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The relationship between the internal oxidation-reduction system and fetal distress on pregnant patients with intrahepatic cholestasis

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Abstract. – OBJECTIVE: To discuss the relationship between the internal oxidation-reduction system and fetal distress in pregnant patients with intrahepatic cholestasis in order to provide a new basis for clinical treatment and research.

PATIENTS AND METHODS: From March 2012 to March 2015, eighty patients with intrahepatic cholestasis of pregnancy (ICP) were selected and divided into two groups: the distressed group (n = 31) and non-distressed group (n = 49). We compared the two groups for differences in MDA, SOD, NO level, GSH level, venous blood and total bile acid level. The relevance of the oxidation-reduction system indicators and the venous blood and total bile acid levels, as well as the differences in the delivery outcome and fetal distress, were compared between the two groups.

RESULTS: The serum MDA level of the distressed group was higher than the non-distressed group while the SOD, NO, and GSH levels were lower than the non-distressed group. All differences were statistically significant ($p < 0.05$). Both the venous blood and total bile acid levels in the distressed group were higher than the non-distressed group and were statistically significant ($p < 0.05$). Based on Pearson's analysis, MDA was positively associated with the venous blood and total bile acid levels while SOD, NO and GSH levels were negatively associated with it. All differences were statistically significant ($p < 0.05$). The death rate of cesarean section and perinatal infant in the distressed group were higher than that of the non-distressed group. The proportion of mild and severe asphyxia was higher than the non-distressed group. However, the neonatal weight of the distressed group was lower. All differences were statistically significant ($p < 0.05$).

CONCLUSIONS: The internal oxidation-reduction system indicators of pregnant patients with intrahepatic cholestasis, which are MDA, SOD, NO and GSH levels, may contribute to the occurrence of fetal distress.

Key Words:
Gestation period, Intrahepatic cholestasis, Oxidation-reduction system, Fetal distress, MDA, SOD, NO, GSH.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) usually appears in the third trimester and is accompanied with typical skin itch and jaundice. The biochemical detection mainly contains an abnormal increase of venous and total bile acid. Most puerperae prognosis is pretty good, but can harm the infant's health[1,2]. Recent studies[3,4] have discovered that an abnormal imbalance of the patients' intrahepatic oxidation-reduction reaction system could result in the cellular metabolic matrix disturbances. This can then lead to a change of placental trophoblastic molecular structure and would weaken and reduce the transport of bile acid. This research investigates whether oxidation-reduction reaction system within the ICP patients' body, MDA, SOD, NO, GSH are related to fetal distress.

Patients and Methods

Patients

Eighty cases of patients diagnosed with ICP from March 2012 to March 2015 in our hospital were selected and divided into two groups: the distressed group (n = 31) and non-distressed group (n = 49). The age range in the distressed group was from 23 to 36 years old with an average age of 25.7 ± 6.3 years old and the gestational age ranged from 31 to 38 weeks with an

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average of 35.4 ± 2.7 weeks. Within the distressed group, there were 18 cases of primiparas and 13 cases of multiparas. The age in the non-distressed group ranged from 22 to 35 years old with an average of 25.8 ± 4.9 years old. The gestational age ranged from 30 to 39 weeks with an average of 35.8 ± 3.1 weeks. And within the non-distressed group, there were 27 cases of primiparas and 22 cases of multiparas. The comparisons and diversities in age, gestational weeks and maternal types between the two groups were statistically insignificant ($p > 0.05$). This research obtained permission from the hospital's Ethics Committee and the informed consent of family members.

The diagnostic criteria of ICP: (1) Clinical features included skin itch, jaundice, no rash, and no digestive tract symptom. (2) Abnormal liver functions were manifested with a direct increase in serum bilirubin and a mild increase in ALT and AST. (3) Skin itch and jaundice faded away rapidly, and liver functions returned to normal promptly after delivery. (4) If the venous blood bile acid ≥ 10.75 μmol/l (the normal value ≤ 5.61 μmol/l) or the total bile acid ≥ 10 μmol/l, a definite diagnosis would follow.

