Clinica Chimica Acta 483 (2018) 135–141

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Diagnostic and therapeutic profiles of serum bile acids in women with intrahepatic cholestasis of pregnancy-a pseudo-targeted metabolomics study

Yue Cui[a], Biao Xu[b], Xiaoqing Zhang[a], Yifan He[a], Yong Shao[c], Min Ding[a,*]

[a] Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, Department of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, PR China
[b] Department of Laboratory, the First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, PR China
[c] Department of Obstetrics and Gynecology, the First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, PR China

ARTICLE INFO

Keywords:
Intrahepatic cholestasis of pregnancy
Serum bile acid
Pseudo-targeted method
Metabolomics
UPLC-triple TOF-MS/MS
Diagnostic and therapeutic profiles

ABSTRACT

Background: Intrahepatic cholestasis of pregnancy (ICP), as a pregnancy-specific liver disorder, obtains increasing recognition due to a series of adverse outcomes. ICP is generally characterized by pruritus and jaundice, and closely related to abnormalities in the metabolism and disposition of bile acids composition. Because of its elusive pathogenesis, ICP has become an intractable issue to be diagnosed and managed for obstetricians. Analysis of metabolic profile could reveal the state of small-molecule metabolites systematically and provide comprehensively metabolic information for diseases. We developed a pseudo-targeted approach to perform metabolomic analysis of bile acids in serum using an ultra-performance liquid chromatography-triple quadrupole time-of-flight tandem mass spectrometry (UPLC-Triple TOF-MS/MS) method.

Methods: We investigated the metabolites of bile acids in 55 healthy pregnant women, 42 women with ICP and 11 women with ICP who persisted to accept ursodeoxycholic acid (UDCA) therapy.

Results: The metabolic profiles of serum bile acids were significantly altered in ICP group compared with the control group. A screened potential combination biomarker, with a high diagnostic efficiency (area under the curve = 0.996, Youden index = 0.940), was superior to total bile acids for the diagnosis of ICP.

Conclusions: The profiles of serum bile acids in women with ICP became more clear under the UDCA therapy, and were fully recovered after the delivery.

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder. It is commonly characterized by pruritus, jaundice, elevated total bile acids (TBA) and/or serum transaminases. ICP increases perinatal risk of adverse fetal outcomes, comprising preterm delivery, asphyxial events, and fetal demise [1,2]. ICP was once considered as a benign disease to women [3,4].However, researchers proposed recently that women with ICP were more vulnerable to pre-eclampsia [2], gestational diabetes [5], and later hepatobiliary diseases [6,7].Even their offspring were more susceptible to metabolic diseases [8].

At present, the diagnosis is primarily based on typical pruritus and abnormal lab detections (increased TBA level above 10 μmol/l and raised transaminases level) as well as spontaneous resolution of discomforts after delivery. Raised fasting serum TBA level was considered to be the most commonly used lab criteria for the diagnosis of ICP [9–11]. However, several researches reported that women who were clinically diagnosed as ICP were frequently absent of elevated TBA level [12,13], leading to the false-negative rate up to 45% [14]. In addition, fasting state influences the detective sensitivity of TBA [15]. On the other hand, the definite diagnosis of some patients might be delayed after parturition according to the postnatal resolution of discomforts.

Abbreviations: ICP, Intrahepatic cholestasis of pregnancy; TBA, total bile acids; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; UPLC-Triple TOF-MS/MS, ultra-performance liquid chromatography-triple quadrupole time-of-flight tandem mass spectrometry; MRM, multiple reaction monitoring; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HDCA, hyodeoxycholic acid; DHCA, dehydrocholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; HCA, hycocholic acid; MCA, muricholic acid; GHCA, glycohycocholic acid; GLCA, glycolithocholic acid; GHDCA, glycohyodeoxycholic acid; GDHCA, glycodehydrocholic acid; THCA, taurohycocholic acid; T-, tauro-; THDCA, taurohyodeoxycholic acid; TDHCA, taurodehydrocholic acid; TLCA, taurolithocholic acid; TBIL, total bilirubin; DBIL, direct bilirubin; PROM, premature rupture of membranes; MSAF, meconium staining of amniotic fluid; IS, internal standard; ESI, electrospray ionization; DP, declustering potential; CE, collision energy; PLS-DA, partial least squares- discriminant analysis; PCA, principal component analysis; AUC, area under the curve; YI, Youden index; VIP, variable importance in the projection; IDA, information dependent acquisition

