ORIGINAL RESEARCH

Do serum Delta-Neutrophil Index and Neutrophil-to-Lymphocyte ratio predict fetal growth restriction?

Seval Yılmaz Ergani[1*], Alperen Aksan[2], Ceren Polat Kamacı[2], Zeynep Şeyhanlı[1], Müjde Can İbanoğlu[2], Yıldız Akdaş Reis[2], Kadriye Erdoğan[2], Şevki Çelen[1]

1 Department of Perinatology, Etlik Zubeyde Hanım Women's Health Training and Research Hospital, Ankara, Turkey
2 Department of Obstetrics and Gynecology, Etlik Zubeyde Hanım Women's Health Training and Research Hospital, Ankara, Turkey

Corresponding Author: Seval Yılmaz Ergani **Email**; dr.svl7@gmail.com

Abstract

Aım

To investigate the prognostic significance of delta-neutrophil index (DNI) and neutrophil/lymphocyte ratio (NLR) in women with intrauterine growth retardation (IUGR) and normal pregnant women.

Methods

Normal pregnant women who delivered at Health Sciences University Etlik Zübeyde Hanım Women's Health Training and Research Hospital between January 2015 and July 2020 and pregnant women with IUGR were included in the study. 486 pregnant women and 400 normally pregnant women diagnosed with IUGR according to Delphi criteria were included in the analysis. Data available at presentation for delivery or within four weeks before delivery were used for analysis of DNI and other laboratory values in both the patient and control groups.

Results

The mean age of study group was 28.3±4.6 and control group was 27.2±5.6 years. There were significant differences between the study and control groups regarding maternal age, prepregnancy BMI, BMI at delivery and gestational age at admission and delivery, hemoglobin(Hb) levels, hematocrit (Hct) values,WBC values, lymphocyte,monocyte,neutrophile,thrombocyte counts, NLRs, DNI values, between the two groups. The median NLRs were 4.6 (range, 1.54±44.29), the mean DNI value of IUGR was --0.26±3.4 and the mean DNI value of control group was -3.7±5.8 ($p<0.001$, $p<0.001$, respectively). The NLR and DNI levels are significantly higher in the IUGR group. The optimal cut-off value for NLR was 3.84, with a sensitivity of 70.6% a specificity of 70.5%, and an area under the receiver operating characteristic curve of 0.780. The optimal cutoff value for DNI was -1.18, with a sensitivity of 53.9%, a specificity of 52%, and an area under the ROC curve of 0.692. The Odds Ratio of the DNI was 1.2, and NLR was 5.7.

Conclusıon

Considering its sensitivity and specificity, the NLR value shows that inflammatory events are much more effective in pregnant women with IUGR than we thought.

Keywords: delta-neutrophil index, intrauterine growth retardation, neutrophil/lymphocyte ratio, pregnant

Introduction

When the developing fetus is exposed to a suboptimal and pathological intrauterine environment during pregnancy, it results in fetal growth restriction (IUGR)[1]. Fetal IUGR occurs in 4% to 7% of live births worldwide each year and can occur alone or in conjunction with other disorders such as preeclampsia[2,3]. IUGR is the second leading cause of perinatal mortality[4]. IUGR not only raises the risk of fetal abnormalities, preterm birth, and stillbirth, but it also increases the risk of numerous neurologic and respiratory problems in infants dramatically[5,6]. Furthermore, there is an increased risk of cognitive impairment in childhood, as well as an increased risk of heart disease, hypertension, and Type 2 diabetes mellitus in adulthood[7-11].

