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Serum levels of IL-37 and correlation with inflammatory cytokines and clinical outcomes in patients with coronary artery disease

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ABSTRACT

Coronary artery disease (CAD) due to atherosclerosis is one of the important reasons for death worldwide. Recent evidence has suggested the essential role of inflammation in the progression of atherosclerosis. Interleukin (IL)-37 is a critical anti-inflammatory member of the IL-1 family which regulates the inflammatory processes. The aim of this study was to compare the serum levels of IL-37 in patients with CAD compared with the control group and its correlation with oxidative stress, cholesterol homeostasis, and inflammation in patients with CAD. A total of 42 patients with CAD and 42 sex-matched and age-matched controls who underwent coronary angiography were included in this study. The serum levels of IL-37 were evaluated via ELISA. Serum levels of biochemical risk factors were determined by enzymatic methods. Serum levels of IL-37 in the CAD group subjects were significantly lower than in the control group and IL-37 was significantly increased in men with CAD than in women with CAD. IL-37 significantly had an inverse correlation with IL-6, tumor necrosis factor- α , IL-32, high-sensitivity C reactive protein, oxidized low-density lipoprotein, and malondialdehyde. Also, IL-37 had a significantly positive correlation with ferric-reducing antioxidant power (FRAP) assay. In addition, IL-37 has positively correlated with *ATP-binding cassette transporter A1* and *G1* gene expression in peripheral blood mononuclear cells and serum levels of the FRAP. A receiver operating characteristic test displayed that IL-37 level ratios were a relatively significant CAD predictor. Our results indicated that decreased serum levels of IL-37 in patients with CAD and its relationship with inflammatory cytokines and reverse cholesterol transport genes are more likely to be associated in the inflammatory process with disease pathology.

INTRODUCTION

Cardiovascular disease (CVD), as a global burden of health and probably one of the biggest non-infectious and multifactorial diseases, is continuously increasing. Various risk factors, such as sedentary lifestyle, psychosocial stress, dyslipidemia, inflammation, hypertension, and genetic

Significance of this study

What is already known about this subject?

- ⇒ Atherosclerosis is a chronic inflammatory disease of large-sized and medium-sized arteries that causes ischemic heart disease, strokes, and peripheral vascular disease.
- ⇒ Interleukin (IL)-37 expression increases in calcified arterial tissue of patients with diabetes mellitus, which has an anti-inflammatory property.
- ⇒ The correlation between the serum levels of IL-37 with serum levels of inflammatory cytokines (IL-6, tumor necrosis factor- α (TNF- α) and IL-32), high-sensitivity C reactive protein (hs-CRP), oxidative stress markers (oxidized low-density lipoprotein (ox-LDL), ferric-reducing antioxidant power assay (FRAP), and malondialdehyde (MDA)) as well as *ATP-binding cassette transporter A1* (*ABCA1*) and *G1* (*ABCG1*) gene expression in peripheral blood mononuclear cells (PBMCs) of patients with coronary artery disease (CAD) is less clear.

What are the new findings?

- ⇒ Serum levels of IL-37 in the CAD group subjects were significantly lower than in the control group.
- ⇒ Serum levels of IL-37 in men with CAD exhibited a more reduction than in women with CAD.
- ⇒ IL-37 significantly had an inverse correlation with IL-6, TNF- α , IL-32, hs-CRP, ox-LDL, and MDA.
- ⇒ IL-37 has positively correlated with *ABCA1* and *ABCG1* gene expression in PBMCs and serum levels of the FRAP.

How might these results change the focus of research or clinical practice?

- ⇒ Decreased serum levels of IL-37 in patients with CAD and its relationship with inflammatory cytokines and reverse cholesterol transport genes are more likely to be associated in the inflammatory process with disease pathology.



