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Can Delta Neutrophil Index Predict The Superimposed Preeclampsia?

Dilek Menekse Beser[1*], Dilek Sahin[2]

[1]Department of Obstetrics and Gynecology, Division of Perinatology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey
[2]University of Health Sciences, Department of Obstetrics and Gynecology, Division of Perinatology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey

ABSTRACT

We aimed to determine the utility of delta neutrophil index (DNI) in predicting superimposed preeclampsia (SPE) in chronic hypertensive pregnant women.
The study included pregnant women diagnosed with chronic hypertension. SPE group was defined as developing acute or worsening hypertension, new-onset proteinuria, and/or significant new end-organ dysfunction after 20 weeks of gestation in a pregnant woman with chronic hypertension. Demographic variables such as maternal age, gravidity, parity, previous history of abortus, gestational age at SPE, birth week, birth weight, and Apgar scores were obtained from medical records. First-trimester laboratory parameters, including maternal aspartate transaminase (AST), alanine transaminase (ALT), platelets, and delta neutrophil index (DNI), 24-hour urine proteinuria level were assessed.
A total of 203 pregnant women with chronic hypertension were included in the study. Eighty-three of them were complicated by SPE. There were statistically significant differences in 24-hour proteinuria levels, the gestational week at birth, birth weight, and Apgar score at 1 minute and 5 minutes in the groups with and without SPE. In addition, the median DNI values were higher in the SPE group (p= <.001). The optimal cut-off value of DNI was 0.15 (76.1% sensitivity, 96.7% specificity, p= <.001). While DNI showed a weak positive correlation with the week patients were diagnosed with SPE (r = .223, p= .043), no significant correlation was found with the level of proteinuria (r =-.113, p= .318).
DNI can be used in predicting SPE in addition to other parameters when used in the first trimester.

Keywords: Chronic hypertension, delta neutrophil index, pregnancy, superimposed preeclampsia

Introduction

Hypertensive diseases are the second leading cause of maternal and fetal morbidity during pregnancy (1). Chronic hypertension, gestational hypertension, preeclampsia-eclampsia, and chronic hypertension with superimposed preeclampsia (SPE) describe hypertensive conditions in pregnancy. Persistent hypertension that worsens or suddenly begins after 20 weeks of gestation, new onset proteinuria, and new end-organ dysfunction indicate superimposed preeclampsia for pregnant women with chronic hypertension (2). Approximately 10-40% of pregnancies in women with chronic hypertension are affected by SPE, which causes higher maternal and perinatal morbidity rates than preeclampsia alone (3). Pregnant women who develop SPE have a greater risk for complications, including preterm birth, fetal growth restriction, cesarean delivery, placental abruption, and stillbirth (4). Early detection of SPE is critical as it is a severe condition that will affect the mother and fetus's life. The cause of these effects is believed to be an excessive systemic inflammatory response that leads to or increases endothelial dysfunction, a significant characteristic of chronic hypertension and preeclampsia (5). Thus, various proinflammatory markers have been investigated for their roles in diagnosing these women.

The DNI, or delta neutrophil index, is a recently developed inflammatory marker that measures the variance between leukocyte differentials calculated in the myeloperoxidase channel and those summed in the nuclear lobularity channel provided by computerized cell analyzers (6). This parameter is believed to indicate the proportion of immature granulocytes to total neutrophils circulating in the body (7). DNI has shown promising potential in predicting and assessing the

***Corresponding Author:** Dilek Menekse Beser, Department of Obstetrics and Gynecology, Division of Perinatology, Turkish Ministry of Health, Ankara City Hospital, 1604th Street, No: 9, Cankaya, Ankara, 06800 Turkey
E-mail: dilekmbeser@gmail.com, Phone: +90 (555) 770 15 75
ORCID ID: Dilek Menekse Beser: 0000-0002-7022-0610, **Dilek Sahin:** 0000-0001-8567-9048
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severity of infectious diseases, including sepsis, acute appendicitis, and bacterial peritonitis (8-10). It has been studied that DNI can serve as a predictive marker for histological chorioamnionitis in pregnant women who have experienced preterm premature rupture of membranes (11). This finding supports the notion that DNI has potential applications across different areas of obstetrics. So, we aimed to determine the role of DNI in predicting SPE.

