A Cross Sectional Study to Assess Neutrophil Leucocyte Ratio as Predictor of Microvascular Complications in Diabetes Mellitus Type II

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ABSTRACT:

Background: Neutrophil-to-lymphocyte ratio (NLR) is a marker/indicator for detecting sub-clinically present inflammatory process which is related to increase in microvascular complications. This increase in NLR has been associated with nephrological and cardiovascular diseases and metabolic syndrome. **Aim:** The aim of this study was to assess neutrophil-leukocyte ratio as predictor of microvascular complications in type II diabetes. **Materials and Methods:** This cross-sectional and prospective study was carried out on 100 diabetic patients with inclusion criteria of a) fulfilling ADA criteria and b) age above 18 years. Exclusion criteria for this study were- Patients diagnosed with an acute infection, leukocytosis, leukocytopenia, chronic types of cardiac, renal or hepatic disorders, auto-immune diseases, malignancy or hematological diseases and those on anti-inflammatory or immunosuppressive therapy. Blood-related parameters such as- neutrophil-leukocyte ratio on total leukocytic count were analyzed. Microvascular changes like were examined using ultrasound imaging. Statistical analysis was performed using descriptive statistics, t test, Chi-square and ANOVA tests. ROC curve analysis was performed for predicting correlation between NLR and cIMT. **Results**: Diabetics with vascular changes showed high glycosylated hemoglobin; high fasting blood glucose, LDL-C, triglycerides, serum creatinine/blood urea nitrogen, estimated glomerular filteration rate (eGFR) and Neutrophil/lymphocyte (N/L) ratio levels that has statistical significance, though less statistical significance for HDL-C was noted. ROC analysis proved NLR as a diagnostic marker for microvascular changes. **Conclusion:** It can be concluded from this study that Neutrophil-lymphocyte ratio may be used as an indicator for assessing microvascular complications in patients with diabetes mellitus.

Key words: NLR, microvascular, diabetes, predictive, marker.

INTRODUCTION:

Diabetes mellitus (type II diabetes) is a health issue all around the world as an increasing number of individuals are being diagnosed in earlier stages.[1]Approximately 92.4 million adults aged 20 years or above i.e., 9.7% of adult population) has diabetes. Of which, 60.7% are not diagnosed while 148.2 million of adult population i.e., 15.5% have prediabetes. Prediabetes is a risk factor for overt diabetes along with Cardio-vascular diseases.[2,3,4] Type II diabetes mellitus is the commonest type of diabetes and accounts for 90 to 95% of all cases of diabetes. This form of diabetes arises as a result of insulin resistance and an inadequate compensatory response. Various etiological factors contributing to this disease are- genetic and environmental factors, age, obesity as well as presence of inflammation. Adipose tissue activation in diabetes might result in release of a variety of inflammatory cell cytokines for example, TNF-α, leptins, endolipids, IL- 6, resistin, monocyte chemotactic protein-1 (MCP-1), serum amyloid A

(SAA), angiotensin and retinol-binding protein-4.[5,6] These inflammatory factors cause induction and maintenance of this inflammatory response and associated damage in diabetes.[6] Previous studies have demonstrated role as well as importance of inflammation-related molecules (for example, adipokines, chemokines, cytokines) and endothelial cell dysregulation in pathogenesis of Type II Diabetes Mellitus as well as its complications.[7, 8] Total leukocyte and differential leukocyte counting have an easy accessibility and are less expensive inflammatory cell markers.[9] Establishing any diagnosis by considering only individual white blood cell count may have bias. On the other hand, 'neutrophil-lymphocyte ratio' (NLR) is dynamic parameter which has higher prognostication.[10] Roles of neutrophils as well as lymphocytes is established as independent cell markers for variety of diseases, specially in complications such as diabetic nephropathy in type II diabetes mellitus.[10, 11]

NLR is a novel and established marker related to chronic inflammation which exhibits balance between two complementary constituents of immunological system- a) Neutrophils: These are active type of non-specific inflammation related mediators and are first line in body's defense and b)Lymphocytes: These are regulatory and protectory constituents of inflammation.[12] Neutrophil-Lymphocyte Ratio is closely related to metabolic syndromes, cardiovascular diseases and diabetic micro-vasculature complications.[13, 14, 15 16, 17] There are only limited numbers of studies that have explored relationship between neutrophil-lymphocyte ratio with type II diabetes. Hence, this study was designed to assess the neutrophil- leukocyte ratio as a predictor of microvascular complications in type II diabetes.

