The relation between neutrophil and platelet to lymphocyte ratios and the evidence of coronary artery disease in myocardial perfusion imaging

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ABSTRACT

Introduction: Various inflammatory markers have been suggested for prognostic purposes and management of coronary artery disease (CAD) patients, with the neutrophil to lymphocyte ratio (NLR) emerging as a promising marker for evaluating CAD. This study aimed to assess the correlation of NLR and platelet to lymphocyte ratio (PLR) with abnormal perfusion findings in single photon computed tomography myocardial perfusion imaging (SPECT MPI) in patients with suspected CAD.

Methods: In this cross-sectional study, 171 patients during the period of 6 months were enrolled and the NLR and PLR were calculated for all of them. Patients with significant valvular or structural heart disease, or inflammatory disease, and those taking anti-inflammatory drugs were excluded. Perfusion and function parameters of SPECT MPI were extracted and compared in patients with NLR≥ 3 and NLR <3. Data were analysed using SPSS software version 20, and P≤0.05 was considered statistically significant.

Results: Individuals with NLR≥ 3 were significantly older (P= 0.046). A comparison of quantitative functional parameters of the SPECT MPI showed that there was a statistically significant difference between NLR≥ 3 and NLR <3 groups in terms of summed motion score (P=0.012), total perfusion deficit at rest (P=0.022), and ejection fraction (P=0.016). However, no significant difference was found when comparing quantitative function and perfusion parameters between high (PLR>180) and low PLR groups.

Conclusions: According to our study, NLR is correlated with perfusion and function abnormalities in SPECT MPI in patients with clinical suspicion of CAD.

Keywords: Coronary artery disease; Inflammatory markers; Myocardial perfusion imaging

INTRODUCTION

Coronary artery disease (CAD) is a common disorder with significant mortality and morbidity [1, 2]. CAD is considered as a chronic inflammatory disease leading to atherosclerotic plaque formation and luminal narrowing of coronary arteries [1, 3]. Inflammatory processes play an important role in the initiation and progression of atherosclerosis as well as the stability of atherosclerotic plaques [4, 5]. The number of white blood cells and their subgroups have been investigated as an indicator for predicting cardiovascular events in several studies [6-8]. In recent studies, the use of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) has been suggested as a predictive marker of cardiovascular events [9, 10]. In the early stages of atherosclerosis, the absorption of inflammatory cells from the blood circulation and their migration to the endothelium occurs. This process is governed by cell adhesion molecules that are expressed on the endothelium and circulating leukocytes in response to inflammatory stimuli [4]. Adhesion is a multi-step process that begins with the rolling of leukocytes on the surface of the endothelium by the selectin receptor. Monocytes and macrophages play an important role in the initiation and progression of atherosclerosis [4, 11]. Adsorption of monocytes to the vessel wall is one of the first events in the process of atherosclerosis. During this process, monocytes transform into macrophages, which are important mediators of inflammation and the primary immune response in atherosclerotic lesions [4]. An increase in inflammatory cytokines and markers have been proposed in acute coronary syndrome (ACS) with ability to predict risk of future cardiovascular disease [6, 12]. The NLR is a very feasible and widely available inexpensive test for evaluation and prognostic application of inflammatory diseases. A meta-analysis conducted, which was the result of a review of 10 cohort studies, showed that NLR was predictive of all-cause mortality and cardiovascular events in patients who underwent cardiac angiography or revascularization [13]. Also, patients with ST –elevation myocardial infarction (STEMI) showed a stronger relationship between NLR and cardiovascular events [13]. Another study examined the relationship between NLR and the prevalence and extent of coronary artery involvement in patients who underwent coronary angiography, finding that a high NLR level was associated with various cardiovascular disease risk factors [10]. Furthermore, NLR demonstrated a direct relationship with both the prevalence and severity of coronary artery disease (CAD) [10].

In the present study, we investigated the relationship between NLR and the platelet-to-lymphocyte ratio (PLR) concerning the presence of perfusion abnormalities and evidence of CAD in patients suspected of having CAD, as assessed by single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI).

