The Egyptian Journal of Medical Human Genetics 18 (2017) 281–287

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Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

Original article

Metabolic derangements in IUGR neonates detected at birth using UPLC-MS

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ARTICLE INFO

Article history:
Received 17 November 2016
Accepted 13 December 2016
Available online 30 December 2016

Keywords:
IUGR
Neonate
Metabolic
Metabolomics
Cord blood
UPLC
Mass spectrometry

ABSTRACT

Background: Intrauterine growth restriction (IUGR) is associated with short- and long-term metabolic consequences which are possibly dictated by in utero programming together with environmental and dietetic manipulation after birth. Early detection of metabolic derangements in these babies through metabolomics approach will help recognition of cases in need for further follow-up and can help future development of therapeutic and preventive strategies for the late consequences.
Objective: To compare amino acids and acyl carnitine levels in neonates with IUGR to normal birth weight controls; as a part of metabolic profiling.
Methods: Cord blood samples were collected at birth from 40 small-for-gestational-age (SGA) neonates and 20 normal birth weight gestational age-matched neonates, for quantification of amino acids and acyl-carnitines using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS).
Results: Significantly elevated acylcarnitine levels especially C18-OH and C16-OH were found in IUGR neonates vs. controls ($p < 0.001$). Specific amino acids that were significantly elevated in IUGR neonates included Histidine, Methionine, Arginine, Aspartic, Valine, Alanine, Leucine, Isoleucine, Glutamic acid, Tyrosine, Ornithine, Phenylalanine, and lastly citrulline. These derangements were recognized to be similar to those found in different disorders.
Conclusion: We conclude that IUGR neonates have unique metabolic derangements detectable by UPLC-MS at birth with similarities to derangements found in certain disorders. These babies should be closely followed up for early detection of the metabolic consequences of IUGR.

1. Introduction

Intrauterine growth restriction (IUGR) or small-for-gestational-age (SGA) is defined as birth weight or birth crown-heel length of less than 10th percentile for gestational age. Both terms are used interchangeably [1].

Prevalence of IUGR in the general population worldwide was estimated to be 7–15%, while in developing countries – including Egypt – it reaches up to 30% and constitutes 50–60% of low birth weight neonates (with birth weight of less than 2500 g) [2,3].

There is growing evidence that IUGR is strongly associated with several short- and long-term complications including, for instance, cognitive and psycho-physical developmental disorders during infancy and the metabolic syndrome during adulthood [4,5]. These consequences are probably related to "Perinatal Programming" and strongly correlated to postnatal dietetic rehabilitation and fast catch-up growth [5,6].

Biomarkers in medicine, particularly in Neonatology, are crucial for defining diagnosis and predicting prognosis of many diseases [7].

Certain biomarkers were previously identified and speculated as being correlated with IUGR such as maternal levels of endothelin-1 and leptin during pregnancy and urinary protein

Abbreviations: AGA, appropriate-for-gestational age; IUGR, intrauterine growth restriction; MS, mass spectrometry; ^{1}H NMR, nuclear magnetic resonance spectroscopy; PCA, principal components analysis; PLS-DA, Partial Least Squares-Discriminate Analysis; SGA, small-for-gestational age; UPLC, Ultra Performance Liquid Chromatography.

Peer review under responsibility of Ain Shams University.

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http://dx.doi.org/10.1016/j.ejmhg.2016.12.002
1110-8630/

S100B in the newborn [8,9]. Furthermore, some of these markers were useful as early predictors for later development of insulin resistance and type 2 diabetes mellitus [10].

However, through the last 2 decades, a new generation of biomarkers has been extensively studied; the metabolites comprising the metabolome, using the metabolomics approach.

Metabolomics is based on full analysis of metabolites in a biological sample using either mass spectrometry (MS) or nuclear magnetic resonance (^{1}H NMR) spectroscopy [11,12].

Being dynamic and highly sensitive to different environmental, dietetic and disease stimuli, the metabolome is a perfect target for diagnostic and prognostic approaches [13]. Identifying the metabolic derangements that occur at the level of metabolome very early in life will help detection of subsequent derangements through regular follow-up. This should help future development of therapeutic interventions or preventive strategies [14].

This study was designed to assess the metabolic fingerprints of IUGR at birth in comparison to normal birth weight neonates, so that we may share our results with the world-wide metabolomic mapping system. Babies with recognized metabolic derangements will be followed-up to detect onset of subsequent derangements known to be associated with IUGR.

2. Patients and methods

This case-control study was conducted in the Maternity Hospital; Ain Shams University, Cairo, Egypt, over a period of 13 months from August 1, 2014 to August 31, 2015.

All SGA neonates, either term or preterm, born during the study period were eligible. Samples from neonates with perinatal asphyxia, intracranial hemorrhage, congenital malformations, or congenital infections were discarded. Informed verbal consents were obtained from parents or caregivers after explanation of the study purpose and planning future visits.

