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Evaluation of predictive value of biochemical markers for adverse obstetrics outcomes in pregnancies complicated by cholestasis

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ABSTRACT

Objectives: Intrahepatic cholestasis of pregnancy (ICP) is significantly more often associated with an abnormal perinatal outcome compared to a group of healthy pregnant women.
The aim of the study was to analyse the correlation between the adverse perinatal outcome and the biochemical parameters in pregnancy complicated by cholestasis, and to assess their predictive value for neonatal complications.

Material and methods: Eighty-six patients with ICP were divided into 3 groups according to their fasting serum bile acid level [group I n = 60, 10–39.90 µmol/L; group II n = 20, 40–99.90 µmol /L; group III n = 6, TBA (total bile acids) ≥ 100.00 µmol/L]. Linear regression models were created to determine the relation of serum TBA, ALT, and AST concentration with total adverse perinatal outcome, defined as an occurrence of at least one perinatal outcome: stillbirth, preterm birth, spontaneous and iatrogenic preterm birth, presence of meconium in amniotic fluid, Apgar score (< 7 in 5th min), pH from umbilical artery (< 7.1), necessity for NICU admission, the presence of breathing disorders, and the need to perform phototherapy.

Results: TBA ≥ 40.00 µmol/L is connected to an elevated risk of the occurrence of total adverse perinatal outcome (OR = 4.17, p = 0.0037, AUC = 0.62, p = 0.046). TBA ≥ 40.00 µmol/L is a predictor of preterm birth (OR 2.3, p = 0.0117), iatrogenic preterm birth (OR 2.5, p = 0.006), admission to NICU (OR 2.38, p = 0.0094), intubation or assisted ventilation (OR 2.16, p = 0.0301), and phototherapy (OR 2.0, p = 0.0438). The threshold value of TBA for the need for phototherapy was 52.7 µmol/L (AUC = 0.67, p = 0.0089) and for preterm birth, 32.1 µmol/L (AUC = 0.62, p = 0.0251).

Conclusions: Pregnant women with ICP and TBA serum level over 40.00 µmol/L have a worse prognosis regarding obstetric outcomes. The concentration of bile acids is a predictor of the occurrence of adverse perinatal outcomes, although the concentration of ALT and AST failed to show such a connection.

Key words: cholestasis; bile acids; adverse obstetric outcomes

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INTRODUCTION

The birth of a healthy, full-term newborn is the most important goal for every obstetrician. This task becomes a challenge especially if the pregnant woman, who was completely healthy, becomes ill during pregnancy. Intrahepatic cholestasis of pregnancy (ICP) is an illness that emerges in 1% of pregnant women in the second or at the beginning of the third trimester [1]. Cholestasis of pregnancy is the most common liver disorder occurring during pregnancy, with symptoms most commonly subsiding shortly after birth [2]. It is a benign liver condition, however, due to the itching that occurs at night, it can be troublesome for a pregnant woman [3]. Cholestasis is manifested by elevated serum bile acid (TBA, total bile acids) and aminotransferases levels. In fewer than 10% of cases, cholestasis is accompanied with jaundice [4]. Although the disease is benign for the pregnant woman, it may be very dangerous for the foetus, because it is significantly more often associated with an abnormal perinatal outcome, including stillbirth as the most serious one, compared to a group of healthy pregnant women [5].

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Among other negative events for the newborn resulting from cholestasis, we observe spontaneous and iatrogenic preterm birth, a worse postnatal condition evaluated on the basis of the pH of the umbilical cord blood and Apgar score, hypoxia, the presence of meconium in the amniotic fluid, and admission to a neonatal intensive care unit (NICU) [6]. Despite numerous hypotheses (placenta microstructure disorders, foetal arrhythmia), the mechanism increasing the risk of such complications, including stillbirth in cholestasis patients, has not been identified yet. Also, no therapies preventing this complication exist [7–9]. The meta-analyses carried out so far have led to a conclusion that the occurrence of adverse obstetrics outcomes, including stillbirth, is associated with the concentration of bile acids in the pregnant woman [10]. Kawakita et al. [11] demonstrated that a concentration of TBA ≥100 μmol/L is correlated with the risk of stillbirth, and a concentration ≥ 40.0 μmol/L is correlated with the presence of meconium in the amniotic fluid. Based on the meta-analysis carried out by Glanza et al., it can be concluded that cholestasis with a concentration of bile acids < 40.00 μmol/L will not impact the increase in risk of foetal complications [12]. However, Chen et al. [13] have shown that an adverse obstetric outcome is affected by a concentration of TBA ≥ 57.55 μmol/L. The mentioned neonatal complications occur unpredictably, without any perceivable preceding symptoms. Currently, the only tool for assessing the risk of an abnormal perinatal outcome is the evaluation of the concentration of bile acids in the pregnant woman's serum.

