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Fetuin B may be a potential marker for predicting maternal and neonatal outcomes in intrahepatic cholestasis: Prospective case-control study

Fetuin B, intrahepatik kolestazda maternal ve neonatal sonlanımı öngörmede potansiyel bir belirteç olabilir: Prospektif olgu kontrol çalışması

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Abstract

Objective: To investigate the levels of serum fetuin B in healthy pregnant women and women with intrahepatic cholestasis of pregnancy (IHCP) and their association with pregnancy outcomes.

Materials and Methods: This was a prospective case-control study, we included sixty singleton pregnant women with IHCP and sixty healthy-matched pregnant women in their third trimester. The serum fetuin B levels of these patients were analyzed. All the patients were followed up prospectively until delivery and data related to maternal, perinatal, and neonatal outcomes were obtained.

Results: Total bile acid levels and liver function tests were significantly higher in the IHCP group than in the control group ($p<0.0001$ and <0.0001, respectively). The serum fetuin B concentrations were higher in the IHCP group than in the control group, without any significant group difference ($p=0.105$). Preterm delivery, iatrogenic preterm delivery, and birth weight ≤2.500 gm are only significantly associated with serum fetuin B levels respectively ($p<0.05$). The diagnostic performance of serum bile acids [area under the curve (AUC)=0.998] was significantly better than that of fetuin B (AUC=0.586) (DeLong's test $p\leq0.001$).

Conclusion: We neither noted a significant difference between the IHCP and control groups concerning the serum fetuin B levels nor could we correlate its levels with adverse maternal and perinatal outcomes except with birth weight, thereby serum fetuin B was not an effective marker for use in shedding light on the pathophysiology of IHCP.

Keywords: Total bile acids, intrahepatic cholestasis of pregnancy, farnesoid X receptor ursodeoxycholic acid, maternal-fetal outcomes

Öz

Amaç: Bu çalışmanın amacı sağlıklı gebelerde ve intrahepatik gebelik kolestazı (IHCP) olan kadınlarda serum fetuin B düzeylerini ve bunların gebelik sonuçları ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: Bu prospektif olgu kontrol çalışmasına, IHCP'li 60 tekil gebeliği olan kadını ve 60 sağlıklı eşleştirilmiş gebeyi üçüncü trimesterde dahil ettik. Bu hastaların serum fetuin B seviyeleri analiz edildi. Tüm hastalar prospektif olarak doğuma kadar takip edildi ve maternal, perinatal ve neonatal sonlanımlara ilişkin veriler elde edildi.

Bulgular: Toplam safra asidi seviyeleri ve karaciğer enzim seviyeleri, IHCP grubunda kontrol grubuna göre anlamlı derecede yüksekti (sırasıyla $p<0,0001$ ve $p<0,0001$). Serum fetuin B konsantrasyonları istatistiksel olarak anlamlı olmasa da IHCP grubunda kontrol grubuna göre daha yüksekti ($p=0,105$). Preterm

PRECIS: We investigated the levels of serum fetuin B in healthy pregnant women and pregnant women with IHCP and its association with pregnancy outcomes in IHCP.

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doğum, iyatrojenik preterm doğum ve ≤2,500 g doğum ağırlığı serum fetuin B düzeyleri ile anlamlı şekilde ilişkili bulunmuştur (p<0,05). Serum safra asitlerinin tanısal performansı [eğrinin altındaki alan (AUC)=0,998], fetuin-B'ninkinden (AUC=0,586) anlamlı olarak daha iyiydi (DeLong testi p≤0,001).

Sonuç: Serum fetuin B düzeyleri açısından IHCP ve kontrol grupları arasında anlamlı bir fark gözlemlemedik ve serum fetuin B düzeylerini doğum ağırlığı dışında olumsuz maternal ve perinatal sonlanımlarla ilişkilendiremedik, bu nedenle serum fetuin B, IHCP'nin patofizyolojisine ışık tutmak için etkili bir belirteç değildir.

