



Serum metabonomics study of pregnant women with gestational diabetes mellitus based on LC-MS

Genxia Li, Wanli Gao, Yajuan Xu, Mingkun Xie, Suhua Tang, Pan Yin, Shuhua Guo, Shuhui Chu, Shaima Sultana, Shihong Cui*

Obstetrics Department, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

ARTICLE INFO

Article history:

Received 2 August 2019
Revised 11 September 2019
Accepted 12 September 2019
Available online 13 September 2019

Keywords:

Gestational diabetes mellitus
Metabolomics
Serum
Liquid chromatography-mass spectrometry
Metabolic pathway analysis

ABSTRACT

Objective: Through metabolomics method, the objective of the paper is to differentially screen serum metabolites of GDM patients and healthy pregnant women, to explore potential biomarkers of GDM and analyze related pathways, and to explain the potential mechanism and biological significance of GDM.

Methods: The serum samples from 30 GDM patients and 30 healthy pregnant women were selected to conduct non-targeted metabolomics study by liquid chromatography-mass spectrometry. The differential metabolites between the two groups were searched and the metabolic pathway was analyzed by KEGG database.

Results: Multivariate statistical analysis found that serum metabolism in GDM patients was different significantly from healthy pregnant women, 36 differential metabolites and corresponding metabolic pathways were identified in serum, which involved several metabolic ways like, fatty acid metabolism, butyric acid metabolism, bile secretion, and amino acid metabolism.

Conclusion: The discovery of these biomarkers provided a new theoretical basis and experimental basis for further study of the early diagnosis and pathogenesis of GDM. At the same time, LC-MS-based serum metabolomics methods also showed great application values in disease diagnosis and mechanism research.

© 2019 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Gestational diabetes mellitus (GDM) is a disorder of glucose metabolism, which usually happens in the third to sixth month or sixth to ninth month of pregnancy and a common pregnancy complication (Weinert, 2010). There is still no clear report on the pathogenesis of GDM. Some research have shown that female with GDM are of higher risk in suffering from type 2 diabetes after childbirth, which may cause insulin resistance (IR) and dyslipidemia, etc. So far, the identified risk factors for GDM are obesity, unhealthy eating habits, and lack of physical activity (Hara et al., 2002). Timely detection of GDM and control of blood glucose levels

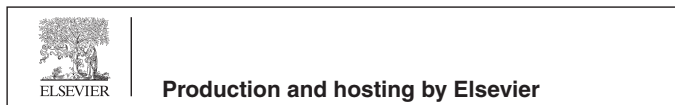
can effectively reduce maternal and child complications (Shaikh et al., 2016). At present, the screening of GDM is mainly through the detection of blood sugar changes before and after the pregnant women taking sugar between 24 and 28 weeks of gestation (Yang, 2013) but this method is relatively late for the diagnosis of patients, which seriously threatens the health of mothers and children. It is therefore necessary to find some new early biodiagnostic markers.

As a metabolic disorder during gestation period, GDM is very suitable for research by adopting metabolomics. Followed by genomics, transcriptomics and proteomics, metabonomics is another important life science research method (Kell, 2004). Metabolomics as a comprehensive research model proposed by Nicholson et al is widely used in the process of diseases pathological, clinical diagnosis, and evaluation of efficacy (Nicholson et al., 2011), which helps people more comprehensively to understand the changing laws of metabolic substances in the development process of disease (Nicholson et al., 2002). Because the blood component contains almost all of the body's metabolites, it can reflect the physiological and biochemical state of the body more comprehensively, so it is

* Corresponding author.

E-mail address: shihongcui@126.com (S. Cui).

Peer review under responsibility of King Saud University.



widely used by researchers in metabolomics analysis (Nicholson et al., 2008). Metabolomic analysis of blood components in GDM patients and normal controls can reveal the changes in metabolites in GDM patients. In the diagnosis of pregnancy diseases, the analysis of metabolite components in the blood also has great clinical significance, for example, adiponectin, reactive protein, etc. can cause hyperglycemia, which further leads to the occurrence of GDM, and to some extent they can make prediction for the occurrence of early GDM (Sawidou et al., 2010).

Clinically existing methods for diagnosing GDM are still incomplete and can easily lead to missed diagnosis and misdiagnosis for patients. Although some literatures have been reported on the application of metabolomics methods in GDM-related fields, and some methods have been used to analyze potential markers of metabolic in GDM, unfortunately results are not completely consistent (Jamil et al., 2016). This study focused on GDM metabolomics research, based on the LC-MS analysis method, to conduct metabolomics analysis of serum of GDM patients and healthy pregnant women. Combined with correlation network analysis, this paper screened differential ions and explored landmark differential metabolites of GDM patients. The metabolic pathways involved were analyzed and the possible mechanism of action and biological significance were explained, which provided data support and theoretical basis for further research on early diagnosis of GDM and exploration of its mechanism.

