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A rapid diagnostic technique based on metabolomics to differentiate between preeclampsia (PE) and chronic kidney disease (CKD) using maternal urine

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ABSTRACT

Similar clinical manifestations between preeclampsia and chronic kidney diseases can lead to potential misdiagnosis. Therefore, it is crucial to investigate effective diagnostic approaches that can reduce misdiagnosis and ensure the well-being of pregnant women. In this study, urine samples collected from 44 individuals with preeclampsia, 37 individuals with chronic kidney disease, and 37 healthy pregnant women were analyzed using metabolomic and proteomic strategies to distinguish between these two diseases. A total of 15 small molecules were tentatively identified as biomarkers to differentiate these two diseases, including potential internally exposed drugs and their metabolites like labetalol and SN-38, metabolites of exogenous substances like 3-phenylpropyl glucosinolate, and endogenous substances related to metabolism such as isoglobotriaose and chitobiose. Metabolic differences between preeclampsia from healthy pregnant women, as well as the differences between chronic kidney disease and healthy pregnant women were also investigated. Major mechanistic pathways were investigated based on the combination of metabolomic and proteomic, amino acid metabolisms and folate metabolism play key roles in distinguishing preeclampsia and chronic kidney disease. Two patients who were initially diagnosed with chronic kidney disease were found to have a closer association with preeclampsia following metabolomic analysis. Subsequent clinical symptoms and manifestations further supported the diagnosis of preeclampsia, and one of patient's pregnancy was ultimately terminated due to severe preeclampsia. Results of this study contribute to a better understanding of the pathogenesis and clinical diagnosis of preeclampsia, offering insights that could potentially improve future diagnostic and management approaches.

Introduction

Preeclampsia is a pregnancy-specific disease characterized by the simultaneous onset of hypertension and proteinuria [5,10]. It affects about 5 % of pregnancies and continues to be a major contributor to maternal and fetal/neonatal mortality and morbidity globally [7,2]. Clinical definition of preeclampsia is the new-onset hypertension and proteinuria, together with/without other organ damages like seizure, breathlessness, severe epigastric pain or massive placental abruption after 20 weeks of gestation. And eclampsia is defined as the occurrence of generalized seizures in a woman with preeclampsia [4,8]. Increasing evidence has confirmed a strong correlation between the incidence of eclampsia arising from preeclampsia and the level of economic and medical development of the pregnant woman and the country in which she resides [7,9,10,2]. Comprehending the underlying pathogenesis of preeclampsia remains an essential yet challenging area of research, and plays key role in ensuring an accurate clinical diagnosis for effectively managing maternal mortality.

The diagnostic criteria for preeclampsia have been redefined to include hypertension with any organ injury, according to the updated guidelines from the American Congress of Obstetricians and Gynecologists [1] and the Society of Obstetricians and Gynaecologists of Canada [6]. Despite this, there is still a risk of misdiagnosing preeclampsia and other hypertension-related disorders, as blood pressure can normalize after childbirth. Furthermore, about 1/10 of women with chronic hypertension exhibit baseline proteinuria (over 300 mg/day) due to

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hypertension-related nephrosclerosis or original chronic kidney disease (American College of Obstetricians and Gynecologists' Committee et al., 2019). Thus the differentiation between preeclampsia and chronic kidney disease may only become evident in hindsight [11]. To accurately and efficiently differentiate preeclampsia from other conditions with similar clinical presentations, biological approaches such as metabolomic and proteomic strategies are valuable in identifying potential biomarkers from pathological samples. This need is particularly pressing for designated hospitals that handle a high volume of complex cases involving critically ill pregnant women where distinguishing between preeclampsia and chronic kidney disease can be challenging. The findings from these studies can not only aid in the diagnosis and management of preeclampsia but also enhance our understanding of the underlying pathogenesis of the condition.

