Original Article

# Stromal Cell-derived Factor-1 and CXC Chemokine Receptor Type-4 are Associated with Cardiovascular Disease in Patients under Hemodialysis

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## Abstract

**Background:** Cardiovascular disease (CVD) is the most common cause of death among patients with end-stage renal disease especially whom under hemodialysis (HD). Stromal cell-derived factor-1 (SDF-1) and its receptor CXC chemokine receptor type-4 (CXCR4) could contribute to CVD. The main aim of this study was to evaluate the association between SDF-1 and CXCR4 with CVD and its related risk factors in patients under HD. **Methods:** Sixty patients under HD and 29 healthy subjects were recruited in the study. The serum levels and relative messenger RNA (mRNA) expressions of SDF-1 and CXCR4 were measured using enzyme-linked immunosorbent assay and real-time polymerase chain reaction in patients and controls, respectively. CVD history of the patients was obtained. **Results:** Twenty patients (33.3%) had a history of CVD. The mean levels of serum and relative mRNA expressions of SDF-1 and CXCR4 were higher in patients than controls and also in patients with a history of CVD than patients without it. The serum levels and relative expressions of SDF-1 and CXCR4 were positively correlated with blood urea nitrogen, parathyroid hormone, and high-sensitivity C-reactive protein and inversely correlated with hemoglobin. The history of CVD was the independent predictor of serum levels of SDF-1 and CXCR4 and also relative mRNA expression of CXCR4. **Conclusions:** The higher levels of serum and relative mRNA expressions of SDF-1 and CXCR4 were associated with CVD in patients under HD. Furthermore, SDF-1 and CXCR4 were associated with several traditional and uremia-related CVD risk factors in such patients.

**Keywords:** *Cardiovascular disease, CXC chemokine receptor type 4, end-stage renal disease, hemodialysis, stromal cell-derived factor-1*

## Introduction

Cardiovascular disease (CVD) is the most common cause of death among patients with end-stage renal disease (ESRD) especially whom under hemodialysis (HD).[1-4] Interaction between several risk factors are involved in CVD development among ESRD patients which can be categorized into three groups as follows: (i) traditional risk factors (diabetes mellitus, smoking, obesity, hypertension, dyslipidemia), (ii) uremia-related risk factors (secondary hyperparathyroidism, hyperphosphatemia, anemia, and hypoalbuminemia), and (iii) emerging risk factors (hyperhomocysteinemia, inflammation, and oxidative stress).[5] Despite management and treatment of these risk factors, CVD is responsible for approximately 45% of mortality among patients under HD;[4,6] hence, discovering the new pathways for understanding the pathophysiology of CVD in such population and eventually finding new treatments are needed.[7]

Stromal cell-derived factor-1 (SDF-1) is a CXC chemokine which is expressed in many tissues, including heart, kidney, endothelium, and leukocytes.[8,9] This chemokine has a role in recruiting endothelial progenitor cells from the bone marrow to the sites of vascular injuries and hypoxic regions.[10,11] CXC chemokine receptor type-4 (CXCR4) is a seven-transmembrane domain G-protein coupled receptor which is one of the receptors of SDF-1, and its stimulation by SDF-1 activates many intracellular signaling.[12] The role of SDF-1/CXCR4 axis in the pathogenesis of CVD including myocardial infarction (MI), atherosclerosis, and heart failure (HF) has been studied.[8,10,13-17] Furthermore, this axis is involved in many kidney diseases such as acute kidney injury, chronic kidney disease (CKD), and glomerular disease.[18-21]



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The SDF-1/CXCR4 axis could connect both kidney diseases and CVD.[22] The current literature on SDF-1 and CXCR4 is mainly focused on their involvement in pathogenesis of CKD or ESRD.[23-28] Almost no data are present on their role in the development of CVD in ESRD patients.[29] Therefore, despite extensive research on the biological function of SDF-1 and CXCR4 in CKD, the relationship between tis axis and development of CVD in ESRD patients is not elucidated yet. The main aim of this study was to evaluate the association between SDF-1 and CXCR4 with CVD and its related risk factors in patients under HD.

## Methods

### Study design and participants selection

This analytical cross-sectional study was conducted on patients under HD at Noor and Ali-Asghar Hospital, Isfahan, Iran, from April 2017 to October 2017. The inclusion criteria were individuals (i) being >18 years of age, (ii) being at least 6 months under HD, and (iii) having complete and clear medical documents. The exclusion criteria were (i) having any rheumatologic diseases including systemic lupus erythematosus, Wegener's granulomatosis, and other vasculitis, (ii) presence of systemic infection, (iii) suffering from malignancies, and (iv) having a history of surgery within 1 month before sampling. Controls were chosen from healthy volunteers who had no history of hypertension, smoking, diabetes mellitus, dyslipidemia, renal disorder, and CVD and were matched by age and gender with patients. After enrolling the eligible individuals, the objectives and the protocol of the study were completely explained and written informed consent was obtained from all participants. The study protocol was evaluated by the ethical committee of Isfahan University of Medical Sciences and also was approved by the ethical committee of AJA University of Medical Sciences (Approval code: IR-AJAUMS. REC.1395.38).

