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Correlation of lipid peroxidation and ATP enzyme on erythrocyte membrane with fetal distress in the uterus in patients with intrahepatic cholestasis of pregnancy

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Abstract. – OBJECTIVE: This paper aims to investigate the correlation of lipid peroxide in erythrocytes and ATP (Adenosine Triphosphate) enzyme activity of erythrocyte membrane with fetal distress in patients with intrahepatic cholestasis of pregnancy (ICP).

PATIENTS AND METHODS: Forty-three patients with ICP treated at Jining No. 1 People's Hospital were enrolled as a study group, and another forty healthy parturient women in the same period were enrolled as a control group, to extract their elbow venous blood and fetal umbilical cord blood. Thiobarbituric acid (TBA) was used to detect superoxide dismutase (SOD) activity of erythrocytes, malondialdehyde (MDA) activity in plasma, Na^+-K^+-ATP enzyme activity and Ca^{2+}-Mg^{2+}-ATP enzyme activity of erythrocytes, which were compared between the study and control groups. The correlation of MDA, Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme activities with fetal distress in the study group was analyzed, and the correlation of MDA with Na^+-K^+-ATP enzyme activity was investigated.

RESULTS: SOD and MDA activities of erythrocytes in maternal blood of the study group were significantly higher than those in the control group ($p<0.05$, $p<0.001$, respectively), but MDA activity in umbilical cord blood of the study group was markedly higher than that in the control group ($p<0.001$). Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme activities of maternal and fetal erythrocytes of the study group were remarkably lower than those of the control group ($p<0.001$). MDA in the fetal distress group was significantly higher than that in the no fetal distress group in the study group ($p<0.001$). Na^+-K^+-ATP enzyme activity was negatively correlated with MDA concentration in maternal and fetal erythrocytes of patients with ICP (both $p<0.001$).

CONCLUSIONS: Lipid peroxidation in patients with ICP will affect ATP enzyme activity of erythrocyte membrane, and the down-regulation of ATP enzyme activity in umbilical cord blood of patients with ICP may cause fetal distress in the uterus.

Key Words
Lipid peroxidation, Intrahepatic cholestasis of pregnancy, SOD, MDA, Na^+-K^+-ATP enzyme, Ca^{2+}-Mg^{2+}-ATP enzyme, Fetal distress.

Introduction

Intrahepatic cholestasis of pregnancy[1] (ICP) is a common liver disease during pregnancy, and often clinically characterized by skin epidermis pruritus or jaundice; hormone changes in the body lead to continuous increase in choleric acid level in pregnant women and fetuses and cause cholestasis. It remains unclear which specific factors are related to the cause of ICP. There is a study[2] believing that ICP may be correlated with race, territorial environment, maternal inheritance and estrogen regulation in the body. The symptoms of pruritus and jaundice in pregnant women gradually disappear after pregnancy. ICP is mainly harmful[3] to the fetus, because excessive cholestasis contaminates the amniotic fluid, and results in fetal distress in the uterus, neonatal asphyxia and preterm labor. Clinical data[4] have shown that ICP has a clinical incidence of 5%-6% and a recurrence rate of 0.7%-13.0%, with an

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extremely high neonatal mortality rate each year around the world. However, the mechanism in which ICP causes adverse reactions in the fetus is still unclear. The experimental results of Husvéth et al[5] show that lipid peroxidation is correlated with ICP. Lipid peroxidation[6] refers that the dynamic equilibrium between oxygen free radical and lipid peroxidation reactions in the body of patients with ICP is broken and a chain reaction of oxygen free radical is formed, which changes the permeability and fluidity of the biological cell membrane, and triggers changes in its function and structure.

ATP (Adenosine Triphosphate) enzyme[7] is closely related to energy metabolism, and ATP enzyme on erythrocytes has a great influence on the nutrient transport, energy exchange and oxygen delivery capacity of erythrocytes. Dose lipid peroxidation cause damage to ATP enzyme on erythrocytes of the blood of patients with ICP? In this study, the correlation of lipid peroxidation and ATP enzyme on erythrocyte membrane with intrauterine distress of perinatal infants in patients with ICP was investigated.

