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Original Research Article

Neutrophil to lymphocyte ratio and red blood cell distribution width as predictors of microalbuminuria in type 2 diabetes mellitus

Sagar Vekariya[1], Arun Bahulikar[1], Deepak Phalgune[2]*, Nandkumar Beke[1]

[1]Department of Medicine, Poona Hospital & Research Centre, Pune, Maharashtra, India
[2]Department of Research, Poona Hospital & Research Centre, Pune, Maharashtra, India

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***Correspondence:**
Dr. Deepak Phalgune,
E-mail: dphalgune@gmail.com

ABSTRACT

Background: Diabetic nephropathy (DN) is a common and feared complication of diabetes mellitus, linked to higher morbidity and mortality rates. Very few studies have assessed the possible clinical value of biomarkers such as neutrophil-to-lymphocyte ratio (NLR) and red blood cell distribution width (RDW) in DN in type 2 diabetes mellitus (T2DM) patients. The present cross-sectional observational study aimed to find the correlation of NLR and RDW with microalbuminuria in T2DM patients.

Methods: One hundred and sixty-nine patients aged ≥40 years of either sex, with duration T2DM >4 years, and diagnosed according to the American Diabetes Association criteria, were included in this cross-sectional observational study. NLR, RDW and urine microalbumin were noted. The primary outcome measures were to find the correlation between NLR and RDW with microalbuminuria, whereas the secondary outcome measures were to study the predictive value of NLR and RDW for microalbuminuria in T2DM patients.

Results: There was a significant positive correlation between NLR and microalbuminuria (r=0.165) and a significant negative correlation between RDW and microalbuminuria (r=-0.159). The mean serum albumin and NLR were significantly higher in patients having microalbuminuria. The sensitivity of NLR and RDW to predict microalbuminuria was 82.4% and 64.7% respectively.

Conclusions: NLR and RDW significantly correlated with microalbuminuria and have good sensitivity for microalbuminuria in T2DM patients. NLR and RDW are cheap and inexpensive methods for detecting DN.

Keywords: Correlation, Diabetic nephropathy, Neutrophil-to-lymphocyte ratio, Red blood cell distribution width

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide, and so are the disease-associated complications.[1] Approximately 540 million adults (one in 11) are living with diabetes worldwide; 20% of these develop diabetic nephropathy (DN), making it a global health concern.[2] The International Diabetes Federation (IDF) estimates that the prevalence of diabetes mellitus will reach 783 million (one in every eight adults) by 2045.[3] According to a report by the National Noncommunicable Diseases Monitoring Survey (NNMS), diabetes prevalence in India stood at 9.3% in 2018.[4] It was reported that the global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.[5]

T2DM is associated with microvascular issues like retinopathy, neuropathy, and nephropathy, as well as macrovascular complications such as myocardial infarction, stroke, and peripheral arterial disease. DN is a common and feared complication of diabetes mellitus

(DM), linked to higher morbidity and mortality rates.[6] It is also one of the leading causes of end-stage renal disease (ESRD) and the need for renal replacement therapy.[7] Microalbuminuria refers to the presence of small amounts of albumin in the urine. It is a marker of kidney damage in both insulin-dependent and non-insulin-dependent diabetics, serving as an early indicator of nephropathy.[8] Early identification and management of microalbuminuria can potentially reduce the risk of developing renal complications, making it a crucial aspect of comprehensive diabetes care. The American Diabetes Association (ADA) has recommended that T2DM patients be tested for albuminuria at the time of initial diagnosis and yearly thereafter.[9]

The neutrophil-to-lymphocyte ratio (NLR) is a reliable biomarker of low-grade inflammation in various clinical conditions such as hypertension, metabolic syndrome, obesity, and lifestyle changes.[10] NLR represents neutrophils and lymphocytes, the two components of chronic inflammatory conditions. A high neutrophil value is a marker of the ongoing, destructive, nonspecific inflammatory process.[11] Red blood cell distribution width (RDW) is a measure of the range of variation of red blood cell volume that is also included in blood tests as part of the complete blood count. Studies showed that RDW might be considered an effective predictive index in the evaluation of DN or diabetes-associated complications.[12]

Previous literature has observed that high levels of NLR and RDW were found in DM patients with microalbuminuria. It seems that the primary cause of the elevations in NLR and RDW levels may be the presence of microalbuminuria and not the level of DM control. Moreover, NLR and RDW have a mild-to-moderate positive predictive value for microalbuminuria in T2DM patients.[13] These parameters are strong inflammatory markers and can be easily measured, providing rapid results and inexpensive costs, which increases the interest in studies related to these parameters. Very few studies have assessed the possible clinical value of these two markers of DN in T2DM patients. The present cross-sectional observational study was conducted to find the correlation of NLR and RDW with microalbuminuria in T2DM patients.

