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MATERNO-FETAL MEDICINE

# Is increased maternal endotelin-1 concentration associated with neonatal asphyxia and preterm delivery in intrahepatic cholestasis of pregnancy?

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**Abstract**
*Objectives* To evaluate plasma endothelin-1 (ET-1) and fetuin-A concentrations in women with intrahepatic cholestasis of pregnancy (ICP) and to determine whether there is any association between these parameters and fetal asphyxia.
*Methods* We carried out a prospective case–control study consisting of 32 women with ICP at third trimester of pregnancy and 32 pregnant women without ICP. Blood samples from maternal peripheral venous circulation were collected and ET-1 and fetuin-A levels were determined from the plasma samples. Pulse-wave Doppler and Apgar scores were also recorded.
*Results* ET-1 concentrations were significantly higher in ICP patients. No difference was observed in fetuin-A levels between the two groups. Six newborns were declared as asphyctic (APGAR score at the 5 min <7). Maternal ET-1 levels did not correlate with the APGAR score at 5 min, total bile acid (TBA) and umbilical artery systolic/diastolic ratio with ICP patients. TBA levels were positively correlated with umbilical artery systolic/diastolic ratio negatively correlated with APGAR score at the 1′ and 5′-Apgar score in all subjects. Plasma ET-1 concentration was higher in the preterm neonates of mothers with ICP compared with normal term neonates of mothers.
*Conclusions* Although these data did not show evidence that maternal ET-1 would be associated with fetal distress, we can speculate that maternal ET-1 may be playing a role in the underlying pathology regarding microvascular dysfunction especially in the preterm neonates of mothers with ICP. Elevated TBA levels may increase the risk of asphyxia whereas fetuin-a (as an anti-inflammation marker) does not seem to have effect in women with ICP.

**Keywords** Intrahepatic cholestasis of pregnancy · Endothelin-1 · Fetuin-A · APGAR score · Umbilical artery systolic/diastolic ratio

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## Introduction

Intrahepatic cholestasis of pregnancy (ICP) not only gives rise to troublesome itching during pregnancy but may also lead to possibly serious complications for the mother and fetus. Although the exact etiology is unknown, the cause of the dysfunction is thought to be a combination of hormonal, genetic, and environmental factors [1]. It is thought that fetal morbidity appears secondary to the limited ability of an immature fetal liver for removing bile acids from the blood and vasoconstricting effect of the bile acids on human placental chorionic veins in vitro [2]. In human umbilical cord blood, plasma endothelin-1 (ET-1) concentration is raised at birth in preeclampsia, intrauterine growth retardation, and ICP [3–5]. One report previously investigated vasoconstrictive effect of the bile acids on the umbilical vein that may be the cause of sudden asphyxial events associated with ICP [5]. Nevertheless, studies have not measured ET-1 levels in maternal peripheral venous

circulation with ICP. We hypothesised that fetal hypoxemia and elevated bile acids in women with ICP may upregulate the ET-1 system in blood vessels. This, in turn, would lead to vasoconstriction and impaired regional blood flow in the neonate and as a precipitating factor in the development of inflammation-associated premature delivery and fetal distress.

The anti-inflammatory property of fetuin-A is supported by different findings in the literature, such as inhibition of cytokine production by macrophages [6], antifibrotic activity [7], and inhibition of apoptosis of vascular smooth muscle cells [8]. The inflammation causes a down-regulation of its concentration [9, 10]. Serum fetuin-A concentration is decreased and reflects at least partly systemic inflammation such as in preeclampsia [11]. However, it remains unknown whether serum fetuin-A is associated with ET-1 levels or fetal distress in ICP patients. To study a possible effect of the concentrations of ET-1, an anti-inflammation marker as fetuin-A in women with ICP and relationship with the fetal distress, we performed pulse-wave Doppler velocimetry of the umbilical artery in 32 patients with intrahepatic cholestasis and 32 normal patients between 34 and 38 weeks of gestation. For these purposes, we investigated umbilical artery systolic/diastolic ratio and 5′-Apgar score on fetuin-A and ET-1 plasma levels in ICP patients.

