

Study of Neutrophil-Lymphocyte ratio (NLR) in diabetes mellitus

Umarani M. K.¹, Keerti Sahi², Bharathi M.³

¹Dr. Umarani M. K, Professor, ²Dr Keerti Sahi, Postgraduate, ³Dr Bharathi M., Professor, and Head; all authors are affiliated with the Department of Pathology, MMC and RI, Mysuru, Karnataka, India.

Corresponding Author- Dr. Keerti Sahi, Postgraduate, Department of Pathology, MMC and RI, Mysuru, Karnataka, India. Email: r.keertisahi@gmail.com

Abstract

Introduction: Diabetes mellitus is a systemic disease having serious microvascular complications. Neutrophil-lymphocyte ratio (NLR) provides insight about subclinical inflammation and thus acts as a prognostic marker for diabetes mellitus and its vascular complications. **Aim:** To study the relationship between neutrophil-lymphocyte ratio with different grades of glucose intolerance. **Materials and Methods** Cases for the present study include known cases of diabetes mellitus and normal subjects from KR hospital, Mysuru. Fasting blood glucose (FBS) and postprandial blood glucose (PPBS) of study subjects are taken into account to grade glucose intolerance under three categories- Normal glucose tolerance, Impaired glucose tolerance, and diabetes mellitus. Neutrophil-lymphocyte ratios are calculated and compared with blood glucose levels. **Results:** NLR showed no significant variation in different grades of glucose intolerance. NLR was increased in patients with neuropathy, glaucoma, and blurring of vision when compared with those patients without any complication. **Conclusion:** NLR is increased in subjects having diabetes mellitus with complications when compared to the subjects with diabetes mellitus under control. Hence NLR can be considered as an early and prognostic marker for microvascular complications in patients with diabetes mellitus.

Keywords: Diabetes mellitus, Neutrophil-lymphocyte, Prognostic marker

Introduction

Diabetes Mellitus is a group of metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiological changes in multiple organ systems that impose a huge burden on individuals with diabetes mellitus and on the health care system. Therefore, early diagnosis and prevention of microvascular and macrovascular complications and thus end-organ failure is a necessary step to be undertaken.

Metabolic dysregulations like hyperglycemia, dyslipidemia, and oxidative stress are considered to be directly involved in tissue-specific inflammation [1]. Many studies have been done to develop the relationship between inflammatory mediators and type 2 diabetes mellitus. It has been stated that chronic inflammation promotes the development and acceleration of micro and

macro angiopathic complications by increasing the various cytokines and chemokines, acute phase proteins such as IL-1, IL-6, IL-8, TGF β , TNF- α [2,3]. Their measurement is not used routinely, rather a neutrophil-lymphocyte ratio (NLR) has been used as a novel marker of inflammation and has been shown to have prognostic values with systemic inflammation [4]. NLR represents a combination of two major components of chronic inflammatory conditions (high neutrophil and low lymphocyte). High neutrophil value is a marker of the ongoing destructive nonspecific inflammatory process. Low lymphocyte value indicates relatively inadequate immune regulation as well as a quiescent immunity pathway [5,6].

Aim: To study the relationship between neutrophil-lymphocyte ratio with different grades of glucose intolerance

Material and Methods

Duration: This study was conducted between December 2018-January 2019

Type of study: Descriptive study

Manuscript received: 4th February 2020

Reviewed: 14th February 2020

Author Corrected: 19th February 2020

Accepted for Publication: 24th February 2020

Sampling methods: Statistical analysis was performed using software (SPSS). Data were presented as Mean \pm SD and compared using one-way analysis of variance (ANOVA). P-value <0.05 was regarded as statistically significant.

Sample size: 104

Inclusion criteria:

1. Known cases of diabetes mellitus
2. Normal subjects

Exclusion criteria: None

Ethical clearance: Informed consent was obtained from all the subjects and ethical committee approval was obtained.

Data collection procedure: In the duration of two months, 104 subjects were included out of which, 76 subjects were found to be known cases of diabetes mellitus. Informed consent was obtained from all the subjects. Fasting blood glucose postprandial blood glucose and a complete blood count of all subjects were done. Cases were classified under the following three categories based on values of fasting blood glucose and postprandial blood glucose

-Normal glucose tolerance

-Impaired glucose tolerance (IGT) and

-Overt diabetes mellitus

Neutrophil-lymphocyte ratio was calculated and correlated with grades of glucose intolerance.

Definitions: Diabetes mellitus is classified based on the WHO consulting criteria (Table 1).

Table-1: WHO consulting criteria.