Anahtar Kelimeler: Toplam safra asitleri, intrahepatik gebelik kolestazı, farnesoid X reseptörü, ursodeoksikolik asit, maternal-fetal sonlanımlar

Introduction

One of the most prevalent pregnancy-induced liver diseases is intrahepatic cholestasis of pregnancy (IHCP), the incidence of this disorder ranges between 1.5% to 4%[1]. The typical features of this disease are pruritus localized to the palms and soles mostly in the second half of the gestation, with raised bile acid levels (≥10 mmol/L), and/or abnormal transaminase levels. IHCP has a relatively nonthreatening course in women with symptoms, usually, liver enzymes and serum bile acids decrease within a few weeks after birth but it is associated with serious fetal complications[2]. IHCP has a varied impact on both mother and fetus. This can manifest upon the fetus leading to preterm birth, meconium-stained amniotic fluid (MSAF), asphyxia or respiratory distress syndrome (RDS), neonatal intensive care unit (NICU) admission, and in extreme cases non-reassuring fetal status and fetal death[2-6]. It appears to have a genetic linkage where in susceptible women develop altered biliary transport and excretion of bile acids due to elevated levels of reproductive hormones[7].

The metabolism of cholesterol in the liver leads to the production of bile acids. The various actions in the gastrointestinal tract like the metabolism of cholesterol, absorption of fat and fat-soluble vitamins, and regulation of the gut microbiome, are regulated by bile acids. Increased serum bile acid in IHCP leads to abnormal metabolic processes. Various pregnancy-related morbidities like preeclampsia, gestational diabetes mellitus, dyslipidemia, and impaired glucose tolerance are quite prevalent among women with IHCP due to a common pathophysiology[8,9]. The proper regulation of bile acid synthesis plays an enormous role in the prevention of the danger effects of bile acids. Hepatocytes and enterocytes express the Farnesoid X receptor (FXR), a member of the nuclear receptor superfamily this is central to the control of multiple metabolic pathways. FXR on its activation by bile acids regulates the synthesis, conjugation, and transport of bile acids and coordinates various aspects of lipid and glucose metabolism[10]. Fetuin B expression is enhanced by the treatment with an FXR agonist. This expression of fetuin B occurs in human primary hepatocytes and in the human hepatoma HepG2 cell line[11]. Fetuin B belongs to the cystatin superfamily of cysteine protease inhibitors documented to have diverse functions. Women with IHCP express higher levels of fetuin B vis-a-vis healthy pregnant women[12]. Thereby we can infer that FXR agonists in human hepatocytes increase fetuin B gene expression[12,13].

Considering the critically important role of FXR, a regulator of the fetuin B gene expression will take part in the regulation of bile acid homeostasis. The concept of serum fetuin B and its use in IHCP is a very recent idea. Hardly any studies are available in the current literature. Based on this information, we set our primary objective to investigate the serum levels of fetuin B in pregnant women who are in good health and pregnant women diagnosed with IHCP. We kept our secondary objectives to determine the association between serum fetuin B, total bile acids (TBAs), and damaging pregnancy outcomes in IHCP and to compare the diagnostic performance of serum fetuin B with serum TBAs in IHCP cases. Based on our data, we speculate if a relationship between serum fetuin B, serum TBAs, and adverse pregnancy outcomes could be established it would prevent many unfavorable maternal and perinatal outcomes by using serum fetuin B as a predictive marker for IHCP similar to serum TBAs.

Materials and Methods

This was a prospective comparative study, conducted in the Department of Obstetrics and Gynecology of a tertiary care hospital and medical college from January 2019 to December 2021. This study was conducted after the approval of the Institutional Ethics Board and after obtaining informed written consent from the participants. We carried out our research according to the Helsinki Declaration 1975. For this study, we included singleton pregnant patients between ≥28 weeks and 40 weeks of pregnancy presented with serious unexplained persistent pruritus in the absence of any primary skin lesions and/or abnormal liver function test (LFT), alanine aminotransferase (ALT >40 IU/L)/aspartate aminotransaminase (AST >40 IU/L) with serum TBA levels ≥10 micromol/liter, who could fit into the category of obstetric cholestasis as a case group (IHCP) and matched pregnant women in good health were recruited as the control group (non-IHCP).

