NEUTROPHIL-LYMPHOCYTE RATIO AND ITS ASSOCIATION WITH GLYCEMIC CONTROL AMONG TYPE-2 DIABETES MELLITUS PATIENTS IN A TERTIARY CARE HOSPITAL, CHENGALPATTU DISTRICT

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ABSTRACT

Objectives: Neutrophil-lymphocyte ratio (NLR) is a novel marker of sub-clinical inflammation which is associated with the worsening of insulin resistance in type-2 diabetes mellitus (type-2 DM) patients. Plasma glycated hemoglobin (HbA1c) is a major investigatory tool for monitoring long-term glycemic control. The objective of this study was to find out the relationship between NLR and HbA1c levels in patients with type-2 DM.

Methods: This was a cross-sectional study carried out in the central clinical laboratory of a tertiary care hospital. Complete blood count and HbA1c laboratory reports of all type-2 DM patients were retrieved from the registers. NLR was calculated by dividing the absolute neutrophil count (ANC) by the absolute lymphocyte count. The patients were categorized into three groups based on the HbA1c values. Data were tabulated and analyzed using the Statistical Package for the Social Sciences v 23.0.

Results: The mean age of the total 143 patients was 49 years with a slight male preponderance (52.4%). The mean NLR was 2.76 and the mean HbA1c value was 8.18%. A statistically significant association was found between HbA1c with NLR ($p<0.001$), ANC ($p<0.001$), and age of the patients ($p<0.001$).

Conclusion: NLR is a simple and cost-effective diagnostic test that can be used as a marker of sub-clinical inflammation and also predict the development of cardiovascular complications in type-2 diabetic patients with poor glycemic control.

Keywords: Absolute neutrophil count, Glycated hemoglobin, Inflammation, Insulin resistance.

INTRODUCTION

Type-2 diabetes mellitus (type-2 DM) is a common metabolic disorder accounting for increased mortality and morbidity worldwide due to its various systemic complications [1]. Obesity and insulin resistance are the main factors that are implicated in the pathogenesis of type-2 DM.

The development of insulin resistance in obese individuals is linked to increased levels of free fatty acids and adipokines [2]. Recently, low-grade chronic inflammation is found to be linked with the development and worsening of insulin resistance in patients with type-2 DM. This sub-clinical inflammation is also attributed to the increased frequency of atherogenesis and vascular complications in patients with type-2 DM [3].

Plasma glycated hemoglobin (HbA1c) has been a major investigatory tool for monitoring long-term glycemic control and assessing the adequacy of treatment in diabetic patients [4]. However, HbA1c cannot provide information regarding the ongoing inflammation in type-2 DM patients.

Neutrophil-lymphocyte ratio (NLR) indicates the ratio of absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) which reflects on the balance between the proportions of neutrophils and lymphocytes in the circulation. It serves as an important marker of inflammation and predicts the prognosis of various diseases including acute myocardial infarction, heart failure, and cerebrovascular accidents [5]. In type-2 DM, NLR is a novel hematological inflammatory marker and serves as a useful indicator of sub-clinical inflammation. Elevated NLR can predict the onset of cardiovascular complications including atherosclerosis in type-2 DM patients at the initial stages [6].

Atherogenesis is directly attributed to the systemic sub-clinical inflammation observed in type-2 DM patients [6]. The various inflammatory biomarkers associated with the pathogenesis of atherosclerosis and type-2 DM include C-reactive protein, interleukin-6, tumor necrosis factor-alpha,etc., with very few studies carried out on the utility of NLR as an inflammatory biomarker in type-2 DM patients.

There are only limited published data on the significance and association between NLR and glycemic control (HbA1c) in type-2 DM patients. Hence, this study was aimed at evaluating the relationship between NLR and HbA1c in type-2 DM patients.

METHODS

Study design

This was a cross-sectional study carried out in the central clinical laboratory of a tertiary care centre during the period of July 2023–December 2023 after getting ethical approval from the Institutional Ethics Committee (IEC Ref No: KIMS/PG/06/09/2023).

Sample size

A purposive sampling technique was used for the selection of the desired samples according to the inclusion criteria and a total of 143 laboratory reports (complete blood count [CBC] and HbA1c) of patients diagnosed with type-2 DM were reviewed.

Inclusion criteria

All the laboratory reports (CBC and HbA1c) of type-2 DM patients of age more than 18 years were included in the study.

