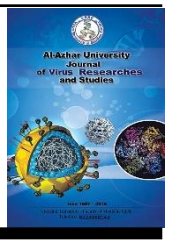
Al-Azhar University Journal
for Virus Research and Studies



Neutrophil-Lymphocyte Ratio as A predictor Inflammatory Marker for Microvascular Complications of Type 2 Diabetes

Mona Gamal Ahmed Mohamed[1], Zeinab Ahmed Yousry Mahmoud[1], Hend Mohamed Tawfeeq[1] and Salwa Ibrahim El shennawy[2]

[1]Department of Internal Medicine, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[2]Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

***E-mail: dr.mona.medo@gmail.com**

Abstract

Retinopathy, peripheral neuropathy, and nephropathy are microvascular consequences of type 2 diabetes mellitus. Numerous investigations have shown an important role for inflammation in the etiology of diabetes complications. In systemic illnesses, the neutrophil/lymphocyte ratio (NLR) is regarded as a predictive inflammatory measure. The paper aims to assess if NLR as an inflammatory bio-maker can be used for the detection of microvascular complications of type 2 diabetes. (retinopathy, peripheral neuropathy, and nephropathy). This is a case-control study with 60 persons 40 of them are diabetic patients divided into 2 groups :(20) with microvascular complications (group 1), the other (20) without microvascular complications (group 2) and twenty individuals with age and sex matched controls group (group 3). All patients had a comprehensive medical history and physical examination, as well as laboratory tests such as complete blood count (CBC), fasting blood sugar, HbA1c, renal function tests (serum urea and creatinine), liver function tests (ALT, AST), lipid profiles (serum cholesterol, Triglycerides (TG), Low-density lipoprotein (LDL), and High-density lipoprotein (HDL), albumin to creatinine ratio in urine, and urine analysis. Additionally, fundus and abdominal ultrasonography were performed. There was a highly statistically significant increase in NLR in diabetic patients with at least one or more microvascular complications (retinopathy, peripheral neuropathy, and nephropathy) in comparison with the control group. NLR may be employed as a diagnostic predictor of inflammatory indicators of diabetic microvascular complications (retinopathy, peripheral neuropathy, and nephropathy).

Keywords: Neutrophil to Lymphocyte ratio (NLR), Complete blood count (CBC) and Diabetes Mellitus (DM).

1. Introduction

Diabetes mellitus (DM) is a metabolic condition marked by a persistently high amount of sugar in the blood. It may be classified as one of the world's major illnesses since it affects a large number of people and has two primary forms, I and II. Possible blindness, renal failure, amputation of a lower limb, and cardiac

arrest are all consequences of diabetes. Okur et al., [1]. Poorly managed hyperglycemia causes a variety of consequences, most of which are vascular. These issues might be microvascular, affecting small arteries, macrovascular, affecting big vessels, or both. Three frequent consequences of diabetes mellitus are caused by microvascular disease: Retinopathy, nephropathy, and neuropathy are all diseases that affect the eyes, kidneys, and nerves Brutsaert et al., [2]. Diabetes and hyperglycemia foster an inflammatory environment that leads to microvascular consequences such retinopathy, nephropathy, and peripheral neuropathy. Dung et al., [3]. Numerous studies investigated the link between systemic inflammation and diabetes complications have shown that chronic inflammation speeds up the progression of diabetic microangiopathy and macroangiopathy in diabetics Fujita et al., [4]. In recent years, NLR, which is just the division of the number of neutrophils by the number of lymphocytes, has gotten a lot of interest in a lot of sectors of medicine. NLR is thought to be a sign of infection or inflammation, thus it may be a better indicator of acute stress than labs that are slower to react (e.g., white blood cell count) Honda et al., [5].

2. Patients and Methods

There were (60) individuals in this prospective and retrospective investigation. They were divided into three categories, as follows: Group 1; (20) type 2 diabetic patients with microvascular consequences, Group 2; (20) diabetic patients without microvascular consequences and the last group included (20) individuals as apparently healthy control group. The research was conducted at Al-Zahraa University Hospital's Internal Medicine and Endocrine outpatient clinics from March to December 2020. Oral and written consents were taken from all individuals who participated in the study and after the approval of the ethical committee of the university.

2.1 Exclusion criteria

1. Type I DM.
2. No pyuria or hematuria.
3. Acute inflammation (bacterial or viral).
4. Patients on steroid or NSAID or immunosuppressive drugs.
5. Patients with cancers, autoimmune disorders, severe liver and kidney conditions.

