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Correlation of lipid metabolism characteristics with bile acid metabolism and placental hypoxia injury in patients with intrahepatic cholestasis of pregnancy

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ABSTRACT

Objective: To study the correlation of lipid metabolism characteristics with bile acid metabolism and placental hypoxia injury in patients with intrahepatic cholestasis of pregnancy (ICP). **Methods:** ICP pregnant women and healthy pregnant women who received antenatal care and delivered in Obstetrics Department of Panzhihua Maternal and Child Health Care Hospital between May 2013 and October 2016 were collected and included in ICP group and control group respectively. Serum lipid metabolism and bile acid metabolism indexes were measured at 20 weeks, 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation; mitochondria damage molecule expression levels in placenta were determined after childbirth. **Results:** Serum TC, LDL-C and HDL-C levels were not different between two groups of pregnant women at 20 weeks of gestation, and serum TC and LDL-C levels of ICP group at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation were significantly higher than those of control group while HDL-C levels were significantly lower than those of control group; serum TBA, ALT and AST levels were not different between two groups of pregnant women at 20 weeks, 24 weeks and 28 weeks of gestation, and serum TBA, ALT and AST levels of ICP group at 32 weeks and 36 weeks of gestation were significantly higher than those of control group; CCO, ATPase, SDH and Bcl-2 protein expression in placenta tissue of ICP group were significantly lower than those of control group while Bax and Caspase-3 protein expression were significantly higher than those of control group. Serum LDL-C levels at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation were positively correlated with TBA, ALT and AST levels in serum as well as Bax and Caspase-3 protein expression in placental tissue, and negatively correlated with CCO, ATPase, SDH and Bcl-2 protein expression in placental tissue. **Conclusion:** Midtrimester lipid metabolism characteristics can early predict the risk of ICP and evaluate the abnormal degree of bile acid metabolism and the placental hypoxia injury.

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a common complication in middle-late pregnancy, which is characterized by intrahepatic cholestasis as well as elevated bile acid and transaminase levels, as well as with or without itchy skin[1,2]. Although the ICP is a benign process for the majority of pregnant women and the symptoms will quickly disappear after childbirth, it will increase the risk of preterm labor, fetal intrauterine hypoxia, fetal growth restriction and other perinatal complications, and it needs early detection and intervention[3–5]. Serum bile acid content is an important indicator for ICP diagnosis and illness evaluation, but the bile acid content increase mostly appears in middle-late pregnancy, and it is not conducive to early prediction of disease risk. Existing study of domestic scholars has confirmed that the there is obvious disorder of lipid metabolism in ICP patients and the abnormality of lipid metabolism indexes appears earlier, so it can provide a basis for the prediction of the onset of ICP[6]. However,

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there is no clear report on the relationship between lipid metabolism characteristics of ICP and illness development. In the following study, the correlation of lipid metabolism characteristics with bile acid metabolism and placental hypoxia injury in patients with intrahepatic cholestasis of pregnancy was analyzed.

2. Case information and research methods

2.1 Case information

The clinical data of ICP pregnant women and healthy pregnant women who received antenatal care and delivered in Obstetrics Department of Panzhihua Maternal and Child Health Care Hospital between May 2013 and October 2016 were collected. ICP inclusion criteria were as follows: (1) with onset in late pregnancy and mainly characterized by skin itch; (2) with total bile acid content more than 10μmol/L; (3) to signing the informed consent. Healthy pregnant women inclusion criteria were as follows: (1) without pregnancy complications after antenatal care; (2) signing the informed consent. ICP patients and healthy pregnant women were enrolled in ICP group and control group of the research respectively. ICP group information was as follows: a total of 54 cases, 27-35 years old, 31 cases of primiparae and 23 cases of multiparae; control information information was as follows: a total of 66 patients, 25-33 years old, 39 cases of primiparae and 27 cases of multiparae. The two groups of pregnant women were not significantly different in general data (P>0.05).

