Is neutrophil-lymphocyte ratio a novel biomarker for macrovascular and microvascular complications of type 2 diabetes?

Walaa H. Mohammad[a], Ahmad B. Ahmad[b], Muhammad H. Al-Maghraby[b], Mohamed Z. Abdelrhman[c], Sanaa Ezzate[d]

[a]Department of Internal Medicine, Nephrology Unit, [b]Department of Internal Medicine, Cardiology and Critical Care Unit, [c]Department of Clinical Pathology, [d]Department of Internal Medicine, Hematology Unit, Assiut University Hospital, Assiut, Egypt

Correspondence to Walaa H. Mohammad, MD, Department of Internal Medicine, Assiut University Hospital, Assiut, 71515, Egypt, Tel: +20 100 522 0944/+20 112 009 6322/+20 882 294 539; e-mails: walaa.hosny2012@gmail.com, walaa.hosny@aun.edu.eg

Background/aim
The neutrophil-to-lymphocyte (N/L) ratio is a biomarker of inflammation. We aimed to test the hypothesis that macrovascular and microvascular complications of type 2 diabetes mellitus (DM) involve an inflammatory process.

Patients and methods
This is a cross-sectional study conducted on patients with type 2 DM. Demographic and clinical examination data of eligible patients were recorded. The blood samples for laboratory variables were collected and measured by the standard methods. A standard ultrasound examination was done to measure both carotid artery intima-media thickness (cIMT), and the averages of three results of each side were recorded.

Results
The current study showed that N/L ratio was an independent predictor of cIMT in patients with type 2 DM (B=0.003, t=8.325, P=0.000), in addition to other conventional cardiovascular risk factors including age, male sex, smoking index, duration of diabetes, estimated glomerular filtration rate, low-density lipoprotein-cholesterol, urinary albumin creatinine ratio, and HBA1c. Moreover, N/L ratio was an independent predictor of albuminuria in patients with type 2 DM (B=0.110, t=3.638, P=0.006), in addition to duration of diabetes, HBA1c, SBP, BMI, and estimated glomerular filtration rate.

Conclusion
N/L ratio was an independent predictor of cIMT and albuminuria. This supports the hypothesis that diabetic macrovascular and microvascular complications involve an inflammatory process. Therefore, NLR may serve as a cost-effective and readily accessible marker of diabetic vascular complications.

Keywords:
diabetic macrovascular and microvascular complications, neutrophil-lymphocyte ratio, novel biomarkers in diabetes

Introduction

Type 2 diabetes mellitus (DM) has many microvascular and macrovascular complications [1]. Carotid artery intima-media thickness (cIMT) is a well-established biomarker of subclinical atherosclerosis and can be used as a predictor for macrovascular events [2]. Albuminuria is an early indicator of diabetic nephropathy (DN), which is one of the DM microvascular complications [3]. Studies have pointed out that inflammation might play an important role in DM and its vascular events [4]. The neutrophil-to-lymphocyte (N/L) ratio was defined as a biomarker of inflammation [5]. However, the relationship between N/L ratio and cIMT and albuminuria in DM has not been fully investigated. The objective of the current study was to investigate the relation between cIMT (as a surrogate of macrovascular predictor) and albuminuria (as a surrogate of microvascular predictor) with N/L ratio in patients with type 2 DM.

