Original Article

Association Between Increased Expression Levels of SDF-1 and CXCR4 on the Platelets of Patients With Coronary Artery Disease and Low LVEF

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ABSTRACT

Background: Since coronary artery disease (CAD) is one of the leading causes of death globally, identifying new risk factors can augment risk assessment. This study aimed to investigate the surface expression of stromal cell-derived factor-1 (SDF-1), CXCR4, and CXCR7 on the platelets of CAD patients and to determine whether there is a correlation between their expressions and left ventricular ejection fraction (LVEF).

Methods: Sixty CAD patients and 60 healthy volunteers as normal controls were studied. The mean fluorescence intensity (MFI) of SDF-1 and its receptor expression was evaluated by flow cytometry. Biochemical markers and platelet parameters were investigated with an AutoAnalyzer and a cell counter, respectively.

Results: The platelets of the CAD group expressed SDF-1 and CXCR4 significantly more than those of the control group (MFI=1112±304 vs 943±131; P=0.042 and MFI=23372±6804 vs 20634±3482; P=0.033, respectively). Nevertheless, no significant difference was found in the platelet expression of CXCR7 between the CAD and control groups (MFI=35256±8706 vs 25053±7270; P=0.061). Notably, increased expression levels of SDF-1 and CXCR4 were associated with decreased LVEF (r= −0.388, P=0.003 and r= −0.431, P=0.001).

Conclusions: Our findings demonstrated that the overexpression of SDF-1 and CXCR4 on platelets could be considered a promising candidate indicating that asymptomatic patients with decreased LVEF may be at the risk of CAD. ***(Iranian Heart Journal 2022; 23(1): 42-53)***

KEYWORDS: SDF-1, CXCR4, CXCR7, Coronary artery disease (CAD), Platelet markers

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The development of novel treatment strategies for coronary artery disease (CAD) formerly evolved an expectation that this fatal disease might be eliminated by the end of the 20th century; however, this optimistic prediction has faced revision and CAD is anticipated to remain as one of the leading causes of death until 2030. [1,2] With approximately 15.4 million individuals older than 20 years annually diagnosed with CAD in the United States, [3] this disease accounts for 1 in 7 mortalities, leading to over 360 000 deaths. [4] Multiple risk factors associated with CAD pathogenesis have been enumerated thus far and categorized into controllable and uncontrollable groups. While hypertension, hyperlipidemia, smoking, diabetes, obesity, physical inactivity, stress, and unhealthy diets could be controllable by an appropriate lifestyle, other risk factors such as age, sex, race, and family history are out of control. [5] Given the inadequacy of these traditional factors in predicting the incidence of coronary events, it is not surprising that the identification of new and emerging risk factors, including chemokines, can help improve risk assessment for CAD patients. [6] The crucial role of chemokines in the pathogenesis of cardiovascular disease has been reported in several investigations. [7] It is well-established that following heart injury, a variety of chemokines are involved in the heart repair processes, including the remodeling of the angiogenesis matrix, the mobilization of fibroblasts, and the stimulation of stem cells. [8] Among these chemokines acting both as a chemoattractant for hematopoietic progenitor cells and a heart remodeling factor, stromal cell-derived factor 1 (SDF-1), also known as CXCL12, has recently attracted tremendous attention. [9] Although SDF-1 is expressed in various tissues, platelets are a major source of this chemokine. [10] The results of several clinical trial studies have shown that the overexpression of SDF-1 in the ischemic myocardium results in cardioprotection and improved left ventricular ejection fraction (LVEF) as an indicator of myocardial function after myocardial infarction. [11, 12] From the first description of SDF-1, CXCR4 was assumed to be the exclusive receptor for this well-known chemokine. CXCR4 is widely expressed on a broad range of cells and tissues, including the immune and central nervous systems, [13, 14] and it is involved in the recruitment of smooth muscle progenitor cells, resting leukocytes, and hematopoietic progenitors to repair vascular tissue. [15] Not so long after the discovery of the fundamental role of CXCR4, it was reported that SDF-1 could also bind to another surrogate receptor, CXCR7, which participates in several biological processes, including scavenging for SDF-1, cell survival, and SDF-1-induced hematopoietic cell migration and homing. [13, 16] Moreover, the expression of this receptor on different organs, including the hematopoietic system, increases its importance in the SDF-1-signaling axis. [17] Given the close association between SDF-1, CXCR4, and CXCR7, we designed the present study to evaluate the surface expression of the aforementioned markers on the platelets of CAD patients.

