Neutrophil/lymphocyte ratio is a useful marker to predict the severity of preeclampsia

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Abstract

Objectives: In this retrospective study, we investigated whether neutrophil/lymphocyte ratio (NLR) in the last trimester among pregnancies with mild preeclampsia, severe preeclampsia, and healthy status has a role in predicting the severity of pre-eclampsia.

Methods: This study involved 933 pregnancies from 1 January 2018 to 30 September 2020, comprising 396 healthy pregnancies, 222 pregnancies with mild preeclampsia, and 315 pregnancies with severe preeclampsia. The relationship between preeclampsia and NLR was analyzed by multiple logistic regression. In addition, maternal placental tissues of three groups were immunohistochemically stained for myeloperoxidase (MPO).

Result: NLR was significantly higher in pregnancies with preeclampsia (including pregnancies with mild and severe preeclampsia.) than that in healthy pregnancies. The NLR level was prominently higher in patients with severe preeclampsia compared with those with mild preeclampsia (p<0.001). The NLR level was significantly positively associated with preeclampsia after adjusting for gestational week at time of blood sampling, BMI, and age (β:1.44; 95%CI:0.01-2.93; p=0.0014 < 0.05). In addition, MPO expressions of placental tissues in preeclamptic groups were significantly increased than these in healthy pregnant controls(P<0.05).

Conclusion: Increased NLR in the last trimester was significantly positively associated with preeclampsia. Hence, NLR plays a role in predicting the severity of preeclampsia. However, further studies are needed on a more extensive population to confirm the relationship between NLR and preeclampsia severity.

1. Introduction

Preeclampsia is the onset of high blood pressure and proteinuria and acutely develops after about 20 weeks of pregnancy; this condition impairs fetal growth and can result in damages to maternal organs, including liver, kidney, and heart[1, 2]. Preeclampsia affects 2–8% of all pregnant women at varying severity, accounting for 10–15% of perinatal mortality and representing the major cause of intra-uterine growth retardation[3, 4]. In some cases, pregnant women with preeclampsia would suffer from postpartum complications, such as cardiomyopathy, hypertension, and long-term renal disease[5, 6]. Given the high incidence and adverse pregnancy outcomes of preeclampsia, urgent prediction of the severity of preeclampsia is necessary to improve pregnancy outcomes and prolong pregnancy.

Changes in the immune system play a crucial role in the pathogenesis of preeclampsia[7, 8]. Hypoxia of the placenta caused by insufficient invasion of the trophoblast[9] will promote the secretion of white blood cells, including neutrophils, lymphocytes, and monocytes[1]. Excessive activation of neutrophils adhered to the endothelium is the key cause of endothelial injury in preeclampsia[10, 11]. When activated neutrophils form neutrocyte extracellular traps (NETs) are excessive or not cleared by the body in time, NETs attach to the endothelium, causing endothelial cell apoptosis and damage to endothelial injury[12]. NLR, a marker of subclinical inflammation, is easy and inexpensive to measure. NLR has gained
significant interest. In addition, the value of NLR in predicting the severity of preeclampsia remains unclear. Therefore, we aimed to determine the relationship between NLR and preeclampsia and explore whether NLR can predict the severity of preeclampsia in this study.