We excluded pregnant women with any alternative causes like acute fatty liver of pregnancy, chronic liver diseases (symptomatic cholelithiasis, cholecystitis, primary biliary cirrhosis, primary sclerosing cholangitis, hepatitis B, hepatitis C), human immunodeficiency virus infection, skin diseases with itching and rashes, addiction to smoking or alcohol, gestational hypertension or preeclampsia or HELLP syndrome, diabetes mellitus, thyroid disease and women who were in labor during recruitment. These exclusions were based on a detailed history, and clinical examination followed by hepatobiliary ultrasound.

Sample Size

Based on a previous study,[14] the un-adjusted odds ratio for the 3 adverse outcomes that is preterm delivery, stillbirth,

and neonatal intensive care admission, lies between 1.81-4.6, by taking an average odds ratio of 3, since we have no data, assuming exposed control by chance of 50%, the power of the study to a minimum of 80%, alpha error of 5%, we got a total sample size of 120, which was equally divided as 60 each in two groups. In one group 60 consecutive pregnant women diagnosed with IHCP were taken as cases and similarly, age and gestational age-matched 60 pregnant women in good health were included as controls.

From the case controls, demographic characteristics, obstetrics details, gestational age at the time of diagnosis of IHCP, and gestational age at biochemical testing were recorded. At 11-13 weeks, fetal crown-rump length was measured for estimation of the gestation age. Before any start of treatment, blood samples were drawn upon admission to the hospital from women with IHCP. Blood samples were obtained from the age and gestational age-matched control group and were tested for LFT, serum bile acids, and serum fetuin B. All the patients in the IHCP group were given symptomatic treatment along with a tablet of ursodeoxycholic acid (15 mg/kg body weight). Till delivery fetal surveillance was performed on a weekly basis with non-stress tests and fetal Doppler. At 37 weeks of gestation, an elective termination was planned for the patients in the IHCP group and the relevant data were collected. Whereas the patients of the control group were followed up prospectively till delivery and data related to maternal, perinatal, and neonatal outcomes were obtained. To determine the incidence of the disease, the total number of deliveries was also noted during the study period.

The serum biochemical test included were LFTs, total serum bile acids, and serum fetuin B tests, which were determined using routine laboratory methods. A commercial enzyme-based colorimetric assay was used for measuring the serum bile acid levels and a standard manufacturer-recommended protocol was followed. Serum preparation for Fetuin B measurement was done by centrifugation of the samples at 1000 g for 20 min followed by freezing at -80 °C for subsequent analysis. The levels of serum Fetuin B were assessed using a Human FETUB (Fetuin B) sandwich ELISA kit, Wuhan Fine Biotech Co. Ltd (catalogue No. EH3055, Detection range: 93.75-6.000 pg/mL, sensitivity: <56.25 pg/mL). However, the test samples were run in 1:50 dilution and the results were obtained by multiplying with 50 (dilution factor).

The parameters studied were the history of IHCP in a previous pregnancy, gestational age at sampling, serum biochemical test (LFT), TBAs and fetuin B level at diagnosis, gestational age at delivery, mode of termination of pregnancy, spontaneous preterm delivery/iatrogenic preterm delivery, birth weight, pathological cardiotocograph (CTG) immediately before delivery, MSAF at the time of delivery, NICU admission, stillbirth, a composite adverse maternal outcome, and neonatal outcome. The definition of composite adverse maternal outcome is the presence of either of the following events like postpartum hemorrhage, prolonged hospital stay, transfusion of blood and blood product, and maternal death. The definition of composite adverse neonatal outcome is the presence of either of the following events like NICU admission, Apgar 1 and 5 min ≤7, meconium aspiration, hypoglycemia, hyperbilirubinemia, RDS, transient tachypnea of the newborn, use of mechanical ventilation, use of oxygen by nasal cannula, newborn pneumonia, and stillbirth.

Statistical Analysis

Excel spreadsheet Program was used for data recording and coding. The data analysis was performed using the statistical package of social sciences (SPSS) version 23 (IBM Corp.). Descriptive statistics were calculated in the forms of mean ± standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables. A graphical representation of the data was chosen for better visualization. Normally distributed variables were compared between groups. The analysis was done using an independent samples t-test and was expressed as mean ± SD. Non-normally distributed data were analyzed with the Wilcoxon test and the chi-square test for categorical data. Fisher's exact test was used if the expected frequency in the contingency tables was found to be <5 for >25% of the cells. All the normally distributed data were analyzed with Pearson's correlation and non-normally distributed data were analyzed with Spearman's correlation. The correlation between continuous and categorical variables was assessed using Point-Biserial Correlation. To determine the predictive cut-off values of TBAs levels and fetuin B levels, receiver operating characteristic (ROC) analysis was used. P values of less than 0.05 were considered statistically significant.

