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First trimester maternal serum marker in intrahepatic cholestasis of pregnancy

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Summary

Purpose of investigation: The authors investigated the difference between the first-trimester maternal serum markers of intrahepatic cholestasis of pregnancy (ICP) cases and uneventful pregnancies, which might be useful in disease prediction. *Materials and Methods:* Twenty patients who had ICP and 40 pregnant women as control group were enrolled and the first-trimester maternal serum markers, maternal and fetal outcomes were compared between two groups. First trimester maternal serum markers: pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG) are the essential components of aneuploidy screening and prediction of adverse obstetric outcome. *Results:* Serum PAPP-A and free β-hCG levels were significantly higher in the ICP group. The mean serum PAPP-A was significantly higher in the ICP than control groups (2.4 ± 0.9 MoM *vs.* 1.2 ± 0.3 MoM: $p < 0.001$, respectively) and the mean of serum free β-hCG was significantly higher in the ICP than control groups (1.8 ± 1.5 MoM *vs.* 1.2 ± 0.6 MoM; $p = 0.036$, respectively). Gestational week at birth and the birth weights were lower in the ICP group ($p < 0.001$ and $p = 0.005$, respectively). *Conclusion:* The first-trimester maternal serum markers were elevated in case of ICP and this might be useful in disease prediction.

Key words: Bile acids; Free β-hCG; Intrahepatic cholestasis of pregnancy; Pregnancy associated plasma protein A.

Introduction

Intrahepatic cholestasis of pregnancy (ICP), the most common pregnancy-related liver disease, is characterised by the elevation of bile acids and liver enzymes in the maternal serum [1]. The cause of ICP is unknown, but genetic, hormonal, and environmental factors are likely involved [2-4]. It is associated with an increased risk of fetal and maternal mortality and morbidity. ICP is associated with an increased risk of fetal complications such as fetal distress, intrauterine fetal demise, and spontaneous premature labor [5]. Measurement of serum fasting bile acids is the most useful test for ICP diagnosis, and the level of >10 μmol/L confirms the diagnosis. The level of serum bile acids is correlated with fetal complication rates. A serum bile acid level ≥ 40 μmol/L can lead to adverse pregnancy outcomes [6]. ICP is not only associated with fetal complications but also with adverse obstetric outcomes such as pre-eclampsia and gestational diabetes [7, 8].

Abnormal maternal serum markers are the essential components of aneuploidy screening programmes. These markers include pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), alpha fetoprotein (AFP), inhibin A, and unconjugated estriol. Apart from fetal anomalies, these serum markers have been studied in the prediction of various obstetrical complications such as preeclampsia and intrauterine growth restriction. Few studies have investigated the relation of ICP with the antenatal serum markers. Prediction of ICP is important for the identification of the high risk pregnancies. Therefore, in this study, the authors' aim was to analyze the relationship between the first trimester biochemical screening tests and ICP, regarding the adverse outcomes of the disease, and compare the pregnancy outcomes of the ICP and control groups.

Materials and Methods

This retrospective cohort study was conducted at the Department of Obstetrics and Gynaecology, Sisli Etfal Research and Training Hospital, Istanbul, Turkey between January 2013 and April 2015. The study was approved by the Ethics Committee of the institution.

All patients with appropriate gestational age, which was measured by ultrasound and last menstrual period, were included in the study. Patients who had viral hepatitis, hypertension, diabetes, history of coronary heart disease, hyper/hypothyroidism, chronic liver diseases, infection, skin diseases, allergic disorders, symptomatic cholelithiasis, choledocholithiasis, gestational diabetes, preeclampsia and HELLP syndrome, and known coagulation abnormalities were excluded from the study. Thus, a total of 60 singleton pregnancies under the age of 35 years were included. Their demographic and maternal outcomes were yielded from the hospital database.

