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The Role of Neutrophils to HDL-C Ratio in Predicting the Severity of Coronary Artery Disease

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ABSTRACT

Background: Coronary artery disease (CAD) is intricately linked to inflammation and atherosclerosis. Neutrophils, key contributors to inflammation, have been associated with CAD development and cardiovascular risk.

Objective: To investigate the potential of the neutrophils to HDL-C ratio (NHR) as a predictor for the severity of coronary artery disease.

Methods: This dual-center, cross-sectional, comparative observational study was conducted at Benha University Hospitals and Heliopolis Hospital from January 2022 to December 2022. The study included 200 patients scheduled for coronary angiography due to CAD-related symptoms. Based on coronary angiography results, patients were categorized into three groups: CAD+ (n=75) with luminal stenosis ≥ 50% in at least one major coronary artery, CAD- (n=25) with luminal stenosis < 50% in one major coronary artery, and a control group (n=100) with normal coronary angiography.

Results: Diabetes, hypertension, and smoking were more prevalent in CAD+ than CAD- and control groups (All P < 0.05). CAD+ had higher BMI (27.99 Kg/m^2), systolic (145.69 mmHg), and diastolic blood pressure (96.53 mmHg) compared to CAD- and control groups (p<0.001). CAD+ had elevated creatinine, triglycerides, uric acid, neutrophils, eosinophils, and neutrophil to HDL-C ratio (p<0.001). NHR predicted severe CAD with AUC=0.907. NHR correlated positively with BMI, triglycerides, neutrophils, eosinophils, and Gensini score. Logistic regression showed BMI and NHR were significant predictors of disease severity.

Conclusions: NHR could serve as a valuable marker in risk stratification and clinical decision-making for CAD patients.

Keywords: Neutrophils, HDL-C Ratio, Severity, Coronary Artery Disease.

INTRODUCTION

Inflammation, the atherosclerotic process, and the development of coronary artery disease (CAD) are closely linked through a variety of complex pathophysiological pathways. Atherosclerosis plays a dominant role in the pathophysiological process of the disease, and studies have shown that CAD and atherosclerosis are closely associated with inflammation [1].

Meanwhile, lipid and inflammatory molecules play a key role in the development of inflammation. Studies have shown that neutrophils contribute critically in the atherosclerotic process and are markers of persistent inflammation as well as predictors of cardiovascular risk [2,3]. Neutrophil counts increase with metabolic changes associated with atherosclerosis development and closely correlate with the extent of early atherosclerosis formation under hyperlipidemic conditions [4].

Epidemiological data have provided broad evidence that low concentrations of high-density lipoprotein (HDL) cholesterol indicate increased cardiovascular risk. Although less consistently, this relationship is even apparent in patients treated with statins. Therefore, raising HDL cholesterol has become a therapeutic target in CAD [5].

Moreover, HDL-C has been found to be closely related to coronary artery stenosis; it has the function of not only reverse cholesterol transport but also oxidation resistance and vascular endothelial function protection, with its concentration being negatively correlated with the coronary heart disease risk. Neutrophils and HDL-C are both important in the atherosclerotic process.

However, few studies have been found to integrate these two indicators [6].

Therefore, the aim of this study is to evaluate the role of neutrophils to HDL-C ratio in predicting the severity of coronary artery disease.

PATIENTS AND METHODS

Study Design: This dual center, cross sectional, comparative observational study was conducted at Benha University Hospitals and Heliopolis Hospital during the period from 1/1/2022 to 30/12/2022.

Patients: This study included 200 patients who were scheduled for coronary angiography due to CAD related symptoms. Patients were classified into 3 groups: Group (1) (CAD+): included patients with luminal stenosis ≥ 50% in at least one of the major coronary arteries (LM, LAD, LCX and RCA) (n=75). Group (2) (CAD-): included patients with luminal stenosis < 50% in one of the major coronary arteries (n=25). Group (3) (control): included patients with normal coronary angiography (n=100).

