

NEUTROPHIL LYMPHOCYTE RATIO (NLR) AS A BIOMARKER IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

Obstetrics & Gynaecology

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ABSTRACT

Introduction: Intrahepatic cholestasis of pregnancy (IHCP) is a reversible liver disorder marked by pruritus and elevated serum bile acid (SBA) levels, typically occurring in late pregnancy. It is linked to adverse maternal and fetal outcomes, making early diagnosis essential. However, SBA testing is costly and not always readily available. The neutrophil-to-lymphocyte ratio (NLR) is being explored as a potential alternative diagnostic marker. **Objectives:** This study aimed to assess the diagnostic utility of NLR in IHCP and its correlation with SBA levels. **Methods:** A prospective observational study was conducted at Dr. KNS Memorial Institute of Medical Sciences, Barabanki, over 18 months including 140 antenatal women (≥ 24 weeks gestation) 70 with IHCP (SBA $> 10 \mu\text{mol/L}$) and 70 healthy controls. Exclusion criteria included pre-existing liver disease, multiple pregnancies, or metabolic disorders. Laboratory tests included complete blood count (CBC) and liver function tests. Statistical methods included chi-square tests, Spearman's correlation, and ROC analysis to evaluate NLR's diagnostic accuracy. **Results:** Most participants were aged 26–30 years, with similar BMI and parity ($p > 0.05$). IHCP cases had significantly higher leukocyte, neutrophil counts, AST, ALT, and ALP levels ($p < 0.001$), and lower lymphocyte counts ($p < 0.001$). NLR was significantly elevated in IHCP cases (≥ 3.25 in 94.3% vs. 32.9% in controls, $p < 0.001$). ROC analysis confirmed NLR's high sensitivity and specificity. **Conclusion:** NLR is a cost-effective, accessible alternative to SBA testing for IHCP diagnosis. An NLR cut-off of ≥ 3.25 showed high diagnostic accuracy, highlighting its potential utility in resource-limited settings.

KEYWORDS

intrahepatic cholestasis of pregnancy, bile acids, neutrophil-to-lymphocyte ratio, diagnostic biomarker, pregnancy complications, liver function

INTRODUCTION

Numerous studies link systemic inflammation to the pathophysiology of IHCP, and several biochemical and hematological markers may be used to measure it. Despite the identification of new disease-specific biomarkers, many of them—such as the measurement of total bile acid (TBA) concentration—are expensive, time-consuming, and not commonly accessible in hospitals. The neutrophil-to-lymphocyte ratio (NLR) has become a promising new inflammatory biomarker in recent years. It has been linked to a number of negative outcomes in various diseases, especially obstetric complications like intrahepatic cholestasis of pregnancy, gestational diabetes mellitus, and preeclampsia. The vast variations in the frequency of these outcomes, however, are probably caused by variations in the definition of IHCP, the small sample size in these studies, and modifications in the treatment of cholestasis throughout time.

A basic complete blood count (CBC) test, which is widely available, reasonably priced, and straightforward to do in a variety of clinical settings, may be used to calculate the NLR. A recent research on the predictive utility of the CBC revealed that the distribution of maternal serum red blood cells, NLR, white blood cell count, absolute lymphocyte count, and absolute neutrophil count might help doctors estimate when a patient would be born if they were at risk of preterm birth. Blood cell subtype ratios, like the NLR, may be useful in prognosticating illnesses linked to persistent low-grade inflammation, according to recent data.

To diagnose IHCP, SBA is required which is an expensive test and not readily available at many centres. As levels of SBA determine the time of delivery of patient and good fetal outcome, it is done at regular intervals, which again is troublesome to patient. Thus, we need a cost-effective test that is easily available and can be used as diagnostic tool to guide clinicians in making a diagnosis of IHCP and appropriate time of delivery. This study thus focuses on finding a cost-effective, easily available biomarker. NLR being an inflammatory marker can be used as a potential alternative biomarker to diagnose chronic low-grade inflammatory conditions like IHCP.

AIM

To evaluate efficacy & diagnostic accuracy of Neutrophil Lymphocyte Ratio in Intrahepatic Cholestasis of pregnancy.