Antenatal screening test results were used for the study. This test was performed between the 11 and 13^{+6} weeks of gestation for the evaluation of maternal serum free-β-hCG and PAPP-A. An immunoassay system was used to measure the serum levels of PAPP-A and free β-hCG. Both results were reported as multiples of the median (MoM), corrected for gestational age and maternal

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Table 1. — *Demographic data of the ICP and control groups.*

	ICP (n=20)	Control (n=40)	*p*-value
Age (years)	29.9 ± 3.75	30.5 ± 4.14	0.542
Gravida (n)	2.1 ± 0.8	2 ± 0.8	0.951
Parity (n)	0.95 ± 0.75	1 ± 0.69	0.704
Diagnosis of ICP (gw)	32.5 ± 2.8		
Cesarean rate	3 (15%)	5 (12.5%)	0.788
Progesterone supplementation in the first trimester	11 (55%)	14 (35%)	0.139

Data are expressed as the mean ± standard deviation or n (%). gw: gestational week.

Table 2. — *Laboratory parameters of the ICP and control groups.*

	ICP (n=20)	Control (n=40)	*p*-value
PAPP-A (MoM)	2.4 ± 0.9	1.2 ± 0.3	< 0.001
Free-β-hCG (MoM)	1.8 ± 1.5	1.2 ± 0.6	0.036
AST (U/L)	128 ± 64	11 ± 2.7	< 0.001
ALT (U/L)	199 ± 164	12 ± 3	< 0.001
LDH (U/L)	406 ± 82	222 ± 23	< 0.001
Direct bilirubin levels (mg/dL)	1.02 ± 0.3	0.7 ± 0.1	0.002
Mean fasting bile acid (μmol/L)	68.65 ± 16.21		

Data are expressed as the mean ± standard deviation.
Free β-hCG: free beta human chorionic gonadotropin;
PAPP-A: pregnancy associated plasma protein A;
AST: aspartate aminotransferase test; ALT: alanine aminotransferase;
LDH: lactic acid dehydrogenase.

weight, at the time of sample collection.

ICP (20 patients) and control group (40 patients) included and the first-trimester maternal serum markers, the maternal and the fetal outcomes, were compared. ICP was diagnosed clinically with unrelenting generalised itching in the absence of any skin rash or systemic illness along with elevated fasting serum bile acids and liver enzymes. Additionally, viral hepatitis markers were measured, and abdominal ultrasound was performed.

The ICP patients were treated with ursodeoxycholic acid three times a day and had weekly fetal Doppler ultrasound. The patients were hospitalised until their liver enzymes and serum bile acid levels returned to normal values. Preterm labor is defined as regular uterine contractions before 37 weeks of pregnancy, resulting in changes in the cervix. Perinatal death is defined as intrauterine deaths and early neonatal deaths (seven days postpartum).

The SPSS software, version 17.0, was used for statistical analysis. The normality of the distribution of continuous variables was assessed by the Kolmogorov–Smirnov test. The chi-square test was used for categorical variables. Student's *t*-test was used for normally distributed continuous variables, and the Mann–Whitney *U*-test was used for non-normally distributed variables. Relative risks (RRs) with 95% confidence intervals (CIs) were calculated. A multivariate analysis was performed to find out whether preterm labor was associated with the fasting bile acid, direct bilirubin level, PAPP-A or free β-hCG. A p-value < 0.05 was considered to indicate statistical significance.

Table 3. — *Neonatal outcomes of the ICP and control groups.*

	ICP (n=20)	Control (n=40)	*p*-value
Gestational week at birth	35.9 ± 2.1	37.8 ± 1.5	< 0.001
Preterm labour (n)	8 (40%)	3 (7.5%)	0.002
Birth weight (gr)	2836 ± 510	3253 ± 518	0.005
NICU (n)	3 (15%)	1 (2.5%)	0.067
Perinatal death (n)	1 (5%)	0	0.154

Data are expressed as the mean ± standard deviation or n (%).
NICU: newborn intensive care unit; n: number of patients.

Table 4. — *Multivariate analysis of risk factors for preterm labour.*

	p-value	OR	95% CI
PAPP-A	0.271	7.3	0.2–260.4
Free β-hCG	0.354	0.1	0.01–1.15
Fasting bile acids	0.287	1.08	0.9–1.2
Serum direct bilirubin level	0.922	1.4	0.1–15.2

OR: odds ratio; CI: confidence interval;
PAPP-A: pregnancy associated plasma protein A;
Free-β-hCG: free beta human chorionic gonadotropin.

Results

The demographic data of the ICP and the control groups are given in Table 1. The mean gestational week for the diagnosis of ICP was 32.5 ± 2.8 weeks. There were no significant differences in terms of age, gravid-parity, and cesarean rates.