Exclusion criteria

Pregnant females, patients with a history of acute infections and chronic illnesses such as autoimmune disorders, malignancy, cardiac diseases, liver diseases, and kidney diseases were excluded. Furthermore,

patients on anti-inflammatory and immunosuppressive drugs were not included in this study.

Data collection procedure

CBC and HbA1c test reports of all the type-2 DM patients processed in automated hematology (PENTRA ES 60) and biochemistry analyzers (tosoh automated glycohemoglobin analyzer-HLC-723GX), respectively, were retrieved from the registers in the central clinical laboratory for the 6 months study period from July 2023 to December 2023. From the CBC test results, NLR was calculated by dividing the ANC by ALC. Based on the HbA1c levels, the patients were divided into three groups: Group A with HbA1c <7%, Group B with HbA1c 7–9%, and Group C with HbA1c >9% [5].

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences 23.0 and results were expressed in terms of frequency and percentage. The correlation between NLR and HbA1c values in the three groups of type-2 DM patients was carried out using Kruskal–Wallis H-test and a p value<0.05 was taken as statistically significant.

RESULTS

In this study, a total of 143 type-2 DM patients were encountered during the study period. The mean age of patients was 49 years with a slight male preponderance (52.4%) and male-to-female ratio of 1.1:1.

The total leukocyte count of the patients varied from 3,900/cu.mm to 35,000/cu.mm with the mean value of 9,238/cu.mm. The mean ANC was 4,271/cu.mm, whereas the mean ALC was 2,366/cu.mm. The mean NLR in this study was 2.76 with a minimum value of 0.70 and a maximum value of 23 among the type-2 DM patients.

The HbA1c values of the patients in this study varied from 4.4% to 15.2% with the mean value of 8.18%. On further sub-grouping, the 143 type-2 DM patients based on the HbA1c values, 62 patients (43.4%) belonged to Group A (HbA1c <7%), 28 patients (19.6%) belonged to Group B (HbA1c 7–9%), and 53 patients (37.1%) belonged to Group C (HbA1c >9%). The mean HbA1c value in Group A was 5.85, Group B was 8.08, and Group C was 10.95 (Fig. 1).

The mean age of the patients in Group A (HbA1c <7%) was 45 years, Group B (HbA1c 7–9%) was 54 years, and Group C (HbA1c >9%) was 51 years. Thus, patients with poor glycemic control (HbA1c >7%) belonged to more than 50 years of age group in this study and the association between mean patients' age and HbA1c values was found to be statistically significant (**p<0.001) (Table 1).

The gender distribution of patients in the three groups revealed that there was a slight male preponderance in Group B (60.75%) and Group C (50.9%) with equal incidence among both genders in Group A.
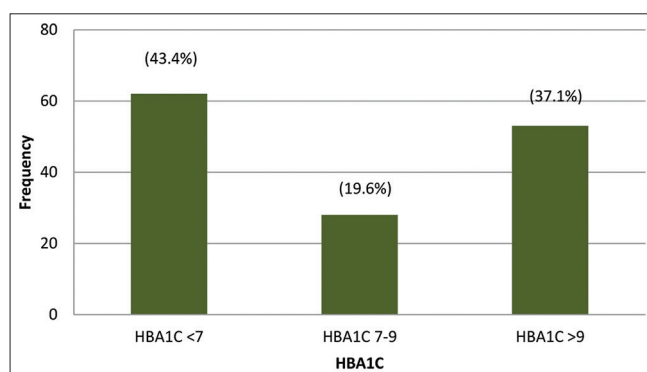

Fig. 1: Graphical representation of hemoglobin A1c (HBA1C), distribution. HBA1C <7 (n)=62 patients, HBA1C 7–9 (n)=28 patients, HBA1C >9 (n)=53 patients

However, there was no significant association of HbA1c values with the gender of patients (p=0.617) in the current study (Table 1).

In this study, the mean total leukocyte count was significantly high in Group C (10,071/cu.mm) followed by Group A (8,856/cu.mm) and Group B (8,507/cu.mm) but on correlating the mean total leukocyte count with HbA1c values, there was no statistically significant association (p=0.06) (Table 1).

The distribution of mean ANC among the three groups revealed that patients in Group C had increased mean ANC value (6,425/cu.mm) when compared to patients in Group B (5,438/cu.mm) and Group A (5,181/cu.mm). A significant association was found between ANC and HbA1c values among the three groups of patients in this study (**p<0.001) (Table 1).