Patients and Methods

Patients

Forty-three patients with ICP admitted to the Obstetrics Department of Jining No. 1 People's Hospital from June 2017 to April 2018 were enrolled as the study group. The age range was from 23 to 38 years old with an average age of 28 years old, and the gestational week was from 37 to 39^{+3} weeks with an average gestational week of (38±2) weeks. Another 40 healthy parturients admitted to the Obstetrics Department of Jining No. 1 People's Hospital in the same period were enrolled as the control group. The age range was from 22 to 37 years old with an average age of 26 years old, and the gestational week was from 36 to 39^{+3} weeks with an average gestational week of (38±1) weeks.

Inclusion and exclusion criteria: Pregnant women admitted to the obstetric department of Jining No. 1 People's Hospital were included. The diagnosis of the study group was based on the international ICP[8], and normal pregnant women without pregnancy complications were included in the control group. In this work, pregnant women with hypertension, hepatitis B virus, gallstone, AIDS (Acquired Immune Deficiency Syndrome) and hemopathy were excluded; pregnant women with malposition, pelvic asymmetry and pelvic gap stenosis were excluded.

Main Reagents and Instruments

Thiobarbituric acid (TBA) kit (Shanghai Meilian Biotechnology Co., Ltd., Shanghai, China), phosphate-buffer, phosphate and sodium chloride (Beyotime, Shanghai, China), fluorescence spectrophotometer (Shanghai Spectrum Instrument Co., Ltd., Shanghai, China), type 721 spectrophotometer (Kenamei Scientific Instrument Co., Ltd., Shanghai, China).

Experimental Procedures

A total of 3 mL of fasting elbow venous blood in heparin was extracted from all subjects enrolled before 9 a.m. in the morning, and 3 mL of umbilical venous blood was extracted from the neonates after the umbilical cord was cut off. The blood samples were stored in a refrigerator at -75°C.

1) During the experiment, 1.5 mL of venous/umbilical cord blood was placed in an anticoagulant tube containing 10 g/dL of $EDTANa_2$ and shaken well, so that the blood concentration was 1 mg/mL.
2) Then, 1 g of absorbent cotton was placed in a syringe with a capacity of 10 mL and compressed into a 6 cm^3 filter candle, which was filtered with normal saline under a negative pressure after 2 mL of anticoagulation was added. The red cell suspension filtered was collected and washed with normal saline 5 times.
3) Finally, 0.2 mL of erythrocytes treated was taken and placed in a test tube, mixed well after 0.8 mL of phosphate-buffer solution (pH7.4) was added, mixed well again with a vortex mixer after 4 mL of trichloroacetic acid (30 g/dL) was added, in an ice bath for 1.5 h and then centrifuged at 3000 r/m for 15 min. Finally, 1 mL of supernatant was aspirated, with 0.1 mL of 0.1 mol/L $EDTANa_2$ and 0.25 mL of 1g/dL TBA added, boiled in boiling water for 20 min and then cooled to room temperature. Colorimetry was carried out in a microcuvette with an optical path of 1 cm and a wavelength of 535 nm. The same operations were performed in the control group.

Statistical Analysis

SPSS17.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software was used to analyze the experimental data. A *t*-test was used for the comparison of the mean between the two groups and linear correlation analysis for correlation analysis. When $p<0.05$, the data difference is statistically significant.

Results

Comparison of Information Between the Study Group and the Control Group

There were no differences between the study group and the control group in terms of age, body mass index, blood routine and thyroid function indication examination (all $p>0.05$), but a significant difference in liver function between the two groups ($p<0.001$) (Table I).

Comparison of Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Two ATP Enzyme Activities of Erythrocytes in Maternal and Umbilical Cord Blood Between the Study Group and the Control Group

1) The SOD activity expression of erythrocytes in maternal blood was (2703.11±568.89) U/gHb in the study group, markedly higher than (2200.95±647.21) U/gHb in the control group ($p<0.05$). The SOD activity expression in umbilical cord blood was (1236.75±270.45) U/gHb in the study group and (1159.76±247.31) U/gHb in the control group, without significant difference between the two groups ($p>0.05$). The MDA concentration activity expression of erythrocytes in maternal blood was (11.18±2.95) nmol/mL in the study group, significantly higher than (6.00±1.27) nmol/m in the control group ($p<0.001$). The MDA concentration activity expression in umbilical cord blood was (10.05±1.86) nmol/mL in the study group, remarkably higher than (5.36±1.60) nmol/mL in the control group ($p<0.001$).
2) The Na^+-K^+-ATP enzyme activity expression in maternal blood was (3.41±1.19) U/gHb in the study group, significantly lower than (5.18±1.61) U/gHb in the control group ($p<0.001$). The Na^+-K^+-ATP enzyme activity expression in umbilical cord blood was (2.48±0.71) U/gHb in the study group, markedly lower than (3.49±1.04) U/gHb in the control group ($p<0.001$). The Ca^{2+}-Mg^{2+}-ATP enzyme activity expression in maternal blood was (16.03±3.34) U/gHb in the study group, remarkably lower than (18.89±3.28) U/gHb in the control group ($p<0.001$). The Ca^{2+}-Mg^{2+}-ATP enzyme activity expression in umbilical cord blood was (14.97±3.02) U/gHb in the study group, significantly lower than (17.97±3.37) U/gHb in the control group ($p<0.001$) (Table II).

