



## Investigating the relationship between the severity of coronary artery disease and inflammatory factors of MHR, PHR, NHR, and IL-25

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### Abstract

**Background:** Coronary artery disease (CAD), as a most common cause of death, is mainly caused by atherosclerosis. Due to the role of inflammation in the process of atherosclerosis, in the present study, the relationship between the severity of coronary artery disease and inflammatory factors of monocyte to HDL-C ratio (MHR), platelet-to-HDL-C ratio (PHR), neutrophil to HDL-C ratio (NHR), and IL-25 was investigated.

**Methods:** In this cross-sectional study, 64 patients with diagnosis of coronary artery disease who were undergoing angiography in Farshchian heart center in Hamadan were studied. For each patient, the count of monocytes, neutrophils, platelet, and HDL-C, and IL-25 were measured from their blood and serum samples. Also, demographic information, such as age, gender, diabetes, smoking, and history of hypertension, was collected using a checklist. Data were described using frequency, percent, mean, and standard deviation. Statistical analysis was performed using independent t test, Mann-Whitney, Wilcoxon, and Spearman rank correlation tests, and multiple linear regression by SPSS version 25.0 (SPSS Inc).  $P < .05$  was considered as significant.

**Results:** The results of this study showed that IL-25 and MHR index has a significant correlation with coronary artery disease and Gensini score ( $P < .001$ ). The PHR index was associated with coronary artery disease. Also, qualitative variables, such as history of hypertension, history of smoking, and gender, have a significant association with the severity of coronary artery disease ( $P < .05$ ).

**Conclusion:** Among the inflammatory markers examined, IL-25 and MHR are stronger markers for assessing the severity of coronary artery disease. Simple and available IL-25 and MHR measurements may be able to, along with common risk factors and lipid profiles, predict the amount of vascular occlusion in treatment centers as an alternative of angiography as well as screening high risk patients prone to cardiovascular disease.

**Keywords:** Coronary Artery Disease, IL-25, HDL-C, MHR, NHR, PHR

**Conflicts of Interest:** None declared

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### Introduction

Coronary artery disease (CAD) is the most common cause of death globally (17.9 million death each year) and

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#### ↑What is “already known” in this topic:

Atherosclerosis, buildup of plaque inside the artery walls, is an inflammatory disease and major cause of coronary artery disease (CAD). IL-25 (Interleukin-25), MHR (monocyte to high-density lipoprotein cholesterol ratio), NHR (neutrophil to high-density lipoprotein cholesterol ratio), and PHR (platelet to high density lipoprotein cholesterol ratio) are recently emerging inflammatory indicator.

#### →What this article adds:

MHR/IL-25/PHR were significantly associated with severity of coronary artery stenosis. MHR and IL-25 were 2 most reliable and powerful inflammatory markers for prediction of CAD severity. These markers have great potential for screening elective patients with stable symptoms.

affects approximately 126 million individuals of the world's population (1.72% or 1,655 per 100,000)(1). Also, CAD is the first leading cause of death in Iranian populations (262 death per 100,000, annually), with approximately an incidence of 12 per 1000 of population (2).

The majority of the CAD is related to atherosclerosis where the progressive occurrence of endothelial dysfunction, vascular inflammation, and thrombosis result in plaque formation and may cause lethal consequence, including acute myocardial infarction (AMI) and unexpected cardiac death. Due to silent atherosclerotic plaque progression, the death rate is high for cardiovascular diseases worldwide. Hence, attempts focus on identification of reliable novel biomarkers to predict the plaque characterization, formation, and diagnosis in early stages and timely treatment, subsequently declining morbidity and mortality of cardiovascular diseases (3).