METHODS

Patients

In this cross-sectional study, we included all patients who were referred to our nuclear medicine department over a six-month period to undergo gated-SPECT myocardial perfusion imaging (MPI) as requested by their cardiologist. Patients with significant valvular or structural heart disease, patients with known underlying inflammatory disease, those taking anti-inflammatory drugs, and patients, who declined to participate in the study, were excluded. In addition, non-gated or rest-only scans and those with insufficient quality or incomplete clinical data were also excluded. The Ethics Committee of Shiraz University of Medical Sciences approved the study's design (IR.SUMS.MED.REC.1397.410). The Declaration of Helsinki, which governs research

involving human beings, is maintained throughout this research. In addition, demographic characteristics of the patients, including gender, age, and CAD risk factors including diabetes, blood pressure, hyperlipidemia, smoking, history of prior CAD or cardiac diseases, and family history of CAD were recorded in the data collection form.

Neutrophil to Lymphocyte ratio (NLR) and Platelet to Lymphocyte (PLR) ratio assay

Blood samples were collected from informed patients. Neutrophil, lymphocyte, and platelet in serum were measured by and after that, NLR and PLR were calculated. Patients were divided into two high-risk (NLR ≥3) and low risk groups (NLR < 3) [14]. In terms of PLR, patients were divided into two groups with high PLR (>180) and normal PLR [15].

SPECT MPI

Two-day stress-rest SPECT MPI protocol was performed for all patients. Depending on the patient's condition, pharmacological or exercise treadmill test (ETT) was considered for them. The pharmacologic stress test was performed with dipyridamole at a dose of 0.56 mg / kg per minute intravenous (IV) infusion within 4 minutes followed by 15-20 mCi ^{99m}Tc-sestamibi (MIBI) injection after 2-4 minutes. ETT was performed according to Bruce protocol. In both stress and rest phases, 15-20 mCi ^{99m}Tc-sestamibi was injected intravenously and imaging was done approximately 15-20 minutes later in exercise and 45-90 minutes later in rest phase or pharmacologic stress, by SPECT method. For SPECT acquisition, 32 projections were acquired over a 180° arc from -45° left posterior oblique (LPO) to 135° right anterior oblique (RAO) view, lasting 30 seconds per projection with a dual head cardiac-dedicated gamma camera. The projections were reconstructed with ordered subset maximization (OSEM) and post filter (Butterworth filter: order 5, cut-off frequency 0.40). Gated-SPECT acquisition was performed in stress phase with 8-frame gating. The left ventricular perfusion quantification was performed using quantitative perfusion SPECT (QPS)/quantitative gated SPECT (QGS). Quantitative data of LV perfusion including summed stress score (SSS), summed rest score (SRS), summed difference score (SDS), total perfusion deficit (TPD) at both phases with delta TPD (dTPD), and LV functional data in post-stress phase including ejection fraction (EF), end-diastolic volume (EDV), end-systolic volume (ESV), summed motion score [16], and summed thickening score (STS) were extracted. All findings were reported based on 17-segment model. For quantitative study, all scans were assessed by a nuclear medicine specialist. In addition, visual interpretation of MPIs as normal or abnormal scan was also made by nuclear a medicine physician.

Statistical analysis

SPSS 18 for Windows (SPSS Inc., Chicago, Illinois) was used for statistical analysis. Data were expressed as mean ± SD for interval and count (%) for categorical variables. Tables were drawn to display the data distribution. Patients based on the NLR, were divided into two groups including NLR<3 and NLR≥3. The prevalence of CAD risk factors and abnormal myocardial scans as well as the mean quantitative MPI parameters were compared between the two groups. The Kolmogorov-Smirnov test was performed to evaluate normal distribution of quantitative variables. For comparison of mean values, Independent Samples T-test was used for variables with normal distribution and Mann-Whitney U test was performed for variables with non-normal distributions. Chi square

test was utilized for comparison of CAD risk factors and abnormal scan prevalence between groups. P value of less than 0.05 was considered statistically significant.

RESULTS

A total of 171 patients participated in this study. Comparison of mean ±SD age the two groups of patients with NLR < 3 (57.91±10.55 years) and NLR ≥ 3 (63.70±10.97 years) revealed that group with NLR≥3 were significantly older (P value: 0.046). According to table 1 which represents the difference of two groups regarding the frequency of other cardiac risk factors only male gender revealed significant differences with higher male to female ratio in the NLR≥3 group.