Inclusion criteria: Patients presented with typical chest pain indicated for coronary angiography, with age older than 18 years. Patients accepted to be included in the study.

Exclusion criteria: Coronary artery bypasses surgery, prior PCI, acute coronary syndrome, liver and kidney disease, inflammatory disease, heart failure, blood

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diseases, rheumatic diseases, malignant tumors and alcohol use.

Methods

All patients were subjected to complete history taking including age, gender and cardiac risk factors (DM, HTN, smoking and family history of CAD), complete physical examination including heart rate, BMI, systolic and diastolic blood pressure, and laboratory investigations including (CBC, lipid profile, HDL-C and LDL-C, neutrophil to HDL ratio, serum creatinine, uric acid, albumin, and total, direct and indirect bilirubin).

Echocardiography: was done using the commercially available systems (Epic 7, Philips ultrasound, USA). Images were obtained with a simultaneous ECG signal. Recordings and calculations of different parameters were performed according to the recommendations of the American Society of Echocardiography (ASE).

Coronary angiography: All patients underwent coronary angiography, performed by experienced interventional cardiologists using standard protocols. Cardiologists visually assessed the stenosis degree, documented the affected coronary locations, including LAD, LCX, RCA, and LM arteries, and determined the number of affected vessels. The findings, recorded for each patient, were crucial for assessing the severity and extent of coronary artery disease in our study population.

Ethical consideration: This study received ethical approval from the Institutional Review Board, Faculty of Medicine, Benha University. All participants provided written informed consents. The study adhered to the ethical guidelines outlined in the World Medical Association's Declaration of Helsinki for research involving human subjects.

Sample Size: Epi Info STATCALC was used to calculate the sample size by considering the following assumptions: - 95% two-sided confidence level, with a power of 80% and an error of 5% odds ratio calculated= 1.115. The final maximum sample size taken from the Epi- Info output was 189. Thus, the sample size was increased to 200 subjects to assume any drop out cases during follow up.

Statistical analysis

The statistical analysis of the collected data was conducted using IBM SPSS Statistics (Version 25.0). To ensure the robustness of the analysis, the following steps were taken: The normality of data distribution was assessed using the Shapiro-Wilk test. Descriptive statistics were employed to summarize the data. Mean and standard deviation were reported for numerical data, while frequency and percentage were utilized for non-numerical data.

Non-parametric two-group comparisons were performed using the Mann-Whitney test. Non-parametric multi-group comparisons were carried out with the Kruskal-Wallis test, and parametric multi-group comparisons were performed using one-way ANOVA. When statistical tests, such as the ANOVA test or Kruskal-Wallis test, indicated significance ($p < 0.05$) in multi-group comparisons, post hoc tests were applied to make pairwise comparisons between each group and every other group. t-test was used compare SV, LVEF, and Gensini score Qualitative Variable Relationships: Qualitative variable relationships were assessed using the Chi-Square test and Fisher's exact test. Correlation analysis (Pearson correlation) was employed to assess associations between quantitative variables. The Receiver Operating Characteristic (ROC) curve was utilized to evaluate the performance of diagnostic measures. Various regression analyses were conducted to predict risk factors and their relationships with the variables under investigation. Significance was established at $p < 0.05$, with a 95% confidence interval [7].

RESULTS

Demographic and clinical data of the studied groups were provided in **table 1**. Regarding laboratory investigations, triglycerides, neutrophils, eosinophils, uric acid, serum creatinine, and neutrophil to HDL-C ratio were significantly higher in CAD+ group than CAD- and control group.

