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First trimester maternal serum PAPP-A levels and associated pregnancy complications in intrahepatic cholestasis of pregnancy

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Summary

Purpose: To investigate first trimester maternal serum pregnancy associated plasma protein A (PAPP-A) multiple of the median (MoM) in cases with intrahepatic cholestasis of pregnancy (ICP). Obstetric complications and relation with PAPP-A MoM were also evaluated. *Materials and Methods:* This was a retrospective case-control study. After exclusions, for each ICP case, two controls with uncomplicated singleton pregnancies were randomly selected. PAPP-A MoM of ICP cases with and without obstetric complications, and the control group were compared with each other. *Results:* Total incidence of ICP was 0.99 % (138/13988). The study included 113 singleton pregnant women. Rates of gestational diabetes mellitus (GDM), preeclampsia (PE), fetal growth restriction (FGR), preterm labor (PTL), and hypothyroidism in cases with ICP were 21.2%, 7.9%, 10.6%, 18.6%, and 5.3%, respectively. Median PAPP-A MoM were 0.93 in ICP group and 1.10 in control group ($p > 0.05$). PAPP-A MoM levels were not significantly different either between the ICP group with complicated pregnancies and the control group or between the ICP group without complicated pregnancies and the control group ($p > 0.05$). *Conclusion:* ICP incidence was similar to other European countries. Rates of obstetric complications expecially GDM were higher than expected in general pregnant population. ICP is not considered as pregnancy complications that have low PAPP-A MoM levels.

Key words: Intrahepatic cholestasis of pregnancy; PAPP-A; Adverse pregnancy outcome.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is primarily a liver disorder, which usually presents in the third trimester of pregnancy. It is characterized by maternal pruritus, elevated liver enzymes, and total bile acid (TBA) levels in the absence of any other skin or liver disease. The sign and symptoms completely resolve after delivery. Although environmental, genetic, and hormonal factors are believed to be responsible from this disorder and the etiology and pathogenesis are not exactly understood [1, 2]. ICP is associated with abnormal biliary transport across the canalicular membrane [3]. Several mutations have been identified in some of these patients, which might lead to dysfunction of bile salt transport proteins. Mutations in ABCB4 gene (ATP-binding casette B4) coding for the phospholipid transport protein MDR-3 (multidrug resistance protein 3) and ABCB11 gene (ATP-binding casette B11) coding for the bile acid transport protein bile salt export pump (BSEP) are the most well defined ones [4].

The ICP incidence in Europe ranges from 0.1% to 1.5% of pregnancies and wide geographical variations are observed throughout the world [5-7]. Maternal prognosis is usually good, but it is associated with some adverse obstetric outcomes, including preterm birth (PTB), meconium staining amniotic fluid, fetal distress, and even stillbirths. Although stillbirth rates were initially reported to be as high as 15%, recent evidence indicates that it is about 3.5% [8]. Antenatal care might be important in improving fetal outcomes, whereas some of the reports are not in accordance with this fact [9, 10]. For the treatment of ICP, ursodeoxycholic acid (UDCA) provides best response in relieving pruritus and reduction of maternal bile acid and liver enzyme levels, and additionally it may have a role in preventing perinatal complications [11, 12]. Induction of labor at 37 to 38 weeks of gestation is generally recommended [9].

Recent reports indicate an increased risk for gestational diabetes mellitus (GDM) as well as preeclampsia (PE) in pregnant women with ICP [12, 13]. Long term risks might include hepatobiliary diseases [14].

Maternal serum biochemical markers used in fetal aneuploidy screening such as pregnancy associated plasma protein A (PAPP-A) human chorionic gonadotropin (hCG), and alfa-fetoprotein (AFP) have been extensively investigated in obstetric risk assesments [15]. Low 11-14 week PAPP-A values are known to predict adverse perinatal complica-

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tions like PE, PTB, and small for gestational age infants, as well as GDM [16, 17]. PAPP-A levels were also investigated in early pregnancy complications such as hyperemesis gravidarum and threatened abortion [18, 19].

PAPP-A is a protease for insulin like growth factor binding protein-4 (IGFBP-4). As it increases the breakdown of IGFBP-4, free insulin like growth factor (IGF) concentration is increased [20]. IGF is important in regulating trophoblast invasion of the decudia. Thus, low PAPP-A levels are associated with decreased free IGF concentrations, leading to impaired trophoblastic invasion, poor placental perfusion, and pregnancy complications such as PE, fetal growth restriction (FGR), and preterm labor (PTL), as shown in many studies [21].

