ORIGINAL PAPER

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Leukocyte count and neutrophil/lymphocyte ratio in the second trimester of pregnancy as a predictor of preeclampsia

Contaje de leucocitos y relación neutrófilos/linfocitos en el segundo trimestre del embarazo como predictor de preeclampsia

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ABSTRACT

Objective: To establish the usefulness of leukocyte count and neutrophil/lymphocyte ratio in the second trimester of pregnancy as a predictor of the development of preeclampsia. **Design:** Case-control study. **Institution:** Hospital Central "Dr. Urquinaona", Venezuela. **Participants:** Pregnant women between 17 and 20 weeks who attended prenatal consultation and were followed up to term. **Methods:** Blood samples were taken from the pregnant women who were followed until delivery to establish whether they developed preeclampsia. **Main outcome measures:** General characteristics of the patients, values of leukocytes, neutrophils, lymphocytes, neutrophil/lymphocyte ratio and prognostic efficacy. **Results:** Of the 504 patients selected, 41 pregnant women developed preeclampsia (group A) and 463 pregnant women were considered as controls (group B). No statistically significant differences were found in overall characteristics at baseline (p = ns). The gestational age at the time of diagnosis of preeclampsia in group A was 35.0 +/- 3.2 weeks. Patients in group A were found to have significantly higher values of leukocytes, neutrophils and neutrophil/lymphocyte ratio along with lower values of lymphocytes compared to patients in group B (p < 0.05). Only the absolute values of neutrophils (area under the curve 0.810) and neutrophil/lymphocyte ratio (area under the curve 0.963) had useful prognostic values for discriminating between patient groups for the development of preeclampsia (p < 0.05). **Conclusion:** The neutrophil/lymphocyte ratio and absolute neutrophil count in the second trimester of pregnancy are useful tools in the prediction of preeclampsia.

Key words: Leukocytes, Neutrophils, Neutrophil/lymphocyte ratio, Preeclampsia, Prediction.

RESUMEN

Objetivo. Establecer la utilidad del contaje de leucocitos y relación neutrófilos/linfocitos en el segundo trimestre del embarazo como predictor del desarrollo de preeclampsia. **Diseño.** Estudio de casos-controles. **Institución.** Hospital Central "Dr. Urquinaona", Venezuela. **Participantes.** Embarazadas entre 17 y 20 semanas que acudieron a la consulta prenatal y fueron seguidas hasta el término del embarazo. **Métodos.** Se tomaron muestras de sangre de las gestantes, que fueron seguidas hasta el parto para establecer si desarrollaban preeclampsia. **Principales medidas de resultado.** Características generales de las pacientes, valores de leucocitos, neutrófilos, linfocitos, relación neutrófilos/linfocitos y eficacia pronóstica. **Resultados.** De las 504 pacientes seleccionadas, 41 embarazadas desarrollaron preeclampsia (grupo A) y 463 embarazadas fueron consideradas como controles (grupo B). No se encontraron diferencias estadísticamente significativas en las características generales al inicio del estudio (p = ns). La edad gestacional en el momento del diagnóstico de preeclampsia en el grupo A fue de 35,0 ± 3,2 semanas. Se encontró que las pacientes del grupo A presentaron valores significativamente más elevados de leucocitos, neutrófilos y relación neutrófilos/linfocitos junto a valores más bajos de linfocitos comparados con las pacientes del grupo B (p < 0,05). Solo los valores absolutos de neutrófilos (área bajo la curva 0,810) y de relación neutrófilos/linfocitos (área bajo la curva 0,963) tuvieron valores pronósticos útiles para discriminar entre los grupos de pacientes para el desarrollo de preeclampsia (p < 0,05). **Conclusión.** La relación neutrófilos/linfocitos y el contaje absoluto de neutrófilos en el segundo trimestre del embarazo son herramientas útiles en la predicción de preeclampsia.

Palabras clave: Leucocitos, Neutrófilos, Relación neutrófilos/linfocitos, Preeclampsia, Predicción.

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Introduction

Preeclampsia is a disorder that affects 4% to 6% of all pregnancies[1]. Although the mechanisms responsible for its etiology have not been clearly defined, inflammation, endothelial dysfunction, altered angiogenesis, inappropriate placentation, oxidative stress, immunologic and genetic factors are essential components for the development of the syndrome[2].