2. Materials and methods

2.1. Main instruments and reagents

The main instruments and reagents used in this study were listed as follows: Xevo G2-XS QTOF mass spectrometer (produced by Waters, UK), 2777C UPLC system liquid chromatograph (produced by Waters, UK), high speed centrifuge (produced by Thermo, USA), ultrapure water preparation system (produced by Milipore, USA), ACQUITY UPLC CSH C18 chromatographic column (100 mm*2.1 mm, 1.7 μm, produced by Waters, UK), isopropanol (produced by Biotech Engineering Co., Ltd.), ammonium formate (chromatographically pure, produced by DIKMA, USA), acetonitrile (chromatographically pure, produced by Yucheng Chemical Plant of Shandong Yuwang Industrial Co., Ltd.), formic acid (chromatographically pure, produced by TEDIA).

2.2. Sample collection

All samples in this study were obtained from the obstetrics department of the Third Affiliated Hospital of Zhengzhou University, including 30 healthy pregnant women (control group/health

group) and 30 GDM pregnant women (GDM group). GDM was diagnosed using the 2010 guidelines of International Association of the Diabetes and Pregnancy Study Group (IADPSG). (1) The first examination of fasting blood glucose during pregnancy was ≥ 5.1 mmol/L, and < 7.0 mmol/L. (2) During 24–28 weeks, 75 g glucose was adopted for 2 h to conduct Oral Glucose Tolerance Test (OGTT): fasting blood glucose ≥ 5.1 mmol/L, the blood glucose after 1 h ≥ 10.0 mmol/L, the blood glucose after 2 h ≥ 8.5 mmol/L. GDM can be diagnosed if one or both of the above two criteria meet or exceed. Blood samples were collected at 24–28 weeks of gestation and then sent to Wuhan BGI Diagnosis Co., Ltd. for metabolomics testing. Outpatient examination information were collected, such as age, BMI and other clinical data, and statistical analysis was conducted, as shown in Table 1. The research was reviewed by the Ethics Committee of the Third Affiliated Hospital, and all participants signed the consent form.

2.3. Sample pretreatment

First, the serum samples were used for metabolite extraction. This experiment adopted the organic reagent precipitation protein method, and the QC sample was prepared, and then the extracted sample was detected (Khattab et al., 2016). There were 2 groups of samples to be tested, and 30 biological replicates in each group. The samples stored at low temperature were thawed and sorted, 40 μL of each sample was sequentially added to the corresponding EP tube, 120 μL of cold isopropanol was added, sealed, and shaken and mixed. After standing for 10 min in room temperature, it was put into a refrigerator at -20 °C overnight. At 2000 g and 4 °C for 40 min, all samples were centrifuged and 25 μL of the supernatant was added to new EP tubes respectively, and then 225 μL of each lipid solution was added for dilution. 20 μL of each experimental sample was mixed into a quality control (QC) sample. 60 μL of all samples were sequentially tested in the order of loading, and one QC sample was inserted into every 10 samples in the separation to evaluate the reliability of the experimental data and the stability of the state of the monitoring instrument.

2.4. Chromatographic conditions

Set column temperature and flow rate set to 55 °C and 0.4 mL/min respectively, then the ACQUITY UPLC CSH C18 chromatographic column was used to perform the separation. In that, mobile phase A was ACN (acetonitrile): H₂O = 60:40, 10 mM ammonium formate and 0.1% formic acid (FA); mobile phase B was IPA (isopropanol): ACN = 90:10, 0.1% FA and 10 mM ammonium formate. The metabolites were eluted as follows: 0–2 min, 40–43% mobile phase B; 2.1–7 min, 50–54% mobile phase B; 7.1–13 min, 70–99%

Table 1
Comparison of general data between the two groups ($\bar{x} \pm s$).

General condition	GDM group (n = 30)	Control group (n = 30)	P value
Age	28.8 ± 3.01	28.53 ± 3.4	0.749
Pre-pregnancy BMI (Kg/m ²)	23.57 ± 1.72	22.54 ± 2.08	0.04
OGTT- fasting blood glucose (mmol/L)	5.71 ± 0.55	4.66 ± 0.24	<0.001
OGTT – blood glucose after 1 h (mmol/L)	10.36 ± 1.77	6.77 ± 1.21	<0.001
OGTT – blood glucose after 2 h (mmol/L)	8.72 ± 1.42	6.27 ± 0.72	<0.001
Fasting insulin (um/L)	10.99 ± 4.72	6.94 ± 1.11	<0.001
Insulin resistance index	2.78 ± 1.20	1.44 ± 0.25	<0.001
glycosylated hemoglobin (HbA1c, mmol/ml)	5.35 ± 0.18	4.95 ± 0.25	<0.001
Triglyceride (TG, mmol/L)	2.73 ± 0.63	2.64 ± 0.9	0.658
Total cholesterol (CHOL, mmol/L)	6.17 ± 1.15	4.88 ± 0.59	<0.001
High density lipoprotein (HDL, mmol/L)	1.83 ± 0.26	2.09 ± 0.31	<0.001
Low density lipoprotein (LDL, mmol/L)	3.13 ± 0.64	2.59 ± 0.55	<0.001

Note: insulin resistance index = fasting insulin × fasting blood glucose ÷ 22.5.

mobile phase B; 13.1–15 min, 40% mobile phase B. Each sample was taken 10 μ L of it out. In the chromatographic separation process, the samples are continuously analyzed in a random sequence to avoid the influence of the experimental instrument detection signal fluctuations on the results.