Table 2 demonstrates the comparison of quantitative perfusion and functional parameters of SEPECT MPI between the NLR ≥3 and NLR < 3 groups. Functional parameters including EF and SMS show significant difference, and only the perfusion parameter of TPDr was significantly different between the two groups (P value: 0.022) (Table 2).

Our result showed that none of male patients showed high PLR; however, there are 7 female patients with predefined high PLR. Among 7 patients with high PLR, only 4 of them also had NLR≥3. Comparison of different cardiac risk factors and quantitative MPI parameters revealed no significant difference between the high PLR and normal PLR groups neither in all patients nor in female group.

DISCUSSION

In the present study, male to female ratio and age was significantly lower in those patients with NLR <3. Comparison of different quantitative perfusion parameters showed that patients with NLR ≥ 3 had significantly higher TPDr and SMS, and lower EF as compared to those with NLR < 3. However, comparing different parameters between groups with high PLR and normal PLR showed no significant difference regarding the perfusion and function parameters of SPECT MPI.

Several previous studies in CAD patients revealed association of NLR with severity of angiographic coronary findings. Kurtul et al. studied 414 patients with non-STEMI who underwent coronary angiography and examined the relationship between NLR and CAD severity based on the SYNTAX score. The patients were divided into three groups of patients with low score, medium score and high score. In this study, NLR in patients with low SYNTAX score was significantly lower than patients with moderate and high SYNTAX score suggesting a direct relationship with CAD severity [17]. Another study by Iranirad et al. on 500 patients with ACS who underwent cardiac catheterization also indicted that patients with one-vessel CAD had the lowest NLR and patients with three-vessel CAD had the highest NLR [18]. In previous studies, known CAD patients were typically evaluated. However, our study demonstrated that in suspected CAD patients, the NLR could differentiate those with a higher rate of perfusion abnormalities on SPECT-MPI. It is important to consider the association of other risk factors in this context. In the current study, age and gender can act as confounding factors, though the majority of conventional CAD risk factors were statistically not affecting the study results as shown in Table 2. Nonetheless, several recent studies in special populations also revealed the independent association of NLR and high-risk markers of CAD [19-21]. In the majority of these studies both age and NLR were independently correlated with coronary artery calcium score [21]. In a recent study that also evaluate the association of NLR and PLR with CAD, age and higher NLR and PLR were independent predictors of significant CAD in a population of physicians [19]. Considering the small sample size of

our study, the independent correlation of NLR and age with perfusion abnormality could not be assessed, in contrast to the mentioned studies when NLR was associated with CAD markers, older age was also another independent risk factor. These coincidental associations between higher NLR and older age with CAD may be suggestive of similar pathophysiologic mechanisms. It also should be noted that small number of our patients in the higher NLR group made gender subgroup analysis less reliable, contrary to recent studies showing the difference in correlation of NLR and CAD between male and female patients [22, 23]. Larger studies are needed for more reliable results. Besides, the statistically significant relationship of high NLR with TPDr may also indicate the role of inflammatory process in patients with previous myocardial infarction (MI). In another study with positron emission tomography (PET), significant 5-fold increase in myocardial perfusion defects were observed when NLR values were in the higher 90^{th} percentile [14]. The current study suggested that NLR was also correlated with functional parameters as well. Chen et al. investigated the relationship between NLR with parameters of cardiac function and extent of cardiac damage in 715 patients after MI [8]. In this study, NLR had a significant positive relationship with cardiac damage and a negative relationship with cardiac function. High NLR was associated with poor cardiac function in all patients and a strong predictor of myocardial injury in MI [8]. Arbel et al. studied 538 STEMI patients who underwent angiography. They observed that higher values of NLR were associated with lower EF [7]. The results of Takahashi et al.'s study also showed that in people with MI, a high level of neutrophils was associated with an increase in ESV and EDV and a decrease in EF [24]. The current study similarly revealed significant association of NLR as an inflammatory marker with lower EF and more abnormal wall motion.

