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Original Article

Internal Medicine Section

A Cross-sectional Study to Assess Neutrophil Lymphocyte Ratio as a Predictor of Microvascular Complications in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Introduction: Neutrophil Lymphocyte Ratio (NLR) is a novel marker of chronic inflammation that exhibits a balance of two interdependent components of the immune system and can be used to predict microvascular complications in diabetes.

Aim: To study the NLR in type 2 diabetes patients and its association with microvascular complications in these patients.

Materials and Methods: This was a cross-sectional study conducted over 18 months at Safdarjung Hospital, New Delhi. Eighty patients of type 2 Diabetes Mellitus (DM) were selected for the study. Detailed history was taken including duration of DM, symptoms suggestive of any complications or co-morbidity and treatment history. Physical examination (height and weight) and systemic examination was performed to check for presence of any diabetic complications. NLR was obtained from the complete blood counts by dividing the absolute number of neutrophils to the absolute number of lymphocytes. Data recorded were analysed using Statistical Package for Social Sciences (SPSS) software version 21.0 Logistic regression analysis and Receiver Operating Curve (ROC) curve were used.

Results: Total 38.7% (31) of the patients had high NLR, with mean±Standard Deviation (SD) of NLR being 3.29±1.49. There was a statistically significant association between poorly controlled diabetes HbA1C >7%, high-sensitivity C-Reactive Protein (hs-CRP) levels and NLR. NLR was found to be the best predictor of diabetic neuropathy and diabetic nephropathy. NLR was also significantly higher in DM patient having more than one microvascular complication.

Conclusion: NLR can be used as a simple parameter to predict presence of diabetic microvascular complications. Thus, an early diagnosis and subsequently targeting inflammatory pathways could possibly be a component of the strategies to prevent and control diabetes related complications.

Keywords: Inflammation, Nephropathy, Neuropathy

INTRODUCTION

India is home to 69.1 million people with DM and is estimated to have the second highest number of cases of DM in the world after China [1]. Type 2 DM due to insulin resistance and an inadequate compensatory insulin secretion, accounts for 90% of all the diabetic cases globally [2]. The microvascular complications of diabetes are retinopathy, nephropathy and neuropathy. Although chronic hyperglycaemia is an important etiological factor leading to complications of DM, the mechanism by which it leads to such diverse cellular and organ dysfunction is still unknown [3]. Glycated proteins cause damage to cells and impair their function, which induces the production of inflammatory cytokines like CRP, Tumour Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6) and free radicals. Activation of inflammatory processes appear to be one of the major mechanisms responsible for vascular damage leading to clinically well recognised complications of DM [4].

Number of leukocytes in the circulation change during the inflammatory response; neutrophilia is associated with relative lymphopenia [5]. An index has thus been subsequently generated to reflect both neutrophilia, which accompanies the acute state of inflammation, and lymphopenia, which is a response to physiological stress. This index, which is the NLR, has been demonstrated to be a reliable indicator of the inflammatory status [6]. NLR is a novel marker of chronic inflammation that exhibits a balance of two interdependent components of the immune system; neutrophils, which mediate the first line of inflammatory defense, whereas lymphocytes are the regulatory and protective component of inflammation [7]. NLR has proven its usefulness in the stratification of mortality in major cardiac events and as a strong prognostic factor in several types of cancers [8,9].

Recent studies have shown that NLR was significantly higher in type 2 DM patients with microvascular complications as compared to those without these complications [10-12]. Thus, due to its easy availability and inexpensive method of determination, NLR is emerging as a simple parameter to assess inflammatory status of a patient as compared to estimation of IL-1, IL-6, TNF-α, etc., which are expensive and cumbersome. Most of the studies have shown NLR association with individual microvascular complications in diabetes and also have not taken into account the cumulative effect of all the three microvascular complications. Thus, this study has been undertaken to evaluate NLR and its correclation with microvascular complications in type 2 diabetic patients.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of 18 months from November 2017 to April 2019 at Safdarjung Hospital, New Delhi, India. Eighty patients of type 2 DM who fulfilled the inclusion criteria were selected for the study.

Consent was taken from the patients after mentioning both verbally and in writing the purpose of the study, the study procedure, maintaining their confidentiality and their right to refuse participation and/or withdrawal from the study at any stage. Institutional Ethics Committee clearance was also obtained (IEC No.- 2017-099).

