Increased Serum Neutrophil Lymphocyte Ratio Raises the Risk for Peripheral Diabetic Neuropathy in Type 2 Diabetes Mellitus Patients

Anak Agung Ayu Agung Pramaswari, M.D*, I Made Oka Adnyana, M.D.*, I Putu Eka Widyadharma, M.D.*, Ketut Suastika, M.D.**

**Department of Neurology, Faculty of Medicine Universitas Udayana, Bali, Indonesia, **Department of Internal Medicine, Faculty of Medicine Universitas Udayana, Bali, Indonesia.*

ABSTRACT

Objective: Peripheral diabetic neuropathy (PDN) is among the most prevalent diabetes mellitus (DM) sequelae. PDN is a severe health issue that represents a huge social and economic burden worldwide, is associated with long-term morbidity, and diminishes the quality of life of those affected. The neutrophil-lymphocyte ratio (NLR) is a mixture of the two primary components of chronic inflammatory diseases (high neutrophils and low lymphocytes) that contribute to the production of PDN. This study aimed to demonstrate high serum NRL levels enhance the risk of PDN in type 2 DM patients.

Materials and Methods: This study employed a case-control design, collecting data from the registers and outpatient medical records of Prof. Dr. IGNG Ngoerah General Hospital type 2 DM patients who satisfied the inclusion and exclusion criteria between January 2018 and December 2019. Based on clinical neuropathy and abnormal electrodiagnostic testing, the PDN diagnosis was established. Serum NLR was collected from laboratory tests recorded by a computer.

Results: The Receiver Operating Characteristic (ROC) curve approach determined the NLR cut-off value of 2.18. High NLR substantially increased the incidence of PDN (OR 10.36; 95% CI 3.69-29.07; p≤0.001). Other characteristics evaluated, including duration of diabetes, usage of anti-diabetic medications, uncontrolled diabetes, obesity, hypertension, and dyslipidemia, were not significantly associated with the incidence of PDN. High serum NLR was an independent risk factor for PDN in type 2 DM patients (adjusted OR=10.36; 95% CI: 3.57-29.07; p≤0.001).

Conclusion: Based on the findings of this investigation, it was determined that elevated serum NLR increases the risk of PDN events in patients with type 2 DM.

Keywords: Diabetes mellitus; neutrophil-lymphocyte ratio; peripheral diabetic neuropathy; risk factors (Siriraj Med J 2023; 75: 622-628)

INTRODUCTION

One of the most prevalent consequences of diabetes mellitus is peripheral diabetic neuropathy (PDN). PDN is a serious health issue representing a large social and economic burden worldwide, causing long-term morbidity and diminished quality of life for those affected. The prevalence of diabetes globally is predicted to be around 382 million people, with the number of diabetics expected to reach 629 million by 2045.[1] PDN was found in about 10% of DM patients within the first year after diagnosis, rosed to 50% after 25 years. Approximately fifty percent of DM patients experience PDN, thirty

Corresponding Author: Anak Agung Ayu Agung Pramaswari
E-mail: agungpramaswari@unud.ac.id
Received 30 May 2023 Revised 14 July 2023 Accepted 18 July 2023
ORCID ID:http://orcid.org/0000-0003-1301-6311
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percent of diabetic patients suffer PDN pain (PDNP), and thirty-nine percent do not obtain treatment or disregard the PDN complaint.[2,3] A lack of understanding of the pathophysiology of neuropathy impedes the management of PDN, resulting in suboptimal therapy that leads to long-term morbidity and diminishes the quality of life for patients.[4] Peripheral diabetic neuropathy causes balance disturbances and increases the risk of falls, especially in the elderly.[5] Foot ulcers are the leading cause of lower limb amputation, affecting around fifty percent of those with neuropathy.[3]

