ORIGINAL RESEARCH

Novel urinary metabolite signature for diagnosing postpartum depression

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Background: Postpartum depression (PPD) could affect ~10% of women and impair the quality of mother–infant interactions. Currently, there are no objective methods to diagnose PPD. Therefore, this study was conducted to identify potential biomarkers for diagnosing PPD.

Materials and methods: Morning urine samples of PPD subjects, postpartum women without depression (PPWD) and healthy controls (HCs) were collected. The gas chromatography-mass spectroscopy (GC-MS)-based urinary metabolomic approach was performed to characterize the urinary metabolic profiling. The orthogonal partial least-squares-discriminant analysis (OPLS-DA) was used to identify the differential metabolites. The logistic regression analysis and Bayesian information criterion rule were further used to identify the potential biomarker panel. The receiver operating characteristic curve analysis was conducted to evaluate the diagnostic performance of the identified potential biomarker panel.

Results: Totally, 73 PPD subjects, 73 PPWD and 74 HCs were included, and 68 metabolites were identified using GC-MS. The OPLS-DA model showed that there were 22 differential metabolites (14 upregulated and 8 downregulated) responsible for separating PPD subjects from HCs and PPWD. Meanwhile, a panel of five potential biomarkers – formate, succinate, 1-methylhistidine, α-glucose and dimethylamine – was identified. This panel could effectively distinguish PPD subjects from HCs and PPWD with an area under the curve (AUC) curve of 0.948 in the training set and 0.944 in the testing set.

Conclusion: These results demonstrated that the potential biomarker panel could aid in the future development of an objective diagnostic method for PPD.

Keywords: postpartum depression, gas chromatography-mass spectroscopy, biomarker, metabolomics

Introduction

Major depressive disorder (MDD) affects ~15% of the population and is a source of substantial financial burden on society and individuals.[1] The mechanisms associated with the pathogenesis of MDD have yet to be completely understood, and the current treatments remain ineffective in a large subset of patients.[2,3] To date, the most consistent findings across studies about MDD might be the disproportionate prevalence in women.[4] Women are more vulnerable to experience MDD during periods of marked hormonal fluctuations, especially during pregnancy and the postpartum. The interactions between gonadal and stress hormones might play an important role in predisposing women to MDD.[5]

Antenatal depression means the depression that starts during the pregnancy, and the postpartum depression (PPD) means the depression that starts after birth. The greatest risk factor for PPD is depression prior to pregnancy, but ~40% of women with PPD have the first episode of depression after birth.[6] A review reported that

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there was moderate evidence that PPD could affect the mother–infant interaction patterns, which in turn might contribute to a disturbed neurobiological development of her child.[7] Meanwhile, Murray et al[8] found that the impairments in mother–infant interactions were related to adverse child outcomes. Previous studies reported that the prevalence of PPD worldwide was estimated to range from 3% to 19%.[9,10] However, there are still no objective methods to diagnose PPD. Using the new *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) to diagnose PPD is very time-consuming and always results in a considerable error rate.[11] Moreover, the DSM-5 criteria could encompass only the first f4 weeks postpartum, but the greatest incidence of new PPD always occurs 2–3 months after parturition.[10,12] Given these facts, it is urgently needed to develop an objective method to diagnose PPD.

Currently, metabolomics and other methods have been developed to identify the potential biomarkers for neuropsychiatric disorders,[13,14] including depression, bipolar disorder and schizophrenia.[15–17] Through metabolomics, previous studies found that there were urinary metabolic abnormalities in patients with MDD.[15,18] Therefore, there might also be divergent urinary metabolic phenotypes between PPD subjects and healthy controls (HCs), and the differential metabolites might be biologically correlated with depression in postpartum women and could be used to develop an objective method for diagnosing PPD. In this study, gas chromatography-mass spectroscopy (GC-MS), one of the main analytical techniques for nontargeted metabolomics mapping, was used to profile 45 PPD subjects, 43 postpartum women without depression (PPWD) and 40 HCs to determine whether this method could effectively separate PPD subjects from PPWD and HCs, and the differential metabolites were further analyzed to identify a potential biomarker panel for diagnosing PPD. Meanwhile, we used 28 PPD subjects, 30 PPWD and 34 HCs to independently validate the diagnostic performance of the identified potential biomarker panel.