## Subjects and methods

The criteria for the diagnosis of ICP were generalized pruritus with or without skin changes starting in the second half of pregnancy and high total bile acid (TBA) concentration with recovery after delivery. Ultrasonography of the abdomen and serological scan of viral hepatitis were carried out for excluding other causes of liver diseases in all patients before enrollment. Active smoker women and women with hypertension, diabetes, history of coronary heart disease, hyper/hypothyroidism, chronic liver diseases, infection, skin diseases, allergic disorders, symptomatic cholelithiasis, choledocholithiasis, pre-eclampsia and HELLP syndrome and known coagulation abnormalities were excluded. None of the patients had received progesterone treatment, antibiotics or cholestyramine before enrolling to the study. For all patients under examination, the treatment was started with ursodeoxycholic acid (UDCA) (500 mg BID) at the time after having taken their blood samples. For each case of ICP, a pregnant woman without ICP, having normal function tests, and matched for parity, trimester of pregnancy, age and geographical region were chosen for control purposes. All patients and controls gave their informed consent for taking part in the study, and the protocol was approved by the local ethical committee.

All blood samples were obtained in the morning between 08:00 and 09:00 hours after an overnight fast from all subjects. Serum liver tests were determined using routine laboratory techniques. Serum TBA was measured by a commercially available kit prepared for kinetic determination (BEN-Biochemical Enterprise S.r.l, Milano, Italy). Bile acids were converted into ketones by 3-α-hydroxysteroid dehydrogenase, in the presence of thio-NAD which reacts with NADH, giving thio-NADH yellow color measured spectrophotometrically at 405 nm [12]. Plasma total cholesterol (TC) and triacylglycerol (Tg) were assayed by enzymatic colorimetric tests, and high-density lipoprotein (HDL) cholesterol was evaluated by an immunoinhibition method on an automated Cobas Integra 800 analyser (Roche, UK). LDL-c was calculated by the Friedewald formula. Blood samples were preserved at −80°C until analysis. Analyses of plasma fetuin-A and ET-1 were performed using commercially available ELISA kits in accordance with the supplier's instructions. For the determination of fetuin-A, an immunoturbidimetric method was used with specific polyclonal goat anti-human fetuin-A antibodies to human fetuin-A (BioVendor Laboratory Medicine, Modreci, Czech Republic). This method was evaluated in a side-by-side comparison with an enzyme-linked immunosorbent assay [intra-assay coefficient of variation (CV) 3.5% and interassay CV 5.4%; BioVendor Laboratory Medicine, Modreci, Czech Republic]. Plasma ET-1 was determined using 100 µl of supernatant per well of a 96-well plate with endothelin EIA kit (Cayman Chemical Company, MI, USA) following supplier's protocol.

Other laboratory tests were measured according to standard procedures. In the 48-h period preceding the expected time of delivery, patients enrolled in the study received Doppler velocimetric evaluation of fetal blood flow and non-stress tests to assess fetal well being. Pulsed-wave Doppler ultrasound of fetuses was performed with the Logic 5 Pro device (General Electric, WI, USA) using 3.5- or 5-MHz probes. All recordings were obtained in the absence of fetal breathing and body movements. Umbilical artery systolic/diastolic ratio was evaluated. Doppler velocimetry results were considered abnormal when, adjusted for gestational age, umbilical artery systolic/diastolic ratio was higher than 95th percentile [13]. Throughout the pregnancy period, fetal status was monitored in the same hospital every week. Pregnancy outcome and newborn status (e.g., term and 1′ and 5′-Apgar score) were assessed by obstetricians and neonatologists. Fetal asphyxia was defined as an APGAR score of less than 7 at 5 min postpartum.

### Statistical analysis

Results are presented as mean ± SD. We used Student's *t* test in order to compare continuous variable distribution. Differences between categorical variables were analyzed by $\chi^2$ test. The relationships between different variables were analyzed by Pearson correlation test. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS, Chicago, IL, USA). A $P$ value of <0.05 was considered to be statistically significant.

## Results

Observed 32 cases of ICP (mean patient age, 27.1 years; range, 18–35 years) were compared with 32 of normal pregnancy followed in our department (Table 1). The baseline anthropometric, hormonal and metabolic features of women with PCOS are shown and compared in Table 1.

When compared to controls, women with ICP had significantly higher concentrations of total serum bile acid levels, ET-1, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase levels, TC, Tg and LDL-c levels and umbilical artery systolic/diastolic ratio. No difference was observed in BMI, age, and fetuin-A levels between groups (Table 1). The mean APGAR score was 5.3 ± 1.8 at 1′ and 7.8 ± 1.3 at 5′ in ICP patients and 7.9 ± 0.6 at 1′ and 9.0 ± 0.3 at 5′ in control subjects. Six newborns were declared as asphytic. No difference was observed in maternal ET-1 and fetuin-A levels between asphytic and non-asphystic subjects (Table 2, $P > 0.05$). None of asphytic neonates were demonstrated in control group.

