

A STUDY OF NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) AND PLATELET TO LYMPHOCYTE RATIO (PLR) WITH GLYCOSYLATED HEMOGLOBIN (HBA1C) AMONG TYPE-2 DIABETIC PATIENTS

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Abstract

Background: Diabetes Mellitus (D.M) is a lifelong disorder associated with serious microvascular and macrovascular complications. As there is ongoing low-grade inflammation in D.M leading to cardiac complications, we need a reliable marker to identify the inflammation at an early stage and predict the CVD (cardiovascular) risk. Neutrophil-lymphocyte ratio (NLR) and Platelet-lymphocyte ratio (PLR) are emerging inflammatory markers. The aim of this study was to evaluate NLR and PLR as inflammatory markers in diabetic patients and to determine the association with HbA1c (Glycosylated Hemoglobin). **Materials and Methods:** This study was done on 105 patients diagnosed with type 2 diabetes mellitus. FBS (Fasting Blood Glucose), HbA1c and CBP (Complete Blood Picture) were performed in all subjects. NLR and PLR were calculated from CBP results. The diabetic patients were divided into 2 groups based on the HbA1c values. Group 1 includes 62 patients with HbA1c value less than 7% and Group 2 includes 43 patients with HbA1c value more than 7%. SPSS software was used for statistical analysis. Unpaired T test was used for continuous variables. A p value < 0.05 was considered statistically significant. **Results:** The mean and S.D values of NLR in group 1 was 2.0079 ± 0.7445 and group 2 diabetic patients was 2.824 ± 1.494 respectively. The mean and S.D values of PLR in group 1 was 114.32 ± 36.149 and group 2 diabetic patients was 132.20007 ± 53.694 respectively. The NLR and PLR values were higher in group 2 when compared to group 1 diabetic patients and it was statistically significant ($p=0.0004$) and ($p=0.04$) respectively. **Conclusion:** In our study, NLR and PLR were found to be significantly associated with poor glycemic control. Type 2 diabetes mellitus patients with raised NLR and raised PLR should be evaluated for CVD risk and other complications of diabetes.

INTRODUCTION

Diabetes is a major health issue that has reached alarming levels. Today, more than half a billion people are living with diabetes worldwide. In 2021, it is estimated that 537 million people have diabetes, and this number is projected to reach 643 million by 2030, and 783 million by 2045. Major concern is that the number of children and adolescents living with diabetes increases annually.^[1] Thus, Diabetes

has emerged to be one of the main threats to human health in the twenty-first century.^[2]

In South-East Asia, 1 in 11 adults (90 million) are living with diabetes. The number of adults with diabetes is expected to reach 113 million by 2030 and 151 million by 2045.1 Further, D.M has important economic implications and thus burdens the healthcare expenditures worldwide. Upto 75 % of individuals with diabetes live in low- or middle-income countries.^[3] The main concern is that over 1 in 2 adults living with diabetes are undiagnosed.^[1]

Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycemia leading to complications. D.M can be due to deficiency of insulin as in Type-1 D.M or resistance to insulin action as in Type-2 D.M.^[2] D.M can lead to serious microvascular complications like Diabetic retinopathy, Diabetic neuropathy, Diabetic nephropathy and macrovascular complications like Stroke, CVD and Peripheral vascular disease.^[3]

It was established that there is a direct relationship between blood glucose concentrations and the risk of microvascular complications.^[2] Thus, monitoring of glycemic control in the early stage can delay the onset of such complications in D.M. Glycosylated Hemoglobin (HbA1c) is established as an index of long-term blood glucose concentrations and also a measure of the risk of microvascular complications. As it is costly and not available widely, it burdens the diabetic patients financially.^[4] Further HbA1c does not predict the macrovascular complications like CVD risk accurately.^[5]

Persons with D.M are at increased risk of CVD, which is the main cause of morbidity and mortality.^[3] As there is ongoing low-grade inflammation in D.M due to Insulin resistance,⁶ leading to cardiac and other complications, we need a reliable marker to identify the inflammation at an early stage and predict the CVD (cardiovascular) risk.³ There are many inflammatory markers like CRP (C-Reactive Protein) and total WBC count.⁷ But as they have certain limitations, there is a need for new and reliable inflammatory markers in D.M to identify the CVD risk at early stage.

Neutrophil-lymphocyte ratio (NLR), a simple ratio between the neutrophil and lymphocyte count from the differential leucocyte count (DLC) is used as an inflammatory marker in various disease like pneumonia, cancers and sepsis.^[8] Many studies found that the inflammatory response is a key mechanism in the pathogenesis of atherosclerosis and its progression. Neutrophils secrete inflammatory mediators that can cause vascular wall degeneration. On the other hand, lymphocytes regulate the inflammatory response and thus have an anti-atherosclerotic role.^[9] Thus, NLR can be a good marker for inflammation and predict CVD risk.

