International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 8, August 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.8.2024.69

ORIGINAL RESEARCH

A case control study to determine the difference of neutrophil to lymphocyte ratio in coronory artery disease patients and healthy persons

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Received date: 15August, 2024 Acceptance date: 22 August, 2024

ABSTRACT

Background: We aimed to compare the predictive value of Neutrophil-to-lymphocyte ratio in patients of CoronaryArteryDisease with healthy persons and to compare the values of NLR among CAD patients with past history of MI and without past history of MI.**Methodology:** This study was an observational analytical case control study, conducted on 75 cases with CAD and 150 healthy controls reporting to Department of Medicine, People's College of Medical Sciences& Research Centre and associated People's Hospital, Bhopal. The study was conducted over the period of 20 months i.e. from 1stNovember 2022 to 30th June 2024. Detailed history was obtained and all the patients were subjected to complete blood examination and NLR was calculated. **Results:** Mean NLR was found to be significantly higher in cases as compared to control group ($p<0.05$).NLR is excellent tool for predicting coronary artery disease (AUC-0.944).The best cut off of NLR for predicting CAD was 2.05 with sensitivity and specificity of 89.3% and 76% respectively.Mean NLR in cases with previous history of MI was found to be significantly higher as compared to cases with no previous history of MI. **Conclusion:** NLR is a simple, cost effective, easily available tool for predicting the risk of CAD. Also, NLR can help in risk stratification in cases with CAD, as observed from high NLR in cases with previous MI as compared to those with no previous history of MI. NLR may help physicians in facilitating early and timely detection of CAD and may aid in initiating early management of the condition.

Keywords- NLR, CAD, MI, risk stratification, inflammation, atherosclerosis

INTRODUCTION

Cardiovascular disease (CVDs) including ischemic heart disease are leading non communicable diseases attributing to significant morbidity and mortality worldwide.[1]World Health Organization factsheet states that around 17.9 million deaths worldwide are due to cardiovascular diseases (CVDs).[2] India is currently facing epidemiologic transition and non-communicable diseases including cardiovascular diseases are rapidly rising in India.[3]

Atherosclerosis plays an important role in onset, progression as well as complications of coronary artery diseases. An initial injury to the endothelium leads to activation of atherogenic process by the activation of macrophage, formation of foam cell and activation of inflammatory cells leading to release of cascade of inflammatory mechanisms.[4] All these factors contribute to release of cytokines and metalloproteinases, leading to destabilization of an atherosclerotic plaque.[5] Therefore, inflammation has a role in both the development and progression of atherosclerosis in coronary artery disease (CAD), and these inflammatory indicators may be used to predict a patient's prognosis. An appropriate inflammatory marker suggesting underlying coronary artery disease may aid in risk stratification of the condition and may have prognostic significance in the evaluation of patients with coronary artery disease, since elevated levels of inflammatory markers are linked to instability of atherosclerotic plaque.[6,7]

Research indicates that there is a high correlation between CAD and a number of inflammatory

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biomarkers.[8-10] Patients with CAD have been found to have higher total leucocyte counts, which may aid in independently estimating the risk of CAD.[1,12,13] While lymphocytes have an antiatherosclerotic function and aid in controlling the inflammatory response, neutrophils secrete inflammatory mediators that contribute to vascular wall degradation and atherogenesis.[14] In light of this, it has been proposed that the neutrophil to lymphocyte ratio (NLR) is a significant inflammatory biomarker for the assessment of patients with cardiovascular disorders.[15]

Numerous studies have been conducted for assessing the role of NLR as a prognostic and diagnostic indicator of CAD and this marker in is found to be elevated in patients with coronary artery disease. Increased NLR is found to be associated with increased long term risk of mortality in stable CAD patients as well as in patients with acute coronary syndrome (ACS) patients.[16,17] With the above background, the present study aimed to compare the predictive value of Neutrophil-to-lymphocyte ratio in patients of CoronaryArteryDisease with healthy persons and to compare the values of NLR among CAD patients with past history of MI and without past history of MI.

