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Tryptophan and purine metabolites are consistently upregulated in the urinary metabolome of patients diagnosed with gestational diabetes mellitus throughout pregnancy: A longitudinal metabolomics study of Chinese pregnant women part 2

Kai P. Law [a,*], Ting-Li Han [a], Xun Mao [a,b], Hua Zhang [a,b,*]

[a] Mass Spectrometry Centre, China-Canada-New Zealand Joint Laboratory of Maternal and Foetal Medicine, Chongqing Medical University, Chongqing, China
[b] Department of Obstetrics and Gynaecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

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ABSTRACT

Background: Gestational diabetes mellitus (GDM) is a pathological state of glucose intolerance associated with adverse pregnancy outcomes and an increased risk of developing maternal type 2 diabetes later in life. The mechanisms underlying GDM development are not fully understood. We examined the pathophysiology of GDM through comprehensive metabolic profiling of maternal urine, using participants from a longitudinal cohort of normal pregnancies and pregnancies complicated by GDM.
Methods: Based on ultra-performance liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry, an untargeted metabolomics study was performed to explore the differences in the urinary metabolome of GDM cases and healthy controls over the course of pregnancy. Multilevel statistical approaches were employed to address the complex metabolomic data obtained from a longitudinal cohort.
Results: The results indicated that tryptophan and purine metabolism was associated with GDM. The tryptophan-kynurenine pathway was activated in the GDM subjects before placental hormones or the fetoplacental unit could have produced any physiological effect. Hypoxanthine, xanthine, xanthosine, and 1-methylhypoxanthine were all elevated in the urine metabolome of subjects with GDM. Catabolism of purine nucleosides leads ultimately to the production of uric acid, which discriminated the subjects with GDM from controls.
Conclusions: The results support the notion that GDM may be a predisposed condition, or prediabetic state, which is manifested during pregnancy. This challenges the conventional view of the pathogenesis of GDM, which assumes placental hormones are the major causes of insulin resistance in GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is a form of hyperglycaemia during pregnancy. It is estimated that 20.9 million or 16.2% of live births to women in 2015 had some form of hyperglycaemia in pregnancy [1]. The highest prevalence of GDM is found in the South-East Asia region with rates as high as 24.2%. The pathophysiology of GDM has not been fully elucidated. Hyperglycaemia during pregnancy usually resolves postpartum, but women with unmanaged gestational diabetes are at increased risk of adverse pregnancy outcomes, and both mother and child are susceptible to develop type 2 diabetes (T2DM) later in life [2]. The development of GDM also induces a state of dyslipidaemia consistent with insulin resistance [3]. Increasing evidence, particularly from genome-wide association studies, has suggested that a strong link exists between GDM and T2DM [4]. The development of GDM may reflect a predisposition to T2DM or a pre-diabetic state, which has been expressed under the metabolic state of pregnancy, in which insulin resistance and hyperinsulinemia occur.

The field of metabolomics has witnessed an exponential growth in the last 2 decades, driven by important applications spanning a range of areas in the basic and life sciences [5]. An important clinical application of metabolomics is to model changes in the metabolome and relate them to health and disease states. Metabolomics studies can be used to identify potential biomarkers of disease, useful for a diagnostic purpose or risk stratification. Additionally, metabolomics studies provide information that can further our understanding of the pathophysiology of a condition. In clinical applications, the measurement of the urinary metabolome has several advantages. Urine is sterile, can be collected non-invasively, is available in large quantities, and provides a rich metabolic profile. Sample treatment is relatively simple compared with

* Corresponding authors at: Mass Spectrometry Centre, China-Canada-New Zealand Joint Laboratory of Maternal and Foetal Medicine, Chongqing Medical University, Chongqing, China.
E-mail addresses: kai.law1@virginmedia.com (K.P. Law), zh2844@gmail.com (H. Zhang).

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other bio-fluids [6]. However, urine poses several analytical challenges for metabolic profiling. A major challenge in urine analysis is that unlike other biological fluids under homeostatic regulation, urine volume can vary widely depending on physiological status and personal hydration status [7]. Other physiological factors, such as gender, age, and body mass index (BMI), also contribute to variation to the urinary metabolome [8]. Furthermore, urine has an enormous chemical diversity [9]. As a biological waste material, urine typically contains metabolic breakdown products from a variety of foods, drinks, pharmaceuticals, environmental contaminants, waste metabolites in the body, and bacterial by-products. At least 3079 metabolites have been found to be present in human urine [10]. The large number of metabolites present in urine, along with their many potential origins, makes data analysis and biological interpretation of the urine metabolome challenging [9].

A handful of metabolomics studies on GDM have been published, mostly investigating the maternal blood metabolome [11–16], few have investigated the excretion profile of maternal urine [17–21]. Among the five studies of the maternal urine, three studies performed comprehensive profiling. A capillary electrophoresis-mass spectrometry study compared the urine profiles from 20 women that developed GDM with 20 healthy controls, at 22–28 weeks of gestation after overnight fast. The profiles contained 72 metabolites. A significantly lower level of carnitine was observed in pregnant women that developed GDM, compared to controls, whereas histidine, glutamine, phenylalanine, tryptophan, and cysteine were augmented in GDM [18]. In an NMR-based metabolomics study [19], a partial least-squares discriminant analysis (PLS-DA) model discriminated GDM subjects from controls. However, the model, based on 29 second-trimester prediagnostic GDM urine samples collected under non-fasting conditions, had a poor predictive power (low Q^2 value). In their follow-up study using ultra performance liquid chromatography-mass spectrometry (UPLC-MS), no significant difference was observed, either in amniotic fluid or in maternal urine, in relation to GDM [20]. In a multi-ethnic population-based NMR study of maternal urine, no reliable GDM classification could be achieved [21]. In summary, although biomarker candidates have been proposed, clinically useful biomarkers are still missing, and significant gaps remain in the understanding of the pathophysiology of GDM.