The mean ALC when compared to ANC showed a slight difference with increased mean value seen in group C (2,591/cu.mm) followed by Group A (2,294/cu.mm) and Group B (2,100/cu.mm) patients. There was no significant association of HbA1c values with ALC (p=0.098) in the current study (Table 1).

The mean NLR was high in Group C (3.70) followed by Group B (3.18) and Group A (2.61). On correlating the mean NLR and HbA1c values among the three groups of patients, a statistically significant association was found (**p<0.001) (Table 1).

DISCUSSION

Type-2 DM is a chronic metabolic disorder that is characterized by persistent hyperglycemia and systemic inflammation. Among the various inflammatory markers, NLR has emerged as a novel diagnostic parameter that can be useful for evaluating the glycemic control and disease progression among type-2 DM patients.

In the present study, the mean age of type-2 DM patients was 49 years which was similar to the study by Akin *et al.* [6]. Male patients constituted around 52.4% of the total cases in the current study which was similar to the study by Jain *et al.* [7] where male patients constituted 50.45% of the total cases. However, female preponderance was seen in studies conducted by Sefil *et al.* [2] and Duman *et al.* [3].

In the current study, the mean total leukocyte count of the patients was 9238/cu.mm in concordance with the study by Da Silva *et al.* [8] where the mean value was 8542/cu.mm. It is believed that leukocytosis is strongly associated with the pathogenesis of type-2 DM and metabolic syndrome [9].

In the study by Varma *et al.* [5], 60% of type-2 DM patients had elevated ANC values of more than 7,000/cu.mm with the mean value of 8,310/cu.mm while 77.14% of patients had normal ALC. However, in the present study, the mean ANC was 4,271/cu.mm, and the mean ALC was 2,366/cu.mm.

NLR is a novel inflammatory marker with superior discriminative and predictive ability compared to total leukocyte count in monitoring the glycemic control and development of microvascular complications among type-2 DM patients [1]. NLR reveals the role of two inevitable mechanisms involved in maintaining the balance of the immune system, one being the neutrophils serving as non-specific acute inflammatory element and the other being the lymphocytes playing a protective role in chronic inflammation [10]. The mean NLR in this study was 2.76 unlike the study by Varma *et al.* [5] where the mean NLR was 6.30.

Among the three subgroups of type-2 DM patients in the present study, the mean HbA1c value in Group A was 5.85, group B was 8.08 and Group C was 10.95. Devamsh *et al.* [1] reported the mean HbA1c values of 6.36 in Group A, 7.82 in Group B and 11.6 in Group C which was almost similar to the present study.

Table 1: Association between HbA1c values and various clinico-pathological parameters

Parameters	HbA1c values			Kruskal Wallis H-test
	Group A (HbA1c <7%)	Group B (HbA1c 7–9%)	Group C (HbA1c >9%)	
Mean age (years)	45.2±13.75	54.3±10.13	51.9±10.80	**p<0.001
Gender				
a. Males	31	17	27	p=0.617
b. Females	31	11	26	
Mean total leukocyte count (cu.mm)	8856±4534	8507±3196	10071±4756	p=0.06
Mean absolute neutrophil count (cu.mm)	5181±3365	5438±3074	6425±4315	**p<0.001
Mean absolute lymphocyte count (cu.mm)	2294±682	2100±707	2591±898	p=0.098
Mean neutrophil-lymphocyte ratio	2.61±2.88	3.18±3.36	3.70±1.69	**p<0.001

Group A (n): 62 Patients, Group B (n): 28 Patients, Group C (n): 53 Patients, HbA1c: Hemoglobin A1c. **Statistically significant association of HbA1c with mean age, mean absolute neutrophil count and mean neutrophil-lymphocyte ratio

In the current study, patients with poor glycemic control (HbA1c >7%) belonged to more than 50 years of age group and there was a statistically significant association between HbA1c values and age of the patients (**p<0.001) unlike the studies by Devamsh *et al.* [1] and Pankaj *et al.* [9]. The gender distribution among the three subgroups of type-2 DM patients in the present study revealed a statistically insignificant association with HbA1c values (p=0.617) similar to the study by Devamsh *et al.* [1].

