Could first- and second-trimester biochemical markers for Down syndrome have a role in predicting intrahepatic cholestasis of pregnancy?

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Abstract

Introduction: The aim of this study is to compare first- and second-trimester Down syndrome biochemical screening markers in intrahepatic cholestasis of pregnancy (ICP) and normal pregnancies.
Material and methods: This observational case-control study was conducted at Health Sciences University Zeynep Kamil Maternity and Children's Health Training and Research Hospital and the Department of Obstetrics and Gynecology at Erciyes University Medical Faculty during 2016–2017. The study included 165 patients, and consisted of 62 women who had been diagnosed with ICP (the ICP-diagnosed group) and 103 healthy pregnant women (the control group). First-trimester free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and second-trimester total β-hCG, estriol (E3), α-fetoprotein (AFP), and inhibin A levels were compared between the two groups.
Results: The mean patient age was 28.67 ±5.96 years, with no significant difference between the groups ($p > 0.05$). Average PAPP-A levels were significantly lower in the ICP-diagnosed group ($p < 0.001$). When the cut-off value for PAPP-A was taken as ≤ 0.93 multiple of median (MoM), the sensitivity and specificity values for ICP were 73.8% and 56.3%, respectively (95% CI, AUC ± SE: 0.663 ±0.042).
Conclusions: The decrease in PAPP-A MoM value indicates an increase in the risk of developing ICP, while changes in other markers were not sufficient to predict ICP.

Key words: intrahepatic cholestasis, pregnancy, Down syndrome biochemical screening markers.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-associated liver disease, and its incidence varies markedly between 0.1% and 15.6% [1]. Intrahepatic cholestasis of pregnancy is

characterized by itching, increased fasting serum bile acid levels and/or abnormal liver function tests. Itching typically occurs at the end of the second or third trimester, is worse at night, and is most noticeable on the palms and soles [2]. Biochemical findings may spontaneously recover at 4–6 weeks postpartum [3]. The disease is associated with an increased risk of preeclampsia, gestational diabetes mellitus (GDM), and adverse fetal outcomes, including spontaneous and iatrogenic preterm delivery, non-reassuring fetal status, meconium staining of the amniotic fluid, and stillbirth [4–6].

Many physiological changes occur during the gestational period [7]. Abnormal biliary transport and excretion as a result of interaction between hormonal changes during pregnancy, genetic, and environmental factors constitute the pathogenesis of ICP [8]. However, there is no method for specifying the risk of ICP, because the cause of this disease is influenced by many factors and its pathogenesis has not yet been fully explained. As part of the first-trimester screening test for Down syndrome, pregnancy-associated plasma protein-A (PAPP-A) has been suggested as an early marker of ICP development [9]. Therefore, the present study evaluated the predictive role of all biochemical parameters of screening tests for Down syndrome (PAPP-A, free and total beta human chorionic gonadotropin (β-hCG), estriol (E3), and inhibin A) in the first and second trimesters of pregnancy.

Material and methods

This retrospective case-control study was conducted at Health Sciences University Zeynep Kamil Women and Children's Health Training and Research Hospital and Department of Obstetrics and Gynecology at Erciyes University Medical Faculty during 2016–2017. This study was approved by the local ethics committee according to the principles outlined by the Declaration of Helsinki.

Diagnosis of ICP was based on the following criteria [1] generalized pruritus without skin lesions during the third trimester of an uneventful pregnancy; [2] plasma levels of alanine transaminase (ALT) > 40; [3] elevated fasting total bile acid (TBA) levels > 10 mmol/l; and [4] spontaneous resolution of clinical symptoms and laboratory findings after delivery.

The hospital records were searched to identify all patients diagnosed with ICP between 2016 and 2017. We excluded 19 patients with ICP in order to reduce possible confounding factors (preeclampsia in 5, GDM in 6, chronic diseases such as hypothyroidism in 3 and multiple pregnancies in 5) and 62 consecutive patients with ICP were recruited for the study group. One hundred and three age-matched healthy pregnant women who applied on the same dates as the ICP patients were selected randomly as the control group. The multiple of median (MoM) values for first-trimester (free β-hCG and PAPP-A) and second-trimester (total β-hCG, E3, and inhibin A) screening tests for all patients were retrospectively obtained from medical records, as were the age, gravidity, parity, and gestational age of the women.