### Patients and study protocol

Overall, 60 patients met the inclusion criteria and were enrolled in the study. All patients were hemodialyzed routinely three times a week for 4 h. Etiologies of ESRD in patients were as follows: diabetic nephropathy (48.3%), hypertensive nephropathy (11.7%), interstitial nephritis (10%), reflux nephropathy and other urological disorders (10%), and unknown etiologies (20%). Twenty-nine age- and sex-matched healthy individuals with no known disease and no consumption of drugs were enrolled as control group. First, demographic features and clinical data of the eligible individuals were collected. In the next step, a fasting venous blood sample was obtained from patients before a HD session and controls. The blood samples were analyzed for serum SDF-1 and CXCR4, relative messenger RNA (mRNA) expressions of SDF-1 and CXCR4 in peripheral leukocytes, and other hematological and biochemical parameters.

### Blood sampling and laboratory evaluation

A sample of whole blood (10 ml) was collected from the peripheral vein after 12.0 h fasting and then divided and transferred into three tubes: (i) 1.5 ml was transferred to a citrated tube for complete blood count, (ii) 1 ml was transferred to an ethylenediaminetetraacetic acid tube for RNA extraction, and (iii) the rest of the sample was transferred to a tube without anticoagulant for other laboratory tests. The blood in the third tube was immediately centrifuged for 20 min at the speed of 3000 rpm and after serum separation was stored at −80°C until the measurements. Serum levels of SDF-1 and CXCR4 were measured by commercially enzyme-linked immunosorbent assay (ELISA) kits (EASTBIOPHARM, Hangzhou Eastbiopharm Co. Ltd., China). High-sensitivity C-reactive protein (hs-CRP) and parathyroid hormone (PTH) levels (normal PTH range: 11.1–79.5 pg/ml) were measured using particle-enhanced immunoturbidimetric assay and electrochemiluminescent immunoassay method on Cobas e411 auto analyzer (Roche Diagnostics International Ltd, Basel, Switzerland), respectively. Other biochemical and hematologic parameters were measured using routine and standard laboratory methods.

### RNA extraction and c-DNA synthesis

RNA was extracted immediately after sampling using total RNA extraction kit (Yekta Tajhiz Azma, Iran) according to the manufacturer's instruction. For each sample, the concentration of RNA was measured using spectrophotometer; overall, the absorption 260/280 ratio for all samples was >1.8. Extracted RNA was stored at −80°C. After that, 4 μg of each RNA sample was treated by 1 U of DNase I RNase-free (1 U/μl) (Sinaclon, Iran). In the next step, complementary DNA (cDNA) of each sample was synthesized using cDNA synthesis kit (BioFACT, 2X RT Pre-Mix, South Korea). For cDNA synthesis, 2 μg of DNase I treated RNA was reverse transcribed in a total volume of 20 μl containing 10 μl 2X RT Pre-Mix and 50 μM oligo dT.

### Real-time polymerase chain reaction

Primers for real-time polymerase chain reaction (PCR) were designed using Allele ID version 7.6 (PREMIER Biosoft, USA) and were purchased from Pishgam Co. (Iran). Table 1 shows the sequences of primers which were used in the real-time PCR. Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was selected as a housekeeping gene. The reaction efficiency of SDF-1, CXCR4, and GAPDH primers was measured using serial dilution of RNA method, and their reaction efficiencies were approximately equal.

SYBR Green-based real-time PCR was performed with a StepOnePlus™ real-time PCR system (Applied Biosystems,

**Table 1: Sequences of primers using for real-time polymerase chain reaction**

| Gene | Forward | Reverse | Product length |
|---|---|---|---|
| SDF-1 | 5'AGATGCTTGACGTTGGCTCT3' | 5'AAGGTCGTGGTCGTGCTG3' | 131 |
| CXCR4 | 5'CTTGTCCGTCATGCTTCTCA3' | 5'GAACCCTGTTTCCGTGAAGA3' | 150 |
| GAPDH | 5'AAGCTCATTTCCTGGTATG3' | 5'CTTCCTCTTGTGCTCTTG3' | 125 |

SDF-1=Stromal cell-derived factor-1, CXCR4=CXC chemokine receptor type-4, GAPDH=Glyceraldehyde-3-phosphate dehydrogenase

USA). Real-time PCR has been done in triplicate in a total volume of 20 µl including 400 ng cDNA, 10 µl SYBR Green PCR Master Mix (2X Real-Time PCR Master Mix, BioFact, South Korea), 7 µl nuclease-free water, and 0.25 µM of each forward and reverse primers. Thermal cycling conditions of real-time PCR were as following: 15 min at 95°C, 45 cycles of the 20 s at 95°C and 60 s at 60°C. Melting curve analysis was performed for determining the specificity of the amplification reaction. For real-time PCR analysis and determining the relative mRNA expression of SDF-1 and CXCR4 in peripheral leukocytes, Pfaffl method was used.[30]

### Retrospective data collection

In addition to biochemical and gene expression evaluations, we retrospectively investigated the medical records of all patients for any history of CVD. CVD which we evaluated included ischemic heart disease (IHD), cerebrovascular accident (CVA), peripheral artery disease (PAD), and HF.

### Definitions

IHD was considered when patients had a history of MI, hospitalization for acute coronary syndrome, and coronary heart disease based on cardiac catheterization. HF was defined as ejection fraction below than 45% which confirmed by echocardiography that had been done by an expert cardiologist. Hypertension was defined as a systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive drugs. Diabetes mellitus was defined as fasting blood sugar (FBS) ≥126 mg/dl or using insulin or antidiabetic medications. Dyslipidemia was considered when one of the following was present: total cholesterol >200 mg/dl, low-density lipoprotein (LDL) >100 mg/dl, high-density lipoprotein (HDL) <40 mg/dl, and triglyceride >150 mg/dl, or statins consumption.