The diagnostic criteria of fetal distress was as follows: (1) The meconium-stained amniotic fluid reached the second or third degree. (2) Auscultate twice or more during the contraction breaks. Fetal heart rate was less than160/min or greater than 120/min (severe abnormity of fetal heart rate is ≤ 100/min or ≥ 180/min) (3) Fetal heart electric monitor slows down, and variable deceleration appears frequently. The NST baseline rate is abnormal, with decreased or no variation, or no response. (4) Fetal movements are less than three times/hour

The exclusion criteria include the following: 1. Primary liver diseases, severe digestive tract diseases, diabetes mellitus, hypertension, placenta previa, premature rupture of the membranes, abnormal amniotic fluid volume, and fetal malformations,

Methods

We compared and analyzed the monoamino oxidase (MOA), superoxide dismutase (SOD), nitric oxide (NO) level, reduced glutathione (GSH) level differences between the two groups, including the relevance between the oxidation-reduction system indicators and the venous blood and total bile acid level. Finally, we analyzed the differences in the delivery outcome and fetal distress between the two groups. In the analysis, thiobarbituric was applied to detect malonyl dialdheyde (MDA); xanthine oxidase to detect SOD; Hafeman micro method to detect GSH and nitrate reductase to detect NO. Latex enhanced immunoturbidimetric assay was used to detect venous blood bile acid while V generation cycle enzyme rate was used to detect the total bile acid. All reagents were provided by Nanjing Jindun Biochemical Reagent Factory, and the instructions were strictly followed.

The Apgar grade, 1 minute after the infants were born, were as follows: normal: 8-10, mild asphyxia: 4-7, severe asphyxia: 0-3.

Statistical Analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The mean value ± the standard deviation was applied to express the quantitative data. The *t*-test was applied to check the comparisons among the groups. The number of cases or (%) were adopted to show the enumeration data. The χ^2 or Fisher exact test were adopted to examine the differences among groups. The Pearson test was used to check the relevance. $p < 0.05$ indicates that the diversities are statistically significant.

Results

The Comparisons of MDA, SOD, NO, GSH Levels Between the two Groups

The serum MDA level of the distressed group was significantly higher than the non-distressed group while the SOD, NO, and GSH levels were significantly lower than the non-distressed group. All differences were statistically significant ($p < 0.05$) Table I.

The Comparisons of the Venous Blood Bile Acid and the Total Bile Acid Level Between the two Groups

Both of the venous blood and total bile acid levels in the distressed group were significantly higher than the non-distressed group. The differences were statistically significant ($p < 0.05$) (Table II).

The Relevance Between the Oxidation-Reduction System Indicators and the Venous Blood and Total Bile Acid Levels

Based on Pearson's analysis, it is known that MDA is positively associated with venous blood

Table I. The comparisons of MDA, SOD, NO, GSH levels between the two groups.

Groups	MDA (μmol/L)	SOD (U/ml)	NO (μmol/L)	GSH (mg/L)
Distress group	4.32±0.41	34.46±6.57	65.72±11.56	416.63±52.45
Normal group	3.24±0.55	46.45±7.26	95.66±16.32	451.52±63.69
t	2.456	3.341	3.825	4.326
p	0.034	0.023	0.019	0.013

Table II. The comparisons of the venous blood bile acid and the total bile acid level between the two groups.

Groups	Bile acid (μ mol/1)	Total bile acid (μ mol/1)
Distress group	15.67±3.62	18.74±4.16
Normal group	11.23±2.85	12.39±3.52
t	3.856	4.521
p	0.029	0.024

bile acid (r = 0.524, $p = 0.036$) and total bile acid level (r = 0.402, $p = 0.043$); SOD was negatively associated with venous blood bile acid (r = -0.328, $p = 0.041$) and total bile acid level (r = -0.516, $p = 0.029$); NO was negatively associated with venous blood bile acid (r = -0.607, $p = 0.029$) and total bile acid level (r = -0.384, $p = 0.038$); GSH was negatively associated with venous blood bile acid (r = -0.394, $p = 0.035$) and total bile acid level (r = -0.537, $p = 0.037$).

The Comparisons of the Delivery Outcome and Fetal Distress Between the Two Groups

The mortality rate of cesarean section and perinatal infant in the distress group were higher that the non-distressed group. Besides, the proportion of mild and severe asphyxia were also higher than the non-distressed group. However, the neonatal weight of the distressed group was lower. All differences were statistically significant ($p < 0.05$) (Table III).