Table 1: Demographic, clinical, and laboratory data				
	CAD+ (n=75)	CAD- (n=25)	Control (n=100)	P
Age (year)	64.39±6.02	65.05±4.68	59.54±5.01	<0.001*
Male gender	27(36%)	9(36%)	34(34%)	0.957
Smoking	30(40%)	5(20%)	12(12%)	<0.001*
Hypertension	38(51%)	10(40%)	30(30%)	0.021*
Diabetes mellitus	30(40%)	6(24%)	12(12%)	<0.001*
BMI (kg/m^2)	27.99±2.08	24.51±1.29	24.23±0.93	<0.001*
SBP (mmHg)	145.69±13.72	119.7±13.35	115.06±9.95	<0.001*
DBP (mmHg)	96.53±11.01	82.29±10.4	76.34±7.51	<0.001*
HR (bpm)	79.14±4.89	75.95±7.98	73.64±9.11	<0.001*
Laboratory investigations				
Creatinine (mg/dL)	0.78±0.08	0.75±0.07	0.74±0.1	0.013*
Albumin (mg/dL)	4.17±0.09	4.43±0.09	4.38±0.09	<0.001*
Total bilirubin (mg/dL)	0.88±0.01	0.95±0.01	0.94±0.01	<0.001*
Direct bilirubin (mg/dL)	0.29±0.01	0.29±0.01	0.29±0.01	1
Indirect bilirubin (mg/dL)	0.58±0.01	0.65±0.02	0.65±0.01	<0.001*
Total cholesterol (mg/dL)	158.82±15.59	154.14±16.66	158.52±16.33	0.423
Triglyceride (mg/dL)	86.62±18.04	70.66±7.95	77.09±19.97	<0.001*
Uric acid (mg/dL)	1.97±0.1	1.89±0.1	1.89±0.01	<0.001*
LDL-C (mg/dL)	109.46±13.46	104.39±15.23	107.81±15.47	0.325
HDL-C (mg/dL)	50.7±6.36	49.75±5.66	49.35±5.6	0.314
Neutrophils (x 10^9/L)	4.67±0.09	3.94±0.1	3.92±0.09	<0.001*
Eosinophil (x 10^9/L)	0.93±0.1	0.09±0.01	0.1±0.01	<0.001*
NHR	3.89±0.52	3.2±0.75	3.18±1.65	<0.001*

BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood pressure; HR= heart rate; LDL-c= low density lipoprotein cholesterol; HDL-c= high density lipoprotein cholesterol; NHR= neutrophil to HDL-c ratio.

Coronary angiography data revealed that LAD and RCA were more commonly affected in CAD+ group. CAD+ group had more patients with 2 vessel disease. Gensini score was significantly higher in CAD+ group (**Table 2**).

Table 2: Echocardiography and coronary angiography data			
	CAD+ n=75	CAD- n=25	**P value**
LVEDD (cm)	4.5±1.02	4.6±1.23	0.574
LVESD (cm)	3.8±2.35	3.9±1.89	0.325
SV (ml)	70.25±0.79	72.63±0.77	0.297
LVEF (%)	64.46±3.97	66.58±2.54	0.162
Affected coronary, n (%)			
LAD	43(57.5%)	6(24%)	0.004*
LCX	24(32%)	12(48%)	0.149
RCA	32(42.66%)	3(12%)	0.005*
LM	17(12%)	4(16%)	0.478
Number of affected vessels, n (%)			
Single vessel disease	16 (21.33%)	25 (100%)	<0.001*
2- vessels disease	46 (61.3%)	0	
3- vessels disease	13 (17.33 %)	0	
Gensini score	39.65±2.61	5.61±0.75	<0.001*

LVEDD= left ventricular end-diastolic diameter; LVESD= left ventricular end-systolic diameter; SV= stroke volume; LVEF= left ventricular ejection fraction; LAD= left anterior descending artery; LCX= left circumflex artery; RCA= right coronary artery; LM= left main artery.