Sample size calculation: Relation of NLR to microvascular complications of DM was studied by Srinivas B et al., [13]. The study observed the sensitivity and specificity of NLR for assessment of microvascular complication to be 71.43% and 78.95%, respectively and out of 40 cases, 21 cases had microvascular complications.

Taking these values as reference, the minimum required sample size with desired precision of 15% and 5% level of significance was 67 patients. To reduce margin of error, total sample size taken was 80.

Inclusion criteria: Patients with type 2 DM, both male and female, above the age of 30 years were selected for the study.

Exclusion criteria: Type 2 DM patients with any of the following conditions: history of any infectious disease within the past one week; any non infectious immunological disease; significant smoking history; Body Mass Index (BMI) >30 kg/m^2; coronary artery disease; heart failure; malignancy; pregnancy were excluded from the study.

Study Procedure

Detailed history was taken including duration of DM, symptoms suggestive of any complications or co-morbidity and complete treatment history. Complete physical examination was done including measurement of height, weight, BMI and detailed systemic examination was performed for the presence of any diabetic complications. Criteria for diagnosing DM in the study was Fasting Plasma Glucose of ≥7 mmol/L (126 mg/dL) or two hour post prandial blood glucose ≥11.1 mmol/L (200 mg/dL) or Glycosylated haemoglobin (HbA1c) ≥6.5% [3]. In these patients, a random spot urine test with protein creatinine ratio of more than 30 unit on two or more occasions three months apart was defined as diabetic nephropathy [3]. For suspected diabetic neuropathy, 10 gm monofilament test was performed to check for sensation and any abnormality was confirmed with Nerve Conduction Velocity (NCV) test [14]. Diabetic retinopathy was screened by fundus examination after dilation with a mydriatic and classified into Non Proliferative Diabetic Retinopathy (NPDR), Proliferative Diabetic Retinopathy (PDR) and macular oedema [15]. Data were recorded in a proforma designed for the study. Routine investigations were performed for these patients - fasting blood sugar levels, postprandial blood sugar levels, HbA1c was measured using A1C Now+®, kidney function tests, liver function tests, serum lipid profile, highly sensitive C-Reactive Protein (Hs-CRP), urine routine and microscopy, urinary albumin excretion was determined using MICRAL-TEST®, fundus examination, electrocardiogram, chest X-ray, NCV (whenever indicated).

Complete blood count with Erythrocyte Sedimentation Rate (ESR), including haemoglobin, total leukocyte count, Differential Leukocyte Count (DLC) and platelet count were measured using automated analyser Sysmex XT-2000i.

The NLR was obtained from the complete blood counts by dividing the absolute number of neutrophils to the absolute number of lymphocytes. A cut-off NLR value of more than 3.53 was considered to be high [16].

STATISTICAL ANALYSIS

The data was entered in Microsoft excel spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented as number and percentage (%) and continuous variables were presented as mean±SD and median. Normality of data was tested by Kolmogorov-Smirnov test. The normality was rejected and non parametric tests were used. Quantitative variables were compared using Independent t-test/Mann-Whitney Test between the two groups. Qualitative variables were correclated using Chi-Square test/Fisher's-Exact test. Spearman rank correlation coefficient was used to assess the association of various parameters with NLR. Receiver operating characteristic curve was used to find out cut-off point of parameters for predicting various microvascular complications. A p-value of ≤0.05 was considered statistically significant.

