

Neutrophil Lymphocyte Ratio (NLR) as a Bio Inflammatory Marker in Pre-eclampsia

Khushboo Singhal¹, Arvind Kumar Pal², Sunita Tiwari³, Renu Singh⁴, Rashmi Kushwaha⁵

ABSTRACT

Introduction: Pre-eclampsia, a systemic disease unique to pregnancy, affects 3–14% of pregnant women. The aim of the present study was to evaluate Neutrophil Lymphocyte Ratio (NLR) as a bio inflammatory marker of pre-eclampsia (PE), a hypertensive disorder of pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation.

Material and Methods: This case control study was carried out over a period of 10 months after informed consent and ethical clearance. The study population included 70 pregnant women (35 pre-eclampsia cases and 35 normotensive pregnant subjects as controls). 3 ml venous blood samples were obtained from both the cases and controls. Complete blood count was done using semi-automated three part haematology analyser which gives the reading of cell counts, NLR was calculated manually.

Results: The case group (subjects with pre-eclampsia) were found to have higher neutrophil to lymphocyte ratio (NLR) than that of the control group. This difference was statistically significant ($p < 0.001$). The receiver operating curve (ROC) analysis showed significant diagnostic accuracy of NLR to discriminate cases and controls (area under the curve [AUC] = 0.73, $P < 0.001$) at cutoff value of ≥ 4.86 , 68.6% sensitivity and 80% specificity.

Conclusion: Unlike many other inflammatory markers, NLR proves to be an inexpensive and readily available biomarkers, obtained from routinely done complete blood counts that may be useful for prediction and diagnosis of pre-eclampsia.

Keywords: Neutrophil Lymphocyte Ratio, Pre-Eclampsia, Pregnancy

INTRODUCTION

Pre-eclampsia is a major cause of maternal morbidity and mortality.¹ Multiple mechanisms, including inflammation, endothelial dysfunction, angiogenesis, inappropriate placentation, oxidative stress, immunological and genetic factors, have been suggested as main components in the development of pre-eclampsia.² The clinical manifestations of PE are associated with generalized endothelial dysfunction, which causes vasoconstriction and end-organ ischemia leading to various hematological abnormalities.^{3,4} They usually manifest as mild and indolent consumptive coagulopathies, including consumption of platelets, clotting factors, and fibrinogen. Changes in the immune system are very important in the background of pre-eclampsia.⁵ Maternal circulating leukocytes are activated in pregnancy and further activated in pre-eclampsia.⁶ Neutrophils are usually thought to be the first line of defense against infection at the site of a wound, but they also infiltrate systemic vascular

tissue in women with pre-eclampsia, thus causing vascular inflammation. Lipoprotein abnormalities may be involved in the pathogenesis of pre-eclampsia. Lipids secreted by the placenta activate the leukocytes which are circulating through the intervillous space. These activated leukocytes re-enter the maternal systemic circulation and are responsible for the vascular dysfunction associated with pre-eclampsia.⁷ Several studies have demonstrated pro-atherogenic lipid profiles in women months before clinical signs of pre-eclampsia. Leukocyte activation is a major pioneer in the pathogenesis of pre-eclampsia and neutrophil activation is considered to be a major part of increased inflammatory response in maternal vascular system during pre-eclampsia.⁸ However, information related to leukocyte count and its differentials is limited in patients with pre-eclampsia. Based on these conflicting data, the studies were conducted in order to compare neutrophil to lymphocyte ratio (NLR) in pregnant women with and without pre-eclampsia.

MATERIAL AND METHODS

This was a case control study, carried out over a period of 10 months in the Department of Physiology, Pathology and Obstetrics and Gynecology of King George's Medical University, Lucknow, Uttar Pradesh from November 2017 to August 2018 after written informed consent and ethical clearance from the Institutional Ethics Committee. Pregnant females more than 18 years of age who reported to the OPD, admitted in ward and labour room of Queen Mary Hospital, KGMU were selected as study population. Total 70 subjects were recruited as the study population out of which 35 were cases of pre-eclampsia who were diagnosed based on the recently revised criteria of the American College of Obstetrics and Gynecology (2013). Normotensive pregnant females of almost same gestational age with singleton

¹Junior resident, Department of Physiology, ²Senior resident, Department of Physiology, ³Professor and Head, Department of Physiology, ⁴Professor, Department of Obstetrics and Gynaecology, ⁵Professor, Department of Pathology, King George's Medical University, Lucknow, UP, India

Corresponding author: Prof. Sunita Tiwari, Professor and Head, Department of Physiology, King George's Medical University, Lucknow, UP, India

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SN	Variables	Cases (n=35)		Controls (n=35)		Total (N=70)	
		Mean	SD	Mean	SD	't'	'p'
1-	Neutrophil	11419.66	3717.76	8731.34	1649.51	3.910	<0.001
2-	Lymphocyte	2123.66	665.91	2115.97	256.67	0.064	0.949
3-	NLR	5.64	1.78	4.19	1.00	4.198	<0.001

Table-1: Between Group Comparison of Hematological Parameters

Test Result Variable(s)	Area	SE	'p'	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
NLR	.739	.062	.001	.617	.861
Neutrophil	.751	.062	.001	.629	.873

Table-2: ROC Curve Analysis for area under curve

SN	Parameter	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
1.	NLR	≥4.86	68.6	80.0	77.4	71.8	74.3
2.	Neutrophil (/cummm)	≥9830	65.7	77.1	74.2	69.2	71.4

Table-3: Projected Cut-off values for different parameters and their corresponding diagnostic efficacy

pregnancy were taken as controls. Controls were followed till delivery and they were found normotensive as no signs and symptoms of pre-eclampsia developed. Women with chronic hypertension, established heart disease, gestational diabetes, thyroid disorders, tuberculosis, acquired infections during early stages of pregnancy, pre-existing vascular disease or malignancy, history of alcohol or tobacco use in any form were excluded from the study. Patients with history of any medication related to inflammatory condition of patient such as corticosteroids which could cause alterations in the maternal NLR values were also excluded from the study. After 8-hour overnight fasting, 3 ml of blood sample was collected and divided into two parts. One part was collected in plain vial for estimation of fasting lipid profile. Second part was collected in EDTA vial for estimation of complete blood count. Complete blood count was done using semi-automated three-part hematology analyzer which gives the reading of cell counts, neutrophil lymphocyte ratio was calculated manually.