The ROC curve analysis was performed to evaluate the predictive ability of neutrophil to HDL-C ratio (NHR) for the presence of coronary artery disease (CAD) with greater than 50% luminal stenosis. The area under the curve (AUC) was calculated as 0.907, indicating a high discriminative power of NHR in distinguishing between patients with CAD and those without. The 95% confidence interval (CI) for the AUC ranged from 0.715 to 0.862, indicating a reliable estimate of the AUC. The AUC was statistically significant, further supporting the predictive ability of NHR for CAD with significant luminal stenosis. The optimal cut-off value for NHR was determined to be 3.62. At this threshold, the sensitivity of NHR in correctly identifying patients with CAD was 89.12 %, indicating a high proportion of true positives. The specificity of NHR was found to be 82.16 %, indicating a high proportion of true negatives (**Figure 1**).

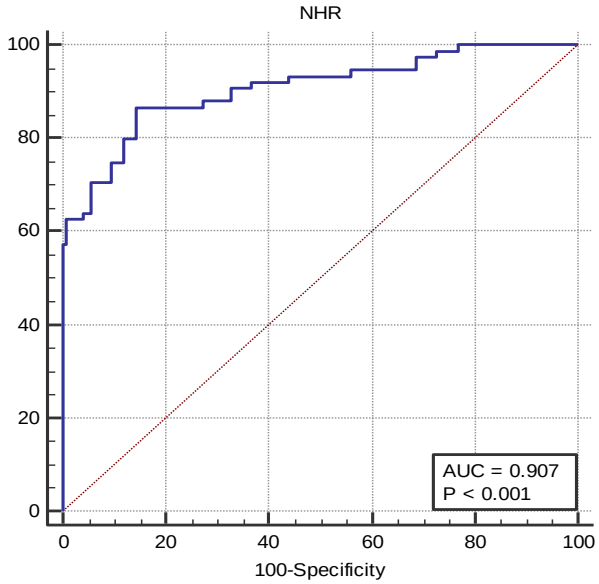

Figure 1: ROC curve analysis for NHR as a predictor of coronary artery disease with >50% luminal stenosis.

The analysis revealed several noteworthy findings. Firstly, strong positive correlations were found between NHR and both neutrophils ($r = 0.588$, $p < 0.001$) and eosinophils ($r = 0.587$, $p < 0.001$), indicating that elevated levels of these immune cells are associated with higher NHR values. Additionally, NHR showed a significant positive correlation with the Gensini score ($r = 0.601$, $p < 0.001$), suggesting that an increased NHR is associated with greater severity of coronary artery disease, as assessed by the Gensini score. Furthermore, there was a positive and significant correlation between NHR and BMI ($r = 0.505$, $p < 0.001$), suggesting that higher BMI values are associated with elevated NHR. Interestingly, no significant correlation was found between NHR and total cholesterol, LDL-C, HDL-C, and LVEF. However, a significant positive correlation was observed between NHR and triglycerides ($r = 0.173$, $p = 0.01$) **(Figure 2).**