2.2 The following were performed on all patients and controls

1- A complete medical history.
2- Clinical examination.
3- Standard laboratory tests (glycated hemoglobin (HbA1c), fasting blood sugar (FBS), CBC, erythrocyte sedimentation rate (ESR), liver function tests, kidney function tests, complete urine analysis, and albumin/creatinine ratio in urine).
4- Lipid profile (total serum cholesterol, TG, HDL and LDL).
5- Fundus examination.
6- Abdominal ultrasonography.
7- Calculation of NLR.

2.3 Statistical Analysis

The statistical system for social sciences, version 20.0, was employed to analyze the data. Frequency and percentage were used to convey qualitative data. The mean ± standard deviation (SD) was used to convey quantitative data. As a result, the significance of p-value was determined as follows: P-values >0.05 were considered inconsequential, P-values < 0.05 were considered significant, and P-values < 0.001 were considered very significant.

3. Results

Table .1 revealed that there were:

- In terms of Hb, HCT, MCV, MCH, MCHC, PLTs, RBCs, and WBCs, there was no statistically substantial variance between the studied groups.

- In terms of NLR, there was a highly statistically significant difference between the studied groups.

- In terms of neutrophils and lymphocytes, there was a substantial variance between the groups investigated.

- In terms of FBS and HbA1c, Table .2 demonstrated that there was a highly relevant difference between the three tested groups.

Table .3 revealed that there were:

-Highly statistically significant difference (p-value< 0.001) between the studied groups as regard ALB/Create ratio.

- No statistically significant difference (p-value< 0.05) between studied groups as regard ALT, AST, Urea and Create.

Table (1): Comparison between studied groups as regard CBC.

		Groups			Stat. test	P-value
		Group I (n = 20)	Group II (n = 20)	Group III (n = 20)		
Hb (g/dl)	**Median ±SD**	12.9±1.53	13.8±1.7	12.7±1.57	F = 2.1	0.131 NS
HCT (%)	**Median**	39.5	43	39	KW = 5.9	0.052 NS
	IQR	36.8 - 42	39 - 45	37 – 43		
MCV (fl/cell)	**Median**	90	90.5	90	KW = 0.65	0.721 NS
	IQR	88 - 91	87.3 - 93.5	84 - 92.8		
MCH (pg/cell)	**Median**	30	30	30	KW = 0.62	0.732 NS
	IQR	28 - 30	28 - 30	28.5 – 30		
MCHC (g/dl)	**Median**	33	32.5	33	KW = 0.89	0.640 NS
	IQR	32 - 33	31 - 33	32 – 33		
PLT ($x10^3/m^3$)	**Median**	210.5	205.5	201	KW = 4.5	0.103 NS
	IQR	203 – 211	199.3 - 217.8	176.3 – 210		
RBCs (m/m^3)	**Mean ±SD**	4.26±0.58	4.65±0.54	4.43±0.54	F = 2.45	0.095 NS
WBCs ($x10^3/m^3$)	**Mean ±SD**	7.67±1.6	7.81±1.49	6.86±1.8	F = 1.93	0.153 NS
Neut. ($x10^3/m^3$)	**Median**	4932	5867	4525	**KW = 6.2**	**0.044 S**
	IQR	4367.8 - 5884	4932 - 6750	2584 – 5737		
Lymph ($x10^3/m^3$)	**Median**	1542.5	2105	2112	**KW = 12.4**	**0.002 S**
	IQR	1406 - 1950	1896 - 2184	1717 – 2787		
NLR	**Median**	3.2	2.95	2.3	**KW = 33.5**	**<0.001 HS**
	IQR	3.01 - 3.4	2.7 - 3.07	1.5 - 2.6		

F: F value of ANOVA, KW: Kruskal Wallis Test.

Table (2): Comparisons between groups as regard FBS and HbA1c.

		Groups			F	P-value
		Group I	Group II	Group III		
FBS (mg/dl)	**Mean± SD**	**242.4±109.1**	**209.2±96.3**	**85.3±8.2**	**19.4**	**<0.001 HS**
$HbA1_C$ (%)	**Mean ±SD**	**9.9±2.3**	**9.3±1.7**	**5.8±0.3**	**30.8**	**<0.001 HS**

Table (3): Comparison between studied groups as regard liver, kidney functions and ALB/Create ratio.