**Table 1** Clinical, hormonal and metabolic features of women with ICP and controls (mean ± SD)

| | ICP | Controls | *P* |
|---|---|---|---|
| Age (year) | 27.1 ± 5.4 | 27.6 ± 5.1 | 0.6 |
| BMI (kg/m$^2$) | 31.6 ± 4.6 | 32.0 ± 2.5 | 0.6 |
| TC (mg/dL) | 277.8 ± 62.9 | 156.1 ± 18.0 | <0.001 |
| Tg (mg/dL) | 246.9 ± 87.8 | 166.4 ± 30.6 | <0.001 |
| HDL-C (mg/dL) | 55.5 ± 20.9 | 54.3 ± 7.0 | 0.7 |
| LDL-C (mg/dL) | 169.1 ± 70.5 | 135.7 ± 17.9 | 0.014 |
| AST (U/L) | 37.9 ± 33.2 | 16.1 ± 3.7 | 0.001 |
| ALT (U/L) | 39.6 ± 45.1 | 11.3 ± 4.1 | 0.001 |
| ALP (U/L) | 351.3 ± 94.0 | 136.1 ± 18.2 | <0.001 |
| TBA (μmol/L) | 25.0 ± 19.1 | 7.1 ± 2.8 | <0.001 |
| Fetuin-A (ng/mL) | 56.0 ± 16.1 | 62.5 ± 23.0 | 0.19 |
| ET-1 (pg/mL) | 38.1 ± 7.0 | 33.8 ± 8.5 | 0.031 |
| APGAR 1 min | 5.3 ± 1.8 | 7.9 ± 0.6 | <0.001 |
| APGAR 5 min | 7.8 ± 1.3 | 9.0 ± 0.3 | <0.001 |
| Umbilical artery systolic/diastolic ratio | 3.9 ± 1.7 | 2.6 ± 0.4 | <0.001 |

**Table 2** Laboratory parameters of asphytic/nonasphytic neonates in ICP group

| | Asphytic ($n = 6$) | Non-asphytic neonates ($n = 26$) | *P* |
|---|---|---|---|
| Fetuin-A (ng/mL) | 46.0 ± 6.0 | 58.3 ± 15.6 | 0.1 |
| ET-1 (pg/mL) | 37.5 ± 8.1 | 38.2 ± 6.9 | 0.8 |
| Umbilical artery systolic/diastolic ratio | 6.7 ± 2.4 | 3.3 ± 0.9 | <0.001 |

Doppler velocimetry results were considered abnormal in only 15 of ICP patients. None of the controls exhibited abnormal umbilical artery systolic/diastolic ratio. Doppler velocimetry levels were high in 15 patients with ICP and in these ICP patients, there were higher TBA levels (25.0 ± 21.0 vs. 13.7 ± 13.2, $P = 0.048$) and lower APGAR scores (7.0 ± 1.5 vs. 8.7 ± 0.4, $P = 0.001$) than other ICP patients.

No difference was observed in ET-1 and fetuin-A levels between groups ($P > 0.05$).

Eight newborns were declared as pre-term delivery in ICP group (<37 weeks). Two of pre-term deliveries were found in control group. While there exist increased circulating maternal ET-1 levels (40.2 ± 7.0 vs. 33.8 ± 8.5, $P = 0.046$), bile acid (20.5 ± 10.5 vs. 7.1 ± 2.8, $P = 0.009$), umbilical artery systolic/diastolic ratio (4.6 ± 2.1 vs. 2.5 ± 0.5, $P = 0.030$) and decreased 5′-Apgar score (7.3 ± 1.3 vs. 8.9 ± 0.2, $P = 0.013$) in patients giving preterm birth, there was no difference between individuals having preterm and term babies in terms of fetuin-a levels.

### Correlations in ICP women

Maternal ET-1 levels, fetuin-A, or TBA did not correlate with the 5′-Apgar score and umbilical artery systolic/diastolic ratio. TBA level was correlated with aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase levels (ALP), TC, and LDL-c levels ($r = 0.50$, $P = 0.004$; $r = 0.42$, $P = 0.016$; $r = 0.34$, $P = 0.05$; $r = 0.45$, $P = 0.009$; $r = 0.55$, $P = 0.001$, respectively).

