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Oxidative stress markers in intrahepatic cholestasis of pregnancy: a prospective controlled study

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Abstract. – OBJECTIVE: Intrahepatic cholestasis of pregnancy (ICP), characterized by skin pruritus and elevation of serum aminotransferase activity and bile acid concentration in the mother, is one of the most common liver disorders in pregnancy. It was proved that ICP might lead to fetal distress by triggering oxidative damage. Total bile acids (TBA) are an established marker for assessment of the severity of ICP. The aim of this study was to explore associations of TBA levels with levels of the oxidative stress markers 8-epimer of prostaglandin F2alpha (8-iso-PGF2α), superoxide dismutase (SOD) and glutathione peroxidase (Gpx) in ICP.

PATIENTS AND METHODS: Maternal plasma levels of 8-iso-PGF2α, SOD and Gpx were examined in ICP patients (n=40) and normal pregnancy controls (n=47) using an enzyme-linked immunosorbent assay (ELISA) analysis.

RESULTS: Plasma levels of 8-iso-PGF2α and Gpx were significantly lower in ICP patients than in controls (p = 0.006 and 0.002, respectively), while no significant difference was observed in SOD levels between the two groups. Levels of 8-iso-PGF2α and TBA were negatively correlated (r = -0.277, p = 0.01, Spearman's correlation coefficient).

CONCLUSIONS: The clinical severity of ICP is closely related to the degree of lipid peroxidation, and the natural antioxidant system might fail to work effectively in the presence of lipid peroxidation damage in ICP.

Key Words:
Oxidative Stress, 8-iso-PGF2α, Cholestasis, Pregnancy.

Introduction

Free radical production and lipid peroxidation increase towards the end of normal pregnancy. These changes are counter balanced by gradual increases in levels of the antioxidants uric acid, vitamin C and and vitamin E during pregnancy[1]. If oxidative damage exceeds the antioxidant capacity of the body, pregnancy complications could occur, such as preterm labor, fetal growth restriction, preeclampsia and miscarriage[2].

Oxidative damage may help explain the clinical consequences of intrahepatic cholestasis of pregnancy (ICP), one of the most common liver disorders in pregnancy in Sichuan province of China. ICP is characterized by skin pruritus and by increases in serum aminotransferase activity and total bile acid (TBA) concentration in the mother. ICP may lead to fetal distress in 27-33% of pregnancies and to fetal loss in 0.23-4.1% of pregnancies[2-4]. TBA levels are considered as a well established marker of ICP severity because serum TBA levels > 40 μmol/L in such women are associated with increased risk of fetal loss[5]. In fact, bile acids could mediate oxidative stress in the placenta. Existing evidence proved that maternal obstructive cholestasis during pregnancy could cause oxidative stress and apoptosis in rat placenta[6]. Ursodeoxycholic acid therapy can ameliorate maternal cholestasis-induced oxidative stress[7].

Bile acids are unlikely to be the only cause of oxidative damage to the placenta in ICP patients. Retrospective analysis of term pregnancies in women with ICP showed that fetal death often occurred after irregular or regular uterine contractions[8,9]. Since uterine contractions during labor repeatedly expose placenta to hypoxia/reoxygenation challenge, we hypothesize that this effect, together with bile acid, aggravates oxidative damage in ICP[10], increasing the risk of adverse pregnancy outcomes[7]. If TBA levels reflect the severity of oxidative damage in ICP, they would be expected to vary significantly with repeated placental hypoxia/reoxygenation. However, this is not the case, which highlights the need for a more sensitive biomarker for oxidative damage.

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Oxidative damage in ICP is typically assessed by measuring the expression levels of superoxide dismutase (SOD) and glutathione peroxidase (Gpx), which are considered first-line defenses in the placental antioxidant system. These enzymes inactivate reactive oxygen species (ROS) and thus changes in their levels could serve as a good index of exposure to ROS and oxidative stress[11]. However, these markers have not been analyzed in depth in ICP so far.

ICP-related oxidative damage can also take the form of lipid peroxidation, reflecting the fact that ICP may be caused in part by dyslipidemia[12]. Peroxidation of arachidonic acid produces the isoprostane 8-iso-PGF2α[13], which was reported to be an excellent predictor of gestational hypertension/preeclampsia[14]. This property, together with the compound's high stability in plasma and urine, renders it a potential candidate marker for measuring the severity of oxidative damage in ICP. In order to gain insight into the pathogenesis of oxidative stress in ICP, we measured the three markers of oxidative stress—SOD, Gpx and 8-iso-PGF2α—in pregnant women with and without ICP, in an effort to explore whether each of these markers could serve as a reliable marker of ICP-related clinical damage, opening the door to improvement in perinatal outcomes in women suffering from this disease.