		Groups			KW	P-value
		Group I (n = 20)	Group II (n = 20)	Group III (n = 20)		
ALT (U/L)	**Median**	22.5	22.5	18.5	1.98	0.370 NS
	IQR	18.3 - 29.5	15.3 - 29	16.2 - 24.8		
AST (U/L)	**Median**	29	26.5	22	4.3	0.112 NS
	IQR	19.3 - 34.8	19 - 32.8	19 - 26.8		
Urea (mg/dl)	**Median**	24.5	24	22	1.25	0.535 NS
	IQR	18.5 – 29	20 - 30.8	20 - 25.8		
Creat (mg/dl)	**Median**	0.6	0.7	0.6	0.24	0.887 NS
	IQR	0.5 - 0.8	0.6 - 0.77	0.6 - 0.8		
ALb / Creat	**Median**	49.7	10.5	7	**29.9**	**<0.001 HS**
	IQR	26 - 481.3	6.25 - 20.6	5 - 10.5		

Table (4): Correlation study between NLR, FBs and HbA1c in all studied groups.

		Groups		P-value in group I	P-value in group II
		Group I	Group II		
NLR Vs FBS (mg/dl)	**R**	**0.61**	**0.075**	**0.004 S**	**0.752 NS**
NLR Vs HbA1C (%)	**R**	**0.51**	**0.013**	**0.019 S**	**0.956 NS**

Table (5): Diagnostic performance of NLR in discrimination of group I and group II.

Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
> 3.02	+0.77	75 %	75 %	75 %	75 %	0.002

Table (6): Relation between NLR and complications in group I.

		Complications		MW	P-value
		Single (N = 13)	Multiple (N = 7)		
NLR	Median	3.1	3.8	16	0.019 S
	IQR	2.7 - 3.3	3.2 - 4.3		

In terms of FBS and HbA1c, Table .4 demonstrated that there was Statistically significant Positive correlation between NLR, FBs and HbA1c respectively in group I (p-value = 0.004) (r= 0.61) & (p-value= 0.019) (r=0.51). Table .5 showed that NLR can be used to discriminate between group I and group II by Using roc curve at a cutoff level of >3.02, with 75% sensitivity 75% specificity, 75% Positive predictive value (PPV) and 75% Negative predictive value (NPV). Table .6 showed that there was statistically relevant variance between patients with single complication and patients with multiple complications as regard NLR in group I.

4. Discussion

Diabetes is a kind of systemic metabolic condition that may cause a variety of micro- and macrovascular problems, such as DR, DN, and neuropathy [6]. Low-grade inflammation is the fundamental pathophysiology in metabolic syndrome and the progression of T2DM. Increased NLR has been shown in many studies to be a marker of cardiovascular disorder and malignancy. NLR is assumed to be the equilibrium of innate (neutrophils) and adaptive (lymphocytes) immune system reactions to low-grade inflammation [7]. In the present investigation, it was shown that there was a highly substantial distinction in HbA1c levels between groups I and II. In accordance with [8] and [9], substantial variations in HbA1c levels across examined groups and a significant association between NLR and HbA1c. HbA1c levels were shown to have a high relationship with NLR in these investigations. HbA1c levels are a measure of blood glucose control, and a higher number may indicate a higher risk of cardiovascular problems in people with type 2 diabetes. In contrast to our findings, [10] found no considerable differences in FBS and HbA1c between two groups. According to neutrophils and lymphocytes, our research found a statistically considerable variance between the groups tested. This conclusion is consistent with that of [10], who found a substantial difference in neutrophil and lymphocyte counts between the normal and DN groups. In terms of serum urea and creatinine, our research revealed that there was not statistically substantial distinction between the groups tested. This was in line with [10], who claimed that there was no change in blood urea and serum creatinine between the two groups. The differences in creatinine and urea between diabetics with problems and diabetics without difficulties were not statistically relevant, according to

[11]. In terms of the Albumin/Creatinine ratio, our research revealed that there was a very considerable distinction between groups I and II, as well as between groups I and III. This was in line with [10], who claimed that there was no change in blood urea and serum creatinine between the two groups. The differences in creatinine and urea between the diabetic group with problems and the diabetic group without difficulties were not statistically relevant, which was in keeping with [11]. In terms of the Albumin/Creatinine ratio, our research revealed that there was a very considerable difference between groups I and II, as well as between groups I and III. This were in concordance with [12] who concluded that there was high statistically considerable increase of NLR, PLR and UACR in group of diabetic patients with nephropathy with macroalbuminuria when compared to groups of, nephropathy with microalbuminuria, diabetic patients without nephropathy and control group.

This increase could be due to metabolic and hemodynamic factors, which are thought to be the primary factors of diabetic nephropathy, inflammation, which influences glomerular functions through distinctions in vascular permeability, vasodilatory and vasoconstrictor processes, extracellular matrix dynamics, and the proliferation of mesangial, endothelial, and vascular smooth muscle cells, as well as the initiation of cytotoxicity, apoptosis, and necrosis [9].

