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Original Article

Pathology Section

Utility of Neutrophil to Lymphocyte Ratio in Hypertensive Disorder of Pregnancy: A Case-control Study

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ABSTRACT

Introduction: Hypertensive Disorders of Pregnancy (HDP), encompasses gestational hypertension, Preeclampsia and eclampsia, remain leading causes of maternal and perinatal morbidity and mortality globally. Among several biomarkers under investigation, the Neutrophil-To-Lymphocyte Ratio (NLR) has emerged as a potential indicator of systemic inflammation, with relevance in predicting disease severity and outcomes in HDP.

Aim: To evaluate the utility of NLR as a biomarker in HDP.

Materials and Methods: This prospective case-control study was conducted in the Department of Obstetrics and Gynaecology, Government Medical College and Rajindra Hospital, Patiala, Punjab, India, from January 2021 to December 2021 (12 months). The study population included 100 pregnant women diagnosed with HDP (gestational hypertension, Preeclampsia, eclampsia, chronic hypertension and preeclampsia superimposed on chronic hypertension) and 100 normotensive pregnant women as controls. Complete Blood Count (CBC) were analysed and NLR was calculated. Data were compiled in Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., USA). Continuous variables were expressed as mean±Standard Deviation (SD), while categorical variables were presented as frequencies and percentages. Comparison between two groups was performed using independent sample t-test for normally distributed variables or the Mann-Whitney U test for non parametric data. Categorical variables were compared using the Chi-square test or Fisher's-exact test, as appropriate. The association between NLR and the severity of HDP was assessed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

Results: The mean NLR was significantly higher in hypertensive pregnant women (mean±SD: 3.69±0.95) compared to normotensive controls (2.81±0.15). Among cases, patients with preeclampsia and eclampsia had higher NLR values (4.23±0.62 and 5.00±0.00, respectively) than those with gestational hypertension (3.00±0.25). A positive association was observed between NLR levels and disease severity (p<0.001), with the highest values seen in severe preeclampsia and eclampsia (4.23±0.62 and 5.00±0.00, respectively).

Conclusion: The NLR is a simple, inexpensive and readily available biomarker that may serve as an early predictive indicator for hypertensive disorders of pregnancy. Its integration into routine antenatal screening could enhance risk stratification and improve clinical management.

Keywords: Biomarker, Eclampsia, Gestational hypertension, Inflammation, Preeclampsia

INTRODUCTION

The HDP remain among the most significant contributors to maternal and perinatal morbidity and mortality worldwide. They encompass a spectrum of conditions, including gestational hypertension, preeclampsia, eclampsia and chronic hypertension with superimposed preeclampsia. Globally, HDP complicates approximately 5-10% of all pregnancies and is responsible for nearly 18% of maternal deaths in developing countries [1].

Preeclampsia is characterised by new-onset hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) after 20 weeks of gestation, accompanied by proteinuria or evidence of end-organ dysfunction. Despite advancements in prenatal care, the exact pathogenesis of preeclampsia remains elusive. However, increasing evidence supports the role of systemic inflammation and endothelial dysfunction in its aetiology [2].

One of the emerging markers of systemic inflammation is the NLR, which is calculated from routine CBC parameters. NLR has been extensively studied as a prognostic marker in various inflammatory and cardiovascular diseases and recent attention has turned toward its role in obstetric complications, especially HDP [3,4].

Neutrophils play a central role in innate immunity and are known to increase in response to stress and inflammation. Conversely, lymphocytes, which are crucial for adaptive immunity, may decrease under stress-induced immunosuppression. Thus, the NLR serves as a composite index reflecting both immune activation and physiological stress response [5].

Several studies have demonstrated that women with preeclampsia exhibit higher NLR values compared to normotensive pregnant women. Moreover, elevated NLR has been associated with disease severity and adverse outcomes, suggesting that it may serve not only as a diagnostic but also as a prognostic biomarker [6,7]. In resource-limited settings, where access to sophisticated laboratory testing is limited, the utility of NLR becomes especially valuable. Since it can be derived from a simple CBC, it offers a cost-effective tool for early identification of at-risk pregnancies, facilitating timely intervention and improving maternal-foetal outcomes [8]. Despite promising preliminary findings, data from Indian populations remain limited.

