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Correlation of Intrahepatic Cholestasis (ICP) with Jaundice and Liver Profile in Pregnant Females: A Cross Sectional Study

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Cholestasis is consequence of a decrease in biliary flow, commonly due to billiary tract obstruction, remove or pinching of the biliary ducts due to structure change or cell death, or bile acid transport at apical membrane of liver cells. The main aim of our study is to rule out prevalence of obstetric cholestasis in pregnant females association always liver profile, as well as also to check out the correlation of ICP and jaundice in pregnant females. In this Cross Sectional study, the samples was collected from 301 pregnant females on the basis of non-random sampling technique from the females of 2nd and 3rd trimester. The serum sample were further processed for the evaluation of LFTs (ALT, AST, GGT, ALP, T. Bill) A/G Ratio and total bile acid level through Roche kit and Alinity TBA reagent kit. Chi square and Pearson correlation was computed to access the statistically results. Of 301 pregnant female samples, the mean age was 28.58. Our clinical data represents that 44.21% females were suffering from cholestasis and 55.79% females were not showing the results of ICP, as well as the only 33% patients have jaundice with the p value <0.0001. The parameters of LFTs were significantly raised in which ALT 65.10 %, AST 55.9%, ALP 96.2% and GGT 6.81%.Our study revealed the relationship between total bile acid level and liver function test in females affected with intrahepatic cholestasis of pregnancy. Overall we concluded that the levels of AST, ALT, ALP, Total Bilirubin and total bile acid level were significantly elevated and the level of A/G ratio were low in second and third trimester of pregnancy. Timely diagnosis can help to prevent the adverse effects of ICP in pregnancy.

Keywords: Total Bile Acid, Intrahepatic Cholestasis, Jaundice, ICP, LFT

INTRODUCTION

Any disruption in the complex sequence of cellular events that eventually result in the entry of bile fluid into the duodenum impairs the bile secretory process and therefore raises a situation descriptively called cholestasis.(Ublick and Meier, 2000) Cholestatic is a collection of common liver diseases that upset bile ducts of all calibres. cholestasis signifies the consequence of reduced bile formation and decrease in bile flow, usually classified as extra and intrahepatic cholestasis.(Jungst et al. 2013)

Obstetric cholestasis is also called intrahepatic cholestasis of pregnancy. It's a syndrome definite to pregnancy characterized by disturbed liver function tests and generalized itching without skin rash both squaring after delivery. Other causes of pruritus and liver damage need to be excepted.(Lo et al. 2007) Obstructive cholestasis is the increase of severe liver injury. It is generally expected that contact of hepatocytes high concentrations of potentially toxic bile acids mainly accountable for cholestatic liver injury.(Allen et al. 2011)

Intrahepatic cholestasis of pregnancy (ICP) is capable of being reversed pregnancy linked liver disease that is categorized by raised serum bile acids and an increased risk of deleterious significances for the fetus, mainly in late second or third trimesters.(Feng et al. 2018) Derangements in bile flow can be caused by environmental triggering factors, antigenic stimuli, xenobiotics, endotoxins and micro-organisms can encourage cholangiocyte reaction that will change in cholestatic state.(Yokoda and Rodriguez, 2020)The levels of progesterone in initial pregnancy can also be a threat factor for ICP. Changes in progesterone metabolism have been showed in ICP patients that display increased urinary excretion and serum levels of total sulphated progesterone metabolites**.**(Arrese et al. 2008) The fact of fundamental genetic mutation to ICP is proposed by the existence of advanced incidence of ICP in female. The

mutation in genes encodings Multidrug resistance protein 3 (MDR3), the amino-phospholipid carrier ATPase Phospholipid Transporting 8B1 (ATP8B1), familial intrahepatic cholestasis type1 (FIC1) and bile salt export pump (BSEP) have diagnosed with ICP. In adding, functional variations of bile salts radar (Farnesoid X receptor), FXR have been initiate in patients with ICP.(Dixon and Williamson, 2016) Accumulation of bile acids is a major mediator of cholestatic liver injury, as humans have a higher percent of glycine conjugated bile acids and increased chenodeoxycholate content, which increases the hydrophobicity index of bile acids. This increase may lead to direct toxicity that kills hepatocytes, and promotes inflammation.(Woolbright et al. 2015)

