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The relationship between first and second trimester biochemical parameters used to screen down syndrome and intrahepatic cholestasis of pregnancy

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ABSTRACT

Objective: To assess the role of first and second-trimester screening biomarkers pregnancy-associated plasma protein-A(PAPP-A), free beta-human chorionic gonadotropin(free ß-hCG), estriol, alpha-fetoprotein and total β-hCG in the early diagnosis of intrahepatic cholestasis of pregnancy (ICP).

Materials and Methods: Patients with ICP admitted to Mersin University Hospital for delivery between 2015 and 2019 and had first and second-trimester aneuploidy screening tests performed in the same facility were retrospectively assessed. Randomly 60 pregnant women without comorbid conditions during the same period were included as controls. Data regarding demographic characteristics, laboratory values including free ß-hCG and PAPP-A in first-trimester screening and total ß-hCG, estriol and alpha- fetoprotein in second-trimester screening were compared.

Results: There were 46 eligible patients with ICP. In first trimester screening, it was found that PAPP-A MoM was significantly lower (0.89±0.55 vs. 1.94±0.73; p=0.035) while free ß-hCG MoM was significantly higher in ICP group when compared to controls (1.84±0.59 vs. 0.99±0.47; p=0.018). In second trimester screening, no significant difference was detected in aneuploidy markers between groups. For prediction of ICP development, first trimester free β-hCG >1.44 MoM was found to have a sensitivity of 50%, a specificity of 80% and positive and negative predictive values of 33% and 88.9% respectively. Similarly first trimester PAPP-A values <1.075 MoM was found to have 80% and 75% sensitivity and specificity with positive and negative predictive values of 75% and 44% respectively.

Conclusion: Low PAPP-A MoM value and elevated free ß-hCG in first trimester seem to be associated with ICP development.

Keywords: Alpha-fetoprotein (AFP), estriol, free beta-human chorionic gonadotropin (free ß-hCG), intrahepatic cholestasis of pregnancy, pregnancy-associated plasma protein-A (PAPP-A)

INTRODUCTION

The intrahepatic cholestasis of pregnancy (ICP) is a common hepatic disorder seen in 0.1% to 15.6% of pregnancies [1]. In ICP, there are elevated hepatic function tests and bile acids levels that result in itching of soles and palms. The itching is more commonly seen at late second trimester and third trimester [2]. The itching shows spontaneous recovery 5 to 6 weeks after delivery [3]. In patients with ICP, poor perinatal outcomes are more common than normal healthy pregnant women because of increased rates of gestational diabetes, pre-eclampsia, meconium in amniotic fluid and preterm delivery [4, 5]. Many physiological alterations occur in organs and the body during pregnancy [6]. The hormonal changes in pregnancy, genetics, and environmental factors affect biliary transport and secretion. Although the etiology of ICP hasn't been fully elucidated, above-mentioned factors have been implied in the etiopathogenesis of the disease [7]. The early diagnosis and treatment are important in the ICP. There are reports suggesting that parameters used in first-trimester screening tests (pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (free ß-hCG) can be used as early markers for ICP [8].

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In our study, we investigated role of first and second trimester screening tests including PAPP-A, free β-hCG, estriol (E3), alpha-fetoprotein (AFP), and total β-hCG in the early diagnosis of ICP.

MATERIAL and METHODS

The study was conducted by reviewing all patients with ICP who were admitted to Obstetrics & Gynecology Department of Mersin University, Medicine School for delivery between 2015 and 2019 and had first and second-trimester aneuploidy screening tests performed in the same facility. Data were extracted from database of the hospital. The following criteria were used as diagnostic criteria for ICP: the presence of general pruritus without skin lesion in late second trimester or third trimester [2]; serum alanine aminotransferase (ALT) level >40 IU [3]; fasting bile acid level >10 mmol/L; and spontaneous recovery of symptoms and laboratory abnormalities after delivery. Patients with multiple pregnancies, those with history of cholecystectomy, those with gestational diabetes mellitus, thyroid disorder or pregnancy-related hypertension, and those with hepatic or biliary diseases were excluded. In addition, we randomly selected 60 pregnant women without the comorbid condition who were admitted to the obstetrics department during the same period as controls.