In terms of NLR, our research found a very statistically relevant variance between the three groups. Patients in their research who were in the highest NLR quartile had a considerably increased incidence of nephropathy and retinopathy, according to [13]. Based on the receiver operating characteristic curve analysis, NLR was the strongest predictor of nephropathy, followed by retinopathy, among the microvascular sequelae.

Similarly, elevated NLR was shown to be strongly linked with DN in another investigation, suggesting that high NLR levels might be a predictor of early-stage DN [14].

NLR, MPV, and PDW were shown to be greater in diabetic individuals with retinopathy or nephropathy in a recent meta-analysis. In addition, MPV was shown to be linked to the severity of DR, while NLR was found to be strongly linked to the degree of DN [15].

Furthermore, NLR was discovered to be higher in diabetic individuals with Retinopathy and linked with the severity of DR [16] and [17] in numerous investigations as a new inflammatory marker. In the opposite of our study, [18] showed that patients with DPN were more susceptible to pain as shown by their pain threshold, NLR may not be elevated but reduced in them and focus should shift to the immunological roles and not just inflammation in the pathogenesis and progression of DPN, knowing that sensory neuropathy (e.g., with abnormal pain sensation) is the first stage of DPN which if well managed will halt the progression of DPN.

In the groups tested, there was no statistically substantial association between NLR and age, according to our findings.

In agreement with [12], no statistically substantial association between NLR and age was found in their research.

Our study found that there was statistically substantial positive correlation ($r = 0.53$) between NLR and DM duration and HbA1C in group 1.

In agreement with [19], participants in the highest NLR have longer duration of diabetes, greater FPG, HbA1c, and greater incidence of cardiovascular disease (CVD), hypertension and DKD.

Our study found that there was statistically significant (p-value =0.018) positive correlation between NLR and ALB/Creat ratio. In agreement with [12], there was positive association between NLR, PLR, HBA1c and UACR in diabetic patients with nephropathy with microalbuminuria group.

Our study showed that NLR can be used to discriminate between group I and group II at a cutoff level of >3.02, with 75% sensitivity 75% specificity.

In line with our findings, [8] in Egypt found that NLR levels in diabetic patients with microvascular problems (retinopathy, neuropathy, and nephropathy) were considerably greater than those in diabetic patients without microvascular issues and healthy controls.

In group I, our research found a substantial difference in NLR between patients with a single issue and those with multiple complications.

In summary, NLR may be a predictor of DPN, with T2DM individuals with higher NLR levels having a greater risk of developing DPN [20]. In Indians with Type 2 Diabetes, NLR is a new and accurate predictor of nephropathy, retinopathy, and coronary artery disorders [13].

Finally, NLR was shown to be the most accurate predictor of diabetic neuropathy and nephropathy. In DM patients with more than one microvascular problem, NLR was also considerably greater. NLR is a simple measure that may be used to predict the occurrence of diabetic microvascular problems. As a result, early detection and management of inflammatory pathways might be a part of preventing and controlling diabetes-related problems [21].

5. Conclusion

NLR and diabetic microvascular problems of type 2 diabetes were shown to have a significant positive connection, with diabetic patients with many issues having higher NLR levels than diabetic patients with a single difficulty. As a result, a high NLR might be used as a prediction inflammatory marker for type 2 diabetes microvascular problems (retinopathy, nephropathy and neuropathy).

Funding Sources: There was no support for this study from any governmental, private, or non-profit organization.

Conflicts of interest: No competing interests.

References

1- Okur ME, Karantas ID, Siafaka PI. Diabetes Mellitus: a review on pathophysiology, current status of oral pathophysiology, current status of oral medications and future perspectives. ACTA Pharmaceutica Sciencia. 2017;55(1).

2- Brutsaert F. Eeica. (2019). Complication of Diabetes Mellitus;456;34-38. Available at: https://www.msdmanuals.com/professional/endocrine-and-metabolic-disorders/diabetes-and-disorders-of-carbohydrate-metabolism/complications-of-diabetes-mellitus#v29299406.

3- Dung V. Nguyen, Lynnc Shaw, and Maria B. Grant. front Endocrinal (Lausanne).2012;3:170.10.3389/fendo. 2012.00170

4- Fujita T, Hemmi S, Kajiwara M, Yabuki M, Fuke Y, et al. Complement-mediated chronic inflammation is associated with diabetic microvascular complication. Diabetes Metab Res Rev. 2013; 29:220–6.