Materials and Methods

The study participants were pregnant women with chronic hypertension who were admitted to the Perinatology Clinic of Ankara City Hospital from September 2019 to March 2023. The study protocol (E2-23-4046) was approved by the Medical Research Ethics Committee of the hospital and the Ministry of Health of the Republic of Turkey.

The determination of SPE was established by referring to the guidelines outlined in the Practice Bulletin issued by the American College of Obstetricians and Gynecologists (2). Based on the given definition, the SPE comprises pregnant women with chronic hypertension who experience acute or aggravated high blood pressure, new-onset proteinuria, and/or notable new end-organ dysfunction after 20 weeks of gestation. The study excluded patients with diabetes, metabolic syndrome, liver or kidney disease, cancer, multiple pregnancies, signs of infection, or missing laboratory values. Excluded patients were shown with a flow chart (Figure 1). We conducted a retrospective analysis of medical records to collect clinical data. The data comprised demographic variables such as maternal age, gravidity, parity, previous history of abortus, gestational age at SPE, birth week, birth weight, and Apgar scores. First-trimester laboratory parameters, including maternal aspartate transaminase (AST), alanine transaminase (ALT), platelets, delta neutrophil index (DNI), and 24-hour urine proteinuria level, were recorded.

To obtain the serum DNI, we utilized the ADVIA 2120 Hematology System, an automated cell analyzer from Siemens Healthcare Diagnostics. The system employs flow cytometry and two independent white blood cell counting methods: the myeloperoxidase channel and the lobularity/nuclear density channel. The calculation of the DNI level involved a simple formula, which is: DNI (%) = (the leukocyte subfraction measured through cytochemical reactions in the myeloperoxidase channel) - (the leukocyte subfraction assayed using the nuclear lobularity channel by reflected light beam measurements).

Statistical Analyses: We used the Statistical Package for Social Sciences software version 17.0 (SPSS Inc, Chicago, IL) for the statistical analyses. Independent t-test and Mann Whitney-U test were performed to compare the clinical data with and without superimposed preeclampsia. Descriptive variables are expressed as means and standard deviations (SDs) or medians and ranges. Error bar was used to show median values of DNI in both groups. We used the Youden index on the receiver operating characteristic (ROC) curve to determine the best cut-off values of DNI in predicting SPE. Pearson correlation coefficient was used to find the possible relationship between DNI values and proteinuria level and gestational week when the pregnant woman had SPE. We considered a two-tailed p-value of less than 0.05 statistically significant.

The sample size was analyzed using the G Power software (version 3.1; Franz Foul, Universität Kiel, Kiel, Germany) (12). An effect size of 0.60 (medium) was determined for the sample size with a p-value (two-tail) of 0.05 and a power of 95%. It was found that there should be at least 148 cases. We planned to include 74 patients in each group.

Results

The study consisted of 203 pregnant women diagnosed with chronic hypertension. Eighty-three of them were complicated by SPE. According to this, the study population was divided into two groups: 83 women with SPE and 120 with chronic hypertension without SPE. In Table 1, the comparison between the demographic and clinical characteristics of both groups is presented. Among the two groups, there were statistically significant differences in 24-hour proteinuria levels, the gestational week at birth, birth weight, and Apgar score at 1 minute and 5 minutes. In addition, the median DNI values were significantly higher in the pregnant women with SPE than those without SPE (0.9 vs. 0,1, p = <.001). Figure 2 demonstrates the distribution of the DNI values in two groups. ROC curve analysis to estimate the performance of DNI values in predicting SPE is shown in Table 2 and Figure 3. The optimal cut-off value of DNI was 0.15 (76.1% sensitivity, 96.7% specificity, p = <.001). Table 3 demonstrates the correlation between DNI with proteinuria level and gestational week for SPE. While DNI showed a weak positive

Table 1: The Clinical Features of The Study Population

	Chronic hypertension (n=203)		
	without superimposed (n=120)	with superimposed (n=83)	p-value
Age	33.1±6.7	34.3±5.7	.187*
Gravidity	3±2	3±2	408*
Parity	2±1	2±1	.475*
Abortus	1±1	1±1	.052*
AST (IU/L)	18.7±7.7	20±13.5	.417*
ALT (IU/L)	16 (13-24)	16 (13-26)	.303**
PLT (10^9)	286±70	275±63	.231*
DNI (%)	0.1 (0.1-0.1)	0.9 (0.2-2.3)	<.001**
24-hour proteinuria (mg)	114 (81-159)	323 (223-544)	<.001**
Birth week	38±2	35±3	<.001*
Birth weight (gram)	3155±587	2660±890	<.001*
Apgar 1st score	7 (7-8)	7 (6-7)	<.001**
Apgar 5th score	9±1	8±1	.004*