Correlation of ATP Enzyme on Erythrocyte Membrane Under Lipid Peroxidation With Fetal Distress in the Study Group

Compared with the no fetal distress group, the fetal distress group had markedly higher MDA in maternal and umbilical cord blood ($p<0.05$), significantly lower Na^+-K^+-ATP enzyme activity ($p<0.05$), and remarkably lower Ca^{2+}-Mg^{2+}-ATP enzyme activity ($p<0.05$). It suggests that fetal

Table I. General clinical baseline data of study group and control group [n (%)]/($\bar{x}$±s).

Groups	Study group (n=43)	Control group (n=40)	*t*	*p*
Age (years)	26.09±8.45	27.41±7.52	0.750	0.456
Body mass index (kg/m²)	18.52±3.12	19.25±2.63	1.400	0.163
Thyroid function examination				
FT3 (pmmol/L)	4.72±0.80	4.90±0.59	1.159	0.250
FT4 (pmmol/L)	11.31±2.73	11.30±4.72	0.012	0.990
TSH (μIU/mL)	1.56±1.15	2.01±0.89	1.983	0.051
TT3 (nmol/L)	1.87±0.40	1.69±0.58	1.656	0.102
TT4 (nmol/L)	124.41±23.49	119.01±24.96	1.015	0.313
Blood routine				
Hb (g/dl)	11.23±1.86	11.63±2.63	0.804	0.424
RBC ($\times10^{12}$/L)	4.28±0.37	4.19±0.35	1.136	0.259
PLT ($\times10^{9}$/L)	148.63±22.78	151.63±25.61	0.565	0.574
Liver function				
ALT (U/L)	11.41±10.43	21.41±7.45	4.993	<0.001
AST (U/L)	12.35±5.63	18.48±8.24	3.981	<0.001

Table II. Comparison of MDA, SOD, Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme activities of erythrocytes in maternal and umbilical cord blood between the two groups ($\bar{x}$±s).

Groups	Study group (n=43)	Control group (n=40)	*t*	*p*
RBC-SOD (U/gHb)				
Maternal blood	2703.11±568.89	2200.95±647.21	3.761	<0.05
Umbilical cord blood	1236.75±270.45	1159.76±247.31	1.350	0.575
MDA (nmol/mL)				
Maternal blood	11.18±2.95	6.00±1.27	10.250	<0.001
Umbilical cord blood	10.05±1.86	5.36±1.60	12.270	<0.001
Na^+-K^+-ATP enzyme (U/gHb)				
Maternal blood	3.41±1.19	5.18±1.61	5.723	<0.001
Umbilical cord blood	2.48±0.71	3.49±1.04	5.199	<0.001
Ca^{2+}-Mg^2-ATP enzyme (U/gHb)				
Maternal blood	16.03±3.34	18.89±3.28	3.392	<0.001
Umbilical cord blood	14.97±3.02	17.97±3.37	4.277	<0.001

distress in patients with ICP is related to the lipid peroxidation system and ATP enzyme on the erythrocyte membrane (Table III).

Correlation of Na^+-K^+-ATP Enzyme With MDA of Maternal and Fetal Erythrocytes of Patients With ICP in the Study Group

In the study group, the Na^+-K^+-ATP enzyme relative expression of erythrocytes in maternal blood of patients with ICP was (3.41±1.19) U/gHb, and the MDA concentration relative expression was (11.18±2.95) nmol/mL; the Na^+-K^+-ATP enzyme relative expression in umbilical cord blood was (2.48±0.71) U/gHb and the MDA concentration relative expression was (10.05±1.86) nmol/mL. In the study group, Na^+-K^+-ATP enzyme was negatively correlated MDA of erythrocytes in maternal blood (r=-0.463, p<0.001), and in umbilical cord blood (r=-0.331, p<0.05) (Figure 1A-B).