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disorder, effectively promote coronary artery disease (CAD) caused by atherosclerosis.¹ Atherosclerosis is a chronic inflammatory condition whose main feature includes the deposition of oxidized lipids in the inner layer of the vessel wall. Immune cells that produce cytokines are involved in the dysfunction of the arterial wall and its inflammatory mechanism.^{2,3} Interleukin (IL)-37 is a novel member of the IL-1 family that can suppress innate and adaptive immunity through many ways and confers protection against several models of inflammatory disease.⁴⁻⁶ IL-37 in immune cells is mainly produced in circulating monocytes, tissue macrophages, dendritic cells (DCs), T cells, B cells, and plasma cells.⁷ IL-37 is expressed and released in the cytosol in its pro-inactive form that requires cleavage to be transformed in its active form, and maturation and secretion are mediated by inflammatory caspases on inflammasome signaling complexes.⁶ Furthermore, IL-37 suppresses nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways by migrating to the nucleus and regulating the expression of proinflammatory genes and cytokines such as IL-1 β and tumor necrosis factor- α (TNF- α).⁸ IL-37 can attenuate inflammation through regulation of the CD4⁺ T lymphocyte activity, including an increase in regulatory T cells, decrease the T helper type 1 (Th1) and Th17 cells, and prevent the maturity of DCs.⁹

Recent studies have reported the significant role of IL-37 in human inflammatory diseases such as chronic periodontitis, lupus erythematosus, allergy, rheumatoid arthritis, Behçet's disease, and acute coronary syndrome.¹⁰⁻¹⁵ Based on the above, the aim of this study was to compare the serum levels of IL-37 in patients with CAD compared with the control group, its correlation with serum levels of inflammatory cytokines (IL-6, TNF- α , and IL-32), high-sensitivity C reactive protein (hs-CRP), oxidative stress markers (oxidized LDL (ox-LDL), ferric-reducing antioxidant power assay (FRAP), and malondialdehyde (MDA)) as well as *ATP-binding cassette transporter A1 (ABCA1)* and *G1 (ABCG1)* gene expression in peripheral blood mononuclear cell (PBMCs) of patients with CAD.

MATERIALS AND METHODS

Study population

This study included 84 consecutive patients from the Hajar Hospital in Shahrekord city, Chaharmahal and Bakhtiari Province, Iran. Samples were taken from patients admitted to the cardiac ward between September and November 2020. All subjects had varying degrees of clogged arteries, and there were no healthy controls in this study. The selection of patients was carried out according to the medical report of a cardiologist using angiography. All patients with CAD were new cases and had 50% or greater arterial stenosis in one or more of the main coronary arteries were included as CAD group. The control group also included participants who had an arterial stenosis of 30% or less. The vessel score in CAD group, which ranges from 1 to 3, is the number of vessels with substantial stenosis (a reduction in lumen diameter of 50% or more) which is named here as coronary stenosis (CS) 1, CS2, and CS3, respectively.¹⁶

Patients with any history of liver and infectious diseases, such as cancer and diabetes, were excluded from the study. Anthropometric data, such as height, weight, body mass

index (BMI), diastolic blood pressure (DBP), and systolic blood pressure (SBP), were also documented. BMI was measured as weight/height² (kg/m²). Blood pressure was evaluated according to the standard protocol (patients examined after 15 min of sitting).

Sample collection and biochemical parameter evaluation

An amount of 5 mL of venous blood was taken from each patient, and serum was immediately isolated and then stored at -80°C . Biochemical markers, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), blood glucose (BG), troponin I (Trop I), and creatine kinase myocardial band (CK-MB), were calculated by Pars Azmoun Company's kit based on spectrophotometric and enzymatic standard methods.

Serum IL-37 evaluation

The concentration of IL-37 in serum was measured by a human IL-37 ELISA kit (Invitrogen, USA). According to the kit's instruction, the kit's sensitivity was 31.3 pg/mL and its detection range was between 31.3 and 2000 pg/mL. Absorption was read via an ELISA reader (Dynex DS2, USA) at 450 nm.

Statistical analysis

Statistical analysis was performed by GraphPad Prism software V.8.4.3 (GraphPad Software, La Jolla, California, USA) and SPSS statistics V.16 (SPSS, Chicago, Illinois, USA). Receiver operating characteristic (ROC) and area under curve (AUC) were considered to assess the potential predictive value of each cytokine for CAD. In addition, measuring the independence of IL-37, hs-CRP, ox-LDL, MDA, FRAP, Trop I, and CK-MB variables in the occurrence of CAD were carried out by multivariate logistic regression test. The quantitative data were evaluated by the independent-samples t-test and one-way analysis of variance (ANOVA) test depends on the data distribution's normality. Pearson's correlation analysis determined the correlation between variables. P values <0.05 (typically ≤ 0.05) were considered to be statistically significant.