Collection of bile acids in hepatocytes leads to mitochondrial damage and ultimately to apoptosis or necrosis.(Yang et al. 2013) Intrahepatic cholestasis of pregnancy (ICP) is a liver disease of pregnancy that affects 0.5%–2% of pregnant women and occurs predominantly during the second or third trimester. The rate has been reported from Pakistan up to 3.1% had intrahepatic cholestasis of Pregnancy. ICP is more common in South Asia, South America, and Scandinavia. ICP increases the risk of an adverse fetal birth. (Geenes and Williamson, 2009) So, the main aim of our study is to rule out prevalence of obstetric cholestasis in pregnant females association with liver profile, as well as also to check out the correlation of ICP and jaundice in pregnant females in Pakistan.

MATERIALS AND METHODS

In this cross sectional study we collected 301 samples of pregnant females during 2nd and 3rd trimester with the complaint of pruritus and itching. Then we separated serum from collected samples at 3000rpm and perform LFTs, TBA (total bile acid), and A/G ratio for the evaluation of biochemical changes in cholestasis patients. LFTs were performed on fully automated Biochemistry analyzer Cobas C111 through Roche kit. TBA samples were run on Integrated Clinical Chemistry and Immunoassay Analyzer Abbot Alinity Ci by utilizing Alinity TBA reagent kit on the wavelength of 405 nm. Both analyzer were fully automated and reagents were ready to use, so we didn't need to prepare any reagents or dilutions. A/G ratio has been calculated as mentioned in the literature. The analysis of the data was done on a software statistical package for social sciences (SPSS) software. Chi square and Pearson correlation was computed to assess the results.

RESULTS

We performed the ALP, ALT, AST, T. Bili, GGT, A/G ratio and TBA on 301 samples of pregnant females and calculate the Max, Mini, mean and St. dev of the concerned data. The demographic analysis revealed that minimum age of the selected female pregnant females was 18 year while the maximum age was 41. The mean and standard deviation of age among this group was 28.59±4.231. Our clinical data represent the frequency of cholestasis among pregnant females in which out of 301 females 133 (44.2%) were suffering from cholestasis and 168 (55.8%) females were showed the no results of ICP.

The cross-tab (1.1) showed that out of 301 patients, 133 have cholestasis while in 133 patients 23 show jaundice (7.70%). In other hand 168 patients present with no cholestasis and they also have no jaundice. These findings suggest an association between cholestasis and jaundice, as patients with cholestasis were more likely to have jaundice than those without cholestasis."(p-value <0.001)

Table 1.1: Comparison of Cholestasis and Jaundice

Cholestasis	Jaundice		Total
	Present	Absent	
Present	23(17.30%)	110(82.70%)	133
Absent	0(0.00%)	168(100.00%)	168
Total	23(7.70%)	278(92.30%)	301

Chi square test 31.46

As out of 133 cholestatic patients (table 1.2), 109 have raised ALT (65.10%) level while 24 have normal ALT. Out of 168 non cholestatic patient 85 have with raised ALT and 83 have normal ALT (p-value <0.001)

Table 1.2: Comparison of Cholestasis and ALT

Cholestasis	ALT		Total
	Raised	Normal	
Present	109(82.00%)	24(18.00%)	133
Absent	85(51.50%)	83(48.50%)	168
Total	194(65.10%)	104(34.90%)	301

Pearson Chi-Square 30.033a

The cross-tab 1.3 showed that 167 pregnant females out of 301 have raised AST (55.9%) level in which 102 female have cholestasis and 65 have normal bile acid. The results have an association between cholestasis and AST (p-value <0.001).

Table 1.3: Comparison between Cholestasis and AST

Cholestasis	AST		Total
	Raised	Normal	
Present	102(76.7%)	31(23.3%)	133
Normal	65(39.2%)	103(60.8%)	168
Total	167(55.9%)	132(44.1%)	301

Pearson Chi-Square 42.191a

The Comparison of cholestasis and GGT levels for 301 patients indicates that out of 133 cholestatic patients, only 9 patients (6.8%) have raised GGT levels, while 124 patients (93.2%) have normal GGT levels. Among the remaining 168 non-cholestatic patients, 12 patients (7.1%) have raised GGT levels, while 156 patients (92.9%) have

normal ALT levels. There is no association between cholestasis and GGT (p-value 0.877). The Comparison of cholestasis and ALP levels for 301 pregnant females indicates that out of 133 cholestatic females, 128 females (96.2%) have raised ALP levels, while 5 females (3.8%) have normal ALP levels. Among the remaining 168 non-cholestatic females, 118 females (70.2%) have raised ALP levels, while 50 females (29.8%) have normal ALP levels. There is an association between cholestasis and ALP (p-value <0.001).