2.5. Mass spectrometry conditions

The high-resolution tandem mass spectrometer, Xevo G2-XS QTOF, was used to collect the small molecules eluted from the chromatographic column in positive and negative ion modes (Li et al., 2019). The cone voltage and capillary voltage were set to 40.0 V and 3.0 kV in positive ion mode and 40.0 V and 2.0 kV in negative ion mode respectively. The centroid data were collected in MSE mode. When the first-level scan is performed, the time was set to 0.2 s, the positive and negative ion ranges were 100–2000 Da and 50–2000 Da, respectively, all the parent ions were fragmented with energy of 19–45 eV and the fragment information was collected.

2.6. Data processing and statistical analysis

The Xevo G2-XS QTOF mass spectrometer was adopted to collect the raw mass spectral data in the samples in different modes, and then the data were imported into Progenesis Q1 software for peak extraction. The operation flow mainly included three steps: peak alignment, peak extraction and peak identification. Data pre-processing was performed using the R software package metaX. to obtain a two-dimensional data matrix of peak area, retention time and mass-to-charge ratio of all ions, and corrected by QC-RLSC method. Then all the data were analyzed by PCA and PLS-DA to screen the differential ions. The PLS-DA model was used to calculate the influence intensity and explanatory ability of each metabolite expression pattern on the classification and discrimination of each group of samples by calculating the VIP value. VIP, also known as Variable Important for the Projection, usually $VIP \geq 1.0$ was regarded as an auxiliary condition for screening for metabolic markers. Identification of biomarkers and analysis of metabolic pathways were performed using the biological databases HMDB and KEGG. Statistical analysis was performed by statistical software SPSS21.0, and *t*-test was used to compare samples, $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Univariate analysis

Univariate analysis of the data was conducted through fold change analysis and independent sample *T* test. After *t*-test analysis, the difference between GDM group and healthy group was of statistical significance ($P < 0.05$). After *t*-test analysis of the genes differentially expressed between the two samples, Volcano plot was drawn with the \log_2 (fold change) regarded as the abscissa and the $-\log_{10}$ (*P* value) regarded as the ordinate. According to the screening condition of Fold change ≤ 0.8333 or ≥ 1.2 , *P* value < 0.05 , differential metabolites were screened. The results in the final positive and negative ion mode were shown in Fig. 1. Each point in the figure represented a metabolite, the red point was a significant differential metabolite, and the remaining points were blue.

3.2. Principal component analysis (PCA)

Unsupervised PCA analysis of the samples of health group and GDM group, the scores of the first two principal components PC1 ~ PC2 obtained in positive and negative ion mode were displayed in Fig. 2. In the positive ion mode, the most of sample points of the two groups overlapped in the two-dimensional PCA dot pattern, but there were still differences in the second principal component, and some abnormal points were separated, indicating that the metabolites of the GDM group and health group had some differences.

Note: One point in the figure corresponded to one sample, blue represented the quality control group, green represented the health group, and red represented the GDM group.

3.3. Partial least squares discriminant analysis (PLS-DA)

For purpose of further verify the statistical difference between GDM group and normal pregnant group, this study used the supervised PLS-DA multivariate method to re-model and analyze the two sets of data, 200 permutation tests were conducted for the model parameters R^2 and Q^2 , to confirm the reliability of the model. The scores of the first two major components PC1-PC2 obtained in the positive and negative ion modes were display in Fig. 3. R^2 and Q^2 respectively represented the interpretation rate

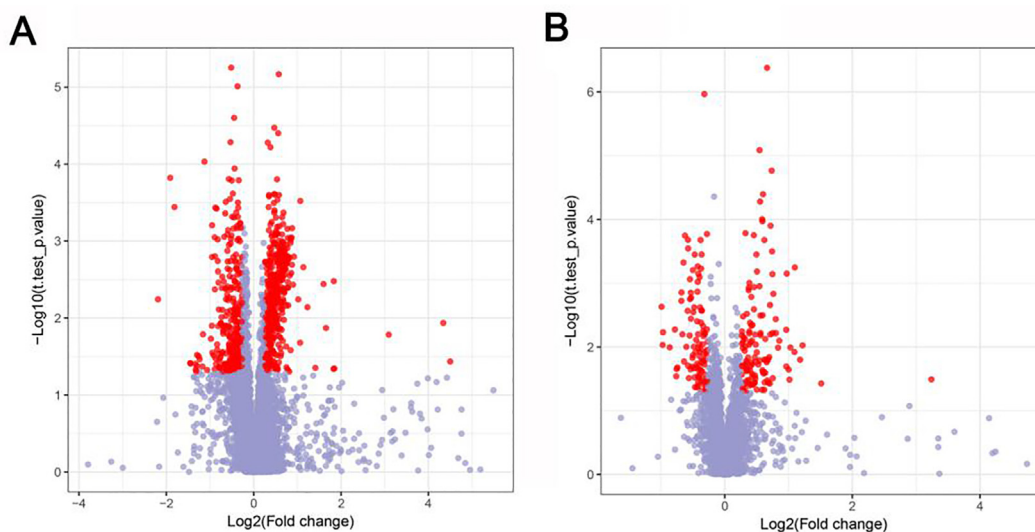


Fig. 1. Volcano plot.