Patients and Methods

Patients

This work was conducted in the Department of Obstetrics and Gynecology of the Second West China Hospital of Sichuan University in Chengdu, China. The protocol was approved by the Ethics Committee of the Second West China Hospital, and all patients gave informed consent. Only women with and without ICP in their third trimester of pregnancy who underwent cesarean section were recruited since vaginal delivery could greatly influence the oxidative status of the pregnant women. ICP was diagnosed on the basis of severe pruritus, the absence of skin rash during the second half of gestation, elevated serum aminotransferase activity and serum TBA concentration (> 10 μmol/L). Because it was hard to collect the placental samples without therapy, women with ICP were admitted into the study regardless of their previous treatment. Patients were excluded if their pruritus could be attributed to non-ICP causes[15,16]. They were also excluded if they had liver dysfunction of any kind, including preeclampsia, HELLP syndrome, liver cirrhosis, viral hepatitis, gallstones, or cholecystitis that might result in biliary obstruction.

Blood Sample Collection

Blood samples were taken from women by peripheric venous puncture before elective cesarean section. Blood (4 ml) was collected in tubes containing 3% citrate as anticoagulant and maintained at 4°C for at most 5 h. Then samples were centrifuged at 1000 *g* for 15 min, and the supernatant was collected and stored at –80°C until use.

Enzyme-linked Immunosorbent Assay (ELISA)

Plasma levels of 8-iso-PGF2α were measured using a sandwich ELISA kit according to the manufacturer's instructions (R&D Systems, Wiesbaden, Germany). Similarly SOD and Gpx levels were measured using an ELISA kit (Cusabio Biotech, Wuhan, China); absorbance was measured using a microplate reader (Model 680, Bio-Rad Laboratories, Sydney, Australia). Samples were diluted with sample buffer as appropriate before absorbance measurements.

Statistical Analysis

Data for levels of SOD and 8-iso-PGF2α were skewed, so they were reported as medians with 25^{th}-75^{th} quartiles in parentheses. Wilcoxon's W test was used to test the significance of differences in SOD and 8-iso-PGF2α levels between ICP and normal pregnancies. Spearman correlation analysis was used to explore possible relationships of SOD or 8-iso-PGF2α with TBA. Since data for Gpx levels showed a normal distribution, results were presented as mean ± SD, and intergroup comparisons were tested for significance using the independent-samples *t* test. Pearson correlation analysis was used to assess the relationship between Gpx and TBA. All statistical analyses were carried out using SPSS for Windows 13.0 (IBM, Chicago, IL, USA). $p < 0.05$ was defined as the threshold of statistical significance.

Results

40 women with ICP and 47 women with normal pregnancies were included. Women with ICP had been treated with ursodeoxycholic acid (UD-

Table I. Maternal and infant characteristics of women with ICP pregnancies or normal pregnancies*.

Characteristic	Pregnancy group		p
	Normal (n = 47)	ICP (n = 40)	
Age, yr	29.63 ± 0.78	28.13 ± 1.23	NS
Gestational age, wk	39.00 ± 0.25	38.07 ± 0.38	NS
Birth weight (g)	3339.38 ± 121.85	3002.5 ± 108.52	NS
Placenta weight (g)	531.25 ± 13.15	506.25 ± 17.52	NS
APGAR score	9.5 ± 0.38	10 ± 0	NS
TBA (μmol/L)	4.3 ± 2.8	30.7 ± 4.65	0.027
ALT (mmol/L)	23.75 ± 6.36	214 ± 34.62	< 0.001
AST (mmol/L)	22 ± 3.48	139.75 ± 24.43	< 0.001
TBIL (mmol/L)	10.13 ± 1.19	18.31 ± 2.43	0.0091
DBIL (mmol/L)	3.06 ± 0.54	8.83 ± 1.85	0.0098

*Values shown are mean ± SD. Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; DBIL: direct bilirubin; NS: non-significant; TBIL: total bilirubin.

CA) at doses of 1250 mg per day for one week in the study. The ICP and control groups were similar in obstetric characteristics such as age, gestational week, birth weight, placental weight and Apgar score. The ICP group showed significantly higher serum levels of TBA, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and direct bilirubin (Table I).

The ICP group showed significantly lower levels of Gpx ($p = 0.006$) and the lipid peroxidation marker 8-iso-PGF2α ($p = 0.002$; Figure 1A, C). In contrast, the two groups showed similar plasma levels of SOD (Figure 1B).

