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Umbilical blood flow ultrasound characteristics of perioperative fetal intrauterine hypoxia and their relationship with maternal and fetal oxidative stress injury

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ABSTRACT

Objective: To study the relationship between umbilical blood flow ultrasound characteristics of perioperative fetal intrauterine hypoxia and maternal as well as fetal oxidative stress injury. **Methods:** 108 puerperae giving birth in our hospital between May 2014 and October 2016 were selected and divided into normal pregnancy group with neonatal Apgar score >7 points and intrauterine hypoxia group with neonatal Apgar score ⩽7 points, color Doppler diasonograph was used to determine umbilical blood flow ultrasound parameters, umbilical cord blood was collected to determine the levels of oxidative stress products, and the placenta was collected to determine the levels of oxidative stress products and related apoptosis molecules. **Results:** During 24–30 weeks, 31–36 weeks and 37–41 weeks of pregnancy, umbilical blood flow resistance index (RI), pulsatility index (PI) and diastolic velocity/systolic velocity (S/D) of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05); malondialdehyde (MDA), oxidized low-density lipoprotein (ox-LDL), 8-isoprostanes (8-iso), and heat shock protein 70 (HSP-70) levels in umbilical cord blood of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05), MDA, oxLDL, 8-ios, HSP-70, Fas, FasL and Bax levels in placenta tissue were significantly higher than those of normal pregnancy group (P<0.05), and Bcl-2 and XIAP levels were significantly lower than those of normal pregnancy group (P<0.05); RI, PI and S/D were positively correlated with MDA, oxLDL, 8-ios and HSP-70 levels in umbilical cord blood and placenta tissue, positively correlated with Fas, FasL and Bax levels in placenta tissue, and negatively correlated with Bcl-2 and XIAP levels in placental tissue. **Conclusions:** The increased umbilical blood flow resistance and decreased flow volume of fetal intrauterine hypoxia are closely related to maternal, fetal and placental oxidative stress injury.

1. Introduction

Fetal intrauterine hypoxia is also known as fetal intrauterine distress, the fetus develops chronic or acute hypoxia in uterus of pregnant women and it harms the fetal life safety[1,2]. In clinical practice, accurate judgment of the existence of fetal intrauterine hypoxia and the level of hypoxia by auxiliary examination means is helpful for early intervening in the disease and avoiding the neonatal viscera function damage caused by continuous ischemia hypoxia. Umbilical cord abnormality is the most common cause of fetal intrauterine hypoxia, more than 50% of the fetal intrauterine distress is associated with abnormal umbilical cord, and the fetal intrauterine distress caused by other factors such as placental abruption and placenta previa will also be accompanied by abnormal blood flow in umbilical cord[3,4]. Therefore, the evaluation of umbilical blood flow characteristics can provide the basis for assessment of fetal intrauterine hypoxia. In the following study, in order to define the

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value of umbilical blood flow characteristics for assessing fetal intrauterine hypoxia, the relationship between umbilical blood flow ultrasound characteristics of perioperative fetal intrauterine hypoxia and maternal as well as fetal oxidative stress injury was analyzed.

2. Materials and methods

2.1. Research subjects

108 puerperae giving birth in our hospital between May 2014 and October 2016 were selected as the research subjects, neonatal Apgar score was used to judge the existence of fetal intrauterine distress, those with neonatal Apgar score >7 points were included in normal pregnancy group and those with neonatal Apgar score ≤7 points were included in intrauterine hypoxia group. Normal pregnancy group included 71 cases, they were 23–34 years old, 48 cases were primiparae and 23 cases were multiparae; intrauterine distress group included 37 cases, they were 25–35 years old, 23 cases were primiparae and 14 cases were multiparae. The two groups were not significantly different in general data (P>0.05).

