

**Original Article**

## Occurrence of coronary artery disease in patients with type 2 diabetes mellitus can be predicted by neutrophil lymphocyte ratio

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### Abstract

Neutrophil lymphocyte ratio (NLR) is the sign of balance between neutrophil & lymphocyte levels in the body and is an indicator of systemic inflammation. NLR is a new predictor for cardiovascular risk and mortality and by which occurrence of coronary artery disease can be predicted in type 2 DM patients. This case control study was conducted in the Department of Clinical pathology in collaboration with Department of Cardiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2014 to February 2015 to assess the NLR as a useful predictive marker for coronary artery disease in type 2 DM patients detected by coronary angiography. Total 134 patients waiting for coronary angiography in the Department of Cardiology, BSMMU were enrolled in this study. Among them, 84 patients were considered as Group A (DM with CAD). All of them had  $\geq 50\%$  coronary artery stenosis on coronary angiography. Fifty patients with DM but without CAD were enrolled in Group B and considered as controls. Statistically significant increased NLR was found in Group A (type 2 DM patients with CAD) compared to Group B (type 2 DM patients without CAD). So, NLR can be used to predict adverse outcome i.e. coronary artery disease in patients with type 2 DM.

**Key words:** Neutrophil lymphocyte ratio, diabetes mellitus, coronary artery disease

### Introduction

Diabetes mellitus (DM) is a major global health problem. According to world health organization (WHO), there were 346 million people suffering from diabetes worldwide in 2011.<sup>1</sup> It is estimated that more than 11 million people of Bangladesh will have diabetes by the year 2030 with diabetic complications.<sup>2</sup> Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease.<sup>3</sup>

Type 2 DM is a chronic disease associated with many complications. There is two to four fold increased risk of cardiovascular disease in type 2 DM patients<sup>3</sup>. About 52% cause of death in type 2 DM is cardiovascular disease.<sup>4</sup> However, diabetes develops over time preceded by several years of gradually worsening insulin resistance<sup>3</sup>. Ideally, persons at risk for diabetes should be identified at this time when strategies to prevent type 2 diabetes and its cardiovascular complications are most likely to be successful.<sup>3,4</sup> Coronary artery disease (CAD) is the most common cause of myocardial infarction.<sup>5</sup> Coronary artery disease progresses before the first onset of symptoms. So, most individual with CAD show no evidence of disease for prolong period.

The current consensus states that if the type 2 diabetes mellitus occurs earlier; it leads to coronary atherosclerosis in a majority of the patients. Several mechanisms are likely to contribute to the accelerated atherosclerosis and increased chronic heart disease (CHD) risk in patients with type 2 diabetes mellitus.<sup>4,5</sup> Multiple hypothesis exist to explain the occurrence of premature coronary atherosclerosis in diabetes but the consensus is that it is multifactorial. Important factors include dyslipidemia, hypertension, hypercoagulability, poor glycemic control, smoking, obesity and lack of physical activity.<sup>6</sup> Most important factors are hyperglycemia affecting the vessel wall, diabetic dyslipidemia, hyperglycemia against dyslipidemia and chronic inflammation in the vessel wall.<sup>7</sup>

Neutrophil lymphocyte ratio (NLR) is the sign of balance between neutrophil lymphocyte levels in the body and an indicator of systemic inflammation.<sup>8</sup> NLR is a new predictor for cardiovascular risk and mortality by which we can predict the adverse outcomes of type 2 DM patients

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with CAD.<sup>9</sup> The relationship between various inflammatory markers and CAD has been definitively established.<sup>10</sup> Among these markers, the levels of white blood cell (WBC) subtypes, confirmed as inflammatory markers, play a crucial role in the pathogenesis of atherogenesis and atherothrombosis and have received significant attention.<sup>11</sup> It has been reported that the WBC count and levels of WBC subtypes not only play an important role in the development of CAD but can also be used to predict the clinical outcomes of patients with CAD.<sup>12</sup> Interestingly, the ratio of the absolute number of neutrophils to that of lymphocytes, a particular WBC parameter, has newly emerged as an inflammatory marker for identifying individuals at risk of CAD.<sup>10-12</sup> With the help of NLR we can take preventive measure and precaution to reduce the risk of coronary artery disease in type 2 diabetic patients.

In this study, we aimed to investigate the association between NLR and CAD detected by coronary angiography in type 2 DM patients and to establish NLR as a useful predictor of coronary artery disease in patients with type 2 DM.

