# Neutrophil to Lymphocyte Ratio as an Indicator of Presence of Coronary Artery Disease in Diabetic Patients

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**ABSTRACT**

**Background and Objectives:** *Cardiovascular disease is the leading cause of death in type 2 Diabetes mellitus (DM). Type 2 DM is a significant risk factor of atherosclerotic coronary artery disease. Neutrophil to Lymphocyte ratio (NLR), an indicator of systemic inflammation has been shown to provide a reliable inflammatory index to be used in prognostic stratification of CAD. The objective of this study is to investigate the relationship between neutrophil to lymphocyte ratio (NLR) and coronary artery disease (CAD) in diabetic patients.*

**Methods:** *100 diabetic patients who had coronary lesion with diameter stenosis of at least 50% and 50 diabetic patients with normal coronary anatomy matched with age and sex were retrospectively selected and classified as CAD(+) and CAD(-) group respectively. Diabetics with chronic infections and inflammation were excluded. NLR in two groups were compared.*

**Results:** *NLR was higher in CAD (+) group compared to CAD (-) group (p = 0.000). ROC curve analysis revealed that NLR value of ≥ 2.26 identified the presence of CAD in diabetic patients with 100% specificity and 92% sensitivity.*

**Conclusion:** *NLR was higher in diabetic patients with angiographically proven CAD compared to those without CAD. NLR may serve as a useful marker to predict the presence of CAD in type 2 diabetic patients.*

**Key words:** *Diabetes mellitus, Coronary artery disease, Neutrophil to Lymphocyte ratio*

## INTRODUCTION

Cardiovascular disease is the leading cause of death in Type 2 Diabetes mellitus in both developed and developing countries[1]. Type 2 DM is a significant risk factor in atherosclerotic cardiovascular disease[2,3] and is also associated with an increased risk of cardiovascular morbidity and mortality[4,5]. Atherosclerosis is a progressive disease which is characterized by the accumulation of lipids and fibrous elements in the large arteries constituting the single most important contributor to the growing burden of cardiovascular disease[6]. Inflammation plays a key role in the development, progression and complications of atherosclerosis. Inflammatory process interacting with endothelial dysfunction within the arterial wall results in the reduction or obstruction of blood supply to the end organs of the body including brain, heart and intra - abdominal organs, causing morbidity and mortality[7-9].

Increased cardiovascular risk has been found to correlate with counts of white blood cells (WBC) and subtypes. Neutrophil-to-lymphocyte (NLR) which is a marker of systemic inflammation has been found to correlate with cardiac events in cardiovascular diseases such as stable coronary artery disease, unstable CAD and acute decompensate heart failure.[10,11] NLR has been shown to provide a reliable inflammatory index to be used in prognostic stratification of CAD.[12,13]

Thus, the aim of this study was to investigate the relationship between Neutrophil to Lymphocyte ratio (NLR) and coronary artery disease (CAD) in DM.

## MATERIALS AND METHODS

### Patient's selection

1. In this cross- sectional study, 150 diabetic patients, diagnosed according to the American Diabetes Association criteria[14], from both sexes of age 30- 70 years who underwent coronary angiogram (CAG) during the period of June 2013 and May 2014 at Sri Ramachandra Medical college and Research Institute hospital were retrospectively examined and selected for the study.
2. Indications for CAG were either the presence of typical angina or positive or equivocal results of non- invasive screening tests for myocardial ischemia.
3. Overall , 100 diabetic patients who had coronary lesion with diameter stenosis of at least 50% were included in CAD(+) group and 50 diabetic patients with normal coronary anatomy were included in CAD(-) group respectively.
4. Diabetics with valvular or dilated heart diseases, acute or chronic infectious diseases, uncontrolled hypertension, cerebrovascular diseases, thrombosis, malignancies, thyroid disorders, hematological disorders and liver or renal insufficiency were excluded from the study.
5. Body mass index (BMI) was calculated as weight/ height$^2$ (kg/m$^2$).

**Laboratory measurements**

1. White blood cell (WBC), neutrophil and lymphocyte counts, total cholesterol (TC), high - density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol. Triglycerides (TG), haemoglobin (Hb), BMI were retrospectively recorded from patients' medical records.
2. NLR was calculated by dividing neutrophil count to lymphocyte count.
3. The whole blood samples for haematological indices were analyzed on an automated blood cell counter (Beckman Coulter LH-780).
4. Biochemical parameters were analyzed using automated analyzer (Siemens Advia 1800).
5. WBC counts more than12,000 cells/$mm^3$ and less than 4000 cells/$mm^3$ were exclusion criteria for the study.