Aim of the study

The aim of the study was to analyse the correlation between the adverse perinatal outcome and the biochemical parameters in pregnancy complicated by cholestasis, and to assess their predictive value for neonatal complications.

MATERIALS AND METHODS

Patients

The analysis included 86 patients with diagnosed Intrahepatic Cholestasis of Pregnancy (ICP), hospitalised in the Gynaecological and Obstetrics Clinical Hospital of Poznan University of Medical Science (GPSK). The research was conducted from January 2017 until December 2018. The protocol of the study was approved by the Bioethics Committee of the Poznan University of Medical Sciences of Karol Marcinkowski in Poznań (1062/16/01.12.2016 and 197/18/01.02.2018). An informed written consent was obtained from each of the patients participating in the study.

The intrahepatic cholestasis of pregnancy was diagnosed on the basis of clinical symptoms (presence of itchiness without skin changes) and abnormal laboratory tests results: elevated concentration of TBA in the serum measured in fasting blood ≥ 10.00 μmol/L, and concentration of aminotransferases: alanine aminotransferase (ALT) > 33 U/L, aspartate aminotransferase (AST) > 32.00 U/L [14]. The exclusion criteria included: other conditions causing pruritus, chronic/acute liver and bile duct diseases (viral or autoimmune hepatitis, primary biliary cholangitis, acute fatty liver, obstructive jaundice, cholecystolithiasis), pre-eclampsia, HELLP syndrome. Each of the women who qualified to participate in the study were Caucasian.

The pregnant women with ICP were divided into three groups, based on the fasting bile acid levels in the serum, reflecting the severity of the illness. Group I, with TBA concentration of 10–39.90 μmol/L, n = 60 (69.77%) with mild cholestasis, group II — TBA 40–99.90 μmol/L, n = 20 (23.25%) presenting ICP with medium severity, and group III — TBA ≥ 100.00 μmol/L, n = 6 (6.98%), comprised of patients suffering from severe cholestasis (Tab. 1) [5].

Treatment

After the diagnosis of cholestasis, all patients were treated with ursodeoxycholic acid (UDCA), starting with a dose of 250 mg three times a day. The medicine's dose was modified subject to the lack of therapeutic effect when treated with a minimal dose (intensified itchiness reported by the patient, elevated TBA level), every few days. The maximal applied dose did not exceed 1500 mg/day.

The concentration of bile acids and aminotransferases was monitored twice a week, or daily, in selected cases.

Every pregnant woman had cardiotocography done four times a day and ultrasonography, along with evaluation of blood flows in the foetal vessels once or twice a week.

Blood from pregnant women suspected of cholestasis was collected from the ulnar vein. The evaluation of bile acids, aminotransferases and bilirubin was performed in GPSK Central Laboratory.

Termination of pregnancy was planned based on TBA levels and the week of pregnancy when cholestasis was diagnosed. In the case of mild cholestasis (TBA < 40.00 μmol/L), the birth took place following the recommendations of PTGiP (the Polish Society of Gynaecologists and Obstetri-

Table 1. Characteristics of patients with cholestasis of pregnancy

Analysed variable	Examined group
Quantity	86
Age [years]	30 (22–46)[a]
Gravidity (number of past pregnancies)	1 (1–6)[a]
TBA 10–39.9 μmol/L	n = 60 (69.77%)
TBA 40–99.9 μmol/L	n = 20 (23.25%)
TBA ≥ 100 μmol/L	n = 6 (6.98%)

[a]median (range)

Table 2. Demographic and laboratory characteristic of pregnant women with mild and severe cholestasis