Materials and methods
Recruiting subjects
This study was reviewed and approved by the Ethical Committee of Linyi People's Hospital. The included PPD subjects met the DSM-IV criteria for depression, and the 17-item Hamilton Depression Rating Scale (HDRS) was used to measure the severity of depression. Two experienced psychiatrists were in charge of recruiting PPD subjects and PPWD by systematically interviewing. Postpartum women with an HDRS score of >8 were included into the PPD

group, and PPWD were included into the PPWD group. All recruited postpartum women were from the Department of Obstetrics and Gynecology of Linyi People's Hospital. Meanwhile, the HCs were recruited from the Medical Examination Center of Linyi People's Hospital. The PPD subjects had no pre-existing physical diseases, psychiatric comorbidities or other mental disorders. The HCs and PPWD had no systemic medical illness, neurological disease and current or previous lifetime history of DSM-IV Axis I/Axis II disorder. All the recruited subjects provided written informed consent. We recruited only women 8–12 weeks postpartum. Finally, 73 PPD subjects, 73 PPWD and 74 HCs were included.

GC-MS acquisition
Morning urine samples of subjects were collected between 9 am and 10 am using a sterile cup and then quickly transferred into a sterile tube. Urine samples were sent to the laboratory under low-temperature condition for centrifugation ($1,500 \times g \times 10$ minutes). After centrifugation, the resulting supernatant was immediately divided into equal aliquots and then quickly stored at $-80°C$ for subsequent analysis. The procedure for GC-MS preparation was performed according to the previous study.[18] Briefly, 1) the 10 µL internal standard solution (L-leucine-13C6, 0.02 mg/mL) and 15 µL aliquot of urine were mixed and vortexed; 2) into the mixed solution, 15 µL urease was added to degrade the urea (37°C, 60 minutes); 3) the mixture was successively extracted using 240 µL and 80 µL of ice-cold methanol, and the obtained mixture was vortexed for 30 seconds; 4) after centrifugation (4°C, 14,000 rpm and 5 minutes), 224 µL supernatant was transferred to a glass vial for vacuum drying at room temperature, and then 30 µL of methoxyamine (20 mg/mL) was used to derivatize the dried metabolic extract (37°C, 90 minutes); 5) 30 µL of N,O-bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane was added into the dried metabolic extract, and then the mixture was heated (70°C, 60 minutes) to obtain trimethylsilyl derivatives; and 6) after derivatization and cooling to room temperature, 1.0 µL derivative was added into the GC-MS system. The GC-MS analysis conditions were injecting 1.0 µL samples at 270°C; solvent delay for 5 minutes; setting appropriate temperature programming (set initial temperature, 85°C; continue it for 5 minutes; increase it to 300°C by 10°C/min; continue 300°C for 5 minutes); interface temperature, 280°C; quadrupole temperature, 150°C; ionization voltage, −70 eV; carrier gas, helium (flow rate, 1.0 mL/min); ion source temperature, 230°C; and full scan was conducted at 50–600 *m/z*. The quantitative information

of urinary metabolites was obtained by mainly referring to the previous study.[19]

Metabolomic data analysis

The normalized peak area percentages of each identified urinary metabolites were imported into the SIMCA-P software (version 14.0; Umetrics, Umeå, Sweden). The orthogonal partial least-squares-discriminant analysis (OPLS-DA) was used to analyze whether the urinary metabolites could separate PPD subjects from PPWD and HCs.[20] There were three parameters (R^2X, R^2Y and Q^2Y) to assess the quality (quantify and predictability) of the built OPLS-DA model. Meanwhile, a 299-iteration permutation test was conducted to rule out the overfitting of the built model; the higher values of the R^2 and Q^2 in the original model than the values from the test could demonstrate that the built model was valid and robust.[21] The variable importance plot (VIP $>$1 equivalent to a $P<0.05$) was applied to select the key differential metabolites responsible for discriminating PPD subjects from PPWD and HCs.

Statistical analysis

The logistic regression analysis was used to further analyze these key differential metabolites, and the Bayesian information criterion (BIC) was used to select the optimal metabolites combination.[22] The receiver-operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of the identified optimal metabolites combination in diagnosing PPD subjects. Student's t-test, nonparametric Mann–Whitney U-test and one-way ANOVA were conducted when appropriate. All these statistical analyses were conducted using SPSS 21.0 (IBM, Armonk, NY, USA). In all comparisons, a $P<0.05$ was considered statistically significant.