MATERIAL AND METHODS:
Study sample:
This cross-sectional prospective study was conducted in outpatient department of the hospital after procuring clearance from the institutional ethical committee. Written informed consent was obtained from all patients. A total number of 100 patients was selected for the study by simple random sampling method.

Inclusion criteria:
for patient selection were- Patients who were diagnosed with type II diabetes mellitus according to ADA criteria and b) age>18 years.

Exclusion criteria:
Patients suffering from acute infections, leukocytosis, leukocytopenia, auto-immune diseases, malignancy or hematological proliferative disorders, chronic cardiac, renal or hepatic diseases, anti-inflammatory and immune-suppressive therapy.
Detailed clinical history was obtained and complete physical examination was conducted.

Studied blood parameters:
After maintaining overnight fasting, venous blood sample collection was done. The total leukocyte count (TLC), neutrophil and lymphocyte count were assessed by an automated hematology analyzer. The Neutrophil-lymphocyte ratio (NLR) was calculated by dividing absolute neutrophil ratio with absolute lymphocyte ratio. Glycosylated hemoglobin (HbA1c) level was measured by using high performance liquid chromatography by an automated ion-exchange method.

Ultrasonic study data:
Carotid artery intima media thicknesses (cIMT) of both of the carotid arteries were measured by using a HDI 5000 (Philips Medical Systems, Washington, USA) which uses a linear probe. The average value from three readings on either side was noted. All patients were then classified into two groups based on cIMT values: a) Group I: Controls with low cIMT (cIMT≤1 mm) and b) Group II: cases with vascular complications who had high cIMT (cIMT>1 mm).

Fundoscopic findings:
Retinopathy was examined by standard direct fundoscopic examination.

Statistical analysis:
was done after calculating means and standard deviations. Statistical software tool (SPSS version XVI.0) was utilized for performing statistical analysis. T-test was employed for calculating continuous variables while Chi-square test was used for categorical variables. 'One-way ANOVA' test was used for comparing the groups. P value of <0.05 was considered as having statistical significance. Linear regression analysis was performed for investigating any direct relationship between HbA1c and NLR and microvascular changes.

RESULTS AND OBSERVATIONS:
A) Demographic as well as clinical data:
Current study showed that cases with vascular complicated diabetic had statistically significant greater duration of diabetes, higher percentage of insulin use, elevated percentage of
anti-hypertensive medications and smoking index (table 1).

B) Laboratory investigation data:
This study demonstrated that diabetic patients with vascular complications demonstrated higher levels of glycosylated hemoglobin (HbA1c); high fasting blood glucose level, LDL-c, triglycerides, serum creatinine/blood urea nitrogen, estimated glomerular filtration rate (eGFR) and Neutrophil/lymphocyte (N/L) ratio which were of statistical significance. But, statistically significant lower levels of HDL-C were seen (Table 1).

Multi-variate linear regression analysis data:
R^2 value of regression models was found to be 84.78%. Independent predictive values of cIMT included- age, male gender, habit of smoking, duration of diabetes, Low Density Lipid levels, HBA1c levels and N/L ratio.

Data from Receiver Operator Characteristic (ROC) curve analysis:
Receiver operator characteristic curve analysis of neutrophil-leukocyte ratio as diagnostic marker of cIMT higher than 1 mm at 2.7 cut- off value demonstrated sensitivity as well as specificity of 100 and 56.89 %, respectively with an area under the curve (AUC)= 0.768 (table 2).