Patients and methods

Patients groups

A total of 240 patients with type 2 DM who were being followed up in the Internal Medicine Department of Assiut University Hospital from May 2017 to May 2018 were enrolled. Overall, 105 patients were excluded. Patients without background retinopathy, cIMT greater than 1 mm, estimated glomerular filtration rate (eGFR) less than 110 ml/min, and urinary albumin creatinine ratio greater than 30 were classified as diabetic control group (63 patients), whereas 62 patients with the previous characteristics and had normal renal US result, no pyuria, no
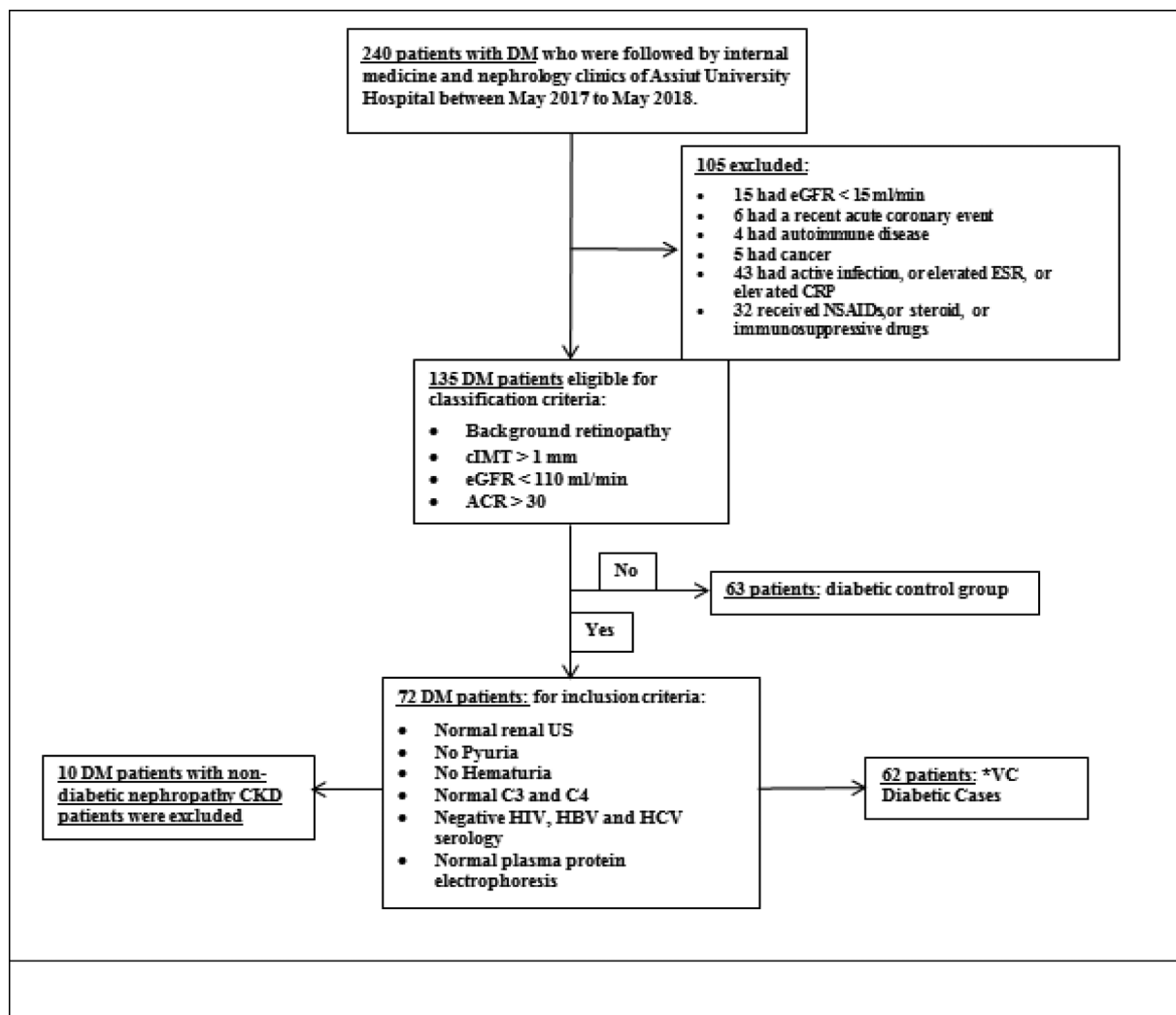
Fig. 1

Patients flow chart.

hematuria, normal C3 and C4, negative HIV, hepatitis B virus and hepatitis C virus serology, and normal plasma protein electrophoresis were classified as vascular complicated diabetic cases group (Fig. 1).