	TBA < 40.0 µmol/L n=60	TBA ≥ 40 µmol/L n=26	p value
Age [years] mean ± SD	31 ± 4	31 ± 5	0.904
Gravidity, median (range)	1 (1–6)	2 (1–4)	0.287
Gestational age at diagnosis [weeks], median (range)	33 (20–39)	33 (13–39)	0.723
TBA at diagnosis [µmol/L], median (range)	17.1 (7.9–37.3)	66.3 (40.1–171.3)	**< 0.001**
AST at diagnosis [U/L], median (range)	97.95 (16.7–339.2)	167.9 (28.7–695.2)	**0.026**
ALT at diagnosis [U/L], median (range)	183.4 (13.5–620.8)	276.7 (29.6–1228.9)	**0.034**

cians), after the foetus' lungs have matured after the 38th week of pregnancy [15]. With TBA ≥ 100.00 µmol/L, the termination of pregnancy took place following the stimulation of maturity of the foetus' lungs, after the 34th week of pregnancy, and with TBA 40–99.9 µmol/L — after the 36th week of gestation. Depending on the level of cervix maturation, labour was preinduced with 3 g of dinoprostone in cervical gel or a Foley's catheter, while oxytocin in infusion pump was used for induction, as per GPSK scheme (5 IU oxytocin with 49 ml of solvent (0.9% NaCl or 5% glucose), infusion starts at 3 mL/h flow, increased by 0.5 mL/h every 30 minutes, up to 6 mL/h).

In the absence of favourable prognostic conditions for natural labour, the pregnancy was ended by caesarean delivery.

Laboratory examination

The concentration of aminotransferases and the total serum bile acid levels were measured using the electrochemiluminescence method on a Cobas 6000 apparatus (Roche, Basel, Switzerland).

Analysed perinatal outcomes

The following obstetrics outcomes were analysed for the specified groups of patients: the total adverse perinatal outcome, stillbirth, preterm birth, spontaneous preterm birth, iatrogenic preterm birth, presence of meconium in the amniotic fluid, Apgar score (< 7 in 5th minute), pH from the umbilical artery (< 7.10), the necessity for NICU admission, the presence of breathing disorders, and the need to perform phototherapy.

The occurrence of at least one of the above analysed perinatal outcomes was considered a total adverse perinatal outcome.

Statistical analysis

For ROC curve analysis, MedCalc Software (Ostend, Belgium) was used. SigmaStat version 3.5 software (Systat Software, Inc., Point Richmond, CA, USA) was used for statistical analysis. The results were analysed using the Mann-Whitney rank sum test for variables with a non-parametric distribution. Linear regression models were created to determine the relationship of serum TBA, ALAT, AspAT concentration with selected obstetric failures. The Chi-square test and the Fisher Exact Test were used for the assessment of the distribution of the tested characteristics. $P < 0.05$ was considered statistically significant.

RESULTS

Perinatal outcomes

The analysed groups of pregnant women did not differ in terms of demographics. The average time in which cholestasis occurred, both mild and severe, was the 33rd week of pregnancy. However, biochemical parameters, such as TBA, AST and ALT concentration, differed significantly statistically between the groups of pregnant women with a mild form and those with a severe form of cholestasis. (Tab. 2)

The patients with TBA ≥ 40.00 µmol/L gave birth on average two weeks earlier in relation to women with mild cholestasis, which was a statistically significant difference (36th vs 38th week, $p = 0.0087$). No statistically significant differences in the manner of pregnancy termination, the percentage of multiple pregnancies, birth weight in the percentage of newborns, whose weight was lower than the 10th and the 3rd percentile were found in both groups of patients. In the examined group of pregnant women with cholestasis, 67 had a single pregnancy, 18 had a twin pregnancy and one had a triplet pregnancy.

Total adverse perinatal outcome

Among the analysed group of 86 pregnant women with intrahepatic cholestasis, an adverse perinatal outcome was found in 50 women, which included 31 of 60 women (52%) with mild cholestasis, and 19 of 26 women (73%) with a severe form of the illness. This difference was statistically significant ($p = 0.0076$). An adverse obstetrics outcome applied to 42 of 72 (58%) newborns from mothers with TBA < 40.00 µmol/L, and 26 of 34 (76%) newborns from mothers with TBA concentrations ≥ 40.00 µmol/L.

