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Neutrophil-to-lymphocyte ratio and microvascular complications in subjects with type 2 diabetes: Pakistan's perspective

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Background/aim: The aim of this study is to find the association between diabetic microvascular complications and the neutrophil-to-lymphocyte ratio (NLR) in subjects with type 2 diabetes.

Materials and methods: This was a retrospective study based on hospital data records from January 2005 to May 2016 at the Baqai Institute of Diabetology and Endocrinology. The eligibility criteria included subjects with type 2 diabetes with their latest complete blood count while subjects with conditions such as chronic inflammation, cancer, heart failure, and end-stage renal disease were not eligible for inclusion. Subjects were divided into two groups: one with any microvascular complications and the other with no microvascular complications. Body mass index, anthropometric measurements, and blood pressure were measured.

Results: Out of 5620 type 2 diabetic subjects, 3202 (57%) were male and (2418) 43% were female. Among these, 3374 diabetic subjects had one or more microvascular complications and 2246 had no microvascular complications. The NLR was found to be 1.14 times higher in diabetic subjects with at least one microvascular complication as compared to diabetic subjects without any complications (4.34 ± 3.32 vs. 3.36 ± 2.67; $P < 0.0001$). Factors likely associated with microvascular complications were high levels of NLR, HbA1c, serum creatinine, and systolic blood pressure and longer duration of diabetes.

Conclusion: According to the results, the NLR is an efficient, cheaper, and readily available marker of inflammation and it is known as an important predictor for the existence of microvascular complications in subjects with type 2 diabetes.

Key words: Neutrophil-to-lymphocyte ratio, type 2 diabetes, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy

1. Introduction

Diabetes is a major public health problem that is approaching epidemic proportions globally. It is increasing at an alarming rate worldwide (1). According to the International Diabetes Federation estimation of 2015, approximately 415 million people or 8.8% of the adult population is suffering from diabetes. It was estimated that 642 million people, or one in ten adults, would have to live with diabetes by the year 2040 (2). People with diabetes usually develop different chronic vascular complications that include macrovascular diseases and microvascular diseases (3). Subclinical inflammation contributes to enhancement of metabolic disturbances, which eventually leads to vascular complications, a major cause of morbidity and mortality in diabetic patients (4,5). The ratio of absolute neutrophil count to absolute lymphocyte count (neutrophil-to-lymphocyte ratio, NLR) is a crucial marker of systemic inflammation (6). A high value of NLR represents a large number of neutrophils with a small number of lymphocytes. When neutrophils are available in large numbers, lymphocyte vascular damage occurs as a result of neutrophilia. Neutrophilia encourages widespread chronic inflammation, which provides an environment suitable for the pathogenesis of diabetes mellitus and its related complications (7). An increase in values of NLR seems to be a significant indicator to diagnose diabetic retinopathy, a major cause of blindness in adults (8,9). The NLR is one of the significant components for chronic kidney disease and the maximum distribution of this ratio is linked as a risk factor for major cardiovascular events (10). Increased NLR may also be associated with type 2 diabetes mellitus, which can be easily measured by

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a simple peripheral blood count, which is simple and less expensive than calculating other inflammatory cytokines, such as IL-6, IL-1β, and TNF-α (11). The chemotactic activity of neutrophils is significantly lower in diabetic patients than in cells from healthy patients (12). There is also decreased bactericidal activity, impairment of phagocytosis, decreased release of lysosomal enzyme, and reduced production of reactive oxygen species, while in leukocytes these activities show a significant correlation with increases in blood glucose level (13). An increase in levels of glucose and ketone bodies decreases polyol production, the enhancer of neutrophil function and metabolic alterations (14). The purpose of the present study is to evaluate the relationship between NLR and microvascular complications in Pakistani subjects with type 2 diabetes.