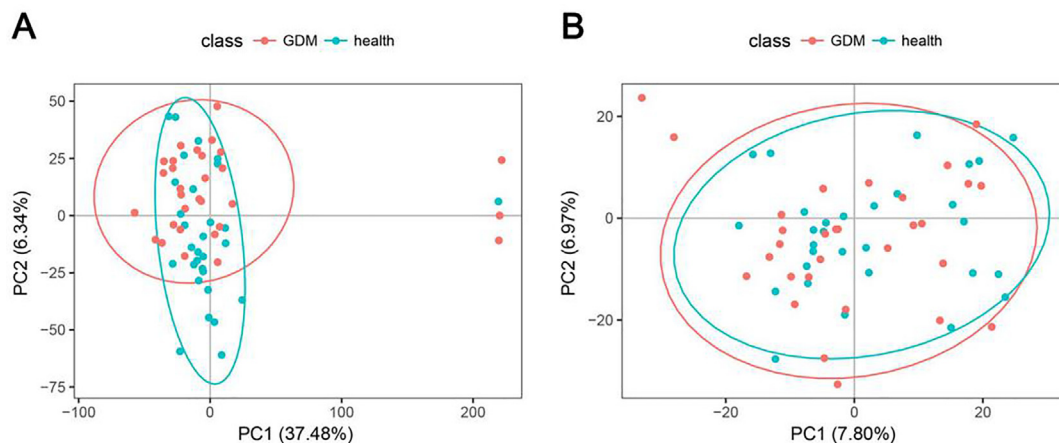


Fig. 2. Principal component analysis model.

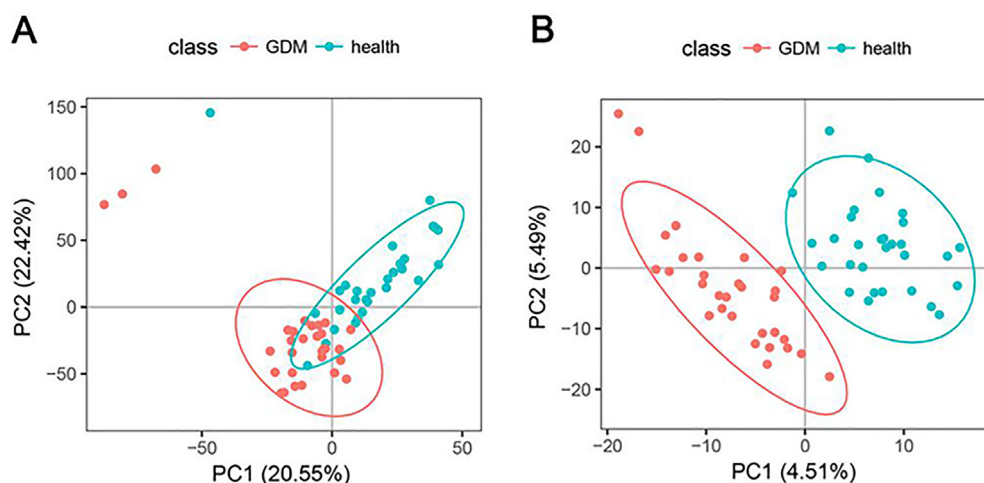


Fig. 3. PLS-DA model. Note: One point in the figure corresponded to one sample, green represented the health group, and red represented the GDM group.

and prediction rate of the model. The results showed that most samples between the two groups were separated in positive ion mode, $R^2 = 0.605$, $Q^2 = 0.208$; in the negative mode, the two groups completely separated, $R^2 = 0.870$, $Q^2 = 0.425$. Theoretically, the closer R^2 and Q^2 are to one, the better the effect is, and the more stable and reliable the model is. However, the clinical samples are large and uncontrollable due to individual differences, especially for large samples, it is acceptable when the values of R^2 and Q^2 are 0.2 approximately. In this study, both R^2 and Q^2 values in the positive and negative modes were greater than 0.2, indicating that the model quality was good and the stable prediction rate was high. Through permutation test, it was found that the model did not show a fitting, which indicated that the model was reliable.

