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Red Cell Distribution Width and Neutrophil-Lymphocyte Ratio as Markers for Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is the foremost cause of end-stage renal disease. Early detection of DN can spare diabetic patients of severe complications. This study aimed to evaluate the diagnostic value of red cell distribution width (RDW) and neutrophil-lymphocyte ratio (NLR) in the detection of DN in patients with type 2 diabetes mellitus (T2DM). This cross-sectional study included a total of 130 patients with T2DM, already diagnosed with T2DM. The albumin creatinine ratio (ACR) in urine samples was calculated for each patient, according to which patients were divided into two groups: with evidence of DN when ACR ≥ 30 mg/g, and those with no evidence of DN when ACR < 30 mg/g. According to multivariate analysis, each of disease duration (OR = 4.43, 95% CI = 1.68-11.68, p = 0.003), HbA1c (OR = 6.4, 95% CI = 2.32-17.65, p < 0.001) and NLR (OR = 13.75, 95% CI = 1.68-11.68, p < 0.001) were independent predictors for DN. Using the receiver operating characteristic (ROC) curve to evaluate the diagnostic value of NLR revealed that the AUC was 0.736 (95% CI = 0.635-0.837), p < 0.001. The sensitivity and specificity of the test at the cut-off value of NLR = 3.35 was 69% and 89%, respectively. These data indicate that NLR is a simple non-expensive test that can be used regularly to investigate diabetic patients for the development of DN. Red cell distribution width (RDW), on the other hand, had no diagnostic value in this regard.

Keywords: Diabetic nephropathy, Red cell distribution width, Neutrophil-lymphocyte ratio, ROC curve.

Introduction

Diabetes mellitus (DM) is a group of chronic progressive metabolic disorder with hyperglycemia triggered by a defect in insulin secretion, insulin activity, or both.[1] Diabetes is a pathology associated with the development of long-term complications.[2] At a microvascular level, T2DM can lead to vision injury (retinopathy), kidney disease (nephropathy) and neuronal impairment (neuropathies).[3]
Diabetic nephropathy develops in 20% to 40% of patients with T2DM.[4] It is the leading cause of end-stage renal disease (ESRD).[5] The overall burden for people with DN is extremely high due to the strong links between DN and cardiovascular disease (CVD) with ESRD.[6]
Identifying and monitoring DN principally involves two diagnostic modalities: evaluation of kidney function in terms of estimated glomerular filtration rate (eGFR) and assessment of kidney injury in terms of albuminuria.[6] However, it was documented that significant glomerular damage has already occurred by the time albuminuria is apparent.[7] Furthermore, a decrease in the renal function of a diabetic patient is not always escorted by augmented albuminuria in the clinic.[8] Thus, it is of paramount importance to find alternative tools for early detection of DN. In order to be feasible, these tools should be simple, easy to perform, and non-expensive.

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Red blood distribution width is originally used as a marker to differentiate different types of anemias. However, during the last few years, this parameter showed high efficiency in the prediction of mortality in many pathologies such as heart failure,[9] coronary heart disease,[10] and stroke.[11] The neutrophil-lymphocyte ratio (NLR) in complete blood count is an inflammatory indicator which was previously studied in many diseases as an inflammatory marker and is used to predict the prognosis of many diseases such as bacterial sepsis and colorectal cancer.[12,13]
The present study aimed to investigate the diagnostic value of RDW and NLR in the detection of DN in a sample of Iraqi patients with T2DM.

Materials and Methods

The study population
This study is a cross-sectional study that was carried out in Al-Imamain Al-Kadhumain Medical City, Baghdad, Iraq from the 1st of April, 2019 to 31st of January 2020. The studied sample included a total of 130 patients aged 40-70 years, already diagnosed with T2DM. Patients with urinary tract infection, pregnancy and gynaecological disorder, history of nephrotic syndrome other than diabetic proteinuria were excluded from the study. The diagnosis of T2DM was performed depending on the American Diabetes Association Guidelines criteria.[14] Socio-demographic and clinical data including, body mass index (BMI), duration of DM, and smoking status were collected through direct interview with the patients or from Hospital records. This study was approved by the review board of Tropical-Biological Research Unit, College of Science, University of Baghdad (No. 112-Sep-2018), and informed consent was obtained from each patient before sampling.