Serum PAPP-A and free β-hCG levels were significantly higher in the ICP group (Table 2). The mean serum PAPP-A was significantly higher in the ICP than control groups (2.4 ± 0.9 MoM *vs.* 1.2 ± 0.3 MoM: $p < 0.001$, respectively) and the mean of serum free β-hCG was significantly higher in the ICP than control groups (1.8 ± 1.5 MoM *vs.* 1.2 ± 0.6 MoM; $p = 0.036$, respectively).

Spontaneous premature labor was significantly higher in the ICP group (40% *vs.* 7.5%; $p = 0.002$). Gestational week at birth and the birth weights were lower in the ICP group ($p < 0.001$; $p = 0.005$, respectively). Perinatal death was not significant among both groups (Table 3).

Multivariate analysis did not show any significant relation between preterm labor and fasting bile acids, PAPP-A, free β-hCG, and direct bilirubin (Table 4).

Discussion

The present study demonstrated higher first trimester PAPP-A and free β-hCG levels in the ICP patients compared to the control group. Preterm labor was increased in the ICP group. The present results suggest that ICP cases were prone to the adverse obstetric outcomes, which are proved by the results of the first-trimester screening test.

ICP is a high risk for the fetus as it is related with unexplained intrauterine fetal demise and premature labor. Laboratory studies showed that bile acid taurocholate, a major bile salt in the gastrointestinal tracts, can cause different types of dysrhythmia in individuals with cardiomyopathies, and with its higher concentrations, the effect is more noticeable [9]. Hence, the prediction of ICP is important to prevent such devastating complications. This led us to investigate whether prediction of the disease could be made with early trimester maternal serum markers.

Despite the abnormal maternal serum markers being mostly used as a part of aneuploidy screening, they are also associated with adverse pregnancy outcomes such as preeclampsia, preterm birth, intrauterine growth retardation, gestational diabetes, and HELLP syndrome [10-12]. Limited studies have been conducted on ICP patients regarding their antenatal serum markers. Two of these studies investigated the serum AFP in ICP. However, the levels of first trimester PAPP-A in ICP cases are not clarified.

Eloranta *et al.* [13] conducted a study in 33 singleton pregnancies affected by cholestasis. They collected the serum samples at the 15th gestational week and measured the maternal serum AFP and hCG concentrations in MoM values. The mean maternal serum AFP was 1.12 MoM, and the mean serum hCG was 0.98 MoM. The median serum AFP and hCG concentrations in the cholestasis group were not significantly different from the control group. In another study, the maternal serum free β-hCG and AFP were measured between 14–18 gestational weeks in 24 cases with ICP [14]. The mean age of the patients was 30.1 years. The mean value for the serum free β-hCG and AFP were 1.29 and 0.94 MoM, respectively. The median values were not significant between the ICP and control groups.

Elevation of the serum bile acids is the most suitable biochemical marker for the diagnosis of the disease. Glantz *et al.* [6] reported that fetal complications were not observed until bile acid levels were ≥ 40 μmol/L. In the present study, the mean bile acid level was estimated to be 68.65 ± 16.21 μmol/L at the time of diagnosis. After the diagnosis, all ICP cases were treated and followed up. Despite the close monitoring of the cases, preterm labor was higher in the ICP group than the control group, but the neonatal intensive care unit (NICU) admission rate was not significant. Moreover, it has been demonstrated that progesterone metabolites impair hepatic bile transport. ICP is associated with supra-physiological levels of the 3 β-sulphated progesterone metabolite [15]. The present authors also compared the rate of patients between the ICP and control groups that received progesterone supplements because of abortus imminens, but did not find any significant differences.

Limitations of the study were its retrospective design and low number of study subjects. This study excluded the diseases presented with ICP such as HELLP syndrome and preeclampsia. However, to the best of the present authors' knowledge, this study is the first to demonstrate the relation of ICP with PAPP-A and free β-hCG.