2. Materials and methods

This was a retrospective study based on hospital data records from January 2005 to May 2016 at the Baqai Institute of Diabetology and Endocrinology (BIDE). Ethical approval was obtained from the institutional review board of BIDE. The eligibility criteria included subjects with type 2 diabetes with their latest complete blood count (CBC) while subjects with conditions such as chronic inflammation, cancer, heart failure, and end-stage renal disease were not eligible for inclusion. The subjects were divided into two groups: one group with at least one microvascular complication (diabetic retinopathy, nephropathy, or nephropathy) and the other group without any microvascular complications. Selected data were obtained from the hospital management system, demographics, anthropometric measurements, and biochemical results; other medical records, family history, duration of diabetes, complications related to diabetes, etc. were noted. Height was measured in centimeters and weight in kilograms. Blood pressure of the participants was monitored by mercury sphygmomanometer in a sitting position using a standard method. Neuropathy was diagnosed after checking pin prick, vibration sense, ankle reflex, and knee reflex. Retinopathy was diagnosed after examining microdots, blot hemorrhage, hard exudates, soft exudates, and new vessel formation. Nephropathy was noted upon finding urinary albumin in detailed urine reports. Body mass index (BMI) is the ratio of weight (kg) to height squared (m^2). CBCs were performed with an automated hematology analyzer (Celltac Alpha MEK-6450, Nihon Kohden) (15). The GOD-PAP method on a Selectra ProS fully automated analyzer was used for determining fasting blood glucose level and triglycerides. Serum total cholesterol was determined by CHOD-PAP method on the Selectra ProS. A homogeneous enzymatic colorimetric method was used for high density lipoprotein (HDL) cholesterol measurement. A direct method was used for low density lipoprotein (LDL) cholesterol measurement. HbA1c was measured by HPLC method on a Bio-Rad D-10 (16).

2.1. Statistical analysis

Statistical analyses were performed with SPSS 20. The data were presented as mean ± standard deviation. Logistic regression was used to investigate the associations of the binary dependent variable "presence of any one microvascular complication" with continuous or categorical independent variables such as age, sex, BMI, blood pressure, duration of diabetes, NLR, and lipid profile. Confidence intervals were presented. ANOVA was also performed to compare differences among groups. Statistical significance was considered at $P < 0.05$.

3. Results

The study included 5620 patients with type 2 diabetes, of which 3202 (57%) were males and 2418 (43%) were females. Basic characteristics and laboratory findings are shown in Table 1. Of these individuals, 3374 diabetic subjects had one or more microvascular complication, whereas 2246 subjects had no microvascular complications. Subjects with microvascular complications were older than subjects without microvascular complications (55.86 ± 10.72 years vs. 52.33 ± 11.05 years), with longer duration of diabetes (15.47 ± 8.47 years vs. 10.89 ± 7.38 years) and lower BMI (27.7 ± 5.84 kg/m^2 vs. 29.07±5.62 kg/m^2). In univariate analysis, significant differences were observed between groups in terms of age, sex, duration of diabetes, BMI, systolic blood pressure, HDL cholesterol, triglycerides, and HbA1c level. The NLR was 1.14 times higher in diabetic subjects with at least one microvascular complication as compared to diabetic subjects without any complications (4.34 ± 3.32 vs. 3.36 ± 2.67, $P < 0.0001$).

Multiple regression analyses confirmed that of all the variables included in univariate analysis (Table 1), namely high NLR ratio, systolic blood pressure, serum creatinine, and HbA1c and longer duration of diabetes, showed increased risk of microvascular complications (Table 2).

Table 3 shows CBC levels among the studied groups. Platelet count, neutrophil count, and total leukocyte count were significantly higher in subjects who had at least one microvascular complication. All other parameters of CBCs were also significantly different among the groups.

4. Discussion

Diabetes mellitus is not only a metabolic disorder. It is now established that certain molecules combined with inflammation play a vital role in the development of diabetes mellitus and diabetes related complications (17). The NLR is a novel potential marker of inflammatory response (9). Neutrophilia or lymphopenia results in

Table 1. Univariate analysis for association between risk factors and the presence of microvascular complications.