### Statistical analysis

Continuous variables except gene expression data were shown as mean ± standard deviation and categorical variables were presented as percentage. Gene expression data were shown as mean (standard error range). For mean comparisons between different groups, independent sample *t*-test and Mann–Whitney U-test were done when appropriate. Categorical variables were compared between different groups using Chi-square test. Correlation analysis for determining associations between the variables was done by Spearman's and Pearson's correlation coefficients and linear regression analysis when appropriate. Multiple regression analysis was performed for determining the independent predictors of serum levels and relative mRNA expressions of SDF1 and CXCR4. Receiver operating characteristic (ROC) curve analysis was performed for evaluating the performance of serum levels and relative mRNA expressions of SDF-1 and CXCR4 as a diagnostic test of CVD. Gene expression analysis was done by REST 2009 (REST version 2.0.7, IBM company, USA, Qiagen, Hilden, Germany). Other statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA), and for drawing the graphs, GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used. $P < 0.05$ considered as statistically significant threshold.

## Results

### Participants' characteristics

Twenty patients (33.3%) had CVD including IHD in 11 patients (55%), CVA in 2 patients (10%), PAD in 3 patients (15%), and HF in 4 patients (20%). Furthermore, among 11 patients with IHD, 6 patients (54%) had combination of IHD and HF. Table 2 summarizes the demographic and clinical characteristic of patients with and without CVD and controls.

### Comparison of serum levels and relative messenger RNA expressions of stromal cell-derived factor-1 and CXC chemokine receptor type-4 in study participants and their subgroups

Table 3 shows the mean levels of serum and relative mRNA expressions of SDF-1 and CXCR4 in controls and patients with and without CVD. According to Table 3, the mean levels of both serum and relative mRNA expressions of SDF-1 and CXCR4 were significantly higher in all patients than controls and also in patients with CVD than without CVD [Figure 1]. There were no significant differences in mean levels of serum and relative mRNA expressions of SDF-1 and CXCR4 in patients with and without hypertension, diabetes mellitus, dyslipidemia, and smoking.

### Correlation analysis

Table 4 shows the results of correlation analysis between serum levels and relative mRNA expressions of SDF-1 and CXCR4 with other variables in the study. For determining the correlation of history of CVD and serum levels and relative mRNA expressions of SDF-1 and CXCR4, linear