### Methods

This case control study was conducted at the Department of Clinical Pathology in collaboration with the Department of Cardiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2014 to February 2015. A total of 134 patients having type 2 DM were enrolled in this study. Among them, 84 patients were considered as Group A (DM with CAD). All of them had  $\geq$  50% coronary artery stenosis on coronary angiography. Fifty patients with DM but without CAD were enrolled in Group B and considered as controls. Adult (18 years and above) patients, patients of both sexes were included in this study. Recurrent myocardial infarction, patients with congenital cardiac disease as shunt or vascular anomalies, pulmonary hypertension (both primary or secondary), acute or chronic infectious diseases, uncontrolled hypertension, known case of patients with cerebrovascular diseases, patients with documented malignancy, hematological disorders, patients with hepatic and renal impairment were excluded from the study.

After selection, all the patients were thoroughly informed about the aims, objectives and procedure of the study and were encouraged for their voluntary participation. Informed written consent was taken from each subject. Detail personal, medical, occupational, educational and smoking histories were recorded in a preformed data sheet and

thorough physical examinations were done and documented.

Two ml of blood was collected in EDTA tube from all the patients prior to coronary angiogram for complete blood count (CBC) using haematology autoanalyzer (SYSMEX 4000i) & rechecked manually. Then NLR was calculated. Fasting plasma glucose (FPG), plasma glucose 2 hours after 75 g glucose (PPBG), glycated haemoglobin (HbA1c), serum lipid profile, serum creatinine, (ALT) were also done.

### Results

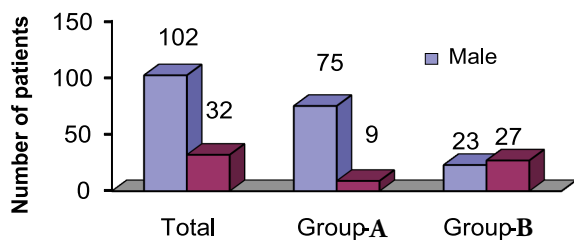
A total 134 type 2 DM patients were included in this study. 84 cases were considered as Group A (DM with CAD) and 50 controls were Group B (DM without CAD) after the assessment of coronary angiography.

The mean age of the DM with CAD (Group A) was  $54.73 \pm 8.94$  years, the mean age of the DM without CAD (Group B) was  $53.10 \pm 9.47$  years. Maximum 34 (40.5%) patients belonged to 51-60 years of age group in Group A and 22 (44.0%) patients belonged to 41-50 years of age in Group B. Lowest number of patients were found in >70 years age group in both groups (2 patients, 2.4% in Group I and 2 patients, 4% in Group II). (Table -I)

**Table - I:** Age distribution of the study population (n=134).

Study group	Group-A n(%)	Group-B n(%)	Total
31-40 years	06(7.1)	04(8.0)	10
41-50 years	28(33.3)	22(44.0)	50
51-60 years	34(40.5)	13(26.0)	47
61-70 years	14(16.7)	09(18.0)	23
>70 years	02(2.4)	02(04.0)	04
Total	84(100)	50(100)	134
Mean $\pm$ SD	54.73 ( $\pm 8.94$ )	53.10 ( $\pm 9.47$ )	Range 35-79 years

It was observed that in Group A (DM with CAD), 75 (89.28%) patients were male and 9 (10.71%) patients were found female. In Group B (DM without CAD) 23 (46%) patients were male and 27 (54%) patients were female. In our country, coronary angiogram is not routinely done. In Group B female is more than male patients because false positive results of exercise tolerance test is more in case of female type 2 DM patients. Overall, male:female ratio was 3.18:1 which was statistically highly significant ( $p < 0.001$ ). (Figure- 1)



**Figure 1:** Sex distribution of the study population (n=134).  
\*Chi-square test was done to measure level of significance (P <0.001).

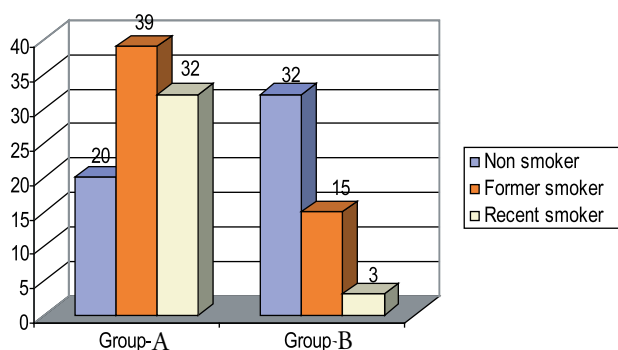
HbA1c (%) was 7.40 ± 1.04 (Mean ± SD) in group A whereas it was 6.13 ± 0.65 (Mean ± SD) in group B. This difference association is statistically highly significant (p <0.001). (Table - II).

**Table II:** Mean difference between Group-A (DM with CAD) and Group-B (DM without CAD) with HbA1c (%) (n=134).

	Group-A Mean ±SD (n=84)	Group-B Mean ±SD (n=50)	P value*
HbA1c (%)	7.40±(1.04)	6.13±(0.65)	<0.001

\* Unpaired t test was done to measure level of significance.