5- Honda T, Uehara T, Matsumoto G, Arai S, Sugano M. Neutrophil left shift and white blood cell count as markers of bacterial infection. Clin Chim Acta. 2016; 457:46-53.

6- Velayutharaj A., Saraswathi R., Muthumani L., Shivakumar R. and Sivakumar M. (2020). Significance of Neutrophil Lymphocyte Ratio and High Sensitivity C - reactive protein in Type 2 Diabetic Patients. International Journal of Current Research, 12(22), 66-69.

7- Moursy, E. Y., Megallaa, M. H., Mouftah, R. F. and Ahmed, S. M. (2015). Relationship between neutrophil lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. Am J Intern Med, 3(6), 250-255.

8- Fawwad, A., Butt, A. M., Siddiqui, I. A., Khalid, M., Sabir, R. and Basit, A. (2018). Neutrophil-to-lymphocyte ratio and microvascular complications in subjects with type 2 diabetes: Pakistan′ s perspective. Turkish journal of medical sciences, 48(1), 157-161.

9- Khandare, S. A., Chittawar, S., Nahar, N., Dubey, T. N. and Qureshi, Z. (2017). Study of neutrophil-lymphocyte ratio as novel marker for diabetic nephropathy in type 2 diabetes. Indian journal of endocrinology and metabolism, 21(3), 387-392.

10- Xu T., Weng Z., Pei C., Yu S., Chen Y., Guo W. and Sun J. (2017). The relationship between neutrophil-to-lymphocyte ratio and diabetic peripheral neuropathy in Type 2 diabetes mellitus. Medicine. 96(45): 1-6.

11- Abdelaziz A. A., El-Sayed E. S. E. M., Emran T. M. and Abdallah A. I. (2018). Study of neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) as a predictor inflammatory marker for diabetic nephropathy in type 2 diabetic patients. Egyptian Journal of Hospital Medicine, 72(7), 4800-4807.

12- Chittawar, S., Dutta, D., Qureshi, Z., Surana, V., Khandare, S. and Dubey, T. N. (2017). Neutrophil-lymphocyte ratio is a novel reliable predictor of nephropathy, retinopathy, and coronary artery disease in Indians with type-2 diabetes. Indian journal of endocrinology and metabolism, 21(6), 864-870.

13- Huang, W., Huang, J., Liu, Q., Lin, F., He, Z., Zeng, Z. and He, L. (2015). Neutrophil–lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy. Clinical endocrinology, 82(2), 229-233.

14- Liu, W. J., Huang, W. F., Ye, L., Chen, R. H., Yang, C., Wu, H. L. and Liu, H. F. (2018). The activity and role of autophagy in the pathogenesis of diabetic nephropathy. European review for medical and pharmacological sciences, 22(10), 3182-3189.

15- Wang, R. T., Zhang, J. R., Li, Y., Liu, T. and Yu, K. J. (2015). Neutrophil– lymphocyte ratio is associated with arterial stiffness in diabetic retinopathy in type 2 diabetes. Journal of Diabetes and its Complications, 29(2), 245-249.

16- Şenel, E., Acar, B. and Demir, E. (2017). Mean platelet volume: A reliable marker of inflammation in recurrent apthous stomatitis and behcet disease? Indian dermatology online journal, 8(6), 468-470

17- Akintoye, O. O., Oniyide, A. A. and Owoyele, B. V. (2018). A study of pain threshold, interleukins and NLR in diabetic polyneuropathy in a selected Nigerian population. Nigerian Journal of Physiological Sciences, 33(2), 151-157.

18- Wan, H., Wang, Y., Fang, S., Chen, Y., Zhang, W., Xia, F. and Lu, Y.

(2020). Associations between the Neutrophil-to-Lymphocyte Ratio and Diabetic Complications in Adults with Diabetes: A Cross-Sectional Study. Journal of Diabetes Research, 1(1), 1-9.

19- Abou Raya, W. M., Abd-El hamed, S. S. and El Salanty, N. H. (2020). Relationship between neutrophil-to-lymphocyte ratio and diabetic neuropathy in type 2 diabetes mellitus. The Scientific Journal of Al- Azhar Medical Faculty, Girls, 4(2), 217-224.

20- Bhattacharyya, S., Jain, N., Verma, H. and Sharma, K. (2021). A Cross-sectional Study to Assess Neutrophil Lymphocyte Ratio as a Predictor of Microvascular Complications in Type 2 Diabetes Mellitus Patients. Journal of Clinical & Diagnostic Research, 15(8), 59-62.