

## Role of Neutrophil-Lymphocyte Ratio (NLR) and Platelet-Lymphocyte Ratio (PLR) as predicting markers in Pre-eclampsia: A retrospective case – control study

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### Abstract:

**Background:** Pre-eclampsia is a disorder of vascular endothelial dysfunction, diagnosed by new onset hypertension after 20 weeks of gestation with proteinuria or end organ dysfunction. Hyperactivation of inflammatory response is proposed in the pathophysiology of pre-eclampsia. **Aim:** The present study aimed to assess the predictive role of simple, cost-effective systemic inflammatory markers Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR) in the diagnosis of pre-eclampsia. **Materials and Methods:** This retrospective case-control study compared the hematological parameters (RBC Count, Total Leucocyte Count, Platelet Count, NLR and PLR) between 20 diagnosed preeclampsia cases and age and gestational age matched 20 healthy pregnant females. Independent t test was used to compare the parameters between cases and controls, The predictive role of NLR was assessed using the ROC curve analysis.  $p < 0.05$  was considered statistically significant. **Results:** The NLR was comparatively higher in the patients with pre-eclampsia ( $5.09 \pm 2.22$ ) compared to healthy controls ( $3.87 \pm 1.17$ ) and it was statistically significant ( $p < 0.05$ ). There was no significant difference between the cases and controls in RBC Count, Leucocyte count, Platelet Count and PLR. The ROC analysis showed significant diagnostic accuracy of NLR to discriminate cases and controls (area under the curve [AUC] = 0.72,  $p < 0.05$ ) at cut-off value of  $\geq 3.59$ , with 80% sensitivity, and 55% specificity. **Conclusion:** NLR may serve as simple, reliable, cost-effective investigation that may help to predict and diagnose individuals with pre-eclampsia, which may help in planning appropriate therapeutic intervention at an early stage.

**Keywords:** lymphocyte, neutrophil, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, normotensives, pre-eclampsia

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### Introduction:

Pre-eclampsia, a disorder of vascular endothelial malfunction represents a common form of pregnancy induced hypertension, with its incidence ranging from 5-15% based on few hospital-based studies in India.<sup>1</sup> Pre-eclampsia is

characterized by a rise in blood pressure and proteinuria that manifests after 20 weeks of gestation.<sup>2</sup>

In addition to the hypothesized dysregulation of immune system in the form of interaction of Natural Killer cells with the paternal HLA (Human

Leucocyte Antigen), hyperactivation of inflammatory responses has also been shown to be a component of pathophysiology of Pre-Eclampsia.<sup>3,4</sup> Neutrophil – Lymphocyte Ratio (NLR) and Platelet – Lymphocyte Ratio (PLR) are simple inflammatory ratios that can be obtained from a complete blood count.

The focus on Neutrophil- Lymphocyte ratio as a prognostic marker for inflammatory load is on a gradual rise in the past decade and its importance as a marker of low grade inflammation has been quoted in cardiovascular diseases, cancer and endometriosis.<sup>5-7</sup>

Platelets and lymphocytes are also essential parameters that reflect cytokine mediated immune surveillance and Platelet-Lymphocyte ratio has been identified to be better marker for systemic inflammation compared to Neutrophil-Lymphocyte ratio in certain conditions.<sup>8</sup> NLR and PLR are simple inflammatory ratios that can be calculated from blood parameters. Complete Blood Count is an easily available and measurable investigation routinely done on all pregnant women. Only few studies exist to support the role of NLR, PLR as predictive markers in pre-eclampsia.

With the identified roles of NLR and PLR as markers of systemic inflammation, this Medical record based retrospective study aimed to assess the predictive role of both NLR and PLR in diagnosed pre-eclampsia individuals and to compare it with age and gestational age matched otherwise healthy pregnant women.

#### **Materials and Methods:**

##### **Type of Study & Study Design:**

Retrospective Case-Control Study

##### **Study Population:**

Healthy Pregnant women and pre-eclampsia individuals who had registered in the Obstetrics

and Gynaecology OPD of our Medical college and Hospital in the period between January 2017 – December 2019.

