

**A Study on Neutrophil-Lymphocyte Ratio as a Marker of Nephropathy and Retinopathy in Type 2 Diabetes Mellitus**S. Murugesan<sup>1</sup>, S. Sujatha<sup>2</sup>, Jeshwin John<sup>3</sup><sup>1</sup>Associate Professor, Department of General Medicine, Government Medical College Hospital, Virudhunagar<sup>2</sup>Assistant Professor, Department of General Medicine, Government Medical College Hospital, Virudhunagar<sup>3</sup>Post Graduate in DNB General Medicine, Department of General Medicine, Government Medical College Hospital, Virudhunagar

Received: 25-07-2023 / Revised: 28-08-2023 / Accepted: 30-09-2023

Corresponding author: Dr. S. Murugesan

Conflict of interest: Nil

**Abstract:**

**Introduction:** The increased burden of diabetes among Indians precedes an exponential increase in diabetes-related end-organ damage and associated morbidity in the next few decades. There is an immediate need for a predictor of the occurrence of diabetes-related end-organ damage among Indians that is cheap and easy to measure. The neutrophil-lymphocyte ratio (NLR), among the multiple parameters of complete blood count, has been studied extensively as an inflammatory marker. Aim of our study is to evaluate the role of Neutrophil-lymphocyte ratio (NLR) as a marker of retinopathy and nephropathy in patients of Type 2 Diabetes Mellitus.

**Methodology:** This study was done at Department of General Medicine, Government Medical College Hospital, Virudhunagar as a cross-sectional study in 110 type 2 diabetes mellitus patients. Patients were considered as nephropathy if there is microalbuminuria, eGFR < 60 mL/min/1.73m<sup>2</sup> and USG-Abdomen: Normal sized kidneys. Normal echoes and cortico-medullary differentiation present. Patients were considered having retinopathy based on ophthalmic examination. Necessary blood investigations particularly neutrophil and lymphocyte levels were done. Univariate regression was done to determine the strength of association among the dependent and independent variable.

**Results:** On USG abdomen 18 (16.4%) had B/L Grade-1 MRD and 9 (8.2%) had B/L Grade-2 MRD. 91 (82.7%) had normal fundus and 19 (17.3%) had retinopathy on funduscopy. ROC Curve for NLR Vs eGFR has an area of 0.867. NLR can predict more true positive patients. In other words, NLR is more accurate. Univariate logistic regression for fundus finding in relation with NLR was done and when there is 1 unit increase in NLR, there is 1.6 times chance of developing fundus Findings.

**Conclusion:** NLR is a simple and easy to calculate. This test is inexpensive and done routinely. NLR can be used as a predictor of diabetic nephropathy in Indians. In a poor resource setting, NLR can be a cheap effective alternative marker to predict diabetes nephropathy. The relationship between NLR and diabetic retinopathy changes were also present.

**Keywords:** NLR, Diabetes, Retinopathy, Nephropathy.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

**Introduction**

The prevalence of diabetes and impaired glucose tolerance has been estimated to be 9.3% and 24.5%, respectively based on the national representative sample of adults aged 18–69-years in the National NCD Monitoring Survey, making India the diabetes capital of the world. Indians have an aggressive clinical phenotype of T2DM. They also have the highest rates of pre-diabetes progressing to T2DM [1,2] Also, the onset of T2DM is nearly two decades earlier when compared to Caucasians. This phenomenon could be explained by: a more aggressive beta cell loss intensified systemic inflammation, increased insulin resistance. The

relationship between the occurrence of micro vascular and macro-vascular complications in diabetes, vascular disease and systemic inflammation has been highlighted by the literatures [3]. The increased burden of diabetes among Indians precedes an exponential increase in diabetes-related end-organ damage and associated morbidity in the next few decades. There is an immediate need for a predictor of the occurrence of diabetes-related end-organ damage among Indians that is cheap and easy to measure. This would help to improve long-term clinical outcomes by instituting preventive therapy targeting these

specific individuals at the earliest.