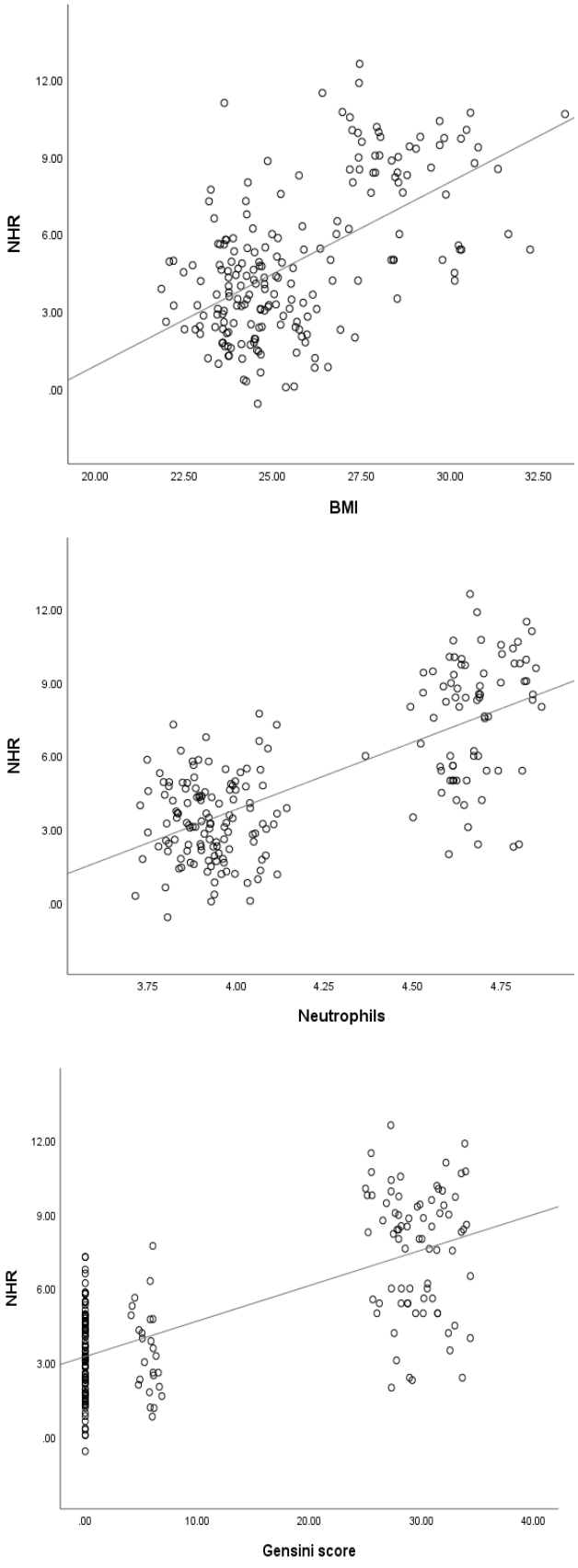

Figure 2: Significant correlation graph's between NHR and other studied variables.

In the univariate analysis, age, male gender, smoking, BMI, triglycerides, and neutrophils to HDL-C ratio (NHR) demonstrated significant associations with CAD. These findings suggest that these factors may play a role in the development and progression of CAD. Upon further analysis using the multivariate model, BMI, and NHR independently contribute to the prediction of CAD with luminal stenosis greater than 50% (**Table 3**).

Table 3: Logistic regression analysis to predict CAD with >50% luminal stenosis

	Univariate			Multivariate		
	p	**OR**	**95% CI**	**p**	**OR**	**95% CI**
Age	0.001*	0.889	0.841-0.940			
Male gender	0.001*	0.314	0.173-0.569			
Smoking	0.002*	0.334	0.165-0.677			
BMI	0.001*	0.234	0.157-0.348	0.001*	0.272	0.163-0.454
Tri-glycerides	0.001*	0.970	0.955-0.986			
NHR	0.001*	0.421	0.333-0.533	0.001*	0.478	0.350-0.654

BMI= body mass index; NHR= neutrophil to HDL-c ratio.

DISCUSSION

In the current study, there was no significant difference between groups as regard age and gender. DM, hypertension and smoking were more prevalent in CAD+ group. CAD+ group had significantly higher body mass index, systolic and diastolic blood pressure. There was no significant difference between groups regarding heart rate. Our results were comparable to **Kou *et al.*** [8] who evaluated the relationship between neutrophils to HDL-C ratio and severity of CAD. They revealed that smoking and hypertension were more prevalent in patients with CAD.

We found that triglycerides, neutrophils, eosinophils, uric acid, and serum creatinine were significantly higher in CAD+ group. Moreover**,** neutrophils to HDL-C ratio was significantly higher in CAD+ group. Similarly, **Kaya *et al.*** [9] showed that serum glucose (P < 0.001) and creatinine levels (P =0.002) were significantly higher in patients with severe atherosclerosis, whereas HDL levels were lower than controls (P < 0.001). Compared with controls, patients with severe atherosclerosis showed a higher leukocyte (P < 0.001) and neutrophil counts (P < 0.001) and lower lymphocyte counts (P = 0.032) while mild atherosclerosis group did not differ. The NLR was significantly higher in severe atherosclerosis group compared with mild atherosclerosis and control groups (4.1±3.0, 2.4±1.2, and 1.9±0.6).

