

# To study association of Neutrophil- Lymphocyte ratio with vascular complications in Type-2 Diabetes



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## ABSTRACT

**Background:** Diabetes is a pro-thrombotic state associated with increased risk of atherosclerosis and inflammation. Neutrophil lymphocyte ratio (NLR) provides information about early and subclinical inflammation and thus may act as a prognostic marker for vascular complications in type 2 diabetes. **Aims and Objective:** To analyze the correlation between Neutrophil- Lymphocyte ratio in diabetics with and without vascular complications. **Materials and Methods:** A total of 111 patients admitted in Victoria hospital and Bowring & Lady Curzon hospital attached to Bangalore Medical College and Research Institute from NOV 2018 to MAY 2020 were studied. The data was collected according to the proforma in terms of history, clinical examination and the necessary investigations. NLR was observed in type 2 diabetic patients and was compared in those with complications and without complications. **Results:** The NLR was higher in diabetics with vascular complications compared to those without complications,  $2.8 \pm 0.7$  fl versus  $6.8 \pm 3.1$  fl ( $P < 0.001$ ), respectively. In this study, Mean N (%), In No Vascular Complications was  $61.7 \pm 10.6$  and with vascular complications was  $79.9 \pm 9.5$ . Mean L (%) in No Vascular Complications was  $23.7 \pm 5.8$ . Mean N (%), In No Vascular Complications was  $61.7 \pm 10.6$  and with vascular complications was  $79.9 \pm 9.5$ . There was a significant difference in mean N (%) mean L (%) and NLR in comparison with respect to Complications. **Conclusion:** This study showed significantly higher NLR in diabetic patients with vascular complications. Hence, NLR can be used as a simple parameter to assess the vascular complications in diabetes.

**Key words:** Neutrophil- Lymphocyte ratio; Type-2 Diabetes; Vascular complications

## INTRODUCTION

Type 2 diabetes mellitus has become a serious threat to global human health because of its vascular complications which are associated with increased disability, frailty and reduced life expectancy, including cardiovascular and cerebrovascular diseases (CVD), diabetic nephropathy, diabetic retinopathy, diabetic foot.<sup>1-3</sup> India is the diabetes capital of the world, with diabetes and pre-diabetes, prevalence of 9% and 11-14%, respectively.<sup>4</sup> This increased burden of diabetes in Indians is a grim precursor of an exponential increase in diabetes-related end-organ

damage and associated morbidity in the next few decades. There is an urgent need for cheap and easy to measure predictors of the occurrence of diabetes related end-organ damage in Indians. This would help in preventing vascular complications in specific individuals to improve long term clinical outcomes.

Chronic inflammation has been considered the potential pathogenesis responsible for the development of diabetic complications.<sup>5-7</sup> Increased white blood count (WBC) is a easily available inflammatory marker, which correlates with several cardiovascular disease risk factors and its sequel with diabetes.<sup>8-11</sup> Apart from WBC count, inflammatory markers

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such as interleukin 1(IL-1), IL-6, IL-8, transforming growth factor  $\beta$ 1, tumor necrosis factor- $\alpha$  have been linked to end organ damage in diabetes.<sup>12-16</sup> Limitations of these markers include lack of availability in routine clinical practice compounded by the associated increased expenses and assay standardization.<sup>17,18</sup> Among multiple parameters complete blood count, neutrophil lymphocyte ratio (NLR) has been studied extensively for the association with cardiovascular risk factors like myocardial infarction, heart failure and stroke.<sup>19,20</sup> NLR stands out as a novel marker of chronic inflammation that reflects a balance between 2 complementary components of the immune system- Neutrophils being the active nonspecific mediator of inflammation, whereas Lymphocytes acting as the protective or regulatory component of inflammation.<sup>21,22</sup> Data are lacking of NLR as a predictor of end-organ damage in Indians with T2DM. This study, therefore, aimed to evaluate the role of NLR has a role in predicting diabetic vascular complications such as retinopathy, neuropathy, nephropathy, diabetic foot, cardiovascular and cerebrovascular complications.

### Aims and objectives

To compare Neutrophil-Lymphocyte ratio in diabetics with and without vascular complications.

## MATERIALS AND METHODS

### Study design

Cross sectional type.

### Study area

The present study was conducted on patients admitted in hospitals affiliated to Bangalore Medical College and Research Institute, Bangalore.

### Inclusion criteria

1. Age >18 years
2. Patient willing to give informed consent
3. Diagnosis as per American diabetes association 2018 guidelines<sup>23</sup>

### Exclusion criteria

1. Patients who did not give informed consent

### Study population

A study population of 111 diabetic patients with and without vascular complications was taken with 56 patients with complications and 55 without complications.

