**Abstract**

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders in pregnancy associated with a number of short- and long-term complications for mother and foetus/child. The major mechanisms responsible for GDM development include increased insulin resistance in pregnancy and inadequate insulin secretion. Nevertheless, the exact mechanisms involved in the pathophysiology of GDM have not been fully elucidated. Moreover, despite the fact that the new diagnostic criteria proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) were approved by WHO in 2013, the protocols for screening and diagnosis of GDM vary considerably between countries.

The aim of the study was to investigate the metabolic alterations in the serum samples of individuals affected by gestational diabetes mellitus, with either exceeded fasting plasma glucose (‘isolated impaired fasting glucose’, ‘iIFG’) or exceeded 1h or/and 2h plasma glucose (‘isolated impaired glucose tolerance’, ‘iIGT’) in comparison to normal glucose tolerance (NGT) pregnant women. The first objective was to determine whether different metabotypes of GDM patients with distinct glycaemic states might reflect different pathophysiological mechanisms responsible for GDM development. Then, novel biomarkers of GDM were investigated in order to assess their potential clinical usefulness to diagnose GDM and/or predict its development in the early pregnancy.

GDM was diagnosed at 24-28 gestational week (gwk) based on IADPSG criteria. Pregnant women were assigned to 3 study groups: NGT (control), GDM with ‘iIGT’ and GDM with ‘iIFG’. Moreover, for the limited set of patients (‘Pre-iIGT’, ‘Pre-iIFG’) who were diagnosed with GDM in the second trimester as well as for the healthy individuals, fasting serum samples in the first trimester (8-14 gwk) were collected. At that period, all selected subjects were characterized by normal fasting glucose level. Eventually, the research project comprised of three study cohorts: discovery cohort 24-28 gwk (n=79), validation cohort 24-28 gwk (n=163) and the additional validation cohort 8-14 gwk (n=92). Firstly, fasting serum samples (discovery cohort) were analysed using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) following an untargeted metabolomics approach. Then, the most promising metabolites according to the experimental data and literature, significantly discriminating ‘iIGT’ from NGT group were selected for quantification in the validation cohorts by a targeted GC-MS analysis. In order to evaluate the clinical usefulness of candidate biomarkers, a ROC curve analysis was performed.

Metabolic fingerprinting resulted in a number of statistically significant metabolites differentiating the study groups. The majority of significant changes were observed between ‘iIGT’ and NGT. Among them a great number of compounds was represented by increased medium- and long-chain fatty acids and organic acids. The statistically significant changes observed in ‘iIFG’ subjects as compared to NGT individuals were minor, revealing mainly decreased level of fatty acid amides and fatty alcohols. The majority of statistically significant compounds distinguishing ‘iIGT’ from ‘iIFG’ belonged to phospholipids and lysophospholipids. Additionally, the alterations in metabolites potentially associated with the gut microbiota metabolism (i.e. p-cresol, cresol sulphate, benzoic acid, fumaric acid, β-indole-3-acteic acid), discriminating ‘iIFG’ and ‘iIGT’ groups, were detected. Validation experiment confirmed the results from discovery cohort for a great number of compounds, i.e. α-HB, β-HB, myristic, lauric, palmitic, oleic, nonanoic and capric acids. ROC curve analysis showed the best diagnostic power considering its sensitivity (73%) and specificity (79%) for the model consisted of α-HB, β-HB and myristic acid (AUC=0.828) to identify ‘iIGT’ individuals in the second trimester, and likewise to identify individuals with (or at risk) of ‘iIGT’ in the first trimester (AUC=0.791, sensitivity 85% and specificity 69%).

The study revealed the complexity of GDM pathophysiology, showing different metabolic alterations among GDM patients with distinct glycaemic states, i.e. ‘iIGT’ or ‘iIFG’. The observed differences demonstrate circulating metabolite patterns that distinguish ‘iIGT’ from ‘iIFG’ among GDM patients. Dysregulated metabolic pathways are involved mainly in lipid metabolism. In ‘iIGT’ individuals altered metabolic pathways may contribute to increased peripheral insulin resistance as a consequence of increased lipolysis in adipose tissue, increased fatty acid uptake, increased liver β-oxidation and subsequent ketogenesis, oxidative stress and pro-inflammatory processes. Moreover, alterations in gut microbiota-related metabolites, discriminating ‘iIFG’ and ‘iIGT’ groups, suggest a potential relationship between gut microbiota and different glycaemic states of GDM patients. A combination of α-HB, β-HB and myristic acid may facilitate diagnosis of GDM in the second trimester by identifying GDM patients with ‘iIGT’ from a fasting serum sample, without the need to perform OGTT. Noteworthy, these biomarkers hold a potential to identify patients with (or at risk) of ‘iIGT’ in the early pregnancy and therefore potentially prevent GDM development by recommending the lifestyle modifications (diet and/or moderate physical activity.