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Impaired fetal adrenal function in intrahepatic cholestasis of pregnancy

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Summary

Background: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-associated liver disease of unknown etiology. The aim of this study was to investigate the change in maternal and fetal adrenal function in clinical and experimental ICP.

Material/Methods: The maternal and fetal serum levels of cortisol and dehydroepiandrosterone sulfate (DHEAS) were determined in 14 women with ICP and in pregnant rats with estrogen-induced intrahepatic cholestasis.

Results: In women with ICP, the fetal serum cortisol and DHEAS levels were significantly higher than those in women with normal pregnancy, after correcting the impact of gestational age at delivery. The relationship between fetal cortisol and maternal cholic acid levels was bidirectional; the fetal cortisol tended to increase in mild ICP, while it decreased in severe ICP. In pregnant rats with estrogen-induced cholestasis, the fetal cortisol level was significantly lower in the group with oxytocin injection, compared with the group without oxytocin injection (191.92±18.86 *vs.* 272.71±31.83 ng/ml, P<0.05). In contrast, the fetal cortisol concentration was increased after oxytocin injection in normal control rats.

Conclusions: The data indicate that fetal stress-responsive system is stimulated in mild ICP, but it is suppressed in severe ICP, which might contribute to the occurrence of unpredictable sudden fetal death. Further studies are warranted to explore the role of impaired fetal adrenal function in the pathogenesis of ICP and the clinical implications.

key words: cortisol • dehydroepiandrosterone sulfate • intrahepatic cholestasis of pregnancy • fetal death • cholic acid • human • rat

Abbreviations: **DHEAS** – dehydroepiandrosterone sulfate; **HPA** – hypothalamic-pituitary-adrenal axis; **ICP** – intrahepatic cholestasis of pregnancy

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BACKGROUND

Intrahepatic cholestasis of pregnancy (ICP), the most common liver disorder unique to pregnancy, is characterized by pruritus and biochemical disturbances in liver functional tests, occurring predominantly during the last trimester of pregnancy [1–6]. The incidence of ICP varies throughout the world, with the highest incidence in South America (Chile and Bolivia, 5–15%) [7]. The incidence of ICP in North American and most European countries is less than 1%. The adverse impact of ICP on perinatal outcome has been well documented, and is associated with increased preterm delivery, intrauterine fetal distress and unpredictable sudden fetal death [8]. Perinatal mortality ranges from 11% to 20% in untreated women with ICP [9,10]. The pathogenesis leading to intrauterine fetal death in ICP is still unclear. There is no convincing evidence that antepartum fetal monitoring with cardiotocography can prevent fetal death associated with ICP, nor is there clear-cut information about specific antepartum fetal monitoring that would be useful for identifying fetuses at increased risk of fetal death in this syndrome [10]. An increase in the levels of bile acids is considered to be the diagnostic hallmark of ICP. Tribe et al. [11] recently reported that measuring the longitudinal profiles of 15 individual bile acids is useful for diagnosis and monitoring of this disorder. ICP was associated with a clear rise in cholic acid conjugated with taurine and glycine from 24 weeks of pregnancy [11].

The etiology of ICP involves genetic, immune and hormonal factors, but is not fully understood [7,12–14]. Hormonal factors may trigger the transient decompensation of the heterozygous state for genes encoding physiologically important hepatobiliary transporters and their nuclear regulators, and thus result in ICP [7,15,16]. These transporters include multidrug resistance protein 3 (ABCB4/MDR3), familial intrahepatic cholestasis type 1 (FIC1/ATP8B1), bile salt export pump (BSEP/ABCB11), and multidrug resistance protein 2 (MRP2/ABCC2), and their expression is tightly regulated by nuclear receptors such as pregnane X receptor (PXR) and farnesoid X receptor (FXR). ABCB4/MDR3 and ABCB11/BSEP are responsible for the biliary secretion of cholephilic compounds [17], and FIC1/ATP8B1 is a transporter for phosphatidylserine. Several studies have found that mutations of the *ABCB4*, *ABCC2*, *ABCB11* and *PXR* genes are associated with ICP [7,15,16,18–21]. Increased hepatic bile acid concentrations during pregnancy in mice are associated with reduced FXR activity [22]. In ICP placenta, 280 genes were found to be up-regulated and 112 were down-regulated [23]. ICP occurs mainly during the third trimester, when serum levels of estrogens and progesterone and their conjugating metabolites reach their peak. ICP is more common in twin (20–22%) or multiple pregnancies that are associated with higher levels of hormones than singleton pregnancies, and in women over the age of 35 years or women receiving *in vitro* fertilization treatment. Women with ICP have a higher incidence of gallstones, more severe and prolonged emesis and higher rates of drug sensitivities. Both estrogens and progesterone and their conjugates can cause direct hepatotoxicity [24]. The activity of several transporters such as BSEP/ABCB11 and MRP2/ABCC2 has been shown to be reduced by higher levels of estrogen glucuronides and progesterone [25,26]. In addition, estrogens down-regulated the expression of Na⁺-dependent taurocholate and organic anion transporters in hepatocytes [27].