RESULTS

Demographic and biochemical parameters

Table 1 indicates the patient's anthropometric data. Patients participated in this study showed no significant difference in terms of age, sex, BMI, TG, HDL, SBP, and DBP between the CAD and control groups. Trop I, LDL, CK-MB, TC, and BG levels in the CAD group were significantly higher than those in the control group subjects. Meanwhile, Trop I and CK-MB concentrations were substantially different from other markers between CAD and control groups.

The serum levels of IL-37 in the control and CAD groups and its relationship with the grade of arterial stenosis

As shown in figure 1A, the serum levels of IL-37 in the CAD group were significantly lower than in the control group (fold change (FC) = -1.46 ; $p < 0.0001$). Ordinary one-way ANOVA test showed that serum levels of IL-37 significantly differed between CS2 and CS1 (FC = -1.08 ; $p = 0.008$),

Table 1 Demographic and biochemical data of study subjects

Variables	Control (n=42)	CAD (n=42)	P value
Age (year)	59.76±12.38	64.69±12.46	0.065
BMI (kg/m ²)	25.58±3.35	25.56±3.48	0.981
Gender (male n (%))	21 (50.00)	21 (50.00)	1.00
SBP (mm Hg)	122.62±14.09	126.74±15.49	0.231
DBP (mm Hg)	75.35±19.20	77.02±16.49	0.579
BG (mg/dL)	107.71±28.95	125.6±27.06	0.004
TC (mg/dL)	139.88±41.54	168.17±39.73	0.02
TG (mg/dL)	145.48±78.33	153.43±107.31	0.613
HDL (mg/dL)	57.19±10.91	55.95±16.28	0.778
LDL (mg/dL)	56.02±38.59	81.53±35.46	0.003
CPK-MB (μg/L)	9.74±4.33	17.64±9.81	<0.0001
Trop I (ng/L)	93.38±73.07	11 753±17 555.72	<0.0001
BUN (mg/dL)	18.31±0.77	20.17±0.93	0.131
Creatinine (mg/dL)	0.898±0.02	1.029±0.03	0.08
Sodium (mEq/L)	139.58±0.58	140.95±0.53	0.212
Potassium (mEq/L)	4.01±0.06	4.14±0.07	0.178

BG, blood glucose; BMI, body mass index; BUN, blood urea nitrogen; CPK-MB, creatine phosphokinase-myocardial band; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; Trop I, troponin I.

CS3 and CS2 (FC=−1.11; p=0.003), and CS3 and CS1 groups (FC=−1.21; p<0.0001) (figure 1B).

Correlation of serum levels of IL-37 with biochemical parameters

Correlation analysis displayed that IL-37 had a significant negative correlation with total cholesterol (r=−0.547; p<0.0001), TG (r=−0.409; p=0.011), and LDL-C

(r=−0.519; p=0.001). IL-37 correlation with other biochemical parameters was not significant (table 2).

Potential ability of IL-37, hs-CRP, and ox-LDL in the prediction of CAD

ROC curves indicate that IL-37 could relatively predict CAD (AUC (95% CI)=0.721 (0.608 to 0.834), p=0.0009). In addition, hs-CRP and ox-LDL serum levels, which were measured in our previous study,¹⁷ could be relatively discriminated between control and CAD groups (figure 2B,C). The results were as follows: hs-CRP (AUC (95% CI)=0.811 (0.715 to 0.907); (p<0.0001) and ox-LDL (AUC (95% CI)=0.748 (0.640 to 0.857); (p=0.0002).

Serum levels of IL-37 in the men and women groups

The serum levels of IL-37 were significantly increased in the men of the control group than in the men of the CAD group (figure 3A: FC=1.65, p=0.0015). Also, the serum levels of IL-37 were significantly increased in the women of the control group than in the women of CAD group (figure 3B: FC=1.16, p=0.021). However, our findings indicated that the serum levels of IL-37 were significantly increased in men with CAD than in women with CAD (figure 3C: FC=1.09, p=0.028). Our results in all subjects showed that serum levels of IL-37 were significantly higher in men than women (figure 3D: FC=1.33, p=0.005).