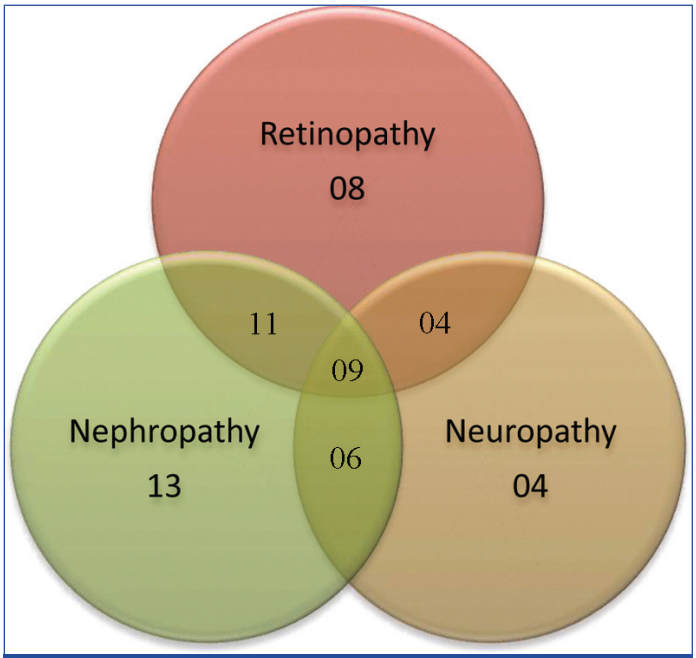
RESULTS

Amongst 80 patients, 47 (58.8%) were male and 33 (41.2%) were female. Minimum age of study population was 37 years and maximum was 84 years. Mean age (SD) was 59.89 (10.9) years. Mean (SD) duration of diabetes was 8.62 (5.33) years, 54 (67.5%) patients had poor glycaemic control (HbA1c >7%), mean (SD) HbA1c was 7.1% (1.1%). Total 45% patients had high hs-CRP >3 mg/L indicating high state of inflammation. The mean (SD) hs-CRP value was 5.135 mg/L (4.46 mg/L).

Microvascular complications were found in 68.75% of patients [Table/Fig-1]. Distribution of microvascular complications in study subjects is demonstrated in [Table/Fig-2]. Out of 39 patients having nephropathy, 30 had microalbuminuria and 9 had macroalbuminuria. NPDR was present in 15 patients, 9 patients had NPDR with macular oedema, while 8 patients had PDR. Out of 23 patients with diabetic neuropathy, 5 had autonomic neuropathy while the rest had polyneuropathy. A total of 38.7% patients had high NLR [Table/Fig-3]. Mean NLR was 3.29±1.49. There was a statistically significant association between poorly controlled diabetes HbA1C >7% and high NLR (p-value of 0.012). A significant positive correlation was also found between high NLR levels and high HsCRP levels (p-value 0.01). There was no correlation between high NLR with age of these patients and duration of diabetes. High NLR was significantly associated with diabetic nephropathy [Table/Fig-4] and neuropathy [Table/Fig-5], but was not associated with retinopathy [Table/Fig-6]. There was a statistically significant association of high NLR with the presence of all three microvascular complications together in these diabetic patients [Table/Fig-7].

Microvascular complications	No. of study subjects	Percentage (%)
No	25	31.25
Yes	55	68.75
Total	80	100.00

[Table/Fig-1]: Microvascular complications of study subjects.



[Table/Fig-2]: Distribution of microvascular complications in study subjects.

NLR	No. of study subjects	Percentage (%)
High	31	38.75
Normal	49	61.25
Total	80	100.00

[Table/Fig-3]: Neutrophil lymphocyte ratio of study subjects.

Logistic regression analysis revealed NLR to be the best predictor of diabetic neuropathy followed by diabetic nephropathy [Table/Fig-8]. The values of area under curve derived from ROC curve for, diabetic nephropathy [Table/Fig-9], diabetic retinopathy [Table/Fig-10], and diabetic neuropathy [Table/Fig-11] were 0.668 (95% CI of

Diabetics with nephropathy	NLR		Total	p-value
	High	Normal		
No	10 (32.26%)	31 (63.27%)	41 (51.25%)	0.007
Yes	21 (67.74%)	18 (36.73%)	39 (48.75%)	
Total	31 (100.00%)	49 (100.00%)	80 (100.00%)	

[Table/Fig-4]: NLR in patients with diabetic nephropathy.
p-value was calculated using chi square test

Diabetics with neuropathy	NLR		Total	p-value
	High	Normal		
No	16 (51.61%)	41 (83.67%)	57 (71.25%)	0.002
Yes	15 (48.39%)	08 (16.33%)	23 (28.75%)	
Total	31 (100.00%)	49 (100.00%)	80 (100.00%)	

[Table/Fig-5]: NLR in patients with diabetic neuropathy.
p-value was calculated using chi square test

Diabetics with retinopathy	NLR		Total	p-value
	High	Normal		
No	15 (48.39%)	33 (67.35%)	48 (60.00%)	0.09
Yes	16 (51.61%)	16 (32.65%)	32 (40.00%)	
Total	31 (100.00%)	49 (100.00%)	80 (100.00%)	