Although preeclampsia is unique to pregnancy in humans and shares pathophysiological characteristics and risk factors (hypertension, diabetes, dyslipidemia and obesity) with cardiovascular disorders in adults, endothelial dysfunction and inflammation are important mechanisms for the onset and development of both conditions[3]. In addition, immune changes are central to the onset and development of the hypertensive syndrome of pregnancy. It has been suggested that excessive activation and exaggerated immune response by neutrophils and lymphocytes result in the release of inflammatory cytokines and autoantibodies leading to inflammation and endothelial dysfunction[4].

In addition to the probable individual effects of neutrophils and lymphocytes in preeclampsia, the neutrophil/lymphocyte ratio (NLR) has been proposed as a new indicator of increased systemic inflammation. Its predictive and prognostic value has been demonstrated in several cardiovascular diseases, such as hypertension, severity of coronary heart disease, long-term mortality in patients undergoing primary percutaneous coronary intervention, and cardiac mortality in patients with stable coronary artery disease[5,6].

NLR has been used to establish the diagnosis and predict the severity of preeclampsia, but so far there are controversial and contradictory results about its role in predicting the syndrome[7]. The aim of the research was to establish the usefulness of the leukocyte count and neutrophil/lymphocyte ratio in the second trimester of pregnancy as a predictor of the development of preeclampsia.

Methods

A prospective, explanatory study was conducted in nulliparous pregnant women with singleton pregnancies who were attended at the outpatient prenatal clinic of the Hospital's Central "Dr. Urquinaona", Maracaibo, Venezuela, between January 2012 and March 2018. The study protocol was approved by the Hospital Ethics Committee prior to the start of the research and written consent was obtained from all patients.

Pregnant women with a diagnosis of polyhydramnios, third trimester hemorrhage (placental abruption, placenta previa), suspected intrauterine growth restriction of the fetus (head circumference, abdominal circumference and femur length less than the 10th percentile reference with postnatal confirmation of weight less than the 10th percentile reference), severe preeclampsia with multiorgan manifestations were excluded; also fetuses with heart rate alterations, multiple gestation, presence of intrauterine or active maternal infection, chronic hypertensive disease (hypertension before 20 weeks of pregnancy), cardiac, hematologic, hepatic, renal or chronic systemic disease, pregestational or gestational diabetes mellitus, smoking, and those pregnant women in whom blood samples could not be obtained were not included. Patients who refused to participate in the research were also excluded.

Preeclampsia was established as systolic blood pressure of 140 mmHg or more or diastolic blood pressure of 90 mmHg or more, confirmed by 6 h or more difference. Blood pressure was measured in a sitting position after 15 min rest using a standard mercury sphygmomanometer with a 14-cm cuff. Systolic and diastolic blood pressure (taken relative to the fifth Korotkoff noise) was located relative to the nearest 2 mmHg point. The palpatory method was used to verify auscultatory readings of systolic blood pressure. Systolic and diastolic blood pressures were calculated from the average blood pressure of each arm. Proteinuria was defined as 300 mg or more of protein in a 24-h urine sample after 20 weeks of gestation.

Once the patients were selected for the study, a data collection form was filled out that included: patient identification, personal and gynecological-obstetrical history, prenatal control, gestational age (by date of last menstrual period or first trimester ultrasound) and leukocyte count values. Gestational age was calculated on the date of the last menstrual period and corrected by ultrasound if measurements during the first trimester showed a difference of more than 7 days. All pregnancies were followed until deliv-

ery and were categorized according to the development of preeclampsia (cases; group A) or not (controls; group B).

Antecubital vein blood samples were collected at the time of routine ultrasound evaluation in all selected pregnant women during the second trimester of pregnancy (17-20 weeks). Total and differential leukocyte counts were measured using an Abbott Cell-Dyn 3700 automated hematology analyzer (Abbott Laboratory®, USA). Absolute counts (cells x 103/mL) of cells were used in the analyses. NLR was determined using these values.