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Sample collection and laboratory investigations

Three (3) mL of fasting peripheral blood and 10 mL of morning mid-stream urine were collected from each participant. Investigations were carried out for each patient through the following procedures: Hematology auto-analyzer (Huroba ABX/India) was used to measure blood parameters, from which the RDW and NLR were calculated. Glycosylated haemoglobin (HbA1c) was measured according to the exchange chromatography method. A ready kit (i-CHROMA^{TM} HbA1c/ i-CHROMA^{TM} /Korea) was used for this purpose according to the manufacturer's instructions.

Urine albumin concentration (UAC) was measured by turbid-meter immunoassay, and the urine creatinine concentration (UCC) was measured based on the Jaffe reaction. Urine albumin concentrations were expressed as urine albumin/creatinine ratio (ACR). This ratio was calculated by dividing UAC in microgram (mg) by UCC in gram (g). Accordingly, nephropathy was considered when ACR $\geq$ 30 mg/g, otherwise the diabetic patient was considered to have no evidence of nephropathy.

Statistical analysis

Statistical Package for Social Sciences (SPSS version 20) was used for data analysis, and Microsoft Excel to generate graphs. Continuous variables were expressed as mean ± standard deviation (SD) and compared with the parametric student t-test. Discrete variables were expressed as frequency and percentages and analyzed with Chi-square. Univariate and multivariate logistic regression were used to calculate the odds ratios (OR) with 95% confidence interval (CI). The receiver operating characteristic (ROC) curve was used for the validity of NLR in the discrimination of ND. The statistical tests were two-sided, and a $p \leq 0.05$ was considered statistically significant.

Results and Discussion

Association of socio-demographic and clinical characteristics with albuminuria

Table 1 shows the demographic and clinical characteristics of diabetic patients with and without evidence of nephropathy. There were no significant differences between the two groups regarding age, sex, BMI, and smoking. However, patients with nephropathy had significantly higher disease duration, HbA1c, WBC count and NLR (7.41 ± 2.48 years, 9.94 ± 1.82%, 9.45 ± 2.31× 10^3/mL and 4.23 ± 2.14, respectively) than patients without evidence of nephropathy (4.36 ± 2.75 years, 8.78 ± 1.91%, 8.28 ± 2.4× 10^3/mL, and 2.53 ± 0.79, respectively). Interestingly, RDW was 14.08 ± 1.98% and 13.86 ± 2.66% in patients with and without evidence of nephropathy, respectively with no significant difference.

Predictors of diabetic nephropathy

Variables that showed a significant variation between patients with and without nephropathy were categorized and entered univariate and multivariate logistic regression. According to multivariate analysis, each of disease duration (OR = 4.43, 95% CI = 1.68-11.68, p = 0.003), HbA1c (OR = 6.4, 95% CI = 2.32-17.65, p < 0.001) and NLR (OR = 13.75, 95% CI = 1.68-11.68, p < 0.001) were independent predictors for DN. On the other hand, WBC count showed a significant association with DN only in univariate analysis (Table 2).

These confirmed the earlier finding that NLR can be used as an inexpensive, simple test for early detection of DN in patients with T2DM. In this regard, Huang *et al*.[15] reported that NLR was significantly higher in diabetic patients with evidence of nephropathy (2.48 ± 0.59) than in those without such evidence (2.2 ± 0.62) or healthy subjects (1.8 ± 0.64). Almost identical results were reported by Akbas *et al*.[16] who suggested a link between inflammation and endothelial dysfunction in diabetic patients with DN. More recently, Moursy *et al*.[17] have shown that NLR was significantly higher in diabetic patients with DN and retinopathy than those in patients without any microvascular complications or in healthy subjects. A Turkish study also has shown that this ratio increased significantly in parallel with albuminuria levels in diabetic patients.[18]

This elevation of NLR in diabetic patients with DN can be explained from two points of view. Pathophysiologically, accumulated evidence suggested that inflammatory process plays an important role in DN. Such inflammation may promote the acceleration of diabetic microangiopathy as well as macroangiopathy in diabetic patients. As the inflammatory process is mainly a function of leukocytes and their subtypes, it is reasonable to assume that these cells or their products influence some body's organs including the kidney. Some studies suggested that there is an elevation in circulating acute-phase proteins and pro-inflammatory cytokines which are associated with the development of what is called inflammation-induced organ dysfunction in diabetic patients.[19] Other studies indicate that the renal glomeruli and the tubulointerstitium are targeted by these mediators with an eventual different degree of damage in these tissues.[20] Thus, proteinuria is not only an important marker of early renal impairment but also it acts as a sign reflecting the changes in the vascular system.[21]

Diagnostic value of NLR in detection of nephropathy

Receiver operating characteristic curve was used to explore the diagnostic value of NLR in the detection of nephropathy (Figure 1). The AUC was 0.736 (95% CI = 0.635-0.837), p < 0.001. The sensitivity and specificity of the test at a cut-off value of NLR = 3.35 was 69% and 89%, respectively.