METHODS

Patients

Sixty patients with symptomatic CAD who were visited at the Cardiovascular Department of Taleghani Hospital were evaluated in our study between 2017 and 2018. This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR. SBMU. RETECH.REC. 1396.717), and all the participants gave informed consent in accordance with the declaration of Helsinki. Before sample preparation, the patients were classified to acute coronary syndrome (ACS;

n=40) and stable angina pectoris (SAP; n=20) with respect to clinical symptoms, myocardial ischemia markers, and electrocardiography (ECG). While SAP was defined as a predictable pattern of chest pain on exertion or emotional stress with negative results for myocardial ischemia markers (troponin and creatinine kinase), ACS was applied to the patients who experienced unstable angina or acute myocardial infarction. As was previously explained, unstable angina is defined as irregular angina with or without ECG changes reflecting ischemia, unelevated troponin levels, [18] and occurrence at rest or lesser degrees of exertion than previous angina. Acute myocardial infarction was defined as a rise or fall in cardiac troponin, accompanied at least by one of the following criteria: symptoms of ischemia, new or presumed new significant ST-segment–T wave (ST–T) changes or a new left bundle branch block, development of pathological Q waves in ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, and identification of an intracoronary thrombus by angiography or autopsy. [19] In our study, the exclusion criteria for the selection of CAD patients were refusal to give informed consent, pregnancy, infectious diseases, and chest pain with non-coronary origins. Sixty healthy volunteers with no history of cardiovascular disease were included as normal controls, and they were matched with the CAD patients regarding age and sex (P>0.05).

Biochemical Markers

Whole blood (5 mL) was taken from the patients and controls in commercially red topped tubes (Becton Dickinson). The sera of all the samples were collected via the centrifugation of the clotted blood at 1500*g* for 10 minutes in a refrigerated centrifuge. The collected sera were analyzed by using a Hitachi 912 AutoAnalyzer (Roche Diagnostics, Canada) to determine the levels of serum C-reactive protein (CRP), troponin, and creatine kinase.

Hematological Analysis

Venous blood samples (2 mL) from the CAD and control groups were collected into vacutainer tubes containing K_2EDTA (Becton Dickinson, USA). Complete blood count (CBC) was performed immediately after blood collection by using an automated cell counter (Sysmex KX21-N; Sysmex Corporation, Kobe, Japan). Platelet parameters, including platelet count, mean platelet volume (MPV), and platelet distribution width (PDW), were evaluated in our study.

Platelet Preparation

Venous blood samples (5 mL) were taken in ACD-A (acid citrate dextrose solution-A) anticoagulant (1:7) from the patients and the controls. Platelet-rich plasma (PRP) was fractionated by blood centrifugation at 200*g* for 15 minutes at room temperature (with no brake applied). Afterward, the PRP was diluted with phosphate-buffered saline (PBS; Gibco, USA) to adjust the platelet concentration at 1×10^7 platelets/mL.

Flow Cytometry Analysis

The samples from the control and CAD groups were analyzed by flow cytometry immediately after collection. For the detection of the surface expression of platelet markers, diluted PRP was incubated with the respective conjugated antibodies or their respective isotype controls for 30 minutes at room temperature. Conjugated monoclonal antibodies were used to determine the expression levels of platelet GPIIIa (CD61; FITC from Dako, Denmark), SDF-1 (CXCL12; PerCP from Novus Biologicals, Littleton, CO, USA), CXCR4 (CD184; PE from Biolegend, San Diego,

California, USA), and CXCR7 (ACKR3; PE from Biolegend, San Diego, California, USA). After the staining process, the cells were fixed with 0.5% paraformaldehyde; then, PBS was added to each sample to reach a volume of 1 mL. Ultimately, the samples were analyzed by using a flow cytometer (Attune NxT; Life Technologies, Carlsbad, CA, USA). CD61-FITC was used to identify the platelet population, and mean fluorescence intensity (MFI) was drawn upon as a quantitative indicator for the surface expression of the indicated proteins.