In all patients, data regarding age, gestational age, hepatic function tests at admission, birth weight and umbilical artery pH values at birth, free ß-hCG and PAPP-A MoM (Multiplies of Median) values in first-trimester screening and total ß-hCG, E3 and AFP MoM values in second trimester, medications and fasting bile acid levels (only for patients with ICP) were extracted from patient files. Demographic data and laboratory results were compared between patients with ICP and controls. In our hospital, first and second-trimester screening tests were studied using Beckman Coulter Dxi 800 analyzer. First-trimester screening is performed between gestational weeks 11 and 14 when CRL (crown-rump length) was measured to be 43-84 mm. In the test, trisomy (trisomy 13, 18 and 21) and age risk is estimated based on free ß-hCG and PAPP-A MoM values calculated according to CRL and NT (nuchal translucency) values. Second trimester screening is performed between gestational weeks 16 and 20 by measuring BPD (biparietal diameter). In the test, risk estimation for trisomy and neural tube defects are estimated based on ß-hCG, E3 and AFP MoM values. In patients diagnosed as ICP, only ursodeoxycholic acid therapy was prescribed based on the recommendation of gastroenterology department.

The delivery decision was made based on worsening in hepatic function tests, intrauterine growth retardation, fetal umbilical Doppler abnormality, severe preeclampsia, and other obstetric indications.

Statistical analysis was performed using SPSS version 22.0 (IBM Corporation, Amork, USA). After testing normal distribution of data, Student's t test was used to compare mean values. ROC curve analysis was performed to determine potential for ICP prediction in parameters found to be significantly higher in patients with ICP. Threshold values, sensitivity, specificity and positive and negative predictive values for ICP development were calculated. A p value <0.05 was considered as statistically significant.

RESULTS

Overall, 46 eligible patients were included. Of the patients, 6 gave birth via vaginal route while 40 gave birth via abdominal route. Of patients with ICP, ursodeoxycholic acid therapy was given to 38 patients. No significant differences were detected between groups regarding mean age, gestational age at admission, fetal weight and umbilical artery pH values at birth (**Table 1**).

When laboratory values were assessed, it was found that ALP, ALT and AST were significantly higher in ICP group while there was no significant difference in GGT level between groups (**Table 1**).

In first trimester screening, it was found that PAPP-A MoM was significantly lower (0.89±0.55 vs. 1.94±0.73; p=0.035) while free ß-hCG MoM was significantly higher in ICP group when compared to controls (1.84±0.59 vs. 0.99±0.47; p=0.018) (**Table 1**).

In second trimester screening, no significant difference was detected in aneuploidy markers between groups (**Table 1**).

The predictive abilities of first and second-trimester PAPP-A and free β-hCG MoM levels for the diagnosis of ICP were depicted in Figure 1 and table 2. Both markers were found to have significant predictive ability for the future development of ICP. First trimester free β-hCG >1.44 MoM was found to have a sensitivity of 50%, a specificity of 80% and positive and negative predictive values of 33% and 88.9% respectively. Similarly first trimester PAPP-A values <1.075 MoM was found to have 80% and 75% sensitivity and specificity respectively with positive and negative predictive values of 75% and 44% respectively (**Table 2**).

Table 1: Characteristics and mean laboratory values in patients with intrahepatic cholestasis of pregnancy and controls

	Patients with intrahepatic cholestasis of pregnancy (n=46)	Controls (n=60)	P
Age (year)	27.1±5.4	29.3±6.3	0.20
Gestational week	32±5	32±7	0.37
Fetal weight (gram)	3035±702	2290±680	0.57
GGT (IU/L)	19±12	27.7±35	0.70
ALP (IU/L)	208±73	137±83	***0.001****
ALT (IU/L)	178±146	52±40	***0.0001****
AST (IU/L)	119±99	44±40	***0.0001****
Bile acid(IU/L)	45±5.6	N/A	N/A
Dose of medication (miligram)	675±38	N/A	N/A
Umbilical Artery Ph	7.32±0.05	7.28±0.09	0.11
PAPP-A (mom)	0.89±0.55	1.94±0.73	***0.035****
Free β-hCG (mom)	1.84±0.59	0.99±0.47	***0.018****
Estriol (mom)	1.12±0.19	0.69±0.21	0.13
β-hCG (mom)	1.1±0.39	0.64±0.21	0.26
AFP(mom)	0.95±0.01	1.19±0.21	0.13

