SJIF IMPACT FACTOR: 5.79 CRDEEP Journals
International Journal of Life Sciences Abdel-Aziz & Mahmoud Vol. 5 No. 3 ISSN: 2277-193x
*International Journal of Life Sciences. Vol. 5 No. 3. 2016.*


*Full Length Research Paper*

# Biomarkers for Diagnosis of Intrahepatic Cholestasis of Pregnancy

**Yasser Rabee Abdel-Aziz[1] and Mahmoud Salah Mahmoud[2]**
[1]*Tropical Medicine Department, Al-Azhar Faculty of Medicine, Egypt.*
[2]*Obstetrics and Gynecology Department, Al-Azhar Faculty of Medicine, Egypt.*

**Article history**
*Received: 08-05-2016*
*Revised: 12-05-2016*
*Accepted: 15-05-2016*

**Corresponding Author:**
**Mahmoud S Mahmoud**
*Obstetrics and Gynecology Department, Al-Azhar Faculty of Medicine, Egypt.*

***Abstract***
*Intrahepatic cholestasis of pregnancy is a common disease that could be associated with fetal morbidity. The diagnosis is based on total bile acids (TBA). However, there is a controversy regarding the use of TBA, and the search of new diagnostic biomarkers is advocated. This study evaluates other biochemical markers and to for diagnosis of intrahepatic cholestasis of pregnancy (ICP) in Egyptian female. Fifty pregnant patients who developed ICP and other 50 healthy pregnant females who did not develop ICP were included. Blood sample was drawn for estimation of liver enzymes, bilirubin, TBA, matrix metalloproteinase (MMP) 2, 9 and Interleukin (IL) 18 and 12 and tumor necrosis factor-alpha (TNF-a). Patients were followed up for delivery and outcome was documented. Total bilirubin, liver enzymes, MMP-2, MMP-9, IL-12, IL-18 and TNF-a were significantly increased, while gestational age at delivery and birth weight were significantly decreased in patients with ICP. The most sensitive biomarker was MMP-2 (100.0%), then MMP-9 (98.0%), IL-12 (96.0%), TNF-a (84.0%) and the least sensitive was IL-18 (68.0%). In addition, each of GA at delivery and birth weight was negatively correlated with total bilirubin, ALT, AST, MMP-2, MMP-9, IL-12, IL-18 and TNF-alpha. On the other hand, each of total bilirubin, AST and ALT was positively correlated with MMP-2, MMP-9, IL-12, IL-18 and TNF-alpha. Matrix metalloproteinase (MMP) 2, 9 and Interleukin (IL) 18 and 12 and tumor necrosis factor-alpha appear to be valuable biomarkers in diagnosis of ICP.*

***Keywords:*** *Cytokines; Interleukin; matrix metalloproteinase; inflammation; cholestasis; pregnancy; hepatic.*

## Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common disease of pregnancy. It usually presented by pruritus, elevation of liver enzymes, and elevation of total bile acids (TBA). Increased total bile acids are considered to be the diagnostic test for ICP. The synonymous of ICP include obstetric cholestasis, obstetric hepatosis, jaundice in pregnancy, or hepatosis gestationalis [1]. ICP incidence ranges between 0.1% and 22%. Women developed gestational diabetes mellitus (GDM), and women who become pregnant after in vitro fertilization had a higher incidence of ICP [2]. Cause of ICP is largely unknown, but it thought to be multifactorial with genetic, environmental, nutritional deficiencies, and fluctuations in hormone levels. Mutations in the hepatocellular transporters of bile acids to the bile canaliculi gene is thought to play a pivotal role in the etiopathogenesis [3,4]. In addition, it had been suggested that, the increased bile acid levels are responsible for maternal and fetal complications, as there is a correlation between TBA values and frequency and severity of these complications [5,6].