**Table 2: Demographic, clinical, hematological, and biochemical parameters of patients and controls**

| Variables | Controls (*n*=29) | All patients (*n*=60) | *P* | Patients with CVD (*n*=20) | Patients without CVD (*n*=40) | *P* |
|---|---|---|---|---|---|---|
| Gender (male/female) | 16/13 | 33/27 | 0.92 | 11/9 | 22/18 | 1 |
| Age (years) | 55.96±12.96 | 57.83±14.02 | 0.54 | 59.15±13.7 | 57.17±14.3 | 0.61 |
| BMI (kg/m$^2$) | 22.82±2.66 | 22.75±5.21 | 0.94 | 23.5±6.16 | 22.37±4.7 | 0.54 |
| SBP (mmHg) | 100±9.25 | 121.91±20.77 | 0.0001 | 124.75±18.88 | 120.5±21.74 | 0.44 |
| DBP (mmHg) | 66.2±5.77 | 72.58±9.36 | 0.0001 | 72.5±8.5 | 72.62±9.87 | 0.93 |
| Hemoglobin (g/dl) | 14.52±1.62 | 10.51±1.34 | 0.0001 | 10.71±1.36 | 10.42±1.34 | 0.43 |
| WBC (×10$^3$ µl) | 6.26±1.6 | 5.94±1.86 | 0.42 | 6.1±2.22 | 5.86±1.67 | 0.64 |
| Neutrophil (×10$^3$ µl) | 3.49±1.17 | 3.83±1.38 | 0.26 | 3.85±1.52 | 3.81±1.32 | 0.92 |
| Lymphocyte (×10$^3$ µl) | 2.14±0.58 | 1.67±0.74 | 0.003 | 1.78±0.96 | 1.61±0.61 | 0.42 |
| Monocyte (×10$^3$ µl) | 0.34±0.1 | 0.12±0.11 | 0.0001 | 0.12±0.07 | 0.12±0.13 | 0.89 |
| Eosinophil (×10$^3$ µl) | 0.18±0.16 | 0.31±0.25 | 0.01 | 0.34±0.17 | 0.29±0.28 | 0.01 |
| Platelet (×10$^3$ µl) | 241.93±53.65 | 169.86±59.92 | 0.01 | 171.45±59.29 | 169.05±61 | 0.88 |
| BUN (mg/dl) | 25.89±6.88 | 63.31±16.44 | 0.0001 | 63.8±20 | 63.7±14.63 | 0.87 |
| Creatinine (mg/dl) | 0.94±0.17 | 7.5±2.35 | 0.0001 | 7.34±2.5 | 7.58±2.29 | 0.71 |
| FBS (mg/dl) | 90.72±7.39 | 96.85±55.85 | 0.55 | 78.7±28.8 | 105.92±63.73 | 0.05 |
| Triglyceride (mg/dl) | 90.31±39.42 | 124.78±85.24 | 0.04 | 122.65±69.97 | 125.85±92.75 | 0.86 |
| Cholesterol (mg/dl) | 140.14±27.41 | 133.43±40.45 | 0.42 | 127.4±45.93 | 136.45±37.68 | 0.41 |
| LDL (mg/dl) | 82±21.63 | 77.95±25.48 | 0.46 | 77.6±27.03 | 78.12±25.02 | 0.94 |
| HDL (mg/dl) | 50.06±10.36 | 36.68±11.37 | 0.0001 | 36.9±10.76 | 36.85±11.8 | 0.89 |
| Phosphorus (mg/dl) | 3.42±0.39 | 5.18±1.1 | 0.0001 | 5.09±0.75 | 5.23±1.24 | 0.64 |
| Calcium (mg/dl) | 9.51±0.24 | 8.79±0.78 | 0.0001 | 8.99±0.56 | 8.69±0.85 | 0.17 |
| Phosphorus × calcium (mg$^2$/dl$^2$) | 32.58±3.83 | 45.47±10.3 | 0.0001 | 45.65±6.54 | 45.38±12.21 | 0.92 |
| PTH (pg/ml) | 47.65±17.35 | 735.88±568.3 | 0.0001 | 781.91±500.52 | 712.87±604.08 | 0.66 |
| Albumin (g/dl) | 4.78±0.29 | 3.79±0.42 | 0.0001 | 3.85±0.38 | 3.76±0.44 | 0.43 |
| hs-CRP (mg/l) | 1.17±2.95 | 15.83±12.9 | 0.0001 | 20.46±8.82 | 13.52±14.04 | 0.04 |
| Duration of hemodialysis (months) | | 73.75±67.5 | | 72.25±62.18 | 74.5±66.76 | 0.8 |
| Kt/V | | 1.46±0.43 | | 1.34±0.42 | 1.52±0.43 | 0.11 |
| Diabetes mellitus, *n* (%) | | 29 (48.3) | | 9 (45) | 20 (50) | 0.71 |
| Hypertension, *n* (%) | | 45 (75) | | 17 (85) | 28 (70) | 0.2 |
| Smoker, *n* (%) | | 9 (15) | | 5 (25) | 4 (10) | 0.12 |
| Dyslipidemia, *n* (%) | | 53 (88.3) | | 20 (100) | 33 (82.5) | 0.04 |
| Medications CCB, *n* (%) | | 28 (46.7) | | 11 (55) | 17 (42.5) | 0.36 |
| β-blocker, *n* (%) | | 19 (31.7) | | 9 (45) | 10 (25) | 0.11 |
| ACEi, *n* (%) | | 5 (8.3) | | 2 (10) | 3 (7.5) | 0.74 |
| ARB, *n* (%) | | 12 (20) | | 6 (30) | 6 (15) | 0.17 |
| Statins, *n* (%) | | 18 (30) | | 10 (50) | 8 (20) | 0.01 |
| Erythropoietin, *n* (%) | | 47 (78.3) | | 19 (95) | 28 (70) | 0.02 |
| Erythropoietin dose intake (IU/Kg/week) | | 187.21±127.1 | | 201.09±162.37 | 178.11±99.75 | 0.54 |

CVD=Cardiovascular disease, BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, WBC=White blood cells, BUN=Blood urea nitrogen, FBS=Fasting blood sugar, LDL=Low-density lipoprotein, HDL=High-density lipoprotein, PTH=Parathyroid hormone, hs-CRP=High sensitivity C-reactive protein, CCB=Calcium channel blocker, ACEi=Angiotensin I converting enzyme inhibitor, ARB=Angiotensin II receptor blocker

**Table 3: The comparisons of serum stromal cell-derived factor-1, relative messenger RNA expression of stromal cell-derived factor-1, serum CXC chemokine receptor type-4, and relative messenger RNA expression of CXC chemokine receptor type-4 levels in controls and patients with, without cardiovascular disease**

| Variables | Controls (*n*=29) | All patients (*n*=60) | *P* | Patients with CVD (*n*=20) | Patients without CVD (*n*=40) | *P* |
|---|---|---|---|---|---|---|
| Serum SDF-1 (ng/ml) | 5.37±2.67 | 7.57±2.57 | 0.0001 | 8.57±2.38 | 7.07±2.56 | 0.03 |
| Relative mRNA expression of SDF-1* | 1 (0.04-25.26) | 5.49 (0.25-96.33) | 0.0001 | 10.56 (0.61-161.68) | 3.96 (0.21-61.39) | 0.0001 |
| Serum CXCR-4 (ng/ml) | 3.78±3.09 | 5.58±3.2 | 0.01 | 6.9±3.01 | 4.92±3.12 | 0.02 |
| Relative mRNA expression of CXCR4* | 1 (0.1-9.19) | 3.85 (0.68-22.16) | 0.0001 | 6.11 (1.19-38.71) | 3.06 (0.57-20.26) | 0.0001 |

*Gene expression data were expressed as mean (SE range). SDF-1=Stromal cell-derived factor-1, CXCR4=CXC chemokine receptor type-4, CVD=Cardiovascular disease, SE=Standard error