Statistical analysis

Statistical Package for the Social Sciences, version 22.0 software (IBM Corporation, Armonk, New York, United States) and MedCalc 14 (Acacialaan 22, B-8400 Ostend, Belgium) programs were used to analyze the variables. Conformity of the data to a normal distribution was evaluated using the Shapiro-Wilk test, and the homogeneity of variance was assessed using the Levene test. The independent-samples *T*-test was used with the bootstrap results, and the Mann-Whitney *U* test was used with the Monte Carlo results. The relationship between the classification and the actual classification of the cutoff estimates calculated according to the variables of the groups was examined and expressed by receiver operating curve analysis with sensitivity (SE) and specificity (SP) values. Quantitative variables were stated as the mean ± standard deviation (SD) and median (minimum/maximum), and categorical variables as the number (*n*) and percentage (%). Variables

Table I. Age and pregnancy characteristics of intrahepatic cholestasis of pregnancy and control groups

Parameter	Control (*n* = 103)	ICP (*n* = 62)	*P*-value
Age [years]	28.70 ±6.34	28.62 ±5.33	0.938
Gravida	2 (1–11)	2 (1–11)	0.477
Parity	1 (0–7)	1.3 (0–4)	0.014
Abortion	0 (0–3)	0 (0–5)	0.453
Live birth	1 (0–5)	1.4 (0–4)	0.004
Gestational age at inclusion [weeks]	38 (30–42)	35 (29–40)	< 0.001

Independent t-test (bootstrap)/Mann-Whitney U test (Monte Carlo). Data are presented as mean ± standard deviation or median (minimum-maximum), as appropriate. ICP – intrahepatic cholestasis of pregnancy.

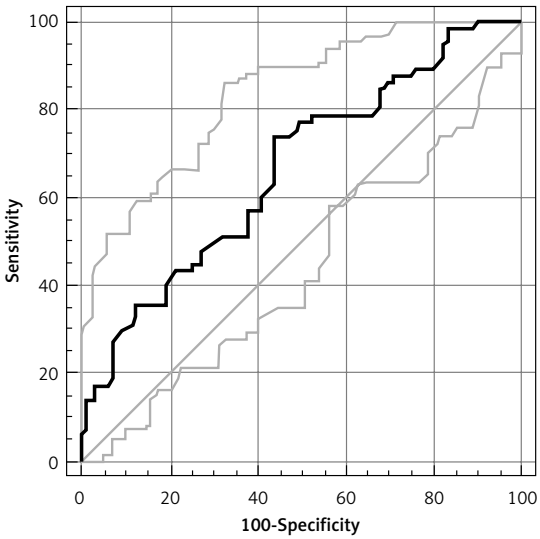

Figure 1. Receiver operating curve (ROC) analysis for the use of pregnancy-associated plasma protein A value observed in the first trimester in the intrahepatic cholestasis of pregnancy group. When the cut-off value for PAPP-A was taken as ≤ 0.93 multiple of median (MoM), the sensitivity and specificity values for ICP were 73.8% and 56.3%, respectively (95% CI, AUC ± SE: 0.663 ±0.042)

were examined at a 95% confidence interval, and a value of $p < 0.05$ was accepted as statistically significant.

Results

The general characteristics of the pregnancies and the outcomes of the ICP-diagnosed and control groups are shown in Table I. The mean patient age was 28.67 ±5.96 years and there was no significant difference between the groups ($p > 0.05$). Parity and live births were higher in the ICP group than in the control group ($p = 0.014$ and $p = 0.004$, respectively). Gestational age at inclusion in the study was significantly lower in the ICP patients than in the control group ($p < 0.001$).

Table II shows the comparison of the third-trimester laboratory results of the healthy and ICP-diagnosed pregnancies. The mean liver function tests and bilirubin values were significantly higher in the ICP group than in the control group ($p < 0.001$). The alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) levels were significantly higher in the ICP group than in the control group ($p < 0.001$, $p = 0.022$, respectively) (Table II).