The multifactorial nature of ICP leads to difficulty in the diagnosis and treatment of ICP difficult [7]**.** Previous studies reported that, during ICP, the total bile acids flux increased from the moth to her fetus 17-19 and subsequently elevated levels of maternal TBA could disrupt hormone production in the placenta and constriction of chorionic blood vessels constriction [8]**.** Thus, higher values of serum TBA level was considered the most sensitive laboratory abnormality in ICP, and levels above 11.0 µM was considered the most accurate early diagnostic biomarker [9]**.** ICP can be categorized into mild or severe according to the levels of TBA, with mild ICP if TBA from 10 to 40 µM, and severe ICP if TBA above 40 µM. But, serum TBA level also have ethnic variations [10, 11]**,** making the clinical use of TBA somewhat insufficient for biochemical diagnosis [12]**.** Thus, in the present work, authors aimed to evaluate other biochemical markers and to for diagnosis of ICP in Egyptian female.

## Materials and Methods

*Methodology*

From January 2015 to January 2016; 50 pregnant patients who developed ICP (clinical and biochemical diagnosis) and other 50 healthy pregnant females who did not develop ICP were included in the study. The study protocol was explained for each female and her informed consent for participation in the study was obtained. The study protocol was approved by the local ethics and research

committee of Faculty of Medicine (Al-Azhar University). For **inclusion** in the study, the female fulfilled the following criteria: female had pruritus and/or jaundice; female had no other dermatological disorders other than lesions occurred by excessive scratching due to pruritus; the concentration of TBA were above 10 µM (the highest accepted cutoff point); female had no viral hepatitis or chronic liver or biliary disease, female had no dilatation of biliary tract on ultrasound examination, females had no signs of fever, preeclampsia, endocardial or urinary tract infection, and finally liver enzymes were returned to normal levels after delivery in females with ICP.

On the other hand, exclusion criteria were autoimmune diseases, smoking, biliary obstruction, multiple gestation and drug addiction or alternative medicine therapy known to precipitate cholestasis. In the present work, diagnosis of ICP was done according to The Royal College of Obstetricians and Gynecologists Green-top Guidelines [13]. Mild and severe ICP were defined according to the study of Glantz et al. [14] and Pradhan & Shao [15]. The standard reference value established for mild ICP (MICP) and severe ICP (SICP) was TBA <40 µmol/L for mild ICP and TBA ≥40 µmol/L for server ICP. Blood sample was drawn for estimation of liver enzymes, bilirubin, TBA, matrix metalloproteinase (MMP) 2, 9 and Interleukin (IL) 18 and 12 and tumor necrosis factor-alpha (TNF-α). Briefly, venous blood sample were collected following an overnight fast in EDTA-containing sterile tubes. The serum was separated by centrifugation immediately and stored at -80 °C until further use of analysis. Serum TBA level was measured using an established enzymatic fluorimetric assay described by Mashige et al. [16] after solid-phase extraction as described by Feldmann et al. [17]. Serum levels of matrix metalloproteinase (MMP)-2 and MMP-9 were measured by the Human MMP-2, IL-18, IL-12, TNF-α and MMP-9 ELISA kits (Sigma-Aldrich) according to manufacturers' instructions.

All patients were followed up for delivery, and pregnancy outcome, with any complications for both mother and her fetus were documented. Statistical analysis was performed with commercially available software; statistical package for social science (SPSS for Windows, version 18.0 (IBM® SPSS® Inc., Chicago, IL, USA). When data were normally distributed, the data were expressed as arithmetic mean and standard deviation (SD); and data of intergroup comparisons were tested using Student's t-test. Categorical variables were presented as relative frequency and percent distribution. A comparison between groups was done by Chi square test. For all statistical analyses, a two-tailed P-value <0.05 was considered statistically significant.