The elevated levels of bile acids in maternal and fetal circulation and amnion fluid are biochemical characteristics of ICP, which are regarded as an unfavorable environmental factor for the fetus [10,28]. The adrenal gland is an important organ of the hypothalamic-pituitary-adrenal axis which is involved in fetal stress responses, and plays a major role in fetal survival under unfavorable conditions [29,30]. As such, we hypothesized that the fetal response to stress *via* this axis might be impaired in ICP, resulting in unfavorable perinatal outcomes. To test this hypothesis, we investigated the perinatal maternal and fetal adrenal function in women with ICP and in pregnant rats with estrogen-induced intrahepatic cholestasis.

MATERIAL AND METHODS

Patient selection and clinical data

This study was approved by the Clinical Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China. From September 2004 to September 2005, 14 pregnant women with ICP were recruited as the ICP group, and 14 paired pregnant women without any complications were recruited as the control group. All subjects had well-dated pregnancies which were documented by ultrasound before 16 weeks' gestation. The diagnostic criteria and management of ICP was as described by Lee et al. [31]. The ICP group terminated pregnancy before 38 weeks' gestation or before onset of labor by lower segment caesarean section. The control group received elective lower segment caesarean section at term. Five milliliters of maternal and cord venous blood from the placental end of the cord were collected immediately after delivery for measurement of dehydroepiandrosterone sulfate (DHEAS) and cortisol.

Estrogen-induced intrahepatic cholestasis in rats

Female Sprague-Dawley rats weighing 190–210 g were obtained from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). The rats were randomly divided into 4 groups: ICP without oxytocin (ICP+/Oxy–, n=8) or with oxytocin (ICP+/Oxy+, n=9), and normal pregnancy without oxytocin treatment (ICP–/Oxy–, n=9) and with oxytocin (ICP–/Oxy+, n=8). Two animals were housed per cage in a light-controlled room maintained at 22°C with a 12 h day/night cycle and were given free access to food and water. Experiments were performed on the 15th day of the pregnancy. ICP was modeled using 17α-ethinyl estradiol (Sigma-Aldrich Chemical Co, St Louis, MO, USA) 2.5 mg/kg body weight per day by subcutaneous injection for 5 consecutive days beginning on the 15th day of gestation. The control rats received only the vehicle (corn oil).

On the 21th day of gestation, all pregnant rats were killed by cervical dislocation between 1:00 p.m. and 3:00 p.m. to obviate effects of the circadian rhythm on corticosteroid secretion. Uterine contractions were induced by thigh muscle injection of oxytocin (3 U/kg body weight) in ICP and normal pregnancy groups 10 min before killing, while 0.9% saline was used as the control. The pregnant rats were killed within 30 sec after removal from their cage and the truncal

Table 1. General clinical characteristics of ICP and healthy control groups.

	ICP group (n=14)	Control group (n=14)	P value
Age	26.4±0.8	28.9±1.1	>0.05
Gravidity	1.6±0.2	1.9±0.3	>0.05
Gestation age at delivery (weeks)	36.2±0.8	38.5±0.2	<0.05
Maternal cholic acid (µg/dl)	2709.74±587.71	177.29±48.65	<0.01
Birth weight (g)	2909±166	3356±102	<0.05
Systolic/diastolic (S/D) ratio of umbilical artery	2.40±0.11	2.22±0.12	>0.05
5-minute Apgar score	9	9	>0.05

Table 2. Maternal and fetal hepatic function in women with ICP and normal control pregnancy.

