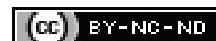


Association of Neutrophil Lymphocyte Ratio and Glycaemic Control in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Neutrophil-Lymphocyte Ratio (NLR) is an easily measurable novel marker of systemic inflammation and is related to cardiovascular disease in patients with type 2 diabetes mellitus. Glycated haemoglobin A (HbA1c) levels are an indicator of blood glucose regulation. However, HbA1c usually do not predict diabetes associated complications accurately.

Aim: To measure NLR and HbA1c values and to determine their association in patients with type 2 diabetes mellitus.

Materials and Methods: This observational analytical study was conducted in a tertiary care centre. Data was collected over a period of six months from June 2020 to December 2020. 100 random patients diagnosed with type 2 diabetes mellitus according to American Diabetes Association (ADA) criteria were included in the study. Patients were divided into two groups, group A with HbA1c ≤ 7

(regulated diabetes) and group B with >7 HbA1c (unregulated diabetes).

Results: Using independent t-test, the NLR ratios were found to be significantly higher in group B when compared to group A (2.41 ± 0.11 verses 1.31 ± 0.57 , respectively). Mean HbA1c (%) levels were 6.67 ± 0.14 and 8.79 ± 0.21 in groups A and B, respectively. Linear regression analysis showed that NLR correlated positively with HbA1c with a p-value < 0.001 .

Conclusion: NLR is an easily available, safe, cost-effective and a simple test. It can also guide the physician in resource limited settings like Primary Health Centres (PHC) to evaluate a patient with type 2 diabetes for microvascular and macrovascular complications. So, it can be used as a disease monitoring tool during the follow-up of diabetics.

Keywords: Glycated haemoglobin A, Macrovascular, Microvascular, Unregulated diabetes

INTRODUCTION

The NLR is an easily measurable novel inflammatory marker and inexpensive laboratory index calculated by simple division of absolute neutrophil count by absolute lymphocyte count in hemogram [1]. Increase in NLR has been demonstrated to be a greater risk factor than increase in total WBC count in the prediction of adverse outcomes in various medical conditions [2-5]. It combines the negative effects of neutrophils on endothelial damage with the anti-atherosclerotic role of lymphocytes [6]. Therefore, considered an appropriate indicator for systemic inflammation and increased risk for cardiovascular events in patients with metabolic syndrome [7-9].

Chronic low-grade inflammation may be involved in the pathogenesis of insulin resistance and type 2 diabetes [10]. HbA1c is used to measure the long-term glycaemic control in patients with diabetes. It has been a major tool in assessing the adequacy of therapy and need for intervention in patients with poorly controlled diabetes mellitus. However, HbA1c values does not predict inflammatory process or diabetes associated complications with precision. Complications due to uncontrolled diabetes are major causes of death in many countries. Cardiovascular disease is the most common complication associated with poor glycaemic control in diabetics and leading cause of death in many populations. Several steps have to be taken in order to control the epidemic of diabetes and to recognise the complications of diabetes at an early stage thereby reducing morbidity and mortality due to diabetes related complications [11]. This study aims to determine the association between HbA1c values and NLR in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

This observational study was carried out in a tertiary care centre . Data was collected over a period of six months from June 2020

to December 2020. Informed consent was taken from the study participants and Institutional ethical committee clearance was obtained (Reference number: PG/537/20).

Inclusion criteria: Patients in the age group of 31-65 years with type 2 diabetes mellitus according to American Diabetic Association (ADA) criteria, within the specified period of six months, were included in the study.

Exclusion criteria: Patients with pregnancy, evidence of active infection, autoimmune disorders, malignancy or haematological proliferative disorder, history of anti-inflammatory or immunosuppressive therapy are excluded from the study.

The ADA's latest guidelines (2018) indicate that the glycaemic target for non-pregnant adults with diabetes is $<7.0\%$ and maintaining this threshold will reduce the risk of complications associated with diabetes. Therefore, a HbA1c level of $<7.0\%$ indicates good glycaemic control for most adults with diabetes.

After overnight fasting, venous samples were collected in Ethylenediamine Tetraacetic Acid (EDTA) vacutainers at room temperature. Within one hour absolute neutrophil counts and absolute lymphocyte counts were measured by automated haematology analyser. NLR were obtained by dividing the absolute neutrophil count by absolute lymphocyte count. HbA1c levels were measured by high performance liquid chromatography using automated ion exchange method from the same blood sample.