**Results**

Results of the present work revealed that both females with ICP and those without ICP were comparable as regard to age at pregnancy (28.86±2.25 vs 28.78±2.08 years respectively). On the other hand, gestational age at delivery was significantly lower in females with ICP when compared to those without ICP (37.48±1.41 vs 39.12±0.84 weeks respectively). In addition, birth weight was significantly lower in females with ICP (2944.52±116.75g) when compared to females without ICP (3181.34±120.22 g). However, total bilirubin, ALT and AST were significantly higher in females with ICP. In addition, both MMP-2, MMP-9 was significantly in females with ICP when compared to females without ICP (255.32±27.26, 844.58±62.36 vs 188.54±10.90, 646.92±17.76 ng/ml respectively). Furthermore, IL-12, IL-18 and TNF-α were significantly increase in females with ICP when compared to females without ICP (36.62±68.12±18.84 and 43.20±5.94 vs 11.72±1.80, 43.80±3.31 and 32.28±3.08 ng/L respectively). Jaundice was reported in 54% of patients who developed ICP compared to none in females without ICP. Finally, both induced vaginal and cesarean delivery were significantly increased in ICP when compared to negative group (28%, 32.0% vs 10%, 10% respectively) (table 1).

**Table (1):** Comparison between patients with and those without ICP as regard to studied variables

| | | With ICP | | Without ICP | | t | p |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | | |
| **Age at pregnancy** | | 28.86 | 2.25 | 28.78 | 2.08 | 0.18 | 0.85(ns) |
| **GA at delivery** | | 37.48 | 1.41 | 39.12 | 0.84 | ***7.01*** | ***<0.001**** |
| **Birth weight** | | 2944.52 | 116.75 | 3181.34 | 120.22 | ***9.99*** | ***<0.001**** |
| **Total bilirubin** | | 1.41 | 0.35 | 0.52 | 0.11 | ***17.06*** | ***<0.001**** |
| **ALT** | | 172.36 | 30.84 | 26.16 | 5.48 | ***32.99*** | ***<0.001**** |
| **AST** | | 147.66 | 38.66 | 32.68 | 8.25 | ***20.56*** | ***<0.001**** |
| **MMP-2 (ng/ml)** | | 255.32 | 27.26 | 188.54 | 10.90 | ***16.08*** | ***<0.001**** |
| **MMP-9 (ng/ml)** | | 844.58 | 62.36 | 646.92 | 17.76 | ***21.55*** | ***<0.001**** |
| **IL-12 (ng/L)** | | 36.62 | 16.16 | 11.72 | 1.80 | ***10.82*** | ***<0.001**** |
| **IL-18 (ng/L)** | | 68.12 | 18.84 | 43.80 | 3.31 | ***8.98*** | ***<0.001**** |
| **TNF-α (ng/L)** | | 43.20 | 5.94 | 32.28 | 3.08 | ***11.52*** | ***<0.001**** |
| **Jaundice (n%)** | **Positive** | 27(54.0%) | | 0(0.0%) | | ***36.98*** | ***<0.001**** |
| | **Negative** | 23(46.0%) | | 50(100.0%) | | | |
| **Mode of Delivery** | **Vaginal (Spontaneous)** | 20(40.0%) | | 40(80.0%) | | ***16.69*** | ***<0.001**** |
| | **Vaginal (Induced)** | 14(28.0%) | | 5(10.0%) | | | |
| | **CS** | 16(32.0%) | | 5(10.0%) | | | |

In the present study, females with mild ICP were comparable to females with severe ICP as regard to age at pregnancy, jaundice, time of appearance of jaundice, ALT and mode of delivery. However, females with severe ICP had younger gestational age at delivery, low