Results

During the study period, the total number of deliveries conducted was 2501, out of which 60 cases of IHCP were diagnosed in women with singleton pregnancies, which fits into the definition of IHCP and as per the inclusion and exclusion criteria. So, the incidence was found to be 2.3%. The baseline parameters and the laboratory values of the study groups are mentioned (Table 1). The demographic parameters were similar however, the gestational week at the time of delivery, history of IHCP, LFT, and the TBAs were significant between the two groups ($p<0.05$). The mean serum fetuin B level was higher in the case group than in the control group, without any significant difference between them ($p=0.105$).

A comparison of obstetric, perinatal, and neonatal outcomes between the two groups is shown in (Table 2). In this study, we found a significant difference in the labor parameters. Among the perinatal outcomes, MSAF, pathological radiography, and admission to the NICU were found to be significant between the two groups.

We also studied the association between the maternal serum bile acids and fetuin B levels with obstetric, perinatal, and neonatal outcomes in the case group. In this study, iatrogenic

Table 1. The demographic and laboratory parameters of the case and control groups

Parameters	Group		p-value
	IHCP Cases (n=60)	Non IHCP Controls (n=60)	
Age (years)	27.63±4.37	26.73±4.82	0.164[1]
BMI (kg/m^2)	24.30±4.13	24.71±3.88	0.385[1]
Gravida			0.577[3]
Primigravida	34 (56.7%)	37 (61.7%)	
Multigravida	26 (43.3%)	23 (38.3%)	
POG at blood sampling (weeks)	35.42±2.35	35.08±2.37	0.510[1]
Past H/O IHCP (yes)***	8 (13.3%)	1 (1.7%)	0.032[4]
POG at delivery (weeks)***	37.18±1.52	38.07±1.51	0.001[1]
Total bile acids (µmol/L)***	26.58±17.54	4.13±2.79	<0.001[1]
ALT (U/L)***	139.52±85.10	26.18±23.32	<0.001[1]
AST (U/L)***	113.88±75.66	22.92±13.86	<0.001[1]
Total bilirubin (mg/dL)***	0.72±0.38	0.48±0.19	<0.001[1]
GGT (U/L)***	32.23±18.37	12.38±16.20	<0.001[1]
S. fetuin B (pg/mL)	77893.85±74855.74	55608.84±38492.48	0.105[1]

***Significant at p<0.05, [1]: Wilcoxon-Mann-Whitney U test, [2]: t-test, [3]: chi-squared test, [4]: Fisher's exact test, BMI: Body mass index, POG: Period of gestation, IHCP: Intrahepatic cholestasis of pregnancy, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase

preterm delivery, NICU admission, MSAF, pathological CTG, and adverse composite neonatal outcomes were significantly associated with serum TBAs levels respectively (p<0.05). Whereas, preterm delivery, iatrogenic preterm delivery, and birth weight ≤2.500 gm are significantly associated with serum fetuin B levels respectively (p<0.05).

A positive Spearman's correlation was detected between TBA levels and gestational age at blood sampling, AST, ALT, and serum total bilirubin levels, respectively. The correlation analysis could not find a correlation between serum fetuin B and gestational age at sampling, serum AST level, ALT level, total bilirubin level, and TBAs levels (Table 3).

On analyzing the Point-Biserial Correlation between serum TBAs, serum fetuin B level with perinatal outcomes, a positive correlation was obtained between serum TBAs and NICU admission, MSAF, pathological CTG, adverse composite maternal outcomes, and adverse composite fetal outcomes with a medium effect size. We did not find any correlation between Serum fetuin B and NICU admission, meconium-stained liquor, pathological CTG, adverse composite maternal outcomes, and adverse composite neonatal outcomes in the case group. Whereas, by Spearman's correlation analysis, we could note a negative correlation of medium effect size between serum TBAs and birth weight and between serum fetuin B levels and birth weight.

