Experimental and Applied Medical Science, 3,3: 410-419, 2022. DOI 10.46871/eams.2022.47

May Neutrophil Lymphocyte Ratio Be A Predictor of Neuropathy in Diabetic Patients?

Hamit Yıldız[1], Deniz Yıldız Pehlivan[2]

[1]Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Gaziantep, Turkey.

[2]İzmir Kâtip Çelebi University, Faculty of Medicine, Department of Physiology, İzmir, Turkey

Abstract

It is known that there are immunological inflammatory mechanisms that play a role in the pathogenesis of type 2 diabetes mellitus (DM), which is a common metabolic disease in adults. Chronic inflammation plays a role in the development of chronic microvascular complications. Neutrophil-lymphocyte ratio (NLR) is a new marker of inflammation that is inexpensive and easy to administer. NLR is accepted as a marker of systemic inflammation and is used as a prognostic marker in patients with heart diseases and malignancies. The aim of this study is to examine whether there is a relationship between NLR and neuropathy, one of the microvascular complications, in patients with type 2 diabetes mellitus in adults. One hundred and nine diabetic patients (40 without microvascular complications and 69 with microvascular complications) were included in the study. Microvascular complications from DM were evaluated with NLR and compared with different inflammatory markers. NLR was higher in patients with diabetic complications compared to the group without (20.40±14.79 vs 5.56±3.46, respectively; p<0.001). Spearmens's correlation analysis revealed NLR was significantly positively correlated with ESR (r:0.633, p:<0.001) and CRP (r: 0.387, p:<0.001). Increased NLR levels may be associated with neuropathy in type 2 diabetic patients.

Keywords: *Type 2 diabetes mellitus, neutrophil lymphocyte ratio, neuropathy, microvascular complication*

***Corresponding Author:** Dr. Hamit Yıldız, e-mail: drhyildiz@hotmail.com, ORCID ID:0000-0001-7858-5123.

Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disease in adults. It is estimated that diabetes mellitus affects 537 million adults worldwide (1). In an analysis of data from the National Health Interview Survey (2016 and 2017), the prevalence of diagnosed type 2 diabetes among adults in the United States was 8.5 percent (2). The main reasons for the increase in prevalence are hypercaloric nutrition, obesity, decreased physical activity and secondary causes (3). DM is a chronic and metabolic disease characterized by high blood sugar levels that can lead to the development of acute, chronic microvascular and macrovascular complications. T2DM usually occurs due to insulin resistance and impaired insulin secretion. In 90% of T2DM patients, there is insufficient insulin secretion from pancreatic beta cells, insulin resistance in tissues, and insufficient compensatory insulin secretion response (4-5). Uncontrolled disease causes hyperglycemia, and the insulin secreted cannot maintain glucose homeostasis. Patients with T2DM are mostly obese or have increased adipose tissue predominantly in the abdomen. In this reduction, adipose tissue supports the development of insulin resistance through various inflammatory mechanisms, including increased free fatty acid (FFA) secretion and adipokine degranulation. In the development of T2DM, pancreas (alpha and beta cells), liver, skeletal muscles, kidneys, brain, small intestines and adipose tissue are affected together (6).

Data obtained from studies show that adipokine tissue dysregulation, inflammation, abnormalities in intestinal microbiota, and immune dysregulation are important factors that play a role in the pathogenesis of the disease (7).

In the case of physical inactivity, which is an important cause of diabetes, glucose uptake into tissues and insulin sensitivity are reduced. Furthermore sedentary life causes inflammation and oxidative stress at the tissue level, which are predisposing factors for T2DM (8). Oxidative stress plays a role in the pathogenesis of many rheumatic diseases besides diabetes mellitus (9-13). Many studies support that chronic moderate inflammation may be associated with insulin resistance and the development of complications (14-16). Studies correlate leukocyte count (WBC) and C-reactive protein (CRP) with diabetes-related microvascular and macrovascular complications (17-18). Blood neutrophil lymphocyte ratio (NLR) is a new marker that shows the body's total inflammation level because it is an inexpensive, easy, and effective marker of subclinical inflammation. NLR has been shown to be an indicator of inflammation and prognostic features in many diseases (19-20). In fact, it is stated that NLR is a better predictor marker than leukocyte count in some diseases (21-22). The aim of the current study is to determine the relationship between the level of NLR, a practical marker of inflammation, and neuropathy in patients with T2DM.