Results

The recruited subjects were randomly segregated into two sets (training set and testing set). The training set was used to identify the potential urinary metabolite biomarkers for PPD, and the testing set was used to independently validate the diagnostic performance of the identified urinary metabolite biomarkers. The age ranged from 24 years to 31 years among the PPD subjects (average age, 27.51±2.17 years), 22 years to 32 years among the PPWD (average age, 26.90±3.13 years) and 21 years to 33 years among the HCs (average age, 27.18±3.67 years). The body mass index (BMI) (8 weeks postpartum) was 22.79±1.98, 23.01±1.83 and 22.61±1.88 in PPD, PPWD and HCs groups, respectively. The full

gestation length was 39.26±1.38 weeks, 39.11±1.41 weeks and 39.14±1.27 weeks in PPD, PPWD and HCs groups, respectively. The child's birth weight was 3.63±0.70 kg, 3.65±0.69 kg and 3.53±0.71 kg in PPD, PPWD and HCs groups, respectively. The three groups were demographically matched. All recruited subjects did not have any mental disorders or pregnancy-related disorders (such as gestational diabetes and pre-eclampsia) before pregnancy. Meanwhile, the PPD subjects did not receive any antidepressants after giving birth. The 33 subjects in the PPD group and 36 subjects in the PPWD group delivered a baby with natural birth. The seven subjects in the PPD group and five subjects in the PPWD group delivered a baby with cesarean section after failing to have a complete natural birth. The remaining subjects among PPD and PPWD groups delivered a baby with cesarean section.

OPLS-DA model

First, the training set (45 PPD subjects, 43 PPWD and 40 HCs) was used to build the OPLS-DA model. The score plots of the built model showed that the PPD subjects were distinguishable from the PPWD and HCs with little overlap. The three parameters (R^2X cum =0.39, R^2Y cum =0.73, Q^2=0.60; Figure 1A) indicated that the built model was positive, implying the significant metabolic differences between PPD subjects and the other subjects (PPWD and HCs). Meanwhile, the results of the permutation test also showed that the built model was valid and positive (Figure 1B). Second, the testing set (28 PPD subjects, 30 PPWD and 34 HCs) was used to independently validate the diagnostic performance of the built model. The T-predicted scatter plot showed that the built model could effectively predict the HCs and PPWD (Figure 2A) and PPD subjects (Figure 2B).