For this study, urine samples from 118 pregnant women were collected. Among these, 44 were clinically diagnosed as preeclampsia (labeled as PE), 37 complicated with chronic kidney disease (labeled as CKD), and another 37 healthy pregnant women (labeled as CON, representing the control group in this study). The urine samples were subjected to metabolomic analysis using UPLC-Q TOF MS to assess their respective conditions. Current research is primarily focused on identifying biomarkers that can improve the differentiation between the onset of preeclampsia and chronic kidney disease by analyzing their variations in metabolomics. The ultimate goal of this research is to enhance outcomes for both women and their infants.

Materials and methods

Sample collection

This was a prospective observational study that included patients from Renji Hospital. This study adhered to the Ethical Review Guidelines for Biomedical Research Involving Humans of the Ministry of Health of the People's Republic of China (2016), the Declaration of Helsinki by the World Medical Association (2013), the International Ethical Guidelines for Biomedical Research Involving Human Subjects by the Council for International Organizations of Medical Sciences (CIOMS, 2002), and the principles of Good Clinical Practice (GCP). Urine sample collection was approved by Shanghai Jiao Tong University School of Medicine, Renji Hospital Ethics Committee with a certification number of KY2021–006. And the analyses of urine samples and data processing was approved by Ethics Committee for Science and Technology Research involving Humans at Shanghai Jiao Tong University with a certification number of B2021116I. The study cohort comprised patients diagnosed with preeclampsia, pregnanct women complicated by chronic kidney disease, as well as normal pregnant women without any health complications, in accordance with the diagnostic criteria outlined in the Guidelines for the Diagnosis and Treatment of Hypertensive Disorders during Pregnancy [3]. Patients with diabetes, immune system diseases, or any other conditions that could lead to proteinuria were excluded from the study.

After obtaining informed consent from each participant, a standardized method was utilized for the collection of 24-hour urine samples. Subsequently, 5 mL of urine was extracted as the experimental specimen. Detailed records were kept, including the patient's name, hospitalization number, experimental identification number, and the precise collection time of each urine sample. All the urine samples were stored at −80 °C before analyses.

Human urine sample preparation

Urine samples were centrifuged at 12,000 g at 4 °C for 10 min. A 100 μL amount of supernatant urine was vortexed with 900 μL of ultrapure water for 30 s and then centrifuged at 9391 g at 4 °C for 10 min. Then the supernatant was transferred into a UPLC vial and injected into UPLC-QTOF-MS for metabolic analyses. Quality control (QC) samples were prepared according to our previous approach. Briefly, 100 μL of each urine sample was pooled and mixed. QC sample was repeatedly injected five injections to verify the system conditions and then injected by twenty samples to confirm analytical consistency.

Metabolic analytical conditions

The UPLC-QTOF MS conditions were the same as those reported by Huang et al. with some modifications in the LC gradients. Specifically, the elution gradient started from 0 to 35 % B, linearly increased to 95 % B in 4 min and stayed for 2 min, and then back to the initial ratio in 2 min to recondition for the next injection. The relative quantification of metabolites was calculated by comparing the absolute peak areas of metabolites in the extracted ion peak mode between the control and the test groups.

Biomarker identification by nontargeted metabonomics

Raw data files collected from UPLC-QTOF MS were processed with MassLynx version 4.1 (Waters, Milford, MA, USA) and Progenesis QI for Metabonomics and Lipidomics version 1.0 (Waters, Milford, MA, USA) for peak detection, alignment, and normalization. Afterward, automatic peak detection with default sensitivity was used. No other limits were applied. Finally, in the peak-picking step, no additional peak-picking criteria were applied other than $p < 0.05$ and fold change > 2. The data matrix established by the accurate mass/retention time pair with corresponding normalized peak areas was analyzed by principal component analysis (PCA). The PCA was used with autoscaling preprocessing and no preprocessing, respectively. The 95 % Hotelling's T2 confidence ellipse were plotted for both positive and negative ion modes. In this study, control (CON), preeclampsia (PE) and chronic kidney disease (CKD) groups were compared and analyzed in pairs. An independent sample t-test between groups was used to evaluate the significant difference ($p < 0.05$) using SPSS Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). Biochemical databases, including HMDB, METLIN, Massbank, and KEGG, were used to identify potential biomarkers. MetaboAnalyst version 3.0 was used to analyze potential metabolite pathways.