The Comparison of cholestasis and AGR levels for 301 pregnant females indicates that out of 133 cholestatic females, 108 females (81.2%) have low AGR levels, 24 females (18.0%) have normal AGR levels, and only 1 female (0.8%) has high AGR levels. Among the remaining 168 non-cholestatic females, 116 females (69.0%) have low AGR levels, 52 females (31.0%) have normal AGR levels, and none of them have high AGR levels. There is an association between cholestasis and ALP (p-value 0.025) (table 1.4).

Table 1.4: Comparison between Cholestasis and AGR

Cholestasis	AGR			Total
	Normal	Low	High	
Present	24(18.0%)	108(81.2%)	1(0.8%)	133
Absent	52(30.1%)	116(69.9%)	0(0.0%)	168
Total	76(24.7%)	224(74.9%)	1(0.3%)	301

Likelihood Ratio 7.356

Total bile Acid (TBA) was inversely related with age (r=-0.156) whereas directly with T Bilirubin (r=0.581), ALT(r=0.579), AST (r 0.735), GGT (r=0.302) and A/G ratio (r -0.022) as showed in table 1.5.

Table 1.5: Correlations of Biochemical parameters with age

	TBA	Age	T. Bilirubin	ALT	AST	ALP	GGT
Age	-.156**						
T Bilirubin	0.581**	-0.219**					
ALT	0.579**	-0.104	0.353**				
AST	0.735**	-0.076	0.453**	0.867**			
GGT	0.302**	-0.061	0.274**	0.245**	0.290**	0.234**	
A/G ratio	-0.022	-0.135*	-0.054	-0.02	-0.042	-.339**	-0.092

**. Pearson Correlation is significant at the 0.01 level.

*. Pearson Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Intrahepatic cholestasis of pregnancy (ICP) is a liver disease of pregnancy that affects 0.5%–2% of pregnant women and occurs predominantly during the second or third trimester. Clinically, pregnant women with ICP present pruritus and hepatobiliary injury with hypercholanemia (elevated serum levels of bile acids) and dyslipidemia.(Padmaja et al. 2010) The current study was based on the frequency of ICP in pregnant females and the results also showed the elevated level of serum bile acid but GGT (p-value 0.877)did not showed the significant results.

We collected 301 sample from second and third trimester of pregnant females, in our study the maximum age was 41years and minimum was 18 years. The mean and standard deviation of age among this group was 28.59±4.231 as Mitsunaga and his colleagues reported in 2021 collected 169 samples which were divided in two groups active n=115 and inactive=54 and the average age was 47.2 ± 17.3 years.(Mitsunaga et al. 2021) As well as Toprak and his fellows represent the demographic data of 50 patients, According to their data the mean age for obstetric cholestasis was 26.6 while for control group the mean age was 25.1. (Toprak and Kafadar, 2021)

The females which were selected for sampling have the high score of pruritus and these samples then proceed for the further biochemical changes (TBA, LFTs, and Total Bilirubin & A/G ratio). Our clinical data presented the maximum values of total bilirubin (342), AST (2335), ALT (1539), and ALP (571) which indicated the intrahepatic cholestasis in the pregnant females. The statistical analysis revealed that 82**%** of females with raised ALT, 76.7% with raised AST and 17%with high level of total Bilirubin. Martinefski and his coworkers described that ICP is characterized by generalized skin pruritus and abnormal liver function in 2012. They studied 38 healthy pregnant women and 32 ICP patients in the third trimester of pregnancy. The biochemical parameters such as determinations of the levels of total bilirubin(0.83± 0.0 8), AST(92.0±13.2), ALT(128±25∗), and ALP(674±50∗∗) were increased in ICP women with high pruritus score.(Martinefski et al. 2012)