Table 1: Table showing multi-variate analysis of independent predictors of cIMT

Parameters	T	P values	95% confidence interval
Age (in years)	8.650	0.000	0.197 to 0.738
Male gender	6.540	0.000	4.712 to 0.534
Smoking	3.671	0.049	0.40 to 1.934
Total duration of type II diabetes mellitus	4.791	0.029	0.512 to 0.457
eGFR (milliliters per minute)	1.456	0.047	−0.003 to 0.002
LDL (milligrams/dl)	4.319	0.029	1.465 to 0.059
HDL (milligrams/dl)	0.689	0.145	−0.002 to 0.001
Triglycerides (milligrams/dl)	0.267	0.649	−0.008 to 0.019
HbA1c (%)	5.603	0.018	0.012 to 0.013
Neutrophil-leukocyte ratio	8.246	0.000	0.001 to 0.004

Table 2: Table illustrating Receiver operator characteristic (ROC) analysis for neutrophil-to-lymphocyte ratio for predicting micro-vascular changes

Cut off value	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Area under curve (AUC)
2.7	100%	56.89%	17.9	100	0.768

Graph 1: ROC curve for neutrophil-leukocyte ratio for predicting microvascular changes
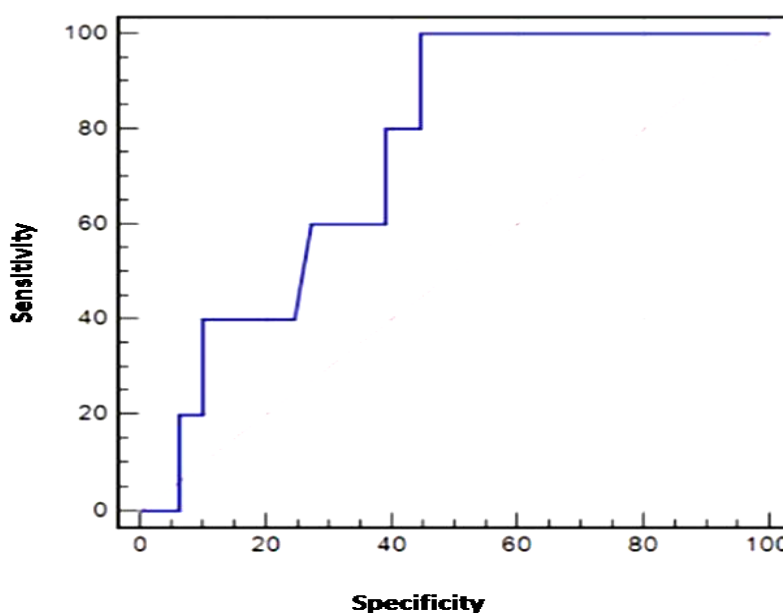
DISCUSSION:

Imaging of blood vessels for identification and quantification of sub-clinical atherosclerosis has been recommended for assessing risk of developing cardiovascular disease.[17] Measurement of cIMT using ultrasound is non-invasive, highly sensitive as well as reproducible method for quantification of microvascular changes.[18] Type II diabetes (Diabetes Mellitus) has two to five times greater risk of developing atherosclerosis that acts as a predisposing factor to various vascular complications which may include- ischemic heart disease, peripheral arterial disease and stroke.[18] High Neutrophil/Leukocyte ratio is a biomarker for micro- as well as macrovascular disease.[19] Present study has shown that Neutrophil-Leukocyte ratio is an independent indicator of coronary artery intima media thickness (cIMT) among patients diagnosed with DM-II. In addition, other there are other conventional risk factors for cardiovascular disease which are- person's age, male gender, habit of smoking, total duration of type II diabetes, levels of LDL and glycosylated hemoglobin(HBA1c). Our study results are similar to other studies which have shown that N/L ratio is an independent indicator for high values of cIMT among patients diagnosed with Diabetes Mellitus.[20] Other studies have also provided evidence of involvement of studied risk factors for instance, age, Body Mass Index, maleness, higher levels of LDL, presence of smoking, diabetes, with an increase incIMT.[21, 22, 23, 24, 25, 26] In accordance with current study, Rahar et al (2021) found higher N/L ratio among diabetics when compared with normal subjects and concluded that Neutrophil-leukocyte ratio correlates with severe presentation of renal impairment.[27] **Mohammad et al** (2018) also observed that N/L ratio must be considered as an independent predictor of cIMT. The investigators concluded that this ratio is involved in micro- as well as macrovascular changes in type II diabetes.[28] Exact mechanisms which relate a high N/L ratio with an increased cIMT in diabetics are still unknown. One possible mechanism may be neutrophilic infiltration of arterial walls which cause a proteolytic enzyme release, thus, speeding up the process.[29] An alternate mechanism may be an autonomic imbalance of vascular bed by high neutrophil-leukocyte ratio. Sympathetic nerve supply causes stimulation of granulocytic cells, while the parasympathetic nerve supply causes stimulation of lymphocytes.[30] Thus, higher N/L ratio might be indicative of high ratio between sympathetic-to-parasympathetic nerve activity. The sympathetic neural supply may increase consumption of oxygen along with an increase in production of pro-inflammatory cytokines, for example, interleukin- 6 and tumor necrosis factor-α.[31]