##### **Selection Criteria:**

##### **Inclusion Criteria:**

18 – 40 years of healthy and pre-eclampsia diagnosed pregnant women registered in the Obstetrics and Gynaecology OPD in the period between January 2017 – December 2019.

Pre-eclampsia was diagnosed based on the standard criteria suggested by ACOG (American College of Obstetricians and Gynaecologists) as Systolic Blood Pressure of 140 mmHg or more, or Diastolic blood Pressure of 90 mmHg or more on 2 occasions of at least 4 hours apart along with proteinuria.<sup>9</sup>

##### **Exclusion Criteria:**

Pregnant women <18years of age, > 40 years of age, Patients with eclampsia, gestational hypertension, any other disorders complicating pregnancy.

##### **Selection of Controls:**

Controls were selected from the age and gestational age matched (1:1 or paired matching) normal pregnant females enrolled in the Obstetrics and Gynaecology OPD in the period between January 2017-December 2019.

##### **Sample Size:**

Being a Medical record based retrospective case control study, it was decided to include the data of normal pregnant females and diagnosed pre-eclampsia patients in the period between January 2017-December 2019.

A total of 184 patients' data (comprising of both healthy and pre-eclampsia) were collected. The number of cases and controls were later tapered

to 20 each, after 1:1 or paired matching of age and gestational age.

#### **Data Collection Procedure:**

Institutional Ethical Clearance was obtained. ID of patients diagnosed with pre-eclampsia were obtained from the registers of Obstetrics OPD of the institution in the time period between January 2017 – December 2019.

Later using the patients ID, the following details were obtained from the medical records department regarding each patient.

1. Age
2. Gestational age at the time of diagnosis
3. Parity index
4. Blood Pressure
5. Blood Parameters - Complete Blood Count parameters which include RBC count, Total Leucocyte Count, Differential Leucocyte Count (Percentage distribution of Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes) and Platelet Count were obtained from the medical records.

#### **Calculation of Neutrophil Lymphocyte ratio and Platelet Lymphocyte Ratio:**

Neutrophil Lymphocyte Ratio and Platelet Lymphocyte Ratio were calculated from the absolute values derived from the automated blood count.

#### **Statistical Analysis:**

Continuous variables were expressed as Mean  $\pm$  S.D. The independent 't' test was used to compare the continuous variables between the control group and patients with pre-eclampsia. Receiver Operating Characteristic (ROC) curve was used to determine the cut-off values for NLR ratio for the diagnosis of pre-eclampsia.

The area under ROC curve with the maximum sum of the sensitivity and specificity was used in the estimation of cut-off value for NLR. A p value  $<0.05$  was considered statistically significant. IBM SPSS Software version 28 for windows was used for statistical analysis.

#### **Observation and Results:**

Around 40 study participants data records were considered for statistical analysis, 20 were diagnosed pre-eclampsia cases and 20 were matched controls. Table 1 shows the baseline values of the study population. The mean age and the gestational age of the study participants were  $25.4 \pm 2.5$  years and  $37.95 \pm 0.87$  weeks respectively.

Table 2 represents the comparison of study variables between the cases and the controls. There was no difference between age and the gestational age in weeks, as matching was done for age and gestational age between the cases and controls.

The Systolic and Diastolic Blood pressure values were significantly higher in cases compared to controls ( $p < 0.05$ ). The NLR was comparatively higher in the patients with pre-eclampsia compared to healthy controls and it was statistically significant ( $p < 0.05$ ). There was no significant difference between the cases and controls in RBC Count, Platelet Count and PLR.

Table 3 shows the data analysis of the ROC curve. The ROC analysis showed significant diagnostic accuracy of NLR to discriminate cases and controls (area under the curve [AUC] = 0.72,  $P < 0.05$ ) at cut-off value of  $\geq 3.59$ , with 80% sensitivity, and 55% specificity.

As there was no significant difference between PLR values in cases and controls, we didn't attempt to analyze the predictive role of PLR.

