± 0.16	0.61 ± 0.18	0.58 ± 0.19	0.58 ± 0.20	*0.035*
Relative NGMD (%)	18.1 ± 5.4	17.8 ± 5.6	16.5 ± 5.2	15.4 ± 5.4	*<0.001*
Dilation (FMD) in proportion of maximal dilation (NGMD) (%)	27.7 [19.9–36.6]	24.4 [15.1–36.4]	23.9 [14.3–35.3]	20.5 [12.4–34.1]	*0.007*

Continuous variables are reported as mean ± standard deviation in case of normal distribution, otherwise as mean [IQR]. Categorical variables are reported as number (%). Statistically significant p-values are presented in cursive.
yrs, years; FMD, flow-mediated-dilation; NGMD, nitroglycerine-mediated dilation.

TABLE 3 Association of age and history of preeclampsia with brachial artery diameter, FMD and NGMD.

	Brachial artery diameter (mm)		Absolute FMD (mm)		Relative FMD (%)	
	β (95% CI), mm/10 yr	*p*-value	β (95% CI), mm/10 yr	*p*-value	β (95% CI), %/10 yr	*p*-value
Unadjusted model						
Age (in deciles)	0.16 (0.13–0.18)	*<0.001*	−0.01 (−0.02–−0.003)	*0.003*	−0.50 (−0.69–−0.32)	*<0.001*
History of PE	−0.01 (−0.07–0.04)	0.602	−0.008 (−0.02–0.004)	0.183	−0.15 (−0.49–0.19)	0.381
Fully-adjusted model						
Age (in deciles)	0.14 (0.10–0.17)	*<0.001*	−0.01 (−0.01–0.003)	0.060	−0.42 (−0.63–−0.21)	*<0.001*
History of PE	−0.06 (−0.12–−0.1)	*0.025*	−0.008 (−0.02–0.003)	0.155	−0.09 (−0.43–0.25)	0.598
	Allometric FMD (%)		**Absolute NGMD (mm)**		**Relative NGMD (%)**	
	β (95% CI), %/10 yr	*p*–value	β (95% CI), mm/10 yr	*p*–value	β (95% CI), %/10 yr	*p*–value
Unadjusted model						
Age (in deciles)	−0.52 (−0.72–−0.33)	*<0.001*	−0.02 (−0.04–−0.01)	*0.001*	−1.20 (−1.58–−0.81)	*<0.001*
History of PE	−0.16 (−0.51–0.20)	0.384	−0.01 (−0.04–0.01)	0.282	−0.36 (−1.05–0.34)	0.312
Fully-adjusted model						
Age (in deciles)	−0.44 (−0.66–−0.22)	*<0.001*	−0.02 (−0.03–−0.002)	*0.024*	−1.02 (−1.48–−0.56)	*<0.001*
History of PE	−0.09 (−0.44–0.26)	0.602	−0.001 (−0.03–0.02	0.951	0.09 (−0.63–0.81)	0.806
	Dilation (FMD) as proportion of maximal dilation (NGMD) (%)					
	β (95% CI), %/10 yr	*p*-value				
Unadjusted model						
Age (in deciles)	−1.47 (−2.67to −0.27)	*0.016*				
History of PE	−0.85 (−3.01 to 1.31)	0.441				
Fully-adjusted model						
Age (in deciles)	1.22 (−2.54 to 0.10)	0.071				
History of PE	−0.87 (−2.97 to 1.24)	0.417				

Fully-adjusted models adjusted for BMI, smoking, anti-hypertensive drug use, MAP, fasting glucose levels, menopausal state and family history of CVD. Regression on FMD and NGMD was additionally adjusted for stress stimulus and regression on absolute FMD and absolute FMD for baseline diameter. Statistically significant p-values are presented in cursive.
β, unstandardized regression coefficient; FMD, flow-mediated-dilation; NGMD, nitroglycerine-mediated dilation; 95% CI, 95% confidence interval.

We also showed that women with a history of PE have smaller brachial artery diameters at baseline, but showed similar age-related diameter and FMD/NGMD changes compared to women with a history of normotensive pregnancies. Physiologically, our study shows that the known PE-linked endothelial dysfunction does not relate to an accelerated endothelial decline with advancing age in former preeclamptic women. Clinically, the future cardiovascular risk after PE seems less likely attributable to an accelerated age-related decline in FMD or NGMD. The absence of an effect of NGMD in addition to the observed difference in diameter suggests smaller arteries in former preeclamptic women, although one would then expect to find differences in relative changes, which is not the case in our study.

Earlier studies found that FMD was diminished in preeclamptic women, even several weeks before diagnosis (6, 25). Also, in the first decade after PE, some studies demonstrated diminished FMD, whilst others did not find any difference in later time periods when compared to control groups (6, 26–30). A meta-analysis of Weissgerber et al. (6) demonstrated a decreased FMD only within the first 3 years after PE, after which no difference in FMD was found up to 10 years postpartum. Our study did not find any difference across all age groups. Severity and/or time of onset of PE may contribute to these conflicting results. Besides, endothelial (dys)function has many dimensions, of which FMD and NGMD are only two. Other endothelial functions might still be altered in former PE women (both independent as in interaction with age), for example, circulating markers that might represent early endothelial dysfunction, including soluble fms-like tyrosine kinase (sFlt-1) and high-sensitivity C-reactive protein, already are elevated after PE up and until 10 years postpartum (27, 31).

Arterial aging in women

With advancing age, arteries demonstrate a systemic, gradual impairment in vascular endothelial function, which is likely due to functional, downregulation of vasodilator pathways (i.e., reduction in endothelial-derived nitric oxide (NO) bioavailability) and/or up-regulation of vasoconstrictor pathways (i.e., increased production of vasoconstrictors like endothelin-1) and structural vessel wall characteristics, amongst diameter and composition (3). The first functional change might specifically affect endothelium-dependent function (FMD), whereas the latter affects endothelium-independent function (NGMD). A lifetime exposure to (CV) risk factors and the susceptibility of individuals to the harmful consequences of these risk factors combined with aging itself may result in decreased arterial function (32). However, as aging and underlying progressive risk factors for disease are interrelated, it is a challenge to separate the so-called biological aging from aging-associated diseases.

The increase in brachial artery diameter over time may, at least partly, reflect a structural basis for an age-related reduction in dilatory capacity. With aging, smooth muscle cells undergo changes that may impact the vascular dilatory capability, such as changes in phenotype and senescence (33–35). As a result of these changes, elastic vessel properties are also altered, shifting the balance from elastin toward collagen. Consequently, arterial mechanical load due to blood pressure is more borne by the stiffer collagen in the arterial wall (3), at the expense of the relative dilatory capacity in response to endogenous or exogenous NO. The magnitude of (NO-mediated) vascular dilation also depends on the ability of the smooth muscle cells to relax which can be quantified by the maximum dilatory response following sublingual NG (3). Therefore, we interpret the age-related decline in FMD and NGMD as reflective of an altered smooth muscle phenotype, either by loss of bioavailability of, or sensitivity to NO, increase vasoconstriction activity, stiffer acting extra-cellular matrix and/or an already stretched vessel wall due to luminal enlargement (34, 36, 37). Unexpectedly, we did not find a fully-adjusted association between age and absolute FMD. Adjusting the absolute FMD by baseline diameter might, however, average out the age-related decline in dilation due to the age-related increase in baseline diameter or suggest that the age-related decline in function is mainly accountable to the age-related increase in brachial artery diameter.

With advancing age the most consistent structural changes include diameter enlargement (i.e., dilation), wall thickening (i.e., remodeling) and changes in wall content (e.g., loss of elastin), with related changes in elastic properties (3). We observed that the baseline diameter increases with advancing age, even after correcting for influencing factors, is in line with the age-related diameter enlargement described previously (23, 24). Interestingly, this increase in baseline diameter itself is independently associated with an increased risk of CVD (14, 38). Since the brachial artery is hardly prone to atherosclerosis, the perceived increase in baseline may more likely be related to age-related structural remodeling of the vessel wall rather than plaque formation. A history of PE was related to a smaller brachial artery diameter compared to normotensive gestation after correcting for confounders. This suggests a so far unknown vascular predisposition after hypertensive-pregnancy complications.