**Ethical clearance**

1. Ethical clearance for this study was obtained from Institutional Ethics Committee (IEC).

**Statistical analysis**

1. Statistical analyses were performed using SPSS software version 15.0.
2. Parametric continuous variables were given as mean ± standard deviation and non-parametic values were given as percentage.
3. Student's t- test was used to compare parametric continuous variables.
4. Two – tailed p- values of less than 0.05 were considered to indicate statistical significance.
5. Receiver - operating characteristic (ROC) curve analysis was performed to detect the cut-off value of the N/L ratio in predicting CAD in diabetic patients.

**RESULTS**

The study population consisted of 150 diabetic patients. The mean age of diabetic patients in CAD (+) group and CAD (-) group were 59. 2 ± 8.05 and 54.56 ± 9.75 respectively and male gender constituted 74% of diabetic patients in CAD (+) group and 36% in CAD (-) group. The diabetic patients in CAD (+) group were relatively older and had higher prevalence of male gender.

Baseline characteristics of diabetic patients with CAD (+) and CAD (-) groups are presented in Table 1. Numbers of diabetic patients affected with different types of lesion in CAD (+) group are shown in Table 2.

**Table 1: Baseline characteristics of the study population**

| Variables | CAD (-) (n=50) | CAD (+) (n=100) | p value |
|---|---|---|---|
| Age (years) | 54.56 ± 9.75 | 59.2 ± 8.05 | 0.002* |
| BMI (kg/$m^2$) | 27.35 ± 4.84 | 25.88 ± 3.56 | 0.037* |
| Fasting plasma glucose (mg/dl) | 165.1 ± 52.18 | 179.5 ± 68.7 | 0.194 |
| Total cholesterol (mg/dl) | 151.4 ± 36.9 | 169.6 ± 43.8 | 0.012* |
| Triglyceride (mg/dl) | 145.7 ± 86.2 | 163.1± 80.4 | 0.224 |
| LDL (mg/dl) | 97.84 ± 28.8 | 116.6 ± 48.6 | 0.013* |
| HDL (mg/dl) | 38.64 ± 8.69 | 37.07 ± 8.08 | 0.271 |
| Total Cholesterol: HDL | 4.01± 1.15 | 4.63 ± 1.24 | 0.004* |
| WBC (cells/$mm^3$) | 8838 ± 1580.7 | 9416 ± 1640.2 | 0.041* |
| **NLR** | 1.71 ± 0.38 | 4.09 ± 1.67 | **0.000**** |

WBC: white blood cell, NLR: neutrophil to lymphocyte ratio, LDL: low- density lipoprotein, HDL: high - density lipoprotein, BMI: body mass index. $p< 0.05$ = statistical significance (*)

**Table 2: Types of lesion in CAD (+) group of diabetic patients**

| Type of lesion | CAD(+) |
|---|---|
| Single vessel disease (SVD) | 18 |
| Double vessel disease (DVD) | 33 |
| Triple vessel disease (TVD) | 49 |
| Total | 100 |

The diabetic patients with CAD had significantly elevated N/L ratio values compared to those without CAD ($p < 0.000$). Significant differences were also observed in terms of white blood cell (WBC) counts, body mass index (BMI), total cholesterol, low- density lipoprotein (LDL), Cholesterol / HDL ratio (Table 1). Glucose, triglyceride, HDL values showed no significant difference. Also, out of 100 diabetic patients in CAD (+) group, 18 of them had single vessel disease (SVD), 33 had double vessel disease (DVD) and 49 had triple vessel disease (TVD), as shown in Table 2.

ROC curve analysis (Fig. 1) was performed in CAD (+) and CAD (-) groups to detect the cut-off value of N/L ratio in predicting the presence of CAD in diabetic patients. The analysis showed that N/L ratio of ≥ 2.26

identified the diabetic patients with CAD with specificity of 100% and sensitivity of 92% and area under the curve (AUC) is 0.997 (95% CI 0.993-1.000) .

