ATHEROSCLEROSIS IS ASSOCIATED WITH NEUTROPHIL LYMPHOCYTE RATIO AS AN INFLAMMATORY MARKER IN TYPE 2 DIABETES MELLITUS PATIENTS WITH CORONARY ARTERY DISEASE

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Abstract

Background: *Increased neutrophil lymphocyte ratio is associated with major adverse outcomes of cardiac events in type 2 diabetes mellitus (DM) patients.*

Objective: *To assess the NLR as an inflammatory marker in atherosclerosis in type 2 DM patients with coronary artery disease (CAD).*

Methods: *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. Total 134 patients were enrolled in this study who were waiting for coronary angiography in the Department of Cardiology, BSMMU; 84 cases were considered as Group I (DM with CAD) and 50 controls were Group II (DM without CAD). A 2 ml of blood was collected in EDTA tube from the patients prior to coronary angiogram for complete blood count (CBC) were measured by haematology autoanalyzer, rechecked manually and NLR was calculated in the Department of Clinical Pathology, BSMMU. Coronary artery disease with 50% coronary artery stenosis and more critical lesion that were diagnosed by coronary angiography were included in this study as cases. Coronary artery disease with less than 50% coronary artery stenosis that was diagnosed by coronary angiography was included in this study as control.*

Results: *NLR was higher in CAD positive group compared to group without CAD negative in type 2 DM patients (2.76 (±0.74) vs. 1.56 (±0.15), p<0.001).*

Conclusion: With the help of *neutrophil lymphocyte ratio* we can take preventive measure and precaution to reduce the *progression of atherosclerosis* in type 2 diabetic patients with *coronary artery disease.*

Key words: *Nutrophil lymphocyte ratio, Diabetes mellitus, Coronary artery disease.*

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Introduction

Neutrophil lymphocyte ratio (NLR) is the sign of balance between neutrophil lymphocyte levels in the body and an indicator of systemic inflammation.[1] NLR is a new indicator for cardiovascular risk and mortality and by which we can predict the risk of coronary artery disease (CAD) in type 2 DM patients.[2] Increased NLR is associated with major adverse cardiac events in type 2 diabetes mellitus patients.[3,4] The relationship between various inflammatory markers and CAD has been established.[5] Among these markers, the levels of white blood cell (WBC) subtypes confirmed inflammatory markers that play a crucial role in the pathogenesis of atherogenesis and atherothrombosis, have received significant attention.[6] It has been established 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.[7]

Diabetes is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[8] 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. About 52% cause of death in type 2 DM is cardiovascular disease.[9] According to WHO report, Bangladesh had 3.2 million of diabetic subjects in 2000 and the number is expected to increase to a staggering 11.1 million by 2030.[10]

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. Important factors of premature coronary atherosclerosis include dyslipidemia, hypertension, hypercoagulability, poor glycemic control, smoking, obesity and lack of physical activity.[11] Most important factors are hyperglycemia affecting the vessel wall, diabetic dyslipidemia, hyperglycemia against dyslipidemia and chronic inflammation in the vessel wall.[12] Coronary artery disease occurs when part of the smooth muscle, elastic lining inside a coronary artery develops atherosclerosis. Inflammation plays a major role in all stage of atherogenesis and participates in the local, myocardial and systemic complications of atherosclerosis.[13] The early phase of atherosclerosis involves in the recruitment of inflammatory cells from the circulation to the arterial wall. Increased number of circulating neutrophils is a well known risk indicator of future cardiovascular disease.[14]

Recently, the differential leukocyte count and elevated NLR has been the subject of interest in predicting the risk for future cardiovascular events. An elevated NLR independently indicates an increased long term risk of mortality in patients with stable CAD.[15,16]

In this study, it was aimed to investigate relationship between atherosclerosis and NLR as an inflammatory marker in type 2 DM patients and to establish NLR as a useful predicator of adverse outcomes of CAD in type 2 DM patients.

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. 134 type 2 DM patients were enrolled in this study; 84 cases were considered as Group I (DM with CAD) and 50 controls were Group II (DM without CAD). Type 2 DM patients with coronary artery disease who have e" 50% stenosis, adults (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. In this study patient's complete blood counts were measured by haematology

autoanalyzer (SYSMEX 4000i), rechecked manually and NLR was calculated in the Department of Clinical Pathology, BSMMU.