MATERIALS AND METHODS

This was a prospective case-control study and conducted on patients of all antenatal patients in Department of Obstetrics and Gynaecology, Dr. KNS Memorial Institute of Medical Sciences, Barabanki in period of 18 months.

INCLUSION CRITERIA

Case: All antenatal patients with IHCP (Total Bile Acid $> 10 \mu\text{mol/L}$)

3,4

Control: All Healthy antenatal women ≥ 24 weeks of gestation.

EXCLUSION CRITERIA

- Presence of genetic disease & congenital fetal anomalies.
- Presence of Chronic hypertension, multiple pregnancies, and type 2 Diabetes.
- Women with underlying skin disorders, allergies, preeclampsia, HELLP syndrome, acute fatty liver of pregnancy, chronic liver diseases (symptomatic cholelithiasis, cholecystitis, primary biliary cirrhosis), and viral hepatitis.
- Women with history of smoking and alcohol intake.

A total of 140 patients among which 70 were cases and 70 were controls were included in this study. The sample size was calculated as per the article by Mansuri et al (Sensitivity = 78%).

Once consent was obtained, a detailed medical history was collected from each subject. Following the detailed history, a comprehensive clinical examination was performed. After the clinical examination, routine antenatal investigations were conducted. These investigations included a series of blood tests, among which Liver Function Tests (LFTs) and Complete Blood Count (CBC) was our main key components. LFTs were performed to evaluate the liver's condition, as liver dysfunction is a significant concern in IHCP. The LFTs provided vital information regarding liver health by measuring bilirubin levels and a number of enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST).

The CBC provided detailed information on various blood components, including neutrophils and lymphocytes, which were crucial for calculating the NLR. The NLR was manually calculated based on the

values obtained from the CBC, with particular attention given to the ratio between neutrophils and lymphocytes. CBC analysis was performed on all collected blood samples using a 5-part hematological analyzer.

For the specific analysis of Neutrophil Lymphocyte Ratio (NLR) and Total Bile Acids (TBA), a fasting blood sample of 4 ml was collected from each participant. The blood sample was then divided into two portions: 2 ml was transferred into an EDTA vacutainer for the estimation of NLR, and the remaining 2 ml was placed in a plain vacutainer for TBA estimation.

In parallel, bile acid analysis was carried out using a Beckman Coulter Clinical Chemistry Analyzer. This analysis involved processing the blood samples to quantify levels of bile acids, an important parameter in assessing IHCP. The data obtained from both the NLR calculation and the bile acid analysis were meticulously recorded and later analyzed to explore the potential of NLR as a biomarker in diagnosing and managing IHCP.

All participants gave their informed permission. The Institutional Ethical Committee granted ethical approval for the project. The privacy of the patients who took part was preserved.

DATA ANALYSIS:

Version 28.0 of the Statistical Package for Social Sciences (SPSS) was used to perform statistical analyses. For precise documentation, every piece of data was painstakingly entered into Microsoft Excel. To give a clear picture of the distribution, the data were summed up as mean and standard deviation. To ascertain the relationship between categorical variables, proportions were compared using the chi-square test. To evaluate the relationship between quantitative variables, Spearman's rank correlation analysis was applied. By setting the criterion of statistical significance at $p < 0.05$, the results were guaranteed to be statistically significant. To further evaluate the precision and dependability of the results, sensitivity, specificity, predictive values, and false-positive and false-negative rates were computed.

RESULTS

Among the 140 antenatal women, the majority in both the case 39 (55.7%) and control 38 (54.3%) groups were aged 26–30 years. Age distribution did not show significant difference between groups across all age categories ($p = 0.560$). Mean age was slightly lower in cases (28.16 ± 3.7 years) compared to controls (28.96 ± 3.2 years), but this difference was not statistically significant ($p = 0.178$). In both groups, the majority of antenatal women had BMI of 25.0 – 29.9 kg/m², with 47 (67.1%) in case group & 51 (72.9%) in control group. The distribution of BMI categories showed no significant difference between groups ($p = 0.744$). The mean BMI was slightly higher in cases (27.13 ± 2.7 kg/m²) compared to controls (26.85 ± 2.2 kg/m²), but this difference was not statistically significant ($p = 0.529$).