In conclusion, the first-trimester maternal serum markers are promising tools in the prediction of adverse maternal and fetal outcomes. ICP is a serious condition and is associated with an increased risk of fetal and maternal adverse outcomes. Genetic predisposition and ICP history are important factors for the disease. Early prediction of disease is important for the pregnancy follow-up and prevention of unwanted outcomes. The first-trimester maternal serum markers are elevated in ICP cases and might be used in the prediction of disease. Larger studies should highlight the importance and accuracy of the present results.

References

[1] Williamson C., Geenes V.: "Intrahepatic cholestasis of pregnancy". *Obstet. Gynecol.*, 2014, *124*, 120.

[2] Gonzalez M.C., Reyes H., Arrese M., Figueroa D., Lorca B., Andresen M., *et al.*: "Intrahepatic cholestasis of pregnancy in twin pregnancies". *J. Hepatol.*,1989, *9*, 84.

[3] Koivurova S., Hartikainen A.L., Karinen L., Gissler M., Hemminki E., Martikainen H., *et al.*: "The course of pregnancy and delivery and the use of maternal healthcare services after standard IVF in Northern Finland 1990-1995". *Hum. Reprod.*, 2002, *17*, 2897.

[4] Heinonen S., Kirkinen P.: "Pregnancy outcome with intrahepatic cholestasis". *Obstet. Gynecol.*, 1999, *94*, 189.

[5] Rioseco A.J., Ivankovic M.B., Manzur A., Hamed F., Kato S.R., Parer J.T., *et al.*: "Intrahepatic cholestasis of pregnancy: a retrospective case-control study of perinatal outcome". *Am. J. Obstet. Gynecol.*, 1994, *170*, 890.

[6] Glantz A., Marschall H.U., Mattsson L.A.: "Intrahepatic cholestasis of pregnancy: relationships between bile acid levels and fetal complication rates". *Hepatology*, 2004, *40*, 467.

[7] Wikström S.E., Marschall H.U., Ludvigsson J.F., Stephansson O.: "Intrahepatic cholestasis of pregnancy and associated adverse pregnancy and fetal outcomes: a 12-year population-based cohort study". *Int. J. Obstet. Gynecol.*, 2013, *120*, 717.

[8] Martineau M., Raker C., Powrie R., Williamson C.: "Intrahepatic cholestasis of pregnancy is associated with an increased risk of gestational diabetes". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, *176*, 80.

[9] Gorelik J., Harding S.E., Shevchuk A.I., Koralage D., Lab M., Swiet M., *et al.*: "Taurocholate induces changes in rat cardiomyocyte contraction and calcium dynamics". *Clin. Sci. (Lond.)*, 2002, *103*, 191.

[10] Conserva V., Signaroldi M., Mastroianni C., Stampalija T., Ghisoni L., Ferrazzi E., *et al.*: "Distinction between fetal growth restriction and small for gestational age newborn weight enhances the prognostic value of low PAPP-A in the first trimester". *Prenat. Diagn.*, 2010, *30*, 1007.

[11] Hourrier S., Salomon L.J., Dreux S., Muller F.: "Screening for adverse pregnancy outcome at early gestational age". *Clin. Chim. Acta*, 2010, *411*, 1547.

[12] Hui D., Okun N., Murphy K., Kingdom J., Uleryk E., Shah P.S.: "Combinations of maternal serum markers to predict preeclampsia, small for gestational age, and stillbirth: a systematic review". *J. Obstet. Gynaecol. Can.*, 2012, *34*, 142.

[13] Eloranta M.L., Heinonen S., Kirkinen P.: "Intrahepatic cholestasis of pregnancy has no effect on maternal serum second trimester alpha-fetoprotein and hCG". *Acta Obstet. Gynecol. Scand.*, 2000, *79*, 548.

[14] Räty R., Anttila L., Virtanen A., Koskinen P., Laitinen P., Mörsky P., *et al.*: "Maternal midtrimester free betaHCG and AFP serum levels in spontaneous singleton pregnancies complicated bygestational diabetes mellitus, pregnancy-induced hypertension or obstetric cholestasis". *Prenat. Diagn.*, 2003, *23*, 1045.

[15] Abu-hayyeh S., Papacleovoulou G., Lovgren-Sandblom A., Tahir M., Oduwole O., Jamaludin N.A., *et al.*: "Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit farnesoid X receptor resulting in a cholestatic phenotype. *Hepatology*, 2013, *57*, 716.

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