	Normal glucose tolerance	Impaired glucose tolerance	Diabetes mellitus
Fasting plasma glucose	<5.6mmol/L <100mg/dl	5.6-6.9mmol/L 100-125mg/dl	>7mmol/L >126mg/dl
2 hours post prandial	<7.8mmol/L <140mg/dl	7.8-11mmol/L 140-199mg/dl	>11.1mmol/L >200mg/dl

Biochemical parameters: FBS and PPBS were measured using a Hitachi Roche Cobas 6000 series based on Hexokinase method

Measurement of NLR: Differential count was assessed using a hematology analyzer (model xn1000; Sysmex). NLR was calculated as the ratio between neutrophil and lymphocyte counts in the study subjects.

Results

A total of 104 subjects were included, of these, 68 subjects were categorized under diabetes mellitus, 22 subjects had impaired glucose tolerance and 14 subjects had blood glucose level under normal limits. These groups were compared for NLR. There was no significant variation of NLR with different grades of glucose intolerance. The mean value of NLR in normal subjects, IGT subjects, and subjects with overt diabetes mellitus were 2.03 \pm 0.88, 2.02 \pm 0.67, and 2.04 \pm 1.22 respectively. And P-value is 0.996 (Table 2).

Table-2: Neutrophil-lymphocyte ratio in subjects with different grades of glucose intolerance

	NLR	P
Normal	2.03 \pm 0.88	0.99
IGT	2.02 \pm 0.67	
DM	2.04 \pm 1.23	

Table-3: Neutrophil-lymphocyte ratio in subjects with diabetes mellitus with complications

Variables	NLR	P
Nil	1.91±0.76	0.113
Neuropathy	2.57±1.9	
Glaucoma	2.84	
Retinopathy	1.96±0.72	

NLR was also compared between the subjects having microvascular complications of diabetes mellitus such as neuropathy, retinopathy, and glaucoma. Out of 104 subjects, 18 had neuropathy, 1 had glaucoma, 3 had retinopathy and the remaining 82 were not diagnosed with any microvascular complications of diabetes mellitus with mean values of 2.57±1.9, 2.84, 1.96±0.72 and 1.91±0.76 respectively (Table 3). NLR is increased in subjects having diabetes mellitus with complications when compared to the subjects with diabetes mellitus under control and P-value is 0.113

A gender difference was also assessed and no difference was observed in NLR.

Discussion

In the current study, it was observed that no significant relationship between NLR and the increasing severity of glucose intolerance. Previously Shiney et al. [1] showed that NLR increases with the severity of glucose intolerance with mean values of 2.2±1.12 in DM, 1.82±0.63 in IGT subjects, and 1.5±0.41 in NGT subjects.

A study done by Xu et al [5] suggested that NLR values of the patients with diabetic peripheral neuropathy were significantly higher than the diabetic Mellitus group. (2.58±0.50 vs 2.18±0.61). A study was done by Khandre, et al [4] showed that the NLR was significantly raised in patients with type 2 diabetes mellitus has increased albuminuria. It was found that NLR is increased in diabetes mellitus with complications like neuropathy (2.57±1.9) and glaucoma (2.84) when compared to the subjects with diabetes mellitus under control (1.91±0.76).

NLR is being used to know the information regarding microvascular complications of diabetes mellitus in terms of prognosis as it is a reliable predictive marker of early-stage diabetic nephropathy, retinopathy, and diabetic foot ulcer [7,8,9]. Studies have shown that chronic inflammation contributes to the occurrence and development of microvascular complications in diabetic patients.

NLR is an important marker of chronic inflammation which exhibits a balance between two interdependent components of the immune system. Neutrophils are the active inflammatory mediator that forms the first line of defense and the high neutrophil count is a marker of the ongoing destructive nonspecific inflammatory process. whereas lymphocytes are the regulatory and protective component of inflammation and low lymphocyte count value indicates relatively inadequate immune regulation [4,10].

Stimuli such as hyperglycemia and oxidative stress increase the expression of Intracellular Adhesion Molecule 1 (ICAM-1), proinflammatory cytokines, and chemokines [11]. The overexpression of ICAM-1 results in the gathering of inflammatory cells [12]. These cascades in inflammatory responses will lead to an increase in neutrophils. Diabetes mellitus and its complications may be associated with lymphopenia which can be attributed to increased oxidative DNA damage and apoptosis in peripheral blood lymphocytes [13,14].

Research has shown that chronic inflammation contributes to the occurrence of diabetic peripheral neuropathy diabetic patients [15,16,17,18]. A study conducted by Ulu et al [19] demonstrated NLR to be a quick and reliable prognostic marker for diabetic retinopathy and its severity. Another study by Ulu et al [20] concluded that NLR can be considered as a predictive and prognostic marker for sensorineural hearing loss in diabetic patients. Another study conducted in the geriatric population also demonstrated that NLR levels are an independent predictor for microvascular complications of DM [21].

