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ORIGINAL RESEARCH ARTICLE

Urine Metabolomics Reveals Overlapping Metabolic Associations Between Preeclampsia and Gestational Diabetes

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Abstract Pregnancy is associated with numerous metabolic adaptations to meet the demands of the growing foetus. These adaptations could be perturbed during pregnancy due to preeclampsia (PE) and gestational diabetes (GDM). As these two obstetric aliments show some overlapping pathophysiology and similar biochemical dysregulation, the present study was undertaken to compare urine metabolome of PE and GDM with normal pregnancy (NT) in all trimesters of gestation using nuclear magnetic resonance spectroscopy-based metabolomics analysis to ascertain and compare metabolome in the study groups. We observed overlapping metabolic perturbations in PE and GDM. Though a study with a small sample size, this is the first report which confirms significantly differential metabolites in urine of both PE and GDM. Dimethylglycine and oxoglutaric acid were decreased while benzoic acid was increased in both the cases in all trimesters. Alanine, aspartate and glutamate metabolism, aminoacyl-tRNA biosynthesis, citrate and butanoate metabolism were the most perturbed pathways in both PE and GDM across pregnancy. These pathways have an association with energy metabolism, glucose homeostasis, insulin sensitivity and oxidative stress which play an important role in the development and progression of PE and GDM. In conclusion, our study showed that urine metabolome could reflect metabolic associations between PE and GDM and also in the identification of biomolecules that could be used as potential biomarker(s) for early detection of the metabolic diseases in pregnancy.

Keywords Gestational diabetes · Insulin resistance · Metabolomics · Oxidative stress · Preeclampsia

Introduction

Preeclampsia (PE) and Gestational Diabetes (GDM) are among the two obstetric ailments affecting pregnancy. Despite its low incidence rate at about 2%, PE is on the radar of clinicians because it directly correlates with both maternal and foetal morbidity and mortality. Predominant features of PE are pregnancy-induced hypertension and proteinuria in which progressive decrease in serum albumin is seen because of loss of protein in urine. Hypoalbuminemia causes decline in plasmatic oncotic pressure and generates extravasation and edema that leads to hypovolemia, increase in sensitivity to angiotensin II, vasoconstriction and increased blood pressure. Numerous studies have grouped PE into two broad groups which include early and late-onset categories. Early PE usually occurs due to placental disorder, and its more severe form might culminate into delivery at ˂34 weeks of gestation. The late-onset is mainly due to vascular and maternal metabolic disorders; as

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the majority deliver at ≥ 34 weeks, which serve cure/treatment. In critical conditions, individuals might suffer from breakdown of red blood cells, decrease in platelet, liver function may be impeded, malfunctioned kidney, oedema, dyspnoea, and blurring of vision.

GDM also affects about 2–14% of expectant mothers. It is characterized by high glucose in the blood as the body is unable to produce sufficient insulin or else the cells of the body become less sensitive to insulin action due to which demand for insulin rises during pregnancy. Individuals suffering from GDM have greater chances of developing PE, depression, and Caesarean sections.

These two obstetric disorders share common pathophysiology like the decline in insulin sensitivity [1], chronic inflammation, unevenness in angiogenic factors and loss of endothelial function [2]. Predisposing factors of both (which include advanced maternal age, nulliparity, multi-foetal pregnancy, and obesity) also overlap [3–5]. It is still not known whether a common aetiology is responsible for both the diseases as mechanisms of disease progression in both is similar. PE is often reported as an upshot of GDM because individuals suffering from GDM have greater chance of developing PE in later stages of pregnancy [6]. Studies suggest similar biochemical dysregulation of various adipokines [7], an increase in oxidative stress [8] and dyslipidemia [9] in both diseases. But the biochemical and metabolic correlation between these two is not well understood.

Metabolic changes during pregnancy are common and it occurs in response to physiological adaption. But in pregnancies affected by PE, GDM and hypertension, metabolic response may not be normal. Metabolites are components of a metabolic pathway that are essential for the normal functioning of a body. Metabolomics deals with the study of these metabolites and a complete metabolome of an individual describes the best phenotype of that organism. It is rapidly being used for early detection and also to study mechanisms of various diseases.

A comparison of the metabolic dysregulation of PE, GDM, and NT may provide a better understanding of the aetiologies, mechanism, and pathogenesis of these diseases. The goal of this pilot study is to evaluate the potential of NMR metabolomics to predict the time-dependent relationship between metabolic profiles of these two obstetric complications that may help in better clinical management of these patients.

Methods

Clinical Samples

Pregnant women who agreed to participate in the study were recruited as subjects. At their first prenatal visit at 10–12 weeks of gestation a detailed form was filled for the present status of pregnancy along with medical or surgical complications if any. Their maternal characteristics, obstetrics history about previous miscarriages, abortions, or ectopic pregnancies and medical history of hypertension, asthma, urinary tract infection and thromboembolism; and family history (in mother and sisters) of medical conditions (hypertension, coronary artery heart disease, cerebrovascular accident, type 1 and 2 diabetes, and venous thromboembolism) was noted. Urine samples were collected in 1st, 2nd and 3rd trimesters at 11^{+0} to 13^{+6}, 22–24 and 30–32 weeks respectively. All the participants of the study were followed up till delivery. Collected urine samples were aliquoted, frozen immediately, and stored at − 80 °C until further processing.

Samples were collected from pregnant women attending antenatal clinic at the department of obstetrics and gynaecology. Individuals were recruited in the study at their prenatal visit (10–12 weeks of gestation) after explaining the purpose of the study. All the study participants were provided with a patient information sheet and written informed consent was taken from each individual. All the participants included in the study were above 18 years of age. Patients with chronic illnesses, diabetes mellitus, and medical renal diseases were excluded from the study.