Materials and Methods

Patients who were referred to the clinic of Deparment of Internal Medicine, Division of General Medicine at Gaziantep University Hospital were included in this cross-sectional study.

All patients were questioned for history of DM (known diabetes treated with diet or drugs or both or either newly diagnosed according to the American Diabetes Association criteria (23).

The first indicator of diabetic nephropathy is microalbuminuria, which is defined as a 24-hour urine albumin level of 30-300 mg in the absence of urinary tract infection and/or uncontrolled hypertension (24). The diagnosis of diabetic neuropathy was defined according to the deterioration in nerve conduction work in patients with sensory impairment/motor weakness or polyradiculopathy (25). The diagnosis of retinopathy was defined as having at least microaneurysm and/or retinal hemorrhage and/or other retinal damage findings (26-27).

Laboratory data were obtained retrospectively from patient files. Complete blood count, fasting plasma glucose, uric acid, creatinine, albumin, globulin, total protein, aspartate amino transferase, alanine aminotransferase, total bilirubin, gamma glutamyl transferase, alkaline phosphatase, lactate dehydrogenase and hemoglobin A1c levels were measured with a hospital autoanalyzer. Complete blood count analysis was measured by Beckman Coulter (High Wycombe, UK) Gen-S automated analyzer. NLR was calculated from differential count by dividing the absolute neutrophil count by the absolute lymphocyte count.

End-stage renal disease, malignant disease, advanced liver disease, presence of active infection and active smokers were excluded from the study. WBC counts more than 11.0 (x103 cells/mm3) and less than 4.0 (x103 cells/mm3) were exclusion criteria for the study.

Informes consent has been obtained, and procedures followed were in acccordance with the institutional ethical standars of the responsible commitee on human experimentation. The study protocol was approved by the Gaziantep University Local Research Ethics Committee.

Patients who were referred to the clinic of Deparment of Internal Medicine, Division of General Medicine at Gaziantep University Hospital were included in this cross-sectional study.

All patientes were questioned for history of DM (known diabetes treated with diet or drugs or both or either newly diagnosed according to the American Diabetes Association criteria(23).

Statistical Analysis

For the statistical analysis of the data, SPSS (Statistical Package for Social Sciences) 22.0 version statistical program was used. All data were entered into a database and were verified by a second independent person. The Shapiro-Wilk test was used to test whether the numerical variables were consistent with the normal

distribution. Paremeters with normal distribution have been evaluated using histograms, variation coefficients, skewness, sharpness, detrended normality graph and Kolmogorov–Smirnov test. Mean standard deviation (SD) values of variables with normal distribution are provided (mean ± SD). Categorical variables are shown as frequencies. Independent samples t-test was used to compare normally distributed variables in two groups. Correlation between ESR, CRP, and NLR was performed with Spearmen's correlation test. Statistical significance level has been defined as $P < 0.05$.

Results

The mean age of 109 patients with type 2 diabetes mellitus included in the study was 61.20± 13.87 years. The mean age of male patients was 61.25±14.50 and the mean age of femalepatients was 61.13±13.10. Demographic properties and laboratory parameters of the study are presented in Table 1. There was no significant difference between the diabetic patients withand without microvascular complications regarding of ESR and CRP.

Table 1. Demographic properties and laboratory parameters of the study populations.

Parameters	DM patients without complications (n:40)	DM patients with complications (n: 69)	***p***
Gender (n,%)			
Male	24 (%60)	39 (%56.5)	
Female	16 (%40)	30 (%43.5)	
Age (yr)	59.5±11.29	62.0±5.23	0.019
NLR	5.56±3.46	10.40±4.79	<0,001
Neutrophil (/mm^3)	6170±482	12010±930	0,019
Lymphocyte (/mm^3)	1275±123	730±66,5	0,002
Glucose (mg/dl)	124.5±62.83	147.10±72.1	0,208
Uric asid (mg/dl)	6.55±3.94	6.10±3.87	0.504
Creatinine (mg/dl)	0.88±1.11	0.80±1.55	0.253
Albumin (g/dl)	2.55±0.52	2.90±2.15	0.273
Globuline (g/dl)	3.03±0.90	3.00±0.79	0.242
Total Protein (g/dl)	6.05±1.09	6.10±5.98	0.149
AST (U/l)	35±5.24	47±54	0.966
ALT (U/l)	22±3.22	29±32	0.978
ALP (U/l)	114±18.4	27±17.1	0.667
GGT (U/l)	56.5±2.29	65±18.4	0.472
Total bilirubin(mg/dl)	0.60±2.29	0.60±1.62	0.087
LDH (U/l)	297±16.8	368±160.4	0.723
WBC (/mm^3)	8470±648	9060±1017	0.123
Hgb (g/dl)	12.78±2.47	12.90±2.34	0.909