The neutrophil-lymphocyte ratio (NLR) combines the two primary components of chronic inflammatory disorders (high neutrophils and low lymphocytes). An increased neutrophil count indicates a continuous, nonspecific, damaging inflammatory activity, whereas a low lymphocyte count suggests comparatively insufficient immunological control and dormant immune pathways. Elevated NLR can show the immune system's functioning during chronic inflammatory events.[6] NLR as an inflammatory biomarker in type 2 complications involving the macrovascular and microvascular systems Diabetes mellitus is an independent predictor of carotid artery intima-media thickening, and albuminuria compared to other cardiovascular risks factors such as age, male sex, smoking history, duration of diabetes, estimated glomerular filtration rate (eGFR), LDL, albuminuria-creatinine ratio, and HbA1c. NLR is also believed to be indicative of an autonomic vascular imbalance. The sympathetic nerves stimulate granulocyte release, whereas the parasympathetic nerves stimulate lymphocyte release. A greater NLR may imply a greater ratio of sympathetic to parasympathetic activity. In addition to increasing oxygen consumption and releasing proinflammatory cytokines such as IL-6 and TNF-, the sympathetic tone also stimulates smooth muscle and interstitial cell proliferation, accelerating the progression of atherosclerosis.[7,8]

According to a literature search undertaken by researchers, there is a lack of domestic literature and study examining the association between NLR and the increased risk of microvascular complications of DM, particularly in PDN instances. NLR examination is simple, non-invasive, available in nearly all level one healthcare institutions, and affordable. Based on this and the reviewed theoretical background, a study on a high NLR is recommended to evaluate an increase in the risk of diabetic neuropathy in type 2 diabetes patients at Prof. Dr. IGNG Ngoerah General Hospital, Denpasar.

MATERIALS AND METHODS

This study used a case-control design. Aim of this study was to prove high NLR as the risk factor of diabetic neuropathy in type 2 diabetes patients. The odds ratio was determined by comparing two groups of subjects with type 2 DM (T2DM) who had PDN (cases) and those without PDN (controls) and then examining the serum NLR in the blood (high or low) recorded in the medical record of neuro polyclinic and internal medicine polyclinic (diabetes center) Prof. Dr. IGNG Ngoerah General Hospital, Denpasar. NLR was measured by dividing the absolute number of neutrophils by the absolute number of lymphocytes. Patients treated from January 2018 to December 2019 were the subjects of the collected data. The study included 85 types 2 DM participants who fulfilled the eligibility requirements and were divided into groups with (42 subjects) and without PDN (43 subjects). This study's sample size fulfilled the minimal sample size requirements, with 39 people in each group.

Inclusion criteria in this research included: (1) patients were diagnosed with type 2 diabetes for under five years, (2) patients were diagnosed with PDN, (3) patients were between 40 and 80 years old, (4) gender, length of DM, type of diabetic therapy, duration of diabetes treatment, diabetes control status, nutritional status, medical history, connected full blood laboratory test, HbA1c examination, and lipid profile were included in the patient's medical record, (5) patients underwent an electrophysiological evaluation at the electro neuro-myography (ENMG) neuro polyclinic, with data indicating a PDN. The exclusion criteria for cases and controls were: (1) patients had history of chronic kidney disease, HIV infection, morbus hansen, systemic lupus erythematosus, cancer, entrapment neuropathy (carpal tunnel syndrome/CTS, cervical root syndrome/CRS), history of the spinal cord and peripheral nerve trauma, blood disease (myeloproliferative and leukemia), coronary heart disease, cerebrovascular disease, diabetic retinopathy, (2) patients had severe illness and were bleeding profusely, (3) antiretroviral, chemotherapeutic, traditional analgesic, anti-inflammatory, and anti-tuberculosis medicines were administered to individuals with neuropathy, (4) patients had history of hazardous exposure, including alcohol, pesticides, mercury, and organophosphates, are at risk for lead poisoning.

Purposive sampling was used to determine research subjects, with the researcher selecting topics based on subjective and practical considerations. Subjects in medical records that met the eligibility criteria were included in the study until the required number of samples was obtained and homogenous. The age and gender matching procedure was conducted in both groups. The Chi-Square test has

been used to test the null hypothesis in bivariate analyses involving categorical nominal independent variables and unpaired dependent variables. If the requirements for the Chi-Square test are not met, Fisher's test will be conducted. The logistic regression method has been used to examine other categorical variables that may have affected the study's results. Variables included in the multivariate analysis are those with a *p*-value≤0.25 in the bivariate analysis. The study data will be statistically examined using Windows SPSS version 20.

RESULTS

The case group included 42 subjects with a mean age of 59.9±1.44 years, while the control group had a mean age of 61.37±1.59 years. In both groups, men predominated, with 69% males in the case group. In the control group, the mean HbA1c was greater than in the case group (8.32±0.38 vs 7.90±0.31%). The case group had a higher median NLR value than the control group (2.99 vs 1.94) (Table 1).

TABLE 1. Fundamental characteristics of research participants.