Systemic markers of immune inflammation derived from peripheral blood cells have recently attracted much attention because they can be easily measured and optimization is readily possible in the same laboratory. According to the studies, NLR has been investigated in cases such as coronary heart disease, and endometriosis, and it has been suggested that it can predict these diseases[12,13]. NLR has become very important in recent years, especially in carcinogenesis, which is thought to be related to inflammation such as colorectal cancer and lung cancer[14,15]. Delta neutrophil index (DNI) is a value that represents polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) and MPO-reactive cells that lack nuclear lobularity and are termed immature neutrophils. Captures immature granulocytes in the blood that are related with infection and inflammation. The serum delta neutrophil index (DNI) has been identified as a novel inflammatory marker. Prior studies revealed that it was increased in patients with severe preeclampsia[16]. It has been shown to be predictive of chorioamnionitis in PPROM patients[17].

Although the mechanism of IUGR is unknown, we wondered if DNI and NLR have predictive significance in IUGR because maternal inflammation from any source could damage the placenta and disrupt the passage of nutrients and oxygen to the fetus.

https://dx.doi.org/10.4314/mmj.v36i3.6

Material and Methods

This study adhered to the rules of the Helsinki Declaration on Human Subjects Research and was approved by the institutional review board from Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital (date: 09/07/2020, approval number: 10/21). Everyone signed a written informed consent form and gave their verbal consent.

The study included healthy pregnant women who delivered at Health Sciences University Etlik Zubeyde Hanım Women's Health Training and Hospital between January 2015 and July 2020, as well as pregnant women with IUGR. The study excluded individuals with multiple pregnancies and fetal malformations, chronic hypertension and pregestational and gestational diabetes, patients with missing DNI levels, and maternal disorders such as liver and renal disease, cancer, hematological and autoimmune diseases. Patients who showed evidence of systemic infection (cystitis, tonsillitis, flu, protracted rupture of membranes, etc.) during delivery were also ruled out, as infections higher DNI levels.

During prenatal follow-up, patients are called for regular follow-up examinations and estimated fetal weight is determined by measurements.

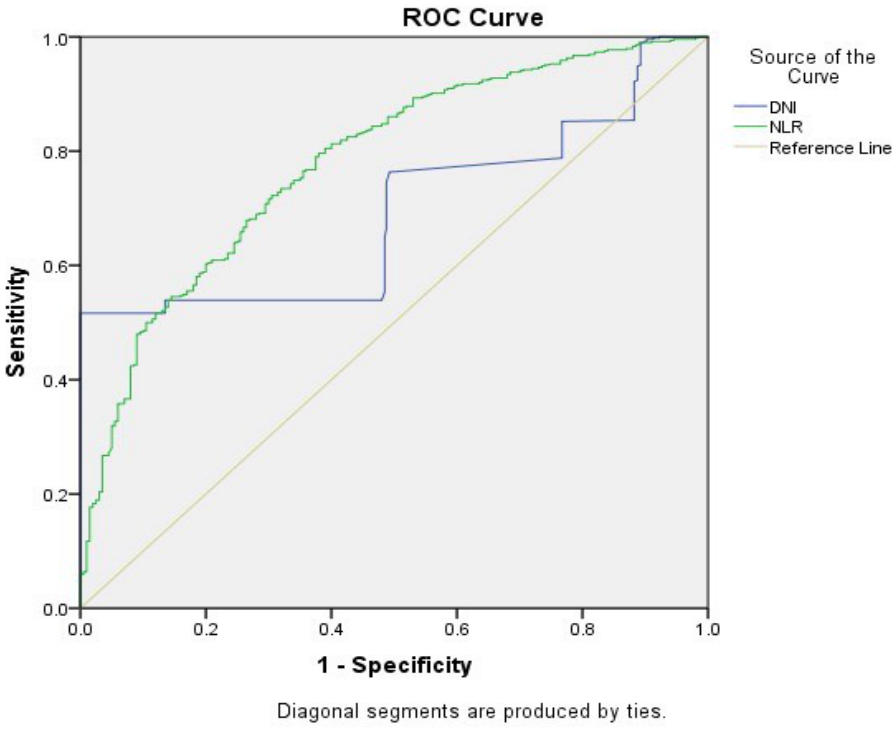
If this estimated fetal weight is < 3rd percentile or < 10th percentile, IUGR is diagnosed by additional Doppler measurements. The diagnosis of IUGR was made according to the Delphi classification, and this classification was < 32 weeks; estimated fetal weight < 3rd percentile or umbilical artery Doppler with absent end diastolic flow/with reverse diastolic flow; or estimated fetal weight < 10th percentile plus umbilical artery Doppler > 95th percentile Pulsatility Index (PI) or uterine artery Doppler > 95th percentile Pulsatility Index (PI) and > 32 weeks; estimated fetal weight < 3rd percentile or at least two out of three of the following: EFW less than 10th percentile or AC less than 10th percentile or umbilical artery (UA) Pulsatility Index (PI) above 95th percentile or cerebroplacental ratio <5th percentile. The study group included patients diagnosed with IUGR by Delphi criteria 18. The diagnosis of IUGR was made by two specialists working in the perinatology clinic (SYE and SC), and the Voluson E6 ultrasound system (GE, Healthcare, Zipf, Austria) was used for fetal morphology screening in our perinatology clinic. All pregnant women with IUGR diagnosed between 23 (limit of viability) and 37 weeks were retrospectively studied. Women with singleton pregnancies who had healthy prenatal care and had healthy neonates after 37 weeks were included in the control group.