Plt (/mm^3)	152500±14850	216000±15166	0.986
Duration of DM	8.37±3.75	6,15±3,18	0.089
HbA1c (%)	6.96±0.44	8,80±1,44	<0,001

DM: diabetes mellitus; NLR: neutrophil/lymphocyte ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gama glutamile transferase; LDH: lactat dehydrogenase; HGB: hemoglobin; WBC: White blood cell; Plt: platelet; HbA1c: glycated hemoglobin

All patients (n:69) with microvascular complications had neuropathy. Of the 69 patients with neuropathy, 21 also had nephropathy and 18 had retinopathy. There were 69 patients with defined microvascular complications. The mean of NLR of patients with microvascular complication was significantly higher than diabetic patients without complications (10.40±4.79 and 5.56±3.46 ,respectively). The NLR of patients id shown in figure 1. NLR was

higher in diabetic patients with nephropathy, neuropathy and retinopathy than in diabetic patients without any of these complications (p:0.021 , p:0.042, and p:0.007, respectively).

There was statistically significant positive correlation between NLR and ESR levels (r: 0.633, p:<0.001). In addition NLR was also positively correlated with CRP levels (r: 0.387, p:<0.001). Correlation analysis results between inflammatory indices are shown in Table 3.

Table 2. NLR of diabetic patients with microvascular complications.

		Mean ± SD	P
Microvascular complications (n:69)	(+)	20.41 ±14.79	<0.001
	(-)	5.57 ± 346	
Nephropathy (n:21)	(+)	14.05 ±14.29	0.267
	(-)	15.89 ±13.60	
Neuropathy (n:69)	(+)	20.40 ±14.79	<0.01
	(-)	5.57 ±3.46	
Retinopathy (n: 18)	(+)	15.02±14.34	0.802
	(-)	14.90±13.61	

