Analysis of NLR in Type 2 Diabetes Mellitus with and without Diabetic Foot Ulcer

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ABSTRACT

Patients with Type 2 Diabetes Mellitus (T2DM) have increased followed by complications including diabetic foot ulcer. Systemic inflammatory conditions in T2DM with diabetic foot ulcers can be assessed by inflammatory markers. Neutrophil Lymphocyte Ratio (NLR) is a good indicator of systemic inflammatory conditions. A retrospective study of the medical record at Dr. Wahidin Sudirohusodo Hospital, Makassar from September 2019–October 2021 involved 120 patients consisting of 60 patients for each group of T2DM with and without diabetic foot ulcers. Leukocytes, neutrophils, lymphocytes, and NLR based on routine blood results using the flow cytometry method. Mann-Whitney test was used for comparison between the two groups on NLR and Kruskal-Wallis test was used for the relationship between NLR and Wagner's classification. There was a significant difference in leukocytes, neutrophils, lymphocytes, and NLR in T2DM patients with diabetic foot ulcers compared to those without 16.2±8.6 and 9.8±4.2 103/μL (p<0.001); 13.3±8.4 and 5.0±3.8 103/μL(p<0.001); 1.6±1.7 and 2.5±2.5 103/μL(p<0.001); 10.0±10.1 and 3.5±4.5, respectively. The relationship between the NLR and Wagner's classification was the highest at Wagner grade 5 (12.87±5.0) and the lowest was at Wagner grade 2 (6.18±7.83) with significant statistical test results (p<0.037). There was increasing NLR in T2DM with diabetic foot ulcers due to systemic inflammation. The NLR integrates different immune pathways, such as neutrophils as an inflammatory response and lymphocytes controlling the inflammatory response. Lymphocytes count and NLR level on T2DM with diabetic foot ulcer were higher than those without diabetic foot ulcer.

Keywords: NLR, diabetes mellitus, diabetic foot ulcer

INTRODUCTION

According to The American Diabetes Association (ADA), Type 2 Diabetes Mellitus (T2DM) is caused by the failure of pancreatic β cells to secrete insulin, resulting in insulin resistance. Type 2 DM is associated with defects in insulin secretion, inflammation, metabolic stress, and genetic factors.[1] Epidemiological studies show a trend of increasing incidence and prevalence of T2DM in various regions worldwide. The International Diabetes Federation (IDF) predicted that the number of DM patients worldwide has increased to 463 million in 2019 and would continue to increase globally from 425 million to 629 million in 2045 and increase from 82 million to 151 million in South East Asia. Indonesia is estimated to have 5.4 million people with DM. The prevalence of T2DM in Makassar is 6.65%. The Basic Health Research (RISKESDAS) report by the Ministry of Health in 2018 showed an increase in the prevalence of DM to 8.5%. Diabetic foot ulcers were found in more than 15% of DM sufferers, which resulted in more than 80,000 amputations per year in Indonesia.[2]

The common complications of T2DM are microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (atherosclerosis, ischemic heart disease, stroke, and peripheral vascular disease, and diabetic foot ulcers, which mostly end in amputation).[3] One of the complications of diabetes is foot ulceration associated with neuropathy in the lower limbs of T2DM patients. The grade of diabetic foot ulcers can be assessed using the Wagner classification. An amputation is required if the ischemic condition cannot be treated by revascularization, a foot infection becomes life-threatening and complications in diabetic foot ulcers cause functional ineffectiveness. Hyperglycemia causes oxidative stress on nerve cells and causes neuropathy. Diabetic foot ulcers are characterized by an increase in the number of leukocytes in the blood as a physiological response to stress in the circulation.[4-6]

The response to circulating leukocytes can be used as an indicator of inflammation. Neutrophils are active inflammatory mediators, which serve as the first line of defense and a high neutrophil count is a nonspecific marker of the inflammatory process. Hyperglycemia and oxidative stress in T2DM increase the expression of Intracellular Adhesion Molecule 1 (ICAM-1), proinflammatory cytokines, and chemokines. This overexpression results in the accumulation of inflammatory cells. This cascade of inflammatory responses will lead to an increase in neutrophils. After the involvement of innate immunity in the body's defense system, adaptive immunity or lymphocytes are activated. Lymphocytes have the ability to control and regulate the inflammatory response as well as anti-atherosclerosis. Systemic inflammatory conditions in diabetic patients, especially in patients with diabetic foot ulcers can be assessed by inflammatory markers such as the neutrophil-lymphocyte ratio (NLR).[7,8]