METHODS

This hospital-based, cross-sectional, observational study was conducted between February 2023 and January 2024 at Poona Hospital and Research Centre, a tertiary care hospital in Pune, India. After approval from the institutional ethics committee (Letter #ADM/ 2022-2023/410), written informed consent was obtained from all the patients. A total of 169 patients aged ≥40 years of either sex, with duration T2DM >four years, and diagnosed according to ADA criteria (HbA1C ≥6.5% /fasting glucose ≥126 mg/dl/ Two-hour glucose ≥200 mg/dl (after 75 gm of anhydrous glucose)/ Random glucose ≥200 mg/dl) were included.[14] Patients with acute infection or inflammation, male patients with haemoglobin (Hb) <12 mg/dl and females with Hb <10 mg/dl, hemoglobinopathies, white blood cell count (<4000 or >11000 cells/µl), chronic kidney injury, ESRD and pregnant women were excluded. A sample size of 169 patients was calculated by a formula, n[15] Ref $=[(Z\alpha+Z\beta)/C]^2 + 3$, where $C = 0.5*\ln[(1+r)/(1-r)]$, $Z\alpha$ is a standard normal variate (at 1% type 1 error ($p<0.05$) it is 1.96, $Z\beta$ is the standard normal deviate for (at β power 80% at type II error) it is 0.842. Assulyn et al reported a correlation between NLR and microalbuminuria as r=0.2146.[13]

Once the patients were enrolled, a thorough history and clinical examination were conducted as per a pre-tested study proforma. A 24-hour urine sample was collected as per the standard protocol. A container containing toluene was provided to the patient. A 24-hour urine protein was tested starting from any time of day. Patients were told that before starting collection void the bladder, discard urine and then start collection. Patients were instructed to take the container to the laboratory within 12 hours after 24 hours of collecting urine in a container.

Demographic, clinical, and laboratory data from patients regarding plasma glucose, HbA1c, serum creatinine, and serum albumin were recorded. Body mass index (BMI) and blood pressure values were recorded. Urine protein was estimated by the biuret method. (Machine: Dimension® EXL™ 200 Integrated Chemistry System, Siemens, Georgia). NLR and RDW were noted by a machine automated cell counter – system XN 1000/XN 330. Urine microalbumin was assessed by the bromocresol purple method (Machine: Dimension® EXL™ 200 Integrated Chemistry System, Siemens, Georgia). HbA1C was assessed by high-performance liquid chromatography (machine: Bio-Rad D-10, BIO-RAD, California). The procedures followed the guidelines laid down in the Declaration of Helsinki.

The primary outcome measures were to find the correlation between NLR and RDW with microalbuminuria, whereas the secondary outcome measures were to study the predictive value of NLR and RDW for microalbuminuria in T2DM patients.

Statistical analysis

Data collected was entered in Excel 2007, and data analysis was done using Statistical Package for Social Sciences for Windows, Version 20.0 from IBM Corporation, Armonk, NY, USA. The data on categorical variables were shown as N (% of cases), and the data on continuous variables were presented as mean and standard deviation (SD). The continuous variables were tested using an unpaired t-test. Receiver-operating characteristic (ROC) curve analysis was performed. The diagnostic efficacy indices, such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy, were determined. Pearson's correlation was

used to find the correlation. The confidence limit for significance was fixed at a 95% level with p value <0.05.

RESULTS

Table 1 depicts the demographic and clinical profile of the study participants. Table 2 shows that the mean serum albumin and NLR were significantly higher in patients with microalbuminuria. The mean age, duration of DM, BMI, HbA1C, mean arterial pressure (MAP), serum creatinine, and RDW were comparable in patients with and without microalbuminuria.

Table 1: Demographic and clinical profile of the study participants.