Strengths and limitations

Several strengths and limitations merit attention in the interpretation of our results. Strengths of this study support internal validity of our findings and include (1) consideration of a longer age-interval than currently published studies and (2) our large sample size powering our study to detect even small effects. In addition, multiple operationalisations of FMD (absolute, relative, allometric) and NGMD (absolute, relative) were included in multivariable analyses and sensitivity analyses, which showed all similar results. This supported the robustness of our findings and further decreased information bias. The most commonly defined measure for FMD and NGMD is the percentage increase in diameter with respect to baseline. Some investigators argue that it is the absolute dilatory response that captures the endothelium-dependent dilatory capacity best, and hence, when using relative FMD, smaller vessels intrinsically show greater FMD (39). There are assumptions that correcting for this vessel diameter dependency by allometric scaling is the best operationalisation of FMD, though it did not affect our results. We found that allometrically-scaled FMD yielded similar results compared to FMD percentage increase, suggesting that differences in baseline vessel diameter did not fully explain age-related decline in FMD in our study population. Furthermore, the scaling exponent of 1.014 justified the use of FMD percentage increase in current study cohort.

Limitations of this study include the cross-sectional design which made it impossible for us to investigate causal pathways. This may have obscured an effect of PE on FMD and NGMD that might had been revealed with repeated measurements within individuals before and after pregnancy. Second, a decline in vascular function due to PE may only be apparent in a subgroup of women with a specific (CV) predisposition, which we, unfortunately could not distinguish in our study. Third, selection bias might have occurred in our control group, which might mitigated the observed effects. Women who perceived higher risk of CVD might have been more willing to participate in a CV study, in which personal advice on risk factors was given to participants. For example, the prevalence of a positive family history for CVD was higher within our study population than expected based on the general population, in which the prevalence ranges between 10 and 16% being depending on one's age (40). Finally, FMD and NGMD measurements are considered susceptible for methodological variability. However, in the hands of experienced sonographers and following a strict protocol, variations can be kept to a minimum (12, 41). Moreover, we used wall tracking software to improve reproducibility and data on the obstetric history was not available for the sonographer.

Conclusion

This study shows that in young- to middle-aged women, vascular aging with respect to endothelium-dependent and -independent vessel dilation testing was similar in women after PE compared to women with a history of normotensive pregnancies, even though former preeclamptic women consistently have smaller brachial arteries. These findings suggest that the increased CV risk in the first decades after PE do not originate from an accelerated decline in endothelial function as measured by FMD or NGMD in conduit vessels. Different site (microvascular) and mode of endothelial action (hemostatic and inflammatory related integrity) might be involved, which remain subject for further investigation.

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 Medical Ethics Committee of the Maastricht University Medical Centre (METC azM/UM). The patients/participants provided their written informed consent to participate in this study.

Author contributions

EJ, VL, MS, and CG-D designed the content of current study. EJ performed the statistical analyses. EJ, MH, and VL wrote the manuscript. All authors critically reviewed the manuscript and approved the final version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.911603/full#supplementary-material

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Early-onset preeclampsia is characterised by an increased vascular tone in internal jugular veins

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Purpose: This study aimed to investigate Doppler characteristics of maternal internal jugular veins in uncomplicated pregnancies vs. those affected by hypertensive disorders.

Materials and methods: Venous pulse transit time and venous impedance index were measured at three different locations (right proximal, right distal, left proximal) of internal jugular veins according to a standardised combined Doppler-Electrocardiogram protocol in five different groups of pregnant women: uncomplicated pregnancy, early-onset preeclampsia, late-onset preeclampsia, gestational hypertension, and normotensive pregnancies with a small for gestational age foetus. Values of both parameters of the latter four groups were plotted against the reference range of uncomplicated pregnancies at corresponding gestation. Linear mixed models with random intercept were used to investigate gestational changes in venous pulse transit time and venous impedance index at the three internal jugular vein locations within and between the different groups.

Results: A total of 127 women were included: 41 had uncomplicated pregnancies, 13 had early-onset preeclampsia, 25 had late-onset preeclampsia, 22 had gestational hypertension, and 26 had normotensive pregnancies with a small for gestational age foetus. Venous pulse transit time values were lower than uncomplicated pregnancy ($p \leq 0.001$) at all three locations in the third trimester of early-onset preeclampsia.

Conclusion: Contrary to late-onset preeclampsia and gestational hypertension, early-onset preeclampsia is characterised by a lower venous pulse transit time at internal jugular veins compared to uncomplicated pregnancy, suggesting increased venous vascular tone.

KEYWORDS

combined Doppler-Electrocardiogram, venous pulse transit time, venous impedance index, preeclampsia, gestational hypertension, venous maternal haemodynamics, eclampsia, internal jugular vein

Introduction

Gestational hypertensive disorders occur in 5–8% of all pregnancies, accounting for both possibly serious maternal and foetal/neonatal morbidity and mortality (1). This obstetric disorder is characterised by cardiovascular maladaptation, not only at the heart and the arterial vascular tree but also in the venous compartment, as was reported for the first time by Bateman et al. (2).

A combined Doppler-Electrocardiogram (CDE) assessment provides a safe, cheap, easily accessible, and non-invasive tool to explore maternal venous haemodynamics (3, 4). Venous pulse waves indirectly reflect right cardiac atrial function and can be detected at supra- and infracardiac levels (5). The atrial contraction (ECG P-top) is responsible for a retrograde rebounce of blood into the venous tree (6) which is reflected by the A-deflection in the pulse waveform (7). The electro-mechanic delay between the ECG P-top and the corresponding Doppler A-deflection can be defined as the P–A interval. This heart rate-dependent interval can be corrected by using the P–A interval/R–R interval ratio also referred to as the venous pulse transit time (VPT). VPT is considered the venous equivalent of the arterial pulse transit time and is inversely related to vascular wall stiffness. In conditions of increased vascular tone or wall stiffness, the propulsion wave is transported faster through the circulation than in conditions of low vascular tone, being responsible for a decrease in VPT (8–10).

The venous impedance index (VI), calculated by X-A/X, is the Doppler equivalent of the arterial pulsatility index (11), representing the intracycle variation of blood flow velocities (12) and can be considered a proxy for distensibility and/or compliance. A large value indicates a strong intravenous rebound of atrial contraction, which counteracts venous drainage from the organs and a decrease of VI is consistent with an increase in compliance (13).

Previous studies have shown that VPT increases and VI decreases (flattening of the pulse waveform) with gestational age at interlobar renal, hepatic and internal jugular veins (IJVs) (3, 14). Uncomplicated pregnancy (UP) is thus characterised by a gradual reduction in vascular tone and an increase in vascular compliance when pregnancy advances. On top of that, Gyselaers et al. have described that short VPT and high VI at hepatic and renal interlobar veins are characteristic features of preeclampsia (PE) (15, 16), presenting more pronounced in early than in late PE (17), but are not observed in GH (18).

Abbreviations: BMI, body mass index; CDE, Combined Doppler-Electrocardiogram; EPE, early-onset preeclampsia; GH, gestational hypertension; IJV, internal jugular vein; LPE, late-onset preeclampsia; PE, preeclampsia; SGA, normotensive pregnancy with small for gestational age baby; VI, venous impedance index; VPT, venous pulse transit time.

PE can include cardiovascular changes, hematologic abnormalities, hepatic and renal impairment, and neurologic manifestations (19). However, >50% of (pre) eclampsia-related maternal deaths are attributed to cerebrovascular events and are thereby by far the most important direct cause of death (20).

Within the complex system of cerebral haemodynamics, the venous heart-brain axis has become the focus of non-obstetric studies in the last few years, not only in confirming its role in cerebrovascular haemodynamics but also in investigating its hypothesised involvement in the pathophysiology of neurovascular and -degenerative disorders (21, 22).

The goal of this study was to evaluate whether VPT and VI at IJVs are different in pregnant women with hypertensive disease relative to the findings in UPs.

Materials and methods

Ethics statement

Ethical approval by the Local Ethics Committee was given before study onset (study reference 2011–12 and 2016–41) and written informed consent was obtained from all participants. All procedures were in accordance with institutional guidelines and adherent to the principles of the Declaration of Helsinki.

