EVALUATING THE DIAGNOSTIC PERFORMANCE OF TOTAL BILE ACIDS AND TRANSAMINASES IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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ABSTRACT

Objective: The objective of this study is to evaluate diagnostic performance of total bile acids and transaminases in intrahepatic cholestasis of pregnancy (ICP).

Material and Methods: This observational cross-sectional study was conducted at Gynecology & Obstetrics ward, Bahawal Victoria Hospital and Pathology Laboratory of Quaid-e-Azam Medical College, Bahawalpur. Thirty pregnant females with intrahepatic cholestasis of pregnancy were included in the study. Thirty age, parity and gestational age matched controls were selected. Total bile acids and alanine transaminases (ALT) were measured in each study subject.

Results: The sensitivity and specificity for both total bile acids and ALT were calculated. Our study results showed a comparable sensitivity and specificity for both tests in patients with ICP. The rise in bile acids and ALT was statistically significant in ICP. The level of transaminases increased in proportion to total bile acids. Also, our study found an increased risk of preterm delivery, low birth weight and delivery by cesarean section in patients with ICP.

Conclusion: It is concluded that ALT can be used alternatively to total bile acids for the diagnosis of intrahepatic cholestasis of jaundice. Total bile acids are expensive and only a few laboratories in Pakistan are offering this service, making its access limited to general population. Whereas ALT is a cost effective and common test, its results are readily available with an additional benefit that no fasting sample is required. Hence, it is a more appropriate diagnostic test for ICP in our resource constraint setup.

Key Words: Bile acids, Liver enzymes, Cholestasis, Pregnancy, Aminotransaminase.

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INTRODUCTION

One of the most common liver disorders that are experienced by women in pregnancy is intrahepatic cholestasis of pregnancy (ICP), with a usual occurrence of 0.1% to 15.6% of pregnant population [1]. It usually encompasses the symptoms of tingling/itching distinctively entailing soles and palms of extremities more at night time. It is biochemically related to altered liver function tests especially elevated total bile acids (TBA) and liver enzymes including alanine transaminase (ALT), aspartate transaminase (AST), Alanine phosphate (ALP) and bilirubin. The disease is pathognomic to appear in the later half of the pregnancy principally after second trimester [2]. The patient is observed through the rest of the pregnancy while governing for the outcomes and customarily the symptoms and the laboratory evidence of normal liver function tests (LFTs) can be witnessed 5-6 weeks after birth. Conventionally the LFTS are first checked on 10th postnatal day [3].

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Intrahepatic cholestasis of pregnancy gains its importance for continuous surveillance throughout the trimesters owing to its fatal fetal and maternal implications. Intrahepatic cholestasis of pregnancy has well known associations to gestational glucose impairment, preeclampsia, silent intrauterine fetal demise, meconium aspiration, preterm birth, still birth, low birth weight and small for gestational age babies. The mothers are also at the risk of iatrogenic preterm delivery, operative delivery and instrumental application in second stage of labour etc. These complications expose the mothers to enhanced risk of PPH too [4-6].

The exact pathogenesis of ICP is not completely understood as many physiological variations are witnessed in pregnancy in the biliary tract which may be responsible for the manifestations of the disease in genetically predisposed pregnant females [7]. Hormonal changes owing to estrogen and progesterone, mutations, raised levels of alkaline phosphatase and gamma-glutamyltransferase (GGT) effect canalicular uptake and transport of bile causing pruritus [8]. Intrahepatic cholestasis of pregnancy is actually a diagnosis of exclusion and is usually named after all other causes of abnormal LFTs in pregnancy have been ruled out. The most

sensitive investigation to label a patient as a case of ICP is the measurement of serum total bile acids [9]. The raised levels of bile acids are thought to proportionate directly to the incidence of sudden intrauterine fetal deaths in pregnancy especially when bile acid is more than ≥40 µmol / L [10]. Cholestasis can also be evaluated by measurement of liver enzymes especially transaminases. The liver enzymes are found to be elevated in ICP owing to the hepatocellular damage due to cholestasis. Therefore, measurement of liver function tests is not only essential in diagnosis and monitoring of these patients but also for the prediction of fetal outcomes. So, we can clinically correlate the patient's symptoms and disease outcomes with biochemistry of bile acids and liver enzymes [11].