The ROC analysis was performed to compare the diagnostic performance of serum fetuin B with serum TBAs in predicting case (IHCP) versus control (Table 4). At a cut-off of serum bile acids (µmol/L) ≥10 microliters predicted a case group with a sensitivity of 100.0%, a specificity of 95%, a positive predictive value (PPV) of 95.2%, and a negative predictive value (NPV) of 100.0%. The area under the ROC curve (AUROC) for serum bile acids (µmol/L) predicting case versus control was 0.998 [95% confidence interval (CI): 0.996-1], thus demonstrating excellent diagnostic performance. This value was statistically significant (p≤0.001).

At a cut-off of serum fetuin B, (pg/mL) ≥45376.8 predicted the case with a sensitivity of 68%, a specificity of 53% a PPV of 59.4%, and a NPV of 62.7%. The AUROC for serum fetuin-B (pg/mL) predicting case versus control was 0.586 (95% CI: 0.483-0.689), thus demonstrating poor diagnostic performance. This was not statistically significant (p=0.105). Therefore, the diagnostic performance of serum bile acids (µmol/L) (AUC=0.998) was significantly better than that of serum fetuin-B (pg/mL) (AUC=0.586) (DeLong's test p≤0.001) (Figure 1).

Discussion

In this study, the incidence of IHCP was 2.3%, which is similar to the incidence mentioned in a few research articles[1,15]. Serum TBAs, serum fetuin B, serum bilirubin, and liver enzymes were raised in the IHCP group when compared with pregnant women with good health. This has also seen in other studies[12,16-18]. We could found a good interrelation between serum TBAs and gestational age at sampling, serum bilirubin, liver enzymes, birthweight of baby, perinatal outcomes like NICU admission,

Table 2. Comparisons of obstetric, perinatal and neonatal outcomes outcome between both the groups

Parameters	Group		p-value
	Case (n=60)	Control (n=60)	
Induced labour (yes)***	37 (61.7%)	25 (41.0%)	**0.023**[3]
Spontaneous labour (yes)***	22 (36.7%)	9 (15.0%)	**0.007**[3]
LSCS (yes)***	53 (88.3%)	42 (70.0%)	**0.013**[3]
VD (yes)***	7 (11.7%)	18 (30.0%)	**0.013**[3]
Elective CS (yes)	18 (30.0%)	21 (35.0%)	0.699[3]
Emergency CS (yes)***	35 (58.3%)	21 (35.0%)	**0.010**[3]
Preterm delivery (yes)	13 (21.7%)	6 (10.0%)	0.080[3]
Iatrogenic preterm delivery (yes)***	10 (16.7%)	3 (5.0%)	**0.040**[3]
Spontaneous preterm delivery (yes)	3 (5.0%)	3 (5.0%)	1.000[4]
Term delivery (yes)	47 (78.3%)	54 (90.0%)	0.080[3]
Birth weight (g)	2735.32±593.93	2846.73±494.20	0.266[2]
Birth weight ≤2500 g (yes)	19 (31.7%)	18 (30.0%)	0.843[3]
Birth weight ≤10th percentile (yes)	18 (30.0%)	21 (35.0%)	0.559[3]
NICU admission (yes)***	13 (21.6%)	3 (5.0%)	**0.007**[3]
MSL (yes)***	23 (38.3%)	13 (21.7%)	**0.048**[3]
Stillbirth (yes)	0 (0.0%)	0 (0.0%)	1.000[3]
Pathological CTG (yes)***	25 (41.7%)	7 (11.7%)	**<0.001**[3]
Composite adverse maternal outcome (yes)	8 (13.3%)	6 (10.0%)	0.570[3]
Composite adverse neonatal outcome (yes)	14 (23.3%)	6 (10.0%)	0.051[3]

***Significant at p<0.05, [1]: Wilcoxon-Mann-Whitney U test, [2]: t-test, [3]: chi-squared test, [4]: Fisher's exact test, LSCS: Lower segment Caesarean section, VD: Vaginal delivery, CS: Caesarean section, PTD: Preterm delivery, NICU: Neonatal intensive care unit, MSL: Meconium-stained liquor, CTG: Cardiotocograph