Participants

A total of five groups of women with singleton pregnancies were included: (1) women with UP and birth of a normal or large gestational age baby according to gender and parity specific customised local reference charts. For inclusion, women with singleton pregnancies presenting in the first trimester at the outpatient antenatal clinic of the Sint Lucas Hospital in Ghent were invited to participate in this study. These women were evaluated prospectively at eight consequent moments during their pregnancy (12-16-20-24-28-32-36 and 38 weeks). (2) For "complicated" pregnancies, women were invited to participate in diagnosis at the outpatient antenatal clinic of the Sint Lucas Hospital in Ghent. These women with a "complicated" pregnancy were categorised into 4 groups: early-onset preeclampsia (EPE), late-onset preeclampsia (LPE), GH, and normotensive pregnancies with small for gestational age baby (SGA). For SGA, women with singleton pregnancies presenting in the outpatient antenatal clinic of the Sint Lucas Hospital in Ghent were invited to participate when the sonographically estimated foetal weight during the course of pregnancy was below the 10th percentile on the foetal growth chart, and when birth weight <P10 was confirmed after birth. Whenever possible, consecutive measurements with an interval ≥ 1 day were performed on the same woman in this group of "complicated" pregnancies. Both for the UP group and the

group of "complicated" pregnancies, women with pre-existing maternal or gestational diseases (e.g., with pre-existing cardiac, renal, liver, hematologic auto-immune diseases or a history of migraine or thyroid, neck, or brain surgery) were excluded.

At birth, data on gestation outcomes were categorised according to the criteria by the International Society for Studies of Hypertension in Pregnancy. PE was defined as new-onset hypertension with proteinuria $\geq$ 300 mg/24 h, other organ dysfunction, or foetal growth restriction, and is labelled as early-onset at clinical presentation $<$ 34 weeks (EPE) and late-onset at presentation $\geq$ 34 weeks (LPE). Next to proteinuria $\geq$ 300 mg/24 h, a urine protein-to-creatinine ratio (mg protein/mg creatinine) higher than 0.3 was also considered to be diagnostic for significant proteinuria. GH was defined as new-onset hypertension without proteinuria, other organ dysfunction, or foetal growth restriction. Women who gave birth to a neonate with birth weight $<$10th percentile according to gender and parity-specific customised local reference charts after a normotensive pregnancy were classified as SGA (23). Line Customised birth weight charts were established from a cohort of 34,684 neonates, born as singletons without congenital anomalies in Sint Lucas Hospital Ghent between 2000 and 2013. Charts were categorised into four groups: primiparous baby girl, primiparous baby boy, multiparous baby girl, and multiparous baby boy. Birth weights were classified per week of gestation, and birth weight percentiles were calculated with an interval of 2.5% between P2.5 and P97.5. According to these population-specific data, the weight at birth of each neonate in the study was expressed as a customised birth weight percentile.

Next to gestation outcome, the following maternal data were collected: age, parity, smoking, pregestational body mass index (BMI) as per medical history, weight gain, and medication (non-cardiovascular, antihypertensive).

Combined Doppler-Electrocardiogram assessment of internal jugular veins

In agreement with the previous study, CDEs of IJVs were performed at three different locations (right proximal, right distal and left proximal according to the previously described standardised protocol with acceptable reproducibility and repeatability (equipment: Toshiba Nemio XG; 6- to 11-MHz linear-array transducer) (24). To summarise, the participant was examined in the supine position and was asked to hold their head stable in a neutral position by facing a focus point at the ceiling during the entire examination. The impact of Valsalva manoeuvres and breathing, thorax wall, and diaphragm movements on blood flow was demonstrated and the importance of breath holding was explained. As soon as the triphasic waveform of the IJV was detected by pulsed Doppler imaging, the participant was instructed to hold her breath at the end of a normal expiration.

In all women, four consecutive measurements were performed at the three IJV locations by the principal investigator (ID). Previous research has shown that averaging four replicates stabilised the CDE IJV measurements and bootstrap analysis suggested that increasing the number of replicates beyond four will not elicit further improvements in the stability of the averaged values (24).

The ultrasound images, with encrypted participant details, were transferred from a portable device to the hard disk of a computer for offline assessment. Irfan View (Version 64 4.41, @ 1996–2016 by Irfan Skiljan, Jajce, Central Bosnia Canton, Bosnia) was used to mark and measure the flow velocities at the Doppler A- and X-deflection, the P-A interval, and R-R interval. VPT and VI were calculated as P–A interval/R–R interval ratio and (X-A)/X for each location at each session.

Statistical analysis

Demographic data are presented as median (IQR) or N (%), and the difference between the five groups was assessed using Kruskal-Wallis's test for continuous variables and Fisher's exact test for categorical variables.

Linear mixed models with random intercepts to account for variation between women and to correct for dependence between different measurements from the same woman were used to investigate gestational changes of VPT and VI at the three IJV locations within and between the different groups. Comparisons between groups (EPE vs. UP, LPE vs. UP, GH vs. UP, and SGA vs. UP) were performed at corresponding UP gestational age. For both outcomes, it was investigated whether there was a linear, quadratic, or cubic evolution during the pregnancy and whether this evolution was different for the two groups. Model selection was done using backward elimination, i.e., non-significant terms were removed from the model in a stepwise manner. All analyses were performed using SAS® version 9.4 (SAS Institute, Cary NC).

Results

Participants

A total of 54 women were assessed for the group UP of which 13 women were excluded. Among the participants, two women developed gestational diabetes and seven women were diagnosed with hypertensive disease during the course of their pregnancy (2 EPE, 2 LPE, and 3 GH). Four women unexpectedly gave birth to an SGA neonate, leaving 41 participants eligible for UP inclusion. For UP, a total of 22/328 (41 participants x 8 moments in pregnancy) assessments were not performed.

13/22 assessments (6 at 12 weeks, 2 at 16, 20, and 24 weeks, and 1 at 36 weeks) were not performed due to interruption of the procedure due to a medical emergency in another patient or practical patient or investigator organisation. 9/22 women delivered between 36 and 38 weeks, leaving 306/328 eligible for inclusion. A total of 86 women with a "complicated" pregnancy were included. Whenever possible, consecutive measurements within the same woman were performed in this latter group: EPE: 29 measurements in 13 women; LPE: 44 measurements in 25 women; GH: 38 measurements in 22 women; SGA: 84 measurements in 26 women. Antihypertensive treatment had already been initiated before the CDE IJV assessment in 19/29 (66%), 18/44 (41%), and 18/38 (47%) participants for EPE, LPE, and GH respectively.

Maternal data and data on gestation outcomes are presented in Table 1. Pregestational BMI was higher in GH, and there were more smokers in SGA than in UP. Women in EPE, LPE, and GH delivered earlier, and the birth weight was significantly lower in EPE, LPE, and SGA than in UP. In LPE, fewer male infants were born compared to UP.

Statistical analysis

For VPT, an increasing trend with conversion to a decreasing trend at approximately 35 weeks was observed during UP at all three IJV locations. Only in EPE, were VPT values significantly lower than UP at all IJV locations ($p \leq 0.001$). EPE showed a linear increasing trend at right proximal and right distal IJV from 31 to 34 weeks of gestation (Figure 1A), no significant trend was found at left proximal IJV. With exception of GH at the right proximal IJV, the VPT trends of LPE, GH, and SGA were similar to UP at the corresponding gestational age (Figures 1B–D). In GH at the right proximal IJV, VPT showed a decreasing trend with conversion to an increasing trend at around 33–34 weeks of gestation.

For VI, during UP there was an increasing trend with conversion to a decreasing trend at ∼22–23 weeks at all three IJV locations. No difference was found between the gestational evolution of VI in EPE, LPE, GH, and SGA compared to UP, with exception of GH at right proximal IJV and SGA at right distal IJV. In GH at the right proximal IJV, an increasing trend with conversion to a decreasing trend at a gestational age of around 33–34 weeks was observed. In SGA at right distal IJV, a decreasing trend with conversion to an increasing trend was observed at a gestational age of around 33 weeks. Absolute values of VI were not different between EPE vs. UP, LPE vs UP, GH vs. UP, and SGA vs. UP, with exception of SGA at left proximal IJV where they were significantly lower ($p = 0.009$).