Significant metabolic differences

To identify the metabolites that were responsible (VIP $>$1.0) for separating PPD subjects from the other subjects, the corresponding OPLS-DA loading plot was analyzed. Totally, there were 68 metabolites used to build the OPLS-DA model, and at last the levels of 22 metabolites were significantly altered in the PPD subjects (Table 1). As compared to the other subjects, the PPD subjects were characterized by the significantly higher levels of formate, succinate, 3-methylhistidine, glutamate, isobutyrate, acetamide, α-ketoglutarate, quinolinic acid, pyroglutamic acid, phenylacetyglycine, hippurate, 1-methylhistidine, valine and m-hydroxyphenylacetate, along with the significantly lower levels of α-hydroxyisobutyrate, N-methylnicotimide, glycolate, acetate, taurine, lactate,
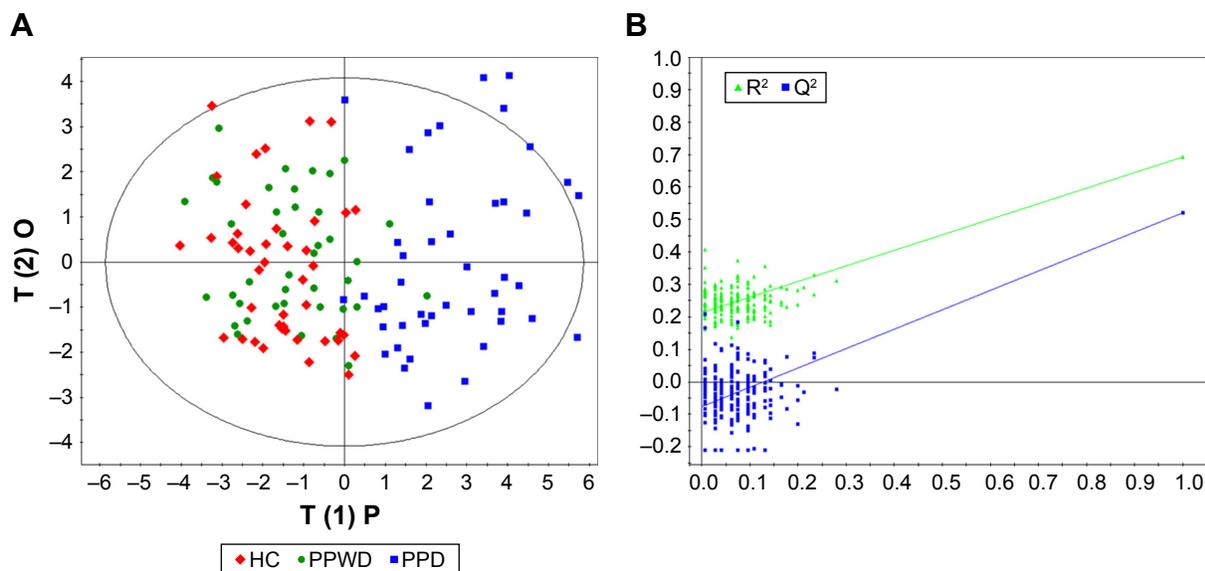
Figure 1 Metabolomic analysis of urine samples and permutation test.
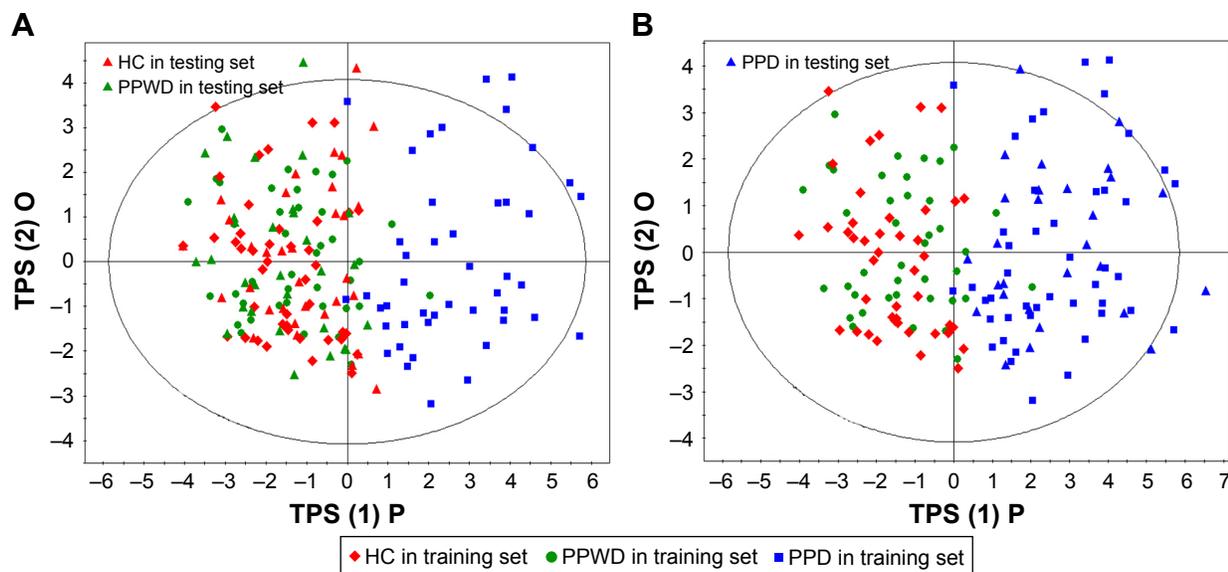**Notes:** (**A**) OPLS-DA score plots; (**B**) permutation test.
Abbreviations: HC, healthy control; OPLS-DA, orthogonal partial least-squares-discriminant analysis; PPD, postpartum women with depression; PPWD, postpartum women without depression.

α-glucose and dimethylamine. Meanwhile, Mann–Whitney *U*-test was used to validate the metabolic alteration identified by the OPLS-DA model, and the 17 of 22 metabolites remained significantly changed (Table 1).