Discussion

In clinical practice, ICP is a malignant disease in pregnancy that easily causes fetal distress in the uterus[10], neonatal asphyxia and fetal death. It is often due to cholestasis that compresses to the fetus, resulting in a series of perinatal adverse reactions[11]. During pregnancy, the metabolic speed of pregnant women in the body changes because of the effect of estrogen regulation[12]. If the original metabolic speed is severely disturbed, the dynamic equilibrium between oxygen free radical and lipid peroxidation reactions will also be broken, which eventually leads to the disequilibrium of the regu-

Table III. Correlation of ATP enzyme on erythrocyte membrane with fetal distress in the study group.

Groups	Fetal distress group (n=9)	No fetal distress group (n=34)	*t*	*p*
MDA (nmol/ml)				
Maternal blood	6.00±1.27	11.18±2.95	10.520	<0.001
Umbilical cord blood	5.36±1.60	10.05±1.86	12.340	<0.001
Na^+-K^+-ATP enzyme (U/gHb)				
Maternal blood	5.18±1.61	3.41±1.19	3.681	<0.001
Umbilical cord blood	3.49±1.04	2.48±0.71	3.431	<0.05
Ca^{2+}-Mg^{2+}-ATP enzyme (U/gHb)				
Maternal blood	16.03±3.34	11.89±3.28	3.355	<0.05
Umbilical cord blood	17.97±3.37	14.97±3.02	2.589	<0.05

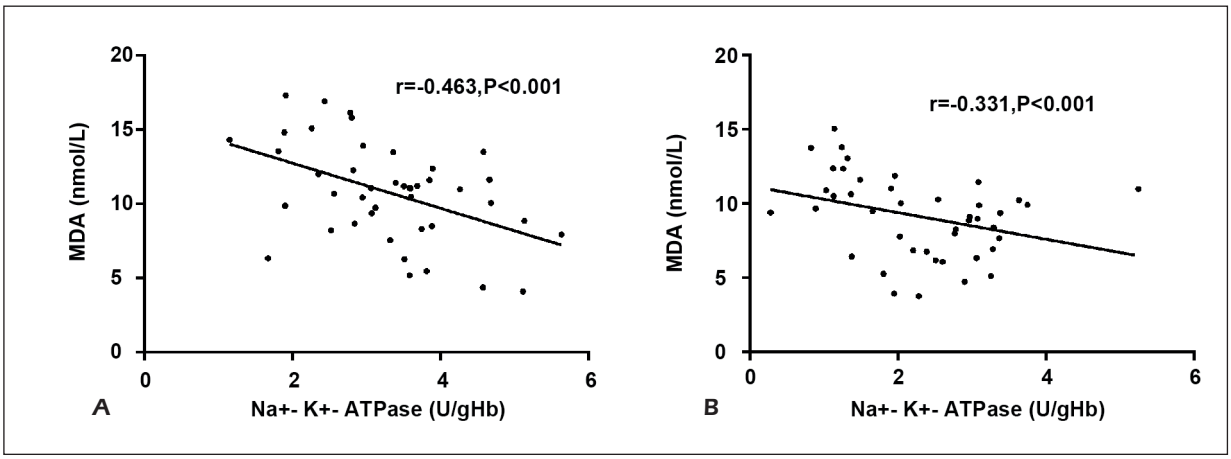

Figure 1. ***A***, Correlation of Na^+-K^+-ATP enzyme with MDA of erythrocytes in maternal blood of patients with ICP in the study group. Na^+-K^+-ATP enzyme was negatively correlated with MDA of erythrocytes in maternal blood of patients with ICP in the study group. **B**, Correlation of Na^+-K^+-ATP enzyme with MDA of erythrocytes in umbilical cord blood of patients with ICP in the study group. Na^+-K^+-ATP enzyme was negatively correlated with MDA of erythrocytes in umbilical cord blood of patients with ICP in the study group.

latory system in the whole body and the pathological pregnancy[13]. In the study by Ajima et al[14], lipid peroxidation affects ATP enzyme on erythrocytes in maternal and umbilical cord blood of patients with ICP, which also causes damage to the structure and fluidity of the biological cell membrane[15], thereby affecting the normal function of it. Lipid metabolism in humans[16] often produces lipid peroxide. Metabolic balance in the body is maintained by enhancing the activity of the biological enzyme or the antioxidant regulation of the defense system[17], but the disturbed metabolism will cause oxidation-antioxidation imbalance and thereby trigger a chain reaction of oxygen free radical[18], eventually causing serious damage to tissue cells in the body. Erythrocytes, a carrier of oxygen transport in the blood, in addition to carrying oxygen, scavenge oxygen free radical, balance acid-base and electrolyte. When lipid peroxidation invades the antioxidant system of erythrocytes, erythrocytes are subject to oxidative damage, which affects oxygen supply to the tissues and organs and microcirculation in the body[19].