The results of the first- and second-trimester screening tests are shown in Table III. Total β-hCG, E3, AFP, and inhibin A levels (MoM), screened in the second trimester, were not significantly different between the ICP and control groups ($p = 0.795$, $p = 0.063$, $p = 0.880$, $p = 0.326$, respectively). With regard to the first-trimester screening results,

Table II. Laboratory findings of third trimester of intrahepatic cholestasis of pregnancy and control groups

Parameter	Control (n = 103)	ICP (n = 62)	P-value
SGOT [IU/l]	16 (6–25)	60 (12–524)	< 0. 001
SGPT [IU/l]	11 (4–32)	95 (9–657)	< 0. 001
Total bilirubin [mg/dl]	0.3 (0.15–0.6)	0.65 (0.1–9)	< 0. 001
Conjugated bilirubin [mg/dl]	0.145 (0.015–10.15)	0.42 (0.01–2.15)	< 0. 001
GGT [IU/l]	12 (7–43)	15 (5–57)	0.022
ALP [IU/l]	89.5 (72–123)	180 (25–512)	< 0. 001
Bile acid	N/A	22 (10–160)	N/A

Independent t-test (bootstrap)/Mann-Whitney U test (Monte Carlo). Data are presented as median (minimum-maximum). ICP – intrahepatic cholestasis of pregnancy, SGOT – serum glutamic oxaloacetic transaminase, SGPT – serum glutamic pyruvic transaminase, GGT – γ-glutamyl transpeptidase, ALP – alkaline phosphatase.

Table III. Results of first- and second-trimester screening tests of intrahepatic cholestasis of pregnancy and control groups

Screening tests (MoM)	Control (n = 103)	ICP (n = 62)	P-value
Total β-hCG	1.16 ±0.52	1.13 ±0.48	0.795
Estriol (E_3)	0.67 ±0.26	0.83 ±0.38	0.063
AFP	0.85 (0.39/2)	0.8 (0.47/1.88)	0.880
Inhibin A	0.52 (0.31/2.05)	0.56 (0.34/2.28)	0.326
PAPP-A	1 (0.2/3.7)	0.8 (0.1/2)	< 0.001
Free β-hCG	0.935 (0.25/2.93)	0.98 (0.59/2.64)	0.265

Independent t-test (bootstrap)/Mann-Whitney U test (Monte Carlo). Data are presented as mean ± standard deviation or median (minimum-maximum), as appropriate. ICP – intrahepatic cholestasis of pregnancy, AFP – α-fetoprotein, hCG – human chorionic gonadotropin, MoM – multiple of the median, PAPP-A – pregnancy-associated plasma protein A.

Table IV. Sensitivity and specificity values of screening tests

Parameter		Control		ICP		AUC ± SE	*P*-value
		n	%	*n*	%		
PAPP-A (MoM)	> 0.93	58	56.3	17	26.2	0.663 ±0.042	< 0.001
	≤ 0.93	45	43.7	48	73.8		

AUC – area under the receiver operating curve, MoM – multiple of the median, PAPP-A – pregnancy-associated plasma protein A, SE – standard error. ■ *Sensitivity,* ■ *Specificity.*

only the PAPP-A (MoM) values were significantly lower in the ICP group than in the control group ($p < 0.001$). No such difference in free β-hCG (MoM) was observed ($p = 0.265$).

The PAPP-A results in the ICP and control groups are shown in Table IV. When the cut-off value for PAPP-A was taken as 0.93 MoM, the SE and SP values for ICP were 73.8% and 56.3%, respectively (area under curve (AUC) ± standard error (SE) r = 0.663 ±0.042 within 95% confidence interval (CI)) (Figure 1).