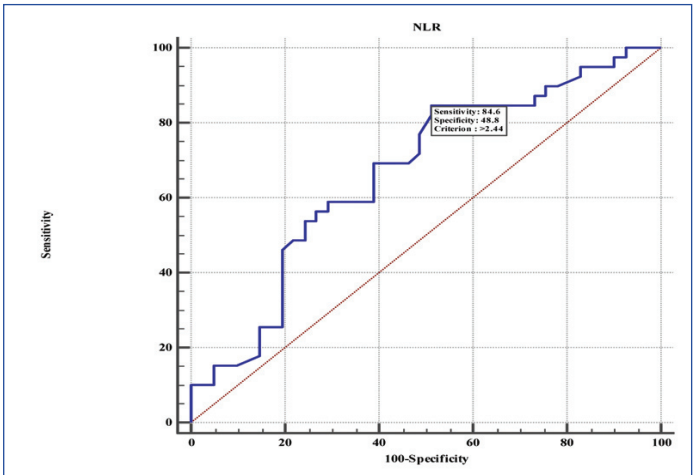
[Table/Fig-6]: NLR in patients with diabetic retinopathy.
p-value was calculated using chi square test

Diabetics with neuropathy, retinopathy and nephropathy	NLR		Total	p-value
	High	Normal		
No	24 (77.42%)	47 (95.92%)	71 (88.75%)	0.024
Yes	07 (22.58%)	02 (4.08%)	09 (11.25%)	
Total	31 (100.00%)	49 (100.00%)	80 (100.00%)	

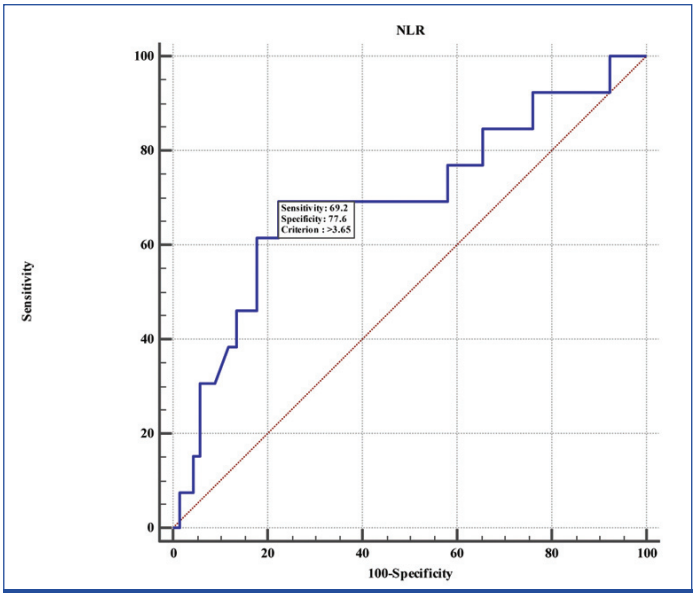
[Table/Fig-7]: NLR in patients with diabetic retinopathy, neuropathy and nephropathy.
p-value was calculated using chi square test

Variables	B	S.E	p-value	Odds ratio	95% CI for odds ratio	
					Lower	Upper
Diabetic nephropathy						
NLR	0.410	0.175	0.019	1.507	1.070	2.124
Diabetic retinopathy						
NLR	0.160	0.155	0.303	1.173	0.866	1.589
Diabetic neuropathy						
NLR	0.627	0.195	0.001	1.871	1.276	2.744

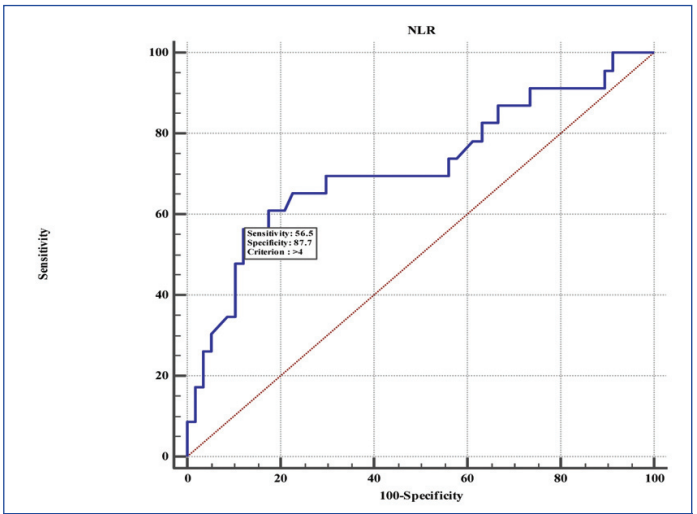
[Table/Fig-8]: Logistic regression analysis of NLR with diabetic nephropathy, retinopathy and neuropathy.
Where B stands for unstandarised beta and represents the slope between predictor; dependent variable predictor is the NLR; dependent variable is the individual microvascular complications; SE stands for Standard error of the regression (represents how much the variable is spread from the regression line)