METHODOLOGY

This was an observational analytical case control study, conducted on 75 cases with CAD presenting with chest painand 150, age and sex matched healthy controls (coming for routine medical fitness and found fit in medicine OPD or Persons accompanying the patient and willing to take part in the study with no history of any chronic disease like hypertension, diabetes mellitus, thyroid disorder, not on any regular medication and no substance use) reporting to Department of Medicine, People's College of Medical Sciences& Research Centre and associated People's Hospital, Bhopal. The study was conducted over the period of 20 months i.e. from 1stNovember 2022 to 30th June 2024. All CAD patients having history of chest pain with abnormal ECG, TMT or echocardiographic findings suggestive of MI were included whereas CAD associated with other comorbidities [like diabetes mellitus, Chronic Kidney disease (sr. creatinine > 1.4mg/ dl)], fever or infectious diseases, sarcoidosis, Connective tissue disorders, autoimmune disorders,hematological abnormality (including malignancy, severe anemia and features suggestive of bone marrow suppression), history of smoking or alcohol, patients on immunosuppressants or steroid use since more than 15 days or TLC > 11000/ cu mm of blood were excluded from the study.

After obtaining ethical clearance from Institute's ethical committee, all patients with chest pain coming to department of Medicine, PCMS & RC, People's Hospital, Bhopal, fulfilling the inclusion and exclusion criteria and willing to give consent were enrolled. All the patients were categorized into 2 groups based upon the diagnosis of coronary artery disease

- Group A- Patients with chest pain diagnosed as coronary artery disease (Case group)
- Group B- Patients or individuals coming for routine medical fitness and found fit in medicine OPD

Detailed data regarding sociodemographic variables such as age and gender along with clinical data such as Chief complaint, comorbidities, past history, medication history, history of substance use (alcohol, smoking, tobacco, any other addiction was obtained and documented. Further all the patients were subjected to detailed general and systemic examination, and findings were documented. All patients were then subjected to ECG and echocardiography. CAD was diagnosed and confirmed based on symptoms of chest pain and any of the following criteria:

- ECG changes indicative of ischemia
- Echocardiographic evidence of RWMA
- Positive exercise stress test
- Past history of MI, acute coronary syndrome, angina, patient on antianginal treatment.

Additionally, under strict aseptic precautions, 2 ml of venous blood was taken in EDTA tubes and submitted for the following blood investigations, which were performed on all patients and controls:

- Total Leucocyte count
- Differential Leucocyte count
- Absolute neutrophile count,
- Absolute Lymphocyte count

Based upon this, Neutrophil-to-Lymphocyte Ratio was calculated.

STATISTICAL ANALYSIS

Data was compiled using MsExcel and analysed using IBM SPSS software version 20. Categorical data was expressed as frequency and proportions whereas continuous data was expressed as mean and standard deviation. The categorical variables between cases and controls were compared using Chi square test whereas continuous variables were compared using Independent t test. To identify the optimal cut off value of NLR for calculating the risk of CAD between cases and controls, ROC curve analysis using Youden's index was performed. P value of less than 0.05 was considered statistically significant.

RESULTS

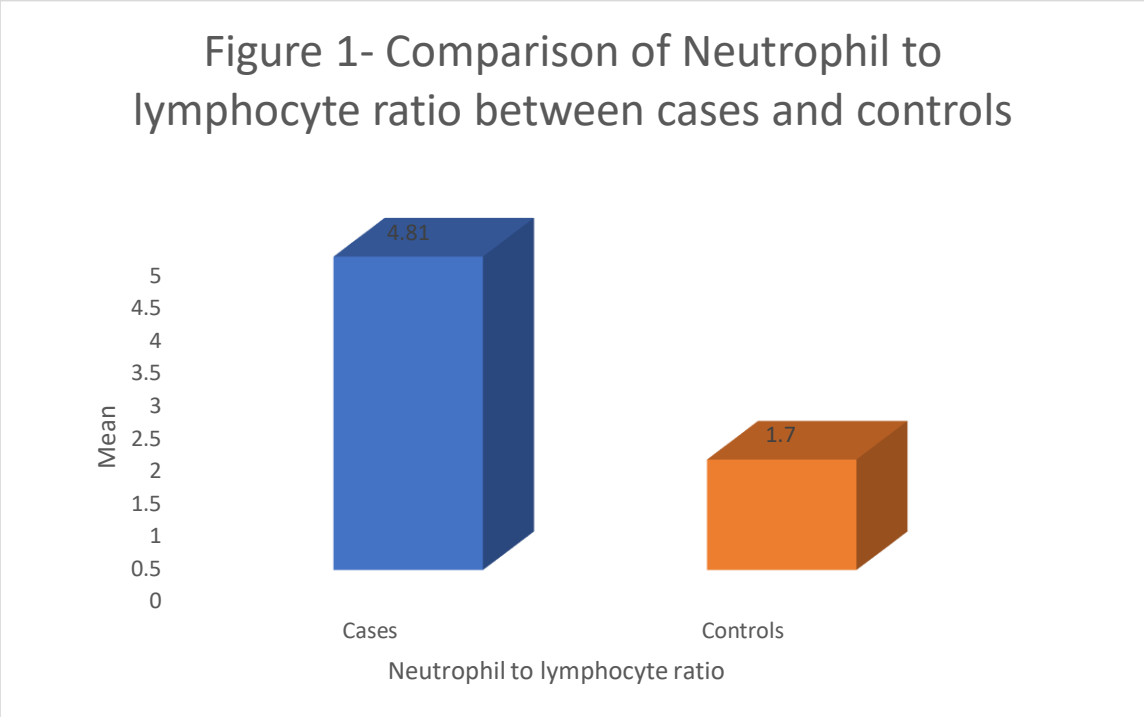
The present study on total of 75 cases with coronary artery disease and 150 age and sex matched healthy controls. Mean age of patients with CAD was 55.04±13.198 years whereas mean age of controls was 52.39±13.20 years.