Discussion

In this paper, characteristics of the jugular venous pulse waveform obtained by CDE in pregnant women with hypertensive disorders are described. To the best of our knowledge, this study is the first to compare Doppler characteristics at IJVs in pregnant women with different clinical types of hypertensive disease relative to the findings in UPs.

TABLE 1 Maternal data and data of gestation outcome among women with UP, EPE, LPE, GH, and SGA.

	UP	EPE	LPE	GH	SGA	*p*-value
n (women)	41	13	25	22	26	
n (assessments)	306	29	44	38	84	
Maternal age, years	30(28–32)	31(27–32)	28(27–31)	31(28–34)	30(26–32)	.488
Nulliparity, *n* (% women)	22(54)	11(85)	19(76)	16(73)	15(57)	0.146
Cigarette smoker, *N* (% women)	0(0)	0(0)	2(8)	0(0)	8(31)*	<0.001
Pregestational BMI, kg/m^2	22(20.9–24.2)	22.8(20.8–26)	24.8(21.3–28.4)	25.7(23.2–28.8)*	21.8(19–24.3)	0.003
Maternal weight gain, kg	11(10–14)	12(9–18)	13(11–16)	13(10–16)	10(9–13)	0.093
Non-cardiovas-cular medication, *N* (% women)	3(7)	1(8)	0(0)	3(14)	1(4)	.353
Antihypertensive medication, *N* (% women)	0(0)	8(62)*	4(16)	9(41)*	0(0)	<.001
Male gender (%)	19(46)	4(30)	3(12)*	11(50)	10(38)	.029
gestational age at delivery, weeks	39.4 (39–40.3)	34 (33.1–35.3)*	37.5 (36,2–39.4)*	38.3 (37.1–39.5)*	38.5 (38–39.8)	<.001
birth weight, G	3,360 (3,100–3,545)	2,005 (1,510–2,400)*	2,600 (2,120–3,465)*	3,080 (2,549–3,373)	2,595 (2,429–2,858)*	<0.001
birth weight, percentile	35 (25–70)	18 (5–55)	13 (3–60)	38 (11–53)	5 (3–8)*	<0.001

Data are presented as n (%) or median (interquartile range). P-value gives the overall significance whereas an * indicates a significant difference compared to UP.

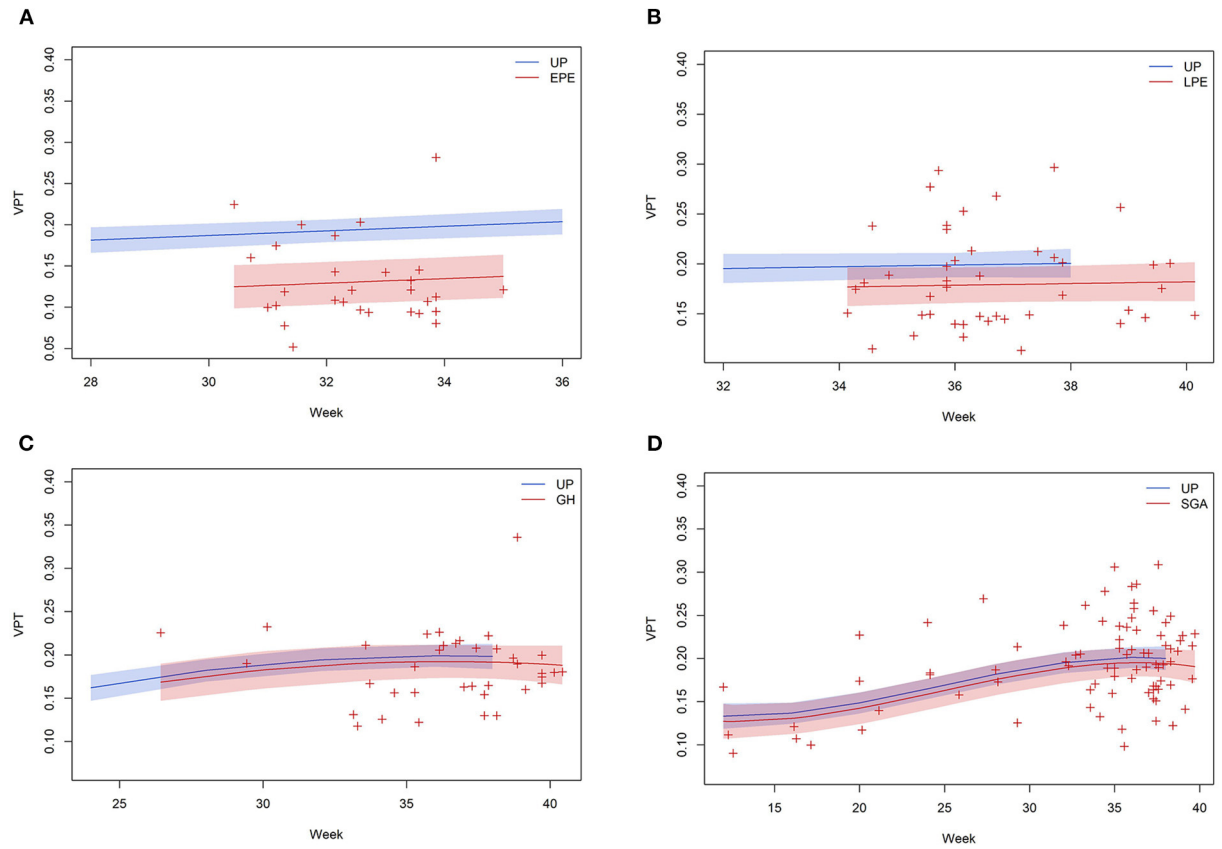

FIGURE 1
Evolution of VPT as measured at right distal IJV in EPE **(A)**, LPE **(B)**, GH **(C)**, and SGA **(D)** relative to gestational age and plotted against the reference values of 41 UPs.

Main findings

For CDE IJV assessment during pregnancy complicated by different clinical types of hypertensive disease, the key findings are:

(1) Only in EPE, absolute VPT values are significantly lower than in UP.
(2) In pregnancies complicated with different clinical types of hypertensive disease, VPT at supracardiac level shows similar patterns to in previous studies on VPT at the infracardiac level.
(3) VI assessments at the supracardiac level seem to be less informative than those at the infracardiac level.

Strengths and limitations

The strengths of this study are the use of a rigid standard protocol for CDE IJV assessment by only one investigator with known intra- and inter-observer correlation (24) and the use of customised population-specific birth weight charts. Thanks to the complete and directly available demographic data, the included women in each subgroup strictly complied with all reported criteria of the International Society for Studies of Hypertension in Pregnancy. Additionally, they were free of complicating maternal or gestational diseases at the time of inclusion.

This study has some limitations. Firstly, we acknowledge the relatively low number of included patients per group. Consequently, the statistical power of this study is <80% and the present findings need to be confirmed by larger prospective studies. Secondly, this technique demands a highly trained investigator and is subject to both patient- and investigator-dependent artefacts, as described previously (3, 24). Thirdly, previous research has shown that cardiovascular adaptations appear to be enhanced by a subsequent pregnancy and that parous women without a history of preeclampsia have a lower peripheral vascular resistance than nulliparous women (25, 26). In this study, the difference in the proportion of nulliparity between UP en EPE is only marginally not significant ($p = 0.057$) and may have been a confounding factor, especially

for VPT values. Larger prospective studies are recommended. Fourthly, this study does not allow drawing any conclusion on the possible impact of starting antihypertensive medication on the characteristics of the jugular pulse waveform. Ideally, the use of antihypertensive medication should be excluded, however, this would reduce the number of inclusions even further as antihypertensive treatment is the standard of care from the moment the blood pressure becomes elevated. Fifthly, the included women were not routinely screened for thrombophilia or auto-immune diseases. Exclusion of this study was only made on the women's medical history. Similarly, in this small observational study, interference from a variety of variables such as smoking, coffee use, and other health behavioural or cultural influences cannot be entirely excluded. For this, a large prospective study is needed. Finally, this study does not allow drawing conclusions about possible interfering variables within the complex functioning of cerebral outflow as reflected in the maternal jugular venous waveform.