2.2. Umbilical blood flow ultrasound assessment methods

During 24–30 weeks, 31–36 weeks and 37–41 weeks of pregnancy, the umbilical blood flow ultrasound characteristics were assessed during antenatal examination respectively, which was as follows: ALOKA-α 10 and SamsungWS80A color Doppler diasonograph were adopted, the probe frequency was 2–6 MHz, the growth of fetal biparietal diameter, placenta, umbilical cord and other structures were measured, then the probe was moved to obtain the umbilical artery rheography, and five consecutive diastolic and diastolic rheography were obtained to detect the resistance index (RI), pulsatility index (PI), diastolic velocity (Vd) and systolic velocity (Vs) and calculate the S/D ratio.

2.3. Placenta tissue collection and detection methods

Proper amount of placenta tissue was collected from the middle maternal surface within 30 min after childbirth, washed with saline to remove all blood, then frozen rapidly in liquid nitrogen, added in RIPA lysis buffer and fully grinded, the tissue suspension was centrifuged in the 4 ℃ centrifuge at 12 000 r/min for 20 min to get the upper clear protein suspension, enzyme-linked immunosorbent assay kits were used to detect heat shock protein 70 (HSP-70), oxidized low-density lipoprotein (ox-LDL), Fas, FasL, Bax, Bcl-2 and XIAP levels, radioimmunoprecipitation kits were used to detect 8-isoprostanes (8-iso) and malondialdehyde (MDA) levels, and BCA kits were used to detect total protein content.

2.4. Statistical analysis

SPSS21.0 software was used to statistically process the experimental data, measurement data analysis between two groups was by routine t test, correlation analysis was by Pearson test and P<0.05 meant statistical significance in differences.

3. Results

3.1. Umbilical blood flow ultrasound parameters of two groups of puerperae

During 24–30 weeks, 31–36 weeks and 37–41 weeks of pregnancy, analysis of umbilical blood flow ultrasound parameters RI, PI and S/D between two groups of puerperae is as follows: umbilical blood flow RI, PI and S/D of intrauterine hypoxia group were significantly higher than those of normal pregnancy group. Differences in umbilical blood flow ultrasound parameters RI, PI and S/D were statistically significant between intrauterine hypoxia group and normal pregnancy group (P<0.05) (Table 1).

3.2. Oxidative stress product levels in umbilical cord blood and placenta tissue of two groups of puerperae

Analysis of oxidative stress products MDA, oxLDL, 8-ios and HSP-70 in umbilical cord blood between two groups of puerperae is shown in Table 2: MDA, oxLDL, 8-ios and HSP-

Table 1

Comparison of umbilical blood flow ultrasound parameters between two groups of puerperae during different gestational weeks ($\bar{x}\pm s$).

Groups	n	Gestational weeks	RI	PI	S/D
Intrauterine hypoxia group		24–30 weeks of pregnancy	0.83±0.10*	1.28±0.14*	3.89±0.52*
	37	31–36 weeks of pregnancy	0.79±0.09*	1.33±0.15*	4.00±0.56*
		37–41 weeks of pregnancy	0.75±0.06*	1.25±0.12*	4.09±0.52*
Normal pregnancy group		24–30 weeks of pregnancy	0.62±0.07	0.84±0.09	3.03±0.45
	71	31–36 weeks of pregnancy	0.60±0.07	0.79±0.08	3.12±0.35
		37–41 weeks of pregnancy	0.63±0.08	0.77±0.05	2.98±0.31

*: compared with normal pregnancy group during same gestational weeks, P<0.05.

70 levels in umbilical cord blood of intrauterine hypoxia group were significantly higher than those of normal pregnancy group. Differences in MDA, oxLDL, 8-ios and HSP-70 levels in umbilical cord blood were statistically significant between intrauterine hypoxia group and normal pregnancy group (P<0.05). Analysis of oxidative stress products MDA, oxLDL, 8-ios and HSP-70 in placenta tissue between two groups of puerperae is also shown in Table 2: MDA, oxLDL, 8-ios and HSP-70 levels in placenta tissue of intrauterine hypoxia group were significantly higher than those of normal pregnancy group. Differences in MDA, oxLDL, 8-ios and HSP-70 levels in placenta tissue were statistically significant between intrauterine hypoxia group and normal pregnancy group (P<0.05). Pearson correlation analysis showed that RI, PI and S/D were positively correlated with MDA, oxLDL, 8-ios and HSP-70 levels in umbilical cord blood and placenta tissue.