All the respondents were divided into three groups according to their smoking habit i.e. nonsmoker, former smoker and recent smoker. It was observed that, majority 64 (75.0%) patients in Group A were smoker (39 patients were former and 25 patients were recent smoker), whereas only 18 (36%) patients were smoker in Group-B (15 patients were former smoker and 3 patients were recent smoker). This association was highly significant (p < 0.001). (Figure 2)



**Figure 2:** Smoking status of the study population (n=134)

Regarding distribution of mean NLR with sex, NLR in male were 2.44 (±0.82) and female 1.92 (±0.76), which was statistically significant (p < 0.008). (Table III)

**Table III:** Distribution of mean NLR with sex (n=134).

Sex	Male Mean ±SD	Female Mean ±SD	P-value*
NLR	2.44(±0.82)	1.92(±0.76)	0.008
Range	1.23-7.0	1.25-5.0	

\* Unpaired sample t test was done to measure level of significance.

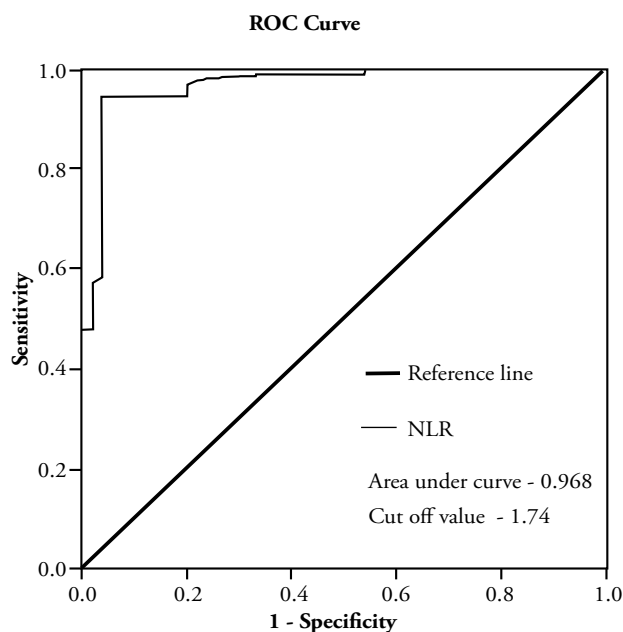
Mean NLR were 2.76 (±0.74) in Group-A patients and 1.56 (±0.15) in Group-B patients and the difference was statistically highly significant (p <0.001). NLR was significantly increased in Group-A and normal or not significantly increase in Group-B. This result reflects that by NLR we can predict the risk of coronary artery disease in type 2 DM patients. In Group-A NLR was more than the calculated cut off value. The range of NLR in Group-A was 2.0-7.0 and the range of NLR in Group-B was 1.23-1.74. (Table IV)

**Table IV:** Mean NLR difference between Group-A and Group-B (n=134).

	Group-A Mean ±SD	Group-B Mean ±SD	P value*
NLR (Ratio)	2.76 (±0.74)	1.56 (±0.15)	<0.001
Range (min-max)	2.0-7.0	1.23-1.74	

\* Unpaired sample t test was done to measure level of significance.

Cut off value of NLR was 1.74. of Sensitivity and specificity of neutrophil lymphocyte ratio was determined by Receiver-operator characteristic (ROC) curve. ROC curves of NLR for prediction of coronary artery disease (CAD) shows that the test result variable(s) NLR (Ratio) (0.968) has at least one tie between the positive actual state group and the negative actual state group; with 95% confidence interval of the difference in lower 0.939 and upper 0.997. Then cut off value was 1.74. (Figure - 3)



Diagonal segments are produced by ties

**Figure 3:** Receiver-operator characteristic curves of NLR and Group-A (DM with CAD) (n=134).

### Discussion

Type 2 DM is a chronic disease associated with many complications.<sup>13</sup> Patients with type 2 DM have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease.<sup>14</sup> Cardiovascular disease accounts for up to 80% of deaths in individuals with type 2 DM.<sup>15</sup> Type 2 DM patients have a threefold higher risk than non-diabetic individuals of developing atherosclerosis and its clinical complications, such as stroke, myocardial infarction (MI) and peripheral vascular disease.<sup>16,17</sup> Despite improvement in primary prevention and treatment, CAD remains the chief cause of death in most developed countries.<sup>18</sup> The incidence is much greater in developing countries.<sup>5</sup>