3.4. Differential ion screening and cluster analysis

The VIP values of the first two principal components of the PLS-DA model were analyzed by multivariate analysis, combined with P values and fold-change (differential multiples) to screen differential metabolites. Screening conditions: (1) P-value < 0.05; (2) $VIP \geq 1$; (3) fold-change ≤ 0.8333 or ≥ 1.2 , taking the intersection of the three to obtain the shared ion, which is the differential ion. Compared with the healthy group, the GDM group identified

719 differential ions in positive ion mode, and 206 differential metabolites were identified by two-stage mass spectrometry; 227 differential ions were identified under negative ions, and 38 differential metabolites were identified by two-stage mass spectrometry. On behalf of more intuitively and comprehensively display the relationship between the samples, and to evaluate the rationality of the candidate metabolites and the differences in the expression patterns of the metabolites in different samples, this paper used a qualitatively significant difference in the amount of metabolite expression to perform hierarchical clustering analysis of each group of samples. Fig. 4 showed the hierarchical clustering of significant differences between two groups in positive and negative ion mode. The results demonstrated that serum differential metabolites between the two groups had a certain clustering trend.

3.5. Identification of potential biomarkers

The above-selected differential ions were searched for the exact molecular weight of the difference by online database HMDB, and the mass spectrometry information was used to identify the substances with significant differences, At the same time, according to the contribution degree of the compound to the group (VIP value), combined with clinical and biological significance, 36 com-

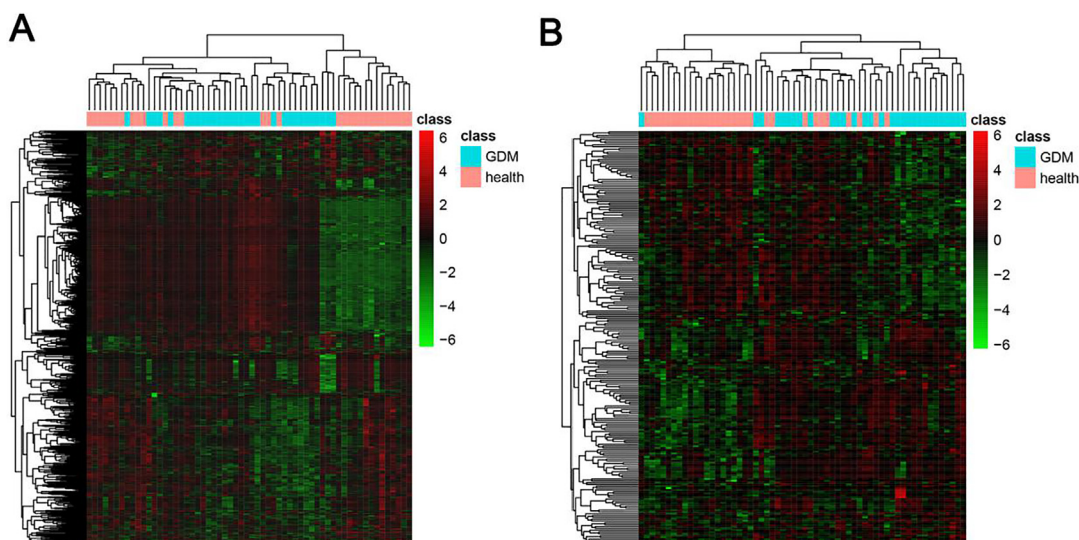


Fig. 4. Differential ion clustering analysis. Note: Each row in the graph represented a differential ion, and each column represented a sample. The different colors represented different intensities, of which the green meant intensity was low and the red meant intensity was high.

Table 2

Identification results of potential biomarkers.

Mode	Retention time /min	m/z	ratio	VIP	Differential metabolites	Class
+	1.18	370.2356	1.48	2.15	TXB2	Fatty acyls
+	1.20	228.1355	1.42	1.91	Traumatic acid	Fatty acyls
+	2.13	357.2045	1.42	1.66	PGC2	Fatty acyls
+	2.13	357.2045	1.42	1.66	PGJ2	Fatty acyls
+	2.13	357.2045	1.42	1.66	PGB2	Fatty acyls
+	2.13	357.2045	1.42	1.66	PGA2	Fatty acyls
+	1.72	425.2542	1.72	3.34	Pravastatin	Fatty acyls
+	2.82	395.2153	1.49	2.13	PGD2-d4	Fatty acyls
+	2.82	395.2153	1.49	2.13	PGE2-d4	Fatty acyls
+	3.64	874.1028	1.68	1.86	Crotonoyl-CoA	Fatty acyls
+	3.64	874.1028	1.68	1.86	Methacrylyl-CoA	Fatty acyls
+	6.12	386.2537	0.66	1.86	PGG2	Fatty acyls
+	6.12	386.2537	0.66	1.86	6-keto PGE1	Fatty acyls
+	6.12	386.2537	0.66	1.86	11-dehydro-TXB2	Fatty acyls
-	0.60	103.0395	1.66	3.34	2S-Hydroxybutanoic acid	Fatty acyls
-	0.60	103.0395	1.66	3.34	D(-)-beta-hydroxy butyric acid	Fatty acyls
-	0.60	103.0395	1.66	3.34	4-hydroxy-butyrac acid	Fatty acyls
-	2.00	329.2477	2.13	2.40	DPA	Fatty acyls
-	2.72	281.2477	1.50	2.90	Oleic acid	Fatty acyls
±	8.31	263.2367	1.31	1.33	Rumenic acid	Fatty acyls
±	8.31	263.2367	1.31	1.33	Linoleic acid	Fatty acyls
+	1.23	347.2215	1.45	2.27	Urocortisone	Sterol lipids
+	1.23	347.2215	1.45	2.27	corticosterone	Sterol lipids
+	1.23	347.2215	1.45	2.27	11-deoxycortisol	Sterol lipids
+	1.28	349.2376	1.23	1.29	Tetrahydrocortisol	Sterol lipids
+	1.34	287.1637	1.53	2.28	2-Hydroxyestrone	Sterol lipids
+	9.58	369.3517	0.69	1.38	Cholesterol	Sterol lipids
+	9.58	369.3517	0.69	1.38	Lathosterol	Sterol lipids
-	1.90	353.1419	1.54	2.14	Dehydroepiandrosterone sulfate	Sterol lipids
-	2.72	49.2352	1.74	2.93	Tetrahydrocorticosterone	Sterol lipids
+	1.69	411.2525	1.71	4.03	LPA (0:0/16:0)	Glycerophospholipids
-	1.01	528.3087	1.35	1.95	LysoPC (20:4)	Glycerophospholipids
+	1.29	461.3335	1.41	2.02	Psychosine	Sphingolipids
+	9.00	880.7184	0.80	1.51	Coenzyme Q10	Prenol lipids
+	6.96	569.4366	0.61	2.85	Lutein	Prenol lipids
+	6.96	569.4366	0.61	2.85	Zeaxanthin	Prenol lipids