**Table 1: Basic Parameters of the study participants**

Variable	Values
N	40
Age (Years)	25.4 ± 2.5
Gestational Age (Weeks)	37.95 ± 0.87
Systolic Blood Pressure (mmHg)	123.37 ± 18.47
Diastolic Blood Pressure (mmHg)	80.97 ± 14.30
RBC Count (x 10 <sup>6</sup> /mcl)	3.77 ± 0.48
Total Leucocyte Count (cells/mcl)	11167.5 ± 2705.30
Platelet Count (x 10 <sup>3</sup> /mcl)	262.25 ± 81.56
NLR	4.48 ± 1.85
PLR	142.37 ± 68.75

Values expressed as Mean ± S.D

**Table 2: Comparison of the study variables between the cases and controls**

Variable	Cases (Pre-eclampsia)	Controls
N	20	20
Age (Years)	25.4 ± 2.58	25.4 ± 2.58
Gestational Age (Weeks)	37.95 ± 0.87	37.95 ± 0.87
Systolic Blood Pressure (mm Hg)	136.4 ± 13.4	110.35 ± 12.7*
Diastolic Blood Pressure (mmHg)	90.5 ± 12.3	71.4 ± 8.76*
RBC Count (x 10 <sup>6</sup> /mcl)	3.75 ± 0.57	3.78 ± 0.37
Platelet Count (x 10 <sup>3</sup> /mcl)	262.20 ± 79.8	262.30 ± 85.2
NLR	5.09 ± 2.22	3.87 ± 1.17*
PLR	160.78 ± 80.42	123.97 ± 50.22

Values expressed as Mean ± S.D, \* p < 0.05

**Table 3: Details of ROC Curve analysis**

Test Variable	Area	SE	p	Cut- off value	Sensitivity	Specificity
NLR	0.720	0.081	0.017*	3.59	80%	55%

\*p < 0.05, SE – Standard Error

**Discussion:**

The present study measured the NLR and PLR between pre-eclampsia patients and normal pregnant females of same age and gestational age. The NLR values were significantly higher in preeclampsia patients compared to the healthy controls which was statistically significant. A systematic review by Zheng WF et al reported an acceptable sensitivity of NLR in the diagnosis of Pre-Eclampsia.<sup>10</sup> Serin S reported a significantly

higher values of NLR in patients with pre-eclampsia and its scope in classifying the severity of Pre-eclampsia.<sup>11</sup> Kushboo Singhal reported a significantly higher value of NLR in patients with preeclampsia compared to the controls and also with a specificity of 80%.<sup>12</sup> A prospective study by Sachan R reported a significant difference in NLR even at early gestation.<sup>13</sup> Dysregulation of immune system<sup>3</sup> and hyperactivation of inflammatory responses have been shown to be a component of

pathophysiology of Pre-Eclampsia.<sup>4</sup> Leukocytes have been shown to be predominantly involved in the pathophysiology of pre-eclampsia.

Neutrophil activation and release of pro-inflammatory cytokines with endothelial dysfunction have been attributed to be the pathophysiological basis for pre-eclampsia.<sup>14</sup> Increase in neutrophil has been attributed to the cause for leukocytosis in patients with pre-eclampsia.<sup>15</sup> Canzoneri et al and Ramma et al reported no change in the number of lymphocytes in patients with pre-eclampsia. These factors suggest an elevated NLR in patients with Pre-eclampsia.<sup>15,16</sup> However, Yavuzcan A and his colleagues did not find a significant difference in the NLR between normal controls and patients with preeclampsia.<sup>17</sup> Mean age of the participants, parity and gestational age are some other factors that may influence these factors.

The ROC analysis showed significant diagnostic accuracy of NLR to discriminate cases and controls at cut-off value of  $NLR \geq 3.59$ , with 80% sensitivity, and 55% specificity, in our study. Gezer et al reported cutoff values of  $NLR \geq 3.08$ , with the area under the ROC as 0.716 and the sensitivity of 74.6% and specificity of 70.1% of NLR in predicting Preeclampsia.<sup>18</sup> In another study ROC analysis showed significant diagnostic accuracy of NLR with area under the curve 0.73, at cut-off value of  $\geq 4.86$ , with 68.6% sensitivity, and 80% specificity.<sup>12</sup>

The ROC showed significant diagnostic accuracy of NLR between controls and non-severe cases area under the curve as 0.75, at cutoff value of  $>3.3$ , 52.9% sensitivity, and 74.5% specificity.<sup>13</sup> These results suggest a significant predictive role of NLR in diagnosing and assessing severity of pre-eclampsia with a reasonable sensitivity and specificity.