Parameters	Without microvascular complications	With at least one microvascular complication	OR (95% CI)	P-value
n	2246	3374	5620	
Age (years)*	52.33 ± 11.05	55.86 ± 10.72	1.03 (1.02 - 1.036)	<0.0001
Sex*				
Female	1065 (47.4%)	1353 (40.1%)	0.742 (0.667–0.827)	<0.0001
Male	1181 (52.6%)	2021 (59.9%)		
Duration of diabetes (years)*	10.89 ± 7.38	15.47 ± 8.47	1.077 (1.069–1.086)	<0.0001
Body mass index (kg/m^2)*	28.96 ± 5.37	27.57 ± 5.46	0.960 (0.949–0.970)	<0.0001
Systolic blood pressure (mmHg)*	129.69 ± 18.62	132.7 ± 20.07	1.008 (1.005–1.011)	<0.0001
Diastolic blood pressure (mmHg)	80.38 ± 10.03	80.92 ± 10.27	1.005 (0.999–1.011)	0.075
High density lipoprotein (mg/dL)*	37.3 ± 9.95	34.47 ± 10.56	0.974 (0.966–0.982)	<0.0001
Low density lipoprotein (mg/dL)	102.55 ± 37.57	100.08 ± 39.9	0.998 (0.996–1.000)	0.109
Triglycerides (mg/dL)	171.18 ± 115.1	164.61 ± 150.37	1.00 (0.999–1.000)	0.248
Cholesterol (mg/dL)*	172.07 ± 47.68	165.85 ± 52.35	0.998 (0.996–0.999)	0.002
Creatinine (mg/dL)*	1.10 ± 0.65	1.35 ± 0.99	1.589 (1.404–1.799)	<0.0001
HbA1c (%)*	9.14 ± 2.27	9.71 ± 2.42	1.110 (1.078–1.143)	<0.0001
Fasting blood sugar (mg/dL)*	179.74 ± 71.63	191.02 ± 90.73	1.002 (1.000–1.003)	0.008
Random blood sugar (mg/dL)	264.06 ± 110.23	265.62 ± 117.83	1.000 (0.999–1.001)	0.784
NLR	3.36 ± 2.67	4.34 ± 3.32	1.146 (1.121–1.171)	<0.0001

Data presented as mean ± standard deviation.
OR= Odds ratio; * = statistically significant at $P < 0.05$.

high NLR while lymphocytosis or neutropenia results in low NLR (11). The present study demonstrated that NLR levels were significantly higher in diabetic patients with microvascular complications (retinopathy and peripheral neuropathy, nephropathy) as compared to other diabetic patients without complications. In the present study, NLR was significantly higher in diabetic subjects with retinopathy and neuropathy ($P < 0.0001$). Similarly, Moursy et al. showed that NLR among subjects with retinopathy was significantly higher than among diabetic patients without retinopathy (4). In certain clinical studies, increased levels of proinflammatory cytokines in the vitreous fluid of subjects diagnosed with type 2 diabetes with proliferative diabetic retinopathy are an indicator of the progression of retinal injury. However, in a few studies diabetic retinopathy severity was not associated with increasing NLR (18). Diagnosis of diabetic peripheral neuropathy relies on the subjective symptoms of neuropathy. According to the Korean Diabetic Association and in most clinical practice guidelines, screening for neuropathy should be done for all diagnosed type 2 diabetes patients (19). In this study NLR was also higher in subjects with diabetic neuropathy as compared to diabetic subjects without neuropathy. Similarly, in an Egyptian study a significant increase was found in NLR between diabetic subjects with and without neuropathy (4). There were no other studies found addressing the relationship between NLR and diabetic peripheral neuropathy, while Polate et al. found the NLR with nonarteritic anterior ischemic neuropathy (20). Furthermore, in this study, no significant difference was found in NLR levels in subjects with or without diabetic nephropathy. Compared to an earlier study, NLR levels among subjects with diabetic nephropathy were significantly higher than among

Table 2. Factors related to microvascular complications according to multivariate logistics regression analysis.