Moreover, **Sahin *et al.*** [10] assessed the relation between the neutrophil to lymphocyte ratio and its association with the severity of coronary artery disease in patients with ST segment elevation myocardial infarction. They showed that NLR was significantly associated with CAD severity in patients with STEMI and that NLR was an independent predictor for Syntax score. Also, **Kaya *et al.*** [9] showed that NLR was significantly associated with both the presence and severity of CAD in patients with stable CAD.

In the current work, Gensini score was significantly higher in CAD+ group. Similarly, **He *et al.*** [11] evaluated 175 patients who were divided into stable angina group (SA, 60 cases), unstable angina group (UA, 60 cases) and acute myocardial infarction group (AMI, 55 cases). The Gensini score was compared among the three groups. They showed that the Gensini score was significantly higher in patients with worst CHD.

In the present study, there was a strong positive correlation between NHR and BMI, triglycerides, neutrophils, and eosinophils. Additionally, NHR showed a significant positive correlation with the Gensini score suggesting that an increased NHR is associated with greater severity of coronary artery disease, as assessed by the Gensini score. There was no significant correlation between NHR and total cholesterol, LDL-C, HDL-C, and LVEF. The multivariate model showed that BMI, and NHR independently contribute to the prediction of CAD with luminal stenosis greater than 50%. Similarly, **Kou *et al.*** [8] reported that the Gensini score was positively correlated with NHR, neutrophils, creatinine, LDL-C, sex, age, cigarette smoking, hypertension, there was a negative correlation between Gensini score and HDL-C, as well as albumin. While Gensini score was not correlated with eosinophil, uric acid, bilirubin, total cholesterol, triglyceride, and diabetes mellitus.

Moreover, **Elamragy *et al.*** [12], evaluated the association between NLR and Syntax score as a measure of CAD severity in patients with NSTEMI. They reported that the Syntax score was strongly and significantly correlated with NLR; while it had intermediately significant correlation with cardiac enzymes and LDL, and a weak correlation with age, HDL, and renal functions.

Similarly, **Huang *et al.*** [13] showed a weak significant positive correlation between NHR and both Gensini score and LDL-C/HDLC. It was found that there was no correlation between monocyte to high-density lipoprotein ratio (MHR) and Gensini score. Furthermore, they showed a correlation between NHR and the extent of coronary artery disease, especially acute myocardial infarction. In addition, they performed regression analysis of predictors of multiple indicators

including Gensini score, Killip class, WBC, hemoglobin, and NHR. Foremost, as a new predictor of CAD, NHR could be calculated from the complete blood count on admission, which is a fast and convenient method.

In our study, the ROC curve analysis was performed to evaluate the predictive ability of neutrophil to HDL-C ratio (NHR) for the presence of coronary artery disease (CAD) with greater than 50% luminal stenosis. The area under the curve (AUC) was calculated as 0.907, and the optimal cut-off value for NHR was determined to be 3.62 indicating a high discriminative power of NHR in distinguishing between patients with CAD and those without.

Huang *et al.* [14] showed the ability of ROC curves of non-HDL-C, NLR, and their combination in predicting coronary artery vulnerable plaques (VPs) in patients with T2DM. Non-HDL-C combined with NLR achieved the highest performance, with AUC 0.825 (95% CI: 0.757–0.887), sensitivity 82.1%, and specificity 70.8%, followed by non-HDLC, with AUC 0.748 (95% CI: 0.676–0.818), sensitivity 0.701, and specificity 0.708, and NLR, with AUC 0.729 (95% CI: 0.650–0.800, sensitivity 0.776, and specificity 0.577).