Parameter	Maternal serum		Fetal serum	
	ICP group	Control group	ICP group	Control group
Alanine aminotransferase (U/L)	130.21±32.39**	40.00±0.00**	16.09±1.35	11.29±1.76
Total bilirubin (µmol/L)	25.26±3.87**	12.14±1.19**	39.91±3.18	39.57±2.06
Conjugated bilirubin (µmol/L)	7.26±1.36**	3.29±0.37**	11.00±1.41	8.50±1.47
Albumin (g/L)	34.86±1.11	37.07±0.85	39.64±1.09	39.93±0.59
Globulin (g/L)	30.29±0.90	26.79±0.75	18.27±0.94	16.00±0.76
Total cholesterol (mmol/L)	6.70±0.39	6.04±0.38	2.29±0.15	1.91±0.13
Triglyceride (mmol/L)	4.02±0.49	3.51±0.33	0.42±0.07	0.35±0.03
Total bile acid (µmol/L)	21.69±3.13**	6.50±3.94**	8.45±1.34*	4.79±0.48*
Glucose (mmol/L)	4.15±0.33*	4.08±0.40*	2.42±0.26	2.18±0.32

* $P<0.05$; ** $P<0.01$.

blood was collected. Caesarean section was performed quickly and the blood of all live fetuses was collected by decapitation. The maternal and fetal blood samples were collected for determination of DHEAS and cortisol.

Determination of serum DHEAS and cortisol

Blood samples were collected in common plastic tubes, and were centrifuged immediately at 3000 g for 10 min. Serum was stored at –70°C until assay. The concentrations of DHEAS and cortisol were evaluated by radioimmunoassay using commercial kits in a single batch done in duplicate. The commercial kits for cortisol and DHEAS were purchased from Northern Biology Technological Research Institute, Beijing, China and Diagnostic Products Corp., Los Angeles, CA, USA, respectively.

Statistical analysis

Data are expressed as means ±SEM. Student's *t* test was used for comparisons between 2 independent groups. In clinical data, a univariate general linear model was used to compare the differences in maternal and fetal cortisol and DHEAS levels between 2 groups using gestation week at delivery as a covariate (because the serum levels of cortisol and DHEAS were increased with increasing gestation age) [32,33]. The

SPSS 12.0 statistical software package (SPSS Inc., Chicago, IL, USA) was used for all data analyses. A P value of <0.05 was considered statistically significant.

RESULTS

The clinical characteristics of the subjects are summarized in Table 1. As expected, the gestational age in the ICP group was significantly lower than the control group (36.2±0.8 *vs.* 38.5±0.2 weeks, P<0.05), and the birth weight was also significantly lower in the ICP group, with higher level of maternal bile acids than the healthy group (body weight: 2909±166 *vs.* 3356±102 g, P<0.05; maternal bile acid level: 21.69±3.13 *vs.* 6.50±3.94 µmol/L, P<0.01). There was no significant difference between the 2 groups regarding maternal age, gravidity, 5-minute Apgar scores after birth, and the systolic/diastolic (S/D) ratio of umbilical artery. There were no cases of neonatal asphyxia or intrauterine fetal death in either group.

The maternal and fetal hepatic functions in the 2 groups are shown in Table 2. The maternal levels of blood alanine aminotransferase, total bilirubin, conjugated bilirubin and total bile acid were significantly higher (P<0.05) in the ICP group than in the control group, but the maternal levels of albumin, globulin, cholesterol and triglyceride were comparable in the ICP and control groups. The fetal blood total

Table 3. Maternal and fetal serum cortisol and DHEAS levels in women with ICP and normal pregnancy.