Results and discussion

Preeclampsia (PE) is a pregnancy-related syndrome characterized by hypertension and proteinuria with various underlying causes. Distinguishing between PE and chronic kidney disease (CKD) during pregnancy can be challenging due to their similar symptoms of hypertension and proteinuria. Accurate differentiation is crucial as they require different management strategies and have varying implications for maternal and fetal health.

Study populations

Based on the study population (Table 1), there were no significant differences in age, gravidity and smoking between the PE group, CKD group and CON group. However, t significant differences were observed in parity, BMI, gestational age at sampling, gestational age at delivery, birthweight, and APGAR score. Upon conducting pairwise comparisons within the groups, it was found that the parity in the CKD group was lower than that in the CON group ($P = 0.039$). Additionally, the average BMI value in the CKD group was lower than that in both the PE group ($P = 0.006$) and the CON group ($P = 0.012$), the gestational weeks at sampling in the CON group were later than those in both the PE group ($P < 0.001$) and the CKD group ($P = 0.001$), the gestational weeks at delivery in the CON group were later than those in both the PE group ($P < 0.001$) and the CKD group ($P < 0.001$). Furthermore, the birthweight in the CON group was greater than that in both the PE group ($P < 0.001$) and the CKD group ($P = 0.001$). The birthweight percentile in the PE

Table 1
Baseline characteristics of the study population.

Characteristic	PE (n = 44)	CKD (n = 37)	CON (n = 37)
Maternal age (y)	31 (28 −35)	31 (29 −33.5)	31 (29.5 −34)
G	2 (1 −3)	1 (1 −3)	2 (1 −3)
P[a]	0 (0 −0)	0 (0 −0)	0 (0 −1)
Body mass index class (kg/m^2)[a]	27.42 (24.12 −27.43)	24.31 (22.44 −27.60)	27.24 (25.00 −28.81)
Smoking	0 %	0 %	0 %
Gestational age at sampling (wk)[a]	32.93 (28.64 −36.64)	34.43 (24.86 −37.21)	38.43 (34.93 −39.14)
Gestational age at delivery (wk)[a]	34.29 (29.29 −37.00)	36.50 (30.18 −38.75)	39.00 (38.29 −40.07)
Birthweight (g)[a]	1935 (1095 −2750)	2770 (1610 −3125)	3350 (2960 −3617.5)
Birthweight percentile[a]	11.4 (2.6 −33.1)	23.7 (12.6 −52.8)	52.4 (19.4 −81.75)
APGAR[a]	10 (7 −10)	10 (10 −10)	10 (10 −10)

Data are presented as median (interquartile range) or number (percentage).
[a] Kruskal-Wallis test or Chi-square test as appropriate (P < .05). G, Gestation; P, Parturition

group was lower than that in the CON group (P = 0.001). Lastly, the APGAR score in the PE group was lower than that in the CON group (P = 0.002), and these differences were statistically significant.

Metabolic and proteomic differences of urine samples from preeclampsia and pregnant women complicated with chronic kidney

The metabolic conditions of 44 PE and 37 CKD urine samples were analyzed with an UPLC-Q TOF MS system. A total of 6381 and 5143 variables were measured in positive and negative ion mode, respectively. Data in positive and negative ion modes are fused and analyzed with multivariate data analyses approaches. The scores plot from unbiased principal component analysis (PCA) was showed in Fig. 1A. CKD samples (labeled in red) were generally clustered on the left side of PC1, whereas most of the PE samples (labeled in green) were on the right side. Above results showed remarkable metabolic differences between urine samples from PE and CKD. But there were 7 CKD and 8 PE samples fell in the opposite region of the PCA scores plot, which may due to individual patient differences and clinical diagnostic bias. The practical implications of the metabolome-proteome binding for clinical differentiation between PE and CKD will be discussed in detail at the end of this section.