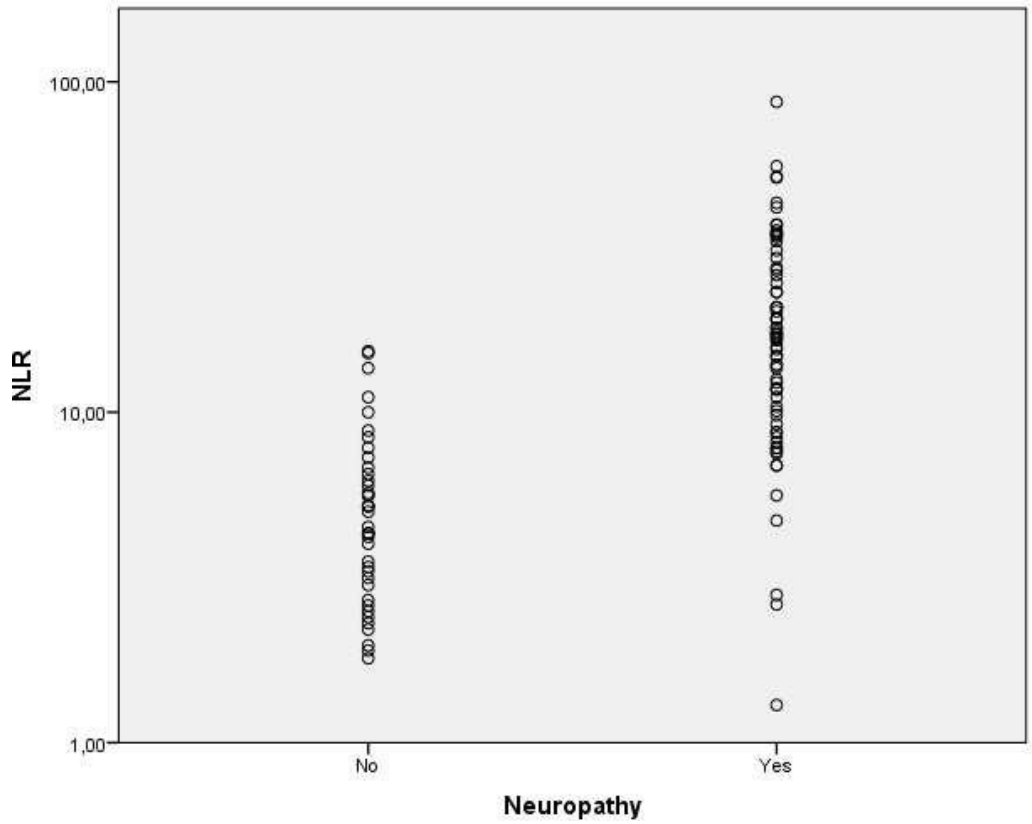
NLR: Neutrophil/lymphocyte ratio

Table 3. Correlation analysis results of inflammatory indices.

Parameters		
NLR-ESR	p: <0.001	r: 0.633
NLR-CRP	p:<0.001	r: 0.387
ESR-CRP	P:<0.001	r: 0.590

NLR: neutrophil/lymphocyte ratio; CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

Figure 1- Relationship between neutrophil lymphocyte ratio and diabetic neuropathy



Discussion

The present study aimed to investigate the relationship of NLR, an innovative marker of inflammation, in the presence of microvascular complications in patients with type 2 diabetes mellitus. It was aimed to detect a noninvasive marker of neuropathy, one of the microvascular complications. Study data revealed that diabetic patients with neuropathy from microvascular complications had higher NLR levels than individuals without neuropathy. In addition, NLR levels were found to be higher in patients with other microvascular complications than in patients with type 2 diabetes mellitus without complications.

Increased free oxygen radicals and decreased antioxidant substances are blamed for the development of macrovascular and microvascular complications related to type 1 and 2 diabetes mellitus (28). It is not known exactly which oxidative stress mechanism causes the development of diabetic complications. Diabetes damage can be thought of as the oxidative damage effects of chronic hyperglycemia in tissues.

Increased intracellular glucose causes the formation of higher oxidative stress molecules than antioxidant systems can buffer. These oxidative molecules cause the activation of protein kinase C (PKC), oxidative stress molecules induced by hyperglycemia, increased hexosamine pathway flux, increased AGEs and increased polyol pathway flux. In particular, activation of the AGEs pathway can damage cells that regulate gene transcription and proteins between the matrix and other tissues.

An important detoxification system of the body, which plays a critical role in defending thiol/disulfide homeostasis, the importance of which has been better understood in recent years, covers all groups in the acute and chronic inflammatory filters (29)). The thiol (-SH) is composed of hydrogen and sulfur atoms containing a sulphidhydryl group. Thiols can bind to oxidative molecules due to the presence of -SH groups that are sensitive to oxidation. Disulfides (-S-S-) are an important class of redox-reactive molecules occurring between thiol groups. Dynamic thiol-disulfide homeostasis is the reversal of the oxidative effect of proteins in our body caused by thiols. It is an important parameter associated with many biochemical processes, including regulation of protein function, stabilization of protein structure, protection of proteins against irreversible oxidation of cysteine residues, chaperone function, regulation of enzyme functions, and transcription (30). Increased oxidative stress as a result of zinc deficiency has been associated with inflammatory diseases (31-32). Zinc in the islet cells of the pancreas is important for the synthesis, storage and secretion of insulin. It is known that large amounts of zinc are excreted from the body in diabetic conditions. Zinc deficiency and increased oxidative stress have an important effect on the pathogenesis of diabetic complications (33).

Studies in the literature show that chronic inflammation plays a role in the pathogenesis of diabetes mellitus (34). In patients with T2DM, inflammatory markers can cause hyperglycemia with insulin resistance and beta cell dysfunction (35). Inflammation alters

endothelial function and causes a decrease in nitric oxide and prostacyclin production in the vascular endothelium (36). Widespread endothelial damage and overexpression of some mediators may lead to the development of diabetic complications. CRP, interleukin 6, and increased WBC levels have been associated with T2DM in some studies. In a meta-analysis, it was revealed that the

risk of diabetes is higher in patients with high CRp levels (37). In addition, it has been shown that there is a positive relationship between interleukin 6 level and new-onset diabetes mellitus due to obesity and insulin resistance (38).