DOI: 10.69605/ijlbpr_13.8.2024.69

Table 1- Comparison of baseline variables between cases and control

Baseline variables		Group				P value
		Cases (n=75)		Controls (n=150)		
		n	%	n	%	
Age	≤30	3	4.0	9	6.0	0.79
	31-40	8	10.7	17	11.3	
	41-50	19	25.3	27	18.0	
	51-60	17	22.7	43	28.7	
	>60	28	37.3	54	36.0	
Gender	Male	55	73.3	103	68.7	0.471
	Female	20	26.7	47	31.3	
Clinical features	Chest Pain	75	100.0	0	0.0	**0.001**
	Breathlessness	64	85.3	0	0.0	
	Abdominal Pain	2	2.7	0	0.0	
	Arm Pain	1	1.3	0	0.0	
	Body Ache	1	1.3	0	0.0	
	Cough	1	1.3	0	0.0	
	Restlessness/ uneasiness	5	6.7	0	0.0	
	Palpitations	14	18.7	0	0.0	
	Pedal Edema	2	2.7	0	0.0	
Comorbid conditions	Hypertension	2	2.7	0	0.0	**0.045**
	CAD/TVD/Post PTCA	1	1.3	0	0.0	0.09
	Cataract	1	1.3	0	0.0	0.09
	COPD	2	2.7	0	0.0	**0.045**
	Hypothyroidism	1	1.3	0	0.0	0.09
	Liver Cirrhosis	2	2.7	0	0.0	**0.045**

As shown in table 1, majority of cases (37.3%) and controls (36%) belonged to more than 60 years of age. We observed male predominance for coronary artery disease. Cases and controls were comparable with respect to age and sex composition (p>0.05). All the cases presented with chest pain and 85.3% cases presented with breathlessness. All the clinical features were reported in significantly higher proportions of cases as compared to controls (p<0.05).We reported hypertension (27% vs 0%), COPD (2.7% vs 0%) and liver cirrhosis (27% vs 0%) in significantly higher proportions of cases as compared to controls (p<0.05). Past history of MI was reported in 9.3% cases.