Medical records were reviewed retrospectively for demographic and clinical data such as maternal age, gravidity, parity, body mass index (BMI), gestational age at delivery, mode of delivery (vaginal delivery or cesarean section), and birth weight, as well as neonatal outcome (neonatal intensive care unit (NICU) admission, APGAR scores, gender), maternal white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), thrombocyte, lymphocyte, neutrophils were used for analysis in both the research and control groups.

A complete blood count is routinely requested from patients when delivery approaches or when they are hospitalized for delivery. The blood parameters considered were those reported at the time of delivery request or within the last 4 weeks before delivery. DNI is an existing finding that automatically appears on a complete blood count. To evaluate the relationship between IUGR and inflammation, DNI values were examined in patients' complete blood counts and NLR values were calculated by dividing the neutrophil count/lymphocyte count.

DNI and NLR values were compared between the study and control groups.

The SPSS 23.0 program was used to do statistical analysis on the study.



The descriptive statistics for the categorical variables in the data set include mean and standard deviation, while the descriptive statistics for the continuous variables include median, minimum, and maximum values. The Shapiro-Wilk test was used to determine if the continuous variables conformed to the normal distribution. For numerical variables, the Mann Whitney U and Student's t-tests were utilized. $p<0.05$ was deemed statistically significant. The Receiver Operating Characteristic (ROC) curve was derived after doing a univariate logistic regression analysis.

Results

The demographic and baseline features of the study and control groups were compared (Table 1). The mean age of study group was 28.3±4.6 and control group was 27.2±5.6 years. The median gestational age at admission of the study group was 37(25-41) and that of the control group was 39 (37-40). There were significant variations in median maternal age, prepregnancy BMI, BMI at delivery, and gestational age

https://dx.doi.org/10.4314/mmj.v36i3.6

Table 1. Clinical characteristics of healthy pregnant women and pregnant women with IUGR

	IUGR (n=486)	Control group (n=400)	P value
Maternal age (year)	28.3±4.6	27.2±5.6	0.002[b]
Gravidity	2(1-7)	2(1-8)	0.059[a]
Parity	0(0-4)	0(0-5)	0.925[a]
Pre-pregnancy BMI (kg/m2)	23.1±5.1	24.1±5.1	0.003[a]
BMI at delivery (kg/m2)	28.2±4.5	29.1±4.3	0.003[b]
Gestational age at admission (weeks)	37(25-41)	39(37-40)	<0.001[a]
Gestational age at delivery (weeks)	37(26-37)	39(37-40)	<0.001[a]
Delivery mode (%)			<0.001[a]
Vaginal delivery	207(42.6%)	262(65.5%)	
Cesarean section	279(57.4%)	138(34.5%)	
Neonatal birth weight (g)	2374±466	3290±414	<0.001[b]
APGAR score at 1 min	9(0-10)	9(7-9)	<0.001[a]
APGAR score at 5 min	10(0-10)	10(8-10)	<0.001[a]
Gender, male (%)	194(39.9%)	204(51%)	0.001[a]
NICU admission (%)	66(7.4%)	0	<0.001[a]