Variables	Case (42 samples) N (%)	Control (43 samples) N (%)	*P*-value
Age mean ± SD (tahun)	59.9 ± 1.44	61.37 ± 1.59	0.490
Sex			
Male	29 (69.0)	31 (72.1)	0.760
Female	13 (31.0)	12 (27.9)	
Level of education			
Elementary	2 (4.8)	6 (14.0)	0.270
Junior high school	12 (28.6)	5 (11.6)	
Senior high school	15 (35.7)	18 (41.9)	
Undergraduate/Diploma	8 (19.0)	9 (20.9)	
Job			
Government employees	10 (23.8)	8 (18.6)	0.270
Private employees	7 (16.7)	15 (34.9)	
Self-employed	12 (28.6)	11 (25.6)	
Farm workers	6 (14.3)	2 (4.7)	
Etc	7 (16.7)	7 (16.3)	
HbA1C levels			
Mean ± SD (%)	7.90 ± 0.31	8.32 ± 0.38	0.220
Neutrophil levels			
Median (103/µL)	6.27	5.01	0.007*
Lymphocyte levels			
Mean ± SD (103/µL)	1.97 ± 0.15	2.57 ± 0.11	0.002*
NLR			
Median	2.99	1.94	<0.001*

Note: *statistically significant $p \leq 0.05$

The total NLR yielded value data with a range of 0.86 to 26.83 and a median of 2.39. The ROC curve demonstrates that the NLR value has a relatively high diagnostic value, as the curve is above the 50% line. The ROC method yielded an AUC value of 77.9% (95% CI: 0.68 - 0.88; p = 0.001). The AUC value of 77.9% indicates appropriate diagnostic capacity from a statistical point of view. The ROC coordinates indicate that the NLR cutoff value of 2.18 utilized in this investigation has a sensitivity of 83.3%, specificity of 67.4%, positive predictive value (PPV) of 71.4%, and negative predictive value (NPV) of 80.5%. The research data were separated into two groups: those with serum NLR levels greater than 2.18 and those lower than 2.18.

Using an NLR cutoff value of 2.18, the relationship between elevated serum NLR as the independent variable and PDN in T2DM patients as the dependent variable was evaluated. The analysis revealed a significant association between high serum NLR and PDN in T2DM patients, with an odds ratio of 10.357 (95%CI = 3.69 – 29.07; $p \leq 0.001$), indicating that high serum NLR in T2DM patients increases the risk of PDN by 10.36 times relative to DM patients with low serum NLR in Table 2. The analysis of each component of NLR (neutrophils and lymphocytes) revealed that lymphocytes significantly increased the risk of PDN events in T2DM patients (OR = 9.07; 95%CI=2.31-34.11; $p \leq 0.001$).

A bivariate analysis was performed between PDN in patients with T2DM and duration of DM, types of anti-diabetic drugs (ADD), uncontrolled DM, obesity, hypertension, dyslipidemia, neutrophil levels, and lymphocyte levels. Of all the variables, no significant relationship was found between other variables and PDN in T2DM patients (Table 3).

Multivariate analysis included serum NLR, dyslipidemia, neutrophil levels, and lymphocyte levels with $p \leq 0.25$ in bivariate analysis. Serum NLR was an independent risk factor for PDN in DM patients, with an odds ratio of 10.36 (95% CI: 3.57-29.07; $p \leq 0.001$) (Table 4).

DISCUSSION

This study performed an age-matching procedure at the outset of the study. However, due to data limitations, an age difference of fewer than 2 years was still included, resulting in a relatively small difference in the mean age between the two groups. This study's findings are consistent with a study by Karki et al.[9] and an investigation by Suwannaphant et al.[10] which found that the prevalence of PDN was highest among individuals aged 51 to 60. The prevalence and incidence of PDN increased and were directly proportional to age, with the prevalence rising by 3.2% in the 20-30 age group, 11.5% in the 40-59 age group, and 20.4% in the over-60 age group.[11,12] It is related to the vascular characteristics of the peripheral nervous system that the prevalence of PDN increases with the age and duration of DM. The peripheral nervous system's reliance on vascular supply makes it susceptible to disruption.[13] In this analysis, the average age of DM patients with PDN was 59-60 years, presumably owing to the significant number of patients over 60 with other comorbid diseases. They were excluded at the outset.