All the detected variables were filtered based on P ≤ 0.05, as well as PE/CKD fold change ≥ 2 or ≤ 0.5. After filtration, significant different variables were searched and identified with Water Nonlinear Progenesis QI software (Waters, Milford, MA).) with databases HMDB and METLIN, and metabolites with identification scores > 50 and fragment scores > 80 were recognized as reliable markers. A total of 489 potential markers were tentatively identified, and most significant markers were labeled in volcano plot (Fig. 1B). Relevant markers have two-fold greater peak intensities in PE than that in CKD were recognized as up-regulated and labeled in red on the right side of the volcano plot. Corresponding markers have less than half peak intensities in PE than that in CKD (two-fold greater in CKD compared with PE) were down-regulated and labeled in blue on the left side. It could be visually noticed that there were greater identified up-regulated markers while comparing PE and CKD. Representative up-regulated and down-regulated markers and their absolute peak intensities in both PE and CKD samples were showed in Fig. 1C. In the upper layer of Fig. 1C, the bar graphs represent the absolute peak intensities of five representative up-regulated markers in PE and CKD samples, including *m/z* 456.1644 ([M-H_2O-H]$^-$, HMDB60464, codeine-6-glucuronide), *m/z* 323.1389 ([M- H]$^-$, HMDB14895, dolasetron), *m/z* 407.1268 ([M-H_2O-H]$^-$, HMDB03556, chitobiose), *m/z* 503.2024 ([M- H]$^-$, HMDB34660, heterophyllin) and