In addition, plasma 8-iso-PGF2α showed an inverse correlation with TBA ($r = -0.277$, $p < 0.01$; Figure 2A) and a with SOD ($r = 0.458$, $p < 0.001$; Figure 2B), while SOD demonstrated no significant correlation with TBA level ($r = -0.16$, $p = 0.143$), based on Spearman's rank correlation test. Plasma 8-iso-PGF2α did not show a significant correlation with GPx, which in turn did not show an association with TBA ($r = 0.169$, $p = 0.12$, Pearson correlation).

Discussion

This study aimed to identify reliable markers of the severity of oxidative damage in women with ICP pregnancies. Our prospective, controlled study showed that maternal plasma 8-iso-PGF2α levels were significantly lower in ICP pregnancies than in normal ones. In fact, plasma 8-iso-PGF2α levels correlated inversely with the

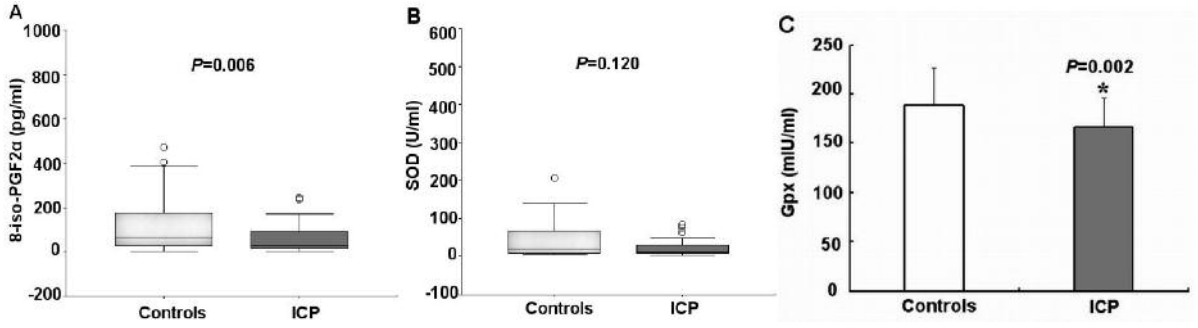

Figure 1. Plasma levels of 8-iso-PGF2α and Gpx are lower in ICP pregnancies than in normal pregnancies. Comparison of concentrations of ***(A)*** plasma 8-iso-PGF2α, ***(B)*** SOD and ***(C)*** Gpx between women with ICP pregnancies (n = 40) and women with normal pregnancies (n = 47). Results for 8-iso-PGF2α and SOD are shown as boxplots displaying medians and 25th-75th quartiles; results for Gpx are shown as a bar plot displaying mean ± SD. Intergroup comparisons were performed using the Wilcoxon's W test ***(A, B)*** or the independent-samples t test ***(C)***.

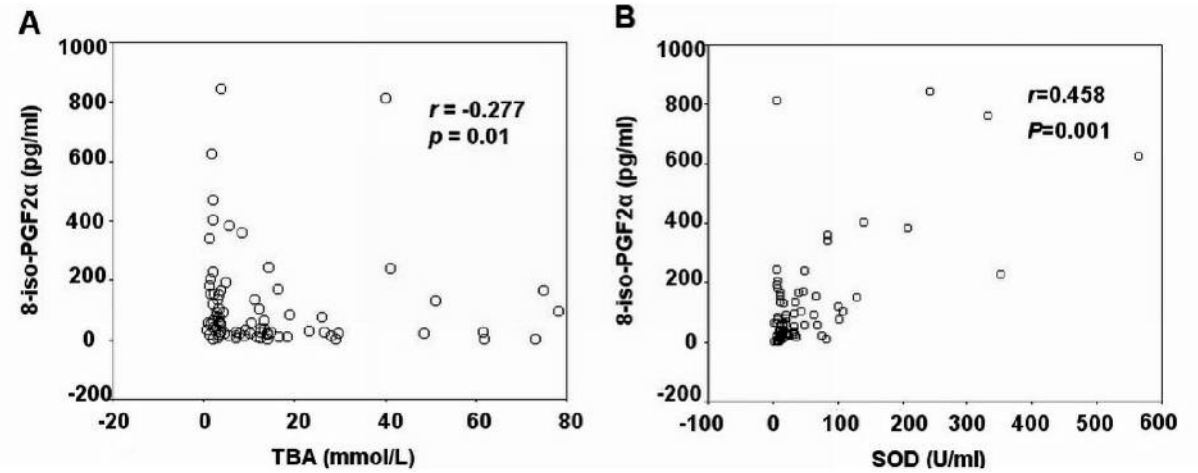

Figure 2. Correlations of oxidative stress markers in ICP. Levels of 8-iso-PGF2α positively correlated with ***(A)*** TBA and ***(B)*** SOD in women with ICP and normal pregnancies based on the Spearman rank correlation test.