The values obtained were presented as mean +/- standard deviation. The Kolmogorov-Smirnov test was used to test the normal distribution of the data ($p > 0.05$). Student's t test for unrelated samples was used for cluster analysis and to compare continuous variables. The accuracy of leukocyte, neutrophil, lymphocyte and NLR values for predicting the development of preeclampsia is presented in terms of sensitivity, specificity, positive predictive value and negative predictive value. Operator-receptor analysis was used to determine the best cutoff value. This was determined by the highest sensitivity and specificity represented by the highest Youden index $p < 0.05$ was considered statistically significant.

RESULTS

Measurement results were obtained for 504 primigravid pregnant women, of whom 41 patients (8.1%) developed preeclampsia (group A) and 463 pregnant women (91.9%) were considered controls (group B). The general characteristics of the 2 groups of pregnant women are shown in Table 1. No statistically significant differences were found in maternal age, gestational age, and systolic and diastolic blood pressure at the time of ultrasound (p = ns). Gestational age at diagnosis of preeclampsia in group A was 35.0 +/- 3.2 weeks. Statistically significant differences were observed in gestational age at delivery, systolic and diastolic blood pressure at delivery, 24-hour proteinuria, and newborn birth weight between the 2 groups of pregnant women ($p < 0.0001$).

Table 2 shows the mean values of leukocyte count and NLR values. It was observed that patients in group A had significantly higher values of leukocytes (8.76 +/- 1.34 cells x 10³/mL compared to

TABLE 1. LEUKOCYTE COUNT AND NEUTROPHIL/LYMPHOCYTE RATIO IN THE SECOND TRIMESTER OF PREGNANCY AS A PREDICTOR OF PREECLAMPSIA. GENERAL CHARACTERISTICS.

Characteristics	Group A Cases (n = 41)	Group B Controls (n = 463)	p
Maternal age, years	22.9 +/- 2.5	22.5 +/- 2.3	ns
Body mass index, kg/m2	28.6 +/- 4.5	27.5 +/- 4.3	ns
Gestational age at time of examination, weeks	18.3 +/- 0.9	18.5 +/- 0.9	ns
Systolic blood pressure at time of examination, mmHg	110.5 +/- 5.3	111.8 +/- 5.2	ns
Diastolic blood pressure at time of examination, mmHg	77.5 +/- 4.2	77.1 +/- 3.9	ns
Gestational age at delivery, weeks	35.0 +/- 3.2	38.3 +/- 1.4	< 0.001
Systolic blood pressure at delivery, mmHg	139.9 +/- 14.4	115.7 +/- 7.2	< 0.001
Diastolic blood pressure at delivery, mmHg	98.6 +/- 5.7	74.2 +/- 8.0	< 0.001
Proteinuria, g/24 hours	4.30 +/- 1.60	0.24 +/- 0.03	< 0.001
Newborn birth weight, grams	2792 +/- 711	3624 +/- 352	< 0.001

TABLE 2. LEUKOCYTE COUNT AND NEUTROPHIL/LYMPHOCYTE RATIO IN THE SECOND TRIMESTER OF PREGNANCY AS A PREDICTOR OF PREECLAMPSIA. COMPARISON IN EACH STUDY GROUP.

	Group A Cases (n = 41)	Group B Controls (n = 463)	p
Leukocytes, x 10³/mL	8.76 +/- 1.34	8.32 +/- 1.21	0.0231
Neutrophils, x 10³/mL	6.61 +/- 1.30	5.67 +/- 1.44	< 0.001
Lymphocytes, x 10³/mL	1.43 +/- 0.44	2.05 +/- 0.62	< 0.001
Neutrophils / Lymphocytes ratio	4.70 +/- 1.25	2.82 +/- 1.20	< 0.001

8.32 +/- 1.21 cells x 10³/mL; p = 0.0231) and neutrophils (6.61 +/- 1.30 cells x 103/mL compared to 5.67 +/- 1.44 cells x 10³/mL; $p < 0.0001$) compared to group B patients. However, patients in group A had lower lymphocyte values (1.43 +/- 0.44 cells x 10³/mL) compared to patients in group B (2.05 +/- 0.62 cells x 10³/mL; $p < 0.0001$). NLR values were significantly higher in group A patients (4.70 +/- 1.25) compared to group B patients (2.82 +/- 1.20; cells x 103/mL; $p < 0.0001$).