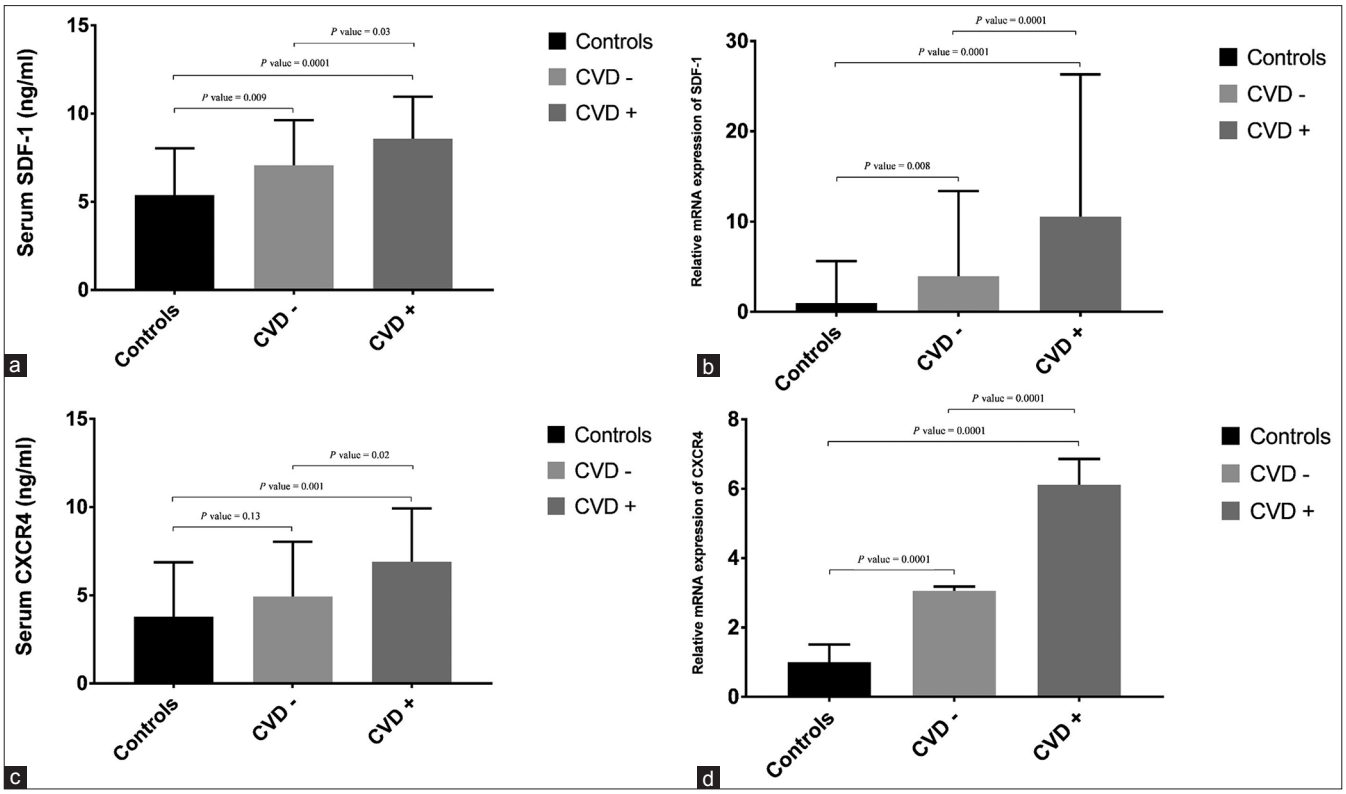


**Figure 1: The comparisons of serum stromal cell-derived factor-1 (a), relative messenger RNA expression of stromal cell-derived factor-1 (b), serum CXC chemokine receptor type-4 (c), and relative messenger RNA expression of CXC chemokine receptor type-4 (d) in controls and patients with, without cardiovascular disease**

regression analysis showed that CVD had a positive correlation with serum SDF-1 (β = 1.5, *P* = 0.03), serum CXCR4 (β = 1.98, *P* = 0.02), and relative mRNA expression of CXCR4 (β = 3.75, *P* = 0.0001), but this analysis did not show significant correlation between history of CVD and relative mRNA expression of SDF-1 (β = 16.68, *P* = 0.32).

### Multiple regression analysis

The variables which were significantly correlated with serum levels and relative mRNA expressions of SDF-1 and CXCR4 were included in multiple regression analysis. Multiple regression analysis [Table 5] showed that history of CVD was an independent predictor of serum SDF-1, serum CXCR4, and relative mRNA expression of CXCR4. The independent predictor of relative SDF-1 expression was SBP.

### Receiver operating characteristic curve analysis

In ROC curve analysis, the area under the ROC curve values of serum SDF-1, relative mRNA expression of SDF-1, serum CXCR4, and relative mRNA expression of CXCR4 in diagnosing CVD were 0.63 (*P* = 0.07), 0.68 (*P* = 0.01), 0.68 (*P* = 0.02), and 0.87 (*P* = 0.0001), respectively.

## Discussion

In this study, we evaluated the association between SDF-1 and CXCR4 which were measured using ELISA and real-time PCR with CVD and several traditional and uremia-related risk factors of them in a population of patients under HD. In our study, the most of traditional and loss of renal function-related CVD risk factors were not significantly different among patients with and without CVD despite high frequencies of them in both subgroups; therefore, this issue supports the idea that for better evaluation and management of CVD in patients with ESRD, the new pathways and novel biomarkers should be discovered.[7] The SDF-1/CXCR4 axis could connect both kidney diseases and CVD[22] which in our study we attempted to assess it in patients with and without both problems comparing each other and also with controls.