Table 1: Demographic and clinical characteristics of the patients

Variables	Without Nephropathy (n = 72)	With Nephropathy (n = 58)	p- value
Age, years	58.91 ± 10.12	61.22 ± 11.7	0.218
Sex			
Male	40 (55.56%)	31 (41.89%)	0.810
Female	32 (44.44%)	27 (58.11%)	
BMI, Kg/m^2	27.73 ± 4.34	28.12 ± 4.2	0.481
Duration, years	4.36 ± 2.75	7.41 ± 2.48	<0.001
Smoking			
Yes	14 (19.44%)	12 (25.68%)	0.860
No	58 (80.56%)	46 (74.32%)	
HbA1c%	8.78 ± 1.91	9.94 ± 1.82	0.001
ACR	13.63 ± 5.92	131.32 ± 37.85	<0.001
WBC× 10^3/ml	8.28 ± 2.4	9.45 ± 2.31	0.006
N/L ratio	2.53 ± 0.79	4.23 ± 2.14	<0.001
RDW (%)	13.86 ± 2.66	14.08 ± 1.98	0.109

BMI: body mass index, FBS: fasting blood glucose, HbA1c: glycated hemoglobin. BMI: body mass index, SD: standard deviation, ACR: albumin: creatinine ration, N/L: neutrophil/ lymphocyte, WBC: white blood cell.

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From the diagnostic point of view, leukocytes, in general, are the classic inflammatory markers that are easy to measure.[22] However, measuring the individual type of cells such as lymphocytes, neutrophils, monocytes or even the total leukocyte count cannot be associate with specific disease condition. On the other hand, NLR, comprises a combination of two markers and it is superior to the other leukocyte parameter due to the stability of the ratio compared with an absolute count that may be altered by various physiological conditions.[23]

According to the current study, NLR had good diagnostic value in discrimination of DN from non-DN diabetes. In accordance with these results, Chittawar *et al.*[24] investigated Indian patients to assess the diagnostic value of NLR in the context of discrimination between patients with and without evidence of DN. At cut-off point of NLR = 2.0, the sensitivity and specificity were 86.4% and 69%, respectively, AUC was 0.888 (95% CI: 0.848–0.929; $p < 0.001$). This slight differences in cut-off values between the current study and the Indian study may be related to the different assays used in the measurement of ACR and ethnic differences.

Correlation of NLR with other variables

In patients without evidence of nephropathy, NLR showed a significant positive correlation only with ACR (r = 0.392, p = 0.009). However, in diabetic patients with nephropathy NLR showed a significant correlation with each of disease duration (r = 0.319, p = 0.024), and HbA1c (r = 0.319, p = 0.024), ACR (r = 0.357, p = 0.011) as shown in Table 3.

Thus, there is a significant positive correlation between NLR and ACR diabetic patients with and without DN. In accordance with these results are that obtained by Afsar who investigated 80 newly diagnosed patients with T2DM and found that 4-hour urinary protein excretion was positively correlated with neutrophil count (r = 0.280, p = 0.012), and NLR (r = 5 0.474, p < 0.001) and negatively with lymphocyte count (r= -0.365, p= 0.001). In stepwise linear regression analysis, 24-hour urinary protein excretion was independently associated with NLR ratio (p = 0.041).[25] It obvious that increase neutrophil count over lymphocyte count (high NLR) is associated with a higher tendency towards inflammation, while a low NLR ratio is usually associated with immune response. As neutrophil is the main source of inflammatory mediators like cytokines and reactive oxygen species (ROS)[26], low-grade chronic inflammatory responses associated with high NLR can produce a widespread vascular damage, endothelial dysfunction, increased oxidative stress and increased production of growth factors and cytokines, thereby causing renal damage and increased ACR.