2. Methods

2.1. Patient and Sample Collection

This retrospective study included 222 pregnancies with mild preeclampsia, 315 pregnancies with severe pre-eclampsia, and 396 healthy pregnancies. The diagnosis of preeclampsia and severe preeclampsia was based on the Guidelines for the Management of Hypertension during Pregnancy (2020). Pregnancies with normal blood pressure (systolic blood pressure below 140 mmHg and diastolic blood pressure below 90 mm Hg) and without complications, such as gestational diabetes mellitus, hypothyroidism, and fetal heart abnormality were considered normal pregnancies. Pregnant women with systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg after 20 weeks of gestation and with urinary protein levels $\geq$ 0.3 g/24 h, urinary protein/creatinine ratio $\geq$ 0.3, or random urinary protein $\geq$ (+) were diagnosed as preeclampsia. Women with gestational hypertension in the absence of proteinuria were diagnosed with preeclampsia if their heart, lungs, liver, kidneys, and other vital organs or the blood system, digestive system, and nervous system have complications. Women were diagnosed with severe preeclampsia if they presented with any of the following features: systolic blood pressure of 160 mm Hg or more and/or diastolic blood pressure of 110 mmHg or higher; new-onset headache, visual disturbances, or abnormalities of the central nervous system; persistent right upper quadrant or epigastric pain; abnormal blood concentrations of liver enzymes mainly manifested by elevated levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST); renal insufficiency: urinary protein quantification $\geq$ 2 g/24 h or serum creatinine concentrations greater than 106 $\mu$mol/L; thrombocytopenia: platelet count less than 100×10⁹/L; pulmonary edema. Women with preeclampsia who have unexplained compulsive convulsions were diagnosed with eclampsia[3, 13]. All these pregnant women were admitted to the Affiliated Hospital of Jining Medical University between 1 January 2018 and 30 September 2019. The exclusion criteria included: (1) pregnancies with any premature rupture of fetal membranes and infection because these infections can affect NLR in pregnant women; (2) pregnancies whose final outcome and clinical data were not tracked down; (3) pregnancies with previous cardiovascular disease, hematopoietic disease, malignancy, and gestational diabetes mellitus as well as those who were currently taking medication that affected their blood routine examination; (4) twin or multiple pregnancy; and (5) miscarriage or stillbirths(Figure1).

We retrieved the demographic and medical history information of the pregnant women from the Haitai medical record information system. To protect the privacy of the pregnant woman, we do not disclose any personal information about them. This research was approved by the Ethics Committee of the Affiliated Medical College of Jining Medical University.

2.2. Immunohistochemistry
Maternal placental tissues of three groups were fixed in neutral formaldehyde, embedded in paraffin, and cut into sections (5 µm). The sections were routinely dewaxed to water and washed twice with buffer solution for 3 min. The slices were incubated in hydrogen peroxide for 10–15 min to block endogenous peroxidase. The sections were incubated sequentially with MPO antibody (Abcam, UK, 1:50) at 4°C for 12 h. The sections were incubated with the secondary antibody at 1:500 dilution at room temperature for 30 min. The sections were mixed with 1 mL of DAB Plus Substrate added with 2 drops of DAB Plus Chromogen and let stand for 15 min. The sections were fully rinsed with tap water, re-dyed, dehydrated, transparent, and sealed. The strength of nuclear, cytoplasmic, membranous and extracellular staining was quantified using standardized semiquantitative histological scoring (H-score) on a 1–4 scale[14].

2.3. Statistical analysis

Continuous variables were presented as mean ± standard deviation (normal distribution) or medium (Min-Max) (skewed distribution). Categorical variables were expressed in frequency or percentage. We used χ² (categorical variables), one-way ANOVA (normal distribution), or Kruskal-Wallis H test (skewed distribution). All analyses were performed with the statistical software packages of R (http://www.R-project.org, The R Foundation) and Empower Stats (http://www.empowerstats.com, X&Y Solutions, Inc, Boston, MA). Categorical data were shown with (number) and percentage (%). Binary logistic regression with single and multi-categorical predictor was used to determine the possible risk factors for preeclampsia. Data were examined at the 95% confidence level, and a p-value of < 0.05 was considered significant.

3. Results

3.1. Baseline characteristics and maternal serum NLR of the three groups

A total of 933 gestational women were included in our study: 396 healthy patients without any pregnancy complications were recruited as controls, 315 pregnant women were diagnosed with severe preeclampsia, and 222 pregnant women were diagnosed with mild preeclampsia. The demographic characteristics, clinical history, and laboratory features, including the values of N/L of the three groups, are shown in Table 1. No significant difference in lymphocyte count was found among the three groups (p = 0.839, p = 0.261 > 0.05). At delivery, the BMI of women severe preeclampsia and mild preeclampsia was significantly higher than that in the control group (p < 0.001, p < 0.001; Table 1). The delivery pregnancy week in pregnant patients with preeclampsia was significantly lower than that in the control group (p < 0.001, p < 0.001). Neonatal weight of patients with severe preeclampsia was 2.22 ± 0.85, which was significantly lower than that of patients with mild preeclampsia (2.64 ± 0.88) and the control group (3.36 ± 0.52; Table 1; P < 0.001). The NLR was higher in the preeclampsia groups than that in women with normal pregnancy, and severe preeclampsia was more pronounced than mild preeclampsia.
### Table 1