### Study period

November 2018 to May 2020.

### Methodology of data collection

This study was conducted among patients admitted under medicine department between November 2018 to May 2020 at Victoria hospital and Bowring & Lady Curzon hospital, Bangalore Medical College and Research Institute, Bangalore, Karnataka, India. Approval and clearance were obtained from the institutional ethics committee on 15/11/2018-BMCRI/PG/124/2018-19. Information was collected from the patients on their duration of T2DM, treatment history, age and sex. Data were collected on the anthropometric parameters and vitals of the patients (height, weight, BMI), Pulse rate and Blood pressure. Both fasting and post prandial blood samples of 5 ml each were collected in plain vacutainer and processed immediately for routine biochemical analysis. Spot urine sample was collected and looked for presence of albuminuria. Such patients underwent ultrasonography abdomen for the evaluation of the kidney echotexture and size. Digital funduscopy was done to assess diabetic retinopathy. Diabetic retinopathy was diagnosed using The Early Treatment Diabetic Retinopathy Study criteria (ETDRS).<sup>24</sup> Patients who had neuropathic symptoms were clinically examined for diabetic neuropathy. Patients who had symptoms and signs of lower limb ischemia were evaluated with respective limb arterial and venous doppler. Patients were screened and evaluated clinically for macrovascular complications and relevant investigations like ECG, 2D ECHO for cardiovascular complications and neuroimaging for cerebrovascular complications. All relevant investigations were done on out-patient and in-patient basis.

## STATISTICAL ANALYSIS

### Statistical methods

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two quantitative variables. ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups for quantitative data.

**Table 1: Age distribution of subjects in the study**

Age	Count	%
<40 years	17	15.3%
41 to 50 years	27	24.3%
51 to 60 years	27	24.3%
61 to 70 years	25	22.5%
71 to 80 years	11	9.9%
>80 years	4	3.6%

**Table 2: Glycemic Profile of subject's comparison with respect to duration of diabetes**

	Duration of Diabetes										P value
	Newly Detected		<5 years		6 to 10 years		>10 years		Total		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
FBS (mg/dl)	202.77	70.93	242.5	85.35	230.44	74.6	249.41	57.67	233.42	77.12	0.13
PPBS (mg/dl)	265.67	63.67	281.38	78.14	276.29	92.39	285.82	62.58	277.95	78.87	0.55
HbA1c (%)	9.42	1.56	9.86	1.93	9.58	2.1	9.34	2.09	9.63	1.97	0.45

**Table 3: Correlation of NLR with respect to duration of diabetes**

	Duration of Diabetes										P value
	Newly Detected		<5 years		6 to 10 years		>10 years		Total		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
N (%)	68.25	6.89	68.93	12.41	71.85	12.93	62.32	11.93	70.9	12.6	0.035
L (%)	20.92	6.44	18.80	7.84	19.12	9.77	21.66	7.21	19.0	8.1	0.128
NL Ratio	3.71	2.39	4.54	3.40	5.39	4.04	3.08	0.75	4.8	3.0	0.045

### Graphical representation of data

MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram and ROC Curve

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

### Statistical software

MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

## RESULTS

This study was conducted among 111 patients admitted in Victoria hospital and Bowring & Lady Curzon hospital attached to Bangalore Medical College and Research Institute.

### Age distribution

In this study Mean age was  $55.66 \pm 13.675$  years (Table 1).

### Sex distribution

Out of 111 patients 63 patients (56.8%) were Male and 48 patients (43.2%) were Female. In this study male patients were more than female.

### Distribution of cases according to duration of diabetes

In the study, duration of diabetes was newly detected in 16.2%, <5 years in 37.8%, 6 to 10 years in 30.6% and >10 years in 15.3%.

### Glycemic profile of subject's comparison with respect to duration of diabetes

There was no significant correlation of FBS, PPBS and HbA1C with the duration of diabetes (Table 2).

**Table 4: Percentage of complications in diabetic subjects**

Complications	Count	%
No Vascular Complications	55	49.5%
Micro complications	25	22.5%
Macro complications	32	28.8%

### Correlation of NLR with respect to duration of diabetes

There was significant correlation of N (%) and NL ratio with duration of diabetes but L (%) did not show significant correlation with duration of diabetes (Table 3).