Platelets and lymphocytes are some essential parameters that reflect cytokine mediated immune surveillance and Platelet-Lymphocyte

ratio has been identified to be better marker for systemic inflammation.<sup>19</sup> However in our study there was no significant difference in the platelet count and PLR between cases and controls. In our study, the values of PLR were slightly higher in pre-eclampsia compared to normal, however it was not statistically significant. Similar results were reported by Yavuzcan A and Cintesun et al.<sup>17,19</sup> All our cases were diagnosed with mild form of pre-eclampsia. Mild nature of the disorder and the low sample size in our cases could be attributed to the lack of statistical difference in PLR values between cases and controls.

In a study by Sadik Sahin S, it is proposed that inflammatory state in pre-eclampsia, promote adherence of platelets and leucocytes to endothelium, which trigger neutrophil recruitment and production of inflammatory cytokines.<sup>20</sup> Systematic review by Zheng WF et al and other studies by Serin S et al, Kushboo Singhal et al and Sachan R et al reported a significant predictive role of NLR in Pre-eclampsia, a handful of studies identified the equal role of PLR as a diagnostic or severity marker for Pre-eclampsia.<sup>10-13, 21,22</sup>

Kim MA et al and Mannaerts D et al studied the clinical significance of PLR in patients with pre-eclampsia and proved it as an effective diagnostic marker for pre-eclampsia.<sup>21,22</sup> Few studies have compared the sensitivity of NLR and PLR in predicting Pre-eclampsia.<sup>23,24</sup> Kholief A did not observe a significant difference in NLR in pre-eclampsia, however observed PLR to be sensitive marker to predict pre-eclampsia compared to the NLR.<sup>23</sup> Toptas M did not observe a significant difference in NLR in pre-eclampsia, but identified a significant association between PLR and severity of pre-eclampsia, compared to NLR.<sup>24</sup>

#### **Strengths and Limitations of the study:**

This study is a case control study, where efforts were made to do paired matching for age and gestational age between cases and controls for

obtaining accurate results. Further the sensitivity obtained with diagnostic accuracy of NLR is optimal.

However, the study results are slightly conflicting with other studies, owing to the low sample size and gestational age. It is recommended that prospective cohort studies are planned to accurately predict the role of NLR and PLR in diagnosis of pre-eclampsia.

#### **Conclusion:**

NLR values are higher in individuals with pre-eclampsia compared to normotensive pregnant women. However, regrading PLR there was a difference in the mean values but was not statistically significant which could be attributed to less sample size. The results of our study show a significant predictive role of NLR with a good sensitivity in the diagnosis of preeclampsia. Hence, we consider NLR as a simple, cost-effective marker of inflammation for predicting the onset and severity of pre-eclampsia. However longitudinal, prospective cohort studies are recommended in large sample to add as a criterion in the diagnosis of pre-eclampsia.

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**Conflict of interest:** Nil

#### **References:**

1. Nobis PN, Hajong A. Eclampsia in India through the decades. *J ObstetGynaecol India*.2016;66:172-6.
2. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy*. 2001;20:IX-XIV.

3. Dekker G, Robillard PY. Pre-eclampsia: is the immune maladaptation hypothesis still standing? An epidemiological update. *J Reprod Immunol*. 2007;76:8–16.
4. Lynch AM, Murphy JR, Gibbs RS, Levine RJ, Giclas PC, Salmon JE, Holers VM. The interrelationship of complement-activation fragments and angiogenesis-related factors in early pregnancy and their association with pre-eclampsia. *BJOG*. 2010;117(4):456-62.
5. Balta S, Celik T, Mikhailidis DP, Ozturk C, Demirkol S, Aparci M et al. The relation between atherosclerosis and the neutrophil-lymphocyte ratio. *Clin Appl ThrombHemost*. 2016; 22:405–411.
6. Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O'Reilly DS et al. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer*. 2011; 47:2633–2641.
7. Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, et al. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. *Fertility and Sterility*. 2008;90(6):2073–9.
8. Turkmen K, Erdur FM, Ozcicek F, Ozcicek A, Akbas EM, Ozbicer A et al. Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-to-lymphocyte ratio in end-stage renal disease patients. *Hemodial Int*.2013; 17:391–396.
9. Gestational hypertension and preeclampsia. ACOG Practice Bulletin No. 222. American College of Obstetricians and Gynecologists. *Obstet Gynecol*. 2020;135:e237–60.
10. Zheng WF, Zhan J, Chen A, Ma H, Yang H, Maharjan R. Diagnostic value of neutrophil-lymphocyte ratio in preeclampsia: A PRISMA-compliant