To compare the mean NLR between pregnant women with hypertensive disorders and normotensive controls; to assess the association between NLR values and the severity of HDP; and to determine the potential role of NLR as a predictive biomarker for identifying severe forms of the disease, such as severe preeclampsia and eclampsia.

In the present study, the potential of NLR as a predictive biomarker for identifying severe forms of HDP was assessed by comparing the mean NLR values across the different clinical categories of HDP, namely gestational hypertension, mild preeclampsia, severe preeclampsia

and eclampsia. A progressive and statistically significant increase in NLR values was observed with increasing disease severity, with the highest values noted among women with eclampsia. This demonstrated a positive association between elevated NLR and worsening disease state. Additionally, comparison between women with and without HELLP syndrome also showed significantly higher NLR values in the HELLP group, further supporting the role of NLR in identifying more severe maternal pathology. Therefore, the study findings confirm that higher NLR values are associated with more severe forms of HDP, indicating that NLR can serve as a useful predictive biomarker for disease severity.

MATERIALS AND METHODS

The present prospective case-control study was conducted in the Department of Obstetrics and Gynaecology at Government Medical College and Rajindra Hospital, Patiala, Punjab, India, over a period of one year from January 2021 to December 2021. The hospital is a tertiary care centre catering to a diverse patient population from both rural and urban regions of North India. The study was approved by the Institutional Ethics Committee (IEC No. BFUHS/2K21P-TH/14847).

The study comprised two groups:

1. Cases: 100 pregnant women diagnosed with HDP, including gestational hypertension, preeclampsia and eclampsia.
2. Controls: 100 normotensive pregnant women matched for gestational age.

Inclusion and Exclusion criteria: The study included pregnant women aged 18-40 years with a gestational age of ≥20 weeks and singleton pregnancies. Exclusion criteria included a history of chronic hypertension, renal disease, diabetes mellitus, autoimmune disorders, pre-existing haematological diseases, multiple pregnancies, any ongoing infection or inflammatory condition and a history of corticosteroid or immunosuppressive therapy within the preceding month.

Sample size calculation: The sample size was calculated assuming the prevalence of hypertensive disorders of pregnancy of 7% (range 5-17%), as per the study by Dhinwa M et al., [9]. The other parameter considered for sample size was calculation was 95% confidence level. The following formula was used for sample size as per the study by Charan J and Biswas T [10]:

$$N = \frac{Z_{1-\alpha/2}^2 P(1-P)}{D^2}$$

Where:

n = Sample size

Z1-α/2 = Z statistic for a level of confidence level= 1.960

P = Expected prevalence/proportion of outcome= 0.07 (7%)

d = Precision= 0.05

The required sample size as per the above-mentioned calculation was 99. To account for a non participation or loss-to-follow-up rate of about 1 %, another 1 , subjects were added to the sample size. Hence, the final required sample size was 100 participants per group, resulting in a total of 200 participants.

Study Procedure

A detailed history, including maternal age, parity, gestational age and presenting complaints, was recorded. Blood pressure was measured using standard sphygmomanometer and proteinuria was assessed by a dipstick test or 24-hour urine protein estimation.

Proteinuria was determined using the reagent strip test, in which the reagent area of the strip is coated with an indicator and buffered to an acidic pH that changes colour in the presence of proteins. Proteinuria with reagent strip test is as: negative, trace, 1+ (30 mg/dL), 2+ (100 mg/dL), 3+ (300 mg/dL) and 4+ (≥2000 mg/dL). Proteinuria is a key diagnostic marker of preeclampsia and helps differentiate it from gestational hypertension. Including it ensured accurate disease classification and allowed assessment of the association between NLR and disease severity.

Hypertensive Disorders of Pregnancy (HDP) was categorised as follows [11]:

a) **Gestational Hypertension:** BP ≥140/90 mmHg after 20 weeks of gestation without proteinuria.

b) **Preeclampsia:** BP ≥140/90 mmHg with proteinuria (≥300 mg/24 hours). Preeclampsia was further categorised as: (i)

Mild (Non severe) Preeclampsia: Systolic BP ≥140 mmHg, diastolic BP 90-110 mmHg and proteinuria 1+.