No stillbirths and no newborn deaths occurred with any of the patients in the examined groups.

In the group with severe cholestasis, pregnancies ended prematurely significantly more often than in the group with

Table 3. Perinatal outcomes of patients with mild and severe cholestasis

	TBA < 40.0 µmol/L; n = 60	TBA ≥ 40 µmol/L; n = 26	p value
Gestation age at delivery [weeks], median (range)	38 (31–41)	36 (29–39)	**0.0087[a]**
Multiple pregnancy, n (%)	12 (20%)	7 (27%)	0.669[b]
Birthweight [g], mean ± SD	2942 ± 639	2688 ± 771	0.0770[c]
Birthweight (percentile) < 10 percentile	6 (8%)	2 (6%)	0.6687[b]
< 3 percentile	4 (5%)	0	N/A
Route of delivery, n (%)			
Vaginal	21 (35%)	15 (58%)	0.0501[b]
Caesarean section	33 (55%)	10 (38%)	0.1589[b]
Vacuum	6 (10%)	1 (4%)	0.3378[b]
Total adverse perinatal outcome (n)	31 patients (52%) 42 newborns (58%)	19 patients (73%) 26 newborns (76%)	**0.0076[b]** 0.0581[b]
Stillbirths	0	0	
Preterm delivery < 37th week of gestation (n) (%)	14 (23%)	14 (54%)	**0.0055[b]**
Spontaneous (n) (%)	5 (38%)	2 (14%)	0.3845[d]
Iatrogenic (n) (%)	9 (62%)	12 (86%)	
NICU admission (n) (%),	23 (32%)	17 (50%)	0.0660[b]
NICU length of stay [days], median (range)	4 (3–42)	5 (3–90)	**0.0472 [a]**
Ventilation (n) (%) or intubation	11 (15%)	10 (29%)	0.082[b]
Phototherapy (n) (%)	11 (15%)	13 (38%)	**0.0066[b]**
Breathing problems (n) (%)	14 (19%)	11 (32%)	0.1337[b]
Presence of meconium — stained amniotic fluid (n) (%)	7 (10%)	8 (24%)	0.0531[b]
Apgar score < 7 at 5th min. after birth (n) (%)	2 (3%)	1 (3%)	0.9531[b]
Umbilical arterial pH < 7.10 (n) (%)	1 (1%)	1 (3%)	0.2111[b]

[a]Mann-Withney Rank Sum Test; [b]chi Sqare; [c]Student U-test; [d]2 × 2 Fisher Exact test

mild cholestasis (54% vs 23%, p = 0.0055). In both groups, the decision to terminate gestation before term was more frequent than the occurrence of premature spontaneous delivery, and was 62% and 86%, respectively, for patients with a TBA concentration of < 40.00 µmol/L and ≥ 40.00 µmol/L. The newborns from mothers with severe cholestasis were hospitalised in NICU compared significantly to the newborns of mothers with mild cholestasis (5 days vs 4 days, p = 0.0472) and required phototherapy more often (38% vs 15%, p = 0.0066). However, the necessity to apply ventilation, the occurrence of breathing disorders, the presence of meconium in the amniotic fluid, the Apgar score in 5th minute, and the number of newborns born with a pH from umbilical artery of < 7.10 did not differ between the groups (Tab. 3).

Biochemical markers

Total adverse perinatal outcome

The average TBA concentration in the group of pregnant women with an adverse perinatal outcome was statistically significantly higher in relation to the women with a normal obstetrics outcome, and was, respectively, 45.2 ± 40.5 vs. 25.7 ± 15.3; p = 0.0028. This correlation was confirmed for single pregnancies p = 0.042. No statistical differences were found in the Aspat and Alat concentrations between women with adverse and normal obstetrics outcomes.

Based on the conducted analysis, it was found that, in the group with more severe cholestasis (TBA ≥ 40.00 µmol/L), the chance of the occurrence of a total adverse perinatal outcome is 4.17 times higher than in relation to the group with mild cholestasis (p = 0.037) (Tab. 4). This relationship was separately confirmed for single pregnancies (OR 3.79, p = 0.0127), but no dependence was found for multiple pregnancies (p = 0.9838) (Tab. 4a). The predictive accuracy of the TBA concentration (> 40.1 µmol/L) for a total adverse perinatal outcome was confirmed by means of a ROC curve (p = 0.046) (Tab. 5).