Ethical considerations
Written consents were obtained from all of the participants; illiterate participants gave their consent by fingerprints. The study was approved by the Ethical Committee of College of Medicine of Assiut University.

Demographic and clinical data
Patients' demographic data and medical history included age, sex, duration of diabetes, type of antidiabetic therapy, use of antihypertension medications, and smoking index (number of cigarette/number of years). BMI was calculated as the weight (kg) divided by the square of height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure of the patients were measured with a mercury sphygmomanometer after 15 min of recumbency.

Laboratory data
Venous blood samples were collected from our patients and analyzed in the Clinical Pathology Department Laboratory. Laboratory measurements, including serum levels of creatinine, blood urea nitrogen, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, fasting blood glucose, HBA1c, and serum albumin were measured using standard kits. eGFR was calculated according to the Modification of Diet in Renal Disease formula. The complete blood count was measured using an automated complete blood cell counter (Cell Dyn Ruby; Abbott, USA), which simultaneously provided values for total white blood cell count, red blood count, hemoglobin, platelet count, absolute neutrophil count, and absolute lymphocyte

count. N/L ratio was calculated as a simple ratio of the absolute neutrophil count to the absolute lymphocyte count.

Ultrasonic data

The cIMT of both carotid arteries was measured by using HDI 5000 instrument (Philips Medical Systems, Bothell, Washington, USA) using linear probe, and the average of three results of each side was used. Diabetic patients were divided into two groups according to the cIMT values: diabetic control group with low cIMT (cIMT ≤1 mm) and vascular complicated diabetic cases group with high cIMT (cIMT >1 mm).

Fundoscopy

Retinopathy was tested by using the standard direct fundoscopic examination method.

Statistical analysis

The statistical analysis was performed using SPSS (version 19.0; SPSS Inc., Chicago, Illinois, USA). The Kolmogorov–Smirnov test was used to test normally. The continuous variables were presented as the means±SD, and categorical variables were reported as number and percentage. χ^2 test was used to compare between categorical variables, and continuous variables were compared using a *t*-test. Multivariate analysis was used to identify independent predictors of cIMT and albuminuria. A receiver operator characteristic curve analysis was performed to identify the sensitivity and specificity of N/L ratio cutoff values in prediction of microalbuminuria, macroalbuminuria, and cIMT. A *P* value less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

The study showed that vascular complicated diabetic cases had statistically significant higher duration of diabetes, percent of insulin use, percent of antihypertension medication use, and smoking index (Table 1).

Laboratory data

The study showed that vascular complicated diabetic cases had statistically significant higher levels of HBA1c, fasting blood glucose, LDL-C, triglyceride, serum creatinine, blood urea nitrogen, eGFR, and N/L ratio, with a statistically significant lower level of HDL-C (Table 1).

Multivariate linear regression analysis data

R^2 of this regression models was 82.68%, which explains most of cIMT changes. The independent

Table 1 Study population characteristics'