[a]Mann Whitney U test and [b]Student's t-test performed. IUGR, intrauterine growth restriction**;** BMI, body mass index; NICU, neonatal intensive care unit. Data is a given as mean± Standard deviation, n(%), median (min-max). Results were accepted as 95% confidence interval and p value <0.05 significant.

Table 2. Laboratory values of healthy pregnant women and pregnant women with IUGR

	IUGR (n=486)	Control group (n=400)	P value
Hb (g/dL)	11.85±4.52	12.68±0.87	<0.001[b]
Hematocrit	35.24±3.93	38.53±2.74	<0.001[a]
WBC (cells/L)	9.36±2.97	8.12±2.33	<0.001[a]
Thrombocyte (x10^3/L)	239.15±65.92	264.70±64.11	<0.001[b]
Neutrophile (cells/L)	8.27±3.04	5.71±1.97	<0.001[a]
Monocyte (cells/L)	0.51±0.33	0.43±0.24	<0.001[a]
Lymphocyte (cells/L)	1.72±0.93	1.87±1.06	<0.001[a]
NLR	4.6(1.54-44.29)	3.1(1.07-11.3)	<0.001[a]
DNI (%)	-0.26±3.4	-3.7±5.8	<0.001[b]

[a]Mann Whitney U test and [b]Student's t-test performed. Hb, hemoglobin; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; DNI, delta neutrophil index . Data is a given as mean± standard deviation, n(%), median (min-max). Results were accepted as 95% confidence interval and p value <0.05 significant.

at admission and delivery, APGAR scores at 1 minute and 5 minutes, and NICU admission between the study and control groups ($p< 0.05$). The study group's mean birthweight was considerably lower than the control group's ($p< 0.001$).

There were substantial differences between the two groups in hemoglobin (Hb) levels, hematocrit (Hct) values, WBC values, lymphocyte,monocyte,neutrophile,thrombocyte counts, NLRs, and DNI values. The median NLRs were 4.6 (range, 1.54±44.29), the mean DNI value of IUGR was --0.26±3.4 and the mean DNI value of control group was -3.7±5.8 ($p<0.001$, $p<0.001$, respectively). The IUGR group has significantly greater NLR and DNI levels.

With a sensitivity of 70.6%, a specificity of 70.5%, and an area under the receiver operating characteristic curve of 0.780, the best cut-off value for NLR was 3.84. With a sensitivity of 53.9%, a specificity of 52%, and an area under the ROC curve of 0.692, the best cutoff value for DNI was -1.18. The DNI Odds Ratio was 1.2, and the NLR was 5.7 (Table 3, Figure 1).

https://dx.doi.org/10.4314/mmj.v36i3.6

Table 3. Area Under the ROC Curve (AUC), OR, sensitivity and specificity by the optimized cut-off values and diagnostic data of DNI and NLR in predicting IUGR

	AUC (95% CI)	Cut off	OR	Sensitivity(%)	Specificity(%)	P value
NLR	0.780(0.750-0.810)	3.84	5.7	70.6	70.5	<0.001
DNI	0.692(0.657-0.727)	-1.18	1.2	53.9	52	<0.001

Logistic regression models obtained from forward stepwise selection. AUC, Area Under the Curve; CI, Confidence interval, OR, Odds ratio; NLR, neutrophil-to-lymphocyte ratio; DNI, delta neutrophil index

Discussion

This is the first study to look into NLR and DNI in pregnancies with complicated IUGR. NLR and DNI values in the IUGR group were substantially higher than in the control group. Given its sensitivity and specificity, NLR, in particular, can be employed for prenatal counseling and management planning.