NMR Spectroscopy

The urine samples collected from pregnant women were processed for ^{1}H NMR spectroscopy for which 100 µl of NMR buffer (0.2 MK_2HPO_4, 0.2 M KH_2PO_4, 2 mM sodium azide, 10% (*w/v*) DSS) was added to 200 µl of samples. All the experiments were done at 298 K using Bruker Avance III spectrometer equipped with a cryogenic triple-resonance TCI probe, operating at the proton field strength of 500 MHz. The Carr–Purcell–Meiboom–Gill (CPMG) echo sequence was used to partially suppress the protein signals. A total CPMG delay of 300 ms was used with an echo time of 200 ms. All one-dimensional ^{1}H NMR spectra were measured with 32 scans with a relaxation delay of 4 s. For each sample, free induction decays (FIDs) were collected with a spectral width of 10,000 Hz and an acquisition time of 3.28 s (t1max). The peaks were processed using Topspin 3.6.1 (Bruker AG, Falländen, Switzerland). DSS at 0 ppm was used for baseline correction; peak alignment and scaling of the peaks. All the peaks between 4.7 and 4.5 ppm regions were removed to eliminate water signals.

Statistical Data Analysis

All statistical and pathway analysis was done using MetaboAnalyst web tool (www.metaboanalyst.ca). All differential metabolites with p value ≤ 0.05 in ANOVA analysis

were considered significant. Supervised multivariate analysis (PLS-DA) analysis was used to predict variance in the study groups. Significantly differentiated peaks were annotated using Human Metabolome Data Bank (HMDB) (www.hmdb.ca), COLMAR and some published data. Metabolic pathways with p value ≤ 0.05 were considered significant.

Results

A total of 10 healthy, 7 with gestational diabetes (GDM) and 4 with early-onset Preeclampsia (PE) pregnant women were recruited to assess variation in their urine metabolites between groups in all trimesters. Women who developed PE showed higher blood pressure and protein albumin ($\geq +1$) in urine dipstick from as early as 20 weeks of gestation as compared to GDM and NT.GDM patients were differentiated from NT and PE, based on the WHO suggested 75 g fasting OGTT with 2 h PPG ≥ 153 mg/dl conducted at 22–24 weeks. Characteristics of study population are shown in Table 1.

Differentially Regulated Metabolites and Pathways Observed in GDM and PE in All Trimester

Partial Least Square Discrimination Analysis (PLS-DA) analysis in 1st trimester revealed clear separation as samples formed three distinct clusters of GDM, NT and PE (Fig. 1). ANOVA analysis showed 20 differential metabolites with p value ≤ 0.05.ANOVA differential metabolites (p value < 0.05) were selected by using the cut-off of PLS-DA Variable Importance in Projection (VIP) score > 1 in (supplementary material Table 1). Pyridoxine, Guanine, Hippuric acid, Benzoic acid and uracil were upregulated in PE and GDM as compared to NT whereas L-Asparagine, Alanine, 3-Hydroxybutyric acid and Citric acid level were reduced in both in @@contrast to NT.

Metabolic pathway analysis showed modification in glucose metabolism through the dysregulation of the Citrate cycle (TCA cycle). Some amino acid metabolic pathways (alanine, aspartate and glutamate metabolism, arginine biosynthesis, beta-Alanine metabolism and Aminoacyl-tRNA biosynthesis), butanoate, pantothenate and coenzyme A (CoA) biosynthesis in PE and GDM in 1st trimester were also dysregulated. All dysregulated pathways are shown in supplementary Table A (p value ≤ 0.05).

ANOVA analysis showed 31 differential metabolites in 2nd trimester with p value ≤ 0.05.Metabolites listed in supplementary material Table 2 have PLS-DA Variable Importance in Projection (VIP) score > 1 and p value ≤ 0.05 in ANOVA analysis. The results indicated more alterations in metabolites in 2nd trimester cross-sectional analysis between groups. A significant decline in the level of Xanthosine and pyridoxine was observed in GDM with the least expression in PE. Thiamine pyrophosphate showed a substantial increase in the level in GDM reaching peak in PE. Pathways showing alterations in both cases in 2nd trimester including Citrate cycle (TCA cycle), Alanine, aspartate and glutamate metabolism and Butanoate metabolism, are listed in Supplementary Table B. All deregulated pathways have p value ≤ 0.05.

Table 1 Characteristics of study participants

n (samples)	PE 4	GDM 7	NT 10
Age (years)	27.5 (29–26)	26.6 (32–20)	28 (35–20)
Weight 11^{+0} to 13^{+6} weeks*	54.5 (58–49)	60 (64–55)	52 (58–45)
Weight 22–24 weeks*	60.6 (62–53)	64.4 (68–59)	56.7 (62–50)
Weight 30–32 weeks*	64 (66.5–57)	68 (72–63)	62.5 (67–55)
BP systolic (mmHg) 11^{+0} to 13^{+6} weeks*	127 (128–126)	125 (128–122)	123 (125–118)
BP systolic (mmHg) 22–24 weeks*	128 (130–122)	111 (125–105)	119 (125–115)
BP systolic (mmHg) 30–32 weeks*	139 (140–134)	125 (128–115)	124 (128–119)
BP diastolic (mmHg) 11^{+0} to 13^{+6} weeks*	80 (85–75)	79 (82–75)	82 (85–81)
BP diastolic (mmHg) 22–24 weeks*	82 (90–76)	82 (84–79)	79 (84–76)
BP diastolic (mmHg) 30–32 weeks*	89 (94–85)	85 (89–81)	83 (85–79)
GTT(0 h)*	84 (92–80)	97 (102–95)	81 (89–76)
GTT(1 h)*	159 (160–145)	198 (205–190)	149 (170–125)
GTT(2 h)	114 (130–100)	170 (180–159)	127 (140–105)
Urine Alb 11^{+0} to 13^{+6} weeks¶	0	0	0
Urine Alb 22–24 weeks¶	1	0	0
Urine Alb 30–32 weeks¶	2	1	0