Table 3. Correlation analysis of TBAs and Serum Fetuin B in IHCP/Case group (n=60)

	Age (years)	BMI (kg/m^2)	GA at sampling (weeks)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	TBAs (µmol/L)	Sr. total bilirubin (mg/dL)
IHCP group (n=60)								
S. TBAs (µmol/L) r	0.08	-0.25	0.51	0.56	0.58	0.077	_	0.51
p-value	0.05	0.54	0.00003[a]	<0.00001[a]	<0.00001[a]	0.595	-	<0.00003[a]
IHCP group (n=60)								
S.Fetuin B (pg/mL), r	0.039	-0.022	0.131	0.037	0.164	-0.082	0.135	0.039
p-value	0.797	0.867	0.153	0.773	0.210	0.503	0.305	0.767

[a]: At the 0.05 level (2-tailed) a significant correlation was established and p values in bold refer to a statistically significant result, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, TBAs: Total bile acids, GA: Gestational age, IHCP: Intrahepatic cholestasis of pregnancy

Table 4. Comparison of the diagnostic performance of serum fetuin B and serum total bile acids in predicting group: case vs control (120)

Predictor	AUROC	95% CI	P	Sn	Sp	PPV	NPV	DA
S. fetuin-B (pg/mL)	0.586	0.483-0.689	0.105	68%	53%	59%	63%	61%
S. bile acids (µmol/L)	0.998	0.996-1	<0.001	100%	95%	95%	100%	98%

AUROC: Area under ROC curve, CI: Confidence interval, P: P-value, Sn: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, DA: Diagnostic accuracy

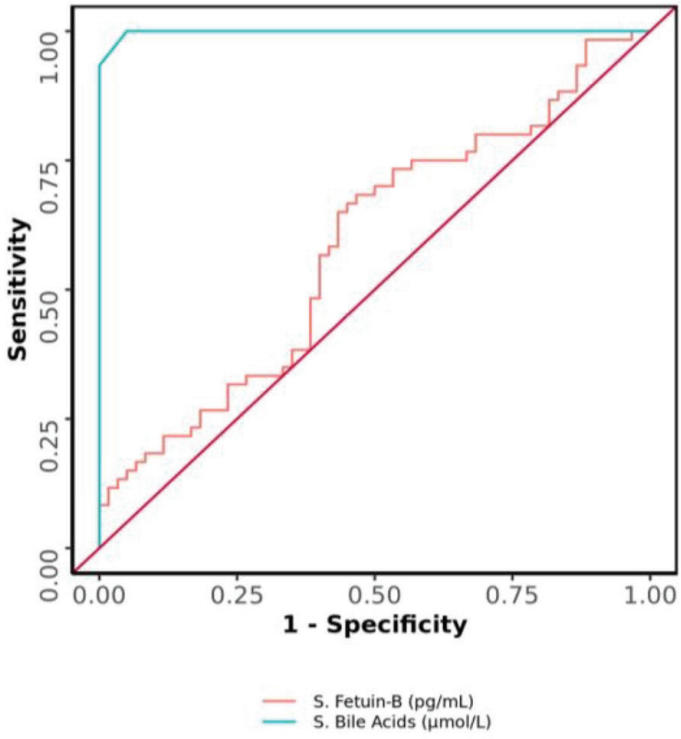

Figure 1. ROC curve showing comparison of diagnostic performance of serum fetuin B and serum bile acids (µmol/L) in predicting cases verses controls (n=120)

ROC: Receiver operating characteristic

MSAF, pathological CTG, composite adverse maternal outcomes, and composite adverse neonatal outcomes. Whereas, we could not establish any interrelation between serum fetuin B and any similar factor except with the birthweight of the baby. In this study, the diagnostic performance of serum bile acids was significantly better than that of serum fetuin B.