Preterm births

The analysis of the relation of elevated TBA (≥ 40.00 µmol/L) with the occurrence of preterm (iatrogenic and spontaneous) birth shown a statistically significant relationship (OR 2.3, p = 0.0117) in relation to pregnant women

Table 4. OR for predictors of adverse perinatal outcomes in intrahepatic cholestasis of pregnancy

Adverse perinatal outcome	Predictor	OR	95% CI	p value
Total adverse perinatal outcome	**TBA[a]**	**4.17**	**1.59–10.93**	**0.0037**
	AST[b]	0.71	0.48–1.39	0.4468
	ALT[c]	0.74	0.39–1.40	0.3509
Preterm births	**TBA[a]**	**2.3**	**1.21–4.49**	**0.0117**
	AST[b]	0.84	0.39–1.79	0.649
	ALT[c]	0.78	0.44–1.40	0.4048
Iatrogenic preterm births	**TBA[a]**	**2.5**	**1.30–4.85**	**0.006**
	AST[b]	0.95	0.42–2.11	0.8922
	ALT[c]	0.90	0.50–1.64	0.7423
Admission to NICU	**TBA[a]**	**2.38**	**1.24–4.58**	**0.0094**
	AST[b]	1.46	0.63–3.39	0.3815
	ALT[c]	2.72	0.90–2.99	0.4723
Ventilation	**TBA[a]**	**2.16**	**1.08–4.34**	**0.0301**
	AST[b]	1.28	0.44–3.76	0.6512
	ALT[c]	1.34	0.61–2.91	0.4647
Phototherapy	**TBA[a]**	**2.00**	**1.02–3.93**	**0.0438**
	AST[b]	1.06	0.41–2.75	0.9014
	ALT[c]	0.89	0.47–1.71	0.7338
Breathing disorders	TBA[a]	1.88	0.97–3.68	0.0634
	AST[b]	1.22	0.47–3.21	0.6805
	ALT[c]	1.23	0.61–2.45	0.5612
Presence of meconium in amniotic fluid	TBA[a]	1.98	0.81–4.86	0.1361
	AST[b]	2.27	0.37–14.06	0.3791
	ALT[c]	2.49	0.62–10.05	0.2005

[a]Reference category is 0–39.9 µmol/L; [b]Reference category is 0 ≤ 40 IU/L; [c]Reference category is 0 ≤ 40 IU/L

with a lower TBA concentration. The chance of preterm labour for a single pregnancy was 2.9 (p = 0.0259), but this dependence was not confirmed for multiple pregnancies (p = 0.3041) (Tab. 4a). Based on the ROC curve analysis, it was found that a TBA concentration of > 32.0 µmol/L is an optimal predictive factor of preterm labour (p = 0.0251) (Tab. 5).

Iatrogenic preterm birth

Higher concentration of TBA (≥ 40.00 µmol/L) was a significant predictive factor for iatrogenic preterm birth, OR 2.5, p = 0.006 (Tab. 4). This correlation was confirmed for single pregnancies OR 4.2, p = 0.0082, whereas a higher TBA concentration was not a predictor of pregnancy ending in multiple pregnancies (p = 0.3082) (Tab. 4a).

Newborn admission to NICU

For the group with the most severe cholestasis, TBA was a predictor for admitting the newborn to the neonatal intensive care unit (OR 2.38, p = 0.0094) (Tab. 4). The chance of admission of a neonate born by a mother with a TBA of ≥ 40.00 µmol/L to NICU for a single pregnancy was 2.6 times higher than for newborns born by mothers with a low TBA concentration (p = 0.0373). This dependence was not confirmed with multiple pregnancies p = 0.2026 (Tab. 4a).

The use of intubation or ventilation

The correlation of TBA concentration with the use of ventilation showed a statistically significant difference between the analysed groups (p = 0.0301). For newborns born by mothers with more severe cholestasis, the chances of the necessity of ventilation were almost twice as high OR 2.16 (Tab. 4).