But measurement of total bile acids is an expensive test and it is not freely available in a resource poor country like Pakistan. The results take on an average of 2-3 working days. The monitoring requires multiple sampling to follow the progression of the disease. Another drawback is that for estimation of bile salts a fasting sample is required as bile salts are affected by meals. Moreover, bile acids are no doubt a more sensitive test for evaluating ICP but it is not a specific marker for cholestasis. So as the access to this investigation is limited because of its high cost and also because not every other lab is doing this investigation. There is a need of a cost effective and readily available test for the diagnosis and monitoring of ICP. In literature, it is proved that bile acids are in close relationship to transaminases in the liver of ICP patients. This alliance is due to damage to secretory phospholipase A2 [12-13].

The purpose of this study is to compare the diagnostic efficacy of total bile acids level and transaminases in patients with intrahepatic cholestasis of pregnancy. So that instead of requesting total bile acids, transaminases can be used for the diagnosis and monitoring of patients with ICP in our resource constrained population.

MATERIAL AND METHODS

This observational cross-sectional study was conducted at Gynecology & Obstetrics ward of Bahawal Victoria Hospital and Pathology Department of Quaid-e-Azam Medical College, Bahawalpur from September 2021 to July 2022 after approval from Institutional Ethical Review Board. Sample size was calculated by WHO sample size calculator taking 95% confidence interval and 5% margin of error with prevalence of ICP in Punjab at 3.1% [14].

Non-probability consecutive sampling technique was used for sample collection. Thirty pregnant women developing intrahepatic cholestasis of pregnancy and presenting with pruritus in the absence of any other liver and biliary disease were included in the study. For control group thirty healthy women were selected randomly and were matched for age, parity and gestational age. Patients with dermatosis, viral hepatitis, autoimmune hepatitis, gall stones or any other liver and biliary disease, cholecystectomy, thyroid disorders, gestational diabetes, hypertensive disorders of pregnancy and patients with twin pregnancies were excluded from the study. Informed consent was taken from all study subjects.

For total bile acids fasting samples was required. 5 ml of venous blood was taken through venipuncture and transferred to plain tube. The serum was separated by centrifugation at 3000 rpm for 5 minutes. Total bile acids and ALT were measured on fully automated chemistry analyzer, Beckman Coulter AU 480 by an expert Chemical Pathologist. The criteria for elevated total bile acids was considered as ≥40 µmol/L and for liver enzymes it was >35 IU/L.

Statistical analysis was performed on the Statistical Package for Social Sciences (SPSS) version 22. The values were expressed in mean and standard deviation and t-test was used as test of significance. p value ≤ 0.05 was taken as statistically significant.

RESULTS

During study period, 30 pregnant women with ICP were studied. There was no significant difference between age, parity and gestational age between two groups. Table-I shows the demographics and results from the laboratory investigations of study population. Levels of total bile acids and ALT were significantly raised in all cases. ALT levels were found to be <20 IU/L in the control group which is lower than the normal reference level of non-pregnant females. Table-II indicates the fetal outcomes in patients suffering from intrahepatic cholestasis of jaundice. We found a higher incidence of preterm delivery, low birth weight, meconium aspiration and delivery by cesarean section.

We compared the sensitivity and specificity of total bile acids and ALT in ICP by constructing Receiver Operating curve (ROC). The parameters indicated comparable sensitivity and specificity of both tests. Table-III shows the ROC results for both bile salts and ALT in patients diagnosed with intrahepatic cholestasis of pregnancy.