A conventional inflammatory marker that correlates well with several cardiovascular disease risk factors, diabetes, and its sequel is an elevated white blood count (WBC). There are also other inflammatory markers such as interleukins (IL-1, IL-6, IL-8), transforming growth factor  $\beta$ 1, tumor necrosis factor- $\alpha$  that have been linked to end organ damage in diabetes, apart from WBC count [4]. The uses of these markers are limited by increased expenses, lack of their availability in routine clinical practice and assay standardization.

The neutrophil-lymphocyte ratio (NLR), among the multiple parameters of complete blood count, has been studied extensively as an inflammatory marker in various cardiac and non-cardiac diseases. In acute myocardial infarction, heart failure, and stroke, NLR has been suggested to be a prognostic marker. NLR reflects a counterbalance between two complementary components of the immune system; neutrophils being the active non-specific mediator of inflammation, whereas lymphocytes acting as the protective or regulatory component of inflammation [5]. In Indians with T2DM, the role of NLR as a marker of end-organ damage, data are lacking.

Therefore, this study is aimed to evaluate the role of NLR in detecting micro vascular complications such as retinopathy and nephropathy among Indians with diabetes mellitus. Based on this aim of our study is to evaluate the role of Neutrophil-Lymphocyte ratio (NLR) as a marker of retinopathy and nephropathy in patients of Type 2 Diabetes Mellitus

### Materials and Methods

This study was done at department of general medicine, Government medical college hospital, Virudhunagar as a cross-sectional study in 110 type 2 diabetes mellitus patients attending out-patient Department of Government Medical College Hospital, Virudhunagar. Study duration was for 15 months. Patients with type 2 diabetes mellitus of both genders and age group between 45 to 65 years of age were included in this study. Whereas patients with type 1 Diabetes Mellitus, recent history of infection, Systemic disorders like coronary artery disease, uncontrolled systemic

hypertension, chronic kidney disease, chronic liver disease, Auto-immune diseases, Malignancy. On chronic drug use like NSAIDs were excluded.

Patients were considered as nephropathy if there is microalbuminuria, eGFR < 60 mL/min/1.73m<sup>2</sup> and USG-Abdomen: Normal sized kidneys. Normal echoes and cortico-medullary differentiation present. Patients were considered having retinopathy based on ophthalmic examination. Necessary blood investigations particularly neutrophil and lymphocyte levels were done.

Data collection will be done using proforma from patients at Government Medical College Hospital, Virudhunagar. Data collected was entered in Microsoft Excel Spread sheet (MS Excel 2010). And then the data was coded and analyzed using SPSS version 24.0 software. Descriptive analysis for all the variables was expressed in frequency and proportions. ROC Curves were drawn to determine the predictability of the true positive cases. Correlation was done to find out the association among variables. Univariate regression was done to determine the strength of association among the dependent and independent variable.

Informed consent was obtained from all the patients and they were assured that the identity of the respondents will be kept anonymous. Ethical approval was obtained from the ethical review board.

### Results

A total of 172 patients were included in the study of which only 110 patients were followed up, others refused to give consent or did not follow up. Of the total 110 patients, Males were 67 (60.9%) and females were 43 (39.1%). Of the total 110 patients, 101 (91.8%) were on Oral Anti-diabetic drugs and 9 (8.2%) were on Insulin. Of the total 110 patients, 63 (57.3%) had no other co morbidities and 47 (42.7%) had Systemic Hypertension. On USG abdomen of the total 110 patients, 83 (75.5%) had normal kidneys on, 18 (16.4%) had B/L Grade-1 MRD and 9 (8.2%) had B/L Grade-2 MRD. Also of the total 110 patients, 91 (82.7%) had normal fundus and 19 (17.3%) had retinopathy on funduscopy. Among those 19 patients severe NPDR was seen in 2 cases, moderate retinopathy in 6 cases and rest had mild retinopathy.