STATISTICAL ANALYSIS

The data analysis was performed using SPSS (Statistical Package for Social Sciences) Version 21.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD. Statistical comparison was done between case and control group with respect to maternal NLR values. The optimal cut-off points of NLR to discriminate case and control groups were evaluated by ROC analysis calculating area under the curve as giving the maximum sum of sensitivity and specificity for the significant test. Sensitivity, specificity, positive and negative predictive values were also calculated at the best cut-off point for NLR. p value < 0.05 was considered as statistically significant.

RESULTS

The demographic characteristics such as age and gestational age of the subjects showed no statistically significant differences between cases and controls (p>0.05). As expected, PE cases had higher diastolic and systolic

blood pressure (p<0.001) as well as urine protein levels in comparison to healthy control group (p<0.001). The anthropometric parameters like height, weight and BMI were also measured and statistically no significant difference was found between two groups (p>0.05). Mean values of hemoglobin were compared and it showed no statistically significant difference among the groups (p>0.05). The mean value of maternal neutrophil lymphocyte ratio was measured and compared, and the difference was significantly higher in cases as compared to controls (5.64±1.78 versus 4.19±1; p<0.001) (Table 1). The receiver operating curve analysis showed significant diagnostic accuracy of NLR to discriminate cases and controls (area under the curve [AUC] = 0.73, P < 0.001) at cut-off value of ≥ 4.86, with 68.6% sensitivity, and 80% specificity (Table 2 and 3).

DISCUSSION

Pre-eclampsia is a hypertensive multisystem disorder with an unclear etiology as yet. Maternal circulating leukocytes are activated in pregnancy and further activated in PE and these activated leukocytes could be responsible for the vascular dysfunction associated with PE. In the present study, diagnostic accuracy for the prediction of PE was determined by ROC analysis. Nearly 68.6% sensitivity and 80% specificity were found at cut-off value 4.86 to discriminate pre-eclampsia cases from healthy pregnant controls. Similar to our results, a study done by Serin et al suggested maternal NLR was statistically significantly higher in PE cases than healthy pregnant females (p = 0.017).⁹ Oylumlu et al also reported NLR > 4.1 had 83.3% sensitivity and 81.5% specificity in accurately predicting a pre-eclampsia (ROC area under curve: 0.925, 95% CI: 0.878–0.973).¹⁰ Another study showed that at the cutoff values of NLR ≥3.08; the area under the ROC was 0.716 for NLR that predicted PE with the sensitivity of 74.6% and specificity of 70.1%.¹¹ Another study showing conflicting results reported no significant difference in NLR among women with severe pre eclampsia and healthy pregnant women.¹² Pre-eclampsia is at the extreme end of a continuous spectrum

of increased inflammatory response that is a hallmark of pregnancy. Leukocytes are important components of the immune system, and play an important role in the pathophysiology of pregnancy-related disorders involving decidua and placenta.¹³ Leukocytes infiltrate systemic vascular tissue in women with pre-eclampsia, thus causing vascular inflammation.¹⁴ The increase in neutrophil numbers is the result of increased levels of colony stimulating factors.¹⁵ In women with pre-eclampsia, neutrophils are activated as they circulate through the intervillous space and are exposed to oxidized lipids secreted by the placenta. Oxidized lipids are potent activators of neutrophils, leading to expression of COX-2 which regulates the release of thromboxane, TNF and superoxide.¹⁶ Thus, neutrophils obtained from pre-eclamptic females express significantly more COX-2 than healthy pregnant females or healthy non-pregnant females.¹⁷

Another theory proposed that the activation of neutrophils occurs in placental circulation under hypoxic conditions that contribute to increased vascular resistance in pre eclamptic pregnancies.¹⁸ It was also found that there was more neutrophil endothelial adhesion and infiltration into intimal space in systemic vasculature while lymphocyte infiltration was not significant.¹⁹

Thus, the findings of the current study taken together with those of the previous studies have added value to the use of maternal NLR levels in the determination of PE. NLR, obtained from complete blood count, an inexpensive and routinely done test, can be used as a predictive and diagnostic tool in patients of pre eclampsia as well as those presenting with masked hypertension. NLR having a good sensitivity will also help us in identifying undiagnosed cases due to subjective error. However, large scale and multicentre prospective studies are needed to determine the optimal NLR value and its predictive and prognostic significance among pre-eclampsia patients.

CONCLUSION

Maternal NLR obtained from complete blood count, an inexpensive and routinely done test, can be considered as a cost effective bio marker in the evaluation of PE. In conclusion, during antenatal follow-up, the measurement of NLR periodically may be useful to predict high-risk pregnancies in terms of pre-eclampsia. However, further research is needed regarding predictive factors for PE, with the purpose of starting preventive treatment in these women, since PE is a pregnancy complication with serious implications on mother and foetus.

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