Expression Analysis of CXCR4 and CXCR7 on Activated Platelets

For the analysis of agonist-induced changes in the surface expression of CXCR4 and CXCR7 on platelets, diluted PRP was treated with the increasing concentrations (20 µM, 100 µM, and 200 µM) of adenosine diphosphate (ADP; Hyphen-BioMed, Andresy, France) for 30 minutes at room temperature and then stained with the indicated antibodies.

Determination of LVEF

LVEF, which is an indicator of LV function, was determined by echocardiography in the Department of Cardiology, Ayatollah Taleghani Hospital. The correlation between LVEF and the platelet expression of SDF-1, CXCR4, and CXCR7 was also evaluated.

Statistical Analysis

All the statistical analyses were performed by using SPSS software, version 21. The Mann–Whitney *U* test and the *t* test were applied to assess differences in terms of SDF-1, CXCR4, and CXCR7 between the CAD and control groups based on their distribution. The correlation analyses of SDF-1, CXCR4, and CXCR7, as well as between the aforementioned markers and LVEF, were assessed by using the Pearson and Spearman tests. Linear regression analysis was conducted to evaluate the association between dependent variables, including SDF1, CXCR4, and CXCR7, and confounding factors. The one-way ANOVA and post hoc tests were employed to compare the platelets treated with different doses of ADP with the untreated platelets. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Platelets of the CAD patients expressed SDF-1 and its receptor, CXCR4, more significantly.

In the present study, we aimed to quantify the surface expression of SDF-1 on the platelets of patients with cardiovascular disease. To this end, we selected 60 healthy volunteers and 60 patients with CAD, including ACS and SAP. The characteristics of the patients and the normal controls (viz, age, sex, CAD type, LVEF, platelet parameters, laboratory tests, cardiovascular risk factors, and co-medications) are summarized in Table 1. Flow cytometry data analysis showed that the expression of SDF-1 on the platelets of the CAD patients was significantly higher than that of the healthy controls (MFI: 1112±304 vs 943±131; *P*=0.042). With respect to the raised level of SDF-1 expression, it was interesting to measure the surface expression of SDF-1 receptors (viz, CXCR4 and CXCR7). As is presented in Figure 1, CXCR4 surface expression was elevated on the platelets of the CAD group as compared with the normal controls (MFI: 23372±6804 vs 20634±3482; *P*=0.033). Nevertheless, the resulting data revealed that the platelet expression of CXCR7 in the CAD patients had no statistically significant difference with that in the control group (MFI: 35256±8706 vs 25053±7270; *P*=0.061) (Fig. 1).

A close correlation existed between the surface expression of SDF-1 and its receptors on platelets.

Having established that the expression of SDF-1 and its receptors was increased in the platelets of the CAD patients, we investigated the possible correlation between the surface expressions of these markers by using bivariate correlation analysis. The results showed that the surface expression of SDF-1 strongly correlated with CXCR4 expression (n=60, r=0.727, P<0.001); however, a moderate association existed between the surface expressions of CXCR7 and SDF-1 (n=60, r=0.370, P=0.002). The results of the bivariate correlation analysis also demonstrated that the surface expressions of CXCR4 and CXCR7 strongly correlated with each other (n=60, r=0.581, P<0.001) (Fig. 2).

Table 1. Characteristics of the CAD and control groups

Characteristics	CAD Patients (n=60)	Normal Controls (n=60)	*P*-value
Age (mean ± SD)	58.14±11.1	53±9.6	0.091
Sex (male/female)	73.7%/26.3%	69.1%/30.9%	0.164
CAD type (ACS/SAP)	66.7/33.3%	-	
EF percentage (mean ± SD)	49.0±11.7	58±2.4%	0.006
Laboratory Results			
Creatine kinase (mean ± SD)	319±101	134±55	0.003
CRP (negative/positive)	80%/20%	90%/10%	0.087
Troponin (negative/positive)	78%/22%	95%/5%	0.071
Cardiovascular Risk Factors			
Diabetes (no/yes)	77%/23%	85%/15%	0.058
History of CAD in the Family (no/yes)	87%/13%	90%/10%	0.645
Hypertension (no/yes)	56%/44%	83%/17%	<0.001
Hyperlipidemia (no/yes)	78%/22%	90%/10%	<0.001
Smoking (no/yes)	77%/23%	83%/17%	0.058
Kidney defect (no/yes)	98%/2%	100%/0%	0.636
Medication on Admission			
Acetyl salicylic acid (no/yes)	61%/39%	90%/10%	<0.001
Clopidogrel (no/yes)	91%/9%	100%/0%	0.007
ACE inhibitor (no/yes)	71%/29%	100%/0%	<0.001
Beta-blocker (no/yes)	70%/30%	100%/0%	<0.001
Statin (no/yes)	71%/29%	95%/5%	<0.001