### Correlations in whole subjects

Maternal ET-1 levels or fetuin-A did not correlate with the 5′-Apgar score and umbilical artery systolic/diastolic ratio. TBA level was positively correlated with AST, ALT, ALP, TC, LDL-c levels, Tg and umbilical artery systolic/diastolic

ratio ($r = 0.61$, $P < 0.001$; $r = 0.54$, $P < 0.001$; $r = 0.61$, $P < 0.001$; $r = 0.65$, $P = 0.001$; $r = 0.59$, $P < 0.001$; $r = 0.49$, $P < 0.001$; $r = 0.42$, $P < 0.001$, respectively) negatively correlated with 1′ and 5′-Apgar score ($r = 0.48$, $P < 0.001$; $r = 0.44$, $P < 0.001$, respectively).

## Discussion

Autopsy specimens in cases of intrauterine fetal loss from ICP are consistent with death from acute intrauterine anoxia. Meconium and bile acids, especially cholic acid, have been indicated to induce vasoconstriction of human placental chorionic veins in vitro, as well as causing acute umbilical vein constriction [14]. Thus, there is some experimental evidence indicating that bile acids are implicated in the mechanisms triggering fetal asphyxia in pregnancies complicated by ICP [15, 16].

In this study, we have shown that ET-1 levels and umbilical artery systolic/diastolic ratio are increased in women with ICP than normal pregnant women. There have been some studies investigating the role of ET-1 in the pathophysiology of intrauterine growth restriction, and most of these studies have correlated with the umbilical artery flow velocity waveforms. High blood ET-1 levels have been reported in perinatal asphyxia [17, 18].

Unfortunately, we could not confirm a relationship between ET-1 and umbilical artery systolic/diastolic ratio and APGAR score for asphyxia. Although a low 5′-Apgar score is not the best marker of perinatal asphyxia, and other signs are more specific (e.g., cord blood acid–base status) [19], a low 5′-Apgar score was found in neonates with maternal or fetal conditions characterized by a variable degree of fetal asphyxia. No difference was observed in ET-1 levels between asphyctic and non-asphyctic subjects.

On the other hand, fetuin-A is an anti-inflammatory mediator that participates in macrophage deactivation. Specifically, fetuin-A enhances the cellular uptake of cationic inhibitors of pro-inflammatory cytokine synthesis by macrophages and hence it prevents the morbid sequelae of infection and trauma that would result from overproduction of pro-inflammatory cytokines [20–22]. The plasma protein and hepatic mRNA levels for fetuin-A transiently fall during the acute phase of a systemic inflammation [23, 24] which classifies fetuin-A as a negative acute-phase protein (APP) [25]. Inflammation has been shown to be leading to reduced fetuin-A levels in serum and in women with ICP may represent different aspects of response to inflammation. However, although it was thought that inflammation might be a cause of this disease, the fact that no difference could be found at the end of the study made us think that there was no inflammation. And this can be connected with the fact that there was no inflammation in kc biopsy in previous studies.

However, we could not demonstrate any differences in women with ICP and healthy control pregnancies, which may be partly explained by the fact that the liver histology in ICP reveals only dilated bile canaliculi with little or no evidence of hepatocellular change and no inflammatory reaction.

Liver biopsy shows cholestasis but no inflammation or necrosis [26, 27]. This suggests that inflammation does not make any significant contribution to the fetal distress, although a prospective study is needed to confirm this observation.

However, in our study, ET-1 and fetuin-A concentrations failed to show fetal asphyxia before delivery; we confirmed the previous reports that total serum bile acid levels appear to be a better predictor of fetal outcome and can be used in antenatal monitoring of fetal well-being and elevated TBA levels increases the risk of asphyxia in fetuses born to ICP mothers [28, 29]. Plasma ET-1 concentration was higher in the preterm neonates of mothers with ICP compared with normal term neonates of mothers. The increased production of ET-1 suggests a possible mechanism for microvascular dysfunction in these subjects.

There are two main limitations in this study. First, we did not determine umbilical pH value which may be much better and more objective than Apgar score. The other limitation is the small sample size in each group. We suggest that lack of such relationship between ET-1 and umbilical artery systolic/diastolic ratio and APGAR score may result from the small sample size. The asphytic group consists of six newborns. The asphytic group is very small, leading to wide standard deviation in statistical analysis and insufficient power to demonstrate the relationship. However, we have shown that women with ICP have increased circulating ET-1 and umbilical artery systolic/diastolic ratio, which may possibly due to fetal hypoxia. We can speculate that ET-1 may be playing a role in the underlying pathology regarding microvascular dysfunction in the preterm neonates of mothers with ICP and fetuin-A (as an anti-inflammation marker) seems not to have effect in women with ICP.

**Conflicts of interest statement** None.

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