According to the result of the current study, RDW has no diagnostic value in the detection of DN. In accordance with this result is a recent study by Zhang *et al.*[27] who reported that the RDW level was not an independent risk factor for renal outcomes in diabetic retinopathy. However, many other studies have shown that the RDW level was significantly higher in patients with DN than those without DN.[28-29] This discrepancy may be attributed to variation in detection of albuminuria and sample size.

The results of this study should be interpreted based on the study's limitations. First, the sample size was relatively small which reduced the statistical power. Second, the estimation of NLR and the RDW level was only performed once at baseline. Frequent measurement during the follow-up undoubtedly increases the validity of the tests. Finally, therapeutic interventions were not considered in the present study. Such intervention might act as additional confounding factors associated with renal damage.

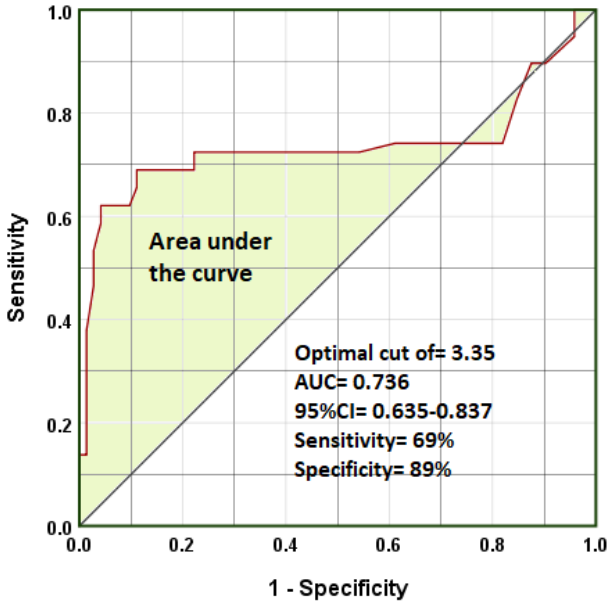

Figure 1: Receiver operating characteristic curve for NLR in the context of discrimination DN from diabetic patients without DN

Table 2: Predictors of diabetic nephropathy

Variables	Without DN (n = 72)	With DN (n = 58)	Univariate analysis		Multivariate analysis	
			p- value	OR (95% CI)	p-value	OR (95% CI)
Duration, y						
<5.5	50 (69.44%)	18 (31%)	<0.001	1.0	0.003	1.0
≥ 5.5	22 (30.56%)	40 (69%)		5.05 (2.39-10.7)		4.43 (1.68-11.68)
HbA1c%						
<9	53 (73.61%)	21 (36.21%)	<0.001	1.0	<0.001	1.0
≥9	19 (26.39%)	37 (63.79%)		4.91 (2.32-10.4)		6.4 (2.32-17.65)
WBC× 10^3/mL						
<8.5	47 (65.28%)	19 (32.76%)	<0.001	1.0	0.372	1.0
≥8.5	25 (34.72%)	39 (67.24%)		3.86 (1.86-8.02)		1.57 (0.58-4.25)
N/L ratio						
<3.5	64 (88.89%)	20 (43.48%)	<0.001	1.0	<0.001	1.0
≥3.5	8 (11.11%)	38 (65.52%)		15.2 (6.1-37.8)		13.75 (1.68-11.68)

DN: diabetic nephropathy, OR: odds ratio, CI: confidence interval, HbA1c: glycated hemoglobin, WBC: white blood cell, N/L: neutrophil/lymphocyte.

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Table 3: Correlation of NLR with other quantitative variables

Variable	Without DN		With DN	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Age, years	-0.095	0.511	0.77	0.594
BMI, Kg/m^2	-0.105	0.469	-0.157	0.296
Duration, years	0.293	0.072	0.355	0.011
HbA1c%	0.239	0.085	0.319	0.024
ACR	0.392	0.009	0.357	0.011
WBC $x10^3$/mL	0.141	0,329	0.267	0.097
RDW (%)	0.199	0.167	0.11	0.446

DN: diabetic nephropathy, BMI: body mass index, HbA1c: glycated hemoglobin, ACR: albumin-creatinine ratio, WBC: white blood cell, RDW: red cell distribution width

Conclusion

The present data suggest that NLR but not RDW is an independent factor that is associated with the development of DN in diabetic patients. Besides the classical tests such as eGFR and ACR, NLR could be a simple cheap and easy tool for regular screening of T2DM patients for development of DN.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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