Inflammation could be as one of the risk factors that contribute to the initiation and progression of atherosclerotic process by promoting plaque growth (4). Interleukin-25 (IL-25 or IL-17E) is related to IL-17 cytokine family and is involved in the induction of T helper 2 (Th2) type immune responses (type 2 immune response) through production of the T helper 2 cytokines, such as IL-13, IL-5, and IL-4 (5). In addition, IL-25 can also prevent the immune responses mediated by Th1/Th17 (6). It has been demonstrated that mature B cells and also endothelial cells and smooth muscle cells (SMCs) of normal/atherosclerotic human arteries in progressive plaque constitutively express IL-25 (7). Furthermore, cytokines of T helper 2 may play a role in local inflammatory response, because they are present in the plaque (5). However, the researches on the role of IL-25 in CAD (protective or plaque stabilizer) is limited and challenging (8, 9).

High-density lipoprotein cholesterol (HDL-C) is a fundamental groups of human lipoproteins that has antiatherogenic properties and reverse relationship with CAD through inhibition of macrophages migration, reduction of low-density lipoprotein cholesterol (LDL-C) oxidation, promotion of cholesterol flow from these cells, and prevention of platelet aggregation (10, 11). On the other hand, blood components such as platelets and circulating white blood cells (WBCs), including monocytes, lymphocytes, eosinophils, and neutrophils, are involved in atherosclerosis inflammatory processes. As known, circulating monocytes interact with endothelial cells and platelets via secretion of different factors and cytokines, and subsequently initiating inflammatory and prothrombotic pathways (12). It has been reported that a recently emerged inflammation-based marker, the monocytes to HDL-C ratio (MHR), could reflect the inflammatory status of a patient and potentially can be used as a novel prognostic and predictor factor for cardiovascular diseases (13, 14). However, platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) has been indicated to be inversely associated with HDL in CAD patients (10, 15).

At the moment, angiography is the gold standard for diagnosing coronary artery disease. Despite its high diagnostic accuracy, angiography has drawbacks and limitations, including aggressiveness, the use of ionizing radiation,

high cost, and the need for special conditions and facilities. Therefore, medical researchers are trying to offer new noninvasive methods for diagnosing coronary artery disease that do not have the mentioned limitations. The above-mentioned markers can potentially provide simple, available, effective, and cheap tools for the prognosis and diagnosis of CAD even in the asymptomatic patients. However, no data are available on the association of MHR, neutrophil count to HDL-C ratio (NHR), platelet count to HDL-C ratio (PHR) inflammatory parameters, and IL-25 with the severity of CAD and atherosclerosis. Thus, in this study, we aimed to investigate these relations to predict coronary artery disease and their potential to be noninvasive diagnostic biomarkers for assessment of severity of coronary artery disease and prognosis.

## Methods

### Patients and Samples

In this cross-sectional study, patients with an initial diagnosis of coronary artery disease who were undergoing angiography, including 43 males and 21 females, and with a mean age of  $55.3 \pm 7.8$  years who referred to the heart hospital of Hamedan University of Medical Sciences between January 2018 and July 2019 were recruited for this study according to the strict inclusion criteria. Based on the reported correlation coefficient ( $r = 0.407$ ) between MHR and Gensini score (12) and considering the 95% confidence level and test power of 90%, the calculated minimum required sample size was approximately 59 patients using the following formula. However, 64 patients were enrolled in study considering about 10% probability of sample loss.

$$n = \left( \frac{z_{1-\frac{\alpha}{2}} + z_{1-\beta}}{0.5 \ln\left(\frac{1+r}{1-r}\right)} \right)^2 + 3$$

Elective patients (nonemergency patients) with stable symptoms were selected based on clinical evidences of coronary artery involvement. Patients with serious heart failure (eg, congenital heart disease, acute infarction, acute coronary syndrome), rheumatic disease, coagulation diseases kidney failure, infectious diseases, connective tissue diseases, malignancies, diabetes and consumption of immunosuppressive drugs were excluded from the study. After a detailed physical examination, the demographic data were recorded. Angiographic data were measured and recorded by an expert cardiologist. Patients were categorized into 4 groups according to coronary angiography, including patients without stenosis or with less than 50% stenosis in one of the coronary arteries (group 1,  $n=16$ ), patients with 50% or more stenosis in one of the coronary arteries (group 2,  $n=16$ ), patients with more than 50% stenosis in 2 coronary arteries (group 3,  $n=16$ ), patients with more than 50% stenosis in 3 coronary arteries (group 4,  $n=16$ ). They were scored through the Gensini score. Peripheral blood samples of patients were collecting before angiography for the following experiments. All patients were enrolled after providing informed consents.