Parameter	Before correction		After correction*	
	ICP group	Control group	ICP group	Control group
Maternal cortisol (ng/ml)	792.3±90.5	795.1±94.3	855.9±96.47	657.2±96.5
Maternal DHEAS (μg/dl)	88.5±10.2	81.3±9.8	97.5±10.0	72.3±10.0
Fetal cortisol (ng/ml)	172.1±31.5	127.8±9.6	204.2±19.5**	95.7±19.5**
Fetal DHEAS (μg/dl)	207.3±37.7	156.1±16.6	252.2±22.1**	111.2±22.1**

* Covariates appearing in the model were evaluated at the following values: gestational age at delivery =37.3429; ** $P<0.01$, compared between ICP group and control group.
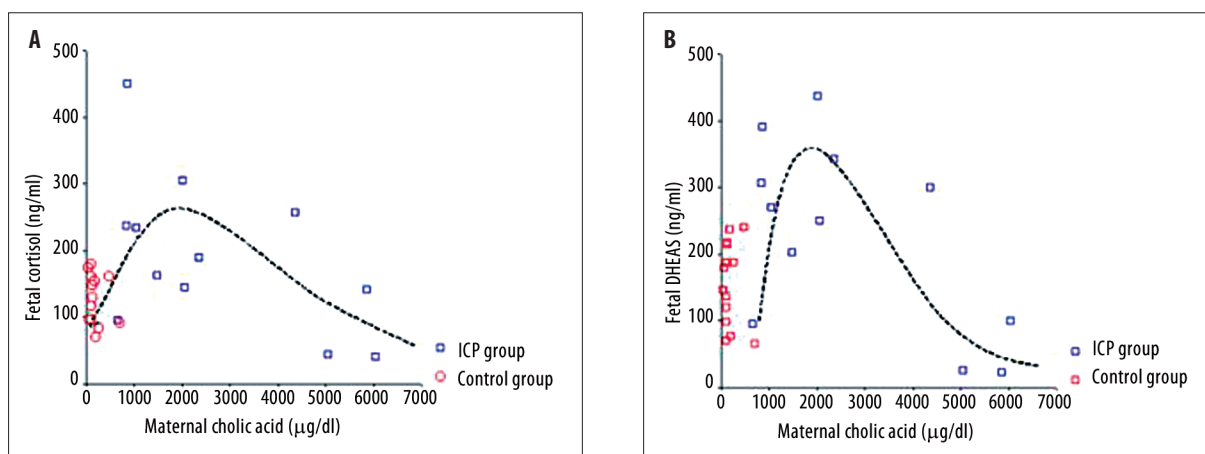
Figure 1. The relationship between maternal cholic acid and fetal adrenal steroid hormone levels. (**A**) fetal cortisol level; (**B**) fetal DHEAS level.

bile acid was also significantly higher in the ICP group than in the control group.

As shown in Table 3, after being corrected for the impact of gestational age at delivery, the fetal serum levels of cortisol and DHEAS in the ICP group were significantly higher than in the control group ($204.2±19.5$ *vs.* $95.7±19.5$ ng/ml, $P<0.01$; $252.2±22.1$ *vs.* $111.2±22.1$ μg/dl, $P<0.01$, respectively). However, the maternal cortisol and DHEAS levels were not significantly different between 2 groups.

Interestingly, we observed a bidirectional relationship between fetal serum cortisol and maternal serum cholic acid levels (Figure 1A). Fetal serum cortisol level increased with the elevation of maternal cholic acid level when maternal cholic acid was <2000 μg/dl. However, fetal serum cortisol level decreased with the elevation of maternal cholic acid level when maternal cholic acid was >2000 μg/dl. A similar relationship was also found between fetal serum DHEAS level and maternal cholic acid level (Figure 1B).

To further explore the impact of ICP on adrenal function, an estrogen-induced intrahepatic cholestasis rat model was constructed. Two pregnant rats in the ICP+/Oxy– group spontaneously labored on the 20th gestational day, all pups were alive, and the pregnancy in other rats was terminated on the 21th gestational day. The pup birth weight in ICP+/Oxy+ group was significantly lower than in the ICP–/Oxy+ group ($4.13±0.30$ *vs.* $4.94±0.34$ g, $P<0.05$). Although a similar trend

was observed between groups of ICP+/Oxy– and ICP–/Oxy– ($4.20±0.25$ *vs.* $4.39±0.13$ g), the difference was statistically insignificant. The maternal body weight and the total alive pup numbers of each litter were insignificantly different among these 4 groups (Table 4).