In the present study, the mean serum levels of SDF-1 and its relative mRNA expression were significantly higher in patients than controls. In the literature, there are some studies which can be compared with our results but in certain aspects have controversies. Two studies which were conducted on patients in different stages of CKD and patients under HD demonstrated that the mean serum level of SDF-1 was significantly higher in patients than controls.[23,24] The relative mRNA expression of SDF-1 was evaluated in a study on children under HD which showed that the value of SDF-1 gene expression in peripheral leukocytes was not significantly different in patients and controls.[31] We also found that patients with ESRD had significantly higher serum level and quantitative mRNA

**Table 4: Correlations between measured markers and other variables in patients under hemodialysis**

| Variables | Serum SDF-1 (ng/ml) | | Relative mRNA expression of SDF-1 | | Serum CXCR4 (ng/ml) | | Relative mRNA expression of CXCR4 | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| Age (year) | 0.03 | 0.71 | −0.07 | 0.47 | 0.08 | 0.45 | 0.16 | 0.12 |
| BMI (kg/m$^2$) | −0.19 | 0.06 | −0.08 | 0.41 | 0.01 | 0.9 | 0.07 | 0.5 |
| SBP (mmHg) | 0.33 | 0.002 | 0.27 | 0.008 | 0.2 | 0.05 | 0.34 | 0.001 |
| DBP (mmHg) | 0.14 | 0.17 | 0.21 | 0.04 | 0.16 | 0.13 | 0.23 | 0.03 |
| Duration of hemodialysis (months) | 0.09 | 0.49 | −0.16 | 0.21 | 0.27 | 0.03 | −0.05 | 0.68 |
| Kt/V | 0.01 | 0.92 | 0.04 | 0.74 | 0.13 | 0.29 | −0.01 | 0.42 |
| SDF-1 (ng/ml) | | | 0.29 | 0.005 | 0.54 | 0.0001 | 0.43 | 0.0001 |
| Relative mRNA expression of SDF-1 | 0.29 | 0.005 | | | 0.24 | 0.02 | 0.35 | 0.001 |
| CXCR4 (ng/ml) | 0.54 | 0.0001 | 0.24 | 0.02 | | | 0.43 | 0.0001 |
| Relative mRNA expression of CXCR4 | 0.43 | 0.0001 | 0.35 | 0.001 | 0.43 | 0.0001 | | |
| Hemoglobin (g/dl) | −0.23 | 0.02 | −0.3 | 0.004 | −0.23 | 0.02 | −0.28 | 0.006 |
| WBC (×10$^3$ µl) | −0.07 | 0.48 | −0.05 | 0.63 | −0.18 | 0.08 | −0.06 | 0.54 |
| Neutrophil (×10$^3$ µl) | −0.01 | 0.91 | 0.004 | 0.97 | −0.12 | 0.23 | −0.01 | 0.9 |
| Lymphocyte (×10$^3$ µl) | −0.18 | 0.07 | −0.1 | 0.31 | −0.09 | 0.48 | −0.16 | 0.13 |
| Monocyte (×10$^3$ µl) | 0.06 | 0.64 | 0.07 | 0.55 | −0.11 | 0.39 | −0.11 | 0.4 |
| Eosinophil (×10$^3$ µl) | 0.21 | 0.1 | −0.1 | 0.32 | 0.01 | 0.91 | 0.03 | 0.76 |
| Platelet (×10$^3$ µl) | −0.19 | 0.07 | −0.2 | 0.06 | −0.19 | 0.06 | 0.05 | 0.7 |
| BUN (mg/dl) | 0.21 | 0.04 | 0.28 | 0.008 | 0.32 | 0.002 | 0.32 | 0.002 |
| Creatinine (mg/dl) | −0.15 | 0.24 | −0.13 | 0.29 | −0.16 | 0.22 | −0.25 | 0.05 |
| FBS (mg/dl) | −0.18 | 0.08 | −0.02 | 0.82 | −0.13 | 0.21 | −0.13 | 0.2 |
| Triglyceride (mg/dl) | 0.04 | 0.65 | 0.02 | 0.84 | 0.02 | 0.8 | 0.03 | 0.75 |
| Cholesterol (mg/dl) | −0.03 | 0.73 | −0.07 | 0.46 | −0.03 | 0.77 | −0.12 | 0.23 |
| LDL (mg/dl) | −0.02 | 0.81 | −0.04 | 0.7 | −0.03 | 0.72 | −0.04 | 0.65 |
| HDL (mg/dl) | −0.22 | 0.03 | −0.14 | 0.18 | −0.09 | 0.37 | −0.26 | 0.01 |
| Phosphorus (mg/dl) | 0.29 | 0.004 | 0.22 | 0.03 | 0.18 | 0.07 | 0.33 | 0.001 |
| Calcium (mg/dl) | −0.16 | 0.13 | −0.11 | 0.26 | −0.17 | 0.09 | −0.18 | 0.09 |
| Phosphorus × calcium (mg$^2$/dl$^2$) | 0.29 | 0.006 | 0.23 | 0.02 | 0.18 | 0.07 | 0.33 | 0.001 |
| PTH (pg/ml) | 0.22 | 0.03 | 0.31 | 0.003 | 0.26 | 0.01 | 0.33 | 0.001 |
| Albumin (g/dl) | −0.26 | 0.01 | −0.24 | 0.02 | −0.06 | 0.36 | −0.35 | 0.001 |
| hs-CRP (mg/l) | 0.3 | 0.004 | 0.25 | 0.01 | 0.35 | 0.006 | 0.41 | 0.0001 |