Potential biomarker panel

In clinical practice, it is not economic and convenient to use 22 metabolites to diagnose PPD. Therefore, the logistic regression analysis was performed to further analyze

these differential metabolites, and the BIC rule was used to identify the optimal biomarker panel. Finally, the results showed that the most significant deviations between PPD subjects and the other subjects could be described by the following five urinary metabolites: formate, succinate, 1-methylhistidine, α-glucose and dimethylamine (Figure 3). The potential biomarker panel consisting of these five metabolites could yield a sensitivity of 91.1% and a specificity of 90.4% in the training set and a sensitivity of 85.7%

Figure 2 T-predicted scatter plot from the OPLS-DA model.
Notes: (**A**) OPLS-DA model was used to predict HC and PPWD in the testing; (**B**) predict PPD in the testing set.
Abbreviations: OPLS-DA, orthogonal partial least-squares-discriminant analysis; HC, healthy control; PPD, postpartum women with depression; PPWD, postpartum women without depression.

Table 1 Metabolites responsible for separating PPD subjects from HC and PPWD subjects

No	Metabolite	P-value[a]	P-value[b]	VIP[c]	Fold change[d]
1	Formate	3.64E–08	2.48E–06	1.486	1.48
2	Succinate	9.28E–07	6.31E–05	1.526	1.19
3	3-Methylhistidine	1.96E–09	1.33E–07	1.213	1.89
4	Glutamate	3.09E–07	2.10E–05	1.281	0.74
5	Isobutyrate	3.85E–08	2.62E–06	1.012	1.06
6	Acetamide	8.73E–04	5.94E–02	1.012	0.65
7	α-Ketoglutarate	2.71E–03	1.84E–01	1.025	0.56
8	Quinolinic acid	6.66E–09	4.53E–07	1.032	1.25
9	Phenylacetyglycine	2.23E–08	1.52E–06	1.036	0.50
10	Pyroglutamic acid	2.54E–03	1.73E–01	1.068	0.54
11	Hippurate	7.29E–09	4.96E–07	1.103	2.63
12	1-Methylhistidine	4.27E–11	2.90E–09	1.132	0.98
13	Valine	4.09E–08	2.78E–06	1.286	0.83
14	m-Hydroxyphenylacetate	7.20E–09	4.90E–07	1.447	1.75
15	α-Hydroxyisobutyrate	8.74E–07	5.94E–05	1.135	–0.77
16	N-Methylnicotimide	3.20E–05	2.18E–03	1.181	–0.52
17	Gycolate	6.99E–04	4.75E–02	1.187	–0.47
18	Acetate	1.29E–07	8.77E–06	1.196	–0.44
19	Taurine	4.80E–10	3.26E–08	1.208	–0.61
20	Lactate	3.74E–07	2.54E–05	1.466	–0.32
21	α-Glucose	1.15E–03	7.85E–02	1.48	–0.85
22	Dimethylamine	1.59E–3	1.08E–01	1.878	–1.08

Notes: [a]P-values were derived from nonparametric Mann–Whitney U-test. [b]P-values were derived from Bonferroni corrections. [c]VIP was obtained from OPLS-DA with a threshold of 1.0. [d]Positive values indicate higher levels in PPD subjects, and negative values indicate lower levels in PPD subjects.
Abbreviations: PPD, postpartum depression; HC, healthy control; PPWD, postpartum women without depression; VIP, variable importance plot; OPLS-DA, orthogonal partial least-squares-discriminant analysis.