Baseline characteristics of subjects

| Characteristic                          | Healthy pregnant controls (n = 396) | Mild preeclampsia (n = 222) | Severe preeclampsia (n = 315) | p-Value | P-value* |
|----------------------------------------|------------------------------------|-----------------------------|-------------------------------|---------|---------|
| Age (years)                            | 29.64 ± 5.26                       | 30.68 ± 5.98                | 31.91 ± 6.38                 | < 0.001 | < 0.001 |
| BMI                                    | 23.01 ± 3.67                       | 23.12 ± 7.17                | 22.99 ± 9.01                 | 0.974   | < 0.001 |
| Gestational week at time of blood sampling (week)* | 37.54 ± 2.05                       | 35.35 ± 3.60                | 33.93 ± 3.95                 | < 0.001 | < 0.001 |
| Delivery pregnancy week (week)*        | 38.76 ± 2.08                       | 36.17 ± 2.87                | 34.08 ± 3.90                 | < 0.001 | < 0.001 |
| Systolic blood pressure (mmHg)         | 120.41 ± 11.23                     | 165.55 ± 14.37              | 179.63 ± 16.69               | < 0.001 | < 0.001 |
| Diastolic blood pressure (mmHg)        | 73.14 ± 8.93                       | 103.47 ± 8.84               | 112.27 ± 10.82               | < 0.001 | < 0.001 |
| Neonatal weight (kg)#                  | 3.36 ± 0.52                        | 2.64 ± 0.88                 | 2.22 ± 0.85                  | < 0.001 | < 0.001 |
| NLR                                    | 68.96 ± 9.64                       | 69.78 ± 7.66                | 72.02 ± 9.72                 | < 0.001 | < 0.001 |
| Lymphocyte count(×10⁹/L)               | 1.97 ± 0.75                        | 1.93 ± 0.64                 | 1.94 ± 1.11                  | 0.839   | 0.261   |
| Neutrophil count(×10⁹/L)               | 6.75 ± 4.25                        | 6.48 ± 4.73                 | 7.02 ± 2.52                  | 0.304   | < 0.001 |

*: mean ± SD, normal distribution of data is presented as mean ± standard deviation; #: median, Min-Max, non-normal distribution of data is presented as median (Min-Max); Results were analyzed by One-way ANOVA (Brown-Forsythe). p*-value: among groups, p-value: within groups, p < 0.05 statistically significant. Statistically significant p values are marked as bold text.

**Abbreviations:** NLR: neutrophil/lymphocyte ratio, BMI: body mass index.

### 3.2. Logistic regression analysis of possible risk factors for preeclampsia

Table 2 lists the results of univariate analyses with some parameters and NLR. The lymphocyte count was not associated with preeclampsia (P > 0.05). In addition, neonatal weight (β: 6.31; 95%CI: 4.65–7.96; p < 0.001) was negatively associated with preeclampsia, whereas NLR (β: 2.15; 95%CI: 0.92–3.38; p < 0.001) was positively associated with preeclampsia. Multivariate logistic regression analysis was performed on NLR (Table 3). Table 3 lists the effect sizes (β) and 95% confidence intervals. In the unadjusted model, multivariate logistic regression analysis showed the positive correlation between NLR
level and preeclampsia. After correction for gestational week at time of blood sampling, BMI, and age, elevated NLR levels were still significantly positively correlated with preeclampsia (β:1.44; 95%CI:0.01–2.93; p = 0.0014 < 0.05; Table 3). Therefore, elevated NLR level may be a positive factor in the pathogenesis of preeclampsia.