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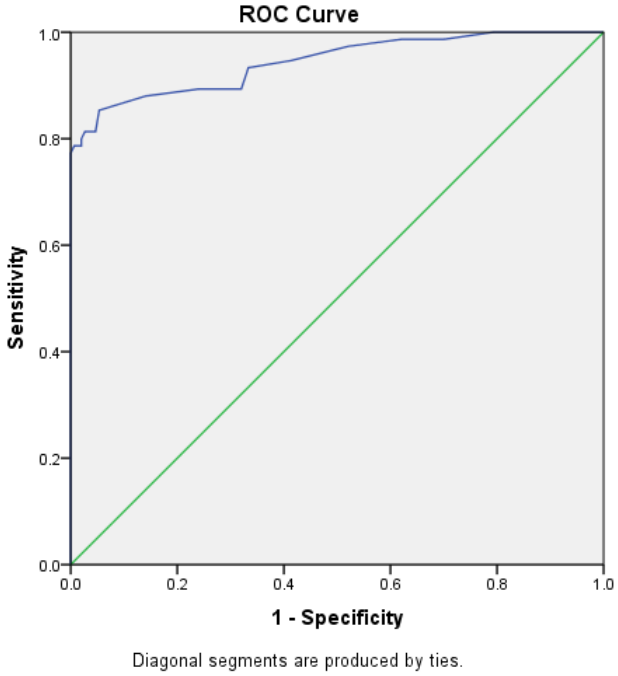
Mean neutrophils to lymphocyte ratio in patients with coronary artery disease was 4.81±1.70 whereas that in control group was 1.70±0.39. Mean neutrophils to lymphocyte ratio was found to be significantly higher in cases as compared to control group ($p<0.05$) (figure 1).

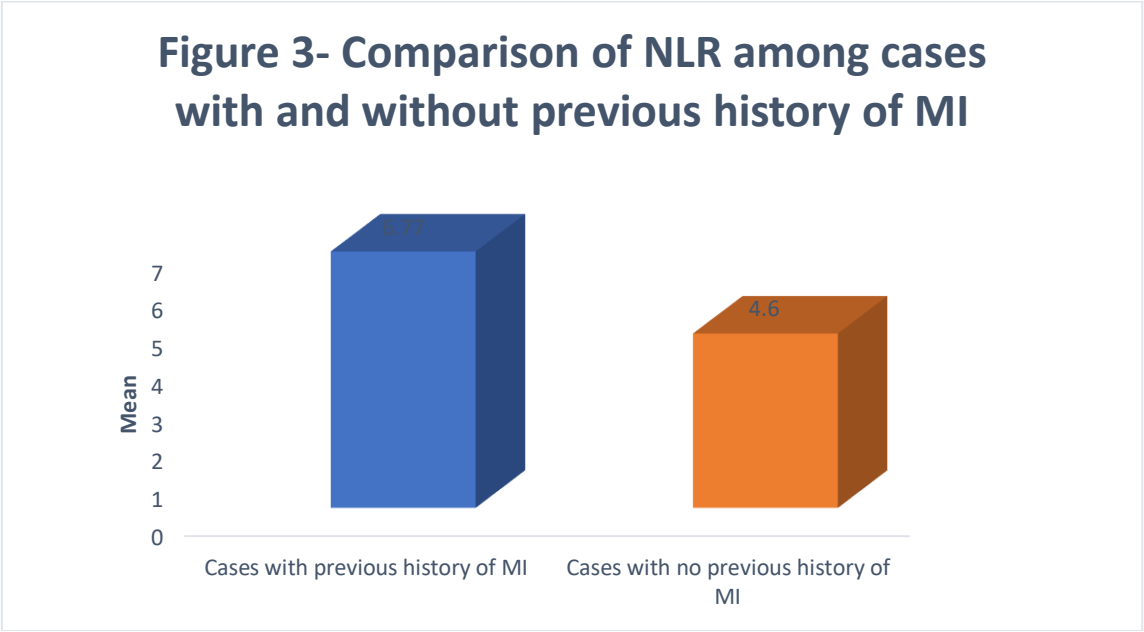
Table 2- Area under curve for NLR in CAD patients as compared to controls

Area	Std. Error	Sig.	95% CI	Cut off	Sensitivity	Specificity
0.944	0.018	0.0001	0.910-0.979	2.05	89.3	76

Neutrophil to lymphocyte ratio is excellent tool for predicting coronary artery disease (AUC-0.944; 95% CI-0.910-0.979; $p<0.05$). The best cut off of NLR for predicting CAD was 2.05 with sensitivity and specificity of 89.3% and 76% respectively (Table and Figure 2)

Figure 2- ROC curve for NLR in CAD patients as compared to controls





Mean NLR ratio in cases with previous history of MI was found to be significantly higher as compared to cases with no previous history of MI (6.77±3.19 vs. 4.6±2.03; $p<0.05$) (Figure 3).