and a specificity of 90.5% in the testing set. To assess the diagnostic performance of this panel, the ROC curve analysis was conducted to calculate the area under the curve (AUC) of this panel in both training and testing sets. This analysis showed that the AUC of this panel was 0.948 and 0.944 in the training and testing sets (Figure 4), respectively.
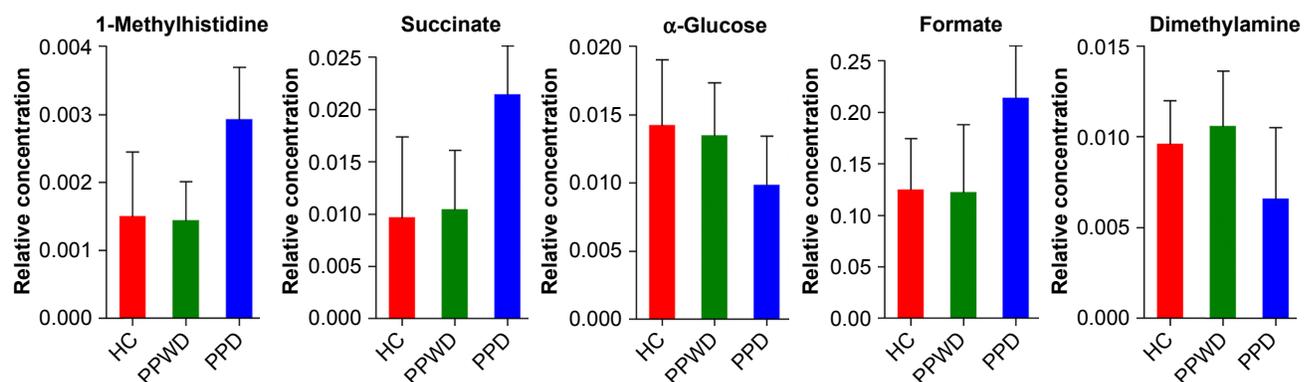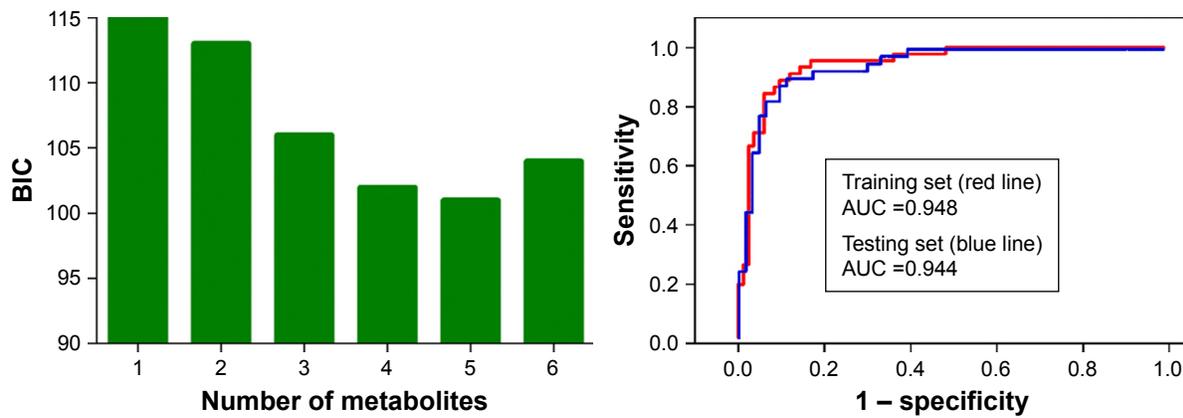
These results indicated that the potential biomarker panel consisting of these five metabolites could be viewed as a potential effective classifier of PPD subjects and other subjects (PPWD and HCs).

Pathway analysis

The online software MetaboAnalyst 3.0 was used to further analyze the biological functions of these differential urinary metabolites.[23] The following criteria were applied to select the significantly altered metabolic pathways: $P < 0.01$ and impact > 0. Finally, seven metabolic pathways were significantly altered: glyoxylate and dicarboxylate metabolism ($P = 0.0004$; impact $= 0.1504$), D-glutamine and D-glutamate metabolism ($P = 0.0027$; impact $= 0.3262$), phenylalanine metabolism ($P = 0.0040$; impact $= 0.0315$), TAURINE and hypotaurine metabolism ($P = 0.0093$; impact $= 0.339$), pyruvate metabolism ($P = 0.0015$; impact $= 0.2370$), propanoate metabolism ($P = 0.0019$; impact $= 0.0013$) and tricarboxylic acid (TCA) cycle ($P = 0.0093$; impact $= 0.1002$) (Figure 5).