*Values are given as mean, statistical p value (≤ 0.05) calculated by One-Way Analysis of Variance

¶Urine Alb. measured with dipstick

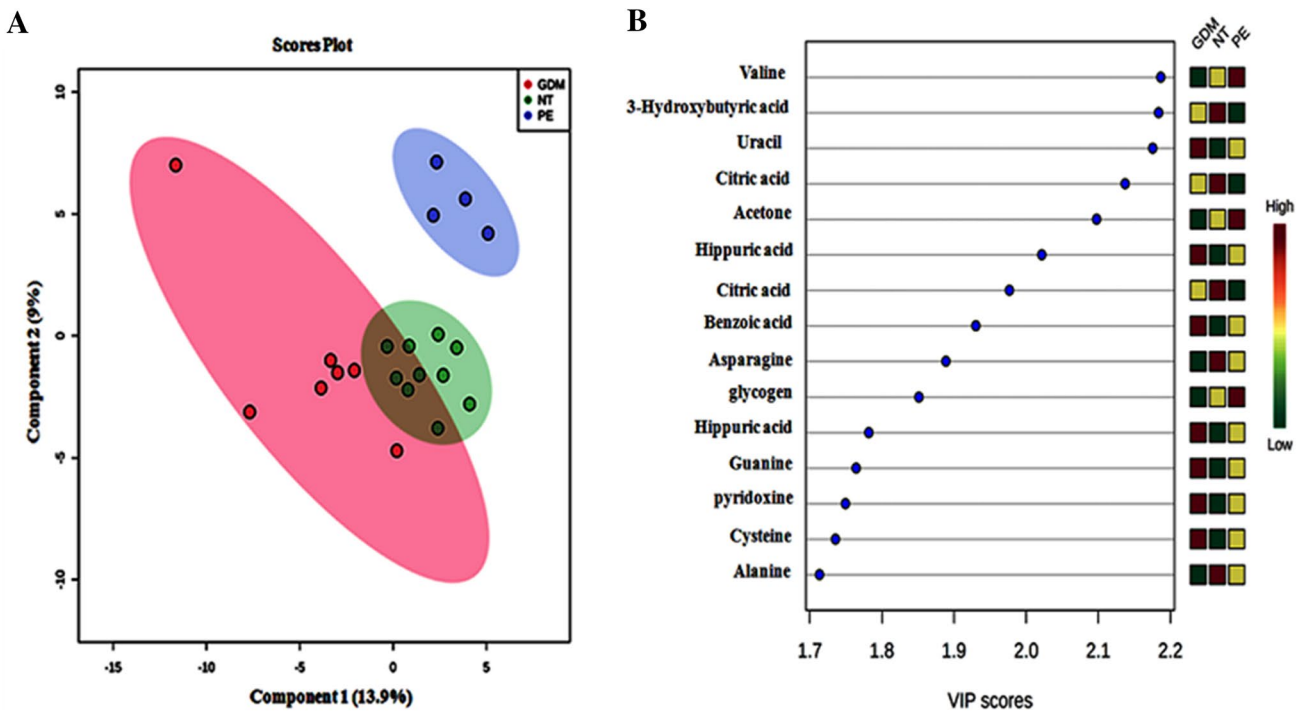


Fig. 1 The figure represents PLS-DA analysis of PE, GDM and NT urine samples in 1st trimester: **A** PLS-DA score plots **B** Variable Importance in Projection (VIP) plot indicating the most discriminating metabolite between PE, GDM and NT in 1st trimester

In 3rd trimester, validated PLS-DA analysis showed separation between all groups (Fig. 2A). 15 metabolites had VIP scores ≥ 1.8. (Fig. 2B). ANOVA analysis showed 38 differential metabolites with p value ≤ 0.05. ANOVA differential metabolites (p value ≤ 0.05) with PLS-DA Variable Importance in Projection (VIP) score > 1 are shown in supplementary material Table 3. All deregulated pathways (p value ≤ 0.05) in 3rd trimester in both ailments are shown in the supplementary Table C.

Longitudinally Perturbed Pathways and Metabolites Observed in all Trimesters in PE and GDM

Uracil, hippuric acid, Guanine (p value ≤ 0.05 in ANOVA and (VIP) score > 1) were altered both in the 1st and 2nd trimester of gestation. Likewise larger panel of altered metabolites showed overlap in the 2nd and 3rd trimester. Gamma-Aminobutyric acid, Xanthosine, 3-methylhistidine, o-Tyrosine, Succinic acid were altered both in 2nd and 3rd trimesters while Glycine was found to be deregulated both in 1st and 3rd trimesters (p value ≤ 0.05 in ANOVA and (VIP) score > 1).

Three metabolites showed longitudinal variation in all trimester. Dimethylglycine, Oxoglutaric acid and Benzoic acid were differentially regulated in PE and GDM across all trimesters. All these metabolites have p value ≤ 0.05 in ANOVA analysis and PLS-DA Variable Importance in Projection (VIP) score > 1 in cross-sectional studies of 1st, 2nd and 3rd trimesters.

Longitudinal perturbation was also observed in few metabolic pathways. Arginine biosynthesis and beta-Alanine metabolism was altered both in 1st and 3rd trimester. Glycine, serine and threonine metabolism and phenylalanine metabolism was found to be perturbed in 2nd and 3rd trimester. A total of four metabolic pathways (Alanine, aspartate and glutamate metabolism, Aminoacyl-tRNA biosynthesis, Citrate cycle (TCA cycle) and Butanoate metabolism) showed longitudinal perturbation (in all three trimesters) in both PE and GDM (Fig. 3). All these pathways showing dysregulation longitudinally in both PE and GDM have p value ≤ 0.05 in pathway analysis in cross-sectional studies of 1st, 2nd and 3rd trimesters.