Severe Preeclampsia: Systolic BP ≥160 mmHg and/or diastolic BP ≥110 mmHg, plus one of the following: proteinuria >1+, headache, visual disturbances, upper abdominal pain, oliguria, thrombocytopenia (<1 lakh/mm^3), or marked elevation of serum transaminases (AST or ALT).

c) **Eclampsia:** Preeclampsia complicated by generalised tonic-clonic seizures.

d) **Haemolysis, Elevated Liver enzymes and Low Platelets (HELLP) syndrome:** A multisystem disorder characterised by evidence of haemolysis, hepatic dysfunction and thrombocytopenia.

A total of 3 mL of venous blood was collected under aseptic conditions into an Ethylenediaminetetraacetic Acid (EDTA) vial. CBC was performed using an automated haematology analyser (Sysmex XP-100). Parameters recorded included Total Leukocyte Count (TLC), Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC). Absolute leukocyte counts were calculated using the formula: percentage of leukocytes × total leukocyte count/mL. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Reference range for NLR was taken as 2.6-3.5 [12].

STATISTICAL ANALYSIS

Data were compiled in Microsoft Excel and analysed using SPSS version 26.0 (IBM Corp., USA). Continuous variables were expressed as mean±SD, while categorical variables were presented as frequencies and percentages. Comparison between two groups was performed using the independent sample t-test for normally distributed variables or the Mann-Whitney U test for non parametric data. Categorical variables were compared using Chi-square test or Fisher's-exact test, as appropriate. The association between NLR and the severity of HDP was assessed using the Chi-square test. A p-value of <0.05 was considered statistically significant. The investigators were blinded to the case-control status during laboratory evaluation.

RESULTS

A total of 200 pregnant women were included in the study, comprising 100 women diagnosed with HDP (cases) and 100 normotensive pregnant women (controls).

The majority of the study population in both the groups fell in 19-29-years age group (cases=65; control=77). The age ranged from 20 to 37 years in cases and from 18 to 39 years in control group. The mean age was 27.79±3.70 years in cases and 26.09±4.16 years in control, respectively. The majority of the cases were primigravida (83%), whereas the majority of control was multigravida (73%). The majority of the women in both the groups have period of gestation more than 36 F weeks (cases=46; controls=64).

The most common clinical feature seen in the cases was headache (96%), followed by visual disturbances (45%). The other clinical features were pedal oedema (24%), seizures (7%), nausea (1%) and other symptoms (6%), like epigastric pain and vomiting.

The mean systolic and diastolic blood pressure in the case group was 158.26±6.39 mmHg and 99.52±13.48 mmHg, respectively. The mean systolic and diastolic blood pressure in the control group was 119.1±7.70 mmHg and 76.11±6.83 mmHg, respectively. The majority of patients in the case group had 2+ proteinuria (75%), whereas no proteinuria was observed in the control group.

Most cases in the study population were diagnosed with mild preeclampsia (77%), followed by severe preeclampsia (10%). The categorisation of the case group in different subgroups given in [Table/Fig-1].

Clinical diagnosis	No. of cases
Gestational hypertension	7
Mild preeclampsia	77
Severe preeclampsia	10
Eclampsia	4
HELLP	2

[Table/Fig-1]: Categorisation of the case group in different subgroup (n=100).

Leukocytosis was observed in cases (mean TLC=20,166 cells/mm^3) compared to control (mean TLC=14,741 cells/cumm). NLR was also higher in cases (mean NLR=3.69) as compared to control (mean NLR=2.81). The haematological parameters of the study population shown in [Table/Fig-2].

Parameters	Case (n=100)	Control (n=100)
	Mean±SD	Mean±SD
TLC (cmm) (Normal= 6000-16000)	20166.00±2854.03	14741.00±11200.25
ANC (cmm) (Normal= 3500-10000)	15890.00±3452.22	9947.30±934.68
ALC (cmm) (Normal= 1000-3600)	4127.40±898.94	3464.50±447.45
NLR (Normal=2.6-3.5)	3.69±0.95	2.81±0.15

[Table/Fig-2]: Haematological parameters of the study population.

The TLC was found to increase with the severity of the disease. As the severity of the disease increased, NLR value also raised with mean NLR values of 3.00, 3.47, 4.23 and 5.00 in gestational hypertension, mild preeclampsia, severe preeclampsia and eclampsia, respectively. The ALC shows a rising trend from gestational hypertension to severe preeclampsia, followed by a decline in eclampsia. The comparison of the various parameters as the severity of the disease increase shown in [Table/Fig-3].