Interpretation

In this study, VPT at IJVs in EPE is significantly lower than in UP. No difference in absolute values was found in LPE, GH, and SGA relative to UP. These observations are in line with previous studies at the infracardiac level. At the level of the kidneys, VPT is short already weeks before the clinical onset of EPE (27). In LPE, borderline reduced VPT has been reported, usually unilaterally and presenting only at the onset of disease (27). At the liver, a significantly short VPT in the clinical stage of EPE is observed (17). The same is true for LPE, but to a much lesser degree (27). GH presents without abnormalities of VPT at renal and hepatic levels (16).

Despite the detailed scanning protocol, VI assessments at IJVs show high variability and absence of significant trends at most of the three IJV locations. This is in contrast with previous studies where VI in liver and kidney were higher in PE compared to UP. Gyselaers et al. reported a higher VI in EPE than in LPE at the infracardiac level, and this higher value of VI was present weeks before the clinical symptoms (17), in contrast with LPE (27). Former studies of this research team at the infradiafragmatic level mainly focused on intraparenchymatous vessels in the liver and kidneys. This prevents external venous compression by the ultrasound probe. Due to anatomic reasons, this problem cannot be tackled at the jugular veins and is responsible for higher intra-and inter-observer variations above than below the diaphragm, larger standard deviations, and measurement ranges as well as more failed samplings (3, 24). This study adds to the reported dysfunction of the venous system in PE, but not in GH, at the supracardiac level. This is in agreement with previous studies at the infracardiac level.

This study does not allow us to draw any conclusions on the possible role of the venous heart-brain axis in the pathophysiology of cerebral complications due to gestational hypertensive disease. Cerebral circulation has an autoregulatory mechanism to maintain a steady cerebral blood flow. Severe hypertension can overwhelm this system and result in blood-brain barrier dysfunction (28) however the degree of hypertension may not always predict the risk of eclampsia (29). In addition to hypertension, recent studies suggest that the cerebrovenous system and cerebral outflow may be important factors in guaranteeing normal brain function. The cerebrovenous system is a complex three-dimensional structure that is often asymmetric and represents a much more variable pattern than the arterial anatomy. The connexion between the right atrium and vena cava is valveless, but dysfunction of IJV valves can cause an increase in the mean value for cerebral venous pressure (30, 31). Intracranial venous hypertension, according to Talbert, may trigger a chain of events involving endothelial dysfunction, increased permeability of the blood-brain barrier, and extravasation of colloids (32). Our results of enhanced venous pulse transit in early-onset preeclampsia are in line with a generalised state of endothelium activation at both the arterial and venous sites.

Conclusion

This study adds to the reported dysfunction of the venous system in PE at both supra- and infracardiac levels. Again, this association is lacking in GH. Short IJV pulse transit time in EPE is consistent with an increased venous vascular tone. This observation opens perspectives to target cerebral venous haemodynamics in the prevention and treatment of preeclampsia-related brain dysfunction.

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 Commissie voor medische ethiek, AZ Sint Lucas Gent, Belgium. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ID: data collection, interpretation results, and wrote the manuscript. CK and LB: statistical analysis of data, preparation figures, and helped to draft the manuscript. WG: design and coordination of the study, interpretation results, and helped to

draft the manuscript. 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.

Publisher's note

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Low-dose aspirin in the prevention of preeclampsia in twin pregnancies: A real-world study

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Background: The use of low-dose aspirin for women with twin pregnancies remains controversial. This study was to describe the frequency of preeclampsia and aspirin use in twin pregnancies in real practice.

Methods: This retrospective cohort study based on real-world data was conducted in the Obstetrics and Gynecology Hospital of Fudan University between 2013 and 2020. Women with twin pregnancies who received prenatal care before 20 weeks of gestational age were included. They were divided into those using low-dose aspirin (LDA group) and those not using aspirin group (N-LDA group). The primary outcome was the frequency of preeclampsia, and secondary outcomes included early-onset and preterm mild and severe preeclampsia.

Results: A total of 2,946 women had twin pregnancies, and 241 were excluded due to missing information. Of 2,705 eligible women, 291 (10.75%) were administered aspirin and the other 2,414 (89.25%) did not. The patients in the LDA group were significantly more likely to be older, have a higher rate of use of ART, have a previous history of hypertension, and have gestational diabetes ($p < 0.05$). In the LDA group, aspirin compliance ≥50% was relatively low (14.43%, 42/291). Preeclampsia occurred in 106 of 291 participants (36.43%) in the LDA group, as compared to 449 of 2,411 (18.62%) in the N-LDA group (OR: 2.15, 95% CI: 1.62–2.82; $p < 0.01$). The association was confirmed (OR: 1.74, 95% CI: 1.26–2.4; $p < 0.01$) in the 1:2 case-matched analysis. Higher odds of ratio in the LDA group were demonstrated (aORs > 1, $p < 0.01$), except for early-onset and preterm mild preeclampsia ($p > 0.05$). This association was confirmed in a subgroup analysis of methods of conception (aORs ≥ 1, $p > 0.05$).

Conclusion: Aspirin prescription of 75 to 100 mg in twin pregnancies was associated with no significant reduction of preeclampsia, which may be due to poor compliance with the aspirin used. Further randomized controlled or prospective cohort studies are required.

KEYWORDS

twin pregnancy, low dose aspirin, preeclampsia, prevention, China

1. Introduction

Twin gestation is associated with a 2–3 times increased risk of preeclampsia, and low-dose aspirin (LDA) prophylaxis is recommended for its prevention (1–4). Available randomized controlled or cohort studies on the effectiveness of LDA, such as ASPERE, ASPIRIN trial, and the APPEC study, were based on singleton pregnancies, where twins were excluded (5–8). Evidence from a well-designed clinical trial for evaluating the effectiveness of aspirin in the prevention of preeclampsia in twin pregnancies is required.

Studies about the effectiveness of LDA in twin pregnancy are indeterminate. Erkan et al. found that 75 mg aspirin daily did not lower the incidence of preeclampsia or gestational hypertension in twin pregnancy (9); however, 100 mg aspirin daily did reduce the risk in an observational study in China (10). Therefore, whether low-dose aspirin is effective for twin pregnancies in reducing the risk of preeclampsia remains controversial.

Herein, we have conducted a retrospective cohort study in China between 2013 and 2020, for the purpose of describing the frequency of PE and aspirin use in twin pregnancies in a real-world study setting. In this study, the largest of its kind ever conducted, we have compared the incidence of preeclampsia between women with twin pregnancies using and not using LDA. In addition, we have conducted a case-matched analysis and subgroup analysis to further explore the association.

2. Methods

2.1. Study design and participants

This was a retrospective cohort study of all women with twin pregnancies who received prenatal care before 20 weeks of gestational age in the Obstetrics and Gynecology Hospital of Fudan University in Shanghai, China, from 1 January 2013 to 31 December 2020. Ethical approval was obtained from the research ethics committee of Fudan University (FE21194).

The inclusion criteria for the study were the following: maternal age ≥ 18 years old, twin pregnancies, and first prenatal visit before 20 weeks of gestational age. The exclusion criteria were as follows: first prenatal visit after 20 weeks of gestational age, missing information on the number of fetuses, gestational age, or delivery week.

This cohort of women was divided into two groups: LDA group (women using aspirin) and N-LDA group (women not using aspirin). Indication for LDA was based on the traditional count of clinical risk factors in China (5): women with twin pregnancies are recommended for aspirin administration at 75–100 mg daily dose from 12 to 20 weeks of gestation until 36 weeks of gestation. According to the Chinese guideline, maternal age ≥40 years old, body mass index ≥28 kg/m^2, use of artificial reproductive technology, and pregnancy interval ≥10 years were considered medium risk factors; and prior history of preeclampsia/fetal growth restriction/placental abruption, renal disease, and hypercoagulable disease were considered high-risk factors. Women with ≥2 medium risk factors or ≥1 high-risk factor were recommended a daily dose of 75–100 mg low-dose aspirin to start between 12 and 20 weeks of gestation.

2.2. Variables

Gestational age was confirmed by the measurement of the fetal crown-rump length of the bigger twin in the first-trimester ultrasound scan. Data were extracted from the electronic medical record database. The exposure variable was the use of aspirin. This information was extracted from the electronic medical records about aspirin prescription at the prenatal visit or during hospitalization. Demographic data including maternal age, nulliparity, body mass index (BMI), chorionicity, methods of conception [natural conception and assistant reproductive technology (ART)], previous history of hypertensive disorders, gestational diabetes, delivery week, and delivery mode were recorded. Detailed electronic medical data sources and preprocessing are described in Supplemental Table 1.