STATISTICAL ANALYSIS

Data was analysed to obtain mean values with standard deviation. SPSS 21.0 (trial version) software was used for statistical analysis. Linear regression analysis was done to determine any direct relationship between HbA1c and NLR. A p-value of <0.05 was considered statistically significant.

RESULTS

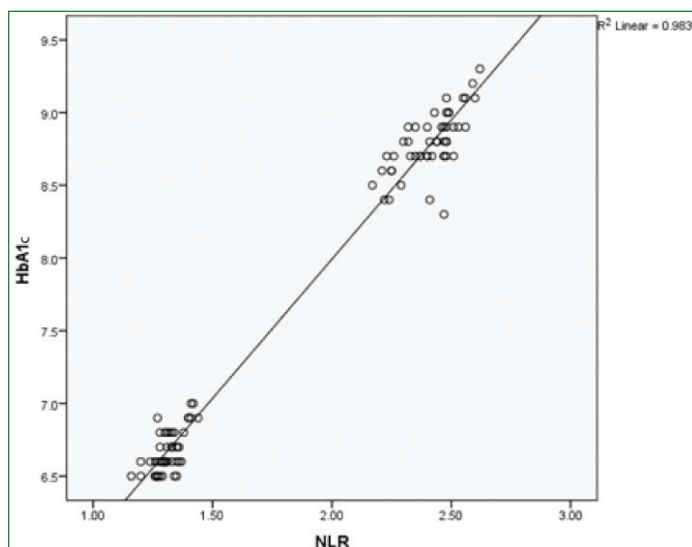
Patients were divided into two groups. Group A with HbA1c ≤ 7 (regulated diabetes) and group B with >7 HbA1c (unregulated diabetes). Age and sex distribution of both groups is represented in [Table/Fig-1]. NLR was significantly higher in group B when compared with group A. Mean HbA1c (%) levels were 6.67 ± 0.14 and 8.79 ± 0.21 in groups A and B, respectively [Table/Fig-2]. Linear regression analysis showed that NLR correlated positively with HbA1c with a p-value < 0.001 [Table/Fig-2] and r-value 0.983 [Table/Fig-3].

Age (years)	Group A	Group B
31-45	4	2
46-55	26	20
56-65	20	28
Mean age in years	48.30 ± 10.95	57.32 ± 6.75
Male to female ratio	1.27:1	1.08:1

[Table/Fig-1]: Age and sex distribution of study population.

Parameter	Group A (HbA1c $\leq 7\%$)	Group B (HbA1c $> 7\%$)	p-value
Absolute neutrophil count	46.82 ± 2.1	66.9 ± 1.7	< 0.001
Absolute lymphocyte count	35.54 ± 1.01	27.67 ± 0.98	< 0.001
Neutrophil lymphocyte ratio	1.31 ± 0.57	2.41 ± 0.11	< 0.001
HbA1c	6.67 ± 0.14	8.79 ± 0.21	< 0.001

[Table/Fig-2]: Parameters (Mean \pm Standard deviation) of study population. p < 0.05 was considered as statistically significant



[Table/Fig-3]: Correlation graph of HbA1c and NLR.

DISCUSSION

The NLR demonstrates role of two inevitable mechanisms simultaneously in maintaining the balance of the immune system one of which is neutrophils acting as the nonspecific inflammatory mediator, other one being lymphocytes playing the protective component of inflammation [12]. There is a kind of correlation between chronic subclinical inflammation, insulin resistance, metabolic syndrome and atherosclerosis [13-15]. Chronic elevated blood glucose levels may result in increased release of reactive oxygen species from neutrophils that eventually lead to endothelial injury [16]. NLR is a dynamic parameter and appears to possess a superior predictive value over total leukocyte count in assessing complications of diabetes mellitus [12]. NLR is cost-effective, economical and widely available more than any other inflammatory marker available in the current scenario. Patients could have increased risk of atherosclerosis related diseases even with normal total leucocyte counts if there is increased NLR [17-19].