### Table 2
Univariate analysis of different variables for preeclampsia

| Covariate                  | β (95%CI)           | P-value  |
|----------------------------|---------------------|----------|
| Systolic blood pressure (mmHg) | 53.40 (51.45, 55.34) | < 0.001  |
| Diastolic blood pressure (mmHg) | 35.49 (34.18, 36.81) | < 0.001  |
| Neonatal weight (kg)        | 6.31 (4.65, 7.96)   | < 0.001  |
| NLR                        | 2.15 (0.92, 3.38)   | < 0.001  |
| Lymphocyte count(×10⁹/L)    | 0.07 (-0.04, 0.18)  | 0.2418   |
| Neutrophil count(×10⁹/L)    | 0.56 (0.17, 0.94)   | < 0.001  |

*p < 0.05 statistically significant, NS; not significant, statistically significant p-values are marked as bold text.

### Table 3
Relationship between NLR and preeclampsia in different models using multivariate linear regression

| Variable | Crude Model | Adjusted |
|----------|-------------|----------|
|          | β (95%CI)   | P-value  | β (95%CI)   | P-value  |
| PIGF     | 2.15 (0.92, 3.38) | 0.0006    | 1.44 (0.01, 2.93) | 0.0014    |

**Logistic regression model (binary logistic regression with single- and multi-categorical predictors) was used to determine the possible risk factors for preeclampsia. Adjusted: adjusted for gestational week at time of blood sampling, BMI, and age.**

*p < 0.05 is statistically significant, NS; not significant. Statistically significant p-values are marked as bold text.

### 3.3. Expression and distribution of neutrophil in placental tissues

Immunohistochemistry was used to detect the expression and distribution of neutrophils and lymphocytes in placental tissues. The expression of MOP in preeclampsia was significantly increased than those in the healthy controls (Fig. 2). Positive staining showed brown-yellow granules in the tissue.
4. Discussion

Preeclampsia is a specific condition of pregnancy and is one of main cause of maternal organ damage and intra-uterine growth retardation\[15, 16\]. In the present retrospective case-control study, we investigated the association between preeclampsia and NLR in whole blood during the third trimester. Our results showed NLR in whole blood and MPO expression in the placental tissues were significantly elevated in pregnancies with preeclampsia compared with healthy controls.

In normal pregnancy, T cells shift toward the anti-inflammatory Th2 phenotype, which can synthesize IL-4, IL-5, IL-6, IL-10, IL-13, and antibody[17]. These factors can neutralize inflammation, inhibit oxidative stress, and reduce cell damage[1]. In preeclampsia, T cells shift toward the Th1 phenotype, which can synthesize IL-12, IL-18, IFN, and TNF[18, 19]. These proinflammatory cytokines can stimulate systemic inflammatory response, leading to apoptosis and reduced trophoblast invasion[20]. As a systemic inflammatory response syndrome, preeclampsia can increase the expression of white blood cells[21]. Neutrophils, the most abundant type of white blood cell in the human circulation system, are the principal cells during inflammatory reactions[22]. Scholars found that in maternal circulation, all classes of leukocytes are activated in women with preeclampsia, but only neutrophils penetrate into the systemic vasculature [21]. Our research also confirmed that neutrophil count in preeclampsia groups was significantly higher than that in the healthy control group (P < 0.05). Neutrophils can lead to the generation of NETs, which are fibrous extracellular lattices containing DNA[23, 24]. In preeclampsia, a large number of NETs are observed in maternal circulation in vivo in the intervillous space, especially within the areas of tissue damage[25–29]. When extracellular trapping nets are not cleared by the body, they lead to damages to endothelial injury and cause preeclampsia[26]. In addition, the level of NETs was positively correlated with the severity of the disease[26, 30]. These NETs serve as a source of elevated levels of cell-free maternal DNA, leading to endothelial damage and enhancing the severity of preeclampsia[27–29]. In the present study, the level of NLR was prominently higher in patients with severe preeclampsia compared with those with mild preeclampsia (p = 0.0006 < 0.05).