Table 3 and Figure 1 show the precision values for the prognosis of preeclampsia of the leukocyte count and NLR elements. Only the neutrophil (area under the curve of 0.810) and NLR (area under the curve of 0.963) values were shown to have area under the curve values that were useful for discriminating between patient groups for the development of preeclampsia. However, the NLR cutoff value of 3.3 was shown to have a higher sensitivity (97.5% compared with 90.2%),
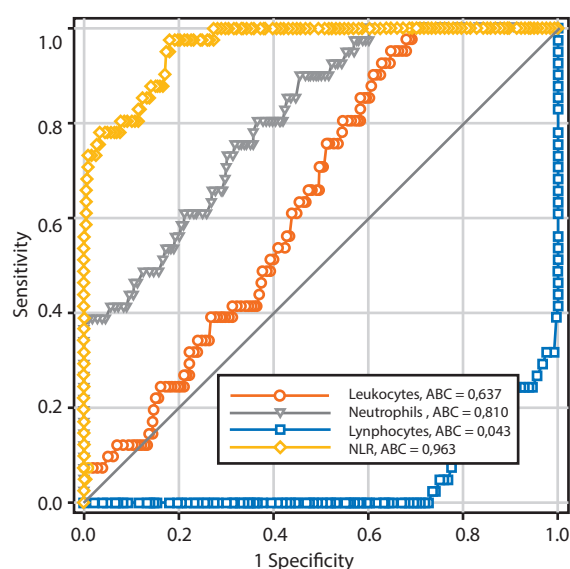
Eduardo Reyna-Villasmil, Duly Torres-Cepeda, Jorly Mejía-Montilla, Nadia Reyna-Villasmil, Martha Rondón-Tapia, Andreina Fernández-Ramírez

TABLE 3. LEUKOCYTE COUNT AND NEUTROPHIL/LYMPHOCYTE RATIO IN THE SECOND TRIMESTER OF PREGNANCY AS A PREDICTOR OF PREECLAMPSIA. PROGNOSTIC EFFICACY.

	Cutoff value	Area under the curve (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Accuracy % (95% CI)
Leukocytes	7,80	0,637 (0,568 - 0,707)	92,6 (80,0 - 98,4)	35,4 (31,0 - 39,9)	11,3 (8,3 - 15,1)	98,2 (94,9 - 99,4)	40,1 (35,9 - 44,4)
Neutrophils	5,80	0,810 (0,747 - 0,873)	90,2 (76,8 - 97,2)	53,3 (48,6 - 57,9)	14,6 (10,8 - 19,5)	98,4 (96,0 - 99,4)	56,3 (52,0 - 50,0)
Lymphocytes	1,71	0,043 (0,001 - 0,105)	15,0 (5,7 - 29,8)	17,4 (14,1 - 21,2)	1,5 (0,7 - 3,3)	70,4 (61,5 - 78,0)	17,3 (14,2 - 20,8)
Neutrophils / Lymphocytes Ratio	3,3	0,963 (0,940 - 0,985)	97,5 (87,1 - 99,3)	79,4 (75,5 - 83,0)	29,6 (22,0 - 37,8)	99,7 (98,6 - 100,0)	81,0 (77,3 - 84,1)

95% CI = 95% confidence interval.

FIGURE 1. RESPONSE OPERATOR CURVE OF THE EFFICACY OF LYMPHOCYTE COUNT AND NEUTROPHIL/LYMPHOCYTE RATIO IN THE PREDICTION OF PREECLAMPSIA.

Pregnancy is a controlled inflammatory condition. The physiologic inflammatory activation that occurs in normal pregnancy is excessively increased in preeclampsia. The most widely accepted abnormalities in the pathophysiology of preeclampsia include endothelial dysfunction, impaired angiogenesis, and low-grade inflammation. Increased concentrations of proinflammatory cytokines produce free radical generation and oxidative stress, leading to endothelial injury[10]. Several factors have been suggested to be involved, including inflammatory cell activation and possible immunological changes, in which both neutrophils and lymphocytes participate, releasing cytokines and autoantibodies[11]. There is evidence that some markers of systemic inflammatory response obtained from routine peripheral blood hematology samples, such as NLR, have prognostic and predictive value in benign and malignant diseases, such as gynecological malignancies and inflammatory diseases in which alterations in immune cell counts are observed[12,13].