In this work, TBA was used to detect Na^+-K^+-ATP enzyme, Ca^{2+}-Mg^{2+}-ATP enzyme, MDA concentration and SOD activity of erythrocytes in the blood of the study group and the control group. The results showed that the study group had markedly higher SOD activity and MDA concentration of erythrocytes in maternal blood than the control group; there was no statistically significant difference in the SOD activity in umbilical cord blood between the study group and the control group, but the MDA concentration was remarkably higher in the study group than that in the control group. In the study by Nukolova et al[20], SOD is a free gene scavenger and MDA is a metabolite of lipid peroxidation; when the MDA activity expression is up-regulated while the SOD activity expression is unchanged, the anti-oxidation ability in the body is reduced and eventually urges the oxygen free radical to react, which has a detrimental effect on the basic mechanism of the cells. Cai et al[21] detected the sensitivity of MDA and SOD to lipid peroxidation of the erythrocyte membrane. The experimental data showed that of 180 patients with ICP (no labor during the study), compared with the normal control group, 120 patients had a higher MDA content, accounting for 66.67%, and 110 patients had a higher SOD content, accounting for 61.11%; patients with ICP had significantly higher MDA and SOD of the erythrocyte membrane than the normal control group. These findings are consistent with our findings in this experiment. The correlation of Na^+-K^+-ATP enzyme with MDA of maternal and fetal erythrocytes in patients with ICP was explored. Compared with the control group, the study group had lower Na^+-K^+-ATP enzyme activity of erythrocytes in maternal and umbilical cord blood; in the study group, compared with the no fetal distress group, the fetal distress group had markedly higher MDA concentration but significantly lower Na^+-K^+-ATP enzyme activity of erythrocytes. Correlation analysis of Na^+-K^+-ATP enzyme activity with MDA of maternal and fetal

erythrocytes in the study group was performed. Na^+-K^+-ATP enzyme activity was negatively correlated with MDA of maternal and fetal erythrocytes in the study group. Preet et al[22] detected activity changes in Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme on erythrocytes under lipid peroxidation. The experimental data showed that in a certain lipid peroxidation environment, the activities of the two ATP enzymes on erythrocytes were significantly down-regulated, which caused damage to the structure and oxygen transport function of the erythrocyte membrane. This is consistent with the results of this study, and better supports the point of view of this paper.

In this work, there were no differences between the study group and the control group in terms of age, weight, thyroid function and blood routine, which reduces the experimental bias to a certain extent and ensures the reliability of the study. However, the specimen collection in this study is relatively small and affected by territoriality, family and ethnicity, so there are some limitations. Regular follow-ups will be conducted based on the patient data from the experimental group and the results will be tested and analyzed, to improve the study and provide further evidence for the results of it.

Conclusions

We found that the study group had higher MDA and SOD activities of erythrocytes in maternal blood than the control group, without significant difference in the SOD activity in umbilical cord blood between the two groups. Patients with ICP had significantly lower Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme activities of erythrocytes than the control group. These two ATP enzymes on erythrocyte membrane under lipid peroxidation were related to fetal distress in perinatal infants. MDA was negatively correlated with Na^+-K^+-ATP enzyme in maternal and umbilical cord blood of patients with ICP. It has been suggested that the decrease in ATP enzyme activity of erythrocytes is a pathological mechanism of fetal distress in perinatal infants in patients with ICP. That is to say, the destruction of Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme activities may lead to the oxygen supply dysfunction of erythrocytes and further aggravate fetal distress in perinatal infants. Therefore, studying the specific mechanism of lipid peroxidation affecting patients with ICP has important clinical significance for the prevention and treatment of ICP.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Jining No. 1 People's Hospital. Patients who participated in this research signed the informed consent and had complete clinical data. Signed informed consents were obtained from the patients and/or guardians.

Competing interests

The authors declare that they have no conflict of interest.

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