Note: mode was ion detection mode, “+” was positive ion mode, “-” was negative ion mode, “±” was common to positive and negative ion mode; ratio was fold change (differential multiple), according to the sample in the file, the ratio between the two groups (GDM group/health group) was obtained, for instance, 1:2, and its ratio = 1/2.

pounds with the largest difference between the groups were selected as potential biomarkers. The identification results of these potential markers such as mass-to-charge ratio and VIP value were

shown in Table 2. These 36 metabolites were mainly classified into several major categories: prenel lipids, sterol lipids, fatty acyls, sphingolipids, glycerophospholipids, etc.

Table 3
Analysis of potential biomarker pathways.

Differential metabolites	Pathway	KEGG. ID	Change direction
TXB2	Arachidonic acid metabolism; bile secretion	C05963	↑
Traumatic acid	Linolenic acid metabolism	C16308	↑
PGC2	Arachidonic acid metabolism	C05955	↑
PGJ2	Arachidonic acid metabolism	C05957	↑
PGB2	Arachidonic acid metabolism	C05954	↑
PGA2	Arachidonic acid metabolism	C05953	↑
Pravastatin	Bile secretion	C01844	↑
PGD2-d4	Arachidonic acid metabolism	C00696	↑
PGE2-d4	Arachidonic acid metabolism	C00584	↑
Crotonoyl-CoA	Amino acid metabolism; Butyric acid metabolism; fatty acid metabolism	C00877	↑
Methacrylyl-CoA	Amino acid metabolism	C03460	↑
PGG2	Arachidonic acid metabolism	C05956	↓
6-keto PGE1	Arachidonic acid metabolism	C05962	↓
11-dehydro-TXB2	Arachidonic acid metabolism	C05964	↓
2S-Hydroxybutanoic acid	Propionic acid metabolism	C05984	↑
D(-)-beta-hydroxy butyric acid	Butyric acid metabolism	C01089	↑
4-hydroxy-butyric acid	Butyric acid metabolism	C00989	↑
DPA	Biosynthesis of unsaturated fatty acids	C16513	↑
Oleic acid	Fatty acid biosynthesis	C00712	↑
Rumenic acid	Linoleic acid metabolism	C04056	↑
Linoleic acid	Linoleic acid metabolism	C01595	↑
Urocortisone	Steroid hormone biosynthesis	C05470	↑
corticosterone	Steroid hormone biosynthesis	C02140	↑
11-deoxycortisol	Steroid hormone biosynthesis	C05488	↑
Tetrahydrocortisol	Steroid hormone biosynthesis	C05472	↑
2-Hydroxyestrone	Steroid hormone biosynthesis	C05298	↑
Cholesterol	Lipid metabolism; bile secretion	C00187	↓
Lathosterol	Steroid biosynthesis	C01189	↓
Dehydroepiandrosterone sulfate	Steroid hormone biosynthesis; bile secretion	C04555	↓
Tetrahydrocorticosterone	Steroid biosynthesis	C05476	↑
LPA(0:0/16:0)	Glycerolipid metabolism; Glycerolphospholipid metabolism	C00416	↑
LysoPC(20:4)	Glycerolphospholipid metabolism	C04230	↑
Psychosine	Sphingolipid metabolism	C01747	↑
Coenzyme Q10	Ubiquinone and other biosynthesis	C00399	↓
Lutein	Metabolic pathway	C08601	↓
Zeaxanthin	Metabolic pathway	C06098	↓

Note: Pathway is the path name, and KEGG. ID is the serial number of the metabolite in the KEGG database.