Discussion

The relationship between the changes in the serum levels of biochemical markers used in the screening of Down syndrome and some adverse maternal and fetal outcomes has been demonstrated. The incidence of preeclampsia is increased in pregnancies with low PAPP-A and higher total β-hCG and inhibin A levels [10]. Also, pregnancies with low PAPP-A levels are associated with higher incidence of GDM and preterm labor [11, 12]. Increase in the adverse fetal outcomes in cases with ICP [13, 14], in addition to the enhanced frequency of pregnancy-associated comorbidities such as GDM, preeclampsia and preterm labor, suggests the presence of commonly shared factors playing a role in the pathophysiology of these diseases [14, 15]. In the light of these findings, we aimed to investigate the levels of these markers and their predictive power in patients with ICP. We found that serum PAPP-A levels were significantly lower in the ICP group than the control group. When the cut-off value for PAPP-A was taken as ≤ 0.93 MoM, the sensitivity and specificity values for ICP were 73.8% and 56.3%, respectively (95% CI, AUC ± SE: 0.663 ±0.042). A significant relationship was not found between other biomarkers (free β-HCG, total β-HCG, E3, AFP, inhibin A) and ICP.

The PAPP-A is synthesized in the placenta, largely in extravillous cytotrophoblasts [16]. Its most important function is to serve as a protease for insulin-like growth factor (IGF)-binding proteins 4 and 5 [17]. Trophoblast invasion plays an important role in the early development of the placenta, and any alteration in this developmental process is critical for the maintenance of a healthy pregnancy. Problems in placentation lead to an increase in the risk of intrauterine growth restriction, pregnancy-related hypertensive disorders and intrauterine fetal death. Studies have shown that low PAPP-A levels may reduce IGF in the circulation, leading to poor pregnancy outcomes such as preterm delivery, preeclampsia, gestational diabetes and intrauterine fetal death [18, 19]. Furthermore, previous studies demonstrated a correlation between IGF levels and bile acid flow where decreased IGF levels were associated with impaired bile flow. It was also reported that in cholestatic rats IGF therapy increases bile acid flow, leading to a therapeutic effect [20, 21]. Therefore, the low PAPP-A levels detected in our study might be related to development of ICP through the IGF pathway.

Hancerliogullari *et al.* found that a decreased PAPP-A (MoM) value was associated with ICP development. They reported that decreased PAPP-A levels in the first trimester should be a warning sign in the prediction of such gestational complications as intrauterine growth restriction, preeclampsia, preterm labor, and cholestasis [9]. In the current study, it was also found that PAPP-A (MoM) values were lower in the ICP group than in the control group. In patients with ICP, decreased PAPP-A levels [10], more frequent detection of preeclampsia, gestational diabetes, and adverse fetal complications [22, 23] might suggest the presence of a common etiopathogenesis as placentation insufficiency. Hancerliogullari *et al.* also observed that free β-hCG levels were higher in patients with ICP [9], but this difference was statistically insignificant in both our study and that of Raty *et al.* [24]. The reason for our inability to obtain a statistically significant difference for free β-hCG levels may be the larger cohort size of the current study.

Raised levels of estrogens and progesterone metabolites temporarily inhibit the bile acid carrier pump in genetically susceptible women and result in the development of toxicity, due to increased bile acid levels [8, 25, 26]. The ICP is most commonly observed in the third trimester when placenta-derived hormones reach their highest level. This is supported by the significantly higher frequency of ICP in women with multiple pregnancies, which cause a marked elevation in gestational hormones [27]. Therefore, E3 may have a role in ICP pathogenesis. However, the ICP and control groups were statistically similar in the level of serum E3 ($p = 0.063$). This may suggest that high E3 levels do not

result in cholestasis per se, but genetic susceptibility or environmental factors predispose women to developing ICP at similar E3 levels.

To the best of our knowledge, this is the first study to evaluate the role of all first- and second-trimester biochemical markers for Down syndrome in patients with ICP.

This study also has some limitations. First, it has a retrospective study design and the study population was relatively small; however, we were still able to demonstrate a significant relationship between the first-trimester Down syndrome screening marker PAPP-A and ICP. Another limitation of this study is the lack of perinatal outcomes of the fetuses.

In conclusion, it has previously been shown that low PAPP-A MoM values in the first trimester were associated with increased ICP risk. Other biochemical screening tests have shown no significance in the prediction of ICP. The results of the present study indicate that careful attention should be paid to the development of ICP in the follow-up of pregnancies with low PAPP-A levels because of perinatal complications. Further prospective studies with a larger number of patients are required in this regard.

Conflict of interest

The authors declare no conflict of interest.

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