2.3. Outcome measures

The primary outcome was the frequency of preeclampsia (PE). According to the bulletin of the American College of Obstetricians and Gynecologists' Committee 2019, (3) preeclampsia was diagnosed as the following criteria: (1) Blood pressure: systolic blood pressure >140 mmHg and/or the diastolic blood pressure should be >90 mmHg on at least two occasions 4 h apart developing after 20 weeks of gestation in previously normotensive women; or systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg; (2) proteinuria: >300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available, or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: thrombocytopenia [platelet count <100,000 × 10 (9)/L], renal insufficiency (serum creatinine concentrations > 1.1 mg/dL), impaired liver function (elevated liver transaminases to twice the normal concentration), pulmonary edema, new-onset headache unresponsive to medications, and not accounted for other diagnoses (1, 2).

Secondary outcomes included mild and severe preeclampsia. Severe preeclampsia was defined as any of the following: systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg, thrombocytopenia [platelet count <100,000 × 10 (9)/L], renal insufficiency (serum creatinine concentrations >1.1 mg/dL, or a doubling of the serum creatinine concentration in the absence of other renal diseases), impaired liver function (elevated liver transaminases to twice the normal concentration, or severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses), pulmonary edema, new-onset headache unresponsive to medications, and not accounted for by other diagnoses. Those patients who did not fit the criteria for the diagnosis of severe preeclampsia were classified into mild preeclampsia. Another secondary outcome was the gestational age (GA) at delivery integrated with the occurrence and type of PE as follows: PE delivery at <34 weeks, PE delivery at <37 weeks, mild PE delivery at <34 weeks, mild PE delivery at <37 weeks, severe PE delivery at <34 weeks, severe PE delivery at <37 weeks.

2.4. Statement about aspirin

The exposure variable in this study was the administration of low-dose aspirin. The daily low dose was 75–100 mg of aspirin to start between 12 and 36 weeks of gestation. Information about aspirin included whether the participant taking aspirin and adherence as well. Adherence to aspirin was evaluated by the actual prescribed dose/total dose required (from starting gestational week to 36

Abbreviations: OR, odds of ratio; BMI, body mass index; ART, assistant reproductive technology; CIs, confidence intervals; aORs, adjusted odd ratios; PSM, propensity score matching; PlGF, placental growth factor; sFlt-1, soluble FMS-like tyrosine kinase-1.

gestational weeks or delivery week) × 100%, and the daily intake is 75–100 mg.

2.5. Statistical analysis

Data are shown as means and standard deviation or numbers (percentages). Differences between the LDA group and N-LDA groups were analyzed using the chi-squared test for categorical variables. A binary logistic regression was employed to evaluate the association, and the results of primary and secondary outcomes were presented as odd ratios (ORs) or as mean differences with 95% of confidence intervals (CIs). The statistically significant variables at the baseline assessment or variables previously reported to be risk factors for preeclampsia were included, such as maternal age, nulliparity, BMI, gestational hypertension, gestational diabetes, gestational week, and ART. In the presence of any significant demographic confounders, adjusted odd ratios (aORs) were calculated after adjusting for the confounders. a p-value of <0.05 was considered statistically significant.

Considering the potential selection bias is likely to lead to a false increased risk of preeclampsia in the LDA group, propensity score matching (PSM) was utilized to reduce the possible bias. PSM was achieved using MatchIt (R package) (11); a conditional logistic regression model was used in 1:2 paired data (after PSM), while an unconditional logistic model was applied in the sensitivity analysis. The variables previously reported to be risk factors for preeclampsia were applied as matching factors, including maternal age, nulliparity, BMI, chorionic type, ART, and previous history of hypertension.

The outcome analysis was calculated using logistic regression, achieved by a generalized linear model (GLM, a R function), with the primary and secondary outcome as the dependent variable and the LDA intake state as the independent variables, and confounding factors that the above factors were matching factors. For the subgroup analysis, we reclassified the data using subgroup metrics (methods of conception) and then examined the association between LDA intake and preeclampsia in a similar way for each subcategory. Missing values in the data were filled with the mean and the plural, respectively, according to the proportion and type of missing values, and details are in the Supplementary material.

2.6. Role of the funding source

The funders played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

3. Results

3.1. Participants

A total of 2,946 women had twin pregnancies and 241 were excluded due to missing information on gestational age or delivery week (Supplemental Table 2). Among 2,705 eligible women, 291 (10.75%) took aspirin (LDA) and the other 2,414 (89.25%) N-LDA did not take aspirin (Figure 1). The patients in the LDA group were significantly more likely to be older, have a higher rate of use of ART, have a previous history of hypertension, and have gestational diabetes ($p < 0.05$). There were no significant differences in parity, BMI, delivery week, and delivery mode between the LDA group and the N-LDA group (Table 1).

In the matched group, BMI, chorionicity, and previous history of hypertension were of no difference between the LDA group and the matched N-LDA group ($p > 0.05$). The rate of ART was observed significantly higher ($p = 0.03$) and maternal age was slightly elder ($p = 0.03$) in the LDA group compared to that in the N-LDA group (Supplemental Table 3). Additionally, aspirin compliance was not satisfactory in the LDA group, since only 14.43% (42/291) of women had compliance of ≥50%, while 85.57% of other women had compliance of <50%.

3.2. Primary outcomes

Preeclampsia occurred in 106 of 291 participants (36.43%) in the LDA group, as compared to 449 of 2,411 (18.62%) in the N-LDA group (OR: 2.15, 95% CI for adjusted OR: 1.62 to 2.82; $p<0.01$) (Table 2). In the 1:2 case-matched analysis, the association was confirmed (OR: 1.74, 95% CI for adjusted OR: 1.26 to 2.40; $p < 0.01$).

3.3. Secondary outcomes

The association for secondary outcomes, quantified as the odds ratio in the LDA group with a 95% CI, is shown in Table 2. Higher odds of ratios in the LDA group were demonstrated, including early-onset preeclampsia (delivery at <34 gestational weeks) and preterm preeclampsia (delivery at <37 gestational weeks) (aORs > 1, $p < 0.01$), except that there was no significant difference in the odds of ratios for early-onset severe preeclampsia ($p = 0.48$) (Table 2).

3.4. Subgroup analysis

A subgroup analysis of methods of conception was conducted to further determine the effect of low-dose aspirin on preeclampsia prevention in twin pregnancy (Table 2). There was a higher risk of preeclampsia in the LDA group regardless of whether ART was used (aORs > 1, $p < 0.05$), together with no difference for mild or severe preeclampsia. In the case-matched analysis, higher odds of ratios were observed in the LDA group for preeclampsia of assistant conception (aORs > 1, $p < 0.05$) and mild preeclampsia of natural conception (OR: 1.99, 95% CI for adjusted OR: 1.20–3.40; $p = 0.02$).

4. Discussion

4.1. Main findings

In this retrospective cohort study, aspirin prescription of 75–100 mg in twin pregnancies was associated with no significant reduction of preeclampsia, including severe or mild preeclampsia. It may be due to poor compliance and/or insufficient dose of aspirin used for twin pregnancy. Thus, further randomized controlled or prospective cohort studies are required.