This study was approved by the research ethics committee, Hamadan University of Medical Sciences (Ethics code: IR.UMSHA.REC.1396.833).

### Biochemical and Hematological Parameters

An automated hematology analyzer (Sysmex Kx-21NL) was used for measuring the total and differential leukocyte counts. Absolute cell counts were confirmed with the smear analyses. A biochemical autoanalyzer (BT 3000, auto-analyzer) was used for high-density lipoprotein cholesterol (HDL-C) measurement. MHR/PHR/NHR were computed as monocyte/platelet/neutrophil counts divided by HDL-C amount. IL-25 levels were measured by separating serum samples from peripheral blood of patients and using ELISA method according to manufacturer's protocol (Eastbiopharm Co).

### Statistical Analysis

Quantitative and qualitative variables are defined as the mean  $\pm$  standard deviation (SD) and frequency (percent), respectively. Independent t test, Mann-Whitney, and Wilcoxon tests were used for comparing the mean/mean ranks of quantitative variables between 2 or more groups. Correlations between quantitative variables were assessed using Spearman rank correlation test. Multiple linear regression analysis was used to predict CAD severity according to independent variables.  $P < .05$  was considered as significant. All statistical analyses were performed using SPSS version 25.0 (SPSS Inc).

### Results

A total of 64 patients with the diagnosis of CAD were enrolled. Patients were classified according to their hypertension, family history of coronary artery disease, and smoking. The demographic, clinical, and laboratory data of the patients are given in Table 1.

Spearman correlation test was used to investigate the relationship between NHR, MHR, PHR, and IL-25 serum levels with the Gensini score. Data analysis results showed a significant positive correlation between MHR

( $r=0.543$ ,  $p<0.001$ ), PHR ( $r=0.374$ ;  $p=0.002$ ), with the Gensini score and negative correlation between IL-25 serum level ( $r= -0.307$ ;  $p=0.014$ ), with the Gensini score (Table 2).

The mean of NHR, MHR, PHR, and IL-25 serum levels were compared between different severity of coronary artery disease using Kruskal-Wallis statistical test (Table 3). Data analysis results revealed that increase of MHR ( $p<0.001$ ), PHR ( $p=0.008$ ) and decrease of IL-25 ( $p=0.009$ ) were significant in the groups with different severity of coronary artery disease. There was no significant difference in the mean of NHR between groups ( $p=0.456$ ).

The Mann-Whitney test was used to compare the mean rank of PHR/MHR/NHR/IL-25 between the 2 levels of explanatory variables of hypertension, family history of coronary artery disease, smoking, and gender. Results of statistical analysis showed that NHR mean was significantly different between males and females ( $p=0.037$ ). Also, MHR mean was significantly different between patients with and without hypertension ( $p=0.020$ ) and between smokers and nonsmoker patients ( $p=0.033$ ). PHR mean was significantly different between males and females ( $p=0.025$ ), between patients with and without hypertension ( $p=0.019$ ), and between smokers and nonsmoker patients ( $p=0.033$ ).

Independent t test was used to compare the Gensini score mean between the 2 levels of explanatory variables of hypertension, family history of coronary artery disease, smoking, and gender. There was a significant difference in the mean of Gensini score between males and females ( $p=0.001$ ), between patients with and without hypertension ( $p=0.013$ ), and between smokers and nonsmoker patients ( $p=0.033$ ).