Variables	N (%)
Mean age in years ±SD	59.2±11.8
Gender	
Males	113 (66.9)
Females	56 (33.1)
The mean duration of DM ±SD in years	8.9±2.7
The mean BMI ±SD in kg/m^2	26.0±3.4
Urine microalbuminuria	
Present	17 (10.1)
Absent	152 (89.9)

SD: Standard deviation, DM: Diabetes mellitus, BMI: Body mass index.

Table 2: Association of microalbuminuria with various factors.

Variables	Microalbuminuria		P value
	Present	Absent	
Mean age ±SD in years	62.2±13.6	58.8±11.5	0.265
Mean duration of DM ±SD in years	9.2±3.0	8.9±2.7	0.628
Mean BMI±SD kg/m^2	26.8±3.7	25.9±3.4	0.312
Mean HbA1C ±SD	7.3±0.8	7.3±0.9	0.96
Mean MAP±SD	94.7±3.4	94.6±3.3	0.869
Mean serum albumin ±SD mg/dl	222.4±72.2	6.4±1.8	0.001
Mean serum creatinine ±SD mg/dl	1.00±0.26	1.03±0.27	0.149
Mean NLR±SD	4.00±1.6	2.6±1.1	0.032
Mean RDW±SD	14.9±2.6	14.2±2.2	0.188

An unpaired t-test was used, SD: Standard deviation, DM: Diabetes mellitus, BMI: Body mass index, MAP: Mean arterial pressure, NLR: Neutrophil-to-lymphocyte ratio, RDW: Red blood cell distribution width.

There was a significant positive correlation between NLR and microalbuminuria (r=0.165) whereas there was a significant negative correlation between RDW and microalbuminuria (r=-0.159). Table 3 depicts the sensitivity, specificity, PPV, NPV, and accuracy of NLR and RDW in predicting microalbuminuria. Figure 1 shows the ROC analysis of NLR for microalbuminuria [Area under curve (AUC) =0.657], whereas Figure 2 shows the ROC analysis of RDW for microalbuminuria (AUC=0.585).

Table 3: Diagnostic accuracy of neutrophil-to-lymphocyte ratio and red blood cell distribution width to predict microalbuminuria.

	Sensitivity	Specificity	PPV	NPV	Accuracy
NLR	82.4	10.5	9.3	84.2	17.8
RWD	64.7	43.5	23.9	95.1	75.7

NLR: Neutrophil-to-lymphocyte ratio, RWD: Red blood cell distribution width, PPV: Positive predictive value, NPV: Negative predictive value.

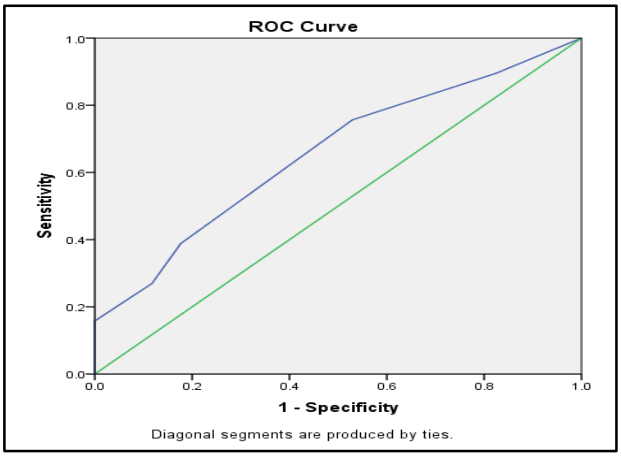

Figure 1: Receiving operative characteristics analysis of neutrophil-to-lymphocyte ratio for microalbuminuria.

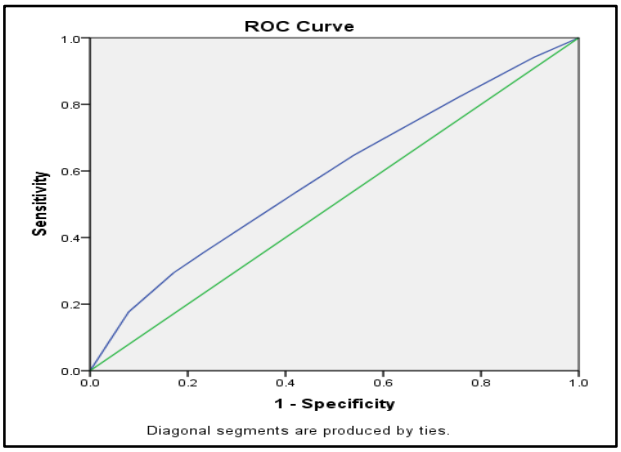

Figure 2: Receiving operative characteristics analysis of red blood cell distribution width for microalbuminuria.