Maternal circulating leukocytes are activated in pregnancy and their activity is even higher in preeclampsia[14]. Therefore, these activated cells could be responsible for the vascular dysfunction associated with the syndrome[15]. Macrophages in the atherosclerotic plaque have a role as foam cells, whereas lymphocytes are part of the adaptive immune system producing antibodies[16]. Neutrophils are the first line of defense against infection at the site of injury, but they also infiltrate systemic vascular tissue in preeclamptic women, causing vascular inflammation[7]. In preeclampsia they are likely to be activated in the intervillous space when exposed to oxidized lipids secreted by the placenta[17]. In addition, neutrophils obtained from preeclamptic women express significantly more cyclooxygenase-2, which regulates the release of thromboxane, tumor necrosis factor-alpha and superoxide, than those

specificity (79.4% compared with 53.3%), positive negative predictive value (28.9% compared with 14.6%), negative predictive value (99.7% compared with 98.4%), and prognostic accuracy (81.0% compared with 56.3%) than the absolute neutrophil count cutoff value of 5.80 cells x 103/mL.

DISCUSSION

Research results show that absolute values of neutrophils and NLR can be useful in the prediction of preeclampsia, as they have a good discriminatory capacity. However, NLR values had a higher predictive ability compared to neutrophil values. It has been proposed that higher circulating neutrophils and decreased lymphocytes are indicators of risk for cardiovascular events[8]. The proposed utility of elevated NLR values is that it combines the predictive ability of two leukocyte subtypes into a single risk factor[9].

obtained from normotensive pregnant women or healthy nonpregnant women[18]. However, the mechanisms responsible for these changes are not fully understood[19].

Canzoneri et al.[20] found that the total leukocyte count was significantly increased in severe preeclamptic women compared to mild preeclamptic and normal pregnant women. This increase in total leukocyte count was mainly due to an increase in the number of neutrophils. It has been reported that the number of neutrophils increases 2.5-fold at 30 weeks of gestation during normal pregnancy and increases even more in preeclamptic women, with no significant differences in monocyte and basophil counts, accompanied by a decrease in absolute lymphocyte count compared to uncomplicated pregnant women[21]. This increase in the number of neutrophils during pregnancy may be the result of increased concentrations of colony-stimulating factors and circulating arachidonic acid[22,23].

It has been considered that NLR could be a marker for predicting the onset and severity of preeclampsia. Although this research demonstrated that its determination can be useful in predicting the onset and development of the syndrome, other studies have reported contrary results regarding both its diagnostic and predictive capacity[7,24-28]. Two studies found that the NLR value was higher in preeclamptic women compared to controls but found no significant differences between the groups[25,27]. These results were contrary to the results of the present investigation. Other investigations have shown that NLR in preeclamptic women was significantly higher compared to controls[24,26,28]. Similarly, there is evidence that the values could predict disease severity[26]. It has also been suggested that the increased NLR value in preeclamptic patients represents an independent predictor of disease severity[28].

There is evidence proposing NLR as a prognostic factor for cardiovascular disorders since significant differences in the increase of this ratio are associated with increased risk of cardiovascular morbidity and mortality[29]. For this reason, it is considered that this inexpensive and noninvasive hematologic marker may be important for risk evaluation and prediction of preeclampsia, as well as a possible prognostic factor for future cardiovascular disease. Further research should be conducted to establish the role of NLR in other inflammatory conditions of pregnancy.

Conclusion

The neutrophil/lymphocyte ratio and absolute neutrophil count in the second trimester of pregnancy are useful tools in the prediction of preeclampsia, as patients who develop the syndrome have significantly higher concentrations in that trimester compared to healthy pregnant controls. However, further studies are needed to confirm these findings.

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Eduardo Reyna-Villasmil, Duly Torres-Cepeda, Jorly Mejía-Montilla, Nadia Reyna-Villasmil, Martha Rondón-Tapia, Andreina Fernández-Ramírez

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