In this survey, it was discovered that men predominated in both categories. In China, the prevalence of PDN among women was greater than among men.[6] Males are four years more likely than females to develop PDN as a complication of T2DM.[14] Compared to previous studies, the variance in this study's proportion of males and females may result from demographic differences

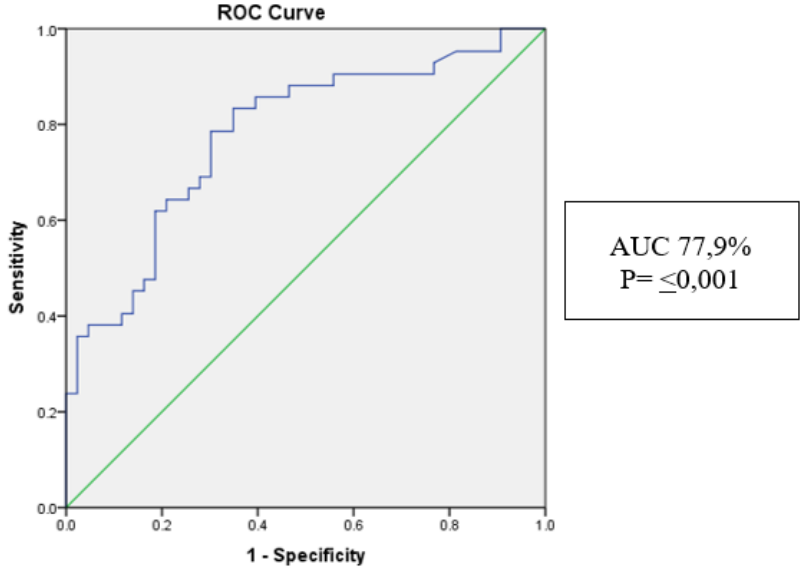

Fig 1. ROC of serum NLR to PDN values.

TABLE 2. Bivariate analysis of NLR, neutrophils, and serum lymphocytes with PDN in DMT2.

Variables		PDN Case N (%)	Control N (%)	OR (CI 95%)	*P*-value
NLR	High	35 (83.3)	14 (32.6)	10.36 (3.69-29.07)	<0.001*
	Low	7 (16.7)	29 (67.4)		
Neutrophil	High (≥7.5)	15 (35.7)	6 (14.0)	3.43 (1.18-9.98)	0.200
	Low-Normal (<7.5)	27 (64.3)	37 (86.0)		
Lymphocyte	Low (<1.5)	17 (40.5)	3 (7.0)	9.07 (2.31-34.11)	<0.001*
	Normal-High (≥1.5)	25 (59.5)	40 (93.0)		

Note: *statically significant $p \leq 0.05$

TABLE 3. Bivariate analysis of other variables and PDN in patients with DMT2.

Variables	PDN Case N (%)	Control N (%)	OR (CI 95%)	*p*-value
Length of diabetes				
3-5 years	13 (72.2)	21 (48.8)	2.72 (0.83-8.97)	0.090
< 3 years	5 (27.8)	22 (51.2)		
Type of diabetics drugs				
Insulin	21 (50.0)	19 (44.2)	1.26 (0.54-2.96)	0.590
Oral	21 (50.0)	24 (55.8)		
Uncontrolled DMT2				
Yes	26 (61.9)	28 (65.1)	0.87 (0.36-2.1)	0.760
No	16 (38.1)	15 (34.9)		
Obesity				
Yes	6 (14.3)	6 (14.0)	1.03 (0.30-3.48)	0.960
No	36 (85.7)	37 (86.0)		
Hypertension				
Yes	22 (52.4)	23 (53.3)	0.96 (0.41-2.24)	0.920
No	20 (47.6)	20 (46.5)		
Dyslipidemia				
Yes	28 (66.7)	34 (79.1)	0.53 (0.2-1.4)	0.198
No	14 (33.3)	9 (20.9)		

Note: DMT2: diabetes mellitus type 2

TABLE 4. Logistic regression multivariate analysis.