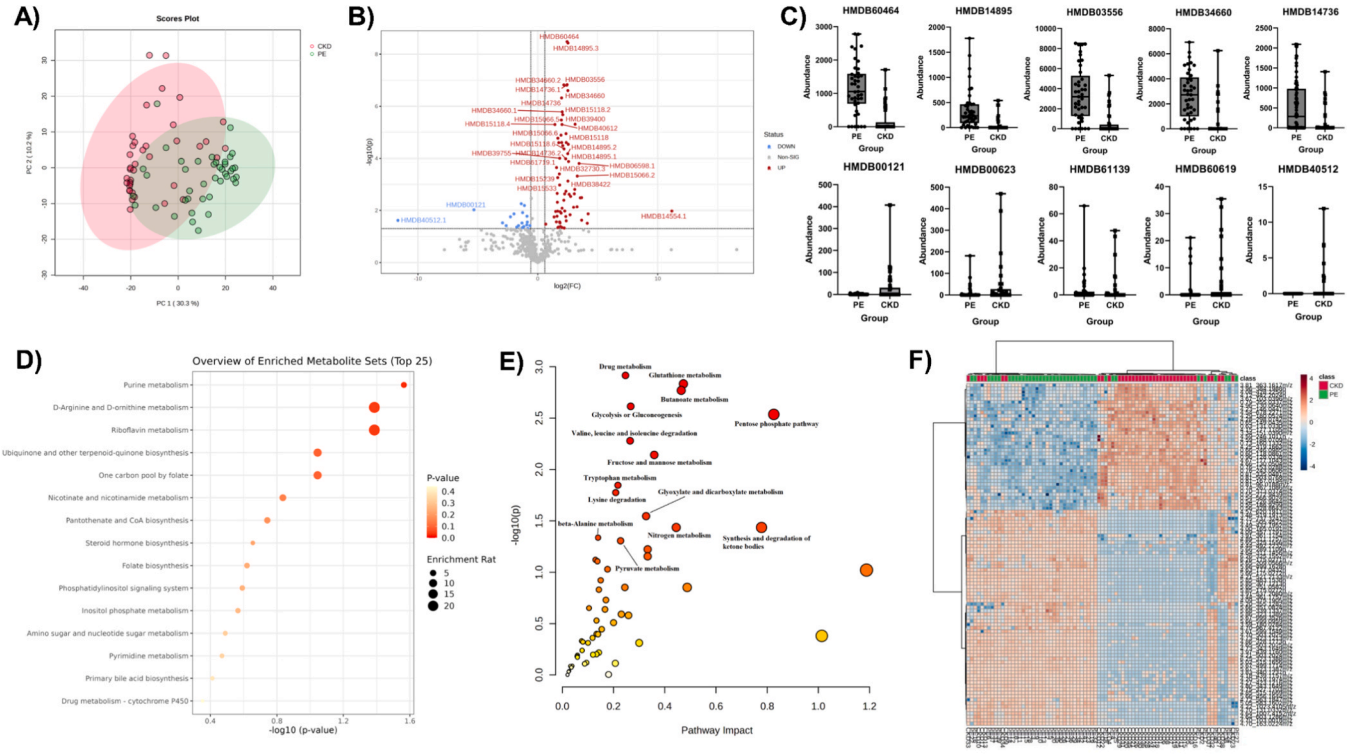


Fig. 1. Metabolic and proteomic differences of urine samples from pregnant women with preeclampsia (PE) or chronic kidney disease (CKD). A) Principal component analysis (PCA) scores plot of potential biomarkers between PE and CKD; B) volcano plot and most remarkable biomarkers in CKD (blue) and PE (red); C) absolute abundances of five representative biomarkers in PE while versus CKD (upper) and five representative biomarkers in CKD while versus PE (lower); D) possible metabolic pathways summarized by identified biomarkers; E) possible pathways summarized by the co-analyzing of metabolic and proteomic results; **F)** heat map of all the populations of PE and CKD samples with top 100 variables.

m/z 327.1703 ([M- H]$^-$, HMDB14736, labetalol). The absolute peak intensities of these five markers were significantly greater in PE samples than that in CKD ones. Actually, although the contents in PE samples varies greatly, these five markers are present in the vast majority of PE samples. So the existences of these five markers have potential to be applied in differentiating PE from CKD urine samples of pregnant women. On contrast, the lower layer of Fig. 1C showed five most significant down-regulated in PE/CKD, including *m/z* 440.1312 ([M- H]$^-$, HMDB00121, folic acid), *m/z* 230.1502 ([M-H_2O-H]$^-$, HMDB00623, dodecanedioic acid), *m/z* 453.1747 ([M-H_2O-H]$^-$, HMDB61139, doxepin N-oxide glucuronide), *m/z* 322.0929 ([M- H]$^-$, HMDB60619, tazarotenic acid) and *m/z* 489.1410 ([M- H]$^-$, HMDB40512, 4′,5,6-trimethylscutellarein 7-glucoside), indicating these markers could not be detected from any PE urine samples, but only from CKD ones. The combination of these selected markers could be furtherly focused in creating an effective model in differentiating pregnancy women with PE from CKD, even with similar hypertension and proteinuria symptoms.

Pathway analyses based on clinical collected urine samples coupled with metabolic and proteomic results could be found in Fig. 1E, all the identified metabolites (with ANOVA $P \leq 0.05$, fold change ≥ 1.5, identified scores ≥ 50) and proteins (false-discovery rate (FDR) ≤ 1 %, exp. q-value ≤ 0.05, fold change ≥ 2) were combined and analyses. Typical metabolic pathways including glutathione metabolism, butanoate metabolism, pentose phosphate pathway, glycolysis or gluconeogenesis, specific amino acid degradation, fructose and mannose metabolism, tryptophan metabolism, and synthesis and degradation of ketone bodies. The significance of linkage in proteins and their related metabolites between PE and CKD urine samples gives the hint about different pathogenic mechanisms of these two syndromes.

Representative biomarkers in differentiating PE and CKD were showed in Table 2. Major biomarkers including ① one potential exposed drugs labetalol, ② metabolites of exogenous substances such as 3-phenylpropyl glucosinolate, divanillyltetrahydrofuran ferulate, dimethylthiambutene, basellasaponin B, hebevinoside X, diosmetin 7-*O*-beta-D-glucuronopyranoside, kaempferol 7-(6''-galloylglucoside) and bisosthenon B, as well as ③ metabolic-related endogenous substances like isoglobotriaose, chitobiose, silica aerogel, tolfenamic acid, gamma-carboxyglutamic acid, heterophyllin and 5-(4-chloro-3-hydroxy-1-butynyl)−2,2′-bithiophene. The existences of representative biomarkers and their biological relations with preeclampsia and/or chronic kidney disease will be discussed.