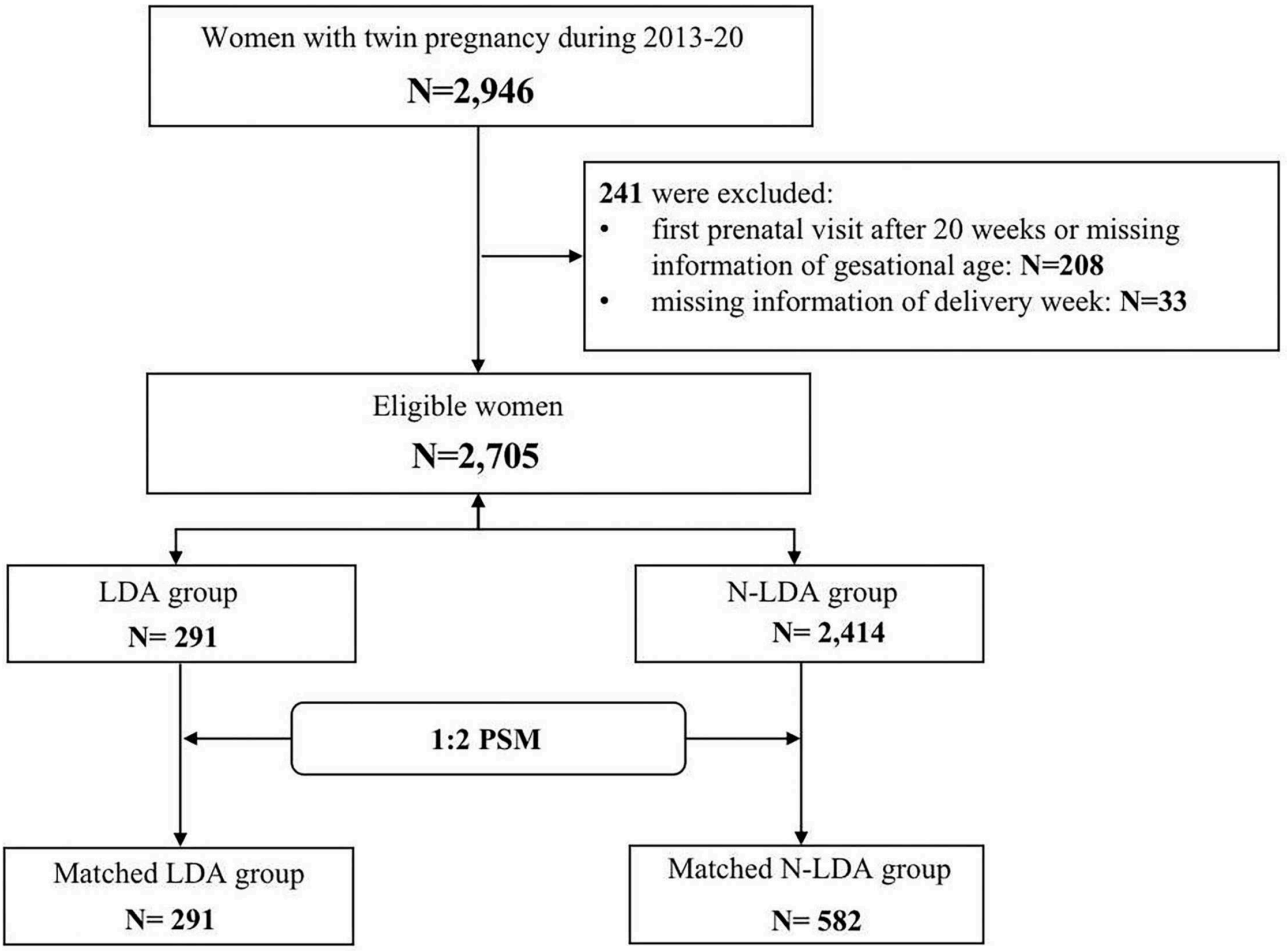

FIGURE 1
A flow diagram of the selection of participants.

4.2. Strengths

The main strength of this study is that it provides a real-world setting for describing the frequency of LDA and preeclampsia in twin pregnancy in a real-world setting and further verification by a 1:2 case-matched analysis and a subgroup analysis, which allowed the reduction of the bias as much as possible. Second, previous studies were limited by significant reporting bias (9, 12–14). In this study, adherence to aspirin was extracted from the medical records with quality control, which is expected to be more objective than that originating from self-reporting or questionnaires. Third, varieties of potential confounders, such as maternal age, BMI, parity, and previous history of hypertension, were extracted from the electronic medical database and were well-adjusted.

4.3. Limitations

This study had some limitations. First, considering that the patients in the LDA group had more risk factors for preeclampsia than the N-LDA group such as older maternal age, higher rate of use of ART, previous history of hypertension and diabetes, and whether the use of aspirin reduces the risk of preeclampsia in twin pregnancy cannot be inferred. A causal relationship could not infer from our retrospective cohort design, and further randomized controlled design is needed. Second, the aspirin compliance was not so satisfactory that 14.43% of women had compliance of ≥50% in the LDA group, and therefore, this insufficient dose of aspirin used for twin pregnancy potentially contributed to the ineffectiveness of aspirin administration for twin pregnancy in reducing the risk of preeclampsia. Whether a sufficient dose of aspirin is effective needs further assessment. Third, regardless of whether patients who initiated aspirin initiated it before 16 weeks of pregnancy is of importance (15–17), however, the initiation gestational week of aspirin was unavailable in our study. Instead, details about whether aspirin was prescribed and the total prescription dosage were extracted from the medical records for the analysis. Additionally, details regarding potential confounders such as placental growth factor (PlGF) and soluble FMS-like tyrosine kinase-1 (sFlt-1) were not available. Further studies are required to take biological markers such as PlGF and sFlt-1 into consideration.

4.4. Interpretation

Our findings indicated that LDA seemed to not lower the risk of preeclampsia in twin pregnancy. Many studies supported that LDA should be used for women at high risk of preeclampsia. Most

10.3389/fcvm.2022.964541

TABLE 1 Demographic characteristics of the participants.

	LDA group ($N = 291$)	N-LDA group ($N = 2,414$)	*P*-value
Maternal age, yr	32.94 ± 4.34	30.88 ± 4.35	<0.01
Primiparity, *N* (%)	255 (87.63)	2,038 (84.42)	0.18
BMI, kg/m^2	27.11 ± 3.82	27.00 ± 3.82	0.75
Chorionicity, *N* (%)		0.51	
Dichorionicity	208 (71.48)	1,669 (69.14)	
Monochorionicity	49 (16.84)	475 (19.68)	
NA	34 (11.68)	270 (11.18)	
Methods of conception, *N* (%)			<0.01
ART	162 (55.67)	680 (28.17)	
Natural	129 (44.33)	1,734 (71.83)	
Previous history of hypertension, *N* (%)	8 (2.75)	11 (0.46)	<0.01
Gestational diabetes, *N* (%)	77 (26.46)	482 (19.97)	0.01
Delivery week, weeks	35.57 ± 1.92	35.58 ± 1.92	0.95
Delivery mode, *N* (%)			0.12
Cesarean section	281 (96.56)	2,269 (93.99)	
Vaginal delivery	9 (3.09)	131 (4.50)	
NA	1 (0.34)	14 (4.81)	
Preeclampsia, *N* (%)	101 (34.70)	439 (18.19)	<0.01
Mild PE, *N* (%)	50 (17.18)	212 (8.78)	<0.01
Severe PE, *N* (%)	51 (17.53)	227 (9.40)	0.03
PE delivery at <34 wk, *N* (%)	16 (5.50)	41 (1.70)	<0.01
PE delivery at <37 wk, *N* (%)	72 (24.74)	301 (12.47)	<0.01
Mild PE delivery at <34 wk, *N* (%)	8 (2.75)	13 (0.54)	<0.01
Mild PE delivery at <37 wk, *N* (%)	34 (11.68)	121 (5.02)	<0.01
Severe PE delivery at <34 wk, *N* (%)	8 (2.75)	28 (1.16)	0.27
Severe PE delivery at <37 wk, *N* (%)	38 (13.06)	180 (7.46)	0.02

ART referred to the use of assistant reproductive technology.

available evidence of aspirin prophylaxis originated from singleton pregnancies, and those women with multiple pregnancies or those pregnant as a result of ART were excluded. Qualified clinical trials of twin pregnancies covering both natural conception and ART are lacking. This study provided evidence for the fact that low-dose aspirin did not reduce the risk of preeclampsia for twin pregnancies. A possible plausible explanation could be increased fetoplacental demand in twin pregnancies instead of reduced uteroplacental blood supply (18, 19). It is because that increased syncytiotrophoblast stress from one larger placenta or two placentas is more likely to be accounted for preeclampsia in twin pregnancies, instead of maternal underlying cardiovascular phenotype (20). Further studies should be focused on the differential pathological mechanisms.