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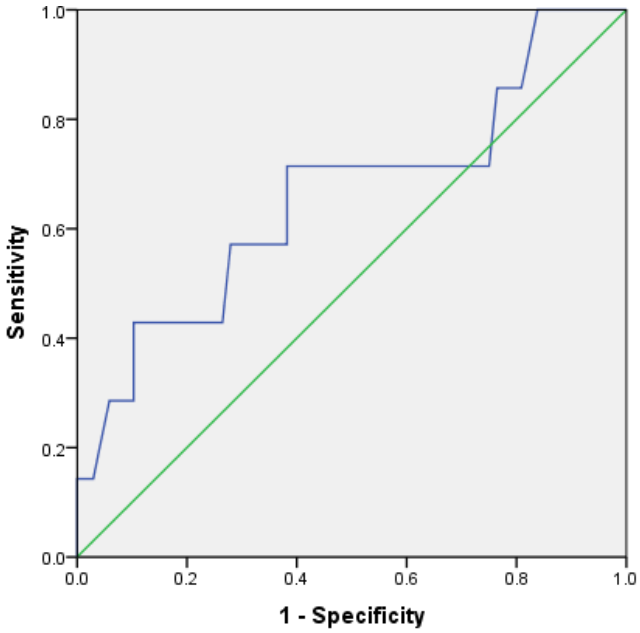
Table 3- Area under curve for NLR in CAD patients with previous MI as compared to cases with no previous history of MI

Area	Std. Error	Sig.	95% CI	Cut off	Sensitivity	Specificity
0.660	0.121	0.166	0.422-0.897	5.30	71.4	61.8

Area under the curve showed satisfactory predictability of NLR for CAD in cases with previous history of MI as compared to those with no previous history of MI (AUC- 0.66; 95% CI- 0.422-0.897, p>0.05). At the cut off of 5.30, the sensitivity was 71.4% and specificity was 61.8% (Table 3 and figure 4)

Figure 4- ROC curve for NLR in CAD patients with previous MI as compared to cases with no previous history of MI



Diagonal segments are produced by ties.

DISCUSSIONS

The inflammation is documented to be one of the major factor in onset, progression as well as complications of coronary artery disease. Elevated levels of the inflammatory markers have been linked with instability of the atherosclerotic plaque. Thus, identification of appropriate inflammatory markers may help in evaluation and determining prognosis in patients with coronary artery disease.[6,7] Literature suggests that Neutrophil to lymphocyte ratio (NLR) can be used as a biomarker of inflammation in cases with CAD as neutrophils secrete inflammatory mediators causing vascular wall degeneration, promoting atherosclerosis whereas lymphocytes are regulators of inflammatory response and are antiatherogenic in nature.[15]

Literature suggests that as compared with healthy individuals, coronary disease patients have significantly higher NLR. NLR and neutrophil level have been linked with the atherosclerotic plaque.[18] Also NLR have been correlated with the severity of CAD and amount of coronary blockage.[19] Increased NLR has been linked to the existence, severity, and extent of atherosclerotic plaques in coronary arteries, according to recent computed CT coronary angiography investigations. The risk of coronary atherosclerosis (OR = 2.30) and critical luminal stenosis (OR = 2.60) increased with an NLR score greater than 2.25.[20] In present study, mean neutrophil counts in cases was 73.46±9.76% and mean lymphocytes count was 18.47±7.55%, however, mean neutrophil: lymphocyte count was 4.81±1.70 in cases with CAD. In contrast, mean neutrophil counts were significantly lower in controls (58.6±6.35%) whereas mean lymphocyte counts were significantly higher in controls (35.2±5.40%) leading to significantly lower neutrophil to lymphocyte ratio in controls (1.70±0.39%) (p<0.05). ROC curve analysis revealed excellent predictive ability of neutrophil to lymphocyte ratio for CAD (AUC-0.944; 95% CI-0.910-0.979; p<0.05). At the cut off of NLR of 2.05, sensitivity was 89.3% and specificity was 76%. The primary involvement of neutrophilia in CAD may be attributed to the release of several inflammatory mediators that damage tissue, including myeloperoxidase, elastase, and oxygen free radicals. The most likely causes of lymphopenia are higher NLR in patients with CAD and increased apoptosis brought on by increased inflammation, which in turn causes reduced production as a result of raised steroid levels brought on by CAD-induced stress. Reduced lymphocyte counts and an increase in neutrophils are

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risk markers for subsequent cardiovascular events. Elevated NLR thereby combines the two leukocyte subtypes' predictive risk into a single risk factor.[13,21]