For cases, the majority were gravida 1 (32.9%), followed by gravida 2 (31.4%), gravida 3 (25.7%), gravida 4 (4.3%), and gravida 5 (5.7%). Similarly, in controls, gravida 1 was the most common (45.7%), followed by gravida 2 (32.9%), gravida 3 (14.3%), gravida 4 (5.7%), and gravida 5 (1.4%). The distribution of gravidas among cases and controls showed no statistically significant differences ($p > 0.05$) across all categories. Hemoglobin levels were similar between both groups (10.68 ± 1.2 vs. 10.74 ± 1.3 g/dL, $p = 0.787$), showing no significant difference ($p > 0.05$). Total leukocyte count, neutrophil count, neutrophil-to-lymphocyte ratio (NLR), AST, ALT, and ALP levels were significantly higher in cases compared to controls ($p < 0.001$). Conversely, lymphocyte count was significantly lower in cases than in controls ($p < 0.001$). Serum bile acid levels were reported only in cases (25.2 ± 8.1 μ mol/L), with no data for controls.

In the case group (figure 1), 66 antenatal women (94.3%) had a Neutrophil Lymphocyte Ratio (NLR) of ≥ 3.25 , while only 23 antenatal women (32.9%) in the control group fell into this category. In contrast, 4 antenatal women (5.7%) from the case group had an NLR of < 3.25 , compared to 47 antenatal women (67.1%) in control group. P-value of < 0.001 highlighted statistically significant difference between 2 groups ($p < 0.05$). The mean serum bile acid level in the NLR category < 3.25 was 16.1 ± 3.1 , while in the NLR category ≥ 3.25 , it was 25.45 ± 8.3 . The p-value of 0.029 indicated that there was significant association between NLR categories and serum bile acid levels, as the value was lesser than 0.05 ($p < 0.05$). The cases that had higher NLR had high S.Bile acid levels.

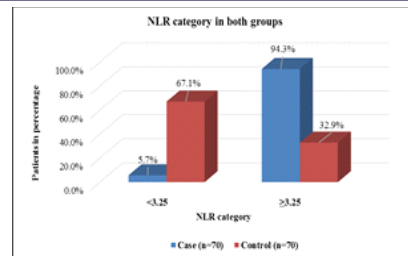


Figure 1 Neutrophil Lymphocyte ratio in Intrahepatic Cholestasis of pregnancy studied antenatal women

The diagnostic accuracy of the Neutrophil-Lymphocyte Ratio (NLR) (table 1, figure 2) for Intrahepatic Cholestasis of Pregnancy (IHCP) showed a cutoff value of 3.25. In figure 7 area under receiver operating characteristic curve (AUROC) was 0.888 (95% CI: 0.835-0.942), with p-value of < 0.001 , indicating high statistical significance. Sensitivity was 84.3%, specificity was 78.1%, and the overall diagnostic accuracy was 88.8%, demonstrating NLR's strong potential as a diagnostic tool for IHCP.

Table 1 Diagnostic accuracy to NLR in Intrahepatic Cholestasis of pregnancy (IHCP)

PARAMETERS	VALUE
CUTOFF	3.25
AUROC	0.888 (0.835-0.942)
P-VALUE	<0.001
SENSITIVITY	84.3%
SPECIFICITY	78.1%
DIAGNOSTIC ACCURACY	88.8%

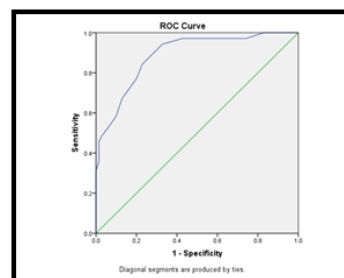


Figure ROC curve analysis showing diagnostic performance of NLR in predicting IHCP in studied antenatal women

DISCUSSION

Cholestasis is characterized by a build-up of bile components in the blood and a disruption in the flow of bile owing to blockage in the bile ducts or hepatocyte excretory failure. Bile flow from the liver to the duodenum is hindered in this scenario. The most prevalent pregnancy-related liver disease is called intrahepatic cholestasis of pregnancy (IHCP), also known as obstetric cholestasis. It is characterized by pruritus, primarily on the palm and sole, elevated serum aminotransferases, and bile-acid levels. It usually begins in the second or third trimester of pregnancy and goes away on its own within four to six weeks after delivery [1]. It can cause problems for both the mother and the fetus and usually manifests as bothersome itching.