* Corresponding author.
E-mail address: dingmin@cqmu.edu.cn (M. Ding).

https://doi.org/10.1016/j.cca.2018.04.035
Received 7 February 2018; Received in revised form 22 April 2018; Accepted 26 April 2018
Available online 27 April 2018
0009-8981/

Thereby, early diagnosis of ICP would be difficult. Castaño and coworkers [4] provided the profile of serum bile acids as a possible solution for the early diagnosis to avoid the intersectional concentration of TBA between healthy pregnant women and women with ICP.

Analysis of metabolic profile could fully reveal the state of small-molecule metabolites and systematically provide metabolic information for biological samples [16]. As the extremely complex small-molecule metabolites, dozens of bile acids were recognized to enrich the metabolic profiles of bile acids. Given the intimate relationship between ICP and deranged metabolism of bile acid profiles [17], it is formal and analytical to diagnose ICP with a holistic profile of bile acids, in contrast to a solely biochemical test of TBA.

Besides the diagnosis, serum bile acid profiles probably provide the dynamic evolution for this disease after therapy. In recent years, ursodeoxycholic acid (UDCA) has become a new favorite medicine in treating women with ICP. UDCA is a naturally hydrophilic bile acid, while lithocholic acid (LCA) is the most hydrophobic and toxic in the monohydroxy bile acids. Recent researches reported that UDCA therapy could decrease TBA concentrations, without causing an increase in LCA level [18]. In addition, UDCA therapy could also ameliorate pruritus, normalize elevated biochemical parameters, and improve the prognosis of pregnant outcomes [19]. Thus, we assumed that serum bile acid profiles in women with ICP were probably altered during the UDCA therapy.

In present study, based on the ultra-performance liquid chromatography-triple quadrupole time-of-flight tandem mass spectrometry (UPLC-Triple TOF-MS/MS) platform, we proposed a novel pseudo-targeted metabolomics analysis, which integrated the advantages of targeted and non-targeted analysis. To figure out metabolic profiles globally, non-targeted analysis has been proved to be an appropriate tool for the study of observing in an unbiased manner as many detectable metabolites as possible [20]. Nevertheless, there are still some deficiencies, such as complicated matrix influence, peak alignment default, and repeatability problem in large scale non-targeted measurement [21]. Triple-quadrupole MS in combination with multiple reaction monitoring (MRM) is a common technology for quantitative analysis of the target. The use of specific precursor ions and product ions transitions facilitates to improve the specificity and sensitivity of quantitative analysis and to reduce the complicated data processing. Based on the ion pairs for the MRM mode, the pseudo-targeted method provided better repeatability and wider linear range than the traditional non-targeted metabolomics method [22]. On the other hand, TOF-based metabolomics analysis could reveal new metabolites, which was not included in the targeted analysis.

Thus, this pseudo-targeted method realized the absolute quantification of confirmed bile acids and relative quantification of identified bile acids simultaneously. Based on the holistic profile of serum bile acids in healthy pregnant women and women with ICP, our aim is to fully illuminate the potential diagnostic biomarker for ICP, and to further clarify the characteristics of bile acid profiles of women with ICP under UDCA therapy.

2. Materials and methods

2.1. Materials

The standards of cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), UDCA, hyodeoxycholic acid (HDCA), dehydrocholic acid (DHCA), LCA, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), glycoursodeoxycholic acid (GUDCA), taurochenodeoxycholic acid (TCDCA), taurocholic acid (TCA), taurodeoxycholic acid (TDCA), and tauroursodeoxycholic acid (TUDCA), formic acid and ammonium acetate were obtained from Sigma. The standards of hycocholic acid (HCA), α-muricholic acid (α-MCA), β-MCA, ω-MCA, glycohycocholic acid (GHCA), glycolithocholic acid (GLCA), glycohyodeoxycholic acid (GHDCA), glycodehydrocholic acid (GDHCA), taurohycocholic acid (THCA), tauro-α-muricholic acid (T-α-MCA), tauro-ω-muricholic acid (T-ω-MCA), taurolithocholic acid (TLCA), taurohyodeoxycholic acid (THDCA), and taurodehydrocholic acid (TDHCA) were purchased from Steraloids. The methanol and acetonitrile were of mass grade (Fisher). Water was purified with a MilliQ™ system (Millipore).