Discussion

Here, the GC-MS-based metabolomic platform was used to study the divergent urinary metabolic phenotypes between PPD subjects and the other subjects (PPWD and HCs). At last, the levels of 22 urinary metabolites in PPD subjects were significantly changed. These differential metabolites could effectively separate the PPD subjects from the PPWD and HCs. Further analysis identified a potential biomarker panel consisting of five potential biomarkers – formate, succinate, 1-methylhistidine, α-glucose and dimethylamine. The diagnostic performance of this panel was similar to the built OPLS-DA model with the 22 differential metabolites. This panel could yield an AUC of 0.948 in the training set and


Figure 3 Five metabolites in the potential biomarker panel.
Abbreviations: HC, healthy control; PPD, postpartum women with depression; PPWD, postpartum women without depression.

Figure 4 Bayesian information criterion rule (**A**) and ROC curve analysis (**B**).
Abbreviations: BIC, Bayesian information criterion; ROC, receiver-operating characteristic; AUC, area under the curve.

0.944 in the testing set, which was significantly higher than that of the currently used methods. These results demonstrated that these differential urinary metabolites might be biologically correlated with depression in postpartum women, and

the panel could be helpful for future development of an objective diagnostic method for PPD.

The underlying mechanisms of PPD are still unclear. Brummelte and Galea[24] reported that the steroid hormones
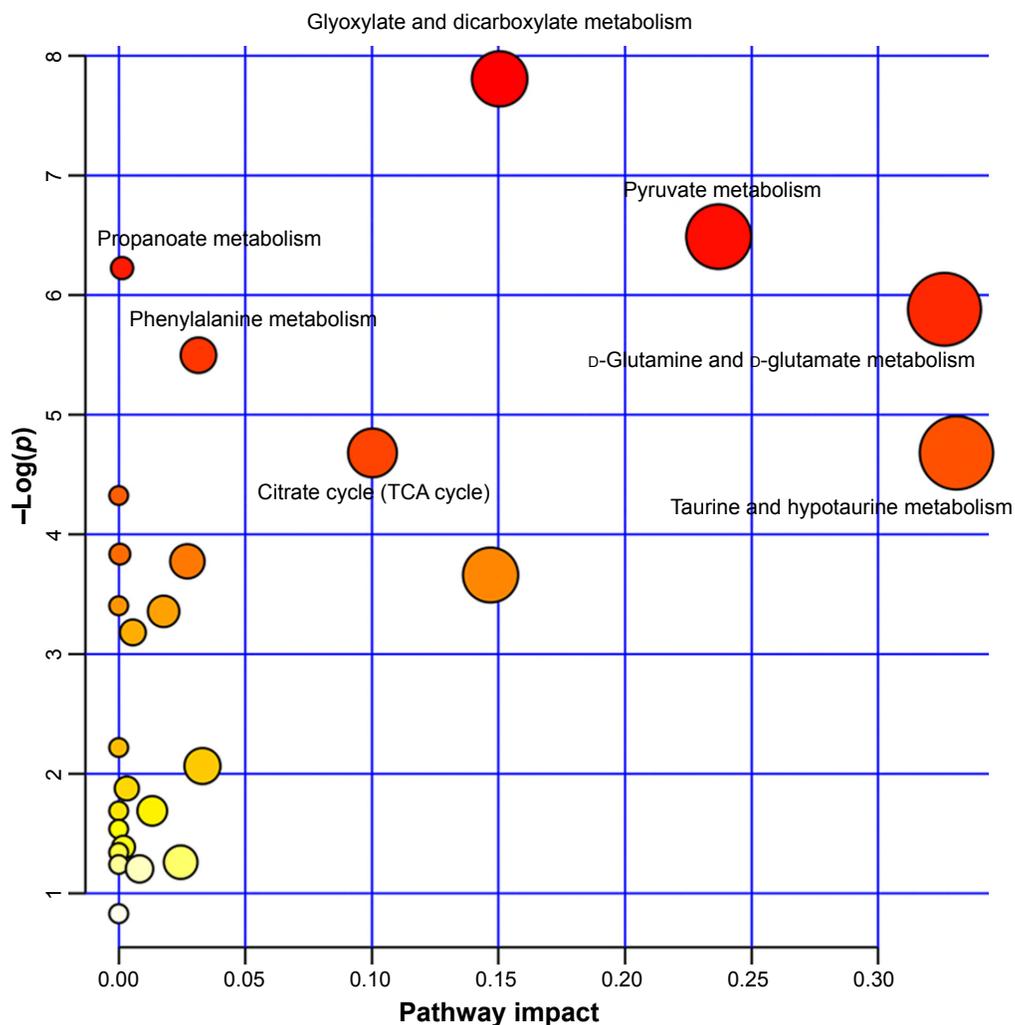
Figure 5 Significantly altered metabolic pathways in PPD subjects.
Abbreviations: PPD, postpartum depression; TCA, tricarboxylic acid.