The implications of current results for subsequent diagnoses to distinguish preeclampsia from chronic kidney disease during pregnancy

The major aim of present study is to investigate potential biomarkers in differentiating PE from CKD patients based on their endogenous small molecule substances in urine samples, then develop more reliable diagnoses approaches to distinguish PE from CKD during pregnancy. As shown in Fig. 1C and Table 2, biomarkers selected based on present model were only present in the PE samples and almost non-existent in the CKD samples, which suggesting that the presence or absence of these biomarkers can be used to determine whether pregnant women with hypertension and/or proteinuria have PE or CKD. Even though, eight out of thirty-seven CKD urine samples still contained varying degrees of certain biomarkers, suggests whether there is a misdiagnosis in the above CKD cases. These eight patients, especially two of them with experimental ID #37 and #105, showed comparable contents of biomarkers as most of PE patients. Seven out of these eight diagnosed-as-CKD pregnant women were diagnosed with both hypertension and proteinuria, with another one diagnosed with abnormal rise in renal function indicators. Half of their CKD diagnoses were confirmed with renal puncture test, and finally six of them were labored in either natural childbirth (1/6) or cesarean (5/6).

The conditions of these two representative cases were concluded. Experimental ID #37 was a 34-year-old patient admitted with a diagnosis of pregnancy at 24 + 2 weeks with persistent elevated blood pressure for over 10 days. Proteinuria was identified during the early trimester, with a 24-hour urine protein level of 0.6 g. Initially, the patient had normal blood pressure and was referred to the Nephrology Department without undergoing renal puncture during pregnancy. Considering the clinical symptoms, chronic nephritis was suspected. However, from the 22nd week of pregnancy, the patient's blood pressure started to rise, accompanied by an increase in proteinuria. As the pregnancy progressed, hypoalbuminemia, pleural effusion, and fetal growth restriction became evident, meeting the diagnostic criteria for severe preeclampsia.

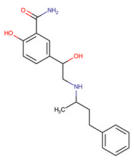
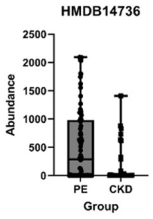
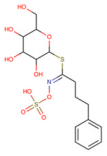
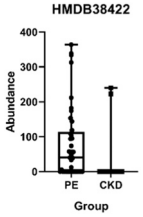
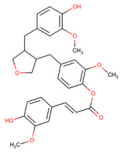
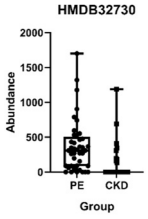
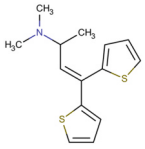
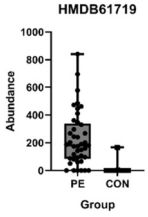
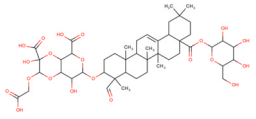
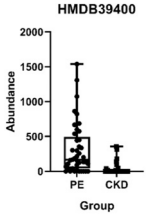
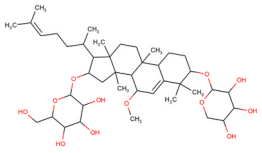
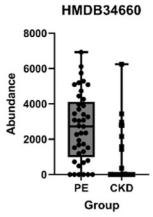
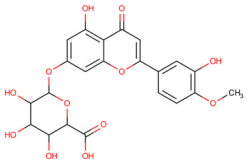
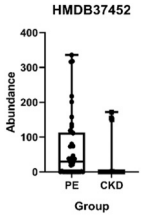
Experimental ID #105 was a 34-year-old patient admitted with a complaint of pregnancy at 16 + 4 weeks with lower limb edema persisting for over two weeks. This pregnancy was achieved through assisted reproductive technology. Proteinuria was detected at 16 weeks, along with a blood pressure reading of 155/93 mmHg. The patient had no prior history of kidney disease. Within two weeks, proteinuria rapidly escalated and couldn't be effectively controlled, accompanied by cardiac impairment. Consequently, a medical induction of labor was performed at 18 + 5 weeks. Since the gestational age was less than 20 weeks, the patient did not meet the diagnostic criteria for preeclampsia. However, postpartum follow-up revealed a rapid decrease in proteinuria after delivery, with complete resolution by 42 days postpartum, and blood pressure returned to the normal range.