DISCUSSION

The present cross-sectional observational study was conducted to find the correlation of NLR and RDW with microalbuminuria in T2DM patients. DN is a common severe complication in patients with DM, but its exact pathogenesis remains unclear.[16] Although microalbuminuria is a strong marker for DN diagnosis and progression, glomerular damage is considered as early sign of DN and precedes the appearance of microalbuminuria.[17] It is known that a cascade of pathological events (glomerular damage gives rise to proteinuria, followed by progressive renal damage, fibrosis, inflammation, and finally loss of functional nephrons) is involved in the development and progression of DN. Accumulated evidence has demonstrated that chronic inflammation plays a key role in the development of DM-associated complications.[18] Several studies have associated DN with chronic inflammation, as various inflammatory molecules such as adipokines, chemokines, adhesion molecules, and cytokines could contribute to the development of DN.[19]

White blood cell counts and their subtypes have been extensively considered as inflammation markers, where neutrophilia and relative lymphocytopenia are independent markers of many diseases, including DM complications such as DN.[20] However, DN diagnosis based on white blood cell, neutrophil, or lymphocyte counts has biases. NLR, a readily available and cheap index calculated by blood routine examination has been considered a novel inflammatory biomarker reflecting both adaptive immune response (mediated by lymphocytes) and innate immune response (mediated by neutrophils) and its stability is better and less influenced by physiological and pathological status.[18] Thus, evaluating the associations between the NLR level and different diabetic complications is important

In the present study, there was no significant association between mean age and microalbuminuria. Ozler et al, and Jaaban et al reported that the mean age was significantly higher in patients with microalbuminuria, whereas Assulyn et al and Tutan et al observed that there was no significant association between mean age and microalbuminuria.[13,21-23] It was observed in our study that no significant association was found between the duration of DM and microalbuminuria. Assulyn et al and Jaaban et al reported that the duration of DM was significantly higher in patients with microalbuminuria.[13,22] In the present study, no significant association was found between BMI and microalbuminuria in type II DM patients. Assulyn et al and Jaaban et al noted similar observations in their studies.[13,22] In our study, no significant association was found between HBA1C and microalbuminuria. This is similar to the studies of Ozler et al, and Tutan et al.[21,23] However, Assulyn et al and Jaaban et al.[13,22] observed that HbA1C was significantly higher in patients with albuminuria. It was observed in the present study that no significant association was found between MAP and microalbuminuria. Similar findings were observed by Assulyn et al.[13] It was observed in our study that no significant association was found between serum albumin and serum creatinine levels with microalbuminuria. This is similar to the study conducted by Tutan et al whereas Assulyn et al, Ozler et al and Jaaban et al reported that serum creatinine was significantly higher in patients with microalbuminuria.[13,21-23] A significant increase in serum creatinine levels, parallel to albuminuria, may indicate an alteration in kidney function. In the present study, the mean serum albumin and NLR were significantly higher in patients having microalbuminuria. Assulyn et al and Jaaban et al observed a statistically significant association between NLR and microalbuminuria.[13,22] Assulyn et al stated that there was a significant association between serum albumin and microalbuminuria.[13] On the contrary, Jaaban et al reported that there was no significant association between serum albumin and microalbuminuria.[22]

The power of the NLR value as an inflammatory factor stems from both a reduction in the lymphocyte counts and an increase in the neutrophil count. Neutrophils rapidly respond to inflammatory stimuli and increase their number in circulation, and there is an increased expression of activation markers like CD11b/CD18 on monocytes and neutrophils in T2DM patients, resulting in increased neutrophil adhesiveness to the endothelium, independent of fasting glucose levels.