In chronic kidney disease, NLR has shown to be a significant indicator of ongoing inflammation [22]. Also in a 3 year follow up study, NLR has proven to be a predictor of worsening renal function [10]. Another study conducted by Akbas et al [23] has shown that NLR was significantly elevated in patients with increased albuminuria depicting a relationship between inflammation and endothelial dysfunction in diabetics with nephropathy. Recent studies have indicated that NLR could be a predictive marker for vascular diseases like cardiovascular disease and NLR was associated with poor survival rates after coronary artery bypass grafting and can also be used as a predictive marker of mortality and morbidity in patients with acute coronary events [24]. Recently, NLR is considered to be

an important marker to determine various disorders as it has a better predictive, diagnostic ability than the total WBC count. Predictive value of NLR is comparable to various other markers such as C-reactive protein, tumor necrosis factor α (TNF α) and interleukin (IL-6) in the detection of subclinical inflammation and endothelial dysfunction and has various advantages over other inflammatory markers because of its low cost, reliability and easy lab detection [25].

Some studies have also been done to demonstrate an association between NLR and postoperative morbidity and have stated that elevated preoperative NLR is also associated with increased morbidity postoperatively, with prolonged hospitalization and increased intensive care admission [26]. Hence NLR should be obtained pre and postoperatively and elevated values can be used to plan postoperative management of patients undergoing surgeries. A small number of study populations and the absence of cardiovascular and renal complications are the limitations of this study. Larger scale study designs are required to provide more definitive results.

Conclusion

The results of the present study have shown that there was a correlation between NLR and microvascular complications of diabetes mellitus, suggesting that inflammation and endothelial dysfunction are the causative factors of these complications. Therefore, NLR can be considered as a predictive and prognostic marker of diabetes mellitus with complications.

What does the study add to the existing knowledge?

NLR is a simple and inexpensive test so it can be used as an alternative for other expensive tests for inflammatory markers. Hence it can be used as a monitoring tool during the follow up of diabetic patients.

Author's contribution

Dr. Umarani M. K: Concept, study design

Dr. Keerti Sahi: Manuscript preparation,

Dr. Bharathi M: Statistical analysis

Funding: No funding sources

Conflict of interest: None declared

Ethical Approval: This study was approved by the Institutional Ethics Committee

References

1. Shiny A, Bibin Y S, Shanthirani C S, Regin B S, Anjana R A, 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(8):524-530. doi: <https://doi.org/10.1089/dia.2013.0264>.

2. Akash MSH, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. *J. Cell. Biochem.* 2013;114(3):525-531. doi: <https://doi.org/10.1002/jcb.24402>.

3. Okyay G U, Inal S, Onec K, Er R E, Pasaoglu O, Pasaoglu H et al. Neutrophil to lymphocyte ratio in evaluation of inflammation in patients with chronic kidney disease. *Renal Fail.* 2012;35(1):29-36. doi: <https://doi.org/10.3109/0886022x.2012.734429>.

4. Khandare SA, Chittawar S, Nahar N, Dubey TN, Qureshi Z. Study of neutrophil-lymphocyte ratio as novel marker for diabetic nephropathy in type 2 diabetes. *Indian J Endocr Metab.* 2017;21(3):387-392. Available from: <http://www.ijem.in/text.asp?2017/21/3/387/205486>.

5. Xu T, Weng Z, Pei C, Yu S, Chen Y, Guo W et al. The relationship between neutrophil-to-lymphocyte ratio and diabetic peripheral neuropathy in type 2 diabetes mellitus. *Medicine* 2017;96(45):e8289. doi: <https://doi.org/10.1097/md.00000000000008289>.

6. Azab B, Daoud J, Naeem FB, et al. Neutrophil-to-lymphocyte ratio as a predictor of worsening renal function in diabetic patients (3 year follow-up study). *Ren Fail.* 2012;34 (5):571-576. <https://doi.org/10.3109/0886022x.2012.668741>.

7. Huang W, Haung J, Liu QLin F, He Z, Zeng Z et al. Neutrophil- Lymphocyte ratio a reliable predictive marker of early stage diabetic nephropathy. *Clin Endocrinol.* 2015;82 (2):229-233. doi: <https://doi.org/10.1111/cen.12576>.

8. Wang RT, Zhang JR, Li Y, Liu T, Yu KJ. Neutrophil lymphocyte ratio is associated with arterial stiffness in diabetic retinopathy in type 2 diabetes complications. *J Diabetes Complications* 2015;29 (2):245-249. doi: <https://doi.org/10.1016/j.jdiacomp.2014.11.006>.

9. Kahraman C, Yumun G, Kahraman NK, Namdar ND, Cosgun S. Neutrophil lymphocyte ratio in diabetes mellitus patients with and without diabetic foot ulcer. *Eur J Med Sci.*2014;1:8-13.