Differentially Regulated Metabolites and Pathways Observed Between GDM and PE

Partial Least Square Discrimination Analysis (PLS-DA) analysis of 1st trimester revealed clear separation as samples formed two groups. Citric acid, Imidazole, 3-Hydroxybutyric acid, alanine and Isoleucine were differentially regulated between PE and GDM in t test (P value < 0.05) and PLS-DA Variable Importance in Projection (VIP) score > 1. Alanine, aspartate and glutamate metabolism, Aminoacyl-tRNA biosynthesis, Synthesis and degradation

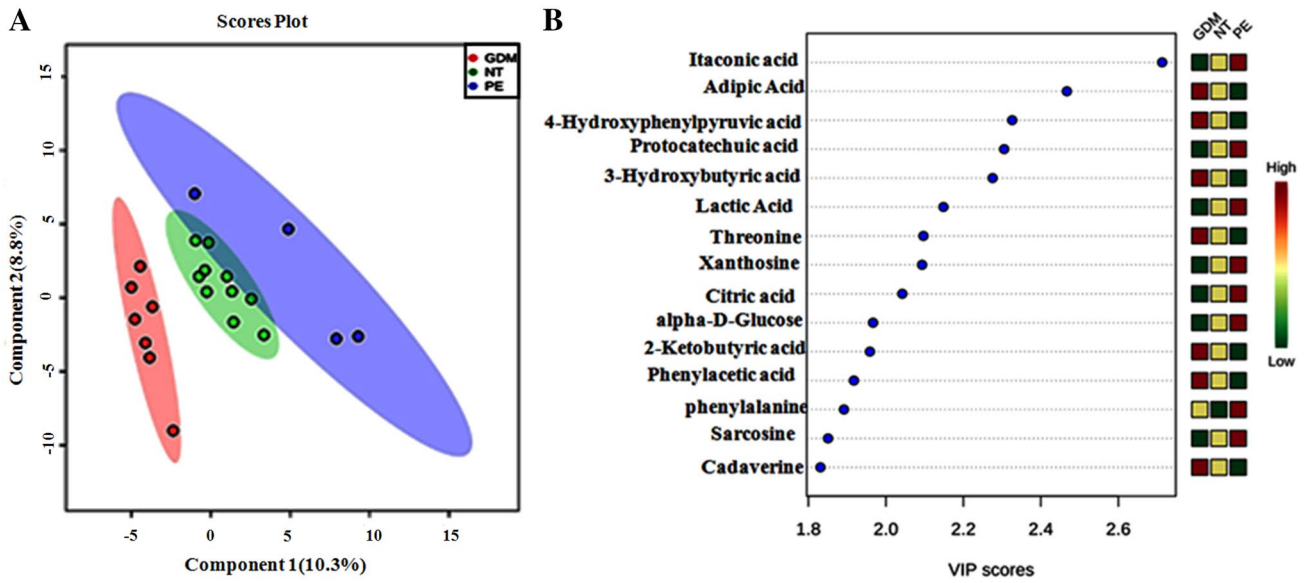


Fig. 2 The figure represents PLS-DA analysis of PE, GDM and NT urine samples in 3rd trimester: **A** PLS-DA score plots PCs. **B** Variable Importance in Projection (VIP) plot indicating the most discriminating metabolite between PE, GDM and NT in 3rd trimester

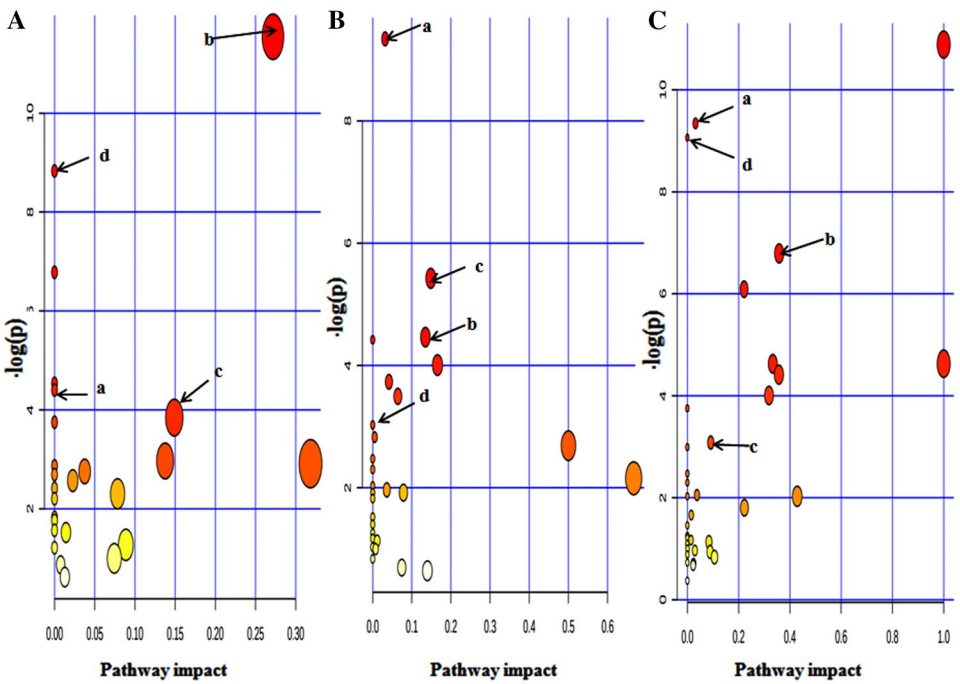


Fig. 3 The figure shows longitudinally aberrant metabolic pathways using differential metabolites from ANOVA analysis (p value ≤ 0.05) and PLS-DA Variable Importance in Projection (VIP) score > 1: **A** 1st trimester, **B** 2nd trimester, **C** 3rd trimester. **a** Represents Butanoate metabolism, **b** Alanine, aspartate and glutamate metabolism, **c** Citrate cycle (TCA cycle), **d** Aminoacyl-tRNA biosynthesis

of ketone bodies, Valine, leucine and isoleucine biosynthesis and Butanoate metabolism showed differential regulation between these two groups (p value ≤ 0.05).