Variables	VC diabetic cases (*n*=62)	Diabetic control (*n*=63)	*P* value
Age (years)	59.57±12.49	56.60±11.65	0.187
Sex: female (%)	46.77	44.91	0.295
Duration of DM (years)	10.78±4.18	7.83±5.81	0.000
Insulin used (%)	55.53	33.87	0.000
Anti HTN (%)	75.48	55.95	0.000
Smoking index	746.46±65.43	576.12±35.95	0.000
BMI (kg/m^2)	27.54±3.56	26.27±7.03	0.198
SBP (mmHg)	128.34±14.43	124.94±15.53	0.765
DBP (mmhg)	88.87±16.94	86.53±13.49	0.876
HBA1c (%)	9.34±2.29	7.34±2.44	0.000
FBG (mg/dl)	128.56±12.36	119.32±28.08	0.048
LDL-c (mg/dl)	136.19±56.89	125.64±38.74	0.038
HDL-c (mg/dl)	34.36±56.89	49.74±58.51	0.022
Triglyceride (mg/dl)	322.48±34.98	287.40±25.46	0.000
Serum albumin (g/l)	37.37±5.58	39.30±4.73	0.063
Creatinine (µmol/l)	367.98±125.32	82.20±18.42	0.000
BUN (mmol/l)	23.15±10.60	6.71±3.25	0.000
eGFR (ml/min/ 1.73 m^2)	89.45±10.60	145.65±22.34	0.000
uACR <30 (mg/g)	62 (100.0)	0 (00.0)	–
30–300	40 (64.51)	0 (0.0)	–
>300	22 (35.48)	0 (0.0)	–
HB (g/dl)	11.92±1.83	12.42±1.96	0.329
WBCs (cell/mm^3)	11.53±5.99	8.36±2.22	0.000
Neutrophils (cell/ mm^3)	68.38±20.10	58.30±11.63	0.000
Lymphocytes (cell/ mm^3)	14.96±9.27	31.81±10.44	0.000
N/L ratio	8.01±7.62	2.10±1.02	0.000
Retinopathy: yes (%)	44 (70.96)	0 (00.0)	0.000
cIMT (mm)	1.2±0.01	0.7±0.02	0.007

Values are expressed as means±SD and number (%). BUN, blood urea nitrogen; cIMT, carotid intima-medial thickness; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HB A1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; N/L ratio, neutrophil-to-lymphocyte ratio; SBP, systolic blood pressure; uACR, urinary albumin-creatinine ratio; VC, vascular complicated; WBCs; white blood cells.

predictors of cIMT include age, male sex, smoking, duration of diabetes, LDL, HBA1c, and N/L ratio (Table 2).

R^2 of this regression models was 80.67%. The independent predictors of albuminuria include duration of diabetes, HBA1c, SBP, BMI, eGFR, and N/L ratio (Table 3).

Receiver operator characteristic curve analysis data

Receiver operator characteristic curve analysis of N/L ratio, as a diagnostic marker for cIMT greater than 1 mm, at a cutoff value of 2.7 showed a sensitivity and specificity of 100 and 55.45%, respectively, with area under the curve (AUC) of 0.748 (Table 4 and Fig. 2).

Table 2 Multivariate analysis of independent predictors of carotid artery intima-media thickness

Model	B	t	P value	95% confidence interval
Age (years)	0.355	7.450	0.000	0.177 to 0.533
Male gender	2.364	5.640	0.000	4.408 to 0.319
Smoking index	1.016	3.452	0.043	0.202 to 1.829
Duration of DM (years)	0.391	4.639	0.022	0.497 to 0.286
eGFR (ml/min)	−0.001	1.387	0.052	−0.005 to 0.003
LDL (mg/dl)	0.677	4.927	0.027	1.287 to 0.068
HDL (mg/dl)	−0.002	0.761	0.283	−0.005 to 0.001
Triglyceride (mg/dl)	0.010	0.239	0.762	−0.007 to 0.027
uACR	−0.001	0.268	0.065	−0.002 to 0.000
HBA1c (%)	0.018	5.219	0.027	0.013 to 0.023
N/L ratio	0.003	8.325	0.000	0.001 to 0.006

Dependent variable: cIMT (carotid intima-medial thickness). DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N/L ratio, neutrophil/lymphocyte ratio; uACR, urinary albumin-creatinine ratio.