2.2 Research methods

2.2.1 Pregnancy serum sample collection and index detection

At 20 weeks, 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, the peripheral venous blood was collected respectively during prenatal care and used for detection of blood biochemical indexes, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), bile acid (TBA), alanine aminotransferase (ALT) and aspartate transaminase (AST).

2.2.2 Placental mitochondrial hypoxia injury-related molecule expression

Within 30 min after childbirth, right amount of maternal surface of placenta tissue was cut off immediately, completely washed with saline, then fully dried with filter paper, transferred into cryopreserved tubes, briefly frozen in the liquid nitrogen for 30 min, then taken out and preserved at -80 ℃; for detection of mitochondrial hypoxia injury-related molecule expression, adequate amount of placenta tissue was taken and added in protein lysis buffer to extract the total protein in the tissue, and the enzyme-linked immunosorbent assay kits were used to determine the expression of CCO, ATPase, SDH, Bcl-2, Bax and Caspase-3 in total protein samples.

2.3 Statistical processing

SPSS 20.0 statistical software was used for data processing, analysis of blood biochemical indexes and placenta molecule expression between two groups was by t test and P<0.05 was the standard of statistical significance in differences between two groups.

3. Results

3.1 Serum lipid metabolism index contents during pregnancy

At 20 weeks, 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, analysis of serum lipid metabolism indexes TC, LDL-C and HDL-C contents between two groups of pregnant women was as follows: (1) at 20 weeks of gestation, serum TC, LDL-C and HDL-C levels were not different between two groups of pregnant women (P>0.05); (2) at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, serum TC and LDL-C levels of ICP group showed increasing trend while HDL-C levels showed decreasing trend, and serum TC and LDL-C levels of control group showed no significant change; (3) at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, serum TC and LDL-C levels of ICP group were significantly higher than those of control group while HDL-C levels were significantly lower than those of control group, and differences in serum TC, LDL-C and HDL-C levels were statistically significant between two groups of pregnant women at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation (P<0.05).

Table 1.

Serum lipid metabolism index contents of two groups of pregnant women during pregnancy (mmol/L).

Groups	n	Gestational weeks	TC	LDL-C	HDL-C
ICP group	54	20 weeks	5.58±0.76	3.42±0.52	1.52±0.18
		24 weeks	6.12±0.84	3.77±0.49	1.45±0.14
		28 weeks	6.92±0.91*	3.91±0.43*	1.13±0.19*
		32 weeks	7.41±0.87*	4.51±0.77*	1.02±0.11*
		36 weeks	7.63±0.93*	5.24±0.78*	0.94±0.12*
Control group	66	20 weeks	5.27±0.65	3.31±0.45	1.55±0.18
		24 weeks	5.74±0.79	3.42±0.62	1.49±0.18
		28 weeks	5.88±0.91	3.51±0.68	1.46±0.12
		32 weeks	6.21±0.83	3.77±0.67	1.50±0.11
		36 weeks	6.32±0.74	3.91±0.61	1.42±0.16

*: comparison between ICP group and control group at the same weeks of gestation, P<0.05.

3.2 Serum bile acid metabolism index contents during pregnancy

At 20 weeks, 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, analysis of serum bile acid metabolism indexes TBA (μmol/L), ALT (U/L) and AST (U/L) contents between two groups of pregnant women was as follows: (1) at 20 weeks, 24 weeks and 28 weeks of gestation, serum TBA, ALT and AST levels were not different between two groups of pregnant women (P>0.05); (2) at 32 weeks and 36 weeks of gestation, serum TBA, ALT and AST levels of ICP group showed increasing trend, and serum TBA, ALT and AST levels of control group showed no significant change; (2) at 32 weeks and 36 weeks of gestation, serum TBA, ALT and AST levels of ICP group were significantly higher than those of control group, and differences in serum TBA, ALT and AST levels were statistically significant between two groups of pregnant women at 32 weeks and 36 weeks of gestation (P<0.05).

Table 2.