10 metabolites were deferentially regulated in 2nd trimester with p value ≤ 0.05 and PLS-DA Variable Importance in Projection (VIP) score > 1.Peturbed metabolites led to deregulation of energy metabolism, few amino acid metabolism, thiamine and butanoate metabolic pathways (p value ≤ 0.05).

Advancement in gestational age led to clear separation between PE and GDM groups with positive R2 and Q2 in all 4 components of PLS-DA analysis (Fig. 4A, B). 21 metabolites showed differential regulation between PE and GDM (t test; p value ≤ 0.05 and VIP scores ≥ 1 in PLS-DA analysis). Few amino acid and butanoate pathways showed differential regulation between two groups. Butanoate

metabolism showed longitudinal differentiation between these two groups.

Discussion

PE and GDM share some aetiology such as upsurge in insulin resistance and oxidative stress. However, the biochemical pathways and the mechanisms linking these pregnancies related pathologies are not yet well understood. Metabolomics is rapidly being used for early detection and a better understanding of disease progression. We have used ^{1}H NMR-based metabonomic approach to study metabolic variations between groups across all trimesters, as looking into longitudinal variations of metabolites and pathways helps in better understanding of disease progression with advancing gestational age in women with PE/GDM as compared to NT. The results from this study demonstrate that GDM and PE show some similarities in urinary metabolome during early pregnancy that could distinguish patients from healthy normal controls. It also provides a snapshot of metabolic changes that accomplish as the disease progresses.

Pregnancy is accompanied with numerous biochemical adaptations including enhanced rate of glucose metabolism to meet excess energy demand with increasing gestational age but energy metabolism shows alteration in PE and GDM patients. Rate of glycolysis and gluconeogenesis rises in PE and GDM like NT, to meet excess energy challenge but reduction in mitochondrial respiration is observed in these aliments [10]. Pyruvate occupies a central position in energy metabolism as it sits at the intersection of glycolysis and TCA cycle. Studies suggests altered pyruvate metabolism in metabolic diseases owing to inhibition of pyruvate dehydrogenase complex by pyruvate dehydrogenase kinase thus impeding conversion of pyruvate to acetyl-CoA which is the start point of Krebs cycle [11]. Scholtens et al. [12] also reported altered Pyruvate metabolism in mothers with abnormal glucose levels during gestation. Upregulation of metabolites of glycolytic pathways and pyruvate metabolism in 2nd trimester and altered expression of few metabolites of TCA cycle (Citric acid in 1st, Succinic acid in 2nd and 3rd and Oxoglutaric acid were downregulated in all trimesters in both the cases) leading to its dysregulation from 1st trimester onwards suggests perturbed glucose metabolism mainly due to attenuated mitochondrial function and reduction in mtDNA content [10, 13].

Vitamins play an important role in various metabolic processes during pregnancy. They act as cofactor in amino acid, carbohydrate as well as lipid metabolism. Level of Pyridoxine (Vitamin B6) was affected in PE and GDM in all trimesters and its metabolism was affected in 3rd trimester (supplementary table 3). Vitamin B6 is known to control transsulfuration of homocysteine. Alteration in the level of homocysteine leads to metabolic disorders such as endothelial dysfunction and insulin resistance, which are responsible for PE and GDM [14]. Pantothenate and Coenzyme A (CoA) biosynthesis was also altered in 1st trimester in both the cases. CoA is an important regulator of energy metabolism and plays an important role, both in synthesis and degradation of lipids and carbohydrates [15]. The involvement

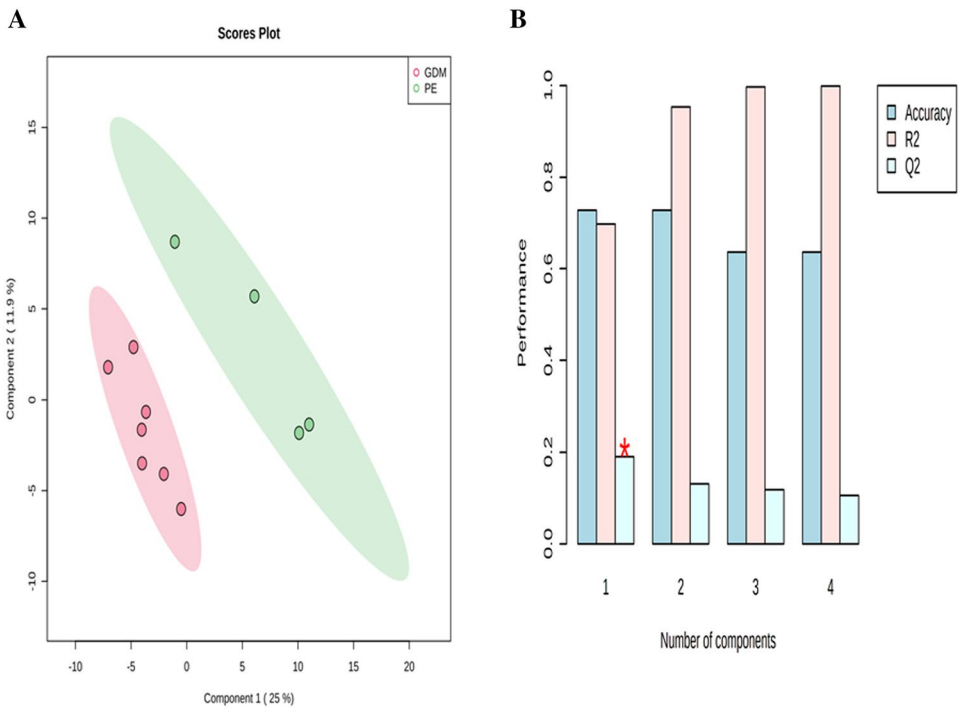


Fig. 4 The figure represents PLS-DA analysis of PE and GDM urine samples in 3rd trimester: **A** PLS-DA score plots, **B** first component best classifies the model shown with asterisk

of these pathways in decreased insulin sensitivity has been reported previously [12, 16].