severity of ICP, as defined based on TBA levels. In contrast, the markers Gpx and SOD gave a less coherent picture: Gpx levels were lower in the ICP group, and SOD levels were similar between the ICP and control groups. These findings suggest that plasma 8-iso-PGF2α levels may be more reliable than the other two oxidative damage markers for assessing ICP severity and predicting risk of perinatal complications.

Our results supported the notion that 8-iso-PGF2α may be a sensitive and reliable marker for estimating lipid peroxidation in biological tissues and fluids in ICP patients[17]. Isoprostane 8-iso-PGF2α forms through the lipid peroxidation of arachidonic acid; this and other F2 isoprostanes are secreted by the placenta during normal pregnancy[18]. In fact, plasma levels of 8-iso-PGF2α are higher in third-trimester pregnant women than in non-pregnant women[19]. Levels of 8-iso-PGF2α in the plasma and placenta are even higher in pregnant women with preeclampsia[18,20]. In contrast to those studies with preeclampsia, we found 8-iso-PGF2α levels to be lower in women with ICP pregnancies than in women with normal pregnancies. This discrepancy presumably reflects the association between intrahepatic cholestasis and dyslipidemia. Pregnant women with ICP demonstrate higher levels of low-density lipoprotein (LDL) and lower high-density lipoprotein (HDL) than women with normal pregnancies do[14]. Since HDL carries plasma F2-isoprostanes[21], this finding would explain the decrease in plasma 8-iso-PGF2α in the ICP group. The reduced levels in our study may reflect the absence of placental hypoxia/reoxygenation due to labor contractions, consistent with the fact that all our study participants elected to undergo cesarean deliveries. Indeed, in a rat model of oxidative injury, 1-h intragastric treatment with carbon tetrachloride increased plasma F2-isoprostane concentrations up to 50-fold[22]. Future studies should examine whether acute hypoxia induces rapid secretion of 8-iso-PGF2α, which may contribute to the oxidative damage in ICP.

In addition, we found 8-iso-PGF2α to correlate inversely with TBA in women with ICP pregnancies or normal pregnancies. We cannot exclude the possibility that our results were influenced by previous UDCA therapy in all the women with ICP in our study. The effects of UDCA on lipid peroxidation in liver disease are controversial. UDCA fails to reduce lipid peroxidation in patients with primary biliary cirrhosis[23], but it does exactly that in patients with cholesterol gallstones[24]. UDCA also suppresses the increase in lipid peroxidation in rats with chronic bile duct ligation[25].

SOD, a scavenger of $O_2 \cdot^-$, is one of the most important enzymes in the antioxidative defense system[26]. It helps ensure that ROS and antioxidants remain in balance to avoid excess ROS that would lead to oxidative stress. Among our participants with ICP pregnancies, plasma 8-iso-PGF2α positively correlated with SOD. This suggests that the antioxidant system may fail to protect effectively against lipid peroxidation damage in ICP. Interestingly, SOD levels were comparable between our ICP and normal groups.

Gpx is another important enzyme that protects cells from oxidative damage. It is present within red blood cells and extracellularly in the plasma. Plasma Gpx, which accounts for nearly all plasma peroxidase activity, is believed to play a key role in the plasma antioxidant defense system[27]. Our finding that Gpx concentration was lower in the ICP group than in the control group is consistent with previous studies showing that plasma Gpx correlates with plasma selenium[28], which in turn is lower in women with ICP pregnancies than in women with normal pregnancies[29].

Conclusions

The present study provides initial insights into the potential role of hepatic bile acid and lipid peroxidation damage in the pathogenesis of ICP. Our results suggest that plasma 8-iso-PGF2α levels may be a suitable indicator of lipid peroxidation and index of ICP severity in pregnant women under. Since we observed similar SOD levels between women with ICP or normal pregnancies, we speculate that the antioxidant system cannot be stimulated as an adaptive response to cholestasis-induced lipid peroxidation. When acute hypoxia, such as during labor, induces lipid peroxidation, this damage probably aggravates and contributes to adverse outcomes in ICP pregnancy. Further studies in both human placenta and animal models under acute hypoxia are needed to explore the functional role of 8-iso-PGF2α in the pathogenesis of ICP.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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