Serum bile acid metabolism index contents of two groups of pregnant women during pregnancy

Groups	n	Gestational weeks	TBA	ALT	AST
ICP group	54	20 weeks	9.58±1.15	17.21±2.24	20.32±3.52
		24 weeks	10.25±1.52	19.04±2.64	19.58±2.35
		28 weeks	10.65±1.41	18.75±2.36	23.57±4.12
		32 weeks	24.52±4.52*	54.26±8.72*	62.35±8.25*
		36 weeks	32.18±5.64*	59.02±8.14*	69.15±8.77*
Control group	66	20 weeks	10.10±1.57	19.02±2.52	21.04±3.76
		24 weeks	9.94±1.25	18.62±2.25	20.57±3.21
		28 weeks	10.59±1.88	17.95±2.33	20.96±3.17
		32 weeks	11.03±1.75	19.59±2.75	22.25±3.27
		36 weeks	10.57±1.46	19.25±2.46	21.29±3.68

*: comparison between ICP group and control group at the same weeks of gestation, P<0.05.

3.3 Placental mitochondrial hypoxia injury molecule expression

After childbirth, analysis of mitochondrial respiratory chain-related enzymes CCO, ATPase and SDH expression in placenta tissue between two groups of pregnant women was shown in Table 3: CCO, ATPase and SDH protein expression in placenta tissue of ICP group were significantly lower than those of control group; analysis of mitochondria apoptosis-related molecules Bcl-2, Bax and Caspase-3 expression in placenta tissue was shown in Table 4: Bcl-2 protein expression in placenta tissue of ICP group was significantly lower than that of control group while Bax and Caspase-3 protein expression were significantly higher than those of control group. Differences in CCO, ATPase, SDH, Bcl-2, Bax and Caspase-3 protein expression in placenta tissue were statistically significant between two groups of pregnant women (P<0.05).

Table 3.

Mitochondrial respiratory chain-related enzyme expression in placenta tissue of two groups of pregnant women (ng/mL).

Groups	n	CCO	ATPase	SDH
ICP group	54	4.28±0.62	3.58±0.41	1.48±0.22
Control group	66	11.36±1.85	8.59±1.31	3.25±0.56
T		13.228	15.028	12.598
P		<0.05	<0.05	<0.05

Table 4.

Mitochondria apoptosis-related molecule expression in placenta tissue of two groups of pregnant women (ng/mL).

Groups	n	Bax	Bcl-2	Caspase-3
ICP group	54	7.51±0.93	3.18±0.52	11.36±1.67
Control group	66	2.52±0.35	6.52±0.87	5.52±0.78
T		22.583	11.285	10.981
P		<0.05	<0.05	<0.05

3.4 Correlation of pregnancy serum LDL–C levels with bile acid metabolism and placenta molecule expression

Pearson correlation analysis showed that serum LDL-C levels at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation were positively correlated with TBA, ALT and AST levels in serum, negatively correlated with CCO, ATPase, SDH and Bcl-2 protein expression in placental tissue, and positively correlated with Bax and Caspase-3 protein expression in placental tissue.

4. Discussion

Abnormal bile acid metabolism is an important characteristic of patients with ICP, which is characterized by elevated serum bile acid content and significant itchy skin. Under physiological conditions, bile acid is generated in liver cells, taken into the intrahepatic bile ducts via hepatic sinusoid, then discharged into the intestinal tract, partially discharged via faeces and partially discharged into the enterohepatic circulation. The bile acid transport in ICP patients is with significant obstacles and deposits in the liver, which on the one hand, affects bile acid excretion and causes the elevated content of serum bile acid, and on the other hand, will cause the liver cell damage and lead to transaminase release into the blood circulation[7,8]. The research subjects of the study were ICP patients with onset in late pregnancy, and the analysis of the content of serum bile acid and transaminase showed that serum TBA, ALT and AST levels were not different between two groups of pregnant women at 20 weeks, 24 weeks and 28 weeks of gestation, and serum TBA, ALT and AST levels of ICP group at 32 weeks and 36 weeks of gestation were significantly higher than those of control group. This means that in the middle pregnancy, there is no abnormal metabolism of bile acid or liver function damage in patients with ICP, the bile acid and transaminase can accurately diagnose ICP and assess the condition, but they are of no significant meaning for early screening for the disease.