Spearman correlation test was used to investigate the relationship between PHR/MHR/ NHR/IL-25/Gensini score and age. Results showed that only the Gensini score was significantly correlated with age ( $r= 0.294$ ;  $p=0.019$ ) (Table 4).

Multiple linear regression was used to predict the Gen-

Table 1. Clinical and laboratory characteristics

Parameters	Total (N = 64)
Age (years)	55.3 $\pm$ 7.8
Male sex [n (%)]	43 [67.2%]
Female sex [n (%)]	21 [32.8%]
Smoking [n (%)]	13 [20.3%]
Hypertension [n (%)]	22 [34.4%]
Family history of CAD [n (%)]	11 [17.2%]
NHR [count.dl/mg]	2.52 $\pm$ 1.3
PHR [count.dl/mg]	2.52 $\pm$ 1.3
MHR [count.dl/mg]	0.15 $\pm$ 0.1
IL-25 [pg/ml]	24.87 $\pm$ 3.76
Gensini score	45.37 $\pm$ 1.3

Table 2. The relationship between inflammatory factors and Gensini score

Variable	Correlation with Gensini score	
	Spearman Correlation (R)	p
NHR	0.207	0.101
MHR	0.543	<0.001*
PHR	0.374	0.002*
IL-25	-0.307	0.014*

**Table 3.** The relationship between inflammatory factors and the severity of coronary artery disease

Variable	Group 1	Group 2	Group 3	Group 4	Kruskal-Wallis Test P-Value
NHR (count.dl/mg)	2.16±0.56	2.86±2.23	2.33±0.55	2.73±1.07	0.456
MHR (count.dl/mg)	0.08±0.05	0.14±0.12	0.14±0.06	0.22±0.11	<0.001*
PHR (count.dl/mg)	7.30±1.89	9.95± 7.34	7.58±1.51	11.50±4.83	0.008*
IL-25 (pg/ml)	26.51±4.15	24.94±3.31	25.64±3.32	22.37±3.19	0.009*

**Table 4.** Comparison of NHR/MHR/PHR/IL-25/Gensini score mean between the two levels of qualitative variables and their relationship with age.

Variable		NHR count.dl/mg		PHR count.dl/mg		MHR count.dl/mg		IL-25 pg/ml		Gensini score	
		Mean±SD/R	p	Mean±SD/R	p	Mean±SD/R	p	Mean±SD/R	p	Mean±SD/R	p
Hypertension	Yes	2.61±0.96	0.110	10.04±4.48	0.019*	0.18±0.11	0.020*	24.97±3.94	0.832	2.78±1.06	0.013*
	No	2.47±1.45		8.58±4.91		0.13±0.09		24.81±3.71		1.87±1.32	
Family history of CAD	Yes	2.39±0.55	0.866	9.48±3.29	0.505	0.15±0.17	0.408	23.70±3.04	0.251	2.79±1.14	0.137
	No	2.5±1.41		9.0±5.05		0.15±0.11		25.11±3.88		2.04±1.31	
Smoking	Yes	3.44±1.41	0.063*	12.77±8.55	0.033*	0.23±0.16	0.033*	24.82±4.21	0.960	2.94±1.09	0.033*
	No	2.29±0.66		8.14±2.61		0.13±0.07		24.88±3.68		1.99±1.28	
Gender	Male	2.68±1.47	0.037*	9.74±5.42	0.025*	0.16±0.11	0.287	24.56±3.75	0.320	2.56±1.14	0.001*
	Female	2.2±0.78		7.74±2.72		0.12±0.05		25.49±3.12		1.42±1.29	
Age		0.091	0.475	0.141	0.266	0.036	0.779	-0.056	0.661	0.294	0.019*