Values are presented as mean± standard deviation and median (IQR (Inter Quartile Ranges))
The bold characters indicated the significant "p" values p < 0.05.
Abbreviations: AST: aspartate transaminase, ALT: alanine transaminase, PLT: platelet, DNI: delta neutrophil index
* Independent t-test
** Mann Whitney U test

Table 2: Receiver Operating Characteristic (ROC) Curve Analysis For The Performance Of Delta Neutrophil Index (DNI) In Predicting Superimposed Preeclampsia

	p-value	AUC (95% CI)	Cut-off	Sensitivity	Specificity
DNI	<.001	.875 (.818-932)	0.15	76.1%	96.7%

Abbreviations: DNI: delta neutrophil index, AUC: the area under the curve
The bold characters indicated the significant "p" values p < 0.05

Table 3: The Correlation Between Delta Neutrophil Index (DNI) With Proteinuria Level and Gestational Week For Superimposed Preeclampsia (SPE)

	Superimposed preeclampsia (SPE) week	Proteinuria
p-value	.043‡	. 318‡
r value	.223	-.113

‡ Pearson test
The bold characters indicated the significant "p" values p < 0.05

correlation with the week patients were diagnosed with SPE (r = .223, p=.043), no significant correlation was found with the level of proteinuria (r = -. 113, p=.318).

Discussion

To date, this was the first study to investigate serum DNI's predictive utility for SPE in pregnant women. This study found that serum DNI levels were significantly higher in women with SPE and had high sensitivity and specificity in predicting this condition.

Pregnant women with SPE are at a higher risk of maternal and perinatal complications than those with preeclampsia alone. It can be challenging to differentiate between SPE and chronic hypertension since the standard criteria for diagnosing preeclampsia, including high blood pressure and proteinuria, could already be present before pregnancy in chronic hypertension cases. Although different rates are observed in the literature, approximately 40.8% of the patients with chronic hypertension are complicated by SPE in our study. This relatively high rate underlines