Although IHCP is relatively mild for the mother, the threat of having fetal complications is very high in pregnancies affected by IHCP. Pregnancy outcomes observed in this study were, women with IHCP were seen to have a high probability for the induction of labor, iatrogenic preterm delivery, increased cesarean delivery rate, and increased emergency cesarean delivery rate, but no stillbirth. The incidence of stillbirth occurs if pregnancy is continued beyond 37 weeks of gestation and with serum bile acid levels ≥100 µmol/L[19,20]. The absence of stillbirth in our study could be due to the reason that most of our patients had late preterm deliveries with serum TBAs not reaching very severe levels and the deliveries were conducted at a tertiary center. For preventing stillbirth in patients with IHCP interventions like treatment with ursodeoxycholic acid, frequent fetal monitoring, and early deliveries at 37 weeks of gestation were conducted. This could have resulted in observations like higher rates of iatrogenic preterm labor, induction of labor, and emergency cesarean section for pathological CTG. Increased spontaneous labor in women with IHCP may be explained by an increased effect of bile acids on uterine contractility through activation of the oxytocin receptor pathway, as suggested by a study conducted on rodents[21,22]. This study depicts an association between IHCP and MSAF, admission to the NICU, and an enhanced rate of pathological CTG. MSAF may be elucidated by an outcome of enhanced colonic peristalsis owing to higher maternal bile acids[4]. Several earlier studies have concluded that MSAF was observed in severe IHCP cases and this finding is consistent with our study[12-23]. Bile acids exert a vasoconstrictive effect on the placental chorionic veins, which can explain the pathological CTG and intrauterine meconium passage[4,24]. Another explanation though unproven for pathological CTG sudden intrauterine fetal death, and arrhythmia, could be the harmful effect of high bile acid levels on cardiocytes[24-26]. There was no antepartum hemorrhage, postpartum hemorrhage, or blood and blood product transfusion in this study.

When comparing IHCP with the control group, this study concludes that the IHCP group recorded composite adverse neonatal outcomes, which were 23.3% in the IHCP group versus 10.0% in the control, without any significant difference. These results may be due to a higher rate of admission to NICU, an earlier birth rate, and an increased rate of RDS in neonates in the cholestasis group. A comparable outcome was seen in a case-control study that showed a high rate of RDS in newborns delivered by IHCP women at two and half times higher (28.6%

vs. 14%) regardless of bile acid levels. The increased neonatal morbidity among newborns delivered by IHCP has been explained by many hypotheses, one probability was, bile acids have inhibitory surfactant activity even authors speculate on bile acid pneumonia due to its direct effect on neonatal lungs[2,27].

In this study, it was found that the mean serum bile acid levels between women IHCP compared to pregnant women in good health were significant. Subsequently, we noticed that the ideal cut-off for serum TBAs levels to differentiate between pregnant women with IHCP from the other group was ≥10 µmol/L.

A triple-arm Indian study conducted in women with IHCP, healthy pregnant women, and non-pregnant women vividly showed raised serum bile acid levels in women with IHCP only. In the same study, taking optimal cut-off levels of serum bile acid to 8.6 µmol/L for differentiating women with IHCP from the other two groups showed a sensitivity of 87.6% and specificity of 93.3%. Upon increasing the cut-off levels of serum bile acid to 10 µmol/L changed the sensitivity and specificity were 83.8% and 95.5% respectively. The authors inferred that the bile acid levels with a cut-off of 8.6 µmol/L or 10.0 µmol/L are very effective in the diagnosis of IHCP in the Indian scenario[28].

Whereas, we couldn't find a significant difference in mean serum fetuin B (pg/mL) between Indian women with IHCP and pregnant women in good health. Further, we identified that the optimal cut-off for serum fetuin B levels to recognize women with IHCP of pregnant women from the pregnant women in good health was ≥45376.8 pg/mL, with a sensitivity of 68.0% and specificity of 53.0%. The area under the ROC analysis (AUROC) for S. fetuin B (pg/mL) predicting IHCP cases was 0.586 (95% CI: 0.483-0.689), thus eliciting a dismal diagnostic performance and it was not statistically significant (p=0.105).