The use of phototherapy

The chances for the necessity of phototherapy for newborns from patients with a higher TBA concentration was twice as high as for mothers with a mild form of cholestasis OR 2.0, which was a statistically significant correlation p = 0.0438 (Tab. 4). Based on the ROC curve analysis, it was found that a concentration of TBA ≥ 52.70 µmol/L is a predictive factor of phototherapy p = 0.0089 (Tab. 5).

Breathing disorders

TBA concentration was not a predictor of the occurrence of breathing disorders, p = 0.0634 (Tab. 4).

Presence of meconium in amniotic fluid

There was no correlation between the concentration of TBA and the presence of meconium in amniotic fluid, p = 0.1361 (Tab. 4).

The concentration of neither AST nor ALT was a predictive factor of any of the analysed adverse perinatal outcomes.

DISCUSSION

In the present study, the correlation of bile acid and aminotransferases levels with an adverse perinatal outcome in 86 pregnant women with intrahepatic cholestasis of pregnancy was analysed. Our study has shown that pregnant women with a TBA of ≥ 40.00 µmol/L serum level experienced an adverse obstetrics result significantly more often than pregnant women with a lower TBA concentration.

In the group of patients suffering from severe cholestasis, over 70% of the women and 76% of the newborns experienced adverse perinatal outcomes, including preterm labour, the presence of meconium in the amniotic fluid, admission of the newborn to NICU, the necessity to intubate, and the use of phototherapy. Our results are consistent with the data presented by other authors. In 2017, Cui et al. presented a meta-analysis of 1,928 patients with cholestasis of pregnancy, in which they assessed the relationship between

Table 4a. OR for predictors of adverse perinatal outcomes in intrahepatic cholestasis divided on single and multile of pregnancy

Adverse perinatal outcome	Predictor	Single pregnancy			Multiple pregnancy		
		OR	95% CI	P value	OR	95% CI	P value
Total adverse perinatal outcome	**TBA[a]**	**3.79**	**1.33–10.82**	**0.0127**	1.01	0.32–4.57	0.9838
	AST[b]	0.44	0.17–1.13	0.0884	n/a[d]		
	ALT[c]	0.60	0.3–1.21	0.1551	n/a[d]		
Preterm births	**TBA[a]**	**2.91**	**1.17–7.40**	**0.0259**	2.17	0.46–9.50	0.3041
	AST[b]	**0.39**	**0.16–0.94**	**0.0364**	n/a		
	ALT[c]	0.53	0.24–1.18	0.1212	0.59	0.12–2.77	0.4998
Iatrogenic preterm births	**TBA[a]**	**4.20**	**1.45–12.12**	**0.0082**	1.73	0.60–4.93	0.3082
	AST[b]	0.50	0.19–1.31	0.1565	n/a[d]		
	ALT[c]	0.90	0.35–2.33	0.8296	0.29	0.06–1.38	0.1196
Admission to NICU	**TBA[a]**	**2.60**	**1.06–6.38**	**0.0373**	2.09	0.67–6.52	0.2026
	AST[b]	1.14	0.42–3.06	0.7957	0.3705	0.09–1.44	0.1536
	ALT[c]	1.30	0.57–3.2	0.5032	n/a[d]		
Ventilation	TBA[a]	**2.16**	**1.08 – 4.34**	**0.0301**	0.80	0.11–5.96	0.8298
	AST[b]	1.28	0.44 – 3.76	0.6512	0.59	0.15–2.31	0.4509
	ALT[c]	1.34	0.61 0–2.91	0.4674	1.02	0.52–4.12	0.9825
Phototherapy	TBA[a]	1.05	0.4–2.8	0.916	2.24	0.83–6.0	0.1095
	AST[b]	0.99	0.37–2.64	0.9833	1.37	0.41–4.54	0.604
	ALT[c]	0.63	0.3–1.32	0.2239	n/a[d]		
Breathing disorders	TBA[a]	1.60	0.58–4.46	0.3671	1.96	0.75–5.16	0.1717
	AST[b]	0.95	0.31–2.87	0.9245	0.58	0.03–10.08	0.7108
	ALT[c]	1.24	0.47–3.27	0.6695	0.96	0.31–2.96	0.9424
Presence of meconium in amniotic fluid	TBA[a]	1.7	0.33–6.47	0.6089	2.55	0.66–9.76	0.1732
	AST[b]	n/a[d]			0.33	0.02–1.19	0.4611
	ALT[c]	1.84	0.27–2.37	0.5294	3.36	0.38–30.08	0.2779