Evidence from many studies suggest that NLR is positively associated with severity of metabolic syndrome [9]. HbA1c level is reliable

indicator of blood glucose regulation, and increased HbA1c levels have shown to be associated with increased risk of cardiovascular complications in patients with type 2 diabetes mellitus [20-22]. In the current study, a positive correlation was shown between HbA1c levels and NLR in type 2 diabetes mellitus. A prominent and reliable role of NLR was seen in various studies conducted on diabetic patients [23].

Devamsh GN et al., in their study found a significant positive correlation between NLR and glycaemic control in accordance with the present study [11]. Shiny A et al., revealed that NLR has strong positive correlation with glucose intolerance and insulin resistance in type 2 diabetic patients [7]. Oh Y et al., and Demirtas L et al., also came to similar conclusions as the present study [24,25]. Akba EM et al., and Huang W et al., have been suggested that high levels of NLR in patients is a safe predictor of early stage diabetic nephropathy [26,27]. In a study performed by Sonmez O et al., a strong association was found between high NLR and severity of coronary artery disease [28]. The relation between incidence of diabetes associated complications and NLR has also become a current issue of investigation. Yilmaz H et al., who investigated the advantages and reliability of NLR in the diagnosis of gestational DM, found a positive correlation between NLR values and incidence of gestational diabetes, and stated that NLR value > 2.93 had a sensitivity of 76% and a specificity of 94% for detection of gestational DM [29]. Ulu S et al., stated that increased NLR in diabetic individuals could be a potential risk factor for sensorineural hearing loss [30].

Limitation(s)

The limitations of the present study include small sample size, certain confounding factors like duration of diabetes, consistency in glycaemic control could not be assessed. Effects of different antidiabetic drug regimens on white cell counts may be a limiting factor.

CONCLUSION(S)

NLR is an easily available, safe and a simple test. NLR is not only a marker of subclinical inflammation but also an indicator of poor glycaemic control in patients with type 2 diabetes. It can also guide the physician in the resource limited settings like PHC to evaluate a patient with type 2 diabetes for microvascular and macrovascular complication. So, it can be used as a reliable marker for monitoring the morbidity during the follow-up of diabetics.

REFERENCES

- [1] Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, et al. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *Journal of Hepatology*. 2013;58(1):58-64.
- [2] Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Atherosclerosis Risk in Communities Study: Low-grade systemic inflammation and the development of type 2 diabetes: The Atherosclerosis Risk in Communities Study. *Diabetes*. 2003;52(7):1799-805.
- [3] Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. *International archives of medicine*. 2012;5(1):01-06.
- [4] Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Kubota K. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer. *Br J Cancer*. 2013;109(2):401-07.
- [5] Sen N, Afsar B, Ozcan F, Buyukkaya E, Isleyen A, Akcay AB, et al. The neutrophil to lymphocyte ratio was associated with impaired myocardial perfusion and long term adverse outcome in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. *Atherosclerosis*. 2013;228(1):203-10.
- [6] Cantor H, Simpson E. Regulation of the immune response by subclasses of T lymphocytes. I. Interactions between pre-killer T cells and regulatory T cells obtained from peripheral lymphoid tissues of mice. *European Journal of Immunology*. 1975;5(5):330-36.
- [7] 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(8):524-30.