The study conducted by Koroglu et al.,[12] showed dissimilar observations. In their study, the serum fetuin B levels in the IHCP group were higher (p<0001). The area under the ROC for serum fetuin B for the diagnosis of IHCP was 0.758 (95% CI: 0.649-0.847). The ideal cut-off for fetuin B serum concentration was 5540.2 pg/mL and serum values greater than this level had 80% sensitivity and 65% specificity for the diagnosis of IHCP, which was different from our study[12]. Lately, it has been reported that serum fetuin B is increased in patients with coronary heart disease, non-alcoholic fatty liver, obesity, diabetes, and metabolic syndrome[8-10,29]. Compared to the study conducted by Koroglu et al.,[12] we have found a higher cut-off level of serum fetuin B for diagnosing cases of IHCP, this may be due to the higher prevalence of coronary heart disease, metabolic syndrome, and diabetes in Asian population. Another possible cause may be the method of measurement used. Serum fetuin B was measured using a Human FETUB (fetuin B) sandwich ELISA kit similar to the study conducted by Koroglu et al.[12] In our study, the test samples were run in 1:50 dilution so the results were obtained by multiplying with 50 (dilution factor) that could be another cause for higher levels of serum fetuin B.

In this study, there was no correlation noted between serum fetuin B and gestational age at sampling, fetal complications, adverse composite maternal outcomes, adverse neonatal outcomes, and biochemical parameters of the LFT. These results match, to some extent, with the study by Koroglu et al.[12] This study is the first that has elucidated the relationship between fetuin B and adverse perinatal outcomes to date.

We could find a medium to strong correlation between serum TBAs and gestational age at sampling, birth weight of the baby, fetal complications, adverse composite maternal outcomes, adverse neonatal outcomes, and biochemical parameters of LFTs including serum fetuin B. This is quite similar to the study by Glantz et al.[23] There was a proportionate increase in fetal complications by one percent to two percent for every mmol/L rise in serum TBA levels. The authors also stated that the risk of MSAF was seen with serum TBA levels of 20 mmol/L and above, whereas serum bile acid levels beyond 40 mmol/L increased the risk of preterm delivery, asphyxia events, and green staining of the placenta and membranes[23].

Inflammation has also been implicated in the pathogenesis of IHCP, and this leads to the production of fetuin B from hepatocytes. It has been substantiated that increased levels were observed in IHCP in the prevailing study, however, it was not statistically significant. Increased fetuin B levels were also related to a disruption in the metabolism of glucose and lipid. The specific pathophysiological mechanism and functions of serum fetuin B remain unclear and further studies need to be conducted to validate such findings.

Study Limitations

This study has some limitations. The sample size is too small and as this is a single-center study, the results can have less generalizability. We also could not examine the serum TBAs and serum fetuin B levels at the different time periods during pregnancy because of cost and irregularities in antenatal check-ups by study participants. The strength of our study is that it is a prospective comparative . The criteria used for diagnosing IHCP were objective, we have used parameters like elevated serum bile acids, and serum transaminase levels combined pruritus during pregnancy. We excluded women tested for other causes of potential liver disease from the case group and we included a control group. We suggest further research with a robust sample size that can determine the definite pathophysiologic method of increased serum fetuin B levels in IHCP and its correlation with adverse maternal and perinatal outcomes.

Conclusion

Our study re-established the increased risks and poor perinatal outcomes among pregnant women with IHCP vis-a-vis pregnant women in good health. Maternal serum TBA levels are related to adverse perinatal outcomes in IHCP. The measurement of serum TBA levels with a cut-off of 10.0 µmol/L is very effective in the diagnosis of IHCP in this population. We did not detect any significant difference between the IHCP group and pregnant

women in the good health group in relation to the serum fetuin B levels. Similarly, we could not correlate its levels with adverse maternal and perinatal outcomes except with the birth weight of the baby. Thereby serum fetuin B is not an effective marker for establishing the pathophysiology of IHCP. The measurement of bile acids for diagnosis and clinical management of this condition remains a hallmark and there should be wider availability of laboratory facilities for measuring it.

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Ethics

Ethics Committee Approval: This study was approved by the All India Institute of Medical Sciences Bhubaneswar (approval number: T/IM-F/18-19/36, date: 16.01.2019).
Informed Consent: All patients who participated in the study signed a consent form.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: J.B., Design: J.B., S.S., M.K.P., Data Collection or Processing: J.B., G.K.S., Analysis or Interpretation: J.B., G.K.S., Literature Search: J.B., S.S., Writing: J.B., G.K.S.
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