



ASSOCIATION BETWEEN NEUTROPHIL TO LYMPHOCYTE RATIO AND LIVER FUNCTION TEST IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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ABSTRACT **Background:** Intrahepatic cholestasis of pregnancy is associated with an increased risk of adverse fetal outcome. Elevated serum bile acid levels trigger an inflammatory response. This study was conducted to find out association between neutrophil to lymphocyte ratio and liver function test in women with IHCP. **Methods:** This prospective case control study was conducted on 80 pregnant women with complain of pruritus. 40 women with IHCP were taken as cases and same number of women without IHCP taken as control. All women were subjected to Liver Function Tests, Serum Bile Acids and CBC. Absolute neutrophil count to absolute lymphocyte count was calculated and neutrophil to lymphocyte ratio was assessed in relation with serum aminotransferase and serum bile acid levels. **Results:** Not only was the mean NLR elevated in the pregnant women with cholestasis when compared to the controls, but it also predicted the severity of the cholestasis. The correlation between transaminase levels, serum bile acid levels and NLR was significant. **Conclusion:** NLR can be used as an alternative marker for diagnosis of IHCP. Although, TBA remains the gold standard, NLR can play a crucial role as initial screening tool.

KEYWORDS : Intrahepatic cholestasis of pregnancy, Neutrophil to lymphocyte ratio

INTRODUCTION

Intrahepatic Cholestasis of Pregnancy (IHCP), previously called Obstetric Cholestasis, is a cholestatic disorder characterized by pruritus without rash, elevated serum aminotransferases and bile acids in 90% cases.^{1,2} It is the most common hepatobiliary disorder during pregnancy with onset in the late second or third trimester of pregnancy and spontaneous relief of signs and symptoms within 48 hours after delivery, with laboratory abnormalities normalizing within 2-8 weeks.^{2,4} IHCP can lead to complications for both mother and fetus, such as meconium stained liquor, antepartum haemorrhage, postpartum haemorrhage, caesarean delivery, fetal distress, sudden fetal demise, preterm labor and perinatal morbidity.^{1,5}

The etiology of the disease is heterogeneous. Multiple factors have been suggested in its pathophysiology, such as dietary insufficiencies, environmental, hormonal and genetic influences.^{2,3} A personal and family history is an independent risk factor with a recurrence risk from 40-92%.⁶

One important pathogenesis responsible for the occurrence of IHCP is inflammation, to which serum bile acids are related. An elevation of serum bile acid concentration is the most sensitive marker of IHCP. They directly affect hepatocytes and stimulate the secretion of proinflammatory mediators, which causes neutrophil accumulation, extravasation, and activation.⁷

Neutrophils, lymphocytes, and monocytes play important roles in inflammation. Also neutrophil to lymphocyte ratio has been evaluated recently as an inflammatory marker in several diseases and several types of cancer. The neutrophil to lymphocyte ratio (NLR) is a marker of systemic inflammation that is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. It is an inexpensive, universal biomarker derived from a routinely performed complete blood count. Therefore it may provide prognostic and diagnostic clues to diseases related to chronic low grade inflammation.⁴ Little is known and has been studied about Neutrophil to Lymphocyte ratio and its relationship with IHCP. So this study was

done as Neutrophil to Lymphocyte ratio might be a promising alternative marker for diagnosis of IHCP.

METHODS:

This prospective case-control study was conducted at the department of obstetrics and gynaecology, SMS Medical College, Jaipur on 80 pregnant women in third trimester with complain of pruritus between Dec 2022 and Feb 2023. The inclusion criteria for cases and controls were women in third trimester with IHCP and without IHCP respectively. The exclusion criteria were pregnant women with pruritus due to preexisting skin disease, viral hepatitis, chronic liver disease, cardiovascular illness and pregnancy specific causes of elevated liver enzymes such as preeclampsia, HELLP and acute fatty liver of pregnancy, women with surgical obstructive jaundice, on drugs that effect LFT, active infection, multiple gestation, chronic inflammatory diseases or in active labour.

After applying inclusion and exclusion criteria women in third trimester with pruritus without rash were subjected to Liver Function Tests, Serum Bile Acids and CBC. The term IHCP was used if the serum transaminase level was ≥ 30 U/L and serum bile acid level ≥ 19 micromol/L with pruritus that could not be explained by any other condition. Absolute neutrophil count to absolute lymphocyte count was calculated and neutrophil to lymphocyte ratio was assessed in relation with serum aminotransferase and serum bile acid levels. Data was collected and compiled, statistical analysis was done.

RESULTS:

The study examined various demographic, socio-economic, and clinical factors among two groups: cases and controls, each consisting of 40 individuals. The study found no significant difference between age groups, gestational age at onset of pruritus, parity and socio-economic status among cases and controls.

Out of 40 cases, 18 were primigravidas while 27 were multiparous women.

Table 1: Demographical parameters in cases and controls

Parameters	Case (N = 40)	Control (N = 40)	P- value
Age (years)	25.9±3.24	26.3±4.37	0.091
Gestational age at onset of pruritus (weeks)	33.60±2.47	34.26±1.91	0.070
Gravida			
G1	13(33%)		0.391
G2	19(48.002%)		
≥G3	8(20%)		
Para			
P0	19(48%)	18(45%)	0.849
P1	18(45%)	15(38%)	
≥P2	3(8%)	4(10%)	
Abortion			
A1	9(23%)	8(20%)	0.917
≥A2	4(10%)	2(5%)	
Socio-economic status	15(37.5%)	12(30%)	0.273

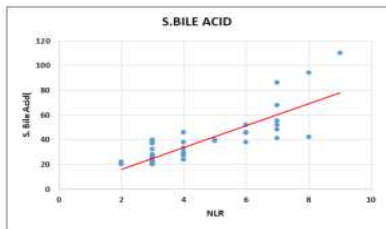
There is no difference between demographic and obstetric data between two groups as shown in Table 1.