SDF-1=Stromal cell-derived factor-1, CXCR-4==CXC chemokine receptor type-4, BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, WBC=White blood cells, BUN=Blood urea nitrogen, FBS=Fasting blood sugar, LDL=Low-density lipoprotein, HDL=High-density lipoprotein, PTH=Parathyroid hormone, hs-CRP=high sensitivity C-reactive protein

**Table 5: Statistical significant predictors of serum stromal cell-derived factor-1, relative messenger RNA expression of stromal cell-derived factor-1, serum CXC chemokine receptor type-4, and relative messenger RNA expression of stromal cell-derived factor-1 using multiple regression analysis in patients under hemodialysis**

| Variables | Predictors | β | SE | *P* |
|---|---|---|---|---|
| Serum SDF-1 (ng/ml) | History of CVD | 1.54 | 0.66 | 0.02 |
| Relative mRNA expression of SDF-1 | SBP (mmHg) | 0.95 | 0.38 | 0.01 |
| Serum CXCR4 (ng/ml) | History of CVD | 1.96 | 0.82 | 0.02 |
| Relative mRNA expression of CXCR4 | History of CVD | 3.63 | 0.54 | 0.0001 |

SDF-1=Stromal cell-derived factor-1, CXCR4=CXC chemokine receptor type-4, CVD=Cardiovascular disease, SE=Standard error, SBP=Systolic blood pressure

expression of CXCR4 than controls. In two studies which were conducted on adults with CKD and children under HD, the CXCR4/CD34+ cells and T-lymphocytes with CXCR4 expression which were measured by flow cytometry were significantly lower in patients than controls, respectively.[23,28] The differences between our results and the mentioned studies most probably due to method of CXCR4 measurements which in mentioned studies CXCR4 was measured in a specific subtype of leukocytes and the other reason was diversity between study populations.

We showed that the serum levels of SDF-1 and CXCR4 were positively correlated with relative mRNA expressions of these two genes. This issue showed that in patients under HD, the increase in gene expressions of SDF-1

and CXCR4 caused their serum elevations, but the correlation between them was not much strong which revealed that the increases in serum levels of SDF-1 and CXCR4 were not completely the result of gene expression increases. In fact, the productions of SDF-1 and CXCR4 in other sources such as lymph nodes, bone marrow, and other tissues were responsible for increasing their serum levels as well as their gene expressions increment in the peripheral leukocytes.[32] One of the possible reasons for increase in measured variables in the patients was uremia which we found positive correlations between blood urea nitrogen (BUN) and serum levels and relative expressions of SDF-1 and CXCR4. However, our result about association between SDF-1 and BUN is argued by findings that were achieved by Ribeiro *et al*., which they showed that SDF-1 expression was significantly lower in human umbilical vein endothelial cells in uremia medium after 6 h than cells in healthy medium.[26] However, in mentioned study, the cells were incubated in a medium with high concentration of urea for a short time,[26] while patients under HD are under uremia condition for a long time, hence, for better evaluation, studies with cells incubation in uremic media with different times and measuring the SDF-1 expression on them are needed. Another reason for higher levels of SDF-1 and CXCR4 in patients with ESRD probably is systemic inflammation. In our study, we found that serum levels and gene expressions of SDF-1 and CXCR4 were positively correlated with hs-CRP and negatively with albumin. Hs-CRP is a marker of inflammation in patients under HD, and also decrease in albumin level is a sign of inflammation in such patients.[33] Similar to our study, Ribeiro *et al*. showed a positive correlation between SDF-1 and interleukin (IL) 8 which is an indicator of inflammatory state[26] and also Mehta *et al*. found a positive correlation between SDF-1 with IL-6, tumor necrosis factor-alpha, and hs-CRP.[29] We did not find any correlation between different types of leukocytes and SDF-1 and CXCR4 expressions, which indicated increasing in expressions of these two chemokine and receptors not due to the numbers of leukocytes, instead for increasing their expressions in every type of leukocytes.

The role of SDF-1 in CVD was evaluated in some studies. However, the results of them were conflicting and most of them were not performed on patients with ESRD. In a study which was done on patients with stable and unstable angina, the plasma level of SDF-1 was significantly lower in patients than controls.[13] In another study which evaluated the correlation between carotid-intima media thickness (c-IMT) as an indicator of atherosclerosis and SDF-1, the level of SDF-1 was negatively correlated with c-IMT.[34] In contrast, other studies have shown that higher levels of SDF-1 are associated with history of CVD[8,29] and prediction of CVD including MI[14,15,29] and HF[16] and also all cause of mortality.[15,16,29] In our study, we found that patients who had a history of CVD had higher serum and relative expression levels of SDF-1, and we also revealed that history of CVD was an independent predictor of serum SDF-1. The discrepancies between studies in this scope were due to multiple reasons including the time of sampling from CVD occurrence: it seems that in acute phase of inflammation such as acute MI, the rapid release of SDF-1 for activating its receptors causes SDF-1 sequestration in receptors which eventually results the decrease in circulating levels of SDF-1;[29] (ii) the type of SDF-1 which was measured; some studies measured SDF-1$\alpha$[13,14] and others including us measured total SDF-1[8,16] or surface SDF-1;[15] and (iii) last but not least, the nature of the SDF-1/CXCR4 axis through having six splice variants and two receptors which causes a complex axis[29] and it is needed to evaluate thoroughly in future studies. We also showed that serum level and relative mRNA expression of CXCR4 were higher in patients with previous CVD. Rath *et al*. found that CXCR4 expression on platelets of patients with coronary artery disease was significantly higher than controls.[10] In addition, Weiberg *et al*. showed that CXCR4 in atherosclerotic plaques was associated with a history of CVD.[35]