Parameters (Mean±SD)	GH (n=7)	Mild PE (n=77)	Severe PE (n=10)	Eclampsia (n=4)
TLC (cmm)	16714.0± 1318.37	19596.0± 2017.45	23420.0± 2615.25	26250.0± 288.68
ANC (cmm)	12372.0± 1149.52	15332.0± 2951.59	18630.0± 2419.38	22312.0± 245.37
ALC (cmm)	4093.70± 372.03	4325.70± 454.27	4416.50± 785.79	3937.50± 43.30
NLR	3.00±0.25	3.47±0.40	4.23±0.62	5.00±0.00

[Table/Fig-3]: Comparison of the various parameters of the study population as the severity of the disease increase.

Comparison of haematological parameters between women with HELLP syndrome and those without HELLP syndrome revealed that NLR values were significantly higher in the HELLP group, with high statistical significance (p<0.001), as shown in [Table/Fig-4].

Parameters (Mean±SD)	Cases without HELLP syndrome (n=98)	Cases with HELLP syndrome (n=2)	p-value
TLC (cmm)	20052.0±2766.97	25750.0±353.55	0.033
ANC (cmm)	15742.0±3323.56	23175.0±318.19	0.002
ALC (cmm)	4159.00±879.79	2575.00±35.36	0.006
NLR	3.58±0.57	9.00±0.00	<0.001

[Table/Fig-4]: Comparison of various parameters between patient with HELLP syndrome and those without HELLP syndrome.
*Data are presented as mean±SD. Comparison between the two groups (HELLP vs. non HELLP) was performed using the Independent Student's t-test for continuous variables and the Chi-square test for categorical variables. A p-value <0.05 was considered statistically significant. p<0.001 indicates highly significant difference.

DISCUSSION

Hypertensive disorders of pregnancy represent a serious global health concern, contributing significantly to maternal and perinatal morbidity and mortality. HDP encompasses a spectrum of clinical conditions, including gestational hypertension, preeclampsia and eclampsia, which are often associated with systemic inflammation, endothelial dysfunction and coagulopathy. In this context, the NLR has gained prominence as a simple yet powerful biomarker reflecting systemic inflammatory response [3-5].

In the present study, TLC was found to increase with the severity of the disease. The ALC shows a rising trend from gestational hypertension to severe preeclampsia, reflecting increasing immune activation, followed by a decline in eclampsia, likely due to immune exhaustion, stress-induced lymphopenia and redistribution of lymphocytes. This dynamic behaviour highlights the complex immunopathogenesis of HDP.

The present study primarily focused on evaluating and comparing haematological parameters, particularly the NLR, across varying severities of HDP. While association between NLR and disease severity was assessed, a formal association analyses, such as logistic regression or multivariate analysis, was not performed. This was mainly because the study design was not aimed at establishing causal or independent associations, but rather to identify haematological trends and explore their potential as supportive biomarkers. Furthermore, although the sample size was adequate for mean comparisons and association testing, it may not have been sufficient to reliably perform multivariate modelling adjusting for potential confounders such as age, parity and gestational age. Future studies with larger sample sizes and longitudinal follow-up are warranted to evaluate independent associations between NLR and adverse maternal and foetal outcomes.

In the present study, significantly elevated NLR values were observed in pregnant women with hypertensive disorders compared to normotensive controls, affirming its potential utility as a diagnostic tool. The mean NLR in the hypertensive group was 3.69±0.95, while the control group had a mean NLR of 2.81±0.15. These findings are consistent with previous studies, including those by Serin S et al., who reported higher NLR values among women with preeclampsia [13].

The association of various haematological parameters, particularly the NLR, was studied between patients with and without HELLP syndrome to evaluate whether NLR could serve as an indicator of disease severity and multisystem involvement. HELLP syndrome (Haemolysis, Elevated Liver Enzymes and Low Platelet count) represents the most severe spectrum of HDP and is characterised by marked endothelial dysfunction, systemic inflammation and hepatic involvement. Since NLR reflects the balance between neutrophil-mediated inflammation and lymphocyte-mediated immune regulation, comparing its levels in HELLP versus non HELLP groups helps to determine its potential role as a marker of disease progression and severity. A significantly higher NLR values observed in the HELLP group (p<0.001) suggests that elevated systemic inflammation and immune dysregulation are closely associated with the pathogenesis of this severe variant of preeclampsia.