**Table 5.** Regression coefficients for Gensini score

Variable	Unstandardized coefficients		Standardized coefficients Beta	t	p
	B	Std. error			
(Constant)	-12.00	54.477	-	-0.220	0.826
Age	1.37	0.498	0.281	2.75	0.008 *
IL-25	-1.91	1.02	-0.198	-1.87	0.067
PHR	3.07	2.70	0.350	1.13	0.262
NHR	-22.64	8.69	-0.702	-2.60	0.012 *
MHR	230.96	56.42	0.696	4.09	0.000 *
Gender (ref=male)	-19.76	8.89	-0.245	-2.22	0.031 *
Hypertension (ref=yes)	-2.83	8.52	-0.036	-0.333	0.741
Family history of CAD (ref=yes)	-6.27	10.91	-0.062	-0.575	0.568
Smoking (ref=yes)	-15.10	11.69	-0.160	-1.29	0.202

sini score using independent variables (NHR, MHR, PHR, serum level of IL-25, age, gender, hypertension, and family history of CAD and smoking). The statistical model for quantitative variables has a P value less than .001 ( $p < 0.001$ ) and adjusted  $R^2$  of 0.398. The variables, NHR (Beta= -0.702;  $p=0.012$ ), MHR (Beta= 0.696,  $p < 0.001$ ), age (Beta=0.281;  $P = .008$ ), and gender (Beta= -0.245;  $p=0.031$ ) can determine the Gensini score. According to standardized coefficients beta, it is concluded that the NHR and MHR are the most important and determinant variable in this regard (Table 5).

## Discussion

Cardiovascular disease has become the most cause of death in global societies. CAD is a widespread heart disease, which is more common in advanced societies and in older people. Since current treatments for CAD are mostly palliative, early detection is more important to prevent disease progression and mortality over the coming years. Hence, introducing novel reliable biomarkers for early detection and prediction of atherosclerosis as a major cause of CAD is required (16-18). HDL, a high-density lipoprotein, is present in the bloodstream and prevents the accumulation of free cholesterol and triglycerides in the vessels and formation of lipid plaques. Monocytes neutrophil and platelets, as inflammatory immune system components, are active in the bloodstream and play an important and essential roles in inflammation through secre-

tion protein substances such as cytokines (12, 19). However, there are limited studies regarding the relationship between inflammatory/anti-inflammatory cytokines and coronary artery stenosis severity in CAD patients.

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The aim of the present study was to find the association between CAD severity with MHR, PHR, NHR (inflammatory markers), and IL-25 (anti-inflammatory cytokine).

We observed a significant correlation between the Gensini score with MHR and PHR indicators and also serum levels of IL-25. In the regression model, NHR, MHR, PHR and serum levels of IL-25 were considered as independent variables for prediction and age of the Gensini score in patients. The analysis showed that MHR index is the strongest predictor in this regard.

In present study, we showed that IL-25 serum level has been declined in CAD patients by progression of severity. Patients with more than 50% stenosis in 3 coronary arteries had the higher level of IL-25. Expression of IL-25 via resident plaque cells and presence of IL-25 positive B cells in progressive plaques were found in patients with atherosclerosis (7). Mantani et al indicated that IL-25 has an atheroprotective role in the apolipoprotein (apo) E deficient mice aortic arch via increasing Th1/Th17-mediated immune responses (9), and also they previously showed that the protective role of IL-25 is due to induction of IL-5, natural antiphosphorylcholine antibody generation by



B1a cells, and expansion of innate lymphoid type 2 cells (ILC2) in atherosclerosis-prone apo-E deficient mice (5). On the other hand, Xu et al reported that IL-25 was significantly higher in coronary arteries of CAD patients when compared with normal coronary arteries, especially in acute myocardial infarction (AMI), and IL-25 was positively correlated with the Gensini score and TNF- $\alpha$  and IL-6 levels ( $r=0.495$ ;  $p<0.001$ ) in CAD patients. Based on their data, macrophages and T lymphocyte are important sources of IL-25 in human atherosclerotic plaques (8), while our data showed that IL-25 is negatively correlated with the Gensini score and there was no relationship between IL-25 expression and risk factor of CAD.