These chemokines cause stimulation of smooth muscles and proliferation of interstitial cells that causes acceleration of the micro-vascular changes in Diabetes Mellitus which are- retinopathic, neuropathic and nephropathic changes. These microvascular changes cause significant changes in 25 to 40% diabetics. These microvascular changes are main reason for end-stage renal failure.[32, 33] However, in contrast to above studies, Mendes et al (2019) found no significant difference in neutrophil to lymphocyte ratio between hyperglycemic and normoglycemic subjects.[34] Hence, there appears to be some conflicting evidence present in literature with regards to neutrophil-lymphocyte ratio and its diagnostic role in diabetes mellitus patients.

CONCLUSION:

Neutrophil/lymphocyte ratio is a cost-effective, is available readily and is simple test that may be performed in all types of health care centres. It is an useful tool for screening microvascular changes that can lead to a variety of diseases such as- nephropathy, hypertension and atherosclerotic changes in diabetic patients. Present study found statistically significant correlation between diabetes mellitus-II and microvascular complications.

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REFERENCES:

1. Mallya NV, Palakal S, Dayanand G. Could neutrophil-lymphocyte ratio be an adjuvant marker for cognitive impairment in patients with Type 2 diabetes mellitus? Natl J Physiol Pharm Pharmacol 2020;10(04):324-9.
2. Yang W, Lu J, Weng J, JiaW, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q et al. Prevalence of diabetes among men and women in China. New England J Med 2010 362 1090–1101.
3. Schmidt MI, Duncan BB, Bang H, Pankow JS, Ballantyne CM, Golden SH, Folsom AR &Chambless LE. Identifying individuals at high risk for diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care 2005 28 2013–8.
4. Levitzky YS, Pencina MJ, D'Agostino RB, Meigs JB, Murabito JM, Vasan RS & Fox CS. Impact of impaired fasting glucose on cardiovascular disease: the Framingham Heart Study. Journal of American College of Cardiology 2008 51 264–70.

5. Steppan CM, Bailey ST, Bhat S. The hormone resistin links obesity to diabetes. Nature 2001;409(6818):307–12.

6. Pop-Busui R, Boulton AJM, Feldman EL. Diabetic neuropathy: a position statement by the American Diabetes Association. Diabetes Care 2017;40(1):136–54.

7. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J ClinEndocrinolMetab2009;94:3171-82.

8. Fujita T, Hemmi S, Kajiwara M, Yabuki M, Fuke Y, Satomura A, *et al*. Complement-mediated chronic inflammation is associated with diabetic microvascular complication. Diabetes Metab Res Rev 2013;29:220-6.

9. 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. Clin Res HepatolGastroenterol2012;36:491-7.

10. Bhutta H, Agha R, Wong J, Tang TY, Wilson YG, Walsh SR. Neutrophil-lymphocyte ratio predicts medium-term survival following elective major vascular surgery: A cross-sectional study. Vasc Endovascular Surg2011;45:227-31.

11. Núñez J, Núñez E, Bodí V, Sanchis J, Miñana G, Mainar L. Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. Am J Cardiol2008;101:747-52.

12. Azab B, Jaglall N, Atallah JP, Lamet A, Raja-Surya V, Farah B. Neutrophil-lymphocyte ratio as a predictor of adverse outcomes of acute pancreatitis. Pancreatology2011;11:445-52.

13. McIntyre NJ, Taal MW. How to measure proteinuria? CurrOpinNephrolHypertens 2008;17:600-3.

14. Buyukkaya E, Karakas MF, Karakas E, Akçay AB, Tanboga IH, Kurt M, *et al*. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. ClinApplThrombHemost2014;20:159-63.

15. Öztürk ZA, Kuyumcu ME, Yesil Y, Savas E, Yıldız H, Kepekçi Y, *et al*. Is there a link between neutrophil-lymphocyte ratio and microvascular complications in geriatric diabetic patients? J Endocrinol Invest 2013;36:593-9.