3.3. Oxidative stress-related apoptosis molecule levels in placenta tissue of two groups of puerperae

Analysis of oxidative stress-related apoptosis molecules Fas, FasL, Bax, Bcl-2 and XIAP in placenta tissue between two groups of puerperae is shown in Table 3: Fas, FasL and Bax levels in placenta tissue of intrauterine hypoxia group were significantly higher than those of normal pregnancy group while Bcl-2 and XIAP levels were significantly lower than those of normal pregnancy group. Differences in Fas, FasL, Bax, Bcl-2 and XIAP levels in placenta tissue were statistically significant between intrauterine hypoxia group and normal pregnancy group (P<0.05). Pearson correlation analysis showed that RI, PI and S/D were positively correlated with Fas, FasL and Bax levels in placenta tissue, and negatively correlated with Bcl-2 and XIAP levels in placental tissue.

4. Discussion

Abnormal umbilical cord is the most common cause of fetal intrauterine hypoxia, and umbilical cord around neck will directly cause the blocked umbilical blood flow and placental ischemia hypoxia; the placental factors such as placenta previa and placental abruption as well as the fetal factors such as congenital cardiovascular anomaly and fetal congenital malformation can also cause abnormal umbilical blood flow and affect the placental blood perfusion[5,6]. Therefore, it is of great significance to assess the umbilical blood flow characteristics to determine the severity of fetal intrauterine hypoxia. In the study, in order to specify the umbilical blood flow characteristics in puerperae with fetal intrauterine hypoxia, the color Doppler ultrasound was used to determine the umbilical blood flow ultrasound parameters, and the results showed that during 24–30, 31–36 and 37–41 weeks of pregnancy, umbilical blood flow RI, PI and S/D of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05). RI, PI and S/D are the more specific and sensitive indicators to assess umbilical blood flow during pregnancy, and in the progression of the pregnancy, the vascular resistance in placenta gradually decreases and the blood flow volume gradually increases, which provide the nutrients needed for fetal growth and development. With the decrease of the placental vascular resistance, umbilical blood flow increases accordingly and the blood flow resistance decreases accordingly, characterized by the decreased RI, PI and S/D. In the progression of fetal intrauterine hypoxia, the rise of RI, PI and S/D indicates the increase in umbilical blood flow resistance and the decrease in blood flow volume, the placenta is in ischemic hypoxic state, and the fetus will develop ischemic hypoxic injury.

The fetal intrauterine hypoxia environment will enhance the oxidative stress responses in fetus, increase the production of oxygen free radicals and cause oxidizing damage in fetus[7,8]. During the continuous generation of oxygen free radicals, the compositions in fetal organs and tissues will undergo oxidation reaction, the oxidation reaction products of lipid and free radicals are MDA and oxLDL, 8-ios is the oxidation reaction product of arachidonic acid, and the MDA, oxLDL and 8-ios levels can reflect the free radical generation[9-11]. HSP70 is an important molecular chaperone

Table 2

Comparison of oxidative stress product levels in umbilical cord blood and placenta tissue between two groups of puerperae ($\bar{x}\pm s$).

Groups	n	Umbilical cord blood				Placenta tissue			
		MDA (μmol/L)	ox-LDL (ng/mL)	8-ios (μmol/L)	HSP-70 (ng/mL)	MDA (μmol/mg total protein)	ox-LDL (ng/mg total protein)	8-ios (μmol/mg total protein)	HSP-70 (ng/mg total protein)
Intrauterine hypoxia group	37	13.48±1.76	23.22±3.05	9.38±1.03	8.56±0.92	7.03±0.89	17.54±2.03	14.52±1.78	22.14±2.58
Normal pregnancy group	71	7.33±0.93	10.49±1.26	4.42±0.58	4.02±0.55	3.41±0.45	9.34±1.15	6.95±0.82	10.59±1.35
t		9.182	12.589	10.892	11.347	10.938	8.389	12.447	13.029
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 3

Comparison of oxidative stress-related apoptosis molecule levels in placenta tissue between two groups of puerperae (ng/mg total protein, $\bar{x}\pm s$).