Some representative biomarkers were only detected and abnormally great in these two induced abortion patients, including methacycline (HMDB0015066), OP-1118 (HMDB0060859), 3-phenylpropyl glucosinolate (HMDB0038422), dimethylthiambutene (HMDB0061719), diosmetin 7-*O*-beta-D-glucuronopyranoside (HMDB0037452), isoglobotriaose (HMDB0006598) and gamma-carboxyglutamic acid (HMDB0041900) (Table 2). Considering the presence of specific biomarkers associated with PE in these two pregnant women initially diagnosed with CKD, along with their subsequent clinical manifestations and the outcome of labor induction, it is conclusive that they should be diagnosed with preeclampsia. And above results further suggest that there is a certain possibility of misdiagnosis in the clinical diagnosis mode of PE and CKD only based on gestational week, hypertension and proteinuria in the past, and the utilization of biomarkers found in present study as a model can effectively avoid the misdiagnosis.

Conclusion

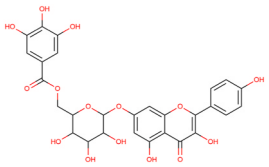
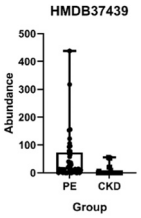
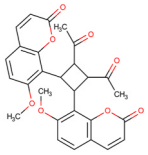
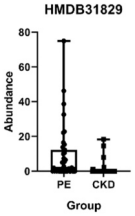
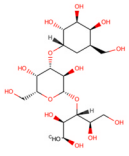
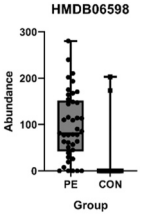
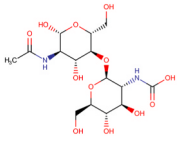
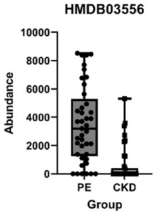
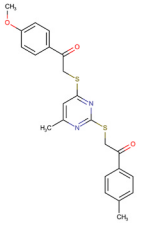
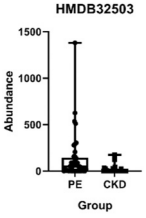
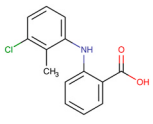
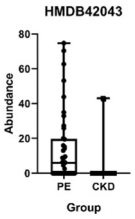
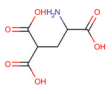
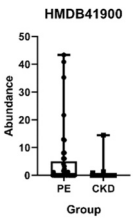
In this study, we conducted a comparative analysis of the metabolic and proteomic differences among preeclampsia, chronic kidney disease, and healthy pregnant women. Accurate differentiation between preeclampsia and chronic kidney disease is of utmost importance in pregnant women presenting with proteinuria and hypertension, as distinct treatment strategies are employed for each condition. The correct diagnosis of either preeclampsia or chronic kidney disease is crucial for ensuring appropriate clinical management and treatment decisions. Furthermore, the biomarkers identified for distinguishing preeclampsia from chronic kidney disease and/or healthy pregnant women could potentially be used in predicting individuals at risk of developing preeclampsia during the first trimester. This early identification would enable timely intervention and treatment for preeclampsia patients, leading to improved prognostic outcomes. Here we identified a total of 15 biomarkers that showed significant differences, including potential internal exposed drug (labetalol), metabolites of exogenous substances (like 3-phenylpropyl glucosinolate and hebevinoside X), and metabolic-related endogenous substances (including isoglobotriaose and chitobiose). The analysis of mechanistic pathways combining metabolomic and proteomic data revealed that amino acid metabolisms and folate metabolism played key roles in distinguishing preeclampsia from chronic kidney disease. By utilizing the biomarkers identified in this study as an adjunct to differentiate between preeclampsia and chronic kidney disease, we can effectively improve the accuracy of clinical

Table 2
Identification results, HMDB IDs, chemical structures and relative abundances of biomarkers in differentiating preeclampsia (PE) and chronic kidney diseases (CKD).