Moreover, **Kaya *et al.*** [9] showed that NLR had significant positive correlations with age ($r = 0.165$, $P = 0.030$) and Gensini score ($r = 0.422$, $P < 0.001$). Using a cutoff level of 2.5, NLR predicted severe atherosclerosis with a sensitivity of 62% and specificity of 69% (ROC area under curve: 0.730, 95% CI: 0.648-0.813, $P < 0.001$). After multivariate analysis, high levels of NLR were independent predictors of severe atherosclerosis (OR: 1.798, 95% CI: 1.348-2.399, $P < 0.001$) together with glucose (OR: 1.010, 95% CI: 1.002-1.017, $P = 0.020$) and HDL (OR: 0.927, 95% CI: 0.884-0.971, $P <0.001$).

CONCLUSION

Neutrophils to HDL-C ratio could serve as a valuable marker in risk stratification and clinical decision-making for CAD patients.

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REFERENCES

1. Wang Q, Jing H, Lin J *et al.* (2022): Programmed prodrug breaking the feedback regulation of P-selectin in plaque inflammation for atherosclerotic therapy. *Biomaterials*,288:121705.

2.Ito F , Ito T (2020): High-Density Lipoprotein (HDL) Triglyceride and oxidized HDL: New lipid biomarkers of lipoprotein-related atherosclerotic cardiovascular disease. *Antioxidants*, 9(5):362.

3. Silvestre-Roig C, Braster Q, Ortega-Gomez A *et al.* (2020): Neutrophils as regulators of cardiovascular inflammation. *Nat Rev Cardiol.,* 17(6): 327-340.

4. Herrero-Cervera A, Soehnlein O, Kenne E (2020): Neutrophils in chronic inflammatory diseases. *Cell Mol Immunol.,* 19:177-191.

5. Kosmas C, Martinez I, Sourlas A *et al.* (2018): High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs Context.,* 7:212525.

6. Sacks F , Jensen M (2018): From high-density lipoprotein cholesterol to measurements of function: Prospects for the development of tests for high-density lipoprotein functionality in cardiovascular disease. *Arterioscler Thromb Vasc Biol.,* 38:487-499.

7. Flechner L , Tseng T(2011): Understanding results: P-values, confidence intervals, and number need to treat. *Indian J Urol.,* 27:532-5.

8. Kou T, Luo H, Yin L (2021): Relationship between neutrophils to HDL-C ratio and severity of coronary stenosis. *BMC Cardiovasc Disord.,* 21:127.

9. Kaya H, Ertaş F, İslamoğlu Y *et al.* (2014): Association between neutrophil to lymphocyte ratio and severity of coronary artery disease. *Clin Appl Thromb Hemost., 20*(1): 50-54.

10. Sahin D, Elbasan Z, Gür M *et al.* (2013): Neutrophil to lymphocyte ratio is associated with the severity of coronary artery disease in patients with STsegment elevation myocardial infarction. *Angiology*,64:423–429.

11. He Q, Zhang P, Li Y *et al.* (2021): The application of Gensini score and IL-1ra in assessing the condition and prognosis of patients with coronary artery disease. *Am J Transl Res.,* 13:10421-10427.

12. Elamragy A, Hussein A, Kaddah A *et al.* (2021): Neutrophil: lymphocyte ratio predicts coronary artery disease severity in non-ST elevation myocardial infarction using syntax score. *BMJ.,* 38:465-473.

13. Huang J, Chen Y, Ji H *et al.* (2020): Neutrophil to high-density lipoprotein ratio has a superior prognostic value in elderly patients with acute myocardial infarction: a comparison study. *Lipids Health Dis.,* 19(1): 1-12.

14. Huang X, Yang S, Zhao Q *et al.* (2022): Predictive value of non-high-density lipoprotein cholesterol and neutrophil-lymphocyte ratio for coronary artery vulnerable plaques in type 2 diabetes mellitus. *Front Cardiovasc Med.,* 9:927768.