Blood NLR is a simple, inexpensive, easy-to-access, low-cost, and non-invasive marker of systemic inflammation that can be obtained from complete blood count. This marker is used to determine prognosis in cardiovascular diseases, malignancies and inflammatory diseases. High NLR is associated with poor prognosis in esophageal, hepatocellular, epithelial ovarian, and nasopharyngeal cancers (39). Recent studies show that it is a strong indicator for predicting cardiovascular outcomes in patients with coronary artery disease (40-41).

The data of this study underline that NLR may indicate a systemic inflammatory state in diabetes mellitus-related complications. In addition, the data obtained within the scope of the study suggest that NLR can be used as an easy and inexpensive test for the early diagnosis of diabetic neuropathy and other microvascular complications. Comprehensive studies with prospective, repetitive NLR measurements will be useful in elucidating the pathogenesis of neuropathy, a microvascular complication in patients with T2DM.

References

1. IDF Diabetes Atlas 2021, 10th edition https://diabetesatlas.org/atlas/tenth-edition/ (Accessed on January 17,2022).
2. Xu G, Liu B, Sun Y, et al. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. BMJ 2018; 362:k1497.
3. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251.) (NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: A pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 2016, 387, 1513–1530.
4. Stumvoll, M.; Goldstein, B.J.; van Haeften, T.W. Type 2 diabetes: Principles of pathogenesis and therapy. Lancet 2005, 365, 1333–1346.
5. Weyer, C.; Bogardus, C.; Mott, D.M.; Pratley, R.E. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J. Clin. Investig. 1999, 104, 787–794.
6. Defronzo, R.A. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 2009, 58, 773–795.
7. Schwartz, S.S.; Epstein, S.; Corkey, B.E.; Grant, S.F.; Gavin, J.R., 3rd; Aguilar, R.B. The Time Is Right for a New Classification System for Diabetes: Rationale and Implications of the beta-Cell-Centric Classification Schema. Diabetes Care 2016, 39, 179–186.
8. Strasser, B. Physical activity in obesity and metabolic syndrome. Ann. N. Y. Acad. Sci. 2013, 1281, 141– 159.
9. Koca I, Tutoglu A, Boyacı A, Pehlivan Y, Yıldız H, Turkbeyler I, Sarıcicek E, Taysi S, Onat AM. An evaluation of oxidative stress and antioxidant capacity in patients with myofascial pain syndrome. Mod Rheumatol. 2014 Nov;24(6):992-6. doi: 10.3109/14397595.2014.886984. Epub 2014 Mar 26. PMID:24670130.
10. Zengin O, Onder ME, Sarica MA, Turkbeyler IH, Kimyon G, Demir ZH, Yildiz H, Kisacik B, Onat AM. Systemic vasculitis in a patient with rhupus syndrome. Reumatismo. 2015 Dec 23;67(4):161-4. doi: 10.4081/reumatismo.2015.858. PMID: 2721518.
11. Apaydın Z. , Yıldız H. , Samin F. , Alaşehirli B. Investigation of Dynamic Thiol Disulfide Homeostasis in Acute Respiratory Failure Patients in Intensive Care Unit. Experimental and Applied Medical Science. 2021;2(2): 164-175.
12. Yıldız H, Yıldız Pehlivan D. Analysıs of thıole/dısulfide homeostasıs ın patıents wıth decubıtus ulcer. Experimental and Applied Medical Science. 2022; 3(2): 345-353.
13. Yildiz H. Successful therapeutic hypothermia in a propofol-related cardiac arrest case: a case report and literature review. Ther Hypothermia Temp Manag. 2018 Dec;8(4):239-244. doi: 10.1089/ther.2018.0009. Epub 2018 Jul 11. PMID: 29993335.
14. Pradhan AD, Cook NR, Buring JE, Manson JE, Ridker PM. C-reactive protein is independently associated with fasting insulin in nondiabetic women. Arterioscler Thromb Vasc Biol 2003, 23: 650-5.
15. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006, 116: 1793-801. Kishi Y, Kopetz S, Chun YS, Palavecino M, Abdalla EK, Vauthey JN. Blood neutrophil-to-lymphocyte ratiopredicts survival in patients with colorectal liver metastases treated with systemic chemotherapy. Ann Surg Oncol 2009, 16: 614-22.
16. Yildiz H. The relationship between neutrophil lymphocyte ratio and 28-day mortality in intensive care patients. Progr Nutr [Internet]. 2019 Sep. 18 [cited 2022 Dec. 15];21(3):566-9. Available from: https://www.mattioli1885journals.com/index.php/progressinnutrition/article/view/8720.
17. Zahorec R. Ratio of neutrophil to lymphocyte counts - rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy 2001, 102: 5-14.
18. Bhutta H, Agha R, Wong J, Tang TY, Wilson YG, Walsh SR. Neutrophil-lymphocyte ratio predicts medium- term survival following elective major vascular surgery: a cross-sectional study. Vasc Endovascular Surg 2011, 45: 227-31.
19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010 Jan;33 Suppl 1(Suppl 1): S62-9. doi: 10.2337/dc10-S062. Erratum