CAD, Coronary artery disease; ACS, Acute coronary syndrome; SAP, Stable angina pectoris; EF, Ejection fraction; CRP, C-reactive protein; ACE, Angiotensin-converting enzyme

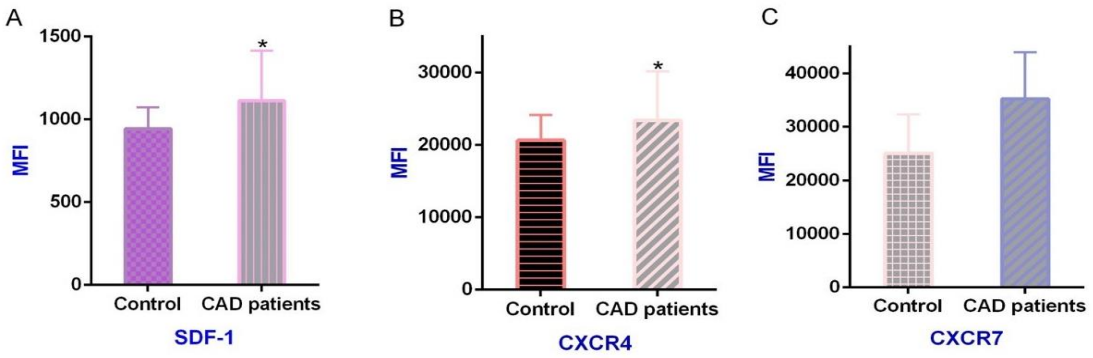

Figure 1. Flow cytometry data analysis shows that the expressions of (A) SDF-1 and (B) CXCR4 on the platelets of the CAD patients are significantly higher than those of the healthy controls. (C) The platelet expression of CXCR7 shows no significant difference between the CAD and control groups.
Data are mean±SD of mean fluorescence intensity from experiments done with 60 healthy controls and 60 CAD patients.
*: A *P*-value< 0.05 represents significant changes from the controls.
CAD, Coronary artery disease; SDF-1, Stromal cell-derived factor-1

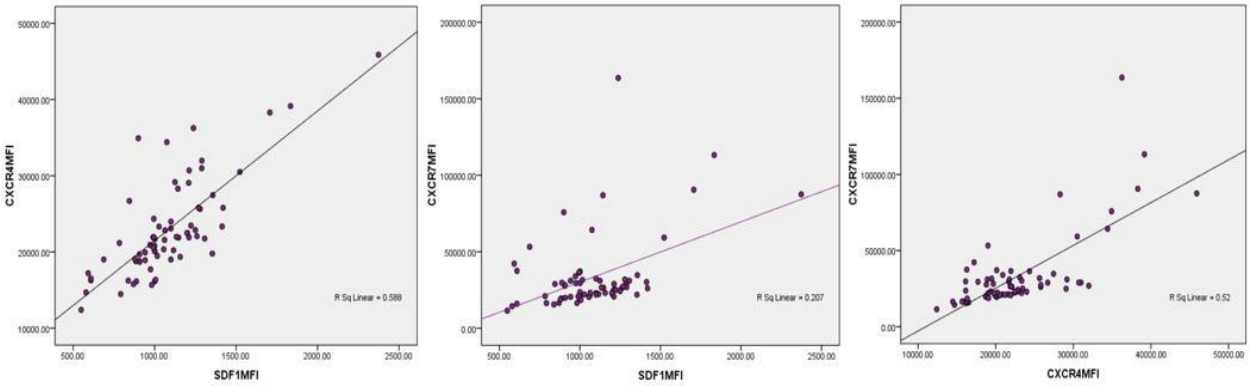

Figure 2. A close correlation can be observed between the surface expression of SDF-1 and its receptors on the platelets of the CAD patients. There is also a significant correlation between CXCR4 and CXCR7.
SDF-1, Stromal cell-derived factor-1

There were significant differences in platelets indices between the CAD and control groups.