In this study, type-2 DM patients with poor glycemic control (HbA1c >9%) belonging to group C showed elevated mean total leukocyte count (10,071/cu.mm), mean ANC (6,425/cu.mm), and mean ALC (2,591/cu.mm). However, mean total leukocyte count (p=0.06) and mean ALC (p=0.098) showed statistically insignificant association with HbA1c values while mean ANC (**p<0.001) showed significant association with HbA1c values among the three subgroups of type-2 DM patients. In the study by Hussain *et al.* [11], HbA1c values showed statistically significant association with total leukocyte count (**p=0.001) and ANC (**p=0.001) while Swathi *et al.* [12] reported significantly elevated total leukocyte count among type-2 DM patients with HbA1c values more than 7% (**p=0.001).

The mean NLR was significantly elevated in patients belonging to Group C with poor glycemic control. Thus, there was a statistically significant association between NLR and HbA1c values in the current study (**p<0.001) in concordance with the studies conducted by Varma *et al.* [5], Devamsh *et al.* [1] and Adane *et al.* [13]. In the studies by Gurmu *et al.* [14] and Khandare *et al.* [15], NLR was significantly elevated in type-2 DM patients with microvascular complications like diabetic nephropathy indicating that NLR could serve as predictive and prognostic biomarker of inflammation and endothelial dysfunction occurring in patients with diabetic nephropathy having poor glycemic control. In the study by Dudani *et al.* [16], type-2 DM patients with fair glycemic control (HbA1c <7%) did not show lower NLR values indicating that strict glycemic control alone may not ameliorate the chronic inflammation seen in diabetic patients until dyslipidemia is corrected. HbA1c is found to have a direct correlation with elevated serum low-density lipoprotein cholesterol and triglycerides [17], thus serving as an useful biomarker along with NLR for predicting cardiovascular complications in type-2 DM patients. DM is the leading cause of kidney failure, heart diseases, and blindless worldwide [18]. Glomerular capillary endothelial damage, mechanical stress, and inflammation contribute to the overt albuminuria seen in diabetic nephropathy patients [19]. Thus, inflammation plays a critical role in the pathogenesis of diabetic microangiopathies including diabetic nephropathy which can be detected by measuring the NLR values.

Limitations

The present study was based on laboratory data. The clinical status of the patients with type-2 DM and the presence of diabetes-related vascular complications were not included in this study. Furthermore, the patients were on different groups of anti-diabetic medications with different mechanisms of glycemic control. Further studies incorporating the clinical data, laboratory data, type of anti-diabetic medications used, presence of various vascular complications, and follow-up information will provide better insight into the relationship between NLR and the clinical severity of type-2 DM.

CONCLUSION

NLR is a valuable marker of systemic inflammation and poor glycemic control in type-2 DM patients**.** A linear association is seen between HbA1c and NLR values, that is, the higher the HbA1c higher the NLR values. Given its strong predictive value, NLR should be considered alongside HbA1c as a routine biomarker for diabetes management**.** Further studies are needed to establish standardized NLR cutoff values for risk stratification and intervention planning**.** Thus, NLR can serve as a simple and cost-effective diagnostic test that can be used as a biomarker of sub-clinical inflammation and also predict the development of cardiovascular complications in type-2 DM patients with poor glycemic control.

AUTHORS' CONTRIBUTION

The concept and study design were carried out by Dr. Karthik Sigamani. Dr. Shimi Sunder Raja Kumar was responsible for data acquisition, data analysis, and interpretation. Dr. Karthik Sigamani and Dr. Shimi Sunder Raja Kumar were responsible for drafting the manuscript and performing statistical analysis. Critical revision of the manuscript and overall supervision were provided by Dr. Karthik Sigamani. Both the authors have contributed equally.

CONFLICT OF INTEREST

The authors declare no Conflict of interest, Financial or otherwise.

REFERENCES

1. Devamsh GN, Parvathi M, Madhumathi R, Raghavan L. Study of neutrophil lymphocyte ratio in patients with type 2 diabetes mellitus and its correlation with glycemic control. Int J Adv Med. 2019;6(5):1637-41. doi: 10.18203/2349-3933.ijam20194233
2. Sefil F, Ulutas KT, Dokuyucu R, Sumbul AT, Yengil E, Yagiz AE, *et al*. Investigation of neutrophil lymphocyte ratio and blood glucose regulation in patients with type 2 diabetes mellitus. J Int Med Res. 2014 Apr;42(2):581-8. doi: 10.1177/0300060513516944, PMID 24567354
3. Duman TT, Aktas G, Atak BM, Kocak MZ, Erkus E, Savli H. Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. Afr Health Sci. 2019;19(1):1602-6. doi: 10.4314/ahs. v19i1.35, PMID 31148989
4. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): Analysis of glucose profiles and HbA(1c) in the diabetes control and complications trial. Diabetes Care. 2002;25(2):275-8. doi: 10.2337/diacare.25.2.275, PMID 11815495