Fetal growth restriction (IUGR) is related with a unique placental phenotype, which is caused by abnormalities in placental transport mechanisms, resulting in fetal malnutrition[19]. Placental infarction, trophoblastic villi inflammation, and vasculitis are prominent histopathological abnormalities in the placentas of IUGR-complicated pregnancies, and they can affect placental elasticity[20,21]. NLR, DNI, WBC, and neutrophil levels were considerably greater in pregnant women with IUGR compared to the control group in our investigation, which is consistent with this study.

Whether this is due to inflammation is still under investigation in the literature. According to a study by Chen et al, inflammation of the placenta due to vitamin D deficiency during pregnancy further impairs placental development and function[22]. In our study, NLR in particular predicted IUGR with a sensitivity of 70.6% and a specificity of 70.5%, suggesting that inflammatory mechanisms, the cause of which we cannot explain with the current literature, play a role in the pathogenesis of IUGR.

Proinflammatory cytokines and apoptotic residues have all been associated with causing maternal endothelial cell activation that characterizes preeclampsia and may explain the pathogenesis, especially in preeclampsia-associated IUGR[23,24]. Because IUGRs associated with preeclampsia were excluded from our study, the change in inflammatory markers in this group is unknown. However, the high NLR and DNI values compared with the normal group suggest that inflammation plays a role in the pathogenesis of IUGR, even in the absence of preeclampsia. Previous studies have shown that a high DNI score along with other inflammatory markers predicts the development of chorioamnionitis in PPROM, whereas a high NLR score predicts preterm labor[17,25]. In our study, the sensitivity and specificity value of DNI for IUGR was found to be lower than NLR in these studies. This suggests that NLR is a stronger marker for the pathogenesis of IUGR than DNI.

Our study has some limitations. First, retrospective design is main limitation of our study.In addition, the status of inflammatory markers in these groups is not known because causes of IUGR such as preeclampsia or pregestational diabetes mellitus are excluded. Another limitation of the study is that other inflammatory markers and placentas were not examined. These groups of interest could be the next research topic.

Conclusion

Although the prediction of IUGR by NLR with a sensitivity of 70.6% and a specificity of 70.5% is the most important finding of the study, we saw in this study that inflammatory events in pregnant women with IUGR are much more effective than we thought. However, further work is needed on this topic.

Acknowledgments

None

Conflict of interest

The author declare no conflicts of interest.

Funding

The study did not receive funding.

Disclosures

Approval was obtained by the institutional review board from Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital on 09/07/2020/10/21.

Authorship Contributions

Conception and design of the study: SYE, Acquisition of data: SYE, AA,ZŞ CPK, Analysis and/or interpretation of data: SYE, YRA, MCİ, Drafting the manuscript: SYE, KE, ŞÇ

References

1.Sutherland MR, Black MJ. The impact of intrauterine growth restriction and prematurity on nephron endowment. Nature Reviews Nephrology. 2023:1-11.

2.Wilson ML, Goodwin TM, Pan VL, Ingles SA. Molecular epidemiology of preeclampsia. Obstetrical & gynecological survey. 2003;58(1):39-66.

3.Cetin I, Foidart J-M, Miozzo M, et al. Fetal growth restriction: a workshop report. Placenta. 2004;25(8-9):753-757.

4.Nardozza LMM, Caetano ACR, Zamarian ACP, et al. Fetal growth restriction: current knowledge. Archives of gynecology and obstetrics. 2017;295:1061-1077.

5.Resnik R. Intrauterine growth restriction. Obstetrics & Gynecology. 2002;99(3):490-496.

6.Albu A, Anca A, Horhoianu V, Horhoianu I. Predictive factors for intrauterine growth restriction. Journal of medicine and life. 2014;7(2):165.