CAD occurs at any adult age, but it is the disease of middle and old age. In our study, the mean age of the patients in Group A (DM with CAD) was 54.73 ( $\pm 8.94$ ) years as compared to 56.59 $\pm 13.63$  years and 58.01 $\pm 12.9$  years, studies done by Assiri A S et al and Al-Saif SM et al.<sup>18,19</sup> It was 52.0 $\pm 8.6$  years in a study reported by Siddique MA et al and 56.6 years by Ullah M et al in Bangladesh.<sup>20,21</sup>

Mean total count of WBC was found to be 9.90  $\pm$  (1.91)  $\times 10^9$ /L in group A and 8.66  $\pm$  (1.37)  $\times 10^9$ /L in Group B. This association was highly significant (p value <0.001). On the other hand, mean ESR was 31.07  $\pm$  (18.25) mm in 1<sup>st</sup>

hour in Group A whereas it was 11.76  $\pm$  (6.20) mm in 1<sup>st</sup> hour in Group B which was statistically highly significant (p value <0.001). Our findings are similar to other study conducted by Selcuk H et al. (2011).<sup>22</sup> They found significantly higher WBC count (8.2 vs 7.4, p value 0.001) and ESR (25.5 vs 12.02, p value <0.001) in CAD group. In another study conducted by Sonmez O et al, they also found higher WBC count in DM with CAD group (7.75 vs 7.01, p value 0.01).<sup>9</sup>

Glycemic control, measured here by HbA1c, was associated with CAD among type 2 DM patients. In our study, it was observed that mean HbA1c % was significantly higher in the DM with CAD group (7.40 $\pm 1.04$  vs 6.13 $\pm 0.65$ ) (p <0.001). Our results reinforce the concept that glycemic control plays an important role in the presence of CAD, at least in this group of type 2 DM patients with high cardiovascular risk.<sup>23</sup> Even in non-diabetic individuals HbA1c can be associated with CAD and its severity.<sup>24</sup>

In this study, we found a relationship between NLR and the presence of coronary artery disease. There is no doubt in cardiovascular medical science that atherosclerosis is a chronic inflammatory disease.<sup>25, 26</sup> In our study mean NLR in Group A was 2.76 ( $\pm 0.74$ ), whereas it was 1.56 ( $\pm 0.15$ ) in Group B. There was statistically significant association between two groups in this regards (p <0.001). Our findings are similar to a study conducted by Sahin S et al.<sup>8</sup> They also found higher mean NLR in patients with CAD group (1.98 $\pm 0.85$  vs 2.69 $\pm 1.74$ ) (p <0.001). Sonmez O et al also found higher mean NLR in patients with coronary artery disease (2.3 vs 1.6) (p <0.001).<sup>9</sup>

Determination of sensitivity and specificity of neutrophil lymphocyte ratio was done by receiver-operator characteristic (ROC) curves. The curve also determines about prediction of coronary artery disease (CAD). In ROC analysis, a cut point of 1.74 was identified for NLR in Group A (DM with CAD patients). Area under curve was 0.968, 95% CI was 0.939-0.997. An NLR value of more than 1.74 demonstrates a sensitivity of 98% and specificity of 58%. Our findings correlate with a similar study conducted by Sonmez O et al.<sup>9</sup> In ROC curve, they found a cut point of 1.95 in angiographic determined CAD patients (area under curve = 0.68, 95% CI 0.60-0.76). They concluded that an NLR value of more than 1.95 revealed a sensitivity of 69% and a specificity of 69%. In another study conducted by He J et al also found similar result.<sup>27</sup> They conducted ROC curve analysis for NLR for myocardial infarction in Chinese people. The area under the curve for NLR was 0.726, 95% CI 0.683-0.769. The optimal NLR

cut-off was 4.22, specificity of 68.8%, sensitivity of 69.3%. These findings concluded that NLR is a strong marker to predict presence of CAD in patients with type 2 diabetes mellitus.

Blood NLR is a new indicator of the overall inflammatory status of the body. It is a simple, inexpensive and useful marker of subclinical inflammation. NLR can be a useful tool to detect and measure the future prognosis and adverse outcomes of CAD in diabetic patients. In this study, we found an association between NLR and CAD detected by angiography in type 2 DM patients and NLR is a useful predictor of adverse outcomes of type 2 DM patients with angiographically proven CAD. But this was a non-randomized single center study that included a relatively small number of patients was subject to selection bias.

Neutrophil lymphocyte ratio (NLR) are a risk factor for developing coronary thrombosis leading to coronary artery disease (CAD) detected by coronary angiography. Adverse outcomes of type 2 DM patients associated with NLR which play an important role in early detection of coronary artery disease (CAD) and beneficial for preventive treatment. The relationship between NLR in type 2 diabetic patients with CAD and NLR in type 2 diabetic patients without CAD were observed. NLR could be used to predict the adverse outcomes of angiographically proven CAD and can take extra measure and preventive intervention to prevent CAD in type 2 DM patients.

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