5. Varma S, Khande M, Gupta PA, Khare RL, Malhotra Y, Lakra D, *et al*. Correlation of neutrophil lymphocyte ratio with HbA1c in patients of type 2 diabetes mellitus. Int J Adv Med. 2021;8(7):952-6. doi: 10.18203/2349-3933.ijam20212407

6. Akin S, Aydin Z, Yilmaz G, Aliustaoglu M, Keskin O. Evaluation of the relationship between glycaemic regulation parameters and neutrophil-to-lymphocyte ratio in type 2 diabetic patients. EMJ Diabetes. 2019;7:91-6. doi: 10.33590/emjdiabet/10311581

7. Jain A, Bhatt K, Purohit CP, Sanadhya HC. Correlation of HbA1c and neutrophil lymphocyte ratio in type 2 diabetes mellitus as a marker of blood sugar control - a case control study. J Cardiovasc Dis Res. 2023;14(02):2057-69.

8. Da Silva TE, Christine I, Djaputra EM. Blood sugar levels with neutrophil-lymphocyte ratio as a marker of diabetes mellitus in elderly. J Widya Medika Jr. 2020;2(3):203-8. doi: 10.33508/jwmj.v2i3.2667

9. Pankaj J, Mohit G, Badri S, Pratap SU. Association of HbA1c and neutrophil-to-lymphocyte ratio in Type 2 diabetic patients: An observational study. Eur J Cardiovasc Med. 2023;13:1020-4.

10. Mahankali P, Nannapaneni S, Vallabhaneni KC, Damera NC, Diddi R. Association of neutrophil lymphocyte ratio and glycaemic control in patients with type 2 diabetes mellitus. Natl J Lab Med. 2021;10(3):70-2.

11. Hussain M, Babar MZ, Akhtar L, Hussain MS. Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycemic control in type 2 diabetic patients. Pak J Med Sci. 2017;33(6):1366-70. doi: 10.12669/pjms.336.12900, PMID 29492060

12. Swathi M, Ramya T, Gangaram U, Kumar KJ, Raj BS. A study of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) with glycosylated hemoglobin (HbAa1c) among type-2 diabetic patients. Int J Acad Med Pharm. 2023;5(4):2753-6556.

13. Adane T, Melku M, Worku YB, Fasil A, Aynalem M, Kelem A, *et al*. The association between neutrophil-to-lymphocyte ratio and glycemic control in type 2 diabetes mellitus: A systematic review and meta-analysis. J Diabetes Res. 2023;3:117396.

14. Gurmu MZ, Genet S, Gizaw ST, Feyisa TO, Gnanasekaran N. Neutrophil-lymphocyte ratio as an inflammatory biomarker of diabetic nephropathy among type 2 diabetes mellitus patients: A comparative cross-sectional study. SAGE Open Med. 2022;10:1-7. doi: 10.1177/20503121221140231, PMID 36505969

15. 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 Endocrinol Metab. 2017;21(3):387-92. doi: 10.4103/ijem.IJEM_476_16, PMID 28553592

16. Dudani S, Poodury S, Mangalesh S. Study of neutrophil-lymphocyte ratio (NLR) in recent onset type 2 diabetes mellitus. Bali Med J. 2021;10(1):11-6. doi: 10.15562/bmj.v10i1.1780

17. Kamalesh DR, Geetha KM. A review on role of markers in diabetes mellitus and associated micro and macrovascular complications. Int J Curr Pharm Res. 2022;14(1):20-6.

18. Yosmar R, Shepany E, Fitria N. A comprehensive analysis of antidiabetic drug interactions in geriatric non-insulin dependent diabetes mellitus patients. Int J Appl Pharm. 2024;16(1):62-5.

19. Sarkar S, Sadhu S, Roy R, Tarafdar S, Mukherjee N, Sil M, *et al*. Contemporary drifts in diabetes management. Int J App Pharm. 2023;15(2):1-9. doi: 10.22159/ijap.2023v15i2.46792