**Table 1: Descriptive Statistics**

	Minimum	Maximum	Mean	SD
Age	46	75	58.17	6.53
Duration Of Diabetes	.17	20.00	5.57	3.67
Body Mass Index	19.00	38.09	26.47	3.34
TC	4800	10900	7940.91	1440.27
Polymorphs	39	75	58.37	7.11
Lymphocytes	20	52	35.30	6.16
NLR	.75	3.65	1.74	.51
FBS	88	290	128.61	35.51

PPBS	109	450	205.01	53.13
Creatinine	.60	1.70	.93	.20
UREA	20	48	31.29	5.98
eGFR	43	121	80.78	18.28
Urine Microalbumin	7.20	142.00	39.87	23.28

Among our study population mean age was 58.17 years and mean duration was 5.57 years. Almost most of the patients were overweight. There was an increase in microalbuminuria levels too. The mean NLR ratio among our study population was 1.74.

We further did correlation of various factors with NLR ratio and other features and parameters.

To start with age has a weak positive correlation with duration of diabetes (r: 0.350) and

Age has a weak negative correlation with Body Mass Index (r: -0.251). Also has a weak negative correlation with Total Lymphocyte Count (r: -0.201), there was weak positive correlation with Blood Urea (r: 0.266) and microalbuminuria (r: 0.190)

Next we correlated duration of diabetes with various parameters. There was a weak positive correlation with age (r: 0.350), weak negative correlation with Body Mass Index (r: -0.251), weak positive correlation with Fasting (r: 0.194) and Post Prandial (r: 203) Blood Sugar, weak negative correlation with eGFR (r: -0.253), also a weak positive correlation with Microalbuminuria (r: 0.326).

Coming to the neutrophil lymphocyte ratio, there was a weak positive correlation with age (r: 0.238), weak negative correlation with eGFR (r: -0.287) with a Confidence Interval of 99% and it is statistically significant

Serum Creatinine has a weak positive correlation with age (r: 0.242), weak positive correlation with Polymorph Count (r: 0.192), weak negative correlation with Lymphocyte Count (r: -0.209), mainly weak positive correlation with NLR (r: 0.217). Blood Urea has a weak positive correlation with age (r: 0.266), strong positive correlation with Serum Creatinine (r: 0.747), weak positive correlation with Microalbuminuria (r: 0.361). E GFR also had similar results.

ROC Curve for NLR Vs eGFR has an area of 0.867. Since the area under the curve is 0.867, NLR can predict more true positive patients. In other words, NLR is more accurate.

Univariate logistic regression for fundus finding in relation with NLR was done and when there is 1 unit increase in NLR (Independent variable), there is 1.6 times chance of developing Fundus Findings (Dependent variable).

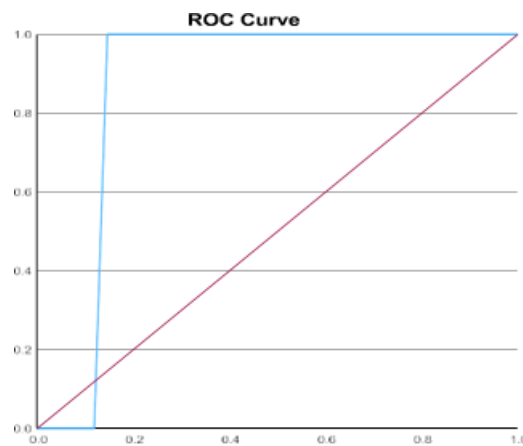


Figure 1:

## Discussion

This study provides evidence about the associations between the NLR level and diabetic complications including diabetic nephropathy and diabetic retinopathy. The main finding was that the NLR level was positively associated with diabetic nephropathy, other than diabetic retinopathy.

The inflammatory molecules and endothelial dysfunction plays a major role in the development of insulin resistance, diabetes and associated micro

vascular and macro vascular complications [6,7]. In previous studies from Caucasians, neutrophilia and relative lymphocytopenia have been shown to be independent markers for micro vascular complications such as nephropathy, neuropathy, and retinopathy.[8-10] Similar data on the utility of NLR among Indians with diabetes is not available. This study highlights that the micro-vascular complications of T2DM can be predicted by a cheap and reliable marker, the NLR. There was a significantly higher occurrence of retinopathy and

nephropathy among the patients with the highest NLR quartile. NLR was the best predictor of nephropathy among the micro vascular complications, based on the receiver operating characteristic curve analysis.