10. 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 diabetes patients (3 year

follow-up study). *Ren Fail.* 2012;34(5):571-576. doi: <https://doi.org/10.3109/0886022X.2012.668741>.

11. Barnes PJ, Karin M. Nuclear factor- kappa B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med.* 1997;336(15):1066-1071. doi: <https://doi.org/10.1056/nejm199704103361506>.

12. Kawamura N, Dyck PJ, Schmeichel AM, Engelstad JK, Low PA, Dyck PJ. Inflammatory mediators in diabetic and non-diabetic lumbosacral radiculoplexus neuropathy. *Acta Neuropathol.* 2008;115(2):231-239. doi: <https://doi.org/10.1007/s00401-007-0326-2>.

13. Gokulakrishnan K, Deepa R, Sampathkumar R, Balasubramanyam M, Mohan V. Association of leukocyte count with varying degrees of glucose intolerance in Asian Indians: the Chennai Urban Rural Epidemiology Study (Cures-26). *Metab Syndr Relat Disord.* 2009;7(3):205-210. doi: <https://doi.org/10.1089/met.2008.0024>.

14. Lum H, Roebuck KA: Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol.* 2001;280(4):C719-C741. doi: <https://doi.org/10.1152/ajpcell.2001.280.4.c719>.

15. Vincet AM, Callaghan BC, Smith AL, Feldman EL. Diabetic neuropathy: cellular mechanisms as therapeutic targets. *Nat Rev Neurol.* 2011;7(10):573-583. doi: <https://doi.org/10.1038/nrneurol.2011.137>.

16. Kampoli AM, Tousoulis D, Briasoulis A, Latsios G, Papageorgiou N, Stefanadis C. Potential pathogenic inflammatory mechanisms of endothelial dysfunction induced by type 2 diabetes mellitus. *Curr Pharm Des.* 2011;17(37):4147-4158. doi: <https://doi.org/10.2174/138161211798764825>.

17. Uceyler N, Rogausch JP, Tokyo KV, Sommer C. Differential expression of cytokines in painful and painless neuropathies. *Neurology.* 2007;69(1):42-49. doi: <https://doi.org/10.1212/01.wnl.0000265062.92340.a5>.

18. Doupis J, Lyons TE, Wu S, Charalambos G, Gardellis, Dinh T, Veves A et al. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. *J Clin Endocrinol Metab.* 2009;94(6):215-263. doi: <https://doi.org/10.1210/jc.2008-2385>.

.....
How to cite this article?

Umarani M. K., Keerti Sahi, Bharathi M. Study of Neutrophil-Lymphocyte ratio (NLR) in diabetes mellitus. *Trop J Path Micro* 2020;6(4):298-302. doi:10.17511/jopm.2020.i04.05

19. Ulu SM, Dogan M, Ahsen A, Altug A, Demir K, Acarturk G, et al. Neutrophil-to-lymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. *Diabetes Technol Ther.* 2013;15(11):942-947. doi: <https://doi.org/10.1089/dia.2013.0097>.

20. 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;271(10):2681-2686. doi: <https://doi.org/10.1007/s00405-013-2734-3>.

21. Ozturk ZA, Kuyumcu ME, Yesil Y, Savas E, Yildiz H, Kepekci Y, et al. Is There a link between neutrophil-lymphocyte ratio and microvascular complications in geriatric diabetic patients. *J Endocrinol Invest.* 2013;36(8):593-599. doi: <https://doi.org/10.3275/8894>.

22. Okyay GU, Inal S, Onec K, Er RE, Pasaoglu O, Pasaoglu H, et al. Neutrophil to lymphocyte ratio in evaluation of inflammation in patients with chronic kidney disease. *Ren Fail.* 2013;35(1):29-36. doi: <https://doi.org/10.3109/0886022x.2012.734429>.

23. Akbas EM, Demirtas L, Ozcicek 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 Clin Exp Med.* 2014;7(7):1794-801

24. Zouridakis E, Avanzas P, Arroyo-Espliguero R, Fredericks S, Kaski JC. Markers of inflammation and rapid coronary artery disease progression in patients with stable angina pectoris. *Circulation.* 2004;110(13):1747-1753. doi: <https://doi.org/10.1161/01.cir.0000142664.18739.92>.

25. Hussain M, Babar M Z B, Akhtar L, Hussain M S. Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycemic control in type-2 diabetic patients. *Pak J Med Sci.* 2017;33(6):6,1366-1370. doi: <https://doi.org/10.12669/pjms.336.12900>.

26. Chung K-P, Chang H-T, Lo S-C, Chang L-Y, Lin S-Y, Cheng A, et al. Severe lymphopenia is associated with elevated plasma interleukin-15 levels and increased mortality during severe sepsis. *Shock.* 2015;43(6):569-575. doi: <https://doi.org/10.1097/shk.0000000000000347>.