Cord blood samples were collected in the delivery room, immediately after cord clamping, from 40 SGA neonates and 20 gestational age-matched healthy, appropriate-for-gestational age (AGA) neonates; as control. From each sample, three drops of blood were put on a filter paper which was immediately analyzed or stored at −80 °C till analysis using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) [ACQUITY UPLC M-Class System, Waters Corporation (NYSE:WAT), Milford, Massachusetts, USA].

Data management and Statistical analysis were done using SPSS 21 and MetaboAnalyst 2.0.

Data processing started with data integrity check, then missing or zero values treatment, and data filtering. Data normalization was done with the generalized log transformation. Data analysis included Univariate analysis (T-test fold change) for preliminary overview of potentially significant features, Correlation analysis, and most importantly, Multivariate analysis including Principal Components Analysis (PCA) and Partial Least Squares-Discriminate Analysis (PLS-DA). Metabolite Set Enrichment Analysis (MSEA) was used to identify meaningful patterns of compounds combination, if any. Pathway analysis was based on KEGG metabolic pathways as the backend knowledgebase.

In this study, we divided the several variables of metabolomic profile into compounds for data reduction using PCA; this aimed at summarization of data into much fewer variables called scores which represent a weighted average of the original variables. The weighting profiles are generally called loadings.

Loadings for any variable in PCA mean the importance of this variable in that component (the higher the loading, the higher is the importance of that variable to explain variability in data).

Then the components were subsequently compared with components contained in the metabolite set library (KEGG).

3. Results

The mean ± SD (min–max) gestational age of SGA neonates was 34 ± 2.4 (30–39) weeks. Their mean birth weight was 1320 ± 300 (800–2400) g, mean crown-heel length was 40 ± 2.6 (35–45) cm, and mean head circumference was 28 ± 1.9 (25–32) cm. They were 18 (45%) males and 22 (55%) females. Eighty percent of them (32/40) were delivered by Cesarean section and 75% (30/40) of their mothers had preeclampsia.

Control group included 20 neonates with mean gestational age of 35 ± 1.4 (32–38) weeks, mean birth weight of 3100 ± 400 (2100–3800) g, mean crown-heel length of 48.5 ± 2.4 (45–51) cm, and mean head circumference of 34.4 ± 1.2 (33–36) cm. They were 12 (60%) males and 8 (40%) females. They were all AGA and 18/20 (90%) of them were delivered vaginally.

The two groups were non-significantly different as regards gestational age and sex distribution ($p > 0.05$), but cases had significantly less birth weight, length and head circumference than controls ($p < 0.001$).

Distinct metabolic profiles were identified for SGA neonates that are different from AGA neonates. Important features identified by t-tests are shown in Table 1. On the basis of individual metabolites, PCA and PLS-DA revealed very high concentrations of acylcarnitines, especially C18-OH and C16-OH acylcarnitines, in cases versus controls as well as significantly different levels – between the two groups – of Histidine, Methionine, Arginine, Aspartic, Valine, Alanine, Leucine, Isoleucine, Glutamic acid, Tyrosine, Ornithine, Phenylalanine, and lastly citrulline.

Coefficients or VIP scores showed details of the variables which were significantly higher or lower in IUGR compared to AGA neonates. C18-OH, C16-OH acylcarnitines, for example, were of highest concentration in patients not controls, while Histidine and Methionine were lower in patients than controls; Fig. 1.

Enrichment analysis showed disorders with highly significant metabolomic similarity to IUGR profiles. Examples are some of urea cycle defects such as Ornithine Transcarbamylase Deficiency (OTC), *N*-acetylglutamate synthetase deficiency and Argininosuccinic Aciduria, Lysinuric Protein Intolerance, Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH-Syndrome), tyrosinemia type I, autism, and diabetes mellitus (MODY); Table 2 and Fig. 2.

Detailed results from pathway analysis are presented in Table 3. It shows that the most significantly altered metabolic pathway in IUGR neonates is the purine metabolism followed by thiamine metabolism, primary bile acid biosynthesis, lysine degradation, pyrimidine metabolism, and lastly glutathione metabolism, among others.

4. Discussion

Metabolomics provide a "snapshot" of metabolic status of a cell, tissue, or organism in relation to genetic variations or external stimuli [14]. During the last decade, many studies in neonates focused on metabolic profiling in different disease states; in order to establish maps for metabolic derangements in different disorders.

In this study, we used UPLC-MS for measurement of amino acids and acyl carnitine profiles in a group of SGA neonates in comparison to a matched group of AGA controls.

UPLC-MS provides significantly more resolution while reducing run times; so it is rapid, simple, and improves sensitivity for the analyses of many compounds [15].