In this study, we also found correlations between traditional and CKD-related risk factors of CVD with SDF-1 and CXCR4. Among traditional risk factors, we showed correlation between SDF-1 and CXCR4 with SBP, DBP, and HDL. Our results are comparable with the studies in the literature. In a study which was carried out on healthy individuals, the level of SDF-1 was inversely correlated with HDL and positively with SBP, LDL, triglyceride, and glucose.[16] In contrast, Matsuoka *et al*. reported no correlation between SDF-1 and traditional CVD risk factors in patients with history of MI.[14] About CXCR4, Weiberg *et al*. showed positive correlation between age, hypertension, and hypercholesterolemia with CXCR4.[35] On the other hand, we did not find any significant differences in SDF-1 and CXCR4 between subgroups with and without hypertension, dyslipidemia, diabetes mellitus, and smoking. In a prospective study which was conducted on 3687 patients with CKD, the plasma level of SDF-1 was significantly higher in patients who had hypertension, diabetes mellitus, and hypercholesterolemia than patients who did not have them.[29] This difference between our study and the mentioned study in this issue most probably is due to our relatively small sample size. Furthermore, we showed that SDF-1 and CXCR4 were correlated with anemia, hyperphosphatemia, $P \times$ Ca product, and hyperparathyroidism among uremia-related risk factors. Despite that uremia-related risk factors have role in CVD including atherosclerosis, arterial stiffness, and HF in patients under HD, the mechanism of CVD development through these risk factors was not well defined.[1] Our results showed correlation between SDF-1/CXCR4 axis and these risk factors could have benefit to elucidate the mechanism. In an animal study, injection of PTH to the

mice 6 days after MI caused increase in SDF-1 levels and also migration of CXCR4 positive cells to the heart which indicated stimulation of SDF-1/CXCR4 axis after PTH increment in blood.[36] On the other hand, stimulation of SDF-1/CXCR4 axis causes activation of signaling pathways such as MAPK, PI3K, and Jak/STAT, which result production of matrix metalloproteinases-2 and 9[12,37] that their roles in atherosclerosis and arterial stiffness have been established and it could be a mechanism for CVD development.[7] Among other risk factors of CVD, we showed positive correlations between SDF-1 and CXCR4 with hs-CRP as a good predictor of CVD incidents and our result in this issue was similar to the studies in the literature.[14,29]

Prevention of CVD in patients with ESRD can be categorized into primary and secondary preventions.[38,39] In primary prevention, decreasing or eliminating the risk factors of CVD which previously were described may help prevent CVD in such patients.[39,40] In the present study, the correlations between SDF-1 and CXCR4 with CVD risk factors were evaluated and established in some aspects. In fact, these correlations could help to describe cellular pathways that CVD risk factors affect them; therefore, by intervention in these pathways through medications, the CVD may be prevented in such patients. For instance, SDF-1/CXCR4 axis could be a target of medications. The inhibitors of dipeptidyl peptidase-4 such as vildagliptin could decrease the circulating level of SDF-1 and finally preventing the CVD.[41] For better evaluation, large cohort studies with using these type of medications are needed. For secondary prevention, finding new biomarkers which can diagnose CVD early in patients under HD might be useful for screening of these patients and early management of CVD on them.[42] In this study, for evaluating the accuracy of the serum levels and relative mRNA expressions of SDF-1 and CXCR4 in discriminating the patients under HD with and without CVD through ROC curve analysis, we showed that relative SDF-1 expression and serum CXCR4 had acceptable predictive values and relative CXCR4 expression had excellent predictive values. In fact, these biomarkers successfully predicted CVD in patients under HD, but for establishing it and using them for CVD screening in such population, prospective studies should be done.

The findings of this study must be interpreted in view of its limitations. First, in this study, we retrospectively evaluated the CVD; therefore, our results included the limitations of this type of evaluation. Given this issue, a prospective study should be done for better evaluation of SDF-1/CXCR4 axis in CVD. Second, we measured relative gene expressions of SDF-1 and CXCR4 in whole white blood cells, so we were not able to determine in which subtypes of leukocytes the gene expressions of them were increased. Thirdly, we did not measure the values of SDF-1 and CXCR4 after HD to compare with the values before HD and therefore to better evaluate the characteristics of this chemokine and its receptor. At last, our relatively small sample size which might affect the results.

## Conclusions

The higher levels of serum and relative mRNA expressions of SDF-1 and CXCR4 were associated with CVD in patients under HD. In addition, SDF-1 and CXCR4 were associated with several traditional and uremia-related CVD risk factors in such patients. In addition, SDF-1 and CXCR4 might be useful for CVD screening in patients with ESRD. Large cohort studies are needed for establishing the SDF-1/CXCR4 roles in CVD development in patients with ESRD and also evaluating their predictive values in CVD diagnosis.

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### Conflicts of interest

There are no conflicts of interest.

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