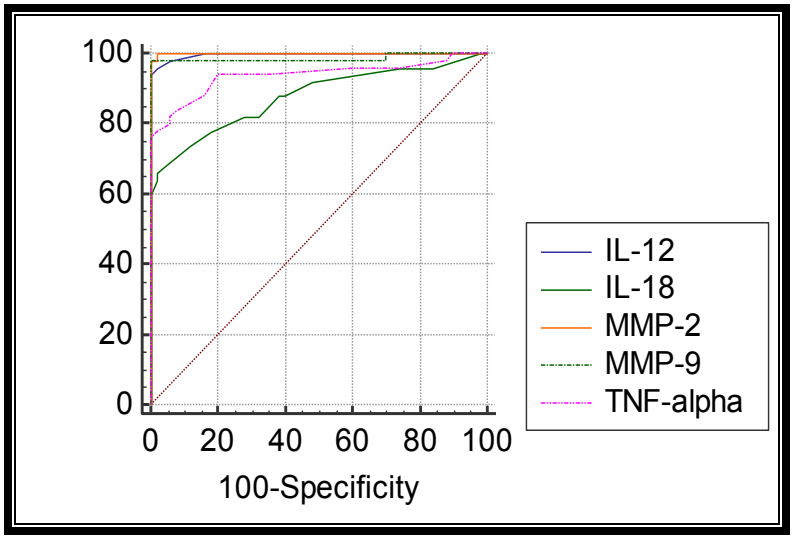
birth weight, and had higher bilirubin, AST, MMP-2, MMP-9, IL-12, IL-18, and TNF-α. The onset of pruritus was significantly earlier in mild when compared to severe ICP (28.05±0.94 vs 28.73±1.25 weeks of gestation respectively) (Table 2). As regard to sensitivity of different biomarkers, the most sensitive was MMP-2 (100.0%), then MMP-9 (98.0%), IL-12 (96.0%), TNF-α (84.0%) and the least sensitive was IL-18 (68.0%) (Table 3; Fig. 1).

**Table (2):** Comparison between ICP mild and severe subgroups as regard to studied parameters

| | | Mild ICP | | severe ICP | | t | p |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | | |
| **Age at pregnancy** | | 28.25 | 2.29 | 29.26 | 2.18 | 1.58 | 0.12 |
| **Onset of pruritus** | | 28.05 | 0.94 | 28.73 | 1.25 | ***2.06*** | ***0.044**** |
| **Appearance of jaundice** | | 30.27 | 1.27 | 30.87 | 1.25 | 1.21 | 0.23 |
| **GA at delivery** | | 38.10 | 1.20 | 37.06 | 1.41 | ***2.67*** | ***0.010**** |
| **Birth weight** | | 3009.05 | 96.26 | 2901.50 | 110.33 | ***3.54*** | ***0.001**** |
| **Total bilirubin** | | 1.28 | 0.25 | 1.50 | 0.38 | ***2.29*** | ***0.026**** |
| **ALT** | | 164.65 | 13.05 | 177.50 | 37.78 | 1.46 | 0.15 |
| **AST** | | 126.25 | 24.75 | 161.93 | 39.96 | ***3.55*** | ***0.001**** |
| **MMP-2 (ng/ml)** | | 243.75 | 25.48 | 263.03 | 26.00 | ***2.58*** | ***0.013**** |
| **MMP-9 (ng/ml)** | | 786.80 | 18.21 | 883.10 | 50.15 | ***8.20*** | ***<0.001**** |
| **IL-12 (ng/L)** | | 19.05 | 2.08 | 48.33 | 9.16 | ***14.00*** | ***<0.001**** |
| **IL-18 (ng/L)** | | 46.90 | 4.06 | 82.26 | 8.46 | ***17.34*** | ***<0.001**** |
| **TNF-α (ng/L)** | | 38.55 | 5.08 | 46.30 | 4.24 | ***5.84*** | ***<0.001**** |
| **Jaundice (n%)** | **Positive** | 11(55.0%) | | 16(53.3%) | | 0.01 | 0.90 |
| | **Negative** | 9(45.0%) | | 14(46.7%) | | | |
| **Mode of Delivery** | **Vaginal (Spontaneous)** | 6(30.0%) | | 14(46.7%) | | 2.58 | 0.27 |
| | **Vaginal (Induced)** | 8(40.0%) | | 6(20.0%) | | | |
| | **CS** | 6(30.0%) | | 10(33.3%) | | | |

**Table (3):** Sensitivity of different biomarkers for diagnosis of ICP

| | Il-12 | Il-18 | MMP-2 | MMP-9 | TNF-α |
|---|---|---|---|---|---|
| **Area under the ROC curve (AUC)** | 0.99 | 0.87 | 1.00 | 0.98 | 0.93 |
| **Standard Error** | 0.00268 | 0.0354 | 0.000566 | 0.0141 | 0.0268 |
| **95% Confidence interval** | 0.95 to 1.0 | 0.79 to 0.93 | 0.96 to 1.00 | 0.93 to 0.99 | 0.87 to 0.97 |
| **Cutt-off** | ***>15*** | ***>49*** | ***>202*** | ***>690*** | ***>36*** |
| **Sensitivity** | 96% | 68% | 100.0% | 98.0% | 84.0% |
| **Specificity** | 98.0% | 96% | 98.0% | 100.0% | 92.0% |