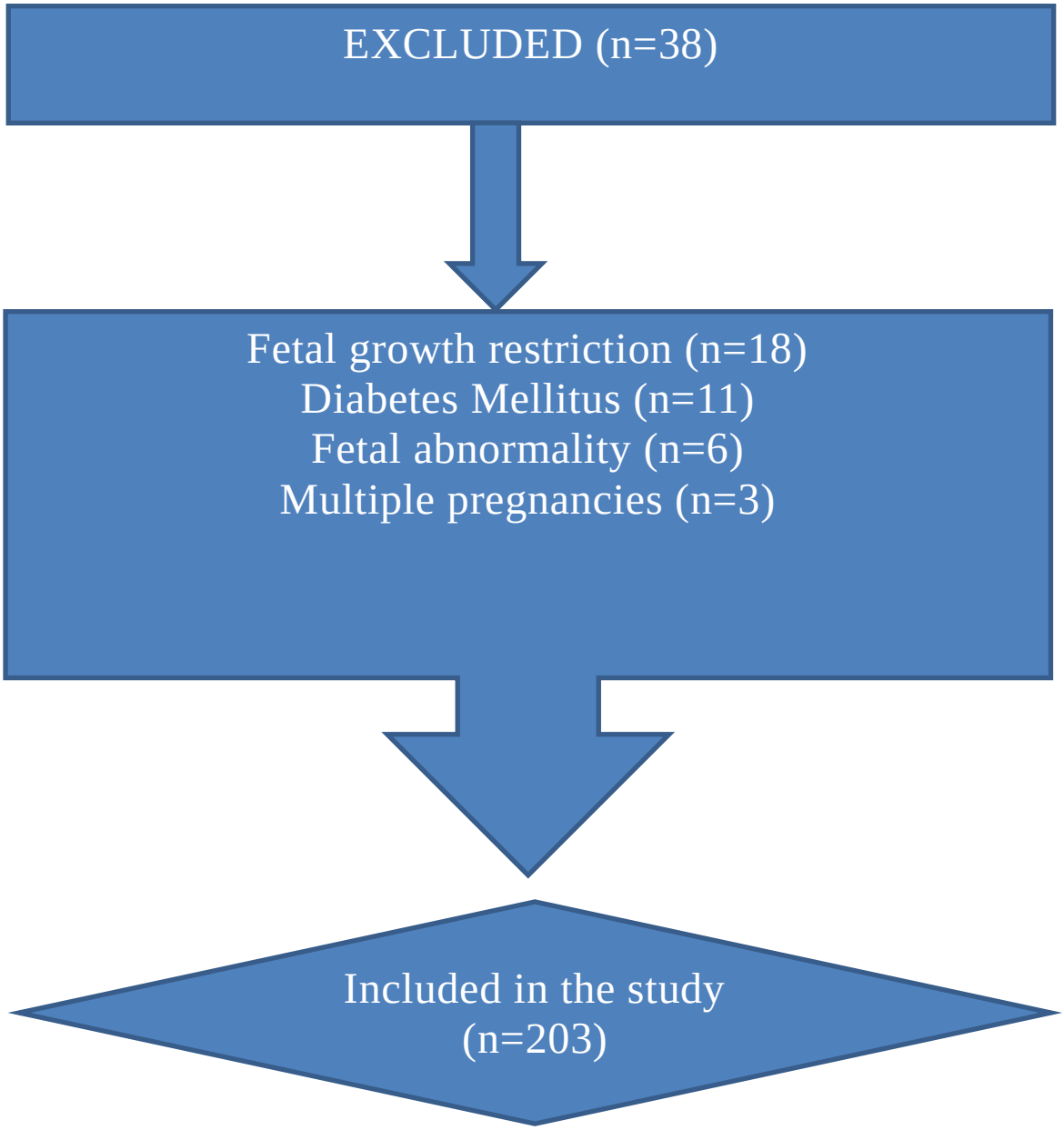

Fig. 1. The flow chart of patients not included in the study

that pregnant women with chronic hypertension should be followed closely regarding SPE.

Preeclampsia is a condition that causes a persistent state of inflammation due to an imbalance in immune functions. The underlying reason for preeclampsia is still uncertain, but it is evident that immunological factors are significant contributors to its pathophysiology. Specific changes have the potential to stimulate chronic inflammation by reducing regulatory immune cells and cytokines while increasing proinflammatory ones. This can lead to an immune imbalance that generates reactive oxygen species and elevates endothelin-1 expression (13). This imbalance begins with insufficient trophoblast invasion, resulting in placental ischemia (14). In addition, antiangiogenic events triggered by an impaired placenta and causing endothelial dysfunction are thought to affect the pathogenesis of the disease substantially. It has been observed that both preeclampsia and chronic hypertension are characterized by an overactive inflammatory response that worsens endothelial dysfunction. As a result, researchers have investigated the usefulness of proinflammatory agents such as tumor necrosis factor-a, interleukin-6, and certain cell adhesion molecules in detecting SPE among women with chronic hypertension (5). In light of this information, we hypothesized the potential use of DNI, a novel inflammatory marker, for predicting SPE.

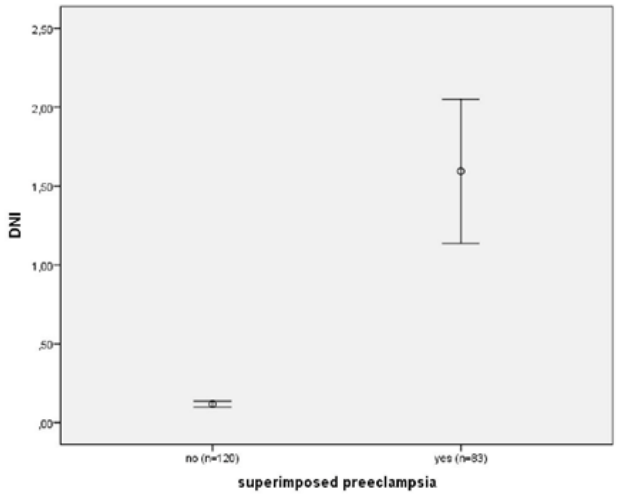

Fig.2. The comparison of the delta neutrophil index (DNI) values between pregnant women with and without superimposed preeclampsia