Our study's ROC analysis of NLR for microalbuminuria displayed an AUC of 0.657, whereas the ROC analysis of RDW for microalbuminuria showed an AUC of 0.585. In the present research, the sensitivity, specificity, PPV and NPV of NLR for microalbumin were 82.4%, 10.5%, 9.3% and 84.2%, respectively. The sensitivity, specificity, PPV and NPV of RDW for microalbumin were 64.7%, 43.5%, 23.9% and 95.1%, respectively. A study conducted in an outpatient clinic at Ziv Medical Center, Safed, and Galilee Medical Center, Nahariya, Israel, during the years 2014-2017 by Assulyn et al. observed ROC curve analysis of inflammatory markers and microalbuminuria prediction showed an AUC of 0.675 for NLR (CI 0.58-0.76, p-value = 0.001) and 0.614 for RDW (CI 0.52-0.70, P = 0.013). The study further reported that the NLR value of 2.54 has 39.7% sensitivity, 78.8% specificity, and 45% PPV, whereas the RDW value of 14.44 has 37.9% sensitivity, 76% specificity, and 41.5% PPV.[13] A study conducted from November 2020 to October 2021 in a tertiary care center in Eastern Odisha by Chollangi et al reported the ROC curve analysis of inflammatory markers and microalbuminuria prediction showed an AUC of 0.69 for NLR and 0.61 for RDW.[24] A study conducted at Al-Assad University Hospital, Damascus, Syria by Jaaban et al observed an ROC curve analysis of inflammatory markers and microalbuminuria prediction demonstrated an AUC of 0.869 for NLR (confidence interval: 0.813–0.926, p value =0.001) and NLR cutoff point of 2.2 has 72.0% sensitivity and 78.0% specificity.[22] A study conducted at the University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital, Istanbul, Turkey by Ozler

et al. concluded that NLR may have prognostic and predictive values for the development and monitoring of DM and complications such as DN and that it is cheap, easily integrated into daily practice, and reliable parameters reinforce their predictive value.[21] A study conducted at Erol Olçok Training and Research Hospital, Turkey, by Tutan et al observed that in the ROC analysis performed to find the cut-off values, 1.93 of NLR had 57.4% sensitivity, 68.5% specificity, 47.3% PPV, 76.5% NPV, and 64.8% test accuracy. The AUC was 0.625 [CI 0.558-0.691] with p value = 0.001. NLR of 1.93 and above had an odds ratio of 2.93 with p value =0.001.[23]

In the present study, there was a significant positive correlation between NLR and microalbuminuria (r=0.165), whereas there was a significant negative correlation between RDW and microalbuminuria (r=-0.159). Assulyn et al. stated a Pearson correlation between NLR and microalbumin was = 0.214 with p value=0.003.[13] A study conducted at the University General AHEPA Hospital of Thessaloniki, Greece by Roumeliotis et al. reported that Spearman's rho analysis showed that RDW correlated with albuminuria (r=−0.17, p value =0.03) and NLR correlated with microalbuminuria (r=−0.06, p value =0.47).[25] Chollangi et al reported that NLR and RDW have a positive correlation with microalbuminuria.[24]

Limitations

Firstly, due to the cross-sectional, observational design, no causality can be established, and our results should be interpreted with caution. Secondly, we cannot draw any definite conclusions regarding the mechanisms underlying the associations that were found, and thirdly, the single center design and the fact that our study did not explore other potential ratios or markers that may be relevant to proteinuria estimation in diabetic patients. Another limitation is the lack of association with relevant outcome data. Moreover, NLR is influenced by genetic and non-genetic factors (sex, age, seasonal conditions, lifestyle and diseases).[26] However, our study showed that information derived from a very simple, cheap and quick examination, the full blood count, might give a rough idea regarding the status of DN. Large-scale prospective longitudinal studies are needed to assess the prognostic significance of NLR and RDW and their ability to predict microalbuminuria and other diabetic complications, as well as to formulate a systematic approach that would validate sufficient sensitivity and specificity and encompass the wide variety of diabetic and other medical complications.

CONCLUSION

There was a significant positive correlation between NLR and microalbuminuria (r=0.165) whereas there was a significant negative correlation between RDW and microalbuminuria (r=-0.159). The mean serum albumin and NLR were significantly higher in patients having microalbuminuria, but there was no significant association between RDW with microalbuminuria. The sensitivity of NLR and RDW to predict microalbuminuria was 82.4% and 64.7 respectively.

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