Platelet parameters such as platelet count, MPV, and PDW were evaluated in the CAD and control groups. The results showed statistically significant differences in MPV and PDW between the CAD patients and the healthy controls. However, no significant difference was found in platelet count between the patients and the controls (Table 2). As is shown in Table 3, the investigation of the correlation between platelet parameters and the surface expression of SDF-1 and its receptors did not show any significant relationship.

Confounding variables did not affect the expressions of SDF-1, CXCR4, and CXCR7 on platelets.

It has been reported that the surface expression of chemokines and their receptors on platelets may be affected by a wide range of confounding variables. To examine the effects of these intervening variables on the surface expression of SDF-1, CXCR4, and CXCR7 in our CAD patients, we applied univariate analysis of covariance on several confounding variables, including hypertension, diabetes, hyperlipidemia, family history of CAD, and smoking. Moreover, we investigated the association between the expressions of SDF-1, CXCR4, and CXCR7 and inflammatory and

ischemic factors such as CRP, troponin, and serum creatine kinase. The results revealed significant interactions between the expressions of SDF-1, CXCR4, and CXCR7 neither with confounding variables nor with inflammatory factors. In addition, linear regression was performed to determine whether the surface expressions of SDF-1, CXCR4, and CXCR7 could be caused by either medications or platelet parameters. The results are summarized in Table 4, which indicates that these confounding factors had no significant effects on the aforementioned markers.

Increased expressions of SDF-1, CXCR4, and CXCR7 were coupled with low LVEF in the CAD patients.

It has been demonstrated that platelet-derived factors could promote cardiac function and may influence LVEF, [20] which serves as an important indicator of efficient blood pumping to systemic circulation. We found that LVEF in the CAD group was significantly lower than that in the control group (49.0±11.7% vs 58±2.4%). Based on the increased expression of SDF-1 on the platelets of the CAD patients, we assume that the lower values of LVEF may correlate with the increased expression of SDF-1. Of particular interest, the resulting data indicated that the surface expression of SDF-1 was higher in patients with low LVEF than in those with normal values (r= −0.388, P=0.003). Based on our results, it was reasonable to hypothesize that there might be a correlation between LVEF and the expression of either CXCR4 or CXCR7. Noteworthy, our results showed a significant correlation between CXCR4 expression and LVEF (r= −0.431, P=0.001). However, there was no significant correlation between CXCR7 expression and LVEF (r= −0.054, P=0.6) (Fig. 3).

ADP increased the surface expression of SDF-1 receptors on the platelets of the healthy volunteers.

ADP is an important primary platelet agonist with the ability to trigger a signaling cascade, which may alter the expression of surface proteins on platelets. To investigate whether ADP could affect the surface expression of CXCR4 and CXCR7, we treated platelets of 5 healthy volunteers with the increasing concentrations of the agonist. Interestingly, flow cytometry analysis revealed that the expressions of CXCR4 and CXCR7 were increased in a concentration-dependent manner following treatment with 20 µM, 100 µM, and 200 µM of ADP, as compared with the expression intensity of the receptors on the untreated platelets (Fig. 4).

Table 2. PLT parameters in the CAD and control groups

Variables	CAD Patients	Controls	*P*-value
PLT count (mean±SD)	234430±69661	221500±48296	0.221
MPV (mean±SD)	10.1±1.0	8.9±1.0	<0.001
PDW (mean±SD)	12.7±2.1	11.5±1.9	0.002

PLT, Platelet; MPV, Mean platelet volume; PDW, Platelet distribution width

Table 3. Correlation between PLT parameters and SDF-1, CXCR4, and CXCR7 MFI

Variables	SDF-1 *r* (*P*-value)	CXCR4 *r* (*P*-value)	CXCR7 *r* (*P*-value)
PLT count	-0.065 (*P*=0.59)	-0.731 (*P*=0.54)	0.120 (*P*=0.321)
MPV	0.051 (*P*=0.67)	0.222 (*P*=0.06)	0.161 (*P*=0.183)
PDW	-0.048 (*P*=0.69)	-0.451 (*P*=0.71)	-0.143 (*P*=0.237)