The pathophysiology of preeclampsia is closely associated with immune maladaptation and chronic inflammation. During normal pregnancy, a shift toward an anti-inflammatory immune milieu is essential for foetal tolerance. However, in preeclampsia,

an exaggerated maternal systemic inflammatory response—characterised by increased neutrophil activation and reduced lymphocyte counts—leads to vascular endothelial dysfunction [14]. As such, NLR becomes an integrated marker of these underlying cellular dynamics.

The present results also demonstrated a progressive increase in mean NLR with the severity of HDP, ranging from gestational hypertension (3.00±0.25) and mild preeclampsia (3.47±0.40) to severe preeclampsia (4.23±0.62) and eclampsia (5.00±0.00). This trend supports the findings of Kurtoglu E et al., who indicated that NLR correlates with disease severity and may serve as a prognostic marker [15]. Similarly, Dadhwal V et al., similarly found elevated NLR values in patients with severe preeclampsia and eclampsia in an Indian cohort [8].

While several biomarkers, such as C-reactive Protein (CRP), Interleukins (IL-6, IL-8), Tumour Necrosis Factor-alpha (TNF-α) and Angiogenic Factors (sFlt-1, PlGF), have been studied in preeclampsia, they are often costly and require sophisticated laboratory infrastructure. In contrast, NLR is derived from a basic CBC, making it especially valuable in low-resource settings [16,17].

The present study's strength lies in its well-defined inclusion and exclusion criteria, uniform methodology and an adequate sample size (n=200). However, it is not without limitations. Being a single-centre, cross-sectional study, it lacks long-term follow-up data. The NLR was assessed only once and serial measurements could provide more dynamic insights. Confounding variables such as nutritional status, Body Mass Index (BMI) and subclinical infections were not controlled for and could influence leukocyte counts.

Comparative studies such as those by Tzur T et al., [18] and Kurtoglu E et al., [15] have advocated for the inclusion of NLR and Platelet-to-Lymphocyte Ratio (PLR) in antenatal risk-stratification protocols. These markers, when used in combination, may enhance predictive accuracy for adverse maternal and foetal outcomes.

Emerging evidence also suggests that elevated NLR may be linked to placental dysfunction and impaired uteroplacental circulation. Histopathological studies have shown neutrophilic infiltration in placental villi and decidua in severe preeclampsia cases, indicating an inflammatory insult at the maternal-foetal interface [19].

Overall, the present study reinforces the growing body of literature supporting the role of NLR as an effective inflammatory biomarker in HDP. Its simplicity, low cost and wide availability make it a practical tool for early detection, risk stratification and monitoring of disease progression in HDP.

Limitation(s)

However, the present study has certain limitations. It was a single-centre study conducted in a tertiary care hospital, which may restrict the generalisability of the findings to broader populations. Although the sample size remained adequately powered, it was relatively small compared with large multicentric studies. NLR was measured only once during pregnancy and serial changes across gestation were not evaluated. Potential confounders such as subclinical infections, inflammatory conditions, or concurrent medications that may influence NLR were not fully accounted for. Furthermore, the study did not include long-term maternal or neonatal outcome measures, which could have provided additional insights into the prognostic value of NLR. Lastly, comparisons with other established biomarkers such as sFlt-1/PlGF ratio, LDH, or uric acid were not performed, limiting the ability to assess the relative predictive strength of NLR.

CONCLUSION(S)

The present study underscores the significant utility of the NLR as an accessible, cost-effective and informative inflammatory biomarker in HDP. The present findings revealed that NLR levels were significantly elevated in women with HDP compared to normotensive pregnant women and that these levels correlate positively with disease severity, progressively increasing from gestational hypertension to preeclampsia and eclampsia.

As NLR is derived from a routine CBC, its inclusion in antenatal screening could enable early identification of high-risk pregnancies, especially in low-resource settings where advanced diagnostic tools may be limited. NLR has the potential to serve not only as a diagnostic aid but also as a prognostic indicator, guiding timely interventions to improve maternal and foetal outcomes.

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