Table-I: Demographics and laboratory results of ICP and control group

	ICP group n=30 (mean±SD)	Control group n=30 (mean±SD)	t value	P value
Age (years)	26.7±5.3	27±4.6	27.7	0.001
Gestational age (weeks)	31.07±2.6	30±3.4	65.4	0.001
ALT (IU/L)	230±112	18.6±3.9	11.2	0.001
Total Bile acids (µmol/l)	88.6±24.5	9.3±1.5	19.7	0.001

*ALT: Alanine transaminase *SD: Standard deviation

Table-II: Comparison of fetal outcomes in ICP and control groups.

	ICP group n=30	Control group n=30
Birth weight (kg)	2.64 ± 0.4	2.8 ± 0.5
Oligohydroamnios	5 (16%)	2 (6.6%)
LSCS	12 (40%)	7 (23.3%)
Instrumental Delivery	3 (10%)	2 (6.6%)
Meconium aspiration	5 (16%)	4(13.3%)
Still birth	1 (3.3%)	0 (0%)
Preterm delivery	5 (16%)	2 (6.6%)

*LSCS: Lower section cesarean section

Table-III: ALT and Total bile acids ROC curve results in ICP

	Sensitivity	Specificity	AUC±SE	95% confidence interval		p value
				Upper	Lower	
ALT	86%	83%	0.82±0.10	0.618	1.000	0.017
Total bile acids	87%	80%	0.81±0.11	0.598	1.000	0.023

*ALT: Alanine transaminase *AUC: Area under curve *SE: Standard error of mean

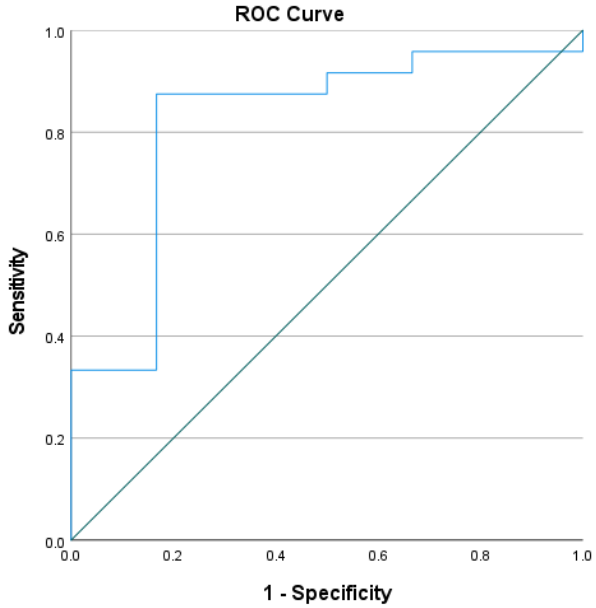

Figure-I: Total bile acids ROC curve in intrahepatic cholestasis of pregnancy.

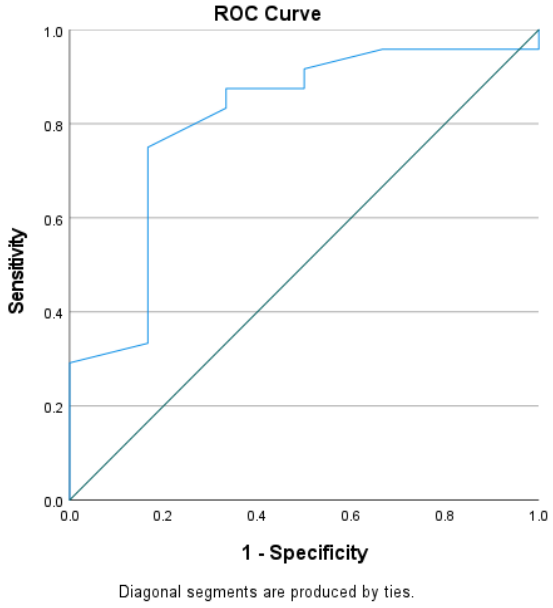

Figure-II: ALT ROC curve in intrahepatic cholestasis of pregnancy.