PLT, Platelet; MPV, Mean platelet volume; PDW, Platelet distribution width; SDF-1, Stromal cell-derived factor-1; MFI, Mean fluorescence intensity

Table 4. Univariate linear regression between SDF-1, CXCR4, and CXCR7 MFI values as dependent variables and clinical factors as confounding factors

Confounding Factors	SDF-1 (*P*-value)	CXCR4 (*P*-value)	CXCR7 (*P*-value)
Cardiovascular Risk Factors			
Hypertension	0.806	0.099	0.261
Diabetes	0.110	0.917	0.953
Hyperlipidemia	0.987	0.674	0.051
History of coronary artery disease in the family	0.078	0.669	0.191
Smoking	0.807	0.247	0.159
Inflammatory and Ischemic Factors			
C-reactive protein	0.753	0.905	0.462
Troponin	0.198	0.405	0.174
Creatine kinase	0.804	0.644	0.313
Medications			
Acetyl salicylic acid	0.833	0.555	0.993
Clopidogrel	0.147	0.165	0.333
ACE inhibitor	0.794	0.560	0.833
Beta-blocker	0.775	0.851	0.424
Statin	0.736	0.947	0.867
PLT Parameters			
PLT count	0.609	0.645	0.360
MPV	0.861	0.142	0.306
PDW	0.628	0.142	0.188

SDF-1, Stromal cell-derived factor-1; MFI, Mean fluorescence intensity; PLT, Platelet; MPV, Mean platelet volume; PDW, Platelet distribution width

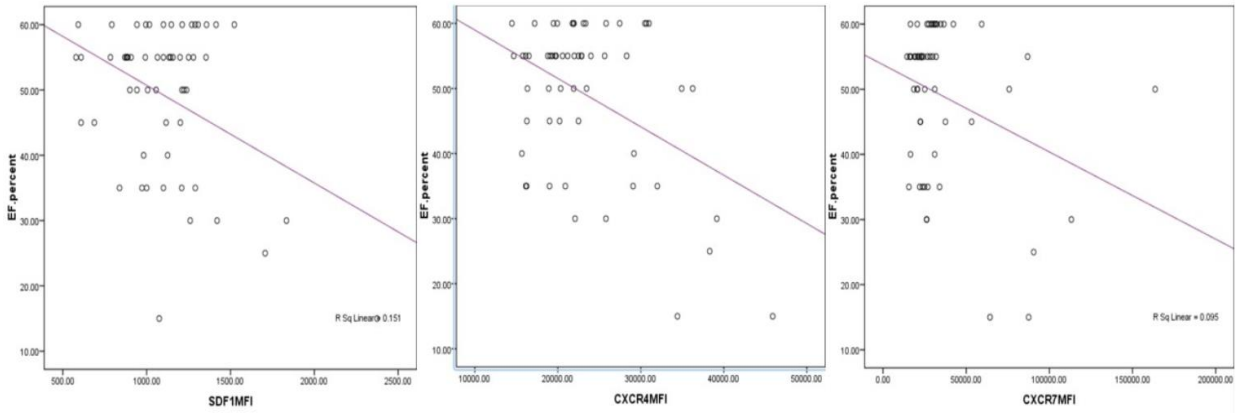

Figure 3. There is a negative correlation between SDF-1 and CXCR4 and LVEF. Increased expressions of SDF-1 and CXCR4 are coupled with low LVEF in the CAD patients. However, no significant correlation exists between CXCR7 and LVEF.

SDF-1, Stromal cell-derived factor-1; LVEF, Left ventricular ejection fraction; CAD, Coronary artery disease