Figure 2 showed maternal and fetal DHEAS and cortisol levels in ICP and normal pregnant rats with and without injection of oxytocin. Except for that the fetal serum cortisol levels in the ICP+/Oxy+ group was significantly lower than in the ICP+/Oxy– group ($191.92±18.86$ *vs.* $272.71±31.83$ ng/ml, $P<0.05$) in rats with ICP, the concentrations of maternal, maternal and fetal DHEAS and cortisol trended to increase in the groups with oxytocin injection in both ICP and normal rats, compared with corresponding groups without oxytocin injection, although the differences were insignificant. In rats injected with oxytocin, maternal and fetal DHEAS levels in ICP groups were significantly higher than in normal groups ($P<0.05$), while the levels of maternal and fetal cortisol in ICP groups were significantly lower ($P<0.05$). In rats without injection of oxytocin, the differences in maternal and fetal DHEAS and cortisol were insignificant between the ICP and control groups.

DISCUSSION

Our above data have confirmed the hypothesis that fetuses have a stress-induced change of corticosteroid secretion in women with ICP, and the fetal adrenal gland might be
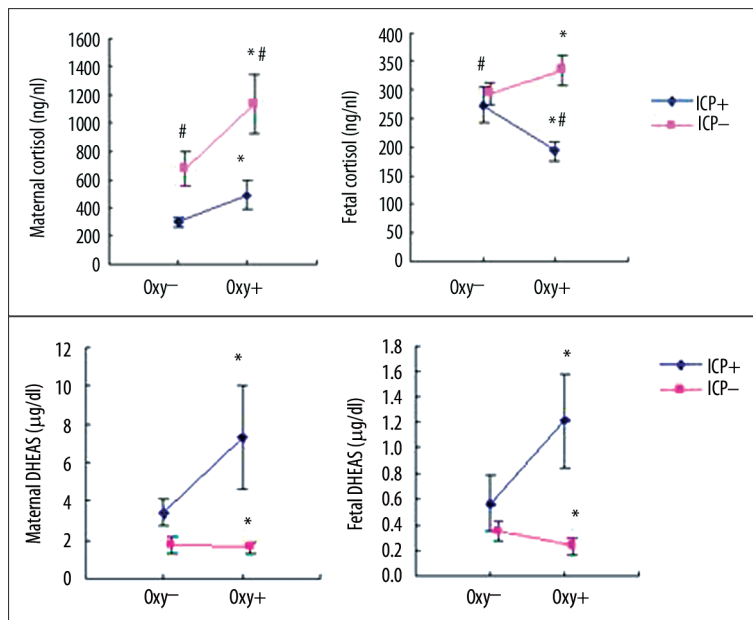
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Figure 2. The maternal and fetal cortisol and DHEAS levels in ICP and normal pregnant rats with or without oxytocin injection. DHEAS, dehydroepiandrosterone sulfate; * P<0.05, ICP+ *vs.* ICP–; # P<0.05 Oxy– *vs.* Oxy+. ICP+, rats with ICP; ICP–, rats with normal pregnancy as control. Oxy+, rats with oxytocin injection, Oxy–, rats without oxytocin injection as control.

Table 4. Comparison of delivery in rats with ICP and normal pregnancy with /without oxytocin injection.

Parameter	ICP+/Oxy– (n=8)	ICP-/Oxy– (n=9)	ICP+/Oxy+ (n=9)	ICP–/Oxy+ (n=8)
Maternal weight (g)	330.6±9.7	327.1±7.7	307.6±8.1	324.5±6.8
Liver weight (g)	14.5±0.5	15.2±0.6	13.3±0.5	14.2±0.6
Birth weight of fetal rats (g)	4.20±0.25	4.39±0.13	4.13±0.30*	4.94±0.34
Pup numbers per litter	13.6±0.9	14.9±0.5	11.3±1.3	13.8±0.9
Fetal death rate of each litter	0.08±0.04	0.00±0.00	0.20±0.08**	0.00±0.00

* P<0.05; ** P<0.01, compared with group ICP–/Oxy+.

impaired in severe ICP with high levels of maternal cholic acid. This may consequently affect fetal stress response to perinatal stressors, resulting in unfavorable perinatal outcomes in ICP, which was supported by our clinical data that the fetal cortisol and DHEAS trended to increase in women with mild ICP, but decrease in women with severe ICP, and that fetal serum cortisol was lowered after injection of oxytocin in rats with ICP.