The distribution of study population based on age revealed that the mean age was slightly lower in cases (28.16 ± 3.7 years) compared to controls (28.96 ± 3.2 years), but this difference was statistically insignificant ($p > 0.05$). Our findings followed the findings of Das S and Roy A [2] who reported a mean age of 25.25 ± 3.61 years in IHCP patients, similarly, Naga VKM et al [3] and Yadav R et al [4] also reported that the mean age of the patients in their study group was 27.40 ± 2.40 years and 26.09 ± 4.90 years respectively. The mean BMI was slightly higher in cases (27.13 ± 2.7 kg/m²) compared to controls (26.85 ± 2.2 kg/m²), but this difference was statistically insignificant ($p > 0.05$).

Multigravida patients made up the majority. When the parity of the two groups was examined, there was no discernible difference between them ($p = 0.221$). With 42 (60.0%) in the case group and 46 (65.7%) in

the control group, the majority of patients in both groups were P0 (nulliparous). The median gravida number of IHCP cases and the control group was 2. In both groups, the median parity number was 1. IHCP patients and the control group had median gestational weeks of 37 and 39.3, respectively, and the difference was not statistically significant ($p>0.05$). Arora S et al [5] and Padmaja M et al [6] said that there was no statistically significant difference between the groups in terms of patient parity or age. According to Mansuri T et al [7], the IHCP patients had a mean POG of 34.5 weeks and were diagnosed between 28 and 39 weeks. Thirty-seven primigravida and fifty-three multiparous women made up the 90 cases.

The present study concluded that, 66 patients (94.3%) from the case group had a Neutrophil Lymphocyte Ratio (NLR) of ≥ 3.25 compared to 47 patients (67.1%) in the control group that had an NLR of <3.25 , ($p<0.001$). A cut-off value of 3.25 was found for the diagnostic accuracy of the Neutrophil-Lymphocyte Ratio (NLR) for Intrahepatic Cholestasis of Pregnancy (IHCP). A p-value of less than 0.001 and an area under the receiver operating characteristic curve (AUROC) of 0.888 (95% CI: 0.835-0.942) indicated significant statistical significance. Sensitivity was 84.3%, the specificity was 78.1%, and the overall diagnostic accuracy was 88.8%, demonstrating NLR's strong potential as a diagnostic tool for IHCP. Our findings were aligned with the findings of Mansuri T et al [8]. He stated that the predictive value of NLR in IHCP was also evaluated using the ROC curve analysis. The sensitivity was 74%, specificity was 73.0%, PPV was 73.6%, and NPV was 74.2% over the optimal cut-off value of 3.3 for predicting IHCP, which was the area under the curve (0.785). Our results are similar to studies done by Kirbas A et al [9] and Silva J et al [10] who also found raised NLR in cases of IHCP. The study by Kirbas A et al [9] further displayed the optimal cut-off value of 2.93. For diagnosing IHCP, the ratio above this amount showed a sensitivity of 91.0%, specificity of 84.0%, positive predictive value of 83.0%, and negative predictive value of 91.0%.

CONCLUSION

The results of this study contribute to the growing amount of data showing that NLR may be used as a substitute marker for IHCP diagnosis. According to the study, ≥ 3.25 was the ideal cut-off value for NLR in the diagnosis of IHCP. A p-value of less than 0.001 and an area under the receiver operating characteristic curve (AUROC) of 0.888 (95% CI: 0.835-0.942) indicated significant statistical significance. Sensitivity was 84.3%, specificity was 78.1%, and the overall diagnostic accuracy was 88.8%, highlighting NLR's strong potential as a diagnostic tool for IHCP. The consistency with existing literature strengthens the validity of this marker, supporting its routine use in diagnosing IHCP. However, to further validate these findings and apply them to the general population, additional research through multicenter trials is recommended.

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