In recent years, research on ICP believes that lipid metabolism is closely related to the occurrence and development of ICP[9,10]. In

the study, the analysis of serum content of lipid metabolism indexes in ICP patients showed that serum TC, LDL-C and HDL-C levels were not different between two groups of pregnant women at 20 weeks of gestation, and serum TC and LDL-C levels of ICP group at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation were significantly higher than those of control group while HDL-C levels were significantly lower than those of control group. This means that the abnormal serum lipid metabolism in ICP patients may appear in the middle pregnancy and at 24 weeks of gestation, the occurrence of lipid metabolism disorder is earlier than that of bile acid metabolism disorder and liver function damage, and the evaluation of lipid metabolism characteristics in pregnant metaphase can predict the risk of late pregnancy ICP. LDL-C is the most important molecule affecting lipid metabolism, and it participates in the transport of cholesterol in the body. In order to further clarify the value of serum LDL-C content in pregnant metaphase for the ICP condition prediction and evaluation, the correlation between LDL-C levels in different time points during pregnancy and bile acid metabolism in late pregnancy was analyzed in the study, and the results showed that serum LDL-C levels at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation were positively correlated with serum TBA, ALT and AST levels. This further confirms that the LDL-C levels in pregnant metaphase can predict the risk of ICP in late pregnancy and evaluate the degree of bile acid metabolism disorder after ICP.

On the basis of cholestasis and liver function damage, ICP will also increase the occurrence risk of fetal intrauterine hypoxia. The placenta is the important maternal-fetal material exchange organ, and mitochondrial intrauterine abnormality in placenta of ICP is an important part causing maternal-fetal material exchange barrier and fetal intrauterine hypoxia. The REDOX reaction and electron transfer process mediated by the mitochondrial respiratory chain can provide energy for placenta sertoli cell metabolism[11,12]. CCO, ATPase and SDH are the key enzymes in mitochondria that catalyze electron transfer and provide energy for oxidation, CCO can catalyze electron transfer from cytochrome C to oxygen molecules, ATPase catalyzes oxidative phosphorylation process and ATP generation, and SDH participates in tricarboxylic acid cycle and electron transfer. In the study, the analysis of mitochondrial respiratory chain-related enzyme expression in placenta showed that CCO, ATPase and SDH protein expression in placenta tissue of ICP group were significantly lower than those of control group. Lower expression of mitochondrial respiratory chain-related enzymes will directly affect the function of mitochondria, and then start the mitochondrial pathway of apoptosis[13]. Bax/Bcl-2 is a pair of molecules regulating mitochondrial pathway of apoptosis, the former has pro-apoptotic effect, the latter has anti-apoptotic effect, and together, they regulate the expression of apoptotic executive molecule caspase-3, and thus affect the mitochondrial pathway of the sertoli cell apoptosis[14,15]. In the study, the analysis of mitochondrial apoptosis molecule expression in placenta showed that Bcl-2 protein expression in placenta tissue of ICP group was significantly lower than that of control group while Bax and Caspase-3 protein expression were significantly higher than those of control group. Further analysis of the correlation between LDL-C levels and mitochondrial damage molecule expression during pregnancy showed that at 24 weeks, 28 weeks, 32 weeks and 36 weeks of gestation, serum LDL-C levels were negatively correlated with CCO, ATPase, SDH and Bcl-2 protein expression in placental tissue and positively correlated with Bax and Caspase-3 protein expression.

Based on the analysis of above blood biochemical indexes and placental mitochondrial hypoxia injury molecules, it is believed that determination of lipid metabolism characteristics in pregnant metaphase has predictive value the risk of ICP in late pregnancy, and lipid metabolism molecules have evaluation value for the degree of bile acid metabolism disorder and placental hypoxia injury.

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