3.6. Analysis of potential biomarker pathways

Metabolic pathway analysis is a very important part of metabolomics research, which helps to understand the signaling pathways and metabolic pathways involved in metabolites, and then related metabolites and genes were explored (Nielsen and Jewett, 2007). This study was based on the KEGG database to analyze the metabolic pathways and content changes of the potential biomarkers obtained, as shown in Table 3. The results showed that the 36 potential markers obtained in GDM group and health group were involved in multiple pathways, mainly in pathways such as fatty acid metabolism, metabolism of amino acid, steroid hormone biosynthesis, metabolism of arachidonic acid, butyric acid metabolism and bile secretion.

4. Discussion

Gestational diabetes mellitus (GDM) is the first type of diabetes discovered or occurred during pregnancy, which is a common medical complication during pregnancy and extremely harmful to mothers and children. Non-targeted metabolomics based on LC-MS technology can analyze the metabolic information and pathogenic mechanism of gestational diabetes from a comprehensive and holistic perspective, providing a new analytical method for the pathogenesis of GDM (Sun, 2017). At present, metabolomics has been increasingly used in the search of biomarkers for the diagnosis of diseases. Although there are more and more studies on pregnancy-related diseases in metabolomics, but only related mar-

ker differential metabolites were selected in most cases. The relevant metabolic pathways of these metabolites have not been studied in depth, and the holistic metabolic network has not been constructed for such disease (Syngelaki et al., 2015). Due to the complex pathogenesis of GDM, there are no known markers for early prediction of GDM, and no single indicator can be used for high-specific screening in patients who meet the GDM diagnostic criteria. In order to increase the ability to predict GDM, most studies have tried to use a combination of serological and clinical indicators, but the effect is still limited (Abell et al., 2015), and as a screening method, it may obviously increase the cost of medical care, which is relative infeasible.

Different researchers described metabolomics analysis of possible GDM marker metabolites, but their results did not show consistency (Liu et al., 2010). Wang argued that the metabolism of tryptophan was affected when GDM occurred, which was very similar to the results of Law, Bentley-Lewis and Daniel studied the early serum of GDM patients and normal pregnant women, respectively, they both found that the amino acid metabolites of GDM patients in serum were different from those of normal pregnant women, but the differential metabolites in these two studies were not completely the same, which may be due to different sample collection periods or different gestational weeks in the two groups. In this study, a metabolomics method based on LC-MS was used to analyze the metabolic profile changes between the samples of GDM group and health group. A total of 36 potential biomarkers and their multiple metabolic pathways involved were obtained by screening differential metabolites and performing KEGG metabolic pathway analysis, which were primarily concentrated in fatty

acid metabolism, metabolism of amino acid, steroid hormone biosynthesis, arachidonic acid metabolism, butyric acid metabolism and bile secretion. It indicated that GDM not only affected the metabolism of organism lipids, amino acids and sugars, but also caused disturbances in other metabolic systems, which was consistent with previous studies (Zhou et al., 2014).

Using isotopically labeled tandem mass spectrometry proposed by Enquobahrie et al. the researchers measured several levels of sterol hormones in the blood during pregnancy and found that levels of 11-deoxycortisol, 17 α -hydroxyprogesterone, and progesterone increased throughout the whole period of pregnancy. Cortisol and androstenedione levels remained stable after an increase in early pregnancy, whereas dehydroepiandrosterone sulfate levels decreased during the third trimester (Enquobahrie et al., 2015). In this study, the level of 11-deoxycortisol in GDM group was also higher than that in normal group, which was consistent with the study. However, the level of dehydroepiandrosterone sulfate was also high in the GDM group, which may be related to the characteristics of the population and the specimen collected from the period of pregnancy, the type of sample, and the applied method were different. Daniel et al. performed metabolomics analysis of differential metabolites in early serum of GDM patients and found 17 differential metabolites including oleic acid and linoleic acid, which mainly involved amino acid and fatty acid metabolism (Daniel et al., 2015). Li tested the dynamic metabolism of serum in pregnant and normal GDM patients, and finally screened 35 differentially different substances, which were derived from steroid hormone, pyruvate, glycerophospholipid, fatty acid and other metabolism, which mainly related to glycolipids metabolism, nucleotide or amino acid metabolism. However, the metabolites in the blood analyzed in this study may be partially different from previous studies because of the reasons of pregnancy (Li et al., 2014). From the overall analysis, GDM had a great influence on phospholipid metabolism, amino acid metabolism, fatty acid metabolism and glucose metabolism. These differential metabolites and related differential metabolic pathways provided some guidance for explaining the pathogenesis of GDM.