In the present study, each of GA at delivery and birth weight was negatively correlated with total bilirubin, ALT, AST, MMP-2, MMP-9, IL-12, IL-18 and TNF-alpha. On the other hand, each of total bilirubin, AST and ALT was positively correlated with MMP-2, MMP-9, IL-12, IL-18 and TNF-alpha (table 4).

**Table (4):** Correlation of studied variables

| | GA at delivery | Birth weight | Total bilirubin | ALT | AST |
|---|---|---|---|---|---|
| **GA at delivery** | 1 | .859** | -.728** | -.636** | -.687** |
| **Birth weight** | .859** | 1 | -.768** | -.735** | -.727** |
| **Total bilirubin** | -.728** | -.768** | 1 | .895** | .929** |
| **ALT** | -.636** | -.735** | .895** | 1 | .925** |
| **AST** | -.687** | -.727** | .929** | .925** | 1 |
| **MMP-2** | -.747** | -.786** | .941** | .880** | .918** |
| **MMP-9** | -.671** | -.748** | .876** | .913** | .952** |
| **IL-12** | -.622** | -.680** | .750** | .753** | .786** |
| **IL-18** | -.594** | -.662** | .716** | .701** | .754** |
| **TNF-alpha** | -.629** | -.678** | .717** | .768** | .757** |

**. Correlation is significant at the 0.01 level (2-tailed).

**Discussion**

ICP is usually associated with increased risk of fetal complications such as prematurity and low birth weight. Thus, it is important to determine sensitive biochemical parameters that aid in diagnosis of ICP. In the present study, authors examined different biomarkers (MMP-2, MMP-9, IL-12, IL-18 and TNF-α) for diagnosis of ICP, in the light of established current gold standard for diagnosis of ICP, the total fasting serum bile salt levels.

In the present work, liver transaminases and total bilirubin were significantly increased in females with ICP. In addition, liver transaminases were negatively correlated with birth weight and gestational age at delivery. These results are comparable to those reported by Kondrackiene et al. [18] who stated that, it is difficult to diagnose ICP only by routine biochemical tests, however, activity of transaminases and bilirubin concentration were considerably higher in ICP patients when compared to normal pregnant females. In addition, results of the present work were in agreement with those reported by Kawakita et al. [19] who found significantly higher liver transaminase, total bilirubin and iatrogenic preterm delivery in patients who developed ICP. Furthermore, Hu et al. [20] reported that, females developed ICP showed significantly higher serum levels of TBA, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and direct bilirubin. In the present study, there was statistically significant decrease of gestational age at delivery in females with ICP. In addition, the incidence of cesarean delivery and induced vaginal delivery was significantly increased in patients with ICP. It had been reported that, labor induction for females with ICP was very common practice. Many researchers advocated the elective early delivery for ICP [2]. This is based on studies revealing that most stillbirths occurred >37 weeks of gestation [21]. However, there is no recommendation on optimal timing of delivery for females developing ICP. There are no randomized studies investigating the optimal timing of delivery. Decision analysis study and large retrospective study showed that delivering at 36 weeks of gestation was optimal strategy considering risk of stillbirth and risk of prematurity [22].

Henderson et al. [23] in his systematic review including 16 articles published between 1986 and 2011 regarding this obstetric controversy (active management of ICP), and were unable to find evidence supporting the practice of active management of ICP. They recommended individualized management that provides informed decision-making guidance for the patient, rather than the routine implementation of an active management protocol. Scientific evidence, including the risks and benefits of the available management options, should be presented to the patient in a clear manner by the health care providers.