The etiology and pathogenesis of preeclampsia are complex. Pregnancy-related immunological changes, in addition to extensive maternal vascular damage, are more important fetal placental changes. This disease is believed to arise owing to defective placentation and eventually resolves when the placenta is removed[31–33]. Thus, preeclampsia is also called ‘placental diseases’. The trophoblast invasion and insufficient remodeling of uterine spiral arteries is considered the main cause of defective placentation[34–36]. Inadequate spiral arteriolar lead to narrow maternal vessels, which will form low flow, low volume capacitance, high-resistance vessels[37]. Placental ischemia and hypoxia can cause intermittent hypoxia and reoxygenation, resulting in oxidative stress of the placenta[38]. Some scholars believe that oxidative stress is the central component of placental and endothelial dysfunction and considered the main cause of preeclampsia[38]. Under oxidative stress, hypoxia/reperfusion with high ROS production provokes the release of sFlt-1 and soluble endoglin via NF-κB [38–40]. NF-κB, a nuclear factor, enters the cell nucleus and promotes the transcription of several proinflammatory mediators[41]. The secretion of proinflammatory cytokines activate the systemic inflammatory response. MPO, an
inflammatory marker, is a heme protein produced and released by activated neutrophils\[42\]. In the present study, the MPO expression in the placental tissues was significantly elevated in pregnancies with preeclampsia compared with that in healthy controls. In addition, the neutrophil counts of pregnant women with preeclampsia were significantly higher than those in the healthy controls. These activated neutrophils in the intervillous space of the placenta of pregnant women with preeclampsia can result in excessive amounts of oxidized lipids\[43\]. These lipids in turn activate neutrophil, leading to expression of cyclooxygenase (COX) \[30\]. COX-2 promotes the secretion of thromboxane prostaglandin E2 TNFα and superoxide, which induce oxidative stress and dysfunction in endothelial cells \[19\]. These explanations are feasible for our results.

A number of reports have shown that white blood cell is associated with atherosclerosis. Various scholars believe that neutrophil count is an objective marker of forecasting cardiovascular events\[44\]. The mechanism of vascular endothelial injury in preeclampsia, a disease of systemic excessive inflammatory response, has been widely recognized. In the present study, preeclampsia is associated with an increase in neutrophil count, which is higher than that in the healthy control group. In addition, the lymphocyte counts in women with preeclampsia did not differ. Increase in neutrophil count and basically unchanged lymphocyte counts could result in increased NLR in the preeclampsia group. In our study, NLR was more meaningful and correlated in this study.

Our study has several limitations. The first limitation is the retrospective nature of the study and the inability to calculate population-based rates. Second, all of the pregnant women in the study are from China; as such, whether this research can be generalized to other ethnic groups should be further investigated.

In conclusion, elevated NLR was positively related to the severity of preeclampsia. Hence, NLR could predict the severity of preeclampsia. Our study and previous works have added value to the use of maternal NLR level in determination of the severity of preeclampsia. We believe that pregnancy outcomes can be improved with optimal antenatal preparation. Nevertheless, scholars should further study the association between NLR level and preeclampsia.

**Abbreviations**

NLR: neutrophil/lymphocyte ratio

BMI: body mass index

MPO: myeloperoxidase

**Declarations**

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**Figures**
Figure 1

Flowchart of the study population. 934 patients from the department of obstetrics, affiliated hospital of Jining medical university in Shandong province of China between 1 January 2018–30 September 2020 were included in the study. Among these patients, 396 healthy pregnancies, 222 mild preeclampsia, and 315 severe preeclampsia according the inclusion and exclusion criteria.
Figure 2

MPO staining of the placental tissues from a healthy control pregnancy (A), a mild preeclampsia pregnancy (B), a severe preeclampsia pregnancy (C). Positive staining showed brown-yellow granules in the tissue (red arrows). The number of MPO was significantly higher in preeclampsia than in healthy controls (p < 0.05). H-score of MPO in three groups (A: healthy control group, B: mild preeclampsia group, C: severe preeclampsia group) (D).