



Prognostic significance of neutrophil lymphocyte ratio in patients with type 2 Diabetes mellitus and it's correlation with glycemic control and complications

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ABSTRACT

INTRODUCTION: White blood cells do play a significant role when there is any deterioration of the vascular wall in diabetes. Thus, the neutrophil-to-lymphocyte ratio (NLR) became widely investigated.^{3,16} The neutrophils are strongly associated with ongoing or impending inflammation and lymphocytes reflect the pathway of a regulated immune response to the inflammation **AIMS AND**

OBJECTIVES: 1. To assess the relationship between neutrophil lymphocyte ratio and various levels of glycemic control in Type 2 Diabetes Mellitus patients. 2. To compare the relationship between neutrophil lymphocyte ratio and HbA1C in patients with excellent control, poor control, and worst diabetes control. 3. To study the correlation between neutrophil lymphocyte ratio and complications due to diabetes. **MATERIAL AND METHODS:** This comparative prospective study was conducted at the L.N. Medical college & research center associated with J.K. Hospital Bhopal during period of one and half year from December 2019 to August 2021. Research study was approved by institutional ethical committee. **RESULTS:** This study was a cross sectional study carried out at a tertiary care center located at urban area. A total of two thousand patients with type 2 diabetes mellitus were screened for their eligibility to be recruited for the study. **CONCLUSION:** . Neutrophil lymphocyte ratio correlates positively with HbA1c and presence of complications

KEYWORDS: NLR, HbA1C, Diabetes Mellitus, Complications

INTRODUCTION:

Global prevalence of diabetes is increasing. As per the International Diabetes Federation, India ranks second in adult diabetic population after China. India accounts for 1 in 7 of all adults living with diabetes in the world. Also notable is that population living in the low and middle-income countries constitutes for three-quarter of the global diabetic population.¹ The chronic micro- and macrovascular complications contribute to the ever-increasing burden of disease-related morbidity and mortality. Chronic subclinical inflammation is responsible for the development of diabetic complications.^{2,3} Inflammation plays a key role underlying the microvascular complications as well.^{4,5}

Glycosylated hemoglobin (HbA1c) is a useful marker for its role in assessing the glycaemic control in patients with type 2 diabetes mellitus (T2DM).⁶ The role of advanced glycation products plays a key role in pathogenesis of insulin secretion and insulin resistance. It is associated with the various micro-and macrovascular complications of diabetes mellitus.^{7,8}



White blood cells do play a significant role when there is any deterioration of the vascular wall in diabetes. After studying the various subtypes of leucocytes, no component could generate as much evidence as the ratio of neutrophils to lymphocytes did. Thus, the neutrophil-to-lymphocyte ratio (NLR) became widely investigated.^{3,9}The neutrophils are strongly associated with ongoing or impending inflammation and lymphocytes reflect the pathway of a regulated immune response to the inflammation. The type 2 diabetes is a state of chronic inflammation, leading to complications. The potential for NLR to be a biomarker is significant.¹⁰

Our study aims to address the relationship between NLR and glycaemic control. It also aims to study if NLR can be used to predict the micro- and macrovascular complications of type 2 diabetes mellitus.

AIMS & OBJECTIVES

AIMS:

To assess the relationship between neutrophil lymphocyte ratio and various levels of glycemic control in Type 2 Diabetes Mellitus patients.

OBJECTIVES :

1. To compare the relationship between neutrophil lymphocyte ratio and HbA1C in patients with excellent control, poor control, and worst diabetes control.
2. To study the correlation between neutrophil lymphocyte ratio and complications

MATERIAL AND METHODS:

This comparative prospective study was conducted at the L.N. Medical college & research center associated with J.K. Hospital Bhopal during period of one and half year from December 2019 to August 2021. Research study was approved by institutional ethical committee

Total 450 patients from OPD and IPD from various clinical departments were enrolled in the study according to inclusion criteria. All enrolled patients surveyed for : Age, gender, history of smoking & alcohol , family history of type 2 Diabetes, any significant past medical history and dietary preference.