In this study, the cord blood DHEAS and cortisol levels were used as an index of fetal adrenal function. Furthermore, the cord blood cortisol level has often been used as an index of fetal adrenal stress response [34]. The fetal adrenal gland was characterized by the presence of the fetal zone, which was the principal localization of DHEAS synthesis, the substrate for estrogen and cortisol synthesis in placenta [35], and the transitional zone that synthesizes cortisol *de novo* after the 28th week of pregnancy [36]. Furthermore, the presence of 11β-hydroxysteroid dehydrogenase (11-HSD2) serves as a barrier enzyme to control the passage of cortisol from mother to fetus [37]. Cortisol in the cord blood is primarily derived from the fetal adrenal gland in the third-trimester [38].

The increased circulating bile acids level, as an adverse environmental stressor, stimulated the stress-induced adaptive response to secrete corticosteroid in mild ICP. ICP has been associated with fetal growth restriction [39] and preterm premature rupture of the membranes [40], and fetal hypoxia [41]. These findings are in accordance with those of Rape et al. [42–45] who reported that the activation of the baboon fetal hypothalamic-pituitary-adrenocortical axis at midgestation is due to estrogen-induced changes in placental corticosteroid metabolism.

Several studies have found that bile acids had a cytotoxic effect, especially on actively metabolized cells, by damaging cell mitochondria, resulting in the disruption of the electronic respiratory chain, which led to ATP insufficiency and cell energy failure [46–48]. More recently, bile acids were thought to cause oxidative damage by stimulating the generation of free oxygen radicals in mitochondria [49–51]. As one of the most actively metabolic organs during pregnancy and the major organ to produce stress response, the fetal adrenal gland might be sensitive to the damaging effects of bile acids.

It has recently become clear that an adverse environment can activate the fetal neuroendocrine stress system, and the

stress axis can deliver environmental signals into developmental responses [52]. Acceleration of maturation might occur in the brain and the lungs, as an adaptation to fetal stress [49]. These adaptive changes could represent a life-saving answer to moderate stress, with resultant earlier birth of a more mature newborn, and increased survival, if the unfavorable fetal environment was moderate [34].

Another interesting finding of this study is that the fetal adrenal gland was insulted, but the fetal stress-responding system was not in compensation, and the fetus could not adaptively respond to the adverse environmental factor. As such, initiative onset of labor in women with severe ICP may make the fetus vulnerable to unfavorable conditions and increase fetal morbidity and mortality. The most stressful fetal experience was birth itself, which was associated with a substantial increase of fetal stress hormones such as cortisol and catecholamines in normal pregnancy [28]. Therefore, we suggest that failure of the fetal stress system in women with severe ICP might contribute to unpredictable sudden fetal death.

Wang et al. [53] reported that 95% of the fetal death and stillbirth in ICP occurred after threatened premature labor and occasional uterine contractions, or at the early stage of labor. Glantz et al. [31,54] found that fetal complications did not arise until bile acid levels were ≥40 mmol/L. Active management by timely delivery at 37 weeks or before onset of labor could significantly reduce the stillbirth rate of ICP, including those with higher bile acids levels and with meconium passage.

There has been an increasing trend toward the active management of ICP [55], although clinicians have yet to discover adequate solutions to avert the morbidities and mortalities associated with this disease. It is believed that treating the clinical symptoms of cholestasis with 2-5 ursodeoxycholic acid will improve maternal symptoms, facilitate the prolongation of pregnancy, and improve fetal outcomes [55].

CONCLUSIONS

In summary, our study indicates that the fetal stress system is excited in mild ICP, but it is suppressed in severe ICP, which might contribute to the occurrence of unpredictable sudden fetal death. These findings may have a therapeutic implication when ICP is handled. Further studies are warranted to explore the role of impaired fetal adrenal function in the pathogenesis of ICP and the clinical implications.

Conflict of interest statement

All authors have no conflicts of interest.

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