Variable	Regression coefficient (β)	OR (95% CI)	P-value
Duration of diabetes	0.059	1.061 (1.048–1.073)	<0.0001
Systolic BP	0.006	1.006 (1.001–1.010)	0.012
Serum creatinine	0.233	1.262 (1.110–1.436)	<0.0001
HbA1c	0.97	1.102 (1.062–1.142)	<0.0001
NLR	0.569	1.766 (1.789–2.093)	<0.0001

OR= Odds ratio.
P < 0.05 was considered statistically significant.

Table 3. CBC level among studied groups.

Parameters	Without microvascular complications	With at least one microvascular complication	Overall	P-value
n	2465	3388	5853	
Platelet count	257.98 ± 99.69	284.52 ± 118.07	273.35 ± 111.47	<0.0001
Neutrophil count	69.23 ± 10	73.01 ± 10.47	71.42 ± 10.44	<0.0001
Lymphocyte count	26.8 ± 9.96	23.09 ± 10.31	24.65 ± 10.32	<0.0001
Total leukocyte count	9.56 ± 4.2	11.19 ± 7.6	10.5 ± 6.44	<0.0001
Red blood cells	4.59 ± 0.69	4.38 ± 0.77	4.49 ± 0.74	<0.0001
Mean corpuscular hemoglobin	27.39 ± 3.24	27.09 ± 3.14	27.22 ± 3.19	<0.0001
Mean corpuscular hemoglobin concentration	32.59 ± 1.96	32.48 ± 1.99	32.53 ± 1.97	0.032
Mean corpuscular volume	83.86 ± 8.24	83.1 ± 7.89	83.42 ± 8.05	<0.0001
Monocyte count	1.77 ± 0.70	1.75 ± 1.01	1.76 ± 0.89	0.01
Hemoglobin	12.66 ± 2.26	12.01 ± 2.35	12.35 ± 2.32	<0.0001
Eosinophil count	2.17 ± 1.81	2.12 ± 1.93	2.14 ± 1.88	<0.0001

Data presented as mean ± standard deviation.
P < 0.05 was considered statistically significant.

diabetic subjects without nephropathy or any other microvascular diabetic complications. Although metabolic and hemodynamic factors are the main causes of diabetic nephropathy, inflammation and inflammatory molecules are believed to affect glomerular functions by alternations in vascular permeability, vasodilatory and vasoconstrictor mechanisms, extracellular matrix dynamics, and the proliferation of mesangial, endothelial, and vascular smooth muscle cells, as well as the induction of cytotoxicity, apoptosis, and necrosis. Akbaş et al. reported that HbA1c and NLR are independent indicators for albuminuria. NLR values greater than 1.7 were found to be associated with albuminuria (20). Afsar et al. also reported that high levels of NLR were independently associated with both 24-h urinary protein and urinary albumin excretion (UAE) (21); likewise, Abdul-Rahman reported that UAE and hemoglobin levels were not associated with each other (22). Glycated hemoglobin is an independent risk factor for increase in urinary albumin, which leads to nephropathy (23). In the present study there is a significant correlation between NLR and HbA1c, which supports the Egyptian study in which HbA1c levels showed a strong correlation with NLR. HbA1c levels are an indicator of blood glucose regulation, and increased HbA1c levels may correlate with increased risk of cardiovascular complications in patients with type 2 diabetes mellitus (24).

The major limitation of this study is its retrospective nature.

In conclusion, NLR was considered as a potent marker of inflammation as it is simple, cheap, and easily available compared to other inflammatory cytokines in diabetic subjects. Based on the results of this study NLR was considered as an essential and stable marker in diagnosing microvascular diabetic complications. In this regard, more extensive and more studies with more type 2 diabetic subjects are needed. Detailed studies are particularly required regarding the association of NLR with individual microvascular complications.

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