Student T test was employed. * p<0,05, ALT – ALT -serum alanine transaminase, AST– serum aspartat transaminase, GGT – g-glutamyl transpeptidase, ALP – alkaline phosphatase, AFP – α-fetoprotein, hCG – human chorionic gonadotropin, MoM – multiple of the median, PAPP-A – pregnancy-associated plasma protein A.AFP-alfa fetu protein,Free Beta hCG-free human corionic gronadodropin

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Table 2: Likelihood of detecting intrahepatic cholestasis of pregnancy according to fFree β-hCG cut-off value

	AUC	Sensitivity	Specificity	PPV	NPV	P
Free β-hCG (MoM) Cut Off >1,44	0.83	50%	80%	33%	88.9%	0.026*
PAPP-A (MoM) Cut Off <1,075	0.88	80%	75%	44%	93.7%	0.01*

*p<0,05, MoM – multiple of the median, Free Beta hCG-free human corionic gronadodropin AUC – area under the receiver operating curve, MoM – multiple of the median, NPV- negative predictive value, PPV- positive predictive value.

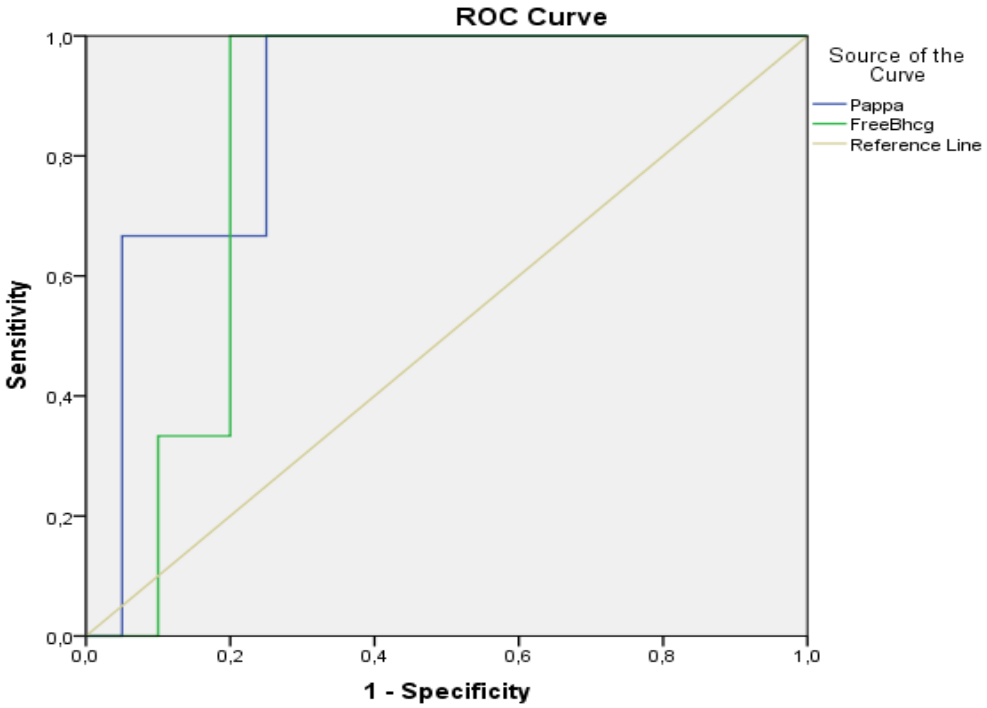

Figure 1: ROC curves for free β-hCG and PAPP-A in intrahepatic cholestasis of pregnancy

DISCUSSION

Intrahepatic cholestasis is the most common liver disease during pregnancy and is associated with adverse maternal and fetal outcomes. It is a diagnosis of exclusion, therefore, it is not always easy to diagnose the disease. Any marker that provide early diagnosis would result in prompt intervention and consequently decrease the adverse outcome risks. In the present study we assessed the roles of first and second trimester aneuploidy markers in the prediction of ICP development and showed that first trimester PAPP-A and free β-hCG MoM levels, but not second trimester markers, may have a comparable predictive potential for the subsequent ICP development throughout the pregnancy. The biochemical markers used for Down syndrome screening can predict poor maternal and fetal outcomes. It was shown that low PAPP-A level is associated with preeclampsia, gestational diabetes mellitus, preterm delivery, intrauterine growth retardation, preterm rupture of membranes and placental ablation [9-12]. A high PAPP-A value was suggested to be associated with a higher rate of meconium-stained amniotic fluid [13] First trimester low hCG levels are reported to be associated with increased risk for intrauterine growth restriction (IUGR), preterm birth, low birth weight (LBW) and low Apgar scores whereas high levels are related with a significantly decreased risk of preterm birth and GDM [14].