7.Barker DJ, Clark PM. Fetal undernutrition and disease in later life. Reviews of reproduction. 1997;2:105-112.

8.Barker DJ. Adult consequences of fetal growth restriction. Clinical

obstetrics and gynecology. 2006;49(2):270-283.

9.Pallotto EK, Kilbride HW. Perinatal outcome and later implications of intrauterine growth restriction. Clinical obstetrics and gynecology. 2006;49(2):257-269.

10.Zhou H, Zhao C, Wang P, Yang W, Zhu H, Zhang S. Regulators involved in trophoblast syncytialization in the placenta of intrauterine growth restriction. Frontiers in Endocrinology. 2023;14.

11.Sacchi C, Marino C, Nosarti C, Vieno A, Visentin S, Simonelli A. Association of intrauterine growth restriction and small for gestational age status with childhood cognitive outcomes: a systematic review and meta-analysis. JAMA pediatrics. 2020;174(8):772-781.

12.Papa A, Emdin M, Passino C, Michelassi C, Battaglia D, Cocci F. Predictive value of elevated neutrophil–lymphocyte ratio on cardiac mortality in patients with stable coronary artery disease. Clinica chimica acta. 2008;395(1-2):27-31.

13.Cho S, Cho H, Nam A, et al. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertility and sterility. 2008;90(6):2073-2079.

14.Walsh S, Cook E, Goulder F, Justin T, Keeling N. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. Journal of surgical oncology. 2005;91(3):181-184.

15.Mandaliya H, Jones M, Oldmeadow C, Nordman II. Prognostic biomarkers in stage IV non-small cell lung cancer (NSCLC): neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR) and advanced lung cancer inflammation index (ALI). Translational lung cancer research. 2019;8(6):886.

16.Cho HY, Jung I, Kim SJ, Park YW, Kim YH, Kwon JY. Increased delta neutrophil index in women with severe preeclampsia. American Journal of Reproductive Immunology. 2017;78(3):e12705.

17.Cho HY, Jung I, Kwon J-Y, Kim SJ, Park YW, Kim Y-H. The Delta Neutrophil Index as a predictive marker of histological chorioamnionitis in patients with preterm premature rupture of membranes: A retrospective study. PLoS One. 2017;12(3):e0173382.

18.Khalil A, Gordijn SJ, Beune IM, et al. Essential variables for reporting research studies on fetal growth restriction: a Delphi consensus. Ultrasound in Obstetrics & Gynecology. 2019;53(5):609-614.

19.Regnault T, Galan H, Parker T, Anthony R. Placental development in normal and compromised pregnancies—a review. Placenta. 2002;23:S119-S129.

20.Vedmedovska N, Rezeberga D, Teibe U, Melderis I, Donders GG. Placental pathology in fetal growth restriction. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2011;155(1):36-40.

21.Akbas M, Koyuncu FM, Artunç-Ulkumen B. Placental elasticity assessment by point shear wave elastography in pregnancies with intrauterine growth restriction. Journal of Perinatal Medicine. 2019;47(8):841-846.

22.Chen YH, Liu ZB, Ma L, et al. Gestational vitamin D deficiency causes placental insufficiency and fetal intrauterine growth restriction partially through inducing placental inflammation. The Journal of Steroid Biochemistry and Molecular Biology. 2020;203:105733.

23.Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. American journal of obstetrics and gynecology. 2018;218(2):S745-S761.

24.Onalan G, Terzi YK, Tohma YA, et al. Placental maspin gene expression pattern may be used to distinguish between preeclampsia and intrauterine growth restriction. Gynecology Obstetrics & Reproductive Medicine. 2021;27(2):99-104.

25.Kim MA, Lee BS, Park YW, Seo K. Serum markers for prediction of spontaneous preterm delivery in preterm labour. European journal of clinical investigation. 2011;41(7):773-780.

https://dx.doi.org/10.4314/mmj.v36i3.6