Furthermore, we have demonstrated the association of MHR/NHR/PHR with the CAD severity alone and in the context of the Gensini score. Our results showed that only MHR and PHR are linked to the Gensini score and CAD severity, so that these inflammation biomarkers are elevated in patients with stenosis in more coronary arteries.

Monocyte migrate into the subendothelial space and uptake lipoproteins and their migration is considerably more in hypercholesterolemic conditions. It seems that monocytes during the CAD and AMI is contributed with plaque progression and high monocyte counts could be an independent marker for CAD and AMI (20). On the other hand, the HDL-C molecule have anti-atherosclerotic properties as a result of a phenomenon named "reverse cholesterol transport" and the HDL-C molecule prevents monocyte activation and recruiting (21). Consistent with our result, in Kundi's study, MHR was significantly higher in patients with high SYNTAX scores and potentially can be considered as a prognostic factor in stable CAD patients, with sensitivity of 66% and a specificity of 65.1% (13). Also, Cetin et al reported that MHR can be a predictor of CAD severity and it is possible to use MHR for identify high-risk patients for major adverse cardiovascular events. In their study, MHR had a significant and positive correlation with CRP, neutrophil to lymphocyte ratio, and SYNTAX, and the Gensini score (12).

Platelets has an important role in the etiology of CAD, and larger MPV (mean platelet volume) is associated with CAD (22). During atherosclerosis, a clot forms in an intact blood vessel that promotes platelet aggregates, and subsequently causing a blockage of blood flow and heart attack. In addition to this, HDL exhibits antithrombotic activity via prohibition of platelet aggregation (23). It has been previously mentioned that platelet-lymphocyte ratio (PLR) is inversely associated with HDL in CAD, and CAD patients having low HDL levels have higher PLR (10). In addition, PLR correlates with the higher CAD severity and poor prognosis in Chinese population, so it can be used as a predictive biomarker of CVD and risk of future CVD events (24). Although the role of platelets in pathogenesis of CAD is well known, the documented relationship between PHR and the Gensini score and intensity of CAD and our data are shown here for first time.

Neutrophils are highly efficient effector cells, short-lived, rather crude, and unrefined phagocytes that mobilize and spread to sites of inflammation. Also, they act as a main protagonist in the first line of immunity under

acute inflammatory conditions. Neutrophils has been rarely detected in atherosclerotic lesions compared to other immune cells, and their contribution was largely unknown (25, 26). Kalay et al found that circulating neutrophil count and NLR were related with progression of atherosclerosis. Patients with high NLR showed higher progression rate (27). Nilsson reported an enhancement of neutrophil count in noncalcified plaques from patients with CAD, although this association was not seen for total plaque burden (28). Chen et al demonstrated mild positive correlations between the NLR and proportions of neutrophils with the Gensini score and suggest the NLR as an independent predictor of the severity of CAD assessed according to the Gensini score (29). Furthermore, according to a study by Zhang et al, NLR of 2.385 or higher predicted high Gensini scores, with a sensitivity of 64% and specificity of 63% (30). However, our result did not show the correlation between NHR, CAD severity, and Gensini scores.

Finally, in our study, IL-25/MHR/PHR (especially IL-25 and MHR) were identified as powerful and reliable markers for diagnosing and estimating the severity of coronary artery stenosis. Also, these markers can potentially be used for disease monitoring and evaluation of response to treatment. However, this hypothesis requires further researches in this regard.

## Conclusion

Among the examined inflammatory markers, MHR and IL-25 were more reliable and powerful for prediction of Gensini scores and CAD severity. Thus, these markers are potentially able to be used in daily clinical practice as an inexpensive and quickly accessible marker for screening elective patients with stable symptoms as an alternative of costly and invasive angiography procedure. We suggest that performances of these markers be investigated in different aspects of CAD disease, including disease progression and monitoring in future studies.

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## Conflict of Interests

The authors declare that they have no competing interests.

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