After selection, all the patients were thoroughly informed about the aims, objectives and procedure of the study and were encouraged for their voluntary participation. Then an informed written consent was taken from each subject. Detail personal, medical, occupational, educational and smoking histories were recorded in a preformed data schedule and thorough physical examinations were done and documented. Tests done in Dept. of Clinical Pathology: CBC with PBF including differential count of WBC, erythrocyte sedimentation rate (ESR) and neutrophil lymphocyte ratio (NLR). Reports collected from patient's file were: plasma glucose- fasting (FPG), plasma glucose 2 hours after 75 g glucose (PPBG), glycated haemoglobin (HbA1c), serum lipid profile, serum creatinine, alanine aminotranferase (ALT).

Results

The mean age of the DM with CAD (Group I) was 54.73±8.94 years, the mean age of the DM without CAD (Group II) was 53.10±9.47 years. It was observed that in Group I (DM with CAD) 75 (89.28%) patients were male and 9 (10.71%) patients were found female. In Group II (DM without CAD) 23 (46%) patients were male and 27 (54%) patients were female (Fig. 1). All the respondents were divided into three groups
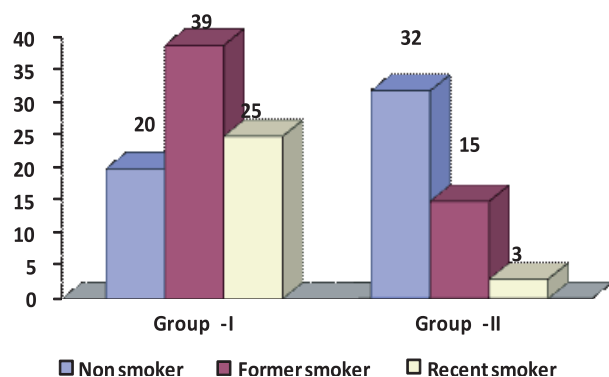

Fig. 1: Sex distribution of the study population (n=134).
*Chi-square test was done to measure level of significance ($P < 0.001$).

according to their smoking habit, namely nonsmoker, former smoker and recent smoker. In this study, it was observed that majority 64 (75.0%) patients in Group I were smoker (39 patients were former and 25 patients were recent smoker), whereas only 18 (36%) patients were smoker in Group-II (15 patients were former smoker and 3 patients were recent smoker). This association was highly significant (p <0.001) (Fig. 2).
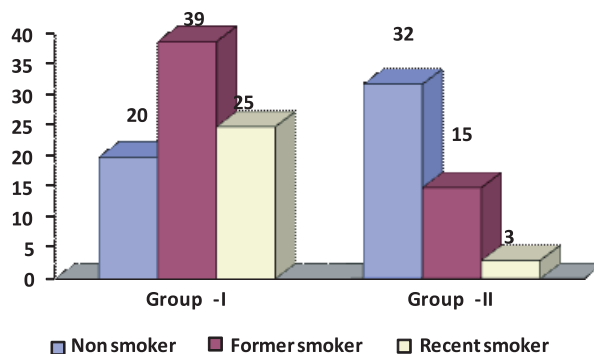

Fig. 2: Smoking status of the study population (n=134).
*Chi-square test was done to measure level of significance ($p < 0.001$).

Mean total count of WBC were found 9.90 ± (1.91) x 109/L in Group I and 8.66 ± (1.37) x 109/L in Group II. This association is highly significant (p <0.001). On the other hand, mean ESR were 31.07 ± (18.25) in Group I, whereas it was 11.76 ± (6.20) in group II which was statistically highly significant (p <0.001) (Table-I). HbA1c (%) was 7.40 ± 1.04 (Mean ± SD) in Group I whereas it was 6.13 ± 0.65 (Mean ± SD) in Group II. This association is statistically highly significant (p <0.001) (Table-II). Table-III shows 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-IV shows mean NLR were 2.76 (±0.74) in Group-I patients and 1.56 (±0.15) in Group-II patients which was statistically highly significant (p <0.001). NLR was significantly increased in Group-I and normal or not significantly increase in Group-II. This result reflects that by NLR we can predict the risk of coronary artery disease in type 2 DM patients. In Group-I NLR was more than the calculated cut off value. The range of NLR in Group-I was 2.0-7.0 and the range of NLR in Group-II was 1.23-1.74.