Informed consent was taken from all patients.

INCLUSION CRITERIA

1. Age >18 years
2. Known case of type 2 diabetes mellitus
3. Able to give a written informed consent.

EXCLUSION CRITERIA

1. Acute or chronic infections and inflammatory conditions for last two weeks
2. Patients with bleeding disorders, hematological disorders , autoimmune diseases, cancers ,severe liver disorder.
3. Type 1 diabetes mellitus.

SAMPLE COLLECTION:

1. **Samples for Complete blood count** – For CBC blood was drawn from the median cubital vein, that allowed technically good sampling. Sample was collected in K3 EDTA vial (Tri- potassium salt of ethylene diamine tetra acetic acid act as an anticoagulant). All samples were processed on automated blood cell counter analyzer (BC-5380 Mindray 5-part hematology analyzer) and results



noted. All tests were conducted within 1 hour of sample collection to minimize variation due to sample aging. Neutrophil lymphocyte ratio(NLR) was calculated by dividing the absolute neutrophil count to absolute lymphocyte count.

2. Samples for HbA1C – For analysis of HbA1C blood sample was collected after an overnight fast. Samples were drawn from the median cubital vein and 2 ml blood was collected in EDTA vial under aseptic conditions.HbA1C estimation was done by using Bio rad d10 analyzer. All samples were processed within 2hours of collection.

STATISTICAL ANALYSIS

Statistical analysis was done by using suitable software. Data was presented as mean \pm standard deviation for continuous variables and percentages for categorical variables. The Mann- Whitney *U* -test for comparing two groups and Kruskal Wallis H Test for comparing 3 groups of continuous variables was used because the distribution of variables was non-parametric. Pearson Chi-square test was used to compare the categorical variables. Spearman's rho was used for correlational analysis in view of non-parametric distribution of variables. Multinomial logistic regression analysis was used since the dependent variable (glycaemic control) had more than 2 outcomes. A multinomial logistic regression was done with complication status being kept as the dependent variable. Regression models are shown as odds ratio (OR) and 95% confidence interval (CIs).

RESULTS:

This study was a cross sectional study carried out at a tertiary care center located at urban area. A total of two thousand patients with type 2 diabetes mellitus were screened for their eligibility to be recruited for the study. A total of 1550 patients had to be excluded from participation due to several reasons discussed here on. Thirty patients refused to consent for participation. 20 patients were known to be suffering from acute inflammation while their lab parameters were being examined. A history of high-grade fever was present in the preceding week for fifty patients. The COVID-19 pandemic was an unexpected factor that fell within our exclusion criterion of acute inflammation. The patients excluded due to an active COVID-19 infection status were one thousand four hundred and fifty. Out of these 1450 patients, were those having various complications of diabetes as well. We were left with 450 patients which were categorised into three groups of glycaemic control as evident from their HbA1c reports. These patients were then our final sample whom we have analysed and reported.

The basic physical profile including age, weight and BMI amongst the patients were not found to have any statistically significant difference when compared amongst the three groups of differing glycaemic control.The demographic variables of gender, diet, current smoking status, current alcohol use, presence of complication and the total duration of diabetes were found to have a statistically significant difference among the three groups.Females were found to have higher number with good glycaemic control and lower numbers of poor and worst glycaemic control.

Studies and meta-analysis have shown that vegetarian diet leads to lower HbA1c levels in diabetic patients, thus leading to a better glycaemic control, although this had a small effect size.Current



smoking status in our study was associated with a higher prevalence of poor and worst glycaemic control. Smoking has been a modifiable risk factor for diabetes mellitus with much associated importance.