DISCUSSION

Intrahepatic cholestasis (ICP) of pregnancy is potentially a detrimental liver disorder that is experienced in the last bisection of the pregnancy. ICP gains its substance because of the consequences it can import to both maternal and fetal life. Hence lays significance in its diagnosis, early consideration of symptoms like itching in pregnancy and ordering investigations. The biochemical indicator commonly used for making a diagnosis of ICP is serum bile acid measurements all over the world [14,15]. The acceptable upper limit of bile acids during pregnancy is 10 µmol/L. Asymptomatic pregnant women without itching may have high bile acid levels on biochemistry as an incidental finding but when followed with weekly levels patients may complain about itching and bile acid levels may gradually raise in 2-3% of the

pregnancies [16]. Serum bile acid measurement is thought to be the most pertinent biochemical marker both in the diagnosis and follow-up of ICP patients [17].

Large prospective trials have proved enough evidence to state that ICP is associated with guarded perinatal outcomes. Spontaneous and iatrogenic both preterm delivery and sudden intrauterine death rates increase escalate rapidly in pregnancies when serum bile acid exceeds 40 µmol/L [18]. Increased circulating bile acid in maternal blood results in bile acid accumulation at fetomaternal unit [19-21]. This leads to the fetuses of ICP patients with more bile acids in intrauterine bronchoalveolar fluid than normal fetuses. The fetuses of ICP mothers are more prone to develop RDS in their early neonatal life [22, 23].

Genes *et al* in the research found that association between serum bile acid and ALT levels with preterm birth that ICP has been shown to link to premature births as is seen in the results of study as well (5). The exact mechanism is not known but studies reflect an enhanced myometrial contractility expression to oxytocin in ICP patients as compared to normal healthy pregnant females [24]**.** Another study shows 14.81% preterm deliveries in ICP group when compared to 6.33% in control group which is similar to our study with 16% and 6.6% preterm births in cases and controls respectively [25]. In another prospective study by Singh G, the numbers of meconium staining of amniotic fluid is 12-22% in controls to 25% in cases which again emphasizes our results that are 13% in controls and 16.6% in cases [25].

An interesting fact is that despite treatments with ursodeoxycholic acid and low bile acid levels, unexplained fetal deaths were not uncommon in patients with ICP. This reflects that the rate and severity of complications are propionate to bile acid levels and a rise in grave outcomes is seen for every 10 µmol/L rise in bile acid levels [26]. In ICP bile acid levels rise concurrently with ALT and AST [19,27]. The previous research data shows that the height of transaminases rise in ICP varies with no definite cut off probably by 2-3 times [11]. ALT is a more appropriate marker than AST which is why we chose ALT for our research variable to study patients and follow the outcomes [27,28]. Statically results of our study are comparable to other studies in reflecting that ALT can be chosen as a reliable marker to diagnose, monitor and follow ICP as compared to bile acids. The rise in ALT is consistent with the rise in bile acids and both comparably reflect the grave outcomes like SGA, LBW, meconium aspiration, pre-eclampsia and intrauterine fetal deaths [25,29].

The advantage of our study is that it can help in up taking ALT as a diagnostic test for ICP instead of bile acids. ALT is an equally sensitive test and is commonly available at all labs. It is pocket friendly and results are available within few hours. The disadvantage of our study is that the sample size is small and more studies are needed to be conducted at a larger scale to generalize the results to whole population.

CONCLUSION

Our study concluded that ALT can be used as an excellent alternative for the diagnosis and monitoring of intrahepatic cholestasis of pregnancy especially in poor, resource constrained areas like Pakistan. This will ultimately lead to timely diagnosis and better management of ICP.

AUTHOR CONTRIBUTION

Saba Nadeem: Conception and design of the article. literature search, drafting

Sara Reza: Statistics, Analysis and interpretation of data, drafting of paper, establishment in selecting the final version to be published

Nimrah Sattar: Helped in data collection and drafting

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