Platelet-lymphocyte ratio (PLR) calculated from Complete Blood Picture (CBP) is an emerging inflammatory marker. Many studies have found the utility of PLR in various inflammatory conditions like cancers, renal disease and CVD.^[10] PLR indicates increased platelets in CBP, leading to platelet aggregation and thus promotes atherosclerosis and decreased lymphocytes, suggestive of inflammation.^[11]

Aim & Objectives

1. To evaluate NLR and PLR in diabetic patients.
2. To determine the association between NLR and PLR with HbA1c in type-2 D.M.

MATERIALS AND METHODS

This study was conducted at Government General Hospital, Nizamabad after approval from institutional ethical committee. The study was done on 105 known diabetic patients from General Medicine Department after the informed consent. A detailed history and general examination were done on all diabetic patients. Patients with any acute or chronic infections, hypertension or chronic heart disease, renal or hepatic disorders, cancers, autoimmune diseases, bleeding / hematological disorders were excluded from the present study. Patients who are on anti-inflammatory or immunosuppressive drugs were excluded from this study.

Study Parameters

After an overnight fast of 12 hours, blood sample was collected from the median cubital vein under aseptic precautions. FBS was estimated by Hexokinase method on Seimens Atellica Auto Analyzer at Biochemistry Laboratory. CBP was done on automated hematology analyzer and HbA1c was measured by enzymatic method on Atellica autoanalyzer. NLR was calculated by dividing absolute neutrophil count to absolute lymphocyte count from the DLC (Differential Leucocyte Count) and PLR was calculated by dividing the platelets to Lymphocyte count. Baseline investigations like serum creatinine and bilirubin were done on all subjects to exclude diabetic patients with severe renal or liver diseases.

The diabetic patients were divided into 2 groups based on the HbA1c values. Group 1 includes 62 patients with HbA1c value less than 7% and Group 2 includes 43 patients with HbA1c value more than 7%.

Statistical Analysis

SPSS software was used for statistical analysis. Unpaired T test was used for continuous variables. A p value < 0.05 was considered statistically significant.

RESULTS

In this study, we included 105 diabetic patients of different age (31- 65 years) and both sexes. The diabetic patients were divided into 2 groups based on the HbA1c values. Group 1 includes 62 patients with HbA1c value less than 7% and Group 2 includes 43 patients with HbA1c value more than 7%. The mean age of group 1 diabetic patients was 46.13 years while group 2 was 46.7 years respectively. There was no statistical significant difference between the age of the 2 groups (p = 0.786)). Figure 1 shows the baseline characters of the group 1.

Figure 2 shows the baseline characters of the group 2.

The mean and S.D values of study parameters of the 2 groups were given in table 1.

The Fasting Blood Glucose (FBS) was higher in group 2 as compared to group 1 diabetic patients and it was statistically significant. ($p < 0.0001$) The total WBC count was high in group 2 diabetic patients when compared to group 1 patients and it was statistically significant. ($p = 0.01$)

The NLR was significantly higher in group 2 with high HbA1c as compared to group 1 diabetic patients with low HbA1c values ($p = 0.0004$) The PLR was statistically higher in group 2 as compared to group 1 diabetic patients and it was statistically significant ($p = 0.04$). Thus, NLR and PLR were positively associated with HbA1c in type-2 diabetic patients.

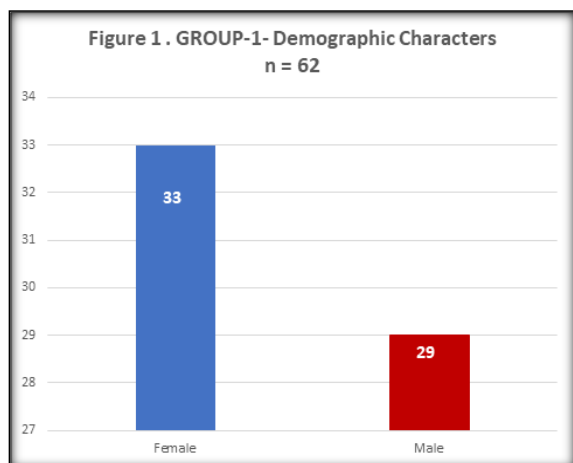


Figure 1: GROUP-1- Demographic Characters

n = 62

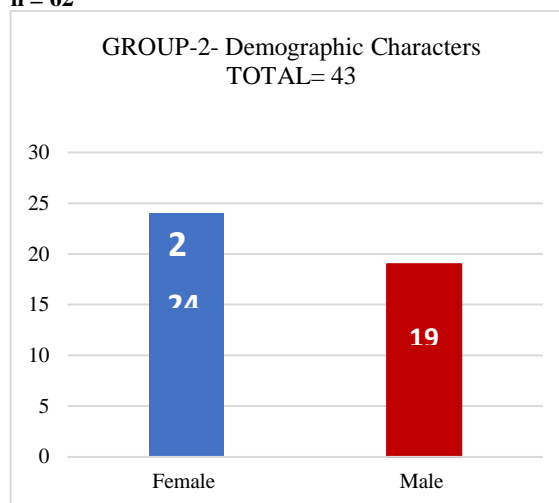


Figure 2: GROUP-2- Demographic Characters
n = 43

Table 1: Comparison of Study Parameters between 2 Groups

Parameter	Group 1	Group 2	P value
HbA1c	5.61 ± 0.66	9.7 ± 2.28	< 0.0001
FBS	104.55 ± 40.22	206.58 ± 83.61	< 0.0001
Total WBC	8552 ± 3410.87	10344 ± 3727.62	0.01
NLR	2.01 ± 0.74	2.82 ± 1.49	0.0004
PLR	114.32 ± 36.15	132.2 ± 53.69	0.04