might have a significant role in PPD.[24] They thought that the dramatically fluctuated steroid and peptide hormones levels during pregnancy and postpartum might contribute to the etiology of PPD. Glynn et al[25] found that the sustained high flattened levels of glucocorticoids was presented in postpartum women. This similar hormonal profile was also observed in depressed patients, which might indicate a possible underlying mechanism of PPD.[7] Recently, researchers have paid much attention to the nonapeptide oxytocin for its potential involvement in depression.[26] Stuebe et al[27] found a lower plasma oxytocin concentration in the last trimester of women at risk of PPD. Besides these findings, an animal study showed that there was disorder and imbalance of metabolites in the brain of rat with PPD.[28] In this study, the urinary metabolic disorder in PPD subjects was found, which could provide new insight and original data for investigating the mechanisms of PPD.

Notably, the levels of α-ketoglutarate, pyroglutamic acid, acetamide, α-glucose and dimethylamine were not significantly perturbed in the univariate analysis. However, the built OLPS-DA model still treated these metabolites as key urinary metabolites. Moreover, α-glucose and dimethylamine was identified as potential biomarkers by logistic-regression analysis. This was done because the multivariate analysis showed that the addition of α-glucose and dimethylamine could result in the highest discrimination power. These results demonstrated that the multivariate statistical approach had an advantage than simple univariate analysis in identifying the subtle yet significantly differential metabolites.[15,29] Additionally, Zheng et al[18] found a potential biomarker panel for diagnosing depression using GC-MS-based metabolomic method. But this panel could only accurately predict 41.1% PPD subjects, which was significantly lower than the prediction accuracy of our panel identified in this study. Two reasons might cause this difference: 1) the panel identified by Zheng et al[18] was used to diagnose depression and 2) all included subjects here were women, but the panel identified by Zheng et al[18] did not take sex-based differences into consideration, since many studies reported that there were sex differences in depression.[30–32]

A previous study reported that the levels of some TCA cycle-associated metabolites were significantly perturbed in patients with MDD.[33] Here, we found that the TCA cycle was significantly altered in patients with PPD. Hasler et al[34] found reduced prefrontal glutamate/glutamine level in depressed patients, which was similar to our findings that the D-glutamine and D-glutamate metabolism was affected in depressed postpartum women. Meanwhile, the other five significantly altered metabolic pathways were associated

with psychiatric disorders,[16,30,35–37] such as depression and bipolar disorder. Additionally, Dowlati et al[38] reported that the dietary supplement of tryptophan and tyrosine in early postpartum could reduce PPD. The abovementioned results showed that the depression could affect the urinary metabolite levels of postpartum women, and these identified metabolic pathways might be potential targets for novel therapeutics for PPD in the future.

Limitations

There were several limitations in this study: 1) the number of recruited subjects here were relatively small; 2) the subjects were recruited from the same place, which might limit the applicability of our conclusions; 3) given that no single analytic method could capture the whole metabolome of a sample,[39] future researchers should apply two or more analytic methods to verify and support our results; 4) future studies are needed to find out whether this panel could effectively separate PPD from postpartum women with other neuropsychiatric disorders; 5) the hypothalamic pituitary adrenal axis is intimately linked to the etiology of depression, then cerebrospinal fluid from PPD subjects is needed to ensure that our urinary biomarkers were physiologically relevant to the disease pathogenesis; and 6) the significantly altered metabolic pathways are needed to be further validated.

Conclusion

This study showed that there was a significantly divergent urinary metabolic phenotype in PPD subjects, and the GC-MS-based urinary metabolomics approach had the potential ability to become an objective diagnostic method for diagnosing PPD. Moreover, a potential biomarker panel consisting of formate, succinate, 1-methylhistidine, α-glucose and dimethylamine was identified, which could aid in the future development of an objective diagnostic method for PPD.

Disclosure

The authors report no conflicts of interest in this work.

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