### Vascular complications in diabetic subjects

In the study patients had no vascular complications in 49.5%, had micro-vascular complications in 22.5% and macro-vascular complications in 28.8%. Out of which patients had Diabetic retinopathy in 34%, diabetic nephropathy in 34.2%, diabetic neuropathy in 0.9%, cerebrovascular Accident in 17.1%, ischemic heart disease in 12.6%, diabetic foot in 6.3% of the individuals (Table 4).

### NLR comparison with complications and glycemic profile in type 2 diabetics

In the study N (%) was  $70.9 \pm 13.6$ , L (%) was  $19 \pm 8.1$  and NL Ratio was  $4.8 \pm 3$  among subjects involved in the study.

In this study, mean N (%) in no vascular complications was  $61.7 \pm 10.6$ , in micro complications was  $80.6 \pm 9.4$  and in macro complications was  $79.5 \pm 9.7$ . There was a significant difference in mean N (%) comparison with respect to complications.

Mean L (%) in no vascular complications was  $23.7 \pm 5.8$ , in micro complications was  $14.1 \pm 6.9$  and in macro

**Table 5: NLR Comparison with respect to complications**

	Complications						F	P value
	No Vascular Complications		Micro complications		Macro complications			
	Mean	SD	Mean	SD	Mean	SD		
N (%)	61.7	10.6	80.6	9.4	79.5	9.7	45.039	<0.001*
L (%)	23.7	5.8	14.1	6.9	14.8	8.0	25.864	<0.001*
NL Ratio	2.8	0.7	7.1	3.5	6.5	2.7	44.522	<0.001*

**Table 6: Correlation between NLR with Glycemic Profile**

		NLRATIO
NLRATIO	Pearson Correlation	1
	P value	
FBS (mg/dl)	N	111
	Pearson Correlation	0.38
	P value	.000039
PPBS (mg/dl)	N	111
	Pearson Correlation	0.49
	P value	<.00001
HBA1C (%)	N	111
	Pearson Correlation	0.29
	P value	.002019
	N	111

**Table 7: Validity of NLR in predicting Vascular Complications**

Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.924
Standard Error	0.0315
95% Confidence interval	0.858 to 0.966
z statistic	13.441
Significance level P (Area=0.5)	<0.0001
Youden index	
Youden index J	0.8565
95% Confidence interval	0.7326 to 0.9282
Associated criterion	>3.9989
95% Confidence interval	3.477203178 to 4.2334

complications was 14.8 ± 8. There was a significant difference in mean L (%) comparison with respect to complications.

Mean NL Ratio in no vascular complications was 2.8 ± 0.7, in micro complications was 7.1 ± 3.5 and in macro complications was 6.5 ± 2.7. There was a significant difference in mean NL Ratio comparison with respect to complications (Table 5, Figure 1).

In the study there was significant positive correlation between NLR and glycemic profile i.e., with increase in FBS, PPBS, HBA1C there was increase in NLR and vice versa, (Table 6).

NLR of >3.998 had highest sensitivity of 89.29%, Specificity of 96.36%, PPV of 96.2% and NPV of 89.8% in predicting vascular complications among diabetics (Figure 2, Table 8).