In the second trimester both low and high β-hCG levels were found to be associated with increased risk for spontaneous abortion, IUGR and preterm birth [14].

The relationship between these aneuploidy markers and the development of ICP has also been assessed in a few studies. Hancerlioglu et al. reported low PAPP-A and high free β-hCG levels were associated with obstetric cholestasis [8]. They included 35 ICP patients and found the mean PAPP-A and free beta hCG MoM levels as 0.76 ± 0.31 and 1.2 ± 0.79 respectively. Contrary to this study, another study from the same country could not find an association between low first trimester PAPP-A levels and ICP [15]. Destici et al. reported median PAPP-A MoM levels as 0.93 in ICP group. In the present study the median first trimester PAPP-A levels were 0.89 which was significantly lower in ICP patients. In ROC analysis, the area under the curve was found to be 0.88, and values >0.8 is considered to be a strong predictor of clinical sensitivity or specificity (16). In their study, Tayyar et al. found PAPP-A <0.93 MoM as a cut-off value for prediction of ICP with 73.8% sensitivity and 56.3 specificities (95% CI, AUC± SE: 0.663 ±0.042). [17]; however, different than our results, they did not find free hCG to be associated with ICP. They also could not find any relationship between second-trimester markers, including Inhibin A, with subsequent ICP

[17]. In the present study PAPP-A 1.075 MoM was found as a cut-off value with substantial sensitivity, specificity, positive and negative predictive values. Therefore low PAPP-A MoM values seem to have a predictive potential for subsequent ICP.

PAPP-A is a glycoprotein secreted from the extravillous trophoblasts of the placenta [18] and it cleaves insulin-like growth factor binding protein (IGFBP)-4 and -5 [19]. By this way it modulates the activity of insulin-like growth factor (IGF)-1 and -2 [20]. Low levels of PAPP-A results in decreased IGF levels due to decreased cleavage of the binding protein. In addition PAPP-A plays a role in the autocrine and paracrine control of trophoblast invasion of the decidua [21] and any change during this period may have a negative effect continuation of pregnancy.

The failure in invasion (due to IGF effect) increases the risk for intrauterine growth retardation, pregnancy-related hypertension, and intrauterine death in advancing periods of pregnancy. In some studies, it was found that low PAPP-A levels reduced blood IGF level. The decreased IGF levels in pregnancy results in poor pregnancy outcomes such as preterm birth, preeclampsia, gestational diabetes mellitus, and intrauterine fetal death [22, 23].

In a study on choleostatic rats, it was found that IGF therapy increased flow of bile acids and improved clinical presentation [24,25], concluding that there is an association between decreased IGF level and biliary flow. In the study, it was found that IGF is important for bile acid homeostasis and secretion and that endogenous and exogenous IGF decreased blood bile acid. The reduction in PAPP-A levels in early pregnancy can lead decrease in protease effect on IGF-4 and IGF-5; thus, ICP development in advancing weeks of pregnancy as a result of decreased IGF. Contrary to first trimester, high levels of third trimester PAPP-A levels have been observed in ICP patients [9].

Therefore obstetric conditions in which there is inadequate trophoblastic invasion in the first trimester would be associated with a low PAPP-A level, whereas hypoperfusion-related stimulation of the production of placental proteins in the second and third trimesters would be associated with a high PAPP-A level [9].

By initiation of pregnancy, trophoblastic cells begin to secrete glycoprotein ß-hCG. The increased free ß-hCG level in fetal and maternal blood has 80% similarity with luteinizing hormone (LH). The increased free ß-hCG in fetal and maternal blood binds to LH/hCG receptors, inducing steroidogenesis. The induced steroidogenesis results in formation of estrogen and progesterone metabolites. The increased estrogen and progesterone metabolites due to elevated free ß-hCG inhibit bile acid transportation in pregnant women. The increased blood bile acid levels due to failure in excretion lead increase in fetal and/or maternal toxicity and complications [7, 26, 27].