2.2. Recruits

This study was performed in total 175 serum samples within 147 pregnant women of the obstetric department of the First Affiliated Hospital of Chongqing Medical University (September 2012 to May 2015). Women with ICP were diagnosed by the second edition of ICP guideline from Royal College of Obstetricians and Gynecologists [23] and confirmed after delivery. The blood samples were taken from 42 women with ICP at the first time visit to take the confirmation diagnosis and 105 healthy pregnant women during the same period. Among the 42 patients, 11 of them were supplied UDCA therapy (Ursofalk capsules, 250 mg, tid, Dr. Falk Pharma Gesellschaft mit beschrankter Haftung, Freiburg, Germany) and taken blood sampling biweekly until delivery. After the delivery, these 11 women were terminated the drug taking and 7 of them supplied their blood samples 2 weeks later. Among the 105 healthy pregnant women, 55 of them were randomly recruited to make control group. And to evaluate the repeatability and stability of this experiment, the sera of another 50 healthy pregnant women with low concentrations of TBA (< 2 μmol/l) were collected and mixed to prepare the quality control (QC) and blank serum. The mixed samples were vortexed, aliquoted and stored at -20 °C before analysis.

All the blood samples were taken from the antecubital vein in a fasting state of recruits in the morning. After centrifugation, one part of serum was collected for clinical biochemical tests by Modular DDP automatic biochemical analyzer system (Roche Diagnostics), including TBA, total bilirubin (TBIL), direct bilirubin (DBIL), alanine transaminase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT). The other part was stored at -80 °C until UPLC-TripleTOF-MS/MS analysis. Obstetric informations of all recruits were assembled, comprising maternal age, maternal blood type, gestational age at delivery, mode of delivery, single/multiple pregnancy, premature rupture of membranes (PROM), meconium staining of amniotic fluid (MSAF), newborn weight, and postpartum hemorrhage. This study was designed conforming to the ethics guidelines given in the Declaration of Helsinki. Written informed consent was obtained from all subjects and authorized by the ethics committee approval of the First Affiliated Hospital of Chongqing Medical University.

2.3. Methods

Thawed serum (300 μl) and 10 μl of 25 μmol/l TDHCA (internal standard, IS) were added into 1 ml of formic acid/water (0.05/99.95, v/v), and mixed thoroughly by vortexing. The sample mixture was loaded onto Bond Elut-C18 cartridge (200 mg/3 ml, Agilent), previously conditioned with 1 ml of methanol and 1 ml of formic acid/water (0.05/99.95, v/v). Loaded cartridges were subsequently washed with 1 ml of water and 1 ml of methanol/water (5/95, v/v) and eluted out with 1.5 ml of methanol/water (90/10, v/v). The eluent was dried at a vacuum system (ISS110 P1, Thermo Fisher Scientific) and dissolved in 50 μl of methanol/water (50/50, v/v). The dissolved solution was sonicated for 2 min, and centrifuged at 13000 $\times g$ for 10 min at 4 °C. Five microliters of supernatant was injected into the UPLC-Triple TOF-MS/MS system.

A Shimadzu CBM20Alite UPLC system coupled online via electrospray ionization (ESI) with a Triple-TOF™ 5600 system (AB Sciex) was used for UPLC-Triple TOF-MS/MS based on pseudo-targeted analysis. The chromatographic separation was performed using a Kinetex XB-C18 column (50 mm × 2.1 mm, 1.7 μm; Phenomenex) linked to a security guard C18 ultra-cartridges (2 mm × 2.1 mm) and ultra-holder