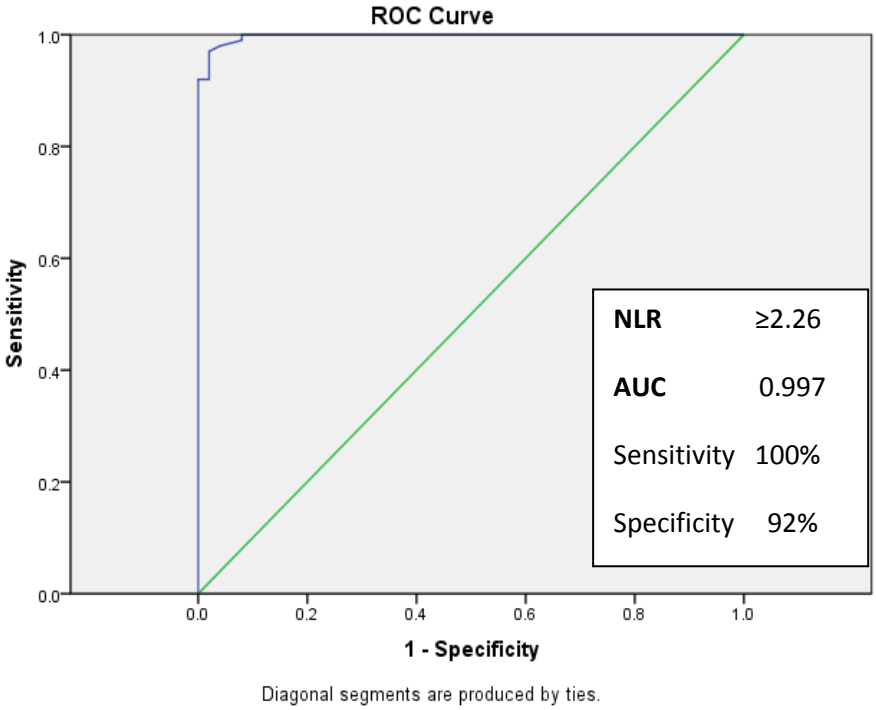


**Fig. 1: Receiver- operating characteristic (ROC) curve analysis of NLR for predicting coronary artery disease (CAD) in CAD (+) and CAD (-) in diabetic patients**

## DISCUSSION

This study showed that a higher N/L ratio (NLR) was associated with presence of coronary artery disease (CAD) in diabetic patients. There was a positive correlation between CAD and NLR. A cut-off value ≥ 2.26 was able to identify the presence of CAD in diabetic patients.

Diabetic patients develop both macro vascular complications such as cor, onary artery disease, stroke, peripheral vascular disease and micro vascular complications such as diabetic nephropathy, diabetic retinopathy and peripheral neuropathy[15]. These vascular complications are mainly due to continuous hyperglycemia leading to endothelial dysfunction and vascular lesions[16,17]. Several studies have shown that chronic, low grade , subclinical inflammation play a major role in the development of insulin resistance which then proceeds to the development of clinically overt DM[18,19]. Several researches have also demonstrated that WBC count and C-Reactive Protein are associated with increased macro vascular and micro vascular complications in type 2 diabetes mellitus[20,21]. This study also showed that WBC counts were higher in coronary artery disease with DM than without coronary artery disease.

Blood NLR is a new indicator of the overall inflammatory status of the body and a simple, inexpensive and useful marker of subclinical inflammation[22,23]. Several studies have shown that increased NLR levels were associated with adverse outcomes and higher cardiovascular morbidity and mortality in diabetic patients[24,25]. Elevated levels of systemic inflammatory markers have been found to be related with the incidence of CVD[26,27].

The main role of neutrophilia in CAD may be explained by secretion of various inflammatory mediators such as elastase, myeloperoxidase and oxygen free radicals which causes tissue damage. The probable cause of lymphopenia include decreased production as a result of increased steroid level due to CAD induced stress and increased apoptosis triggered by increased inflammation thereby resulting in elevated NLR in CAD (+) group[28-29].

Increased number of neutrophils and decreased lymphocytes are risk indicators for future cardiovascular events. Therefore, elevated NLR integrates the predictive risk of the two leukocyte subtypes into a single risk factor. Papa et al, in his study also demonstrated that increased NLR, widely recognized as an indicator of systemic inflammation has been associated with higher mortality rates[30]. NLR has remained as a predictor of all - cause mortality in acute coronary syndrome and cardiac revascularization patients with normal WBC counts[31]. In another study conducted by Gibson et al, elevated levels of NLR were also found to be associated with

poor survival of patients undergoing coronary artery bypass graft[32]. Moreover, Kaya et al have shown that NLR has been found to correlate with the severity and complexity of CAD in stable patients with CAD[33]. Sonmez et al showed that higher NLR at baseline was found to be independently associated with greater coronary complexity of CAD[34]. Present study showed that NLR was associated with CAD in diabetic patients which were consistent with previous studies.

Coronary artery disease (CAD) in diabetic patients could be identified by more sensitive and specific cardiovascular imaging modalities. However, these tools are expensive and time consuming with potential unwanted effects such as exposure to radiation. Therefore, NLR, which is cheap and easily obtainable, could be used as an initial filter criterion, especially in small centers to determine the need for further imaging modalities in the assessment of CAD.

## LIMITATIONS

Inflammatory markers such as CRP, MMP, IL-6 and TNF-α were not analyzed and not compared to NLR. However such inflammatory biomarkers are expensive and are not immediately available in everyday practice. Also the relation between severity of CAD and NLR was not studied due to the relatively small sample size in each subtype of CAD (+) group which can result in low statistical power for equivalency testing. Hence large scale further studies are needed to support the findings.

## CONCLUSION

NLR was higher in diabetic patients with angiographically proven CAD compared to those without CAD. NLR which is a simple, inexpensive and useful marker could be used as an initial filter criterion, especially in small centres; to determine the need for further imaging modalities to detect the presence of CAD in type 2 diabetic patients.

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