5. Conclusion

In this study, 36 structural potential biomarkers were identified between the GDM group and the normal group by compound structure identification and analysis, combined with clinical and biological significance. These potential biomarkers were mainly classified as prenol lipids, sterol lipids, fatty acyls, sphingolipids, and glycerophospholipids. KEGG metabolic pathway analysis showed that these differential metabolites involved in multiple metabolic pathways, mainly in pathways such as fatty acid metabolism, steroid hormone biosynthesis, arachidonic acid metabolism, butyric acid metabolism, amino acid metabolism and bile secretion. The consequence of this study indicate that LC-MS-based serum metabolomics can well distinguish between GDM patients and normal pregnant women, and find specific differential substances, which is a good reference for early diagnosis and prognosis risk assessment for GDM patients. At the same time, the corresponding metabolic pathway analysis was carried out, which provided a theoretical basis for the future investigation of GDM pathogenesis. It also proved that the LC-MS-based serum metabo-

lomics method had great application value in disease diagnosis and mechanism research.

Declaration of Competing Interest

The authors declare that there is no conflict of interest in the content of this paper.

Acknowledgment

The work was financially supported by Key R&D and Promotion Projects in Henan Province (Grant No. 182102410020).

References

- Abell, S.K., Courten, B.D., Boyle, J.A., et al., 2015. Inflammatory and other biomarkers: role in pathophysiology and prediction of gestational diabetes mellitus. *Int. J. Mol. Sci.* 16 (6), 13442–13473.
- Daniel, A., Denis, M., Tadesse, M.G., et al., 2015. Maternal early pregnancy serum metabolites and risk of gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.* 100 (11), 4348–4356.
- Enquobahrie, D.A., Denis, M., Tadesse, M.G., et al., 2015. Maternal early pregnancy serum metabolites and risk of gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.* 100 (11), 4348–4356.
- Hara, K., Boutin, P., Mori, Y., et al., 2002. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51 (2), 536–540.
- Jamil, S., Khan, R.A., Afroz, S., Ahmed, S., 2016. Phytochemistry, brine shrimp lethality and mice acute oral toxicity studies on seed extracts of *vernonia anthelmintica*. *Pak. J. Pharm. Sci.* 29, 2053–2057.
- Kell, D.B., 2004. Metabolomics and systems biology: making sense of the soup. *Curr. Opin. Microbiol.* 7 (3), 296–307.
- Khatab, H., Wazzan, M., Al-Ahdab, M.A., 2016. Nephroprotective potential of artichoke leaves extract against gentamicin in rats: antioxidant mechanisms. *Pak. J. Pharm. Sci.* 29S, 1775–1782.
- Li, J.X., 2014. New Strategy Research on Prenatal Screening and Diagnosis. ChongQing Medical University.
- Li, W., Jia, M., Wang, J., Lu, J., Deng, J., Tang, J., 2019. Association of MMP9-1562C/T and MMP13-77A/G polymorphisms with non-small cell lung cancer in Southern Chinese population. *Biomolecules* 9, 107.
- Liu, J., Hu, W.H., Sun, L.Y., et al., 2010. Metabolic markers in serum of patients with gestational diabetes. In: National Conference on Spectroscopy.
- Nicholson, J.K., Wilson, I.D., Lindon, J.C., 2011. Pharmacometabonomics as an effector for personalized medicine. *Pharmacogenomics* 12 (1), 103–111.
- Nicholson, J.K., Connelly, J., Lindon, J.C., et al., 2002. Metabonomics: a platform for studying drug toxicity and gene function. *Nat. Rev. Drug. Discov.* 1 (2), 153–161.
- Nicholson, J.K., Lindon, J.C., 2008. Systems biology-metabonomics. *Nature* 455 (7216), 1054–1056.
- Nielsen, J., Jewett, M.C., 2007. The role of metabolomics in systems biology. Springer, Berlin, Heidelberg, vol. 18, pp. 1–10.
- Sawidou, M., Nelson, S.M., Makgoba, M., et al., 2010. First-trimester prediction of gestational diabetes mellitus: examining the potential of combining maternal characteristics and laboratory measures. *Diabetes Care* 59, 3017–3022.
- Shaikh, A.S., Li, W.L., Yuan, G.Y., Gao, M.M., Geng, C.M., Guo, N., Guo, R.C., 2016. Simple, rapid and highly sensitive HPLC method for measurement of lamotrigine in human plasma and its clinical applications. *Pak. J. Pharm. Sci.* 29, 2245–2250.
- Sun, X.F., 2017. Study on TCM Syndrome of Gestational Diabetes Based on Metabolomics. Guangzhou University of Chinese Medicine, Guangzhou.
- Syngelaki, A., Kotecha, R., Pastides, A., et al., 2015. First-trimester biochemical markers of placental screening for gestational diabetes mellitus. *Metabolism* 64 (11), S002604951500195X.
- Weinert, L.S., 2010. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy: comment to the international association of diabetes and pregnancy study groups consensus panel. *Diabetes Care* 33, (7) e97.
- Yang, Q.H., 2013. Current status and countermeasures of diagnosis and treatment of gestational diabetes in China. *Chinese J. Pract. Gynecol. Obstetr.* 4, 241–243.
- Zhou, L., Shen, G.P., Xu, J.J., et al., 2014. Application of metabolomics method in the study of gestational diabetes. *J. Int. Obstetr. Gynecol.* 3, 213–217.