Names of biomarker	HMDB ID	Molecular weight (*m/z*)	Molecular formula	Chemical structure	Fold change (PE vs CKD)	Relative abundance (PE vs CKD)
Labetalol	HMDB0014736	327.1703 [M-H]$^-$	$C_{19}H_{24}N_2O_3$		4.51	
3-Phenylpropyl glucosinolate	HMDB0038422	418.0687 [M-H_2O-H]$^-$	$C_{16}H_{23}NO_9S_2$		5.67	
Divanillyltetrahydrofuran ferulate	HMDB0032730	519.1976 [M-H]$^-$	$C_{30}H_{32}O_8$		2.98	
Dimethylthiambutene	HMDB0061719	244.0628 [M-H_2O-H]$^-$	$C_{14}H_{17}NS_2$		3.09	
Basellasaponin B	HMDB0039400	967.4122 [M-H]$^-$	$C_{47}H_{68}O_{21}$		6.49	
Heterophyllin	HMDB0034660	503.2025 [M-H]$^-$	$C_{30}H_{32}O_7$		4.94	
Diosmetin 7-O-beta-D-glucuronopyranoside	HMDB0037452	475.0913 [M-H]$^-$	$C_{22}H_{20}O_{12}$		5.64	

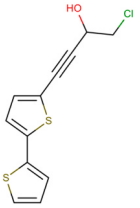
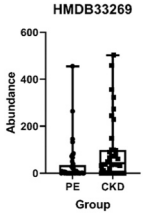
(*continued on next page*)

Table 2 (*continued*)

Names of biomarker	HMDB ID	Molecular weight (*m/z*)	Molecular formula	Chemical structure	Fold change (PE vs CKD)	Relative abundance (PE vs CKD)
Kaempferol 7-(6''-galloylglucoside)	HMDB0037439	599.0981 [M-H]$^-$	$C_{28}H_{24}O_{15}$		6.54	
Bisosthenon B	HMDB0031829	469.1275 [M-H_2O-H]$^-$	$C_{28}H_{24}O_8$		5.61	
Isoglobotriaose	HMDB0006598	501.1867 [M-H]$^-$	$C_{19}H_{34}O_{15}$		9.71	
Chitobiose	HMDB0003556	407.1268 [M-H_2O-H]$^-$	$C_{15}H_{26}N_2O_{12}$		4.54	
Silica aerogel	HMDB0032503	419.0901 [M-H_2O-H]$^-$	$C_{23}H_{22}N_2O_3S_2$		23.04	
Tolfenamic acid	HMDB0042043	242.0362 [M-H_2O-H]$^-$	$C_{14}H_{12}ClNO_2$		5.47	
gamma-Carboxyglutamic acid	HMDB0041900	190.0367 [M-H]$^-$	$C_6H_9NO_6$		11.28	

(*continued on next page*)

Table 2 (*continued*)

Names of biomarker	HMDB ID	Molecular weight (*m/z*)	Molecular formula	Chemical structure	Fold change (PE vs CKD)	Relative abundance (PE vs CKD)
5-(4-Chloro−3-hydroxy−1-butynyl)−2,2′-bithiophene	HMDB0033269	248.9582 [M-H_2O-H]$^-$	$C_{12}H_9ClOS_2$		3.33	

diagnosis and further enhance our understanding of the pathogenesis and clinical diagnosis of preeclampsia in the future.

CRediT authorship contribution statement

Xin Lv: Validation, Writing – original draft. **Boyan Gao:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft. **Xu Zhuang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Jingli HOU:** Investigation.

Declaration of Competing Interest

Authors declare that there are no competing interests.

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