Another possible reason could be that women with twin pregnancies may require better compliance and a larger dosage to achieve effective prevention of preeclampsia. The different dosage of aspirin is always one of the key concerns. Our study provided evidence for the fact that low-dose aspirin did not reduce the risk of preeclampsia for twin pregnancies. One of the earliest RCTs of aspirin in twin pregnancy, dated 1989, showed that birth weight was increased among 15 women using aspirin compared to 12 who were not using (21). In the 1993 Italian study of 1,106 twin pregnancies, a greater proportion of hypertensive diseases during pregnancy and intrauterine growth restriction was observed in the group of 50 mg/day aspirin, but the difference was insignificant (8). The difference between 75 vs. 100 mg of aspirin, as well as initiation before and after 16 gestational weeks, was not compared since our local guideline recommends a dosage of 75–100 mg before 20 weeks. Aspirin has been demonstrated to be beneficial when given before 16 weeks of gestation and has preventive effect with a 100–150 mg dosage (1–4), and therefore, whether a cutoff of 16 weeks or a larger dose would achieve effective prevention for twin pregnancy requires further validation.

5. Conclusion

In conclusion, our findings indicated that an aspirin prescription of 75–100 mg in twin pregnancies was associated with no significant reduction of preeclampsia. This may be due to poor compliance

TABLE 2 Outcomes of total participants and case-matched cases.

	Total participants						Case-matched analysis					
	LDA group	N-LDA group	Crude OR (95%CI)	*P*	Adjusted OR (95%CI)*	*P*	LDA group	N-LDA group	Crude OR (95%CI)	*P*	Adjusted OR (95%CI)	P
Total participants												
PE	101 (34.7)	439 (18.2)	2.39 (1.83–3.10)	<0.01	2.15 (1.62–2.82)	<0.01	101 (34.7)	137 (23.5)	1.73 (1.27–2.35)	<0.01	1.74 (1.26–2.40)	<0.01
Mild PE	50 (17.2)	212 (8.8)	2.15 (1.56–3.06)	<0.01	1.87 (1.27–2.62)	0.01	51 (17.5)	72 (12.8)	1.47 (1.02–2.20)	0.04	1.48 (0.97–2.12)	0.02
Severe PE	51 (17.5)	227 (9.4)	2.05 (1.21–2.48)	<0.01	1.88 (1.32–2.64)	<0.01	50 (17.2)	65 (11.2)	1.69 (1.33–2.51)	<0.01	1.66 (1.01–2.50)	0.01
Subtype of PE												
PE delivery at <34 wk	16 (5.5)	41 (1.7)	3.37 (1.81–5.97)	<0.01	3.80 (1.40–6.30)	<0.01	16 (5.5)	20 (3.4)	1.63 (0.82–3.20)	0.15	2.83 (1.24–6.35)	0.01
PE delivery at <37 wk	72 (24.7)	301 (12.5)	2.31 (1.71–3.08)	<0.01	2.17 (1.30–2.53)	<0.01	72 (24.7)	91 (15.6)	1.77 (1.25–2.51)	<0.01	1.89 (1.27–2.73)	<0.01
Mild PE delivery at <34 wk	8 (2.8)	13 (0.5)	5.22 (2.05–12.5)	<0.01	6.04 (2.06–17.2)	0.06	8 (2.8)	5 (0.9)	3.26 (1.08–10.90)	0.04	6.07 (1.70–25.70)	<0.01
Mild PE delivery at <37 wk	34 (11.7)	121 (5.0)	2.51 (1.66–3.71)	<0.01	2.38 (1.54–3.59)	<0.01	34 (11.7)	38 (6.5)	1.89 (1.20–3.18)	<0.01	1.98 (1.20–3.20)	<0.01
Severe PE delivery at <34 wk	8 (2.8)	28 (1.2)	2.41 (1.02–5.10)	0.03	2.26 (0.85–5.48)	0.08	8 (2.8)	15 (2.6)	1.07 (0.43–2.50)	0.88	1.42 (0.51–3.82)	0.48
Severe PE delivery at <37 wk	38 (13.1)	180 (7.5)	1.86 (1.27–2.68)	0.01	1.69 (1.13–2.48)	<0.01	38 (13.1)	53 (9.1)	1.50 (0.96–2.33)	0.07	1.54 (0.96–2.44)	0.07
Subgroup analysis of conception method												
ART												
PE	66/162 (40.7)	183/680 (26.9)	1.87 (1.30–2.66)	<0.01	1.73 (1.18–2.52)	<0.01	66/162 (40.7)	79/278 (28.4)	1.73 (1.15–2.61)	<0.01	1.59 (1.05–2.43)	0.03
Mild PE	32/162 (19.8)	90/680 (13.2)	1.61 (1.02–2.50)	0.03	1.43 (0.88–2.27)	0.14	32/162 (19.8)	38/278 (13.7)	1.55 (0.93–2.60)	0.09	1.51 (0.88–2.60)	0.13
Severe PE	34/162 (21.0)	93/80 (13.7)	1.08 (1.07–2.58)	0.02	1.62 (1.01–2.54)	0.04	34/162 (21.0)	41/278 (14.8)	1.54 (0.93–2.54)	0.09	1.49 (0.88–2.51)	0.14
Natural conception												
PE	35/129 (27.1)	256/1,734 (14.8)	2.15 (1.41–3.21)	<0.01	2.03 (1.31–3.09)	<0.01	35/129 (27.1)	58/304 (19.1)	1.58 (0.97–2.55)	0.04	1.60 (1.20–2.60)	0.03
Mild PE	18/129 (14.0)	122/1,734 (7.0)	2.14 (1.22–3.56)	<0.01	1.99 (1.20–3.40)	0.02	18/129 (14.0)	34/304 (11.2)	1.29 (0.69–2.35)	0.41	1.38 (0.72–2.60)	0.31
Severe PE	17/129 (13.2)	134/1,734 (7.7)	1.81 (1.02–3.03)	0.03	1.72 (0.96–2.91)	0.06	17/129 (13.2)	24/304 (7.9)	1.77 (0.90–3.40)	0.08	1.81 (0.90–3.56)	0.09

Data were present as *N* (%).

*adjusted for maternal age, nulliparity, BMI, gestational hypertension, gestational diabetes, gestational week, and ART.

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10.3389/fcvm.2022.964541

and/or an insufficient dose of aspirin used for twin pregnancy. Further well-designed randomized controlled or prospective cohort studies are required.

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 Research Ethics Committee of Fudan University, and written informed consent was obtained from all participants (FE21194). The patients/participants provided their written informed consent to participate in this study.

Author contributions

QZ and XL have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, study concept and design, administrative, technical, or material support, and study supervision. QZ, XZ, and XL: drafting of the manuscript and obtained funding. QZ, XZ, JB, and XL: critical revision of the manuscript for important intellectual content. QZ, XZ, X-MZ, and XL: statistical analysis. All authors: acquisition, analysis, or interpretation of data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.964541/full#supplementary-material

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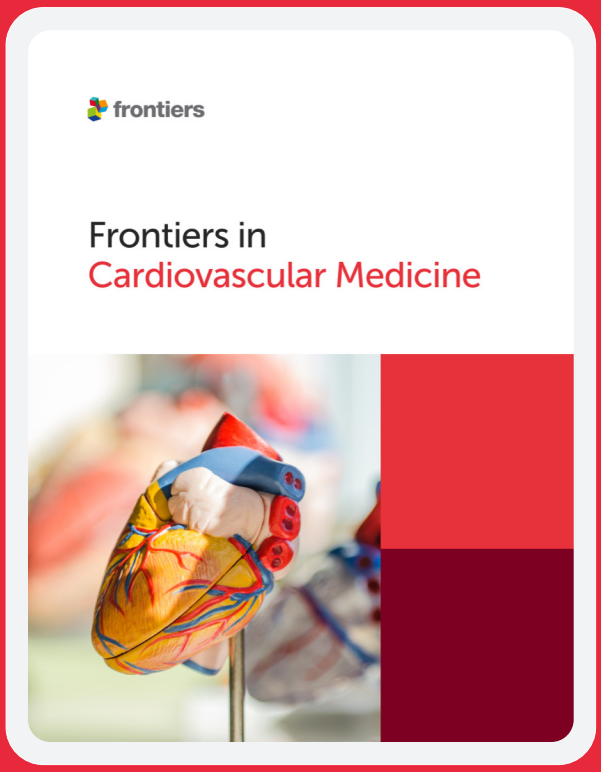

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