in: Diabetes Care. 2010 Apr;33(4): e57. PMID: 20042775; PMCID: PMC2797383.

20. Farag YM, Al Wakeel JS. Diabetic nephropathy in the arab gulf countries. Nephron Clin Pract 2011, 119:c317-22.

21. Ko SH, Cha BY. Diabetic peripheral neuropathy in type 2 diabetes mellitus in Korea. Diabetes Metab J 2012,36: 6-12.

22. Fong DS, Aiello L, Gardner TW, et al. Diabetic retinopathy. Diabetes Care 2003, 26: 226-9.

23. Jindal S, Gupta S, Gupta R, et al. Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications. Hematology 2011, 16: 86-9.

24. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev. 2009;89(1):27–71.

25. Yildiz H. Thiol/disulphide homeostasis in intensive care unit patients with sepsis and septic shock. Turk J Med Sci. 2020 Jun 23;50(4):811-816. doi: 10.3906/sag-1905-148. PMID: 32233178; PMCID: PMC7379464.

26. Erel Ö, Erdoğan S. Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. Turk J Med Sci. 2020 Nov 3;50(SI-2):1728-1738. doi: 10.3906/sag-2003-64. PMID: 32233181; PMCID: PMC7672356.

27. Tudor R, Zalewski PD, Ratnaike RN. Zinc in health and chronic disease. J Nutr Health Aging. 2005;9(1):45-51. PMID: 15750665.

28. Yildiz H. Is it given over importance to serum zinc level in patients with sepsis? Zinc and sepsis. Progr Nutr [Internet]. 2019 Sep. 18 [cited 2022 Dec. 15];21(3):605-10. Available from: https://www.mattioli1885journals.com/index.php/progressinnutrition/article/view/8672.

29. Barman S, Srinivasan K. Diabetes and zinc dyshomeostasis: Can zinc supplementation mitigate diabetic complications? Crit Rev Food Sci Nutr. 2022;62(4):1046-1061. doi: 10.1080/10408398.2020.1833178. Epub 2020 Oct 14. PMID: 33938330.

30. Pitsavos C, Tampourlou M, Panagiotakos DB, et al. Association between low-grade systemic inflammation and type 2 diabetes mellitus among men and women from the ATTICA Study. Rev Diabet Stud 2007, 4: 98-104.

31. Sattar N. Biomarkers for diabetes prediction, pathogenesis or pharmacotherapy guidance? Past, present and future possibilities. Diabet Med 2012, 29: 5-13.

32. Nakanishi N, Sato M, Shirai K, Suzuki K, Tatara K. White blood cell count as a risk factor for hypertension; a study of Japanese male office workers. J Hypertens 2002, 20: 851-7.

33. Dehghan A, Kardys I, de Maat MP, et al. Genetic variation, C-reactive protein levels, and incidence of diabetes. Diabetes 2007, 56: 872-8.

34. Wannamethee SG, Lowe GD, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. Diabetes Care 2007, 30: 1200-5.

35. Öztürk ZA, Kuyumcu ME, Yesil Y, Savas E, Yıldız H, Kepekçi Y, Arıoğul S. Is there a link between neutrophil-lymphocyte ratio and microvascular complications in geriatric diabetic patients? J Endocrinol Invest. 2013 Sep;36(8):593-9. doi: 10.3275/8894. Epub 2013 Mar 19. PMID: 23511196.

36. Muhmmed Suliman MA, Bahnacy Juma AA, Ali Almadhani AA, Pathare AV, Alkindi SS, Uwe Werner F. Predictive value of neutrophil to lymphocyte ratio in outcomes of patients with acute coronary syndrome. Arch Med Res 2010, 41: 618-22.

37. Azab B, Zaher M, Weiserbs KF, et al. Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. Am J Cardiol 2010, 106: 470-6.