Table 3 Multivariate analysis of independent predictors of albuminuria

Model	β	t	P value	95% confidence interval
Duration of DM (years)	0.090	5.428	0.000	0.043 to 0.137
HBA1c (%)	0.133	4.848	0.027	0.008 to 0.259
SBP (mmHg)	0.855	3.922	0.032	0.320 to 1.390
BMI (kg/m^2)	0.497	3.362	0.022	0.486 to 0.508
eGFR (ml/min)	−0.064	5.427	0.006	−0.102 to −0.027
Serum albumin (g/l)	−0.946	0.765	0.125	−2.338 to 0.445
N/L ratio	0.110	3.638	0.006	0.195 to 0.026

Dependent variable: albuminuria. DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; N/L ratio, neutrophil-lymphocyte ratio; SBP, systolic blood pressure.

N/L ratio at a cut-off value of 3.7 showed sensitivity and specificity of 62.12 and 96.30%, respectively, with AUC of 0.863, when used as a diagnostic marker for uACR greater than 30 (Table 5 and Fig. 3).

N/L ratio at a cutoff value of 7 had sensitivity and specificity of 75.00 and 95.00%, respectively, and AUC of 0.830, when used as a diagnostic marker of macroalbuminuria (uACR >300) (Table 6 and Fig. 4).

Discussion

Use of vascular imaging to identify and quantify the presence of subclinical atherosclerosis is recommended to refine cardiovascular (CV) risk assessment [6]. Measuring cIMT with ultrasound is a noninvasive, sensitive, and reproducible technique for quantifying atherosclerotic burden [7]. Type 2 DM has twofold to fivefold increased risk of atherosclerosis which

Table 4 Receiver operator characteristic analysis for neutrophil-to-lymphocyte ratio as a predictor of carotid artery intima-media thickness

| Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
>2.7	100.00	55.45	16.9	100.0	59.2	0.748

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

predispose to its macrovascular complications, including ischemic heart disease, stroke, and peripheral artery disease [8]. A high N/L ratio is a predictive biomarker of progression of atherosclerosis [9]. The current study showed that N/L ratio is one of the independent predictor of cIMT in patients with type 2 DM in addition to other conventional CV risk factors, including age, male sex, smoking, duration of diabetes, LDL, and HBA1c. These results are consistent with previous studies that showed that N/L ratio was an independent predictor for high cIMT in patients with DM [10] as well as a predictive biomarker of progression of atherosclerosis [9]. Other studies have supported that conventional risk factors such as age, BMI, male sex, systolic BP, higher level of LDL-C, smoking [11,12,13], diabetic state [14], and uACR [15,16] were associated with increased cIMT.

However, the exact mechanisms of high N/L ratio being related with increased cIMT in diabetes patients are as yet unknown. One mechanism is that neutrophil infiltration of the arterial wall plays an important role in the progression of atherosclerosis and makes plaques more vulnerable through the release of proteolytic enzymes [17]. Another possible mechanism is that N/L ratio can reflect an autonomic imbalance of the vascular bed. Sympathetic nerves stimulate granulocytes, whereas parasympathetic nerves stimulate lymphocytes [18]. Therefore, a higher level of N/L ratio may indicate a high sympathetic-to-parasympathetic activity ratio. Sympathetic tone increases oxygen consumption and increases production of proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α [19]. These cytokines stimulate smooth muscle and interstitial cell proliferation, which could accelerate atherosclerosis development [20].

The microvascular complications of DM classically include retinopathy, neuropathy, and nephropathy. DN leads to significant problems in 25–40% of diabetic patients and is the major cause of end-stage renal failure [21]. Albuminuria is the basic finding of renal damage and is linked to the progression of kidney diseases [22]. Albuminuria serves as a biochemical biomarker for DN development and progression [23].