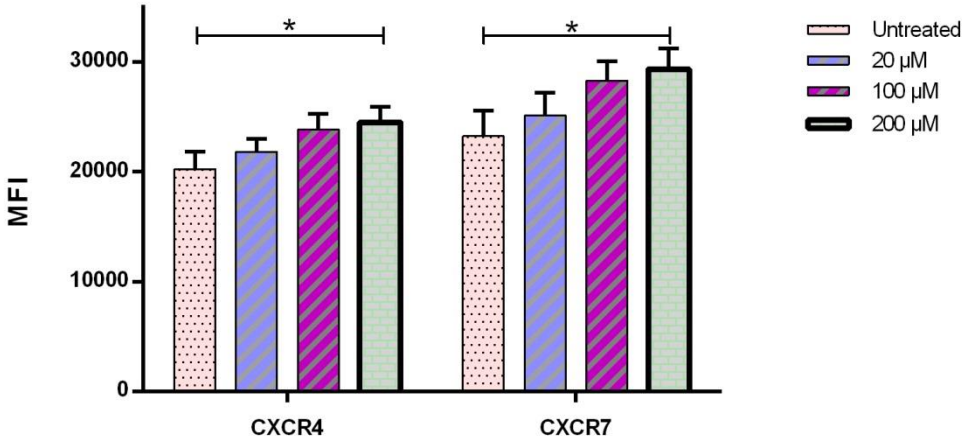

Figure 4. ANOVA analysis and LSD post hoc comparisons between the groups show that the surface expression of CXCR4 and CXCR7 are increased in a dose-dependent manner after treatment with ADP as an agonist. There is also a significant difference between the 2 groups in all pairwise comparisons with LSD. Data are mean ± SD of MFI from experiments done with 5 healthy controls. *: $P< 0.05$
MFI, Mean fluorescence

DISCUSSION

Platelets constitute a major source of stromal SDF-1 and store it as part of their α-granules. [10] SDF-1 expression is increased on activated platelets and induces the adherence and differentiation of $CD34^+$ progenitor cells to endothelial cells. [15] Increasing evidence demonstrates that the recruitment and migration of progenitor cells could contribute to the restoration of heart tissue and improve cardiac function. [21] To investigate the correlation between the expression of SDF-1 and its receptors (viz, CXCR4 and CXCR7) and cardiac function, we evaluated the surface expression of these molecules on platelets of CAD patients. Notably, our results showed that the expression levels of SDF-1 and CXCR4 were significantly increased in the CAD patients in comparison with the control group, which is in line with previous studies. [20,22,23] In a recent study, Stellos et al [22] indicated that patients with ACS displayed a significantly enhanced SDF-1 expression level on admission when compared with patients with SAP. Correlation analysis also outlined a tight correlation between SDF-1 and its receptors. Taken together, our results suggest that platelets probably play a key role in progenitor cell homing to damaged myocardium and thereupon contribute to myocardial restoration and survival in CAD patients.

Several studies have reported that different confounders could influence the SDF-1/CXCR4/CXCR7 axis. [24-26] However, the data in the present study did not show any significant relationship between the SDF-1, CXCR4, and CXCR7 expression and intervening factors, including cardiovascular risk factors, medications, inflammatory factors, and platelet parameters. Elevated platelet indices have been suggested as a risk factor for CAD patients. [27] Larger platelets have greater mass, more active metabolism, and more granules and adhesive receptors than smaller platelets, increasing their activation. [28] Still, it is controversial whether increased platelet indices are the cause or the outcome of CAD. [29, 30] In the present study, we found a significant increase in MPV and PDW between our CAD and control groups, which is compatible with the results of

several studies. [31, 32] Of particular interest, our results delineated that there was no significant correlation between platelet parameters and the surface expression of SDF-1/CXCR4/CXCR7. Overall, our findings suggest that increased platelet indices are not necessarily associated with the augmented surface expression of the indicated markers on platelets.

Most clinical studies, as well as the current investigation, have demonstrated that LVEF is decreased in patients with CAD in comparison with normal controls. [33, 34] Our correlation analysis showed a negative, albeit significant association, between SDF-1 and CXCR4 and LVEF. Chiming in with our results, other researchers have suggested that in CAD patients with decreased LVEF, the surface expression of SDF-1, CXCR4, and CXCR7 on platelets is increased to induce cardiac improvement. [22] Alterations in the surface expression of multitude proteins upon the agonist-induced activation of platelets have been reported previously. [35] In contrast to the results reported by Rath et al, [20] we found that the activation of platelets with ADP resulted in a significant increase in the surface expression of CXCR4 and CXCR7.

CONCLUSIONS

Identifying and introducing novel biomarkers as predictive markers of cardiovascular disease is of great importance. Accordingly, our results showed that the increased expression of SDF-1 and CXCR4 on platelets could be considered a promising candidate indicating that asymptomatic patients with decreased LVEF are at the risk of CAD.

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Conflict of Interest

The authors hereby declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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