The DNI value has been researched in many various situations before. Studies have demonstrated that it increases in complicated acute appendicitis and is a valuable prognostic factor in assessing the 30-day mortality rate for cases of spontaneous bacterial peritonitis (8, 15). Moreover, It has been concluded that it is an effective marker for identifying and prognostic evaluating patients diagnosed with sepsis (9). It has also been assessed in various situations in obstetrics. According to a study, pregnant women experiencing severe preeclampsia have higher serum DNI levels than those with mild preeclampsia and healthy pregnant women (16). Preeclampsia occurs due to an immune function imbalance caused by insufficient trophoblast invasion, resulting in chronic inflammation (17). This leads to an uncontrolled state of inflammation characterized by increased pro-inflammatory immune cells and cytokines and decreased regulatory ones. Studies have recently discovered that neutrophils are activated by oxidized lipids secreted by the placenta in the intervillous space in preeclampsia patients (14, 17). These activated neutrophils then cause vascular inflammation by infiltrating the systemic vascular tissue in pregnant women. This suggested that neutrophil indices were related to the severity of preeclampsia. Moreover, another study indicates that DNI can serve as a predictive marker for chorioamnionitis in pregnant women with preterm premature rupture of membranes (11). A study comparing the DNI levels in normal pregnancies and diabetic pregnant women revealed high DNI levels in pregnant women with gestational diabetes mellitus (18). On the contrary,

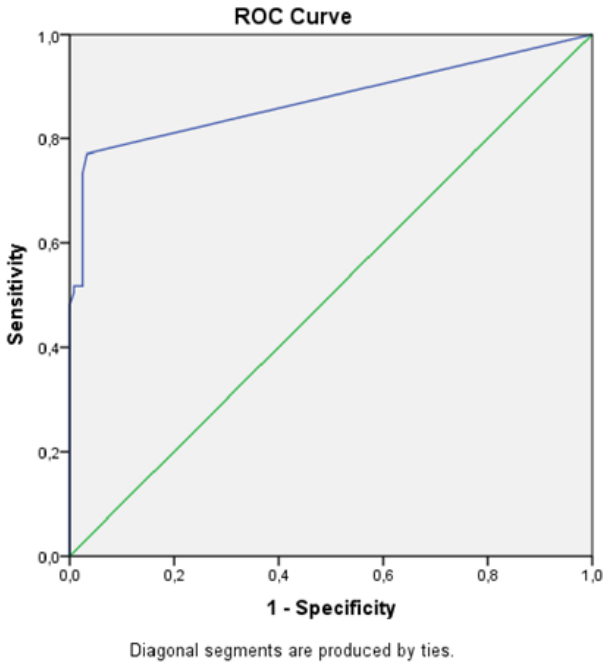

Fig. 3. Receiver operating characteristic (ROC) curve predicting superimposed preeclampsia in pregnant women with chronic hypertension

a study investigating the hematological parameters of patients with hyperemesis gravidarum showed that DNI did not differ significantly between the groups (19). Still, neutrophil count and neutrophil-lymphocyte ratio were higher in pregnant women with hyperemesis gravidarum. Another study investigating the effectiveness of blood parameters in predicting gestational hypertension and preeclampsia showed that the DNI and neutrophil-lymphocyte ratio were insufficient but underlined the need for further prospective studies (20). Our study found 76.1 % sensitivity and 96.7% specificity for prediction in addition to high DNI levels in the SPE group. We also analyzed the correlation between DNI values and proteinuria levels and the gestational week in pregnant women with SPE, as the values of DNI were notably elevated in this group. While there was no correlation between DNI values and proteinuria level, the significant correlation between superimposed gestational weeks supported that this parameter could be used to predict the disease.

The study's strengths are that DNI is a simple method that can be easily obtained and acquired from routine blood tests without additional intervention. Our study also has some limitations. The limitations include the lack of analysis of other inflammatory parameters and the relatively small number of patients. Future studies should be

planned prospectively with a larger number of patients.

In conclusion, DNI can be used in predicting SPE in addition to other parameters when used in the first trimester.

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Conflicts Of Interest: The authors have no conflicts of interest.

Synopsis: DNI can be used in predicting superimposed preeclampsia in addition to other parameters when used in the first trimester.

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