Results of the present work reveled that, MMP-2, MMP-9, IL-12, IL-18 and TNF-α were significantly increased in females developed ICP; and there was higher significant increase in severe ICP when compared to mild ICP. These results are comparable to previous studies reported that IL-18 is involved in the pathogenesis of liver dysfunction. IL-18 can stimulate NK cells by different mechanisms, which may lead to liver cells apoptosis and subsequent liver dysfunction. In addition, IL-12 can enhance the effect of IL-18, and encourage NK cell production and toxicity [24, 25]. In addition, it has been revealed that TNF-α, IL-12, IL-18 and IL-6 serum values were significantly increased in patients with ICP [26, 27]. In addition, results of the present work are in agreement with the study of Shao et al. [28] who reported that, the serum levels of IL-12, IL-18 and TNF-α were significantly higher in ICP patients when compared with controls. It had been reported that, TNF-α can directly destroy hepatic cells and lead to liver cell peroxidation by induction of mitochondria to yield more oxygen-free radicals. It could also stimulate the apoptosis of placental trophoblasts, impair function of placenta, and cause injury to the fetus [29]. Furthermore, Chen et al. (7) have evaluated serum levels of MMP-2 and MMP-9 in patient with ICP. Their results revealed that serum values of both MMPs are consistently increased in ICP patients, compared with healthy pregnant females. Moreover, even among females with ICP, their serum values of both MMPs were sensitive enough to reflect the severity of the disease, as evident by strong and positive correlations of both MMP levels with results of liver function tests.

**Conclusion**

Results of the present study revealed the sensitivity of different studied chemicals in diagnosis of ICP. The most sensitive was MMP-2 (100.0%), then MMP-9 (98.0%), IL-12 (96.0%), TNF-α (84.0%) and the least sensitive was IL-18 (68.0%). In addition, these

variables were negatively correlated with gestational age at delivery and birth weight, and positively correlated with liver transaminase and total bilirubin. Thus, these biomarkers can share or even replace the old biomarkers in diagnosis of ICP. However, due to small number of included females, results should be treated cautiously and future works including huge number of females is advocated before generalization of the results of the present study.