Selcuk et alalso found significantly low lymphocyte counts in patients with CAD (2031±741 cells/μL) as compared to patients without CAD(2392± 611 cells/μL) whereas mean neutrophils as well as neutrophil to lymphocyte ratio was found to be significantly higher in patients with CAD (2.86±1.57 vs 2.04±1.01, p<0.05).[22]

Our study findings were concordant with the findings of Chen et al, in which the authors documented mean neutrophils as well as median NLR to be significantly higher whereas significantly lower mean lymphocyte counts in patients with CAD as compared to control group with no CAD (3.82±1.28 vs. 3.64±1.30,: 1.99 vs 1.83; 1.87±0.60 vs.1.94±0.62 respectively, with p value of less than 0.05).[23]Sharma et al found mean neutrophil levels to be significantly higher in patients with CAD (9184.2±4532.9% vs 7284.2±3924.9%; p<0.05). However, they found no significant difference in mean lymphocyte levels between cases with CAD and cases without CAD (2191.0±922.6% vs. 2265.6±951.9%; p>0.05). The NLR was documented to be significantly higher in cases with CAD as compared to individuals without CAD (5.6±4.5 vs. 4.3±3.8; p<0.05). Sensitivity and specificity of NLR was 83.64% and 63.46% respectively at the cut off of 2.13 (AUC-0.823; p<0.05).[24]

Nepal et al found NLR to be best predictor of CAD with AUC of 0.815, and sensitivity of 97.4%, specificity of 83.3% at the best cut off of 1.785.[25]In a study of Bhuiyan et al, mean NLR in CAD group was 2.76±0.74 whereas that in patients with no CAD was 1.56±0.15, and the observed difference was statistically significant (p<0.05). ROC curve analysis revealed excellent predictive ability of NLR for diagnosis of CAD (AUC0.968;p<0.05). At the cut off of 1.74, the sensitivity was 98% whereas specificity was 58%.[26]

In our study, about 9.3% cases had past history of MI and mean neutrophil to lymphocyte ratio in CAD cases with previous history of MI (6.77±3.19) was found to be significantly higher in cases with no previous history of MI (4.6±2.03) (p<0.05). However, the predictive ability of NLR for CAD in cases with previous history of MI was satisfactory (AUC- 0.66; 95% CI- 0.422-0.897, p>0.05), with sensitivity and specificity of 71.4% and 61.8% at the cut off of 5.30.To best of our knowledge, none of the previous studies assessed the role of NLR in CAD cases with and without previous MI. Dziedzic et alassessed the role of NLR in all the CAD cases with previous history of MI and categorized patients into two groups i.e. patients with stable CAD and patients with acute coronary syndrome. The median NLR in cases with stable CA was 2.3 whereas that in cases with ACS was 2.8, and the difference was statistically significant (p<0.05).[27] Song et al documented NLR to be a strong predictor of mortality in cases with NSTEMI i.e. NLR of more than 4.7 was associated with approximately three fold higher risk of mortality as compared to NLR of less than 3.[28] Similarly, Ipek et al documented NLR of more than 3.7 in cases with STEMI to have a prognostic significance for identification of serious complications such as ventricular free wall rupture.[29]

Our study had certain limitations, first the sample size of the study was small and the group of patients were mainly from urban area lacking the demographic diversity. Secondly, the study was conducted as an observational comparative study and follow up of patients was not done limiting the causal analysis of various factors.Third, the influence of comorbidities and their medications on NLR was not taken into consideration.

CONCLUSION

NLR is a simple, cost effective, easily available tool for predicting the risk of CAD. NLR levels are significantly high in CAD as compared to controls and ROC curve revealed excellent predictive ability of NLR for CAD at cut off of 2.05 with sensitivity and specificity of more than 75%. Also, NLR can help in risk stratification in cases with CAD, as observed from high NLR in cases with previous MI as compared to those with no previous history of MI. NLR may help physicians in facilitating early and timely detection of CAD and may aid in initiating early management of the condition. We recommend that neutrophil to lymphocyte ratio, a ratio that can be derived from a simple, universally available and cost-effective investigation i.e. complete blood count can be utilized for predicting coronary artery disease with excellent predictive ability and thus NLR must be assessed in every case presenting with history suggestive of CAD with or without previous history of MI.

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