Though there is a positive link between NLR and Diabetic Retinopathy, the data are insufficient to comment on their significance as a predictive marker.

Complete blood count and its components are commonly available and inexpensive to measure, classic inflammatory markers [11]. NLR, a readily available and inexpensive 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). NLR, being a dynamic parameter has a higher predictive value compared to total WBC Count, neutrophil, or lymphocyte individually. The prognostic value of NLR in various malignancies has been demonstrated.[12,13]

NLR also has a prognostic significance in various vascular diseases and cardiovascular events as highlighted by the recent studies [14]. It is also linked with hypertension, the severity of glucose intolerance, and insulin resistance [15].

Huang et al. found that diabetic patients with evidence of nephropathy had a significantly higher NLR as compared to those without nephropathy [16]. Similarly, in another study done by Akbas et al. [17], the association between inflammation and endothelial dysfunction in diabetics with nephropathy was described by establishing the significantly increased NLR in patients with increased albuminuria. Afsar B conducted a study that relates NLR to diabetic nephropathy and also correlates as a predictor of end-stage renal disease [18]. They also followed up diabetic patients for another 3 years and found that NLR has a prognostic significance to declining renal function.

Moursy et al. have also shown that, in diabetic patients with nephropathy ( $P < 0.001$ ), NLR values are significantly higher than those of diabetic patients without any microvascular complications and healthy control subjects [19]. In recent studies, it was published that in Turkish patients with albuminuria, it correlated significantly with NLR [38]. Our study also shows that NLR has a high sensitivity to predict diabetic nephropathy through ROC Curves and correlation analysis. NLR Was significantly higher when the eGFR Value was below 70 mL/min per 1.73 m<sup>2</sup>. Thus indicating its significance as a predictor of diabetic nephropathy.

At present, the association between the NLR level and the prevalence of DR is controversial. A hospital-based cross-sectional study indicated that

NLR could be recommended as an inexpensive diagnostic biomarker for diabetic retinopathy. However, the result of the study conducted by Ciray et al. showed there was no independent association between NLR and diabetic retinopathy [20], which our result is consistent with. In our study, the association between NLR and fundus findings in diabetic retinopathy is not statistically significant ( $p: 0.207$ ) based on Univariate logistic regression. In yet another study done by He et al.[21] 2772 subjects were included in the study and it was concluded that NLR levels correlated with an elevated risk of DR. NLR positively correlated with DR when its value was less than 4.778. Further studies are required with larger study population to find the association.

### Conclusion

NLR is a simple and easy to calculate. This test is inexpensive and done routinely. NLR can be used as a predictor of diabetic nephropathy in Indians. In a poor resource setting, NLR can be a cheap effective alternative marker to predict diabetes nephropathy. The relationship between NLR and diabetic retinopathy changes were also present. Further research is recommended with larger sample size to establish the relationship between NLR and diabetic retinopathy. Also, further research is needed to shed light on the lack of association of NLR with diabetic neuropathy in Indians.

### References

1. Dutta D, Mondal SA, Kumar M, Hasanoor Reza AH, Biswas D, Singh P, et al. Serum fetuin-A concentration predicts glycaemic outcomes in people with prediabetes: A prospective study from Eastern India. *Diabet Med.* 2014.
2. Dutta D, Choudhuri S, Mondal SA, Maisnam I, Reza AH, Ghosh S, et al. Tumor necrosis factor alpha -238G/A (rs 361525) gene polymorphism predicts progression to type-2 diabetes in an Eastern Indian population with prediabetes. *Diabetes Res Clin Pract.* 2013.
3. Rudiger A, Burckhardt OA, Harpes P, Müller SA, Follath F. The relative lymphocyte count on hospital admission is a risk factor for long-term mortality in patients with acute heart failure. *Am J Emerg Med.* 2006.
4. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, et al. White blood cells count and incidence of type 2 diabetes in young men. *Diabetes Care.* 2013.
5. Sampath kumar Rangasamy, Paul G. McGuire, and Arup Das. *Diabetic Retinopathy and Inflammation: Novel Therapeutic Targets.* 2012.
6. N Kashihara et al. *Oxidative stress in diabetic nephropathy.* 2010.
7. Pitsavos C, Tampourlou M, Panagiotakos DB,