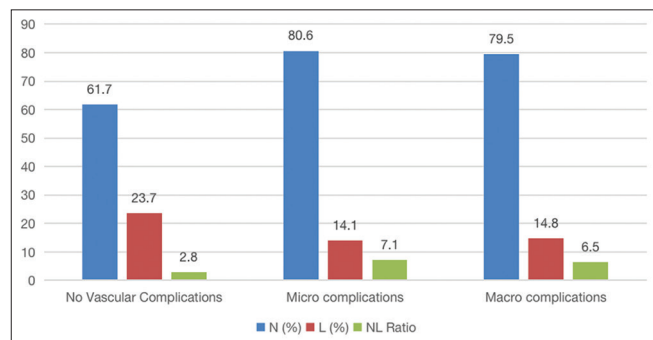


Figure 1: Bar diagram showing NLR comparison with respect to complication

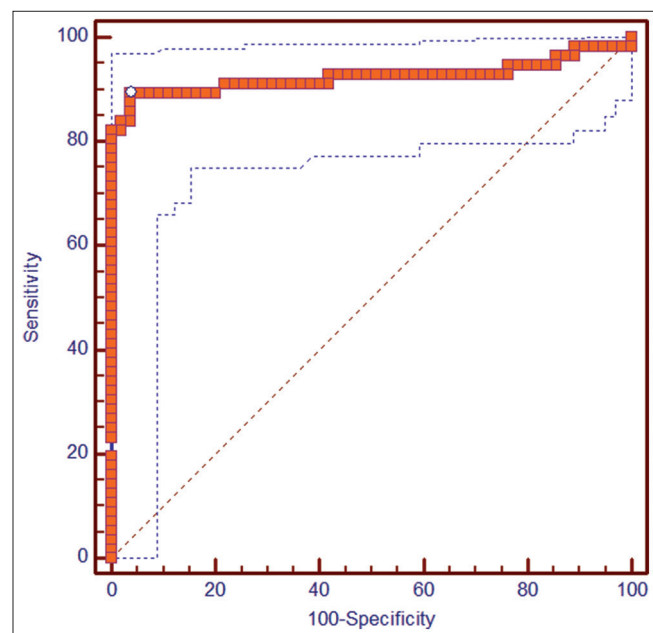


Figure 2: ROC Curve showing Validity of NLR in predicting Vascular Complications

## DISCUSSION

Diabetes is a growing health problem associated with increased risk of micro and macro-vascular complications. With the easy availability of various blood tests such as complete blood count (CBC) efforts are made to identify and prove their utility to act as bio-markers for early detection of diabetic complications.

This is a cross sectional study conducted over a period of 2 years from Nov 2018- May 2020 to study association

**Table 8: Criterion values and coordinates of the ROC curve**

Criterion	Sensitivity	95% CI	Specificity	95% CI	+PV	95% CI	-PV	95% CI
>3.9989	89.29	78.1 - 96.0	96.36	87.5 - 99.6	96.2	86.8 - 99.5	89.8	79.2 - 96.2

of NLR with vascular complications in Type 2 DM. 111 patients were included in this study, where 56 patients were diabetics with vascular complications and 55 patients were diabetes without vascular complications.

### Age

In this study the most common age group was 51-60 (22 patients) with mean age of  $55.66 \pm 13.67$ . Most of the patients were of 40-70 years. The IDF Diabetes Atlas which tracks the global impact of diabetes also shows the more common age group of diabetes is between 20-79 years of age.<sup>25</sup> This is important because of increasing risk within population and failure to control factors such as obesity and poor diets. This would further cause a rising prevalence as each person getting diagnosed at a earlier age stays longer.

### Sex

In this study there was male predominance i.e., 56.8% of patients and female patients were 43.2%, which was similar to study done by Mohammed Haghghatpanah et al, Correlation of glycosylated hemoglobin levels with fasting and postprandial glucose in South Indian Type 2 Diabetic patients.<sup>26</sup>

### Duration of diabetes

In this study, Duration of Diabetes was Newly Detected in 16.2%, <5 years in 37.8%, 6 to 10 years in 30.6% and >10 years in 15.3%. Ours being a tertiary care center most of the patients were incidentally diagnosed patients and duration more than 6 years who were on regular follow up for their medications.

There was no significance in mean FBS, mean PPBS, mean HBA1C compared with the duration of the diabetes.

In this study, Duration of diabetes had significant correlation with N (%) with p value (0.035) and NL ratio with p value (0.045) but the L (%) did not show significant correlation.

Study done by Sachin chittawar et al. showed that patients in higher NLR quartiles had significantly higher diabetes duration.<sup>27</sup>

### Glycemic profile

In this study, there was a significant positive correlation found in between NLR and FBS, NLR and PPBS and NLR and HBA1C.

Study done by Mazhar hussain et al. also showed that NLR was significantly high in diabetic patients with poor glycemic control.<sup>28</sup>

### NLR with respect to vascular complications in type-2 diabetes

Study done by Moursy et al. showed NLR is an important predictor for the presence of microvascular complications like diabetic nephropathy, retinopathy and neuropathy.<sup>29</sup>

In this study there was a significant difference in NL-Ratio comparison with respect to vascular complications in patients with Type 2 DM. Thus, this study highlighted the importance of a routine assessment of Neutrophil-Lymphocyte ratio which can be easily calculated from a simple peripheral blood count in patients with diabetes to predict the vascular complications.

## CONCLUSION

From this study we can conclude that NLR is significantly increased in diabetic patients with complications compared to diabetics without complications showing that increment is more in diabetics with poor glycemic control and longer duration of the disease. NLR would be a useful marker of vascular complications in diabetes both micro and macro-vascular. Hence, it can be shown that NLR can be used as a simple and cost-effective tool to monitor the progression and control of DM and its vascular complications.

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**Author's Contribution:**

**STA** - Concept and design of the study; coordination; **SHK** - Interpreted the results; reviewed the literature and manuscript preparation; **RKR** - Review of literature and manuscript preparation; coordination; **RMG** - Prepared first draft of manuscript Statistically analyzed and interpreted, preparation of manuscript and revision of the manuscript.

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