Alcohol use, in our study population was associated with a higher prevalence of poor and worst glycaemic control. We had more than 62% of our population with a duration of diabetes more than 5 years. The risk of complication related mortality and poor glycaemic control was linked to a longer duration of diabetes. The incidence of micro- as well as macrovascular complications in high with poorer glycaemic control as evidenced in literature

Laboratory Parameters across the three groups

Among clinical laboratory parameters all 450 patients, the total leucocyte counts varied by levels of glycaemic control. Our study found increasing mean ranks of TLC as the glycaemic control worsened, in line with another study having the same cut offs for glycaemic control. As with TLC, the neutrophil counts were significantly higher, reasons for which per the literature could be many. The role of a chronic sub-clinical inflammation may be highlighted. NLR was found to significantly increase as the glycaemic control worsened. Our study found the diabetic patients with excellent control, poor control, and worst control to have NLR of 1.9, 2.1 and 2.7. We found relative lymphopaenia

Laboratory parameters compared by status of complications

a) Among the population with *no complications*, we found TLC, Neutrophil %, Lymphocyte %, Absolute Neutrophil Count and NLR to be significantly different across three groups..

b) The same parameters were found to vary significantly among patients across the three groups with *diabetic retinopathy* and in line with results seen in multiple studies.

c) No parameters were found to vary between the three groups for patients diagnosed with diabetic nephropathy or diabetic neuropathy

d) Those with cardiovascular complications had a significant difference in TLC levels across the three groups whereas no differences could be appreciated with individual components of the leucocytes.

Correlations between NLR and Complications

The results showed that the two main parameters of our study, HbA1c and NLR significantly and positively correlated with each other. We infer that NLR raises with increasing levels of HbA1c and could be used as a marker for monitoring glycaemic control

DISCUSSION:

This study was done with a background that type 2 diabetes mellitus had a high burden of disease and India was unfortunately among the leading countries in the world in terms of prevalence.¹ The understanding of this disease has revealed pathophysiology in terms of an underlying systemic inflammation as well as the impaired glycaemic control.

The diagnosis is based on the WHO classification and long-term disease monitoring with HbA1c as the marker of long-term glycaemic control, informing us of an average blood glucose over 2 – 4



months.⁶The importance of this marker cannot be stressed enough but there are areas where it is not as robust as any marker could be. This begins with the most distressing consequences of having long term uncontrolled type 2 diabetes, where various macro- and microvascular complications set in.^{10,11} Although HbA1c significantly correlates with prevalence of such complications, it falls short on being a marker with sensitivity enough to pick up the beginning stages of the complications. It also relatively expensive for those belonging to the south Asian sub-continent. The underlying pathophysiology has chronic sub-clinical inflammation at its core and therefore researchers focused on inflammatory markers like CRP, S. Fibrinogen., TLC, Neutrophil %, NLR and S. Albumin. NLR has shown promising results for being a marker for various micro- as well as macrovascular complications.^{2,12,13}

NLR has also has prognostic significance in the outcome of treatments for the complications as well as risk of developing such complications. There is the need to study this marker and see if it may serve as reliable marker for predicting complications due to diabetes as well as glycaemic control.¹⁰ There are few studies conducted in India with cross sectional design.¹⁴⁻¹⁹

Our study was a cross sectional observational study. We screened two thousand patients and selected four hundred and fifty patients that fit our inclusion criteria. We grouped them in three categories based on their glycaemic control guided by the ADA criteria.¹⁸ The three groups were of good control (HbA1c < 7%), poor control, and worst control. Similar categorization has been used in various other studies where 3 groups were made according to the HbA1c level.¹⁹ .Our study also well correlated with few studies done in the past.

CONCLUSION:

We deduce that patients with complications due to type 2 diabetes mellitus, glycaemic control may be stratified by a simple test such as complete blood count, when looking at the ratio of neutrophil% to lymphocyte% which is an easily accessible test, inexpensive and informative. The aim of the study was to assess the relationship between neutrophil lymphocyte ratio and various levels of glycemic control in Type 2 Diabetes Mellitus patients as well as its correlation with various micro- and macrovascular complications. In the study, it was found that NLR differs across various levels of glycaemic control. NLR correlates positively with HbA1c and presence of complications

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