Lipid metabolism is usually perturbed during pregnancy. In NT an increase in lipid synthesis and fat storage occurs in initial trimesters to fulfil increasing energy demands of the growing foetus but at later stages, catabolic processes take lead to supply substrates to the growing fetus. But, PE and GDM show abnormalities in lipid metabolism known as dyslipidaemia which might be due to placental oxidative stress. Dyslipedemia is often associated with alterations in metabolism of non-essential fatty acids and ketone. Altered expression of 3-Hydroxybutyric acid both in PE and GDM in the present study from 1st trimester onwards suggests perturbation in ketone metabolism which is responsible for dyslipedemia, frequently reported in both aliments. Butanoate is a short chain fatty acid. Its metabolism showed alterations in all three trimester (Fig. 4). Butanoate pathway is associated with human gut microbiota, which go through changes during pregnancy hence leads to various metabolic changes in the host as well [17]. Fermentation product of the gut microbiota, ligand for free fatty acid 2 receptor (*Ffar2*) play an important role in maintaining gestational glucose homeostasis, thus alteration in butanoate metabolism leads to hyperglacemia [18]. Our findings on altered lipid metabolic pathways corroborate with previous metabolomics, lipidomics and transcriptomic analysis of blood and urine samples from patients with increased insulin resistance [19].

Protein metabolism plays an important role in foetal growth and synthesis of both foetal and maternal tissue. Altered amino-acid metabolism occurs as a result of insulin resistance. Variation in aminoacyl-tRNA biosynthesis in all trimesters in both cases affects glucose responsiveness and insulin secretion. Alteration in this pathway has been previously reported in GDM [20]. Aminoacyl-tRNA biosynthesis has been reported to be involved in early development of type 2 diabetes (T2DM) [21] and it is among the pathways targeted by Gao et al. [22] for treatment of T2DM. Leucine and Valine are essential branched-chain amino acids (BCAA). Catabolism of BCAA is a two-step process, a reversible process which may be cytosolic or mitochondrial and an irreversible process which takes place in mitochondria where they enter TCA cycle. As women with PE and GDM show defect in Krebs cycle, metabolism of these BCAA also gets perturbed [23]. Dysregulation of Valine, Leucine and Isoleucine degradation pathway in 2nd trimester might be due to altered TCA cycle in PE and GDM. We observed that glycine, serine and threonine metabolism was dysregulated in the 2nd and 3rd trimester in both conditions which corroborate with Liu et al. [16] Downregulation of glycine as observed in both 1st and 3rd trimesters is in congruent with earlier study that reported glycine levels as negative correlates of insulin sensitivity and an early indicator of insulin resistance [24]. Observed reduced expression of Dimethylglycine (DMG) in all trimesters in both cases might be linked to oxidative stress and metabolic diseases like insulin resistance as earlier report had shown close relationship between these metabolites and the aforementioned conditions [25]. From the foregoing, **DMG** might be an important player in insulin resistance and it can be a biomarker for early detection of PE and GDM (Fig. 5).

L-glutamine is metabolized to gamma-Aminobutyric acid (GABA) and then to L-aspartate via the Krebs cycle [26]. Alterations in the TCA cycle lead to incomplete oxidation of L-glutamine and an increase in the level of **GABA** observed in the 2nd and 3rd trimester. As TCA cycle is attenuated in PE and GDM leading to decline in generation of reducing equivalents (NADH and $FADH_2$).It decreases cellular ATP:ADP ratio resulting in opening of KATP channels, membrane repolarisation and efflux of Ca^{2+} which prevents exocytosis of insulin granules [27]. Deregulation of alanine, aspartate and glutamate metabolism observed in all trimesters might be responsible for the increase in blood glucose level, because of poor exocytosis of insulin granules. Essential amino acid L-histidine is a scavenger of reactive oxygen species (ROS). Lower level of histidine in the 3rd trimester of both PE and GDM in this study is similar to a recent report that showed a decline in its level in plasma of obese and in urine of women with spontaneous abortion [28]. This is thus related to inflammation and increased burden of ROS. This also suggestive of elevated oxidative stress which is known to be involved in the pathophysiology of both cases. Glew et al. [29] reported increase in concentrations of aromatic amino acid, phenylalanine in serum of PE and perturbation in its metabolism. But we found increase in urinary levels of phenylalanine in 3rd trimester and alteration in its metabolism in 2nd and 3rd trimester in both PE and GDM which suggests increased glomerular permeability conferred by increased insulin resistance. Tyrosine, phenylalanine, and tryptophan biosynthetic pathways was perturbed in 3rd trimester, arginine biosynthesis and beta-Alanine metabolism were perturbed in the 1st and 3rd trimesters respectively. Alterations in these pathways have been previously linked with hyperglycemia [12] as amino-acids under appropriate conditions participate in regulation of insulin secretion by islet cells [30]. Dysregulation of Purine metabolism in 2nd trimester in this study have also been reported in previous studies to be strongly associated with decreased insulin sensitivity [16].