Fig. 2
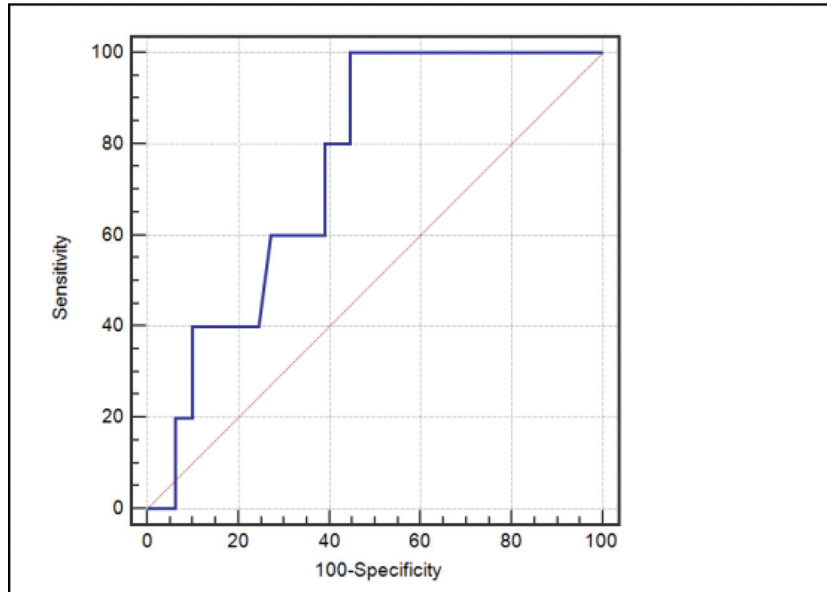
Receiver operator characteristic curve for neutrophil-to-lymphocyte ratio as a predictor of carotid artery intima-media thickness.

Fig. 3
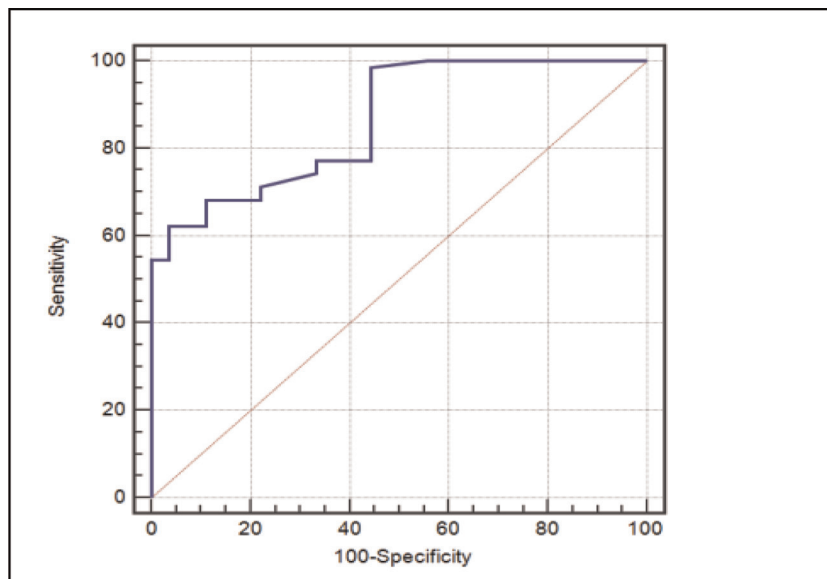
Receiver operator characteristic curve for neutrophil-to-lymphocyte ratio as a predictor of albuminuria (albumin creatinine ratio > 30).

Table 5 Receiver operator characteristic analysis for neutrophil-to-lymphocyte ratio as a predictor of albuminuria (albumin creatinine ratio >30)

Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
>3.7	62.12	96.30	95.3	67.5	77.5	0.863