[a]Reference category is 0–39.9 µmol/L; [b]Reference category is 0 ≤ 40 IU/L; [c]Reference category is 0 ≤ 40 IU/L; [d]not applicable. All patients represented the same category

Table 5. Results of ROC curves analysis of TBA predictive values for negative neonatal outcomes

Adverse perinatal outcome	AUC [95% CI]	Cut Off [µmol/L]	Sensitivity (true positive rate) [95% CI]	Specificity (true negative rate) [95% CI]	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value	p
Total adverse perinatal outcome	0.62 [0.51–0.71]	40.1	41.43 [29.8–53.8]	87.88 [71.8–96.5]	3.42	0.67	87.9	41.4	**0.046**
Phototherapy	0.67 [0.58–0.76]	52.7	54.17 [32.8–74.4]	84.34 [74.7–91.4]	3.46	0.54	50.0	86.4	**0.0089**
Preterm births	0.62 [0.53–0.72]	32.1	55.56 [40.0–70.3]	72.58 [59.8–83.1]	2.03	0.61	59.5	69.2	**0.0251**

the TBA serum level and the risk of an abnormal perinatal result. The authors concluded that an increase of the TBA level to ≥ 40.00 µmol/L is related to a significantly increased risk of the occurrence of a total abnormal perinatal outcome, preterm labour, the presence of meconium in amniotic fluid, hypoxia, and breathing disorders in newborns [6].

In our study, we found a significantly higher percentage of preterm births in the group of patients with more severe cholestasis. In a study of 106 pregnant women with cholestasis, Chen et al. showed that a concentration of TBA ≥ 40.15 μmol/L is connected to an almost fourfold increased risk of preterm delivery as compared to pregnant women whose TBA concentration is lower than this value. In pregnancies complicated with cholestasis, the number of preterm births grows with the increase of the concentration of bile acids [16]. The results from collective data from meta-analyses also indicate an elevated risk of spontaneous preterm delivery (OR = 3.47) and iatrogenic preterm delivery in pregnant women with cholestasis [5].

In our research, the high percentage of preterm births was due to the high rate of occurrence of iatrogenic preterm labours for both TBA concentration ranges, and was, respectively, 62% and 68% for the group with mild and severe cholestasis. Such high percentages of interventions result from the high proportion of multiple pregnancies (20% and 27%, respectively, in the group with mild and severe cholestasis), and, or primarily to avoid the most serious complication of cholestasis, which is intrauterine foetal death. None of the patients with cholestasis who gave birth in GPSK during this period had intrauterine foetal death or neonatal death. In 2019, Ovadia et al. published a meta-analysis of data 5557 patients with intrahepatic cholestasis of pregnancy, concerning the relationship of bile acid serum concentration with stillbirth. Interpretation of the results obtained allowed the formulation of conclusions that only a concentration of bile acids of over 100.00 μmol/L is related to an increased risk of stillbirth [5]. The risk of intrauterine foetal death increases regardless of pregnancy advancement. The concentration of bile acids does not exceed 100.00 μmol/L in most of the pregnant women suffering from cholestasis. In this group of patients, the risk of stillbirth is comparable with the risk for the general population of pregnant women, in both ranges, 40–100 μmol/L, and < 40 μmol/L.