In a study performed by Muravska *et al.*, patients with ICP (n=15) had increased serum levels of PAPP-A compared to controls [22]. As far as the present authors know, there is no published data with a larger study population, thus the relation of PAPP-A and ICP is unclear. The present authors' aim to perform this study was to determine the incidence of ICP in their pregnant population, and to investigate the first trimester maternal serum PAPP-A levels in their ICP cases, dividing them further into two groups with and without any pregnancy complications.

Materials and Methods

This retrospective case-control study was conducted at Başkent University School of Medicine, Department of Obstetrics and Gynecology. The study group included patients with the diagnosis of ICP between the years 2007 and 2013 inclusive, in Ankara, Istanbul, and Adana hospitals. The following criteria were required for the diagnosis of ICP: pruritus and elevated fasting serum TBA (>ten μmol/L) with exclusion of any other liver or skin disease.

Antenatal records of the cases were evaluated and maternal age, gravidity, parity, clinical symptoms, liver enzymes (ALT, AST), and TBA levels, gestational age at diagnosis, gestational age at delivery, delivery route, birthweight, sex of the newborn, associated pregnancy complications such as GDM, PE, FGR, preterm premature rupture of the membranes (PPROM), and PTL as well as medical problems such as hypothyroidism, were noted. Normotative data for fetal growth based on birthweight was used for diagnosis of FGR with the cut-off of 10th percentile

Multiple pregnancies, pregnancies with fetal chromosomal or structural anomalies, and those whose deliveries did not take place in Başkent University Hospitals were excluded from the study. After exclusions, for each case with ICP, two maternal age, gravida and parity matched controls were randomly selected among singleton deliveries without any pregnancy complications.

PAPP-A values of the cases and the controls were obtained from routine first trimester aneuploidy screening tests performed in the present hospitals between 11-14 weeks of the pregnancy. An immunoanalyzer with PAPP-A kits was used to measure maternal serum levels of PAPP-A. Gestational age was determined according to the fetal crown-rump length (CRL) measured on the day of serum sampling. Maternal serum PAPP-A levels were adjusted for ethnicity and body mass index (BMI), and then expressed as multiples of the gestational age specific median (MoM), by using the prenatal screening program PRISCA 4.0.

Statistical analyses were performed using SPSS program. Parameters were compared between groups by using Mann Whitney U test. Chi-square test was used for the statistical analyses of the cross-tables. Results were accepted to be significant when p value was < 0.05.

Table 1. — *Maternal characteristics and laboratory findings in cases of ICP*.*

	Mean ± SD	Range
Gravida	1 ± 1.3	1-7
Parity	0 ± 0.86	0-6
Maternal age (years)	31.26 ± 5.44	20-53
Gestational age at diagnosis (weeks)	33 ± 4.57	13-40
AST (IU/L)	49.08 ± 68.37	10-576
ALT (IU/L)	82.36 ± 136.78	7-987
Bile acid (μmol/L)	35.06 ± 45.57	10.2-366
Gestational age at birth (weeks)**	37 ± 2.27	26-40

* Singleton pregnancies delivered at Başkent University Hospitals, n=113.
** Excluding one case who aborted at the 15th gestational week, n=112.

Results

There were 143 patients diagnosed with ICP during the years 2007 and 2013 inclusive in Başkent University Ankara, Istanbul, and Adana hospitals. Among those, 116 were singleton and 27 were multiple pregnancies. Deliveries of three singleton and two multiple pregnancies were not in Başkent University Hospitals. The incidence of ICP among the deliveries performed in the present hospitals during the years 2007 and 2013 inclusive was 0.99% (138/13988). This value was 0.86% for singletons (112/13186) and 3.12% for multiples (25/802). Out of 138 ICP cases whose deliveries took place in the present hospitals, 38 were assisted reproduction pregnancies including 21 multiple pregnancies.

Multiple pregnancies and those whose deliveries that did not take place in Başkent University Hospitals were excluded from the study group with ICP. There were no pregnancies with fetal chromosomal or structural anomalies. The control group of these 113 cases with ICP included 226 pregnancies randomly selected among singleton deliveries without any pregnancy complications.

The study included 64.6% cases that were nulliparous (73/113). The mean maternal age was 31.26 ± 5.44 years ranging between 20 and 53 years. The median gestational age at diagnosis of ICP was 33 weeks, ranging between 13 and 40 weeks. The mean serum TBA level at diagnosis was 35.06 ± 45.57 μmol/L; it ranged between 10.2 and 366 μmol/L. Excluding one case who aborted at the 15th gestational week, the median gestational age at birth was 37 weeks, ranging between 26 and 41 weeks. (Table 1).