Conclusion

As PE and GDM show association in their risk factor profile as well as in pathophysiology and aetiology. Our study revealed that even the urinary metabolomic profile of PE and GDM shows overlap in all three trimesters. However, the

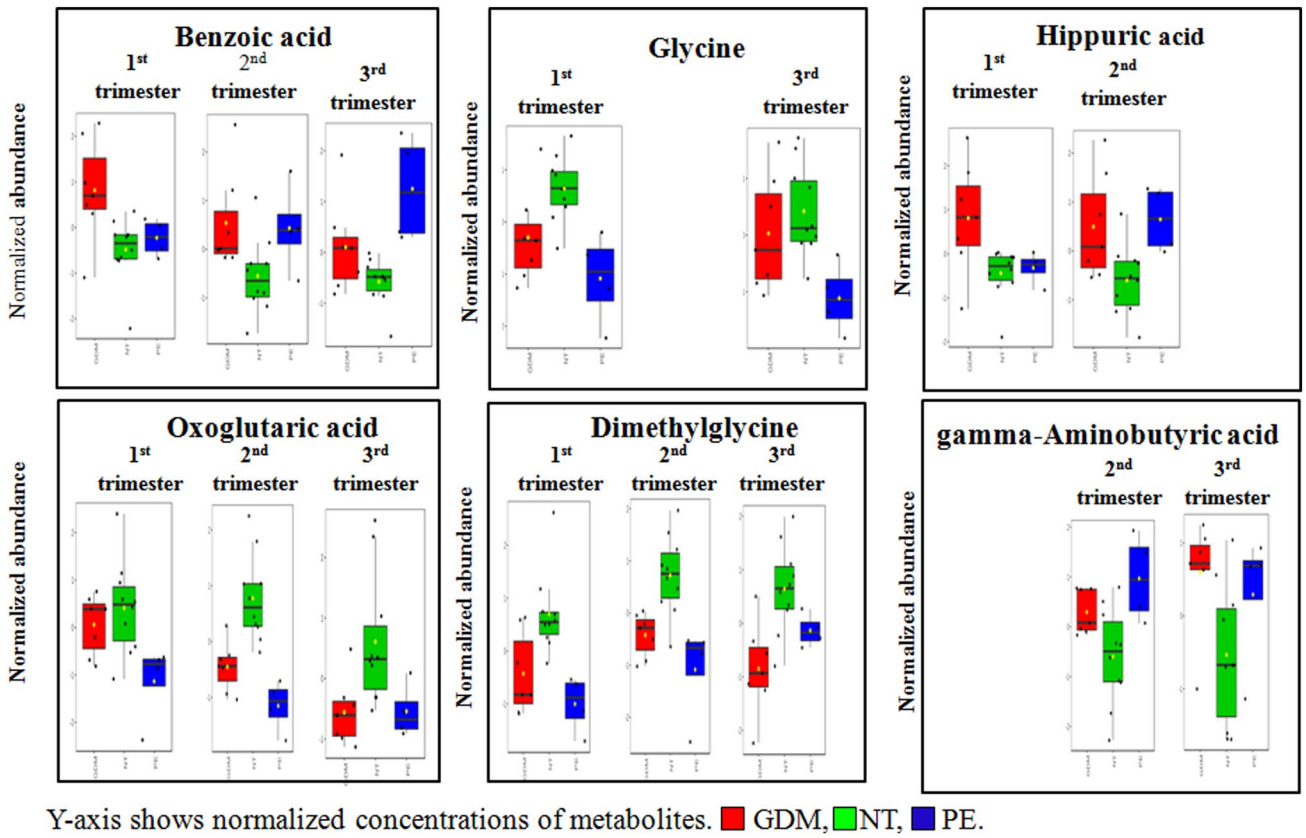


Fig. 5 Box-and-whisker plots illustrating metabolite discrimination in GDM (red), PE (blue) and NT (green). The horizontal line in the middle portion of the box, median; bottom and top boundaries of boxes, 25th and 75th percentiles, respectively; lower and upper whiskers, 5th and 95th percentiles, respectively. The *y*-axis shows the normalized concentrations of the metabolites

sample size in the present study was very small and hence large-scale and multi-centre study needs to be performed in the future, which will aid in better understanding of common and distinct biochemical and metabolic pathways affected in PE and GDM. It was a pilot study to evaluate the potential of metabolomics to predict PE/GDM from urine in early pregnancy and further studies would help in early screening and better clinical outcomes.

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Author Contributions NSB and NRA designed the research. GK and NRA were involved in patient recruitment and sample collection. NRA and BFO performed the research and analyzed the data. NRA, NSB and GK wrote the paper. The research work was supervised by GK and NSB.

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Declarations

Conflict of interest The authors report there are no competing interests to declare.

Ethical Approval and Consent to Participate The study was approved by the Institutional ethical review board of AIIMS (IEC-512/01.09.2017) and ICGEB (ICGEB/IEC/2017/07). The study was also approved by Institutional Biosafety committee (IBSC) of ICGEB (64th meeting, 15/12/2017). The study was performed adhering to approved IEC and IBSC guidelines. All the study participants were provided with a patient information sheet and written informed consent was taken from each individual.

Consent for Publication Written informed consent was taken from each individual.

References

1. Roberts JM. Endothelial dysfunction in preeclampsia. Semin Reprod Endocrinol. 1998;16(1):5–15.
2. Carpenter MW. Gestational diabetes, pregnancy hypertension, and late vascular disease. Diabetes Care. 2007;30:S246–50.