DISCUSSION

Neutrophil lymphocyte ratio (NLR) is a simple ratio and easily calculated from CBP. The NLR value indicates the balance between two aspects of the immune system: acute and chronic inflammation (as indicated by the neutrophil count) and adaptive immunity (lymphocyte count). Many studies have established that NLR is an emerging inflammatory marker in conditions like acute M.I, Heart failure, stroke, respiratory failure, sepsis and cancer.^[12] Minkyong et al., studies have reported that higher NLR was significantly associated with overall mortality due to cardiovascular disease.^[13] NLR test has various advantages over other inflammatory markers because of its wide availability, low cost, reliability and easy lab detection.^[14]

Platelet-to-lymphocyte ratio (PLR) is an emerging inflammatory biomarker. It is calculated from Platelet count divided by lymphocyte count from the CBP result. As a new prognostic marker, PLR was the combination of the two indexes which provides the concept of platelet aggregation and

inflammatory pathways.^[11] Many studies demonstrated the utility of PLR as a predictor for the inflammatory conditions like cancers, CVD, sepsis and renal diseases.^{10,15,16} Further, it was found that PLR predicts mortality in cardiac related diseases.^[11] Thus PLR has emerged as an informative marker revealing shifts in platelet and lymphocyte counts due to acute inflammatory and prothrombotic states.^[16]

Cardiovascular disease (CVD) is one of the leading causes of mortality and morbidity in people with DM. It was found that more than 50% of diabetic patients die of a cardiovascular event. Diabetic patients had a 10% higher risk of CVD, a 53% higher risk of MI, a 58% higher risk of stroke, and a 12% higher risk of heart failure than the non-diabetic population.^[17] The risk of CVD is constantly increasing by increasing fasting plasma glucose levels, even before they reach a sufficient level to diagnose DM.^[17] Thus, there is a need for a reliable marker to identify the CVD risk in D.M and to start the early intervention. It was found that certain combinations of biomarkers can lead to

moderate improvements in cardiovascular disease risk prediction in diabetes.^[18]

In our study, we found that NLR and PLR, the inflammatory markers were positively associated with HbA1c and FBS. Many studies found that NLR has strong diagnostic as well as prognostic potential for CVD.^[9,12] Even patients with increased NLR but normal TLC count could have increased risk of atherosclerosis related diseases.^[14] It was also revealed that increased NLR has strong association with glucose intolerance and insulin resistance in type 2 diabetic patients. NLR has a potential to be used to assess inflammatory state and the complications associated with diabetes.^[14] Devamsh et al found a significant positive correlation between NLR and glycemic control in their study.^[19] Mazhar et al found that increased NLR is associated with elevated HbA1c and poor glycemic control in type 2 diabetes patients.^[20] Few studies have consistently shown that NLR is associated with complications like diabetic nephropathy.^[21,14] and depression in diabetes.^[22]

Heidarpour et al. and Guangyao Zhai et al., established the role of PLR as an inflammatory marker in CVD.^[10,11] Few studies have found that both PLR and NLR were elevated in diabetes patients with retinopathy.^[23] and nephropathy.^[24] PLR is found to be elevated in many cancers, CVD and other inflammatory conditions.^[10,15,16] Atak B et al found that PLR was significantly and positively correlated with HbA1c and FBS.^[25]

Thus, NLR and PLR from routine CBP test were reliable markers of inflammation and predict the CVD risk in D.M. In the present study, elevated NLR and PLR were also associated with poor glycemic control.

Limitations of the Study

Small sample size, other comorbid conditions like obesity and hypertension, use of different anti diabetic drugs by patients and lifestyle measures by patients.

CONCLUSION

Diabetes and its complications are major causes of early death in most countries. Cardiovascular disease accounts for 50% or more of deaths due to diabetes. HbA1c test is a routinely used measure of glycemic control but with limitations.

Both NLR and PLR are simple, widely available and cost-effective tests and easily calculated from routine CBP test available in any peripheral setting. NLR and PLR are emerging markers of subclinical inflammation and also associated with poor glycemic control in patients with type 2 diabetes. Thus, NLR and PLR should be routinely assessed during follow-up of diabetes patients. They may predict the CVD risk and other complications in D.M. Thus NLR and PLR are simple assessment tools in Type-2 D.M patients and to initiate the

necessary preventive measures at an early stage and decrease the disease burden.

Recommendation

NLR and PLR from routine CBP is widely available and cost effective than HbA1c. These tests should be done routinely during the follow-up of diabetic patients to assess the glycemic control and mainly to identify the at-risk patients.

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