The NLR integrates different but complementary immune pathways in the circulating blood. It is calculated by dividing the neutrophil count by the lymphocyte count. An increase in NLR can be a manifestation of an increased number of neutrophils, which adhere to endothelial cells, cause vascular damage, and lead to extensive chronic inflammation. The number of lymphocytes in diabetic foot ulcer patients who are chronically infected can be associated with immunological stress. Infection induces the production of polymorphonuclear cells (PMNs), which has the potential to cause relative suppression or increase lymphocyte apoptosis resulting in a decrease in lymphocytes.[9-11]

A study by Kahraman showed an increase in NLR in T2DM patients with diabetic ulcers compared to T2DM patients without diabetic ulcers not only due to local inflammation but also due to systemic inflammation.[3] Altay stated that patients with high NLR had more severe diabetic foot ulcers, increased risk of amputation, prolonged hospital stay, and required more aggressive therapy.[12] Demirdal stated that NLR could be a predictor of the need for amputation in patients with diabetes. In addition, this study also found that NLR was higher in patients who needed surgery compared to patients who only needed ordinary wound care.[13]

METHODS

This research was a retrospective study with a cross-sectional method, which was carried out by collecting secondary data on patients diagnosed with T2DM with and without diabetic foot ulcers from patient medical records from September 2019 to October 2021 at the medical records installation of Dr. Wahidin Sudirohusodo Hospital, Makassar.

The study population was patients with DM at Dr. Wahidin Sudirohusodo Hospital. The research subjects were patients with complete medical record data who were diagnosed with T2DM by a clinician. The research subjects were those who met the inclusion criteria, namely patients aged >18 years with T2DM with and without diabetic foot ulcers who had data on the results of complete routine blood tests using a hematology analyzer. Exclusion criteria in this study were patients with hematological malignancy and/or patients with a history of treatment for hematological malignancy.

Laboratory data collected in this study were leukocyte count, absolute neutrophil count, and absolute lymphocyte count. Neutrophil lymphocyte ratio values were obtained by dividing absolute neutrophil count by absolute lymphocyte count.

Data were analyzed using Statistical Product and Service Solutions (SPSS) computer software version 25. Calculation of frequency distribution and statistical tests were also used for data analysis. The normality of numerical data was analyzed using the Kolmogorov-Smirnov test because the number of samples was > 50 and the p-value > 0.05 indicated that the data were normally distributed. Descriptive analysis was presented in numerical and categorical data. Analysis of categorical data was presented in percentage (amount). The relationship between the 2 groups of numerical variables was carried out using Mann-Whitney due to abnormal distribution. The relationship of numerical variables for more than 2 groups was carried out using Kruskal-Walis due to abnormal distribution. Categorical variables with a table size of 2 x 2 were analyzed using the Chi-Square test or Fischer's exact depending on the normality of the data distribution. Research permission was obtained from the Ethics Commission for Health Research, Faculty of Medicine, Hasanuddin University, Hasanudin University Hospital, Dr. Wahidin Sudirohusodo Hospital with number: 665/UN4.6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

The total sample obtained in this study met the inclusion criteria: patients aged > 18 years diagnosed with T2DM with and without diabetic foot ulcers totaling 120 patients.

Type 2 DM patients with diabetic foot ulcers were more predominated by male subjects (65%), and the most age range was 56-65 years (31.7%). There was no significant difference in the distribution of gender and age in both groups (p > 0.05).

Table 1. General characteristics of subjects based on T2DM with and without diabetic ulcer

Characteristics	T2DM without Diabetic Foot Ulcer (n=60), n (%)	T2DM with Diabetic Foot Ulcer (n=60), n (%)	p*
Gender			
Male	30 (50)	39 (65)	0.097*
Female	30 (50)	21 (35)	
Age (years)			
32-45	10 (16.7)	8 (13.3)	
46-55	16 (26.7)	17 (28.3)	0.806*
56-65	15 (25.0)	19 (31.7)	
>65	16 (26.7)	19 (31.7)	

* Chi-Square test

Table 2. Difference of leukocyte count, absolute neutrophil count, absolute lymphocyte count, and NLR in T2DM patients with and without diabetic ulcer