**References**

1. Geenes V, Chappell LC, Seed PT, et al. (2014) Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. Hepatology 59:1482-91.
2. Williamson C, Geenes V (2014) Intrahepatic cholestasis of pregnancy. Obstet Gynecol 124: 120-33.
3. Lee RH, Kwok KM, Ingles S, et al. (2008) Pregnancy outcomes during an era of aggressive management for intrahepatic cholestasis of pregnancy. Am J Perinatol 25: 341-5.
4. Rook M, Vargas J, Caughey A, Bacchetti P, et al. (2012) Fetal outcomes in pregnancies complicated by intra-hepatic cholestasis of pregnancy in a Northern California cohort. PLoS One 7: e28343.
5. Puljic A, Kim E, Page J, et al. (2015) The risk of infant and fetal death by each additional week of expectant management in intrahepatic cholestasis of pregnancy by gestational age. Am J Obstet Gynecol 212:667.1-5.
6. Brouwers L, Koster M, Kemperman H, et al. (2015) Intrahepatic cholestasis of pregnancy: maternal and fetal outcomes associated with elevated bile acid. Am J Obstet Gynecol 212:100. e1-7.
7. Chen Z, Shen Z, Hu L, Lu M, Feng Y (2016) Identification of Matrix Metalloproteinase-2 and 9 as Biomarker of Intrahepatic Cholestasis of Pregnancy. Ann Hepatol 16(2):291-296.
8. Meng LJ, Reyes H, Axelson M, et al. (1997) Progesterone metabolites and bile acids in serum of patients with intrahepatic cholestasis of pregnancy: effect of ursodeoxycholic acid therapy. Hepatology 26: 1573-9.
9. Brites D, Rodrigues CM, van-Zeller H, et al. (1998) Relevance of serum bile acid profile in the diagnosis of intrahepatic cholestasis of pregnancy in a high incidence area: Portugal. Eur J Obstet Gynecol Reprod Biol 80: 31-8.
10. Mays JK (2010) The active management of intrahepatic cholestasis of pregnancy. Curr Opin Obstet Gynecol 22: 100-3.
11. Pathak B, Sheibani L, Lee RH (2010) Cholestasis of pregnancy. Obstet Gynecol Clin North Am 37:269-82.
12. Huang WM, Gowda M, Donnelly JG (2009) Bile acid ratio in diagnosis of intrahepatic cholestasis of pregnancy. Am J Perinatol 26: 291-4.
13. Kenyon AP, Girling JC (2011) Obstetrics cholestasis; RCOG green-top guidelines. London, RCOG , No 43.
14. Glantz A, Marschall HU, Mattsson LA (2004) Intrahepatic cholestasis of pregnancy: relationships between bile acid levels and fetal complication rates. Hepatology 40:467-4.
15. Pradhan M, Shao Y (2013) Clinical Grading of Intrahepatic Cholestasis Pregnancy. J Nepal Med Assoc 52:413-419.
16. Mashige F, Imai K, Osuga T (1976) A simple and sensitive assay of total serum bile acids. Clin Chim Acta 70: 79-86.
17. Feldmann D, Fenech C, Cuer JF (1983) Evaluation of a sample preparation procedure for bile acids in serum and bile. Clin Chem 29: 1694.
18. Kondrackiene J, Zalinkevicius R, Sumskiene J, et al. (2016) Sensitivity and Specificity of Biochemical Tests for Diagnosis of Intrahepatic Cholestasis of Pregnancy. Ann Hepatol. 16(4):569-573.
19. Kawakita T, Parikh LI, Ramsey PS, et al. (2015) Predictors of adverse neonatal outcomes in intrahepatic cholestasis of pregnancy. Am J Obstet Gynecol 213(4):570.1-8.
20. Hu YY, Liu JC, Xing AY (2015) Oxidative stress markers in intrahepatic cholestasis of pregnancy: a prospective controlled study. Eur Rev Med Pharmacol Sci 19(17):3181-6.
21. Williamson C, Hems LM, Goulis DG, et al. (2004) Clinical outcome in a series of cases of obstetric cholestasis identified via a patient support group. BJOG 111:676-81.
22. Lo JO, Shaffer BL, Allen AJ, Little SE, Cheng WY, Caughey AB (2014) Intrahepatic cholestasis of pregnancy and timing of delivery. J Matern Fetal Neonatal Med 28:1-5.
23. Henderson CE, Shah RR, Gottimukkala S, et al. (2014) Primum non nocere: how active management became modus operandi for intrahepatic cholestasis of pregnancy. Am J Obstet Gynecol 211: 189-196
24. Finotto S, Siebler J, Hausding M, et al. (2004) Severe hepatic injury in interleukin 18 (IL-18) transgenic mice: a key role for IL-18 in regulating hepatocyte apoptosis in vivo. Gut 53: 392-400.
25. Agaugué S, Marcenaro E, Ferranti B, Moretta L, Moretta A (2008) Human natural killer cells exposed to IL-2, IL- 12, IL-18, or IL-4 differently modulate priming of naive T cells by monocyte-derived dendritic cells. Blood 112:1776-1783.
26. Cao LQ, Qu GD, Wang DM (2012) Expression profiles of IL-10, TNF-a, and SOCS3 in placenta of pregnant women with intrahepatic cholestasis. Zhonghua Gan Zang Bing Za Zhi; 20:935-938.
27. Zhang Y, Hu L, Cui Y, et al. (2014) Roles of PPARγ/NF-κB signaling pathway in the pathogenesis of intrahepatic cholestasis of pregnancy. PLoS One 9.
28. Shao Y, Chen J, Zheng J, Liu CR (2016) Effect of Histone Deacetylase HDAC3 on Cytokines IL-18, IL-12 and TNF-α in Patients with Intrahepatic Cholestasis of Pregnancy. Cell Physiol Biochem ; 42(4):1294-1302.
29. Ai Y, Liu SY (2004) Influence of transforming growth factor-beta2 on production of tumor necrosis factor-alpha and interferon-gamma from placenta of intrahepatic cholestasis of pregnancy. Zhonghua Fu Chan Ke Za Zhi 39:322-325.