Our study results indicate a correlation between a higher NLR and evidence of myocardial infarction, as well as lower EF and impaired wall motion. These findings suggest that inflammatory process may play a role in MI-related cardiomyopathy and CAD patients with poorer cardiac function. Additionally, these results could reflect more severe CAD, as previously suggested in angiographic studies linking NLR to severity of the [25]. The findings of a meta-analysis demonstrated that high NLR values increased the risk of coronary artery disease (CAD) 1.62-fold and the risk of stroke 3.86-fold [26]. Although the atherosclerosis is a multifactorial disease, inflammation has been suggested as one of the most important pathophysiologic causes [27]. Subtypes of white blood cells play an important role in creating an immune response in the process of atherosclerosis [28]. Neutrophils lead to the secretion of large amounts of inflammatory mediators such as elastase and myeloperoxidase and the creation of oxygen free radicals and are involved in the regulation of inflammatory responses related to tissue damage [28]. Studies have also shown that neutrophils play an important role in causing coronary artery lesions and their destructive activities lead to destabilization of coronary artery lesions. As a result, atherosclerotic lesions are exposed to rupture, which leads to thrombosis, coronary artery occlusion, and clinical events such as MI [14]. A high level of neutrophils has been predictive of MI in patients with STEMI [29]. On the other hand, lymphocytes play a role in regulating the immune system. Low levels of lymphocytes have been associated with adverse events in MI. Also, low level of CD4 cells has been associated with low cardiac output, re-stroke and increased mortality in post-MI patients [29]. Higher NLR may also affect the LV function and dilation via impairment of microvascular integrity that has been previously suggested as one of the important contributor factors [30, 31].

Though in the current study PLR showed no statistically significant relationship with abnormal perfusion and function parameters of MPI, more dedicated studies with larger sample size are recommended. Platelets also have a key role in the process of atherosclerosis, and increase in response to inflammation [32]. Another study by Ozdemir et al. also showed no significant association between SPECT MPI parameters with PLR and other platelet indices [33]. In our study, interestingly, no male patient was in the group of high PLR thus PLR values and the PLR analysis in this study was limited to only female patients.

The main limitation of this study is the relatively small sample size, which makes the subgroup analysis less accurate, particularly regarding evaluation of PLR. Nonetheless, both NLR and PLR are inexpensive and readily available inflammatory markers that can be used to predict and stratify the risk and severity of CAD, along with other useful measures. Further studies with larger sample size and the combination of other diagnostic modalities in patients with different stages of CAD risk are recommended.

CONCLUSION

In this study, a higher NLR was found to be associated with a greater frequency of abnormal perfusion and reduced systolic function in gated-SPECT MPI. Further research with a larger sample size is needed to validate the role of increased NLR as a high-risk factor during interpretation of SPECT MPI.

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Table 1. Comparison of CAD risk factors in NLR<3 and NLR≥3 groups

CAD risk factors	NLR<3 (N=151) N(%)	NLR≥3 (N=20) N(%)	P value
Male gender	47(31.1%)	12(60%)	0.012
Diabetes Mellitus	43(28.5%)	3(15%)	0.203
Hypertension	75(49.7%)	9(45%)	0.696
Dyslipidemia	65(43%)	6(30%)	0.267
Smoking	31(20.5%)	5(25%)	0.646
Family history of CAD	32(21.2%)	4(20%)	0.902
Prior history of CAD	23(15.2%)	4(20%)	0.531

Table 2. Mean value of SPECT MPI parameters between the NLR≥3 and NLR <3 groups

SPECT MPI parameter	NLR<3 (N=151) Mean±SD	NLR≥3 (N=20) Mean±SD	P value
SSS	3.68±4.59	6.15±8.87	0.090
SRS	2.19±3.42	4.00±7.23	0.383
SDS	1.50±2.46	2.15±3.34	0.288
TPDs	5.09±5.97	9.90±14.85	0.071
TPDr	1.75±4.80	4.90±9.99	0.022
dTPD	3.34±3.06	5.00±6.89	0.189
SMS	3.32±6.41	10.21±12.44	0.012
STS	1.23±2.79	4.64±6.91	0.098
ESV	27.75±18.08	43.29±31.80	0.082
EDV	73.69±29.73	93.86±45.08	0.066
EF	64.90±9.49	57.14±11.36	0.016

SPECT MPI: Single-photon emission computed tomography myocardial perfusion imaging; SSS: Summed stress score; SRS: Summed rest score; SDS: Summed difference score; TPDs: Total perfusion deficits at stress; TPDr: Total perfusion deficits at rest); dTPD: Delta of total perfusion deficit scores; SMS: Summed motion score; STS: Summed thickening score; EDV: End diastolic volume; ESV: End systolic volume, EF: Ejection fraction.
P value <0.05 is considered as statistically significant