3. Schneider S, Freerksen N, Röhrig S, Hoeft B, Maul H. Gestational diabetes and preeclampsia—similar risk factor profiles? Early Hum Dev. 2012;88(3):179–84.
4. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ. 2005;330(7491):565.
5. Hollander MH, Paarlberg KM, Huisjes AJ. Gestational diabetes: a review of the current literature and guidelines. Obstet Gynecol Surv. 2007;62(2):125–36.
6. Vambergue A, Nuttens MC, Goeusse P, Biausque S, Lepeut M, Fontaine P. Pregnancy induced hypertension in women with gestational carbohydrate intolerance: the diagest study. Eur J Obstet Gynecol Reprod Biol. 2002;102(1):31–5.
7. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11(2):85–97.
8. Genc H, Uzun H, Benian A, Simsek G, Gelisgen R, Madazli R, et al. Evaluation of oxidative stress markers in first trimester for assessment of preeclampsia risk. Arch Gynecol Obstet. 2011;284(6):1367–73.
9. Nasioudis D, Doulaveris G, Kanninen TT. Dyslipidemia in pregnancy and maternal-fetal outcome. Minerva Ginecol. 2019;71(2):155–62.
10. Shi Z, Long W, Zhao C, Guo X, Shen R, Ding H. Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. PLoS ONE. 2013;8(5):e64351.
11. Lee IK. The role of pyruvate dehydrogenase kinase in diabetes and obesity. Diabetes Metab J. 2014;38(3):181–6.
12. Scholtens DM, Bain JR, Reisetter AC, Muehlbauer MJ, Nodzenski M, Stevens RD, et al. Metabolic networks and metabolites underlie associations between maternal glucose during pregnancy and newborn size at birth. Diabetes. 2016;65(7):2039–50.
13. Crovetto F, Lattuada D, Rossi G, Mangano S, Somigliana E, Bolis G, et al. A role for mitochondria in gestational diabetes mellitus? Gynecol Endocrinol. 2013;29(3):259–62.
14. Liu Z, Li P, Zhao ZH, Zhang Y, Ma ZM, Wang SX. Vitamin B6 prevents endothelial dysfunction, insulin resistance, and hepatic lipid accumulation in apoe (−/−) mice fed with high-fat diet. J Diabetes Res. 2016;2016:1748065.
15. Leonardi R, Zhang YM, Rock CO, Jackowski S. Coenzyme A: back in action. Prog Lipid Res. 2005;44(2–3):125–53.
16. Liu T, Li J, Xu F, Wang M, Ding S, Xu H, et al. Comprehensive analysis of serum metabolites in gestational diabetes mellitus by UPLC/Q-TOF-MS. Anal Bioanal Chem. 2016;408(4):1125–35.
17. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;150(3):470–80.
18. Fuller M, Priyadarshini M, Gibbons SM, Angueira AR, Brodsky M, Hayes MG, et al. The short-chain fatty acid receptor, FFA2, contributes to gestational glucose homeostasis. Am J Physiol Endocrinol Metab. 2015;309(10):E840–51.
19. Dudzik D, Zorawski M, Skotnicki M, Zarzycki W, García A, Angulo S, et al. GC-MS based gestational diabetes mellitus longitudinal study: identification of 2-and 3-hydroxybutyrate as potential prognostic biomarkers. J Pharm Biomed Anal. 2017;144:90–8.
20. Sakurai K, Eguchi A, Watanabe M, Yamamoto M, Ishikawa K, Mori C. Exploration of predictive metabolic factors for gestational diabetes mellitus in Japanese women using metabolomic analysis. J Diabetes Investig. 2019;10(2):513–20.
21. Zhang AH, Sun H, Yan GL, Yuan Y, Han Y, Wang XJ. Metabolomics study of type 2 diabetes using ultra-performance LC-ESI/quadrupole-TOF high-definition MS coupled with pattern recognition methods. J Physiol Biochem. 2014;70(1):117–28.
22. Gao K, Yang R, Zhang J, Wang Z, Jia C, Zhang F, et al. Effects of Qijian mixture on type 2 diabetes assessed by metabonomics, gut microbiota and network pharmacology. Pharmacol Res. 2018;130:93–109.
23. Zhang S, Zeng X, Ren M, Mao X, Qiao S. Novel metabolic and physiological functions of branched chain amino acids: a review. J Anim Sci Biotechnol. 2017;8:10.
24. Yan-Do R, MacDonald PE. Impaired "Glycine"-mia in type 2 diabetes and potential mechanisms contributing to glucose homeostasis. Endocrinology. 2017;158(5):1064–73.
25. Magnusson M, Wang TJ, Clish C, Engström G, Nilsson P, Gerszten RE, et al. Dimethylglycine deficiency and the development of diabetes. Diabetes. 2015;64(8):3010–6.
26. Fernández-Pascual S, Mukala-Nsengu-Tshibangu A, Martín Del Río R, Tamarit-Rodríguez J. Conversion into GABA (gamma-aminobutyric acid) may reduce the capacity of L-glutamine as an insulin secretagogue. Biochem J. 2004;379(Pt 3):721–9.
27. Jitrapakdee S, Wutthisathapornchai A, Wallace JC, MacDonald MJ. Regulation of insulin secretion: role of mitochondrial signalling. Diabetologia. 2010;53(6):1019–32.
28. Liu X, Wang X, Sun H, Guo Z, Liu X, Yuan T, et al. Urinary metabolic variation analysis during pregnancy and application in Gestational Diabetes Mellitus and spontaneous abortion biomarker discovery. Sci Rep. 2019;9(1):18003.
29. Glew RH, Melah G, El-Nafaty AI, Brandt Y, Morris D, VanderJagt DJ. Plasma and urinary free amino acid concentrations in preeclamptic women in northern Nigeria. Clin Chim Acta. 2004;342(1–2):179–85.
30. Newsholme P, Brennan L, Bender K. Amino acid metabolism, β-cell function, and diabetes. Diabetes. 2006;55(Supplement 2):S39–47.

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