The rates of associated obstetric complications and medical problems in cases with ICP were as follows: GDM – 24/113 (21.2%), PE – 9/113 (7.9%), FGR – 12/113 (10.6%),

Table 2. — *Comparison of PAPP-A MoM levels between ICP cases and controls.*

	Cases (n=53) mean±SD (median) (range)	Controls (n=106) mean±SD (median) (range)	*p**
PAPP-A (MoM)	1.04 ± 0.59 (0.93) (0.26 - 2.89 MoM)	1.11 ± 0.48 (1.10) (0.35 - 2.54 MoM)	0.182 (NS)

* Mann Whitney U Test. NS: nonsignificant.

Table 3. — *Comparison of PAPP-A MoM levels between ICP cases with and without any pregnancy complications.*

	Complication (+) (n=20) mean±SD (median) (range)	Complication (-) (n=33) mean±SD (median) (range)	*p**
PAPP-A (MoM)	0.99 ± 0.59 (0.83) (0.29 - 2.12 MoM)	1.06 ± 0.59 (0.99) (0.26 - 2.89 MoM)	0.620 (NS)

* Mann Whitney U Test. NS: non-significant.

PPROM – 2/113 (1.8%), PTL – 21/113 (18.6%), and hypothyroidism – 6/113 (5.3%). Meconium stained amniotic fluid was observed in four cases (4/113, 3.5%). Stillbirth was observed in two cases (2/113, 1.8%). Large for gestational age (LGA) newborn was observed in four cases (4/113, 3.5%). UDCA was used in 56/113 cases (49.6%).

Out of 113 study cases with ICP, 53 had first trimester PAPP-A MoM levels; therefore those levels were compared to the levels of 106 control women matched for those 53 cases. Median PAPP-A MoM levels were 0.93 and 1.10, respectively. There was no statistically significant difference between the two groups ($p > 0.05$) (Table 2).

Twenty out of 53 ICP cases whose first trimester PAPP-A MoM levels were available had at least one of the following pregnancy complications: GDM, PE, FGR, PPROM or PTL. Abortion or stillbirth was not observed among these 53 cases. The PAPP-A MoM levels of this group was compared to the group of ICP cases without any pregnancy complications. Median PAPP-A MoM levels were 0.83 and 0.99, respectively. However there was no statistically significant difference between the two groups ($p > 0.05$) (Table 3). PAPP-A MoM levels were not significantly different either between the ICP group with complicated pregnancies and the control group ($p = 0.198$) or between the ICP group without complicated pregnancies and the control group ($p = 0.393$).

Fifty-seven female and 56 male babies were present among the 113 ICP cases with singleton pregnancies whose deliveries took place in Başkent University Hospitals. When this group was compared to the group including 226 matched controls, sex of the babies did not show any significant difference ($p = 0{,}141$). Out of 113 women with ICP, 87 had delivered by cesarean section (C/S). As the rate of C/S on maternal request is high in Turkey, the ICP and control groups were not compared with respect to the delivery route.

Discussion

PAPP-A is expressed in placenta both in villous and extravillous cytotrophoblasts. Its levels increase gradually in maternal serum throughout the pregnancy. PAPP-A gene expression has been documented in a variety of other cell types such as human fibroblasts and human coronary artery smooth muscle cells after stimulation by proinflamatory cytokines like tumor necrosis factor-α (TNF-α) [20]. PAPP-A facilitates the breakdown of IGFB-4, resulting in release of IGF. IGF is a small peptide, similar to insulin and believed to play a key role in the regulation of trophoblast invasion of the decidua. Low PAPP-A levels may result in low free IGF levels and may yield to poor placental development and several pregnancy complications [20, 21]. In the Hanita *et al.* study, it was concluded that lower PAPP-A value in threatened abortion was associated with pregnancy failure [18]. Conversely hyperemesis gravidarum was associated with elevated PAPP-A levels in the Derbent *et al.* study [19].

There are several studies in the literature showing that when there is no fetal chromosomal abnormality, low PAPP-A levels observed in the first trimester, aneuploidy screening might predict a high risk for adverse pregnancy outcome including PE, FGR, PTB, and GDM [17, 23]. Muravska *et al.*, have investigated the relation of maternal serum PAPP-A levels with PE, threatening PTL, FGR, and ICP in a total of 165 women. Patients with ICP (n=15) had increased serum levels of PAPP-A compared to controls [22]. In the present cases with ICP (n=53), first trimester maternal serum PAPP-A levels were not significantly different from those of uncomplicated control pregnancies (n=106). Even in ICP cases with obstetric complications (n=20), either GDM, PE, FGR, PPROM or PTL, which are known to be associated with low first trimester PAPP-A levels, median PAPP-A level was 0.83 MoM, not significantly different from that of the ICP group without any obstetric complications (0.99 MoM) ($p > 0.05$) or than that of the control group (1.10 MoM) ($p > 0.05$). Therefore ICP is not considered as pregnancy complications which have low PAPP-A MoM levels. The results may be more accurate in larger study groups.