Table-I

Association between Group-I and Group-II with mean total WBC count and ESR (n=134)

	Group-I Mean ±SD	Group-II Mean ±SD	P value*
TC of WBC(10^9/L)	9.90±(1.91)	8.66±(1.37)	
Range (min-max)	5-13.50	6-13.0	<0.001
ESR(mm/1st hour)	31.07± (18.25)	11.76± (6.20)	<0.001
Range (min-max)	5-120	3-28.0	

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

Table-II

Mean difference between Group-I (DM with CAD) and Group-II (DM without CAD) with HbA1c (%) (n=134)

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

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

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 (min-max)	1.23-7.0	1.25-5.0	

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

Table IV

Mean NLR difference between Group-I and Group-II

	Group-I Mean ±SD	Group-II 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.

Discussion:

Type 2 DM is a chronic disease associated with many complications.[17] Patients with type 2 DM have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease.[18] Cardiovascular disease accounts for up to 80% of deaths in individuals with type 2 DM.[19] 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.[12,20] Despite improvement in primary prevention and treatment, CAD remains the chief cause of death in most developed countries.[21] The incidence is much greater in developing countries.[22]

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 I (DM with CAD) was 54.73 (±8.94) years as compared to 56.59±13.63 years and 58.01±12.9 years, studies done by Assiri et al. (2012) and Al-Saif et al. (2011). It was 52.0±8.6 years in a study reported by Siddique et al. (2010) and 56.6 years by Ullah M et al., (2011) in Bangladesh.[21,23-25]

Mean total count of WBC was found 9.90 ± (1.91) x 10^9/L in Group I and 8.66 ± (1.37) x 10^9/L in Group II. This association is highly significant (p <0.001). On the other hand, mean ESR was 31.07±18.25 mm in 1st hour in Group I whereas it was 11.76±6.20 mm in 1st hour in Group II which was statistically highly significant (p <0.001). Our findings are similar to other study conducted by Selcuk et al. (2011).[26] They found significantly higher WBC count (8.2vs7.4, p value 0.001) and ESR (25.5vs12.02, p value <0.001) in CAD group. In another study conducted by Sonmez et al. (2013), they also found higher WBC count in DM with CAD group (7.75 vs 7.01, p value 0.01).[2]

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±1.04 vs 6.13±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.[27] Even in non-diabetic individuals HbA1c can be associated with CAD and its severity.[28]

In this study, we found a relation of NLR with the presence of coronary artery disease. There is no doubt in cardiovascular medical science that atherosclerosis is a chronic inflammatory disease.[13,29] In our study, mean NLR in Group I was 2.76 (±0.74), whereas it was 1.56 (±0.15) in Group II. There was statistically significant association between two groups in this regards (p <0.001). Our findings are similar to a study conducted by Sahin et al. (2013).[1] They also found higher mean NLR in patients with CAD group (1.98±0.85 vs 2.69±1.74) (p <0.001). Sonmez et al. (2013) also found higher mean NLR in patients with coronary artery disease (2.3 vs 1.6) (p <0.001).[2]

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 atherosclerosis in diabetic patients with CAD. In this study, we found a relationship between NLR and atherosclerosis in CAD

proven with coronary angiography and NLR is a useful predicator of atherosclerosis in type 2 DM patients with CAD.

Limitations
The main limitation of our study was the small sample size and this was a non-randomized single center study. We did not compare the prognostic value of NLR with other inflammatory markers such as: CRP, interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF- ± etc.

Conclusion
By the assesment of NLR we can take preventive measure and precaution to reduce the progression of atherosclerosis in type 2 diabetic patients with CAD. NLR is a simple, cost effective, readily available test and could be calculated easily to predict the atherosclerosis and can take extra measure and preventive intervention to prevent the atherosclerosis in type 2 DM patients with CAD.

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