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.
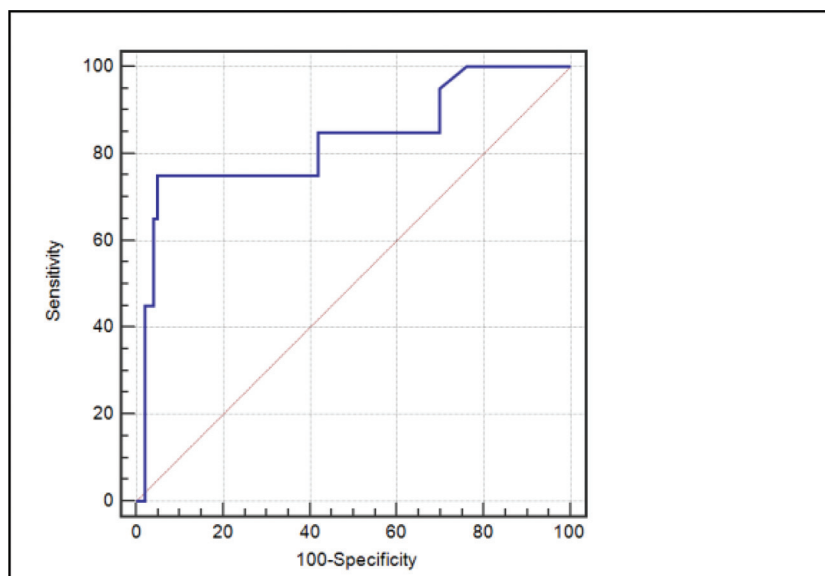
Table 6 Receiver operator characteristic analysis for neutrophil-to-lymphocyte ratio as a predictor of macro-albuminuria (albumin creatinine ratio > 300)

Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
>7	75.00	95.00	75.0	95.0	91.7	0.830

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

A growing evidence has emphasized the critical role of inflammation in the pathogenesis of DN, which results in elevated circulating levels of pro-inflammatory cytokines [24] such as tumor necrosis factor-α and IL-1β [25], IL-18, interferon gamma induced protein (IP-10), monocyte chemo-attractant protein 1 (MCP-1) [26], granulocyte colony-stimulating factor (G-CSF) [27], eotaxins (regulated on activation, normal T cell expressed and secreted), RANTES or CCL-5, and orosomucoid [28]. These cytokines change

Fig. 4

Receiver operator characteristic curve for neutrophil-to-lymphocyte ratio as a predictor of macroalbuminuria (albumin creatinine ratio > 300).

leukocyte levels. However; the biological mechanisms by which leukocytes and their subtypes play a role in mediating increased protein and albumin excretion and retinal injury are not fully known. The current study showed that N/L ratio is independently associated with albuminuria in patients with type 2 DM in addition to duration of diabetes, HBA1c, SBP, BMI, and eGFR.

Previous studies have shown that N/L ratio is independently associated with proteinuria in patients with type 2 DM [29,30,31], even after adjustment for other known endothelial and CV risk factors (age, hypertension, dyslipidemia, diabetes, or altered GFR) [32]. N/L ratio has been associated with proteinuria in older type 2 diabetic patients [33,34], and in newly diagnosed patients with type 2 DM [30,35,34] mentioned a negative correlation between granulocytic count and GFR.

Moreover, previous studies have shown that proteinuria is significantly correlated with total leukocytes, neutrophil, and monocyte counts [29] and N/L ratio [36] in patients with CKD regardless of the etiology. Previous studies also have mentioned that HBA1c [37,31], BMI [38], and BP [39] were independent factors of albuminuria. However, the relation between eGFR and proteinuria is not usually predictable and varies according to the presence and severity of proteinuria [40].

Conclusion

The current study showed that N/L ratio is an independent predictor of cIMT in patients with type 2 DM in addition to other conventional CV risk factors. Moreover, N/L ratio is an independent predictor of albuminuria in type 2 diabetic patients in addition to duration of diabetes, HBA1c, SBP, BMI, and eGFR.

Study limitations and recommendations

The main limitation was the limited study size. Future studies are required to clarify these relationships with a larger study sample. Ours was a monoethnic cross-sectional study, and we suggest a multiethnic prospective study to be carried out to identify clinical outcome. We recommend the use of N/L ratio as a cost-effective and readily accessible novel biomarker for macrovascular and microvascular complications of type 2 DM.

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