As placental hormone levels are increased by advancing gestational age, ICP is most frequently seen in third trimester. In multiple pregnancies, ICP is more common since placental hormones were higher [28]. In a study by Hancerlioglu et al., a relationship was found between high levels of ß-hCG and ICP [8]. However, no such relationship was found in some studies [29].

In our study, it was found that free ß-hCG level in first trimester was significantly higher in ICP group when compared to controls. In ROC analysis, a relatively higher AUC value was found and a cut-off value of 1.44 MoM was calculated for free ß-hCG. It was also found that the cut-off value can predict ICP by the sensitivity of 80% and specificity of 50%, indicating that first trimester free ß-hCG level can be a potential marker for ICP.

Alpha-fetoprotein (AFP) is a glycoprotein secreted from fetal liver and yolk sac. It is a parameter of second trimester testing used for Down syndrome screening between gestational weeks 16 and 20. There are reports suggesting that low AFP MoM values are associated with gestational DM while high AFP and ß-hCG MoM values were associated with preeclampsia [30, 31].

In previous studies, no relationship was found between the second-trimester screening test and ICP [17, 32, 33]. In a study by Yuan et al., it was concluded that the increased maternal AFP and estriol (E3) levels lead to an increase in risk for ICP in the third trimester [34]. In the study, it was also found that placental anomalies such as placental previa and placental abruption were more common among patients with elevated AFP. The authors concluded that serum AFP level is increased in women with placental dysfunction; as a result, ICP can be seen more commonly in third trimester. In our study, no significant difference was found in AFP between ICP and control groups in agreement with literature.

In previous studies, ICP was more frequently seen in patients with impaired estrogen metabolism [35]. It was found that serum bile acids were increased in non-pregnant women using oral contraceptives with high estrogen and progesterone content [36]. In an in vitro study, it was found that progesterone metabolites, not progesterone itself, impaired bile acid transport [27].

The increased ß-hCG level during pregnancy leads to an elevation in progesterone and its metabolite E3. The elevated E3 levels impair bile acid transport, increasing the risk for ICP. In a study by Raty et al. it was concluded that elevated ß-hCG level is associated with ICP [29]. In two distinct study by Eloranta et al. and Raty et al., no relationship was detected maternal AFP and ß-hCG levels and ICP [29, 33].

However, in the study by Raty et al., ß-hCG levels were increased in the ICP group but did not reach statistical significance. In a study by Leslie et al., no significant difference was found in E3 level between ICP and control groups [37].

In our study, AFP levels in ICP groups were comparable with controls. However, both ß-hCG and E3 levels were higher in ICP group than controls, but the difference did not reach statistical significance. The elevated E3 and ß-hCG levels alone may not result in cholestasis. However, genetic sensitivity or environmental factors can lead ICP in patients with similar E3 and ß-hCG levels.

Although GGT was found to be high in patients with ICP in some previous studies [12, 38] GGT levels were normal in patients with ICP in many studies in agreement with our results [32]. In patients with ICP, the extent of ALT increase was higher than AST [38, 39].

In our study, it was also found that ALT increased more than AST. Since ALP is mainly released from placenta, the use in ICP diagnosis is limited.

Our study is one of the rare studies that evaluated first and second-trimester biochemical markers together in patients with ICP. In our study, the diagnosis and treatment were conducted by the same healthcare providers in a single-center. Major limitations are retrospective design and small sample size. In addition, as it is the case in the studies using biochemical parameters, the values obtained are dependent to study population, laboratory and analyzer used. Thus, it will be appropriate to take these factors into consideration.

CONCLUSION

In conclusion, it was found that low PAPP-A MoM value, one of the Down syndrome screening tests in first trimester, and elevated free ß-hCG were associated with ICP development. Although other biochemical parameters were found to be higher in ICP group, no relationship with ICP was found. The early recognition of ICP is important as perinatal complications are more common in patients with ICP. ICP development can be predicted by low PAPP-A and high free ß-hCG MoM values in the first trimester. If women are already being screened for Down syndrome using the first-trimester aneuploidy test markers, which is routinely performed, the same markers can be employed to screen for ICP, offering a simple and low-cost opportunity to identify groups at high risk of developing this disorder. There is a need for prospective studies with a larger sample size for introduction to routine practice.

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