Discussion

It has been found in the survey of epidemiology[5] that the high-risk factors of ICP are advanced age, abnormal liver functions, multiple pregnancy and family history. ICP will cause severe complications such as fetal growth restrictions, premature rupture of the membranes, spontaneous premature delivery, meconium-stained amniotic fluid, neonatal asphyxia, perinatal death, intracranial hemorrhage, and neural sequel of the newborn. At present, the research on ICP pathogenesis involves internal secretion[6] (including estrogen, progestin, thyroid hormones, chorionic gonadotropin), genetic factors[7,8] (including BSEP gene, also called ABCBLL gene, MDR3 gene, farnesoid X receptor gene, ABCC2 gene, OATP-A gene, familial intrahepatic cholestasis gene), environmental factors, immune factors (including Th1 cell factor, Th2 cell factor, IFN-γ, anticardiolipin antibody), cell apoptosis, HCV, and leptin. All factors involved can give explanations to the clinical features to some extent.

Table III. The comparisons of the delivery outcome and fetal distress between the two groups [percent (%)].

Groups	Cases	Cesarean section	Perinatal infant	Mild asphyxia	Severe asphyxia	Neonatal weight (kg)
Distress group	31	9 (29.03)	3 (9.68)	6 (19.35)	4 (12.90)	2.59±0.71
Normal group	49	3 (6.12)	1 (2.04)	3 (6.12)	2 (4.08)	3.17±0.53
t (χ^2)		5.386	4.957	4.127	5.026	3.524
p		< 0.001	< 0.001	< 0.001	< 0.001	0.018

The change of the oxidation-reduction system that causes the generation of many free radicals is the main factor that damages the tissue proteins, nucleic acid, and fat. At the same time, it will produce lipid peroxide magnifying the oxidation damage further and generate a series of reactions resulting in the complications of patients, thus, inducing fetal distress. MDA is the product of lipid peroxide with the effect of O_2. The content can be used as the widest index to reflect free radicals in our bodies. The MDA level increases in the plasma of the distressed group during this study. The SOD and GSH are the main substances that clear free radicals in our bodies. SOD can react with free radicals directly, and the mechanism is to disproportionate O_2 into H_2O, forming free radicals with strong oxidative activities by impeding decomposition and, then, generating peroxidation with unsaturated fatty acid. Thereby, the damage and degradation of NO by free radicals would be interdicted, and NO would remain active. As a result, its half-life period would be extended[11-13]. GSH is one type of important enzymes which exists widely in our bodies. Its main function is to catalyze the decomposing reaction of hydrogen peroxide. Some toxic substances such as hydrogen peroxide, superoxide anion, lipid peroxide could turn into low toxic substances by catalysis and protect biological membrane and biomacro molecules from the damage of free radicals[14-16]. Reduced form of GSH is a polypeptide compound that activates the oxidation-reduction system, thereby, detoxifying, and activating the SH enzyme. GSH will facilitate toxic substances' conversion of non-toxic substances, promote metabolic disorder and reduce bile acid level effectively. It can also relieve the symptoms of intrahepatic cholestasis. According to this research, SOD and GSH levels decreased in the distressed group.

NO is an unstable gas with small molecules. Its half-life period is pretty short and is 20-30s in general[19]. NO compounds many biological activities with protein such as platelet aggregation, intestines and stomach activities, neural information delivery, immune and so on. Its biological functions can only be developed with the help of redox reactions. NO can also maintain the flabby of the uterine smooth muscle during the pregnancy period[20,21]. It has been found in the clinical research that[22,23] the NO content will decrease during the pregnancy period, and it may cause the occurrence of uterine contraction. Consequently, the fetal distress may also appear. According to this research, NO level decreased in the distressed group.

Fetal distress means that the fetus' health is threated because of acidosis and anoxia. It is one of the important reasons causing perinatal mortality and neonatal asphyxia. Until now, the definition of fetal distress and the judgment of acidosis and anoxia are not clear. Therefore, examinations during the pregnancy period are essential to understand the mechanism of fetal distress. It has been found in this research that both the venous blood and total bile acid levels in the distressed group were significantly higher than the non-distressed group. MDA was positively associated with the venous blood and total bile acid levels while the SOD, NO, and GSH levels were negatively associated with it. The death rate of cesarean section and perinatal infant in the distressed group were higher than that of the non-distressed group. However, the proportion of mild and severe asphyxia were higher than the non-distressed group and, the neonatal weight of the distressed group was obviously lower. All differences were statistically significant ($p < 0.05$).

Conclusions

The internal oxidation-reduction system indicators of pregnant patients with intrahepatic cholestasis, such as MDA, SOD, NO and GSH levels, may contribute to the occurrence of fetal distress. This provides an important guideline in diagnosing and treating fetal distress.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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