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;82:229-33.

17. Taylor AJ, Merz CNB, Udelson JE. 34th Bethesda Conference: executive summary − can atherosclerosis imaging techniques improve the detection of patients at risk for ischemic heart disease? J Am CollCardiol2003;41:1860–2.

18. Preis SR, Hwang S-J., Coady S, Pencina MJ, D'Agostino RB, Savage PJ et al. Trends in all-cause and cardiovascular disease mortality among women and men with and without diabetes mellitus in the Framingham Heart Study, 1950 to 2005. Circulation 2009; 119:1728–35.

19. Kalay N, Dogdu O, Koc F, Yarlıoglues M, Ardic I, Akpek M, et al. Hematologic parameters and angiographic progression of coronary atherosclerosis. Angiology 2012; 63:213–7.

20. Li X, Shen J, Lu Z, Chen M, Fang X, Wang G. High neutrophil-tolymphocyte ratio is associated with increased carotid artery intima-media thickness in type 2 diabetes. J Diabetes Investig. 2017; 8:101–7.

21. Raitakari OT, Juonala M, Kähönen M, Taittonen L, Laitinen T, Mäki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima media thickness in adulthood: the cardiovascular risk in young finns study. JAMA 2003; 290:2277.

22. Mannami T, Konishi M, Baba S, Nishi N, Terao A. Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. Stroke 1997; 28:518–25.

23. Oren A, Vos LE, Uiterwaal CSPM, Grobbee DE, Bots ML. Cardiovascular risk factors and increased carotid intima-media thickness in healthy young adults: the atherosclerosis risk in young adults

(ARYA) study. Arch Intern Med 2003; 163:1787.

24. Brohall G, Oden A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. Diabet Med 2006; 23:609–16.

25. Mykkanen L, Zaccaro DJ, O'Leary DH, Howard G, Robbins DC, Haffner SM. Microalbuminuria and Carotid Artery Intima-Media Thickness in Nondiabetic and NIDDM Subjects: The Insulin Resistance Atherosclerosis Study (IRAS). Stroke 1997; 28:1710–6.

26. DehdashtiShahrokh H, Zakerkish M, Jenabi A, Hanafi MG, Rahim F, Rezazadeh A, et al. Association of microalbuminuria and estimated glomerular filtration rate with carotid intima-media thickness in patients with type 2 diabetes mellitus. Jentashapir J Health Res. 2015; 6:2.

27. Rahar S, Marwah S, Kulshreshtha B. Neutrophil lymphocyte ratio (NLR) in type 2 diabetes mellitus and its correlation with renal function: An institutional experience. J NTR Univ Health Sci2021;10:82-7.

28. Mohammad WA, Ahmad AB, Al-Maghraby MH, Abdelrhman MZ, Ezzate S. Is neutrophil-lymphocyte ratio a novel biomarker for macrovascular and microvascular complications of type 2 diabetes? Egypt J Intern Med 31:1–7.

29. Eriksson EE, Xie X, Werr J, Thoren P, Lindbom L. Direct viewing of atherosclerosis in vivo: plaque invasion by leukocytes is initiated by the endothelial selectins. FASEB J Off Publ Fed Am SocExpBiol2001;15:1149–57.

30. Abo T, Kawamura T. Immunomodulation by the autonomic nervous system:therapeutic approach for cancer, collagen diseases, and inflammatory bowel diseases. TherApher Off J IntSocApherJpnSocApher2002;6:348–57.

31. Tracey KJ. The inflammatory reflex. Nature 2002; 420:853–9.

32. Bhagat K, Vallance P. Effects of cytokines on nitric oxide pathways in human vasculature. CurrOpinNephrolHypertens. 1999; 8:89–96.

33. Ritz E, Rychlík I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: a medical catastrophe of worldwide dimensions. Am J Kidney Dis Off J Natl Kidney Found 1999; 34:795–808.

34. Mendes BB, Oliveira ACR, Alcantara AC. Comparison of the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in normoglycemic and hyperglycemic subjects.einstein(SãoPaulo).2019;17(1):eAO4403.http://dx.doi.org/10.31744/einstein_journal/2019AO4403.