Despite multiple studies having shown the values and applications of urinary metabolomics for the diagnosis of T2DM [22–25], metabolic profiling of maternal GDM urine has thus far revealed no, or minor differences from healthy pregnancies. A possible reason for the lack of findings might be due to the small sample sizes used in the former studies [19,20], resulting in a low predictive power of their discriminatory models. In the latter study [21], the multi-ethnic cohort introduced a very large intrinsic variation between the experimental subjects, but no measure was taken to lessen the genetic and dietary effects. The cohort they investigated was by definition not a "longitudinal cohort", but subjects recruited at different stages of pregnancy (the number of subjects was 667, 671 and 573 from the first, second and third visits, respectively). The data was not paired analysed, for example, using multilevel analysis [26,27]. Variables were normalised to creatinine concentration, without consideration that creatinine excretion is affected by many exogenous factors, including diabetes [28] and GDM [29]. Regardless, creatinine concentration might not be suitable for normalisation of all metabolites in urine [30]. In addition, urine samples contain many xenobiotic substances [31] that might obscure principal components analysis (PCA) modelling. Furthermore, the authors applied 2 distinctive sets of diagnostic criteria for the classification of the participants, one of which based on the World Health Organization 1999 criteria [32], and the other was based on more recently introduced International Association of Diabetes and Pregnancy Study Groups (IADPSG) 2010 recommendations [33]. However, the diagnosis for GDM was not consistently applied to all participants of their study. All these factors detrimentally affected the precision and confidence of their measurements and so the conclusions of their study! A recent NMR-based study of maternal urine, which normalised their data by probabilistic quotient normalisation, found the metabolomic analysis of maternal urine to be useful in the clinical management of GDM [34]. 3-Hydroxyisovalerate, choline, hippurate, creatine, phenylacetylglutamine, galactose, lysine and threonine were found to be significantly different between the GDM subjects compared to controls. Still, NMR-based approaches are limited by their bias towards the abundant metabolites, making the effects of less abundant metabolites under-represented.

We hypothesised that by employing suitable analytical measures, and applying appropriate data processing and statistical methods, the subtle differences between women that developed GDM and the women that did not develop GDM would be identified. The primary objective of this study was to investigate maternal urine from a longitudinal cohort of healthy pregnancies, and pregnancies complicated by GDM, with the aim to advance our knowledge of GDM. The second objective was to determine the trajectories of the urinary metabolome throughout the pregnancy, both in the healthy pregnancies, and pregnancies complicated by GDM. To take into account the repeated measurements of the same subject at different stages of pregnancy (the longitudinal data set), as in part 1 [35], multilevel multivariate statistics [26,27] and multivariate empirical Bayes analysis (MEBA) [36] were used to identify the changes of the urinary metabolome induced by GDM.

2. Material and methods

2.1. Study design and the longitudinal cohort

The clinical study has been reported previously [35]. In brief, this study was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (University Hospital) and was performed in accordance with the guidelines and regulations of the University Hospital. Participants consisted of pregnant women who attended their first prenatal visit in the outpatient unit of the obstetric department at the University Hospital, during their first 10–14 weeks of gestation in 2013. An obstetrician informed the eligible participants of the nature of the study and invited them to participate. Samples were collected from each participant after obtaining an informed consent. A senior obstetric nurse was instructed to follow up the eligible women in according to a set of standardized operating procedures. Participants were asked to complete questionnaires containing questions on their anthropometric, social-demographic, lifestyle (e.g., smoking habit, alcohol consumption), reproductive and medical histories, at their antenatal visits. Participants' weight, blood pressure, and intake of supplements were monitored. All participants were scheduled for three antenatal visits, one in each trimester. Participants were followed up until giving birth, eventually defining sample groups according to their clinical characteristics. 61 participants completed their antenatal care with us and formed the final longitudinal cohort for this study. Of the 61 participants, 34 had normal glucose tolerance (controls), and 27 met the diagnostic criteria for GDM (see Section 2.2). Their clinical characteristics have been described previously [35]. Age, BMI, and parity were considered as confounding factors in this study.

2.2. Diagnostic criteria

The diagnostic criteria for GDM has been described previously [35]. In brief, GDM was diagnosed based on the IADPSG recommendations [33]. Participants underwent a fasting plasma glucose test at the first prenatal visit and they were recognised as having normal glucose intolerance. A routine oral glucose tolerance test (OGTT) was performed at 24–28 weeks of gestation, after an overnight fast. The test was performed as described by the World Health Organization [37]. Women diagnosed with GDM were referred to nutritionists and were given advice on dietary and lifestyle intervention for controlling their blood glucose.