- systematic review and meta-analysis. *Medicine (Baltimore)*. 2019 Dec;98(51):e18496.
11. Serin S, Avci F, Ercan O, Köstü B, Bakacak M, Kiran H. Is neutrophil/lymphocyte ratio a useful marker to predict the severity of pre-eclampsia? *Pregnancy Hypertens*. 2016 Jan;6(1):22-5
  12. Khushboo Singhal, Arvind Kumar Pal, Sunita Tiwari, Renu Singh, Rashmi Kushwaha. Neutrophil lymphocyte ratio (NLR) as a bio inflammatory marker in preeclampsia. *International Journal of Contemporary Medical Research*. 2019;6(4):D1-D3.
  13. Sachan R, Patel ML, Vandana, Sachan P, Shyam R. Diagnostic accuracy of neutrophil to lymphocyte ratio in prediction of nonsevere preeclampsia and severe preeclampsia. *J Curr Res Sci Med*.2017;3:79-83.
  14. Laresgoiti-Servitje E, A leading role for the immune system in the pathophysiology of preeclampsia, *J. Leukoc. Biol.* 2013; 94:247–257.
  15. Canzoneri BJ, Lewis DF, Groome L, Wang Y. Increased neutrophil numbers account for leukocytosis in women with preeclampsia. *Am J Perinatol*. 2009 Nov;26(10):729-32.
  16. Ramma W, Buhimschi IA, Zhao G, et al. The elevation in circulating anti-angiogenic factors is independent of markers of neutrophil activation in preeclampsia. *Angiogenesis*. 2012, 15 (3), 333-340.
  17. Yavuzcan A, Çağlar M, Ustün Y, Dilbaz S, Ozdemir I, Yildiz E, Ozbilgeç S, Kumru S. Mean platelet volume, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in severe preeclampsia. *Ginekol Pol*. 2014 Mar;85(3):197-203.
  18. Gezer C, Ekin A, Ertas IE, Ozeren M, Solmaz U, Mat E, Taner CE. High first-trimester neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios are indicators for early diagnosis of preeclampsia. *Ginekol Pol*. 2016;87(6):431-5.
  19. Çintesun E, IncesuÇintesun FN, Ezveci H, Akyürek F, Çelik Ç. Systemic inflammatory response markers in preeclampsia. *J Lab Physicians*. 2018 Jul-Sep;10(3):316-319.
  20. Sadik Sahin S, Ozakpinar OB, Eroglu M, Tetik S. Platelets in Preeclampsia: Function and Role in the Inflammation. *MUSBED*. 2014;4(2):111-116.
  21. Kim MA, Han GH, Kwon JY, Kim YH. Clinical significance of platelet-to-lymphocyte ratio in women with preeclampsia. *Am J Reprod Immunol*. 2018 Jul;80(1):e12973.
  22. Mannaerts D, Heyvaert S, De Cordt C, Macken C, Loos C, Jacquemyn Y. Are neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and/or mean platelet volume (MPV) clinically useful as predictive parameters for preeclampsia?. *J Matern Fetal Neonatal Med*. 2019;32(9):1412-1419.
  23. Kholief A, Swilam R, Elhabashy A, Elsherief R. Neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and c-reactive protein as markers for severity of preeclampsia. *Res OpinAnesth Intensive Care*.2019;6:1-8.
  24. ToptasM, Asik H, Kalyoncuoglu M, Can E & Can M. Are Neutrophil/Lymphocyte Ratio and Platelet/Lymphocyte Ratio Predictors for Severity of Preeclampsia?. *Journal of Clinical Gynecology And Obstetrics*.2016;5(1):27-31.