Total bile acid is an important marker for the evaluation of intrahepatic cholestasis so, in our research study the max valueof TBA (342.00) depicts the presence of intrahepatic cholestasis with mean ± standard deviation (17.9369±27.83) in pregnant females. These Findings were supported by Piechota and Jelski who conducted

their study in 2020 and described that the most sensitive biochemical marker used in the diagnostics of intrahepatic cholestasis of pregnancy is the level of total bile acids, which may be the first or the only laboratory-detected symptom. In healthy pregnant women, TBA levels are slightly and insignificantly higher than in non-pregnant women.(Piechota and Jelski, 2020) According to the past studies patients with early-stage cholestasis were typically asymptomatic and may only exhibit elevated serum alkaline phosphatase (ALP) and gamma-glutamyl transferase levels (GGT). As the condition worsens, hyperbilirubinemia can happen, which can cause liver cirrhosis, liver failure, or even death.(Lu, 2022) So, if we talk about the effect of biochemical parameters on ICP in our findings, the ALP have (p-value <0.001). The maximum value of GGT was (344) but according to our results the GGT have no significant correlation with ICP. The insignificant level of GGT would be due to any environmental and hormonal factors in concerned pregnant females but the previous study which were conducted by Luo and their fellows in 2022 revealed that, the level of GGT was significantly high in females with ICP.(Luo et al. 2022)

Out of 301 samples our statistical analysis represents the prevalence rate of ICP in which 133 (44.2%) females were diagnosed with intrahepatic cholestasis of pregnancy while the rest of 168 (55.8%) females were showed no results of ICP. While in 133 patients 23 showed jaundice (7.70%), On the other hand 168 patients present with no cholestasis and they also have no jaundice. These findings suggest an association between cholestasis and jaundice, as patients with cholestasis were more likely to have jaundice than those without cholestasis (p-value <0.001). These findings were supported by Padmaja and her research fellows who collected 550 samples of females from their institute in 2010. The mean age of these women was 28 year (20-37), in which 45(8 .2%) females were diagnosed with ICP and show no case of jaundice in which 71% diagnosed with ICP in third trimester.(Padmaja et al. 2010) Amita and co-workers in 2009 collected 892 samples of females during the period of april-2003 to march-2005 in which 83 (9%) females were diagnosed with obstetric cholestasis and the remaining 809(91%) females were showed the no results of obstetric cholestasis.(Amita et al. 2009)

There was a significant correlation of cholestasis with LFT's parameters in our research work as about 82% females with ICP were presented with high ALT, 76.7% females were diagnosed with elevated AST and ALP was raised in almost 96.2% affected females(p-value <0.001). But if we talk about the AGR during cholestasis it becomes low as compare to normal so our results have an association (p-value 0.025) between AGR and cholestasis because only one female have the high value of AGR and 108 females (81.2%) have low AGR levels.

As Joanna and his colleagues in 2020 concluded in their research that the elevated levels of biochemical markers depict the presence of intrahepatic cholestasis of pregnancy. They suggested that the TBA was diagnostically most significant to detect the presence of ICP in different trimesters of pregnancy as it was significantly high in patients possessing ICP and also indicated the abnormality in the liver function test. An approximately 2-15 folds increase in serum SGPT level was also observed in almost 60-85% cases of ICP. The elevation in serum alkaline phosphatase level was not much significant due to its production from bones and placenta.(Martinefski et al. 2012)

CONCLUSION

On the basis of our findings, we concluded that the raised level of total bile acid is a remarkable finding for the diagnosis of ICP. The hormonal changes in pregnant females would be a causative agent of ICP that also change the biochemical parameters like LFT's, A/G ratio and TBA. Our studies showed a significant correlation of ICP with jaundice and LFT's in pregnant females as well as ICP cause a decrease level in A/G ratio because of the harmful effect on liver. So, this scientific research would be a lead mark in the early diagnosis of ICP in pregnant females. Due to lack of resources we did not extend our study till hormonal profile. Further studies in 1st trimester with larger sample size and association with other parameters like hormones were suggested.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MM Write-up, ZS Sample Collection & Lab work, MSA Write-up & Lab Work, HS Write-up, RJ Proofread, HA Conceptualization and Proofread, TM Statistical analysis
All authors read and approved the final version.

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