- Skoumas Y, Chrysohoou C, Nomikos T, et al. Association between low-grade systemic inflammation and type 2 diabetes mellitus among men and women from the ATTICA study. *Rev Diabet Stud.* 2007.
8. Núñez J, Núñez E, Bodí V, Sanchis J, Miñana G, Mainar L, et al. The usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. *Am J Cardiol.* 2008.
  9. Ulu S, Bucak A, Ulu MS, Ahsen A, Duran A, Yucedag F, et al. Neutrophil-lymphocyte ratio as a new predictive and prognostic factor at the hearing loss of diabetic patients. *Eur Arch Otorhinolaryngol.* 2014.
  10. Torun S, Tunc BD, Suvak B, Yildiz H, Tas A, Sayilir A, et al. Assessment of neutrophil-lymphocyte ratio in ulcerative colitis: A promising marker in predicting disease severity. *ClinRes Hepatol Gastroenterol.* 2012.
  11. Azab B, Daoud J, Naeem FB, Nasr R, Ross J, Ghimire P, et al. Neutrophil-to-lymphocyte ratio as a predictor of worsening renal function in diabetic patients (3-year follow-up study) *RenFail.* 2012.
  12. Lee YY, Choi CH, Kim HJ, Kim TJ, Lee JW, Lee JH, et al. Pretreatment neutrophil: Lymphocyte ratio as a prognostic factor in cervical carcinoma. *Anticancer Res.* 2012.
  13. Mallappa S, Sinha A, Gupta S, Chadwick SJ. Preoperative neutrophil to lymphocyte ratio >5 is a prognostic factor for recurrent colorectal cancer. *Colorectal Dis.* 2013.
  14. Akpek M, Kaya MG, Lam YY, Sahin O, Elcik D, Celik T, et al. Relation of neutrophil/lymphocyte ratio to coronary flow to in-hospital major adverse cardiac events in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. *Am J Cardiol.* 2012.
  15. Shiny A, Bibin YS, Shanthirani CS, Regin BS, Anjana RM, Balasubramanyam M, et al. Association of neutrophil-lymphocyte ratio with glucose intolerance: An indicator of systemic inflammation in patients with type 2 diabetes. *Diabetes Technol Ther.* 2014.
  16. Huang W, Huang J, Liu Q, Lin F, He Z, Zeng Z, et al. Neutrophil-lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy. *ClinEndocrinol (Oxf)* 2015.
  17. Akbas EM, Demirtas L, Ozcecek A, Timuroglu A, Bakirci EM, Hamur H, et al. Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy. *Int J ClinExp Med.* 2014.
  18. Afsar B. The relationship between neutrophil lymphocyte ratio with urinary protein and albumin excretion in newly diagnosed patients with type 2 diabetes. *Am J Med Sci.* 2014.
  19. Moursy EY, Megallaa MH, Mouftah RF, Ahmed SM. Relationship Between neutrophil-lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. *AmJ Intern Med.* 2015.
  20. Ciray H., Aksoy A.H., Ulu N., Cizmecioglu A., Gaipov A., Solak Y., Nephropathy, but not angiographically proven retinopathy, is associated with neutrophil to lymphocyte ratio in patients with Type 2 diabetes. *Experimental and Clinical Endocrinology & Diabetes.* 2015.
  21. He X, i S, Zhang X, Pan J. The relationship between the neutrophil-to-lymphocyte ratio and diabetic retinopathy in adults from the United States: results from the National Health and Nutrition Examination Survey. *BMC Ophthalmol.* 2022 Aug 17;22(1):346.