While the exact cause of ICP is not known, abnormal biliary transport across the canalicular membrane is thought to be the major defect in this process. High estrogen levels may play additional role and inhibit the sulfation and the transport of bile acids by exerting effects upon NTCP (Na-taurocholate co-transporting polypeptide) and BSEP [24]. As estrogen concentrations markedly increase with advancing pregnancy, the abnormality in the transport mechanism becomes more obvious and the clinical symptoms as well as the laboratory findings, that is, increased materal serum TBA, liver enzyme and even bilirubin levels, are observed. Although the underlying pathophysiologic mechanism is probably there initiating from the beginning of the

pregnancy, ICP as a disease, becomes apparent in late gestation.

Impaired bile acid metabolism and transport across the placenta leads to increased TBA accumulation in the fetal circulation similar to which occurs in the maternal circulation. Elevated maternal TBA levels affect placental hormone production, chorionic vessel constriction, placental transport, and increase myometrial sensitivity to oxytocin [25, 26]. The defect in the bile acid transport system most probably is present even in early pregnancy, either because of genetic mutations or environmental factors or hormonal effects especially those of increasing estrogens. Gradually accumulating bile acids in placental and fetal compartment might have an adverse effect on the trophoblastic invasion process leading to poor placental development. Indeed, pathological examinations of the placental tissue in ICP have revealed increased terminal villous surface area and capillary vessels together with increased syncytial knots indicating chronic hypoxia [27]. This may be the most logical explanation for the increased risks of obstetric complications observed in women with ICP, such as PE, FGR, and PTB. Although it is expected that these complicated pregnancies should have significantly lower first trimester PAPP-A MoM levels, the present study results did not indicate to be so. The reason may be an increased inflammatory reaction in the placental compartment due to gradually accumulating toxic bile acids. Increased proinflamatory cytokines like TNF-α and interleukin-1β (IL-1β), in turn, might stimulate PAPP-A gene expression within the trophoblasts. Thus first trimester PAPP-A MoM levels in ICP may not be as low as expected even in those cases with adverse obstetric outcomes. This fact may also explain the increased PAPP-A levels in ICP observed in the study of Muravska *et al.* [22]. Of course further investigations are necessary to see whether toxic bile acids begin to accumulate in the placental compartment in the first trimester or not in ICP cases.

Recent studies on ICP indicate an increased risk for GDM and PE in these patients [12,13]. In one report evaluating the ICP cases, the incidences of GDM and PE were found to be 7.2% and 5.1%, respectively [12]. The study of Shemer *et al.*, including 5,477 cases within a 12-year period revealed that the incidence of GDM was 1.3% in ICP compared to 0.4% in the control group. These values were 1.1% and 0.6% for PE. Both differences were statistically significant [6]. In the present study GDM was present in 21.2% of 113 ICP cases. PE was present in 7.9% of the cases. The incidence of GDM observed in ICP patients in the present study was quite high compared to the 3-5% rate expected in general pregnant population [12, 13]. The incidence of PE was in accordance with the expected rate, that is 2-8% [12, 13]. Additionally, in the present study group composed of 113 ICP cases, the rates of FGR (10.6%), PTL (18.6%), and stillbirth (1.8%) were higher than the expected rates of 3-7%, up to 12% and 0.5-0.6% [28, 29] respectively. Previous reports have also revealed increased risk for FGR, PTL, and birth as well as stillbirth in ICP [6, 7, 12]. On the other hand, in the present ICP cases, the rates of PPROM (1.8%) and hypothyroidism (5.3%) were similar to those observed in general pregnant population. LGA, in a recent study, was observed in 4.48% of the pregnancies [30]. LGA rate we have observed in ICP (3.5%) may be considered to be similar to that rate.

ICP incidence varies significantly with geographic and ethnic variations. It is reported to be as high as 15% in Chile and Bolivia and less than 1% in Europe [7,8]. Advanced maternal age, a history of ICP or hepatobiliary disease and multiple pregnancies increase the risk for ICP [8]. To the present authors' knowledge, this study is the first to report the incidence of ICP among 13,988 deliveries from three regions of Turkey: Ankara- Central Anatolia Region, Istanbul- Marmara Region, and Adana- Mediterranean Region. The incidence of ICP in the present study, that is 0.99%, is in accordance with the figures observed in European countries [7].

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