Groups	n	Fas	FasL	Bax	Bcl-2	XIAP
Intrauterine hypoxia group	37	5.28±0.89	7.24±0.79	13.28±1.58	6.02±0.78	4.52±0.56
Normal pregnancy group	71	3.02±0.45	3.87±0.55	6.24±0.77	14.35±1.78	9.88±1.05
t		7.938	9.918	10.757	10.326	11.425
P		<0.05	<0.05	<0.05	<0.05	<0.05

in cells, it is highly expressed as compensation in the process of cellular damage caused by oxidative stress, it is of important value for maintaining the normal protein function in cells and the intracellular homeostasis, and its content can reflect the extent of oxidative stress reaction[12]. Umbilical cord blood is the channel that connects the fetal and maternal material exchange, the levels of MDA, oxLDL, 8-ios, HSP-70 and other oxidative stress products can reflect the degree of fetal and maternal oxidative stress injury, and analysis of the above oxidative stress products in the study showed that MDA, oxLDL, 8-ios and HSP-70 levels in umbilical cord blood of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05) and positively correlated with umbilical cord blood RI, PI and S/D. At the same time, the fetal intrauterine hypoxia is mostly accompanied by the reduced placental blood perfusion and ischemia hypoxia, the placenta can also undergo corresponding oxidative stress reaction, and analysis of the corresponding oxidative stress products in placenta showed that MDA, oxLDL, 8-ios and HSP-70 levels in placenta tissue of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05) and positively correlated with umbilical cord blood RI, PI and S/D. This means that the changes in umbilical blood ultrasound parameters are closely related to the activation of fetal, maternal and placental oxidative stress reaction.

In the progression of fetal intrauterine hypoxia, the placental tissue is in a hypoxic low perfusion state, both hypoxic and oxidative stress stimuli can induce trophocyte and endothelial cell apoptosis in the placenta, which affect the placental growth and are not conducive to the maternal and fetal material exchange. Death receptor apoptosis pathway and mitochondrial apoptosis pathway are the important mechanisms that regulate cell apoptosis, Fas/FasL is involved in the regulation of death receptor apoptosis pathways, and Bax/Bcl-2 participates in the regulation of mitochondrial apoptosis pathways. Fas is a member of the tumor necrosis factor receptor superfamily, and its combination with ligand FasL can activate the cascade apoptosis signal mediated by a variety of downstream caspase, eventually activate caspase-3 and cause apoptosis[13,14]; Bax and Bcl-2 are the key molecules adjusting the mitochondrial membrane permeability to cytochrome C, the former can increase the release of cytochrome C, the latter can antagonize the role of Bax in promoting cytochrome C release, and the cytochrome C that enters into the cytoplasm can also induce apoptosis by caspase cascade activation[15,16]; XIAP is an important anti-apoptotic molecule in cells, and can antagonize the apoptosis mediated by the cascade activation of a variety of caspase[17]. In order to define the effect of fetal intrauterine hypoxia on cell apoptosis in the placenta tissue, the levels of above oxidative stress-related apoptosis molecules were analyzed in the study, and the results showed that Fas, FasL and Bax levels in placenta tissue of intrauterine hypoxia group were significantly higher than those of normal pregnancy group (P<0.05) and positively correlated with umbilical cord blood RI, PI and S/D, and the Bcl-2 and XIAP levels were significantly lower than those of normal pregnancy group (P<0.05) and negatively correlated with umbilical cord blood RI, PI and S/D. This means that the changes in umbilical blood ultrasound parameters are closely related to the degree of oxidative stress-related cell apoptosis in placenta.

To sum up, it is believed that the umbilical blood flow of fetal intrauterine hypoxia is characterized by the increased resistance and decreased flow volume; in the progression of fetal intrauterine hypoxia, the maternal, fetal and placental oxidative stress as well as the oxidative stress-related cell apoptosis in placenta is closely related to the changes in umbilical blood flow parameters.

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