Variable	OR	CI 95%	*P*-value
Step 1			
NLR	9.83	3.47-27.88	<0.001*
Length of diabetes	1.65	0.59-4.58	0.330
Dyslipidemia	0.73	0.23-2.30	0.590
Step 2			
NLR	10.11	3.58-28.59	<0.001*
Length of diabetes	1.72	0.63-4.72	0.290
Step 3			
NLR	10.36	3.69-29.07	<0.001*

Note: *statically significant p<0.05

in the sampling location. The same was discovered for education in secondary school in our study. The difference in results may be because most subjects in this study reside in urban areas and generally have at least a high school education. Those with a high level of education are more aware of complications of DM, such as a PDN, and therefore are more likely to seek medical attention. Work and education are not directly related to the incidence of diabetes mellitus or neurodegenerative disease but rather to the existence of urban residents, the majority of whom are urban residents who have a lifestyle-related association.[15] PDN severity correlates linearly with age, body mass index (BMI), and duration of DM, but not with HbA1c in our study.[16] In this study, obesity was determined by BMI values and the results did not indicate a statistically significant increase in the risk of adverse events. In previous studies, the BMI had no statistically significant effect on the incidence of PDN in T2DM patients.[17,18] Similarly, precise results were obtained for hypertension, possibly due to the large number of DM patients with hypertension at our hospital who had other complicating conditions and thus did not satisfy the criteria for sample collection.

Neutrophils are integrally connected to chronic inflammation, whereas lymphocytes reflect immune regulation pathways. The literature explaining the direct relationship between lymphocytes and the formation of PDN is scarce. Chronic hyperglycemia in DM will increase the release of reactive oxygen species (ROS) from neutrophils and decrease lymphocyte levels. Chronic hyperglycemia also directly causes diminished lymphocyte proliferation. In patients with uncontrolled type 2 diabetes, decreased lymphocyte proliferation is more prevalent. In type 2 diabetes, the decrease in lymphocyte proliferation is due to the low expression of IL-2 receptors. CD25 deficiency is the cause of the reduced level of IL-2 receptor expression. CD25 is crucial for stimulating the IL-2 receptor to produce T cells. CD25 serves a vital role in expanding T cell cloning after antigen discovery. Antigen stimulation increases IL-2 expression because T cells are the primary proliferating cells in specific immune responses. Low levels of IL-2 inhibit lymphocyte proliferation and differentiation.[10] NLR is an independent risk factor for PDN associated with diabetic microangiopathy, which impairs the nutrient supply to neuronal and Schwann cells, resulting in peripheral neuropathy due to nerve degeneration. NLR combines the two primary components of chronic inflammatory disorders (high neutrophils and low lymphocytes).[6] High neutrophils were more prevalent in the PDN group, but there was no significant association with an increased risk of PDN.

In contrast, low lymphocytes substantially increased the risk of a PDN incident. Similar lymphopenia has been observed in numerous clinical and experimental studies of people with diabetes with microvascular, macrovascular, and other complications. It may be the result of increased oxidative DNA damage and lymphocyte apoptosis. Diabetes patients exhibited decreased lymphocyte proliferation due to lower IL-2 receptor expression levels. It causes a decrease in lymphocytes and an increase in neutrophils, thereby increasing the NLR.[6]

This study's strength is the lack of prior research on NLR and the incidence of PDN. NLR has extensively

studied DM microvascular complications such as peripheral arterial disease (PAD), diabetic retinopathy, and diabetic nephropathy.[18] Still, its relationship to PDN has not been extensively studied, particularly in Indonesia so this study will add future research references. This study was also conducted by matching by design on confounding variables so that other factors contributing to the incidence of PDN can be controlled and the results of high serum NLR as an independent risk factor for PDN in patients with T2DM can be strengthened. Blood assays for evaluating NLR (a marker of ongoing destructive nonspecific inflammatory processes) or lymphocytes (relatively inactive or insufficient immune regulation) alone are less stable indicators of the functional status of the immune system than the ratio.

The limitation of this study is there were differences in treatment between the two groups, with ENMG examination not being performed in the control group, so a diagnosis of subcutaneous PDN (no visible signs of neuropathy but abnormalities in nerve conduction velocity or abnormal small fiber neuropathy examination) could not be ruled out (no visible symptoms of neuropathy but irregularities in nerve conduction velocity or abnormal small fiber neuropathy examination). In addition, the sample size is limited to a single region, so it cannot be used to characterize the population as a whole. Large-scale research is required to further evaluate the implementation of NLR in predicting PDN.

CONCLUSION

Neutrophil-lymphocyte ratio can be one of the predictors of high risk PDN in T2DM patients. Compared to T2DM patients with low serum NLR, T2DM patients with high blood NLR levels (2.18 mg/dL) had a 10-fold greater chance of developing PDN.

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