Parameter	T2DM without Diabetic Foot Ulcer (n=60) Mean±SD	T2DM with Diabetic Foot Ulcer (n=60) Mean±SD	p*
Leukocyte (10^3 cells/µL)	9.8 ± 4.2	16.2 ± 8.6	<0.001*
Neutrophil (10^3 cells/µL)	6.27 ± 3.87	13.13 ± 8.45	<0.001*
Lymphocyte (10^3 cells/µL)	2.5 ± 1.02	1.74 ± 0.80	<0.001*
NLR	3.5 ± 4.5	10.0 ± 10.1	<0.001*

*Mann-Whitney test

Table 3. Relationship between NLR and Wagner's classification

Wagner Grade	Minimum	Maximum	Median	Mean	SD	n	p*
2	1.96	25.40	2.49	6.18	7.83	12	
3	3.24	55.88	6.84	12.52	13.56	19	0.037*
4	1.67	38.70	6.05	9.60	8.50	25	
5	7.08	18.02	13.20	12.87	5.00	4	

*Kruskal-Wallis test

Table 2 shows an increase in leukocyte count, absolute neutrophil count, and NLR value as well as a significantly lower absolute lymphocyte count in T2DM with diabetic foot ulcers compared to T2DM without diabetic foot ulcers (p<0.001).

Table 3 shows that the NLR value was significantly higher in grade 5 (12.8.7±5.0) and lowest in grade 2 (6.18±7.83) (p<0.05), indicating that there was a significant relationship between NLR and Wagner's classification.

According to the characteristics of the research subjects in Table 1, T2DM patients with diabetic foot ulcers were found to be higher in males (65%) than females with diabetic foot ulcers (35%). This was followed by the study of Dinh *et al.*, which stated that females have a lower risk than males for diabetic foot ulcers due to the influence of the low severity of neuropathy, increased joint morbidity, and foot pressure related to more active physical activity in

males. Between patients with diabetic foot ulcers and without diabetic foot ulcers a mean difference of 55-65 years of age was found; most of which had diabetic foot ulcers according to the research of Chomi *et al.* Diabetic foot ulcers were more common in the 5th and 6th decade of life due to decreased inflammatory response, decreased proliferation, delayed angiogenesis, and lower collagen synthesis compared to its degradation. Research conducted by Rabean *et al.* also showed that the prevalence of diabetic ulcers increased with age.[14]

This study showed an increased leukocyte count in T2DM patients with diabetic foot ulcers. This might be due to activation by Advanced Glycation End-products (AGE), oxidative stress, and angiotensin II, which are produced due to hyperglycemia leading to the release of TNF alpha and IL-1, which are involved in the pathogenesis of chronic complications in T2DM. A study by Mordai

et al. revealed that high leukocyte counts were associated with chronic complications in T2DM related to metabolic disorders and insulin resistance, which are responsible for pathophysiological changes in various organs of the body.[15]

The NLR represents the balance of neutrophils and lymphocytes in-vivo. Neutrophils are closely related to the inflammatory response and lymphocytes reflect the immune regulatory pathways involved in inflammation. The results of our study showed that the neutrophil count, lymphocyte count, and NLR values had significant differences in T2DM patients with diabetic foot ulcers compared to T2DM patients without diabetic foot ulcers. This was in line with a study by Goornavar *et al.* showing an increase in NLR values to predict sub-clinical inflammation and complications such as diabetic foot ulcers.[16]

Diabetic foot ulcers occur due to neuropathy (sensory, motor, autonomic) and ischemia. The condition is then exacerbated by infection. The degree of diabetic foot ulcers can be assessed by Wagner's classification consisting of Grade 0 (no lesions); Grade 1 (ulcer on the skin surface); Grade 2 (ulcer deep into the tendon and joint capsule); Grade 3 (deep ulcer with abscess, osteomyelitis or joint infection); Grade 4 (local gangrene, finger, sole or instep) and Grade 5 (gangrene of the whole foot). This study showed the highest NLR values in patients with grade 5 and the lowest in grade 2 diabetic foot ulcers. In a study by Eren *et al.*, the NLR was significantly lower in the grade 2 group (2.8±0.9) than in the grade 3 group (6.0±5.2; p=0.017) and the grade 4 group (6.9±5.3; p=0.01). NLR is associated with peripheral vascular disease and its severity.[17]

CONCLUSIONS AND SUGGESTIONS

The leukocyte count and NLR values in T2DM with diabetic foot ulcers were higher compared to those of T2DM without diabetic foot ulcers.

The researcher encourages upcoming studies to use a larger sample size and add other infection markers.

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