The group of 6 pregnant women whose bile acid concentrations exceeded 100.00 μmol/L seems, from a clinical perspective, the most interesting one. The average age in that group was 29.6 years (28–32 years). For five of them, it was the second pregnancy, and only one woman had cholestasis in the previous pregnancy. In this group, two patients were in twin pregnancy. Two patients gave birth in completed the 37th week of pregnancy, and four prematurely (respectively, in the 31st, 33rd, 34th, and 36th week of pregnancy). In this group of patients, the average TBA serum concentration was 135.20 μmol/L (102.00–171.30 μmol/L), AST 230.77 U/L (36.00–350.60 U/L), ALT 356.72 U/L (52.30––521.90 U/L). Caesarean section was performed on 5 of the 6 patients. Although the presence of meconium in the amniotic fluid was present twice, all newborns had normal pH in the umbilical cord blood (> 7.3) and the Apgar score in 5th minute ranged from 7 to 10. Three newborns were admitted to NICU: the twins born in the 31st week due to breathing disorders and the necessity to apply mechanical ventilation, and one of the twins from the 33rd week for the same reason. These three newborns also required phototherapy. In both twin pregnancy cases, the decision to terminate the pregnancy was based on very high concentrations of bile acids, which increased despite treatment. In the pregnancy which ended in the 31st week of pregnancy, HELLP syndrome developed additionally.

In our study, neonatal problems significantly more often concerned babies of mothers with severe cholestasis. These newborns were significantly more frequently prematurely born, stayed longer in NICU and required phototherapy more often. Similar results were presented by Garcia-Flores et al. [17] who, in a group of 52 newborns from 47 pregnant women with cholestasis, found significantly more frequent adverse neonatal outcomes, including the presence of meconium in the amniotic fluid, admission to NICU, and neonatal global morbidity.

Our analysis of the relation between adverse obstetrics outcomes and serum bile acids and aminotransferases level showed that a TBA concentration of over 40.00 μmol/L is connected to an elevated risk of the occurrence of total adverse perinatal outcome (OR = 4.17, p = 0.0037, AUC 0.62, p = 0.046). A TBA of ≥ 40.00 μmol/L was also a predictor of preterm labour, iatrogenic preterm labour, admission to NICU, intubation, assisted ventilation, and phototherapy. The correlation was confirmed for single pregnancies, but was not visible in multiple pregnancies, probably due to the small number of patients within the group. In the paper from 2017, Chen et al. [13] showed that a concentration of TBA ≥ 57.55 μmol/L was a significant predictor of the occurrence of an adverse obstetrics outcome (OR = 3.214). The results obtained by Celik et al. [18] show that both preterm births and admission to NICU take place significantly more often if the concentration of TBA in the serum exceeds 34.00 μmol/L. Our analysis of ROC curves showed that a TBA of > 32.00 μmol/L was a significant predictor of preterm delivery, while a TBA of > 52.70 μmol/L was a significant predictor for phototherapy. The above-cited authors showed that the presence of meconium in the amniotic fluid, and foetal distress occur significantly more often if cholestasis occurs before the 34th week of pregnancy, regardless of the concentration of bile acids [18]. Our study fails to confirm the results obtained by the above authors. In our material, the average time of onset of both mild and severe cholestasis was the 33rd week of pregnancy. A study published by Oztas et al. showed that a TBA concentration of ≥ 51.00 μmol/L is a predictor of a low Apgar score in pregnant women with cholestasis. We did not confirm this relationship [19].

Unfortunately, adverse outcomes of pregnancy complicated by cholestasis may occur despite the treatment and reduction of the bile acid serum level [20]. Based on a randomised controlled trial (PITCHES), whose objective was to evaluate whether the application of ursodeoxycholic acid reduces the percentage of adverse perinatal outcomes in women with intrahepatic cholestasis of pregnancy, it was found not to have such an impact. Serious adverse events took place in both the treated group and the placebo group. In both groups, intrauterine foetal death occurred (twice in the placebo group and once in the UDCA- treated group). The stillbirths took place in the 35th and the 37th weeks of pregnancy [21].

The monitoring of pregnant woman with cholestasis should include systematic tests of bile acid serum level and active proceedings, involving elective early termination of pregnancy, in particular with high (> 100.00 µmol/L) concentrations of bile acids. Therefore, these women should be hospitalised in centres where bile acid concentrations are routinely tested, ready for immediate termination of pregnancy and specialist care for premature newborns.

The analysis we presented confirms that a higher TBA concentration is connected with adverse obstetric result in ICP patients. It may be concluded, following the analysis, that pregnant women with a TBA serum level over 40.00 µmol/L, have a worse prognosis regarding obstetric outcomes. The concentration of bile acids is a predictor of the occurrence of adverse perinatal outcomes, although the concentration of ALT and AST failed to show such a connection.

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