- [8] Gibson PH, Cuthbertson BH, Croal BL, Rae D, El-Shafei H, Gibson G, et al. Usefulness of neutrophil/lymphocyte ratio as predictor of new-onset atrial fibrillation after coronary artery bypass grafting. *Am J Cardiol*. 2010;105(2):186-91.
- [9] 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. *Clin Appl Thromb Hemost*. 2014;20(2):159-63.
- [10] Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes*. 2002;51(2):455-61.
- [11] Devamsh GN, Parvathi M, Madhumathi R, Raghavan L. Study of neutrophil lymphocyte ratio in patients with type 2 diabetes mellitus and its correlation with glycaemic control. *Int J Adv Med*. 2019;6(5):1637-41.
- [12] 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 Surg*. 2011;45(3):227-31.
- [13] Tsai JC, Sheu SH, Chiu HC, Chung FM, Chang DM, Chen MP, et al. Association of peripheral total and differential leukocyte counts with metabolic syndrome and risk of ischemic cardiovascular diseases in patients with type 2 diabetes mellitus. *Diabetes Metab Res Rev*. 2007;23(2):111-18.
- [14] Stulc T, Ceska R, Marinov I, Skrha J. The effect of simvastatin and fenofibrate on the expression of leukocyte adhesion molecules and lipopolysaccharide receptor CD14 in type 2 diabetes mellitus. *Neuro Endocrinol Lett*. 2012;33(suppl 2):73-77.
- [15] Eckel RH, Wassef M, Chait A, Sobel B, Barrett E, King G, et al. Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group II: pathogenesis of atherosclerosis in diabetes. *Circulation*. 2002;105(18):e138-43.
- [16] Saito Y, Takahashi I, Iwane K, Okubo N, Nishimura M, Matsuzaka M, et al. The influence of blood glucose on neutrophil function in individuals without diabetes. *Luminescence*. 2013;28(4):569-73.
- [17] Ozturk C, Balta S, Balta I, Demirkol S, Celik T, Turker T, et al. Neutrophil-lymphocyte ratio and carotid-intima media thickness in patients with Behcet disease without cardiovascular involvement. *Angio*. 2015;66(3):291-96.
- [18] Balta S, Celik T, Mikhailidis DP, Ozturk C, Demirkol S, Aparci M, et al. The relation between atherosclerosis and the neutrophil-lymphocyte ratio. *Clinical Applied Thrombosis/Hemostasis*. 2016;22(5):405-11.
- [19] Balta S, Kurtoglu E, Kucuk U, Demirkol S, Ozturk C. Neutrophil-lymphocyte ratio as an important assessment tool. *Expert Review Cardiovasc Ther*. 2014;12(5):537-38.
- [20] Fernández-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev*. 2003;24(3):278-301.
- [21] Andersson C, van Gaal L, Caterson ID, Weeke P, James WP, Coutinho W, et al. Relationship between HbA1c levels and risk of cardiovascular adverse outcomes and all cause mortality in overweight and obese cardiovascular high-risk women and men with type 2 diabetes. *Diabetologia*. 2012;55(9):2348-55.
- [22] Yousefzadeh P, Wang X. The effects of dipeptidyl peptidase-4 inhibitors on cardiovascular disease risks in type 2 diabetes mellitus. *J Diabetes Res*. 2013;2013:459-821.
- [23] Hussain M, Babar MZM, Akhtar L, Hussain MS. Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycaemic control in Type-2 diabetic patients. *Pak J Med Sci*. 2017;33(6):1366-70.
- [24] Oh Y, Kwon GC, Koo SH, Kim J. Association between glycaemic control and haematological indices in type 2 diabetic patients. *Lab Med Online*. 2016;6(3):134-39.
- [25] Demirtas L, Degirmenci H, Akbas EM, Ozcicek A, Timuroglu A, Gurel A, et al. Association of haematological indices with diabetes, impaired glucose regulation and microvascular complications of diabetes. *Int J Clin Exp Med*. 2015;8(7):11420-27.
- [26] Akbas EM, Timuroglu A, Ozcicek A, Ozcicek F, Demirtas L, Gungor A, et al. Association of uric acid, Atherogenic index of plasma and albuminuria in diabetes mellitus. *Int J Clin Exp Med*. 2014;7(12):5737-43.
- [27] 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. *Clin Endocrinol (Oxf)* 2015;82(2):229-33.
- [28] Sönmez O, Ertaş G, Bacaksız A, Tasa A, Erdoğan E, Asoğlu E, et al. Relation of neutrophil-to-lymphocyte ratio with the presence and complexity of coronary artery disease: An observational study. *Anadolu Kardiyol Derg*. 2013;13(7):662-67.
- [29] Yılmaz H, Celik HT, Namuslu M, Inan O, Onaran Y, Karakurt F, et al. Benefits of the neutrophil-to-lymphocyte ratio for the prediction of gestational diabetes mellitus in pregnant women. *Exp Clin Endocrinol Diabetes*. 2014;122(1):39-43.
- [30] 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):2681-86.

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PLAGIARISM CHECKING METHODS: ^[Jain H et al.]

- Plagiarism X-checker: Jan 16, 2021
- Manual Googling: May 05, 2021
- iThenticate Software: May 08, 2021 (24%)

ETYMOLOGY: Author Origin**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Jan 13, 2020**Date of Peer Review: **Apr 04, 2021**Date of Acceptance: **May 06, 2021**Date of Publishing: **Jul 01, 2021**