Table 2: NLR and Transaminase levels and Serum bile-acid levels in cases and controls

	Case	Control	P-value
NLR	4.63±1.89	3±1.09	<0.007
NLR category			
≤3.5	16(40%)	29(72.50%)	<0.007
>3.5	24(16%)	11(27.50%)	
ALT(U/I)	226.83±106.04	23.43±5.63	<0.001
AST(U/I)	213.9±108.73	22.35±5.52	<0.001
Serum Bile-acid	39.39±20.27	4.50±2.20	<0.001

The neutrophil-to-lymphocyte ratio (NLR) was significantly different, with mean NLR of 4.63 ± 1.89 in cases compared to 3 ± 1.09 in controls (p<0.001). Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels were significantly higher in cases (p<0.001 for both), with mean SGOT levels of 213.9 ± 108.73 U/L in cases versus 22.35 ± 5.52 U/L in controls and mean SGPT levels of 226.83 ± 106.04 U/L in cases versus 23.43 ± 5.63 U/L in controls. Mean serum bile acid levels were significantly higher in cases (39.39 ± 20.27 micromol/L) than in controls (4.50 ± 2.20 micromol/L) (p<0.001). The study found significant association between NLR and transaminase levels, with higher values in cases indicating potential clinical relevance. The study also found significant association between NLR and serum bile acid levels as shown in figure 1.

Figure 1: Scatter diagram depicting positive correlation between Serum Bile Acid levels and NLR among cases and controls



The ROC curve analysis was also performed to assess the predictive value of NLR in IHCP. The area under curve was 0.766 (Figure 2). The best cut off value in predicting IHCP was 3.5, with sensitivity of 60%, specificity 72.5%, PPV 60% and NPV 72.5% are shown in Table 3.

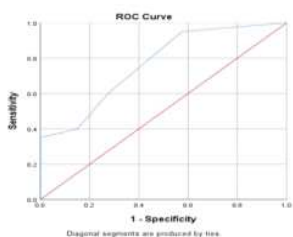


Figure 2: ROC curve analysis showing diagnostic performance of NLR in predicting IHCP in cases and controls (n=80).

Table 3: Parameters of NLR with 95% of Confidence Level among Cases and Controls

Parameters	Value (95% CI)
Cutoff (p value)	≥3.5 (<0.001)
AUROC	0.766 (0.664 - 0.868)
Sensitivity	60%
Specificity	72.5%
PPV	60.0%
NPV	72.5%
Positive Likelihood Ratio (LR+)	2.18
Negative Likelihood Ratio (LR-)	0.55
Diagnostic odds ratio	3.95

DISCUSSION:

IHCP is a liver disease of pregnancy that increases maternal and fetal morbidity therefore, early diagnosis and assessment of the severity of the disease is important to improve maternal and fetal outcome. In this study, the associations between NLR with the severity of IHCP was studied. A cutoff value of 3.5 (pvalue <0.001) was identified as significant for distinguishing cases from controls. The AUROC value reaffirms the good discriminative power of NLR. Sensitivity is 60%, while specificity is 72.5%. The positive predictive value (PPV) and negative predictive value (NPV) are 60.0% and 72.5%, respectively. The positive likelihood ratio (LR+) is 2.18, suggesting that women with the condition are about twice as likely to have a high NLR, whereas the negative likelihood ratio (LR-) is 0.55, indicating a moderate decrease in the probability of the condition when NLR is below the cutoff. The diagnostic odds ratio of 3.95 means that the odds of having the condition are almost four times higher with a positive NLR test.

Mansuri T et al¹ in 2022 performed the ROC curve analysis to assess the predictive value of NLR in IHCP. The area under curve was 0.785 and the best cut off value in predicting IHCP was 3.3, above which the sensitivity of 74%, specificity 73%, PPV 73.6%, NPV 74.2% which is also in line with the study done by Kirbas A et al⁸ in 2014. The study conducted by Kirbas A et al⁸ in 2014 showed the best cutoff value of NLR 2.93. The ratio above this value had sensitivity 91% sensitivity, 84% specificity, 83% positive predictive value and 91% negative predictive value for the diagnosis of IHCP.

Similar to above studies, in present study the ROC curve and associated tables demonstrate that the Neutrophil-Lymphocyte Ratio (NLR) is a valuable diagnostic tool with a good ability to discriminate between cases and controls. An AUC of 0.766, along with significant sensitivity (60%) and specificity (72.5%), confirms the reliability of NLR as a marker. The high diagnostic odds ratio further supports its diagnostic utility. These findings suggest that NLR can be effectively used in clinical settings to differentiate between patients with and without the condition, with a cutoff value of 3.5 providing optimal diagnostic performance.

CONCLUSION:

The neutrophil to lymphocyte ratio (NLR) is a marker of systemic inflammation. As per results of the study, with a cut off value of 3.5, NLR can be used as an initial screening tool for the diagnosis of IHCP. Whilst, TBA remains the gold standard for IHCP, NLR can play a crucial role as initial screening tool due to its high sensitivity, easy accessibility and cost effectiveness and thereby early intervention can reduce adverse pregnancy outcome. The sample size of the study was small for the result to be significant enough to be applicable to the general population, so to improve the reliability and applicability of the findings, more multi-centric trials need to be done.

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