[Table/Fig-9]: ROC curve predicting diabetic nephropathy with NLR.
A NLR cut-off value of 2.44 had a sensitivity and specificity of 84.62% and 48.78%, respectively, in predicting diabetic nephropathy



[Table/Fig-10]: ROC curve predicting diabetic retinopathy with NLR.
For Diabetic retinopathy, NLR had an area under the receiver operating characteristic curve of 0.578 (95% CI: 0.462 to 0.687; p-0.2475). A NLR cut off value of 3.65 had a sensitivity and specificity of 69.24% and 77.61%, respectively, in predicting diabetic retinopathy in our study population



[Table/Fig-11]: ROC curve predicting diabetic neuropathy with NLR.
A NLR cut-off value of 4 had a sensitivity and specificity of 56.52% and 87.72%, respectively, in predicting diabetic neuropathy

0.554 to 0.769, p-value 0.0062), 0.578 (95% CI: 0.462 to 0.687, p-value 0.2475) and 0.718 (95% CI: 0.606 to 0.813, p-value 0.0019), respectively. Thus, it can be seen that the strongest association was seen between NLR and diabetic neuropathy.

DISCUSSION

Hyperglycaemia in diabetes leads to generation of glycated proteins which damage the cells in various ways, including impaired cellular function, and subsequently induces the production of inflammatory cytokines like CRP, TNF-α, IL-6, etc., [3]. This inflammation contributes to development of microvascular complications. NLR is an inexpensive and easily available marker to assess the inflammatory status in these patients. This study was conducted to assess NLR in diabetic patients and its correlation with age, duration of diabetes, HbA1c and microvascular complications. The statistically significant association found between high NLR and high HbA1c could be corroborated with the fact that maintaining a good HbA1c can help in reducing inflammation in diabetes and subsequent microvascular complications.

In this study, out of 39 patients with nephropathy, 53.84% had a high NLR and this correlation was found to be statistically significant. A study by Zhang J et al., found increased NLR to be associated with worsening renal function and corresponded with increased severity of histological lesions in patients with diabetic nephropathy

[17]. Indian studies by Khandare SA et al., and Chittawar S et al., also found high NLR values to be significantly associated with diabetic nephropathy. Inflammatory and oxidative pathways lead to intraglomerular haemodynamic abnormalities, alteration of extracellular matrix and glomerular basement membrane ultimately leading to development of diabetic nephropathy [12,18].

Amongst 32 patients with diabetic retinopathy 50% had a high NLR. No statistically significant association was found between diabetic retinopathy and high NLR in the study. Similarly, a study conducted by Ciray H et al., also showed no significant association of high NLR between diabetics having retinopathy and those without retinopathy [19]. In contrast Ulu SM et al., demonstrated NLR to be a quick and reliable prognostic marker for diabetic retinopathy and its severity [20]. The exact reason behind the contrasting findings in these studies are not known but may be due to variations in baseline glycaemic status of the study population.

In this study, out of 23 diabetics with neuropathy, 65.21% had a high NLR. A highly significant correlation was found between high NLR and diabetic neuropathy. Moursy EY et al., also found a significant association between high NLR and diabetic neuropathy [10]. A study by Xu T et al., showed that diabetic peripheral neuropathy was significantly associated with high NLR [21]. Ischemic reperfusion injury induced inflammatory response in diabetic nerves is believed to be responsible for the pathogenesis of diabetic neuropathy [12]. In diabetics having all the three microvascular complications, 77.77% of 9 patients had a high NLR and this association was also found to be statistically significant. From regression analysis, high NLR was found to be the best predictor for diabetic neuropathy followed by diabetic nephropathy.

Limitation(s)

This study is limited by the fact that it was cross-sectional without any follow-up of the study subjects.

CONCLUSION(S)

It is clear that high NLR was associated with microvascular complications in type 2 DM patients and can be used as a reliable predictor. It can be a cheaper and simpler alternative to IL-6, TNF-α and Hs-CRP for assessing inflammation in diabetic patients. To the best of our knowledge this is the first Indian study to find NLR as an independent predictor of diabetic neuropathy and also to study the correlation of NLR in diabetics having more than one microvascular complications.

Based on the findings of this study, NLR offers a relatively simpler tool for predicting future development of microvascular complications in diabetes patients. Such patients can be screened more extensively and frequently. Early diagnosis and subsequently targeting inflammatory pathways could possibly be a component of the strategies to prevent and control diabetes related complications.

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