Correlation of serum levels of IL-37 with hs-CRP and oxidative stress markers (FRAP, MDA, and ox-LDL) in the patients with CAD

Our previous study revealed that serum levels of ox-LDL, hs-CRP, and MDA were significantly higher, and serum levels of FRAP were significantly lower in the CAD group than in the control group.¹⁷ The Spearman's correlation

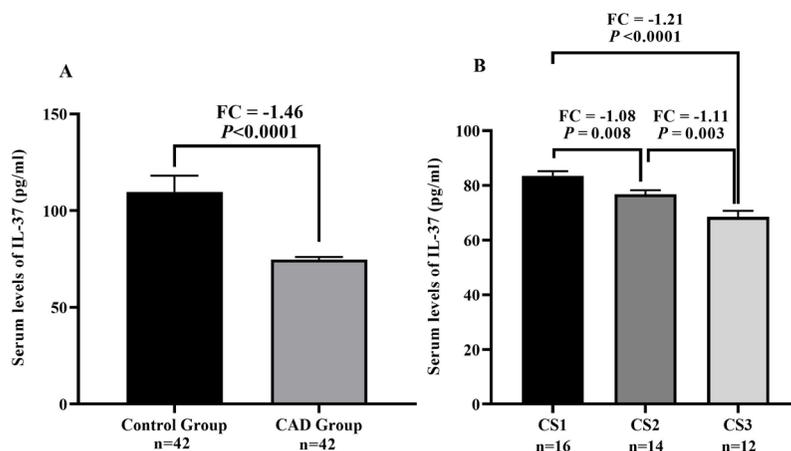


Figure 1 The comparison of serum levels of IL-37 between CAD and control groups and its level in groups with different grades of arterial stenosis. (A) Serum levels of IL-37 in the CAD group (76.7 pg/mL) was significantly lower than the control group (107.7 pg/mL) by −1.4-fold (p=0.0006). (B) Serum levels of IL-37 significantly differed between control and CS1 (FC=−1.37; p=0.022), control and CS2 (FC=−1.40; p=0.012), control and CS3 groups (FC=−1.42; p=0.010). However, no significant difference was detected between CS1, CS2, and CS3 groups. Parametric unpaired t-test was used to compare the IL-37 concentration between control and CAD groups. An ordinary one-way analysis of variance test was used to compare the IL-37 concentration between all groups. P value ≤0.05 was considered as a significant value. Results are expressed as the mean±SEM. CAD, coronary artery disease; CS, coronary stenosis; IL, interleukin.

Table 2 Pearson's correlation between IL-37 and biochemical parameters in patients with CAD

Variable	IL-37
Trop I	
Correlation coefficient	-0.047
Sig. (two-tailed)	0.778
N	84
TC	
Correlation coefficient	-0.547
Sig. (two-tailed)	<0.0001
N	84
TG	
Correlation coefficient	-0.409
Sig. (two-tailed)	0.011
N	84
HDL-C	
Correlation coefficient	0.207
Sig. (two-tailed)	0.213
N	84
LDL-C	
Correlation coefficient	-0.519
Sig. (two-tailed)	0.001
N	84
CK-MB	
Correlation coefficient	-0.173
Sig. (two-tailed)	0.3
N	84
BG	
Correlation coefficient	-0.095
Sig. (two-tailed)	0.57
N	84

BG, blood glucose; CAD, coronary artery disease; CK-MB, creatine kinase-myocardial band; HDL-C, high-density lipoprotein-cholesterol; IL, interleukin; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; Trop I, troponin I.

analysis between these parameters and IL-37 indicated that there is a significant relationship between them. As shown in figure 4A–D, our results demonstrated that IL-37 was negatively correlated with hs-CRP ($r=-0.407$; $p=0.013$), ox-LDL ($r=-0.552$; $p=0.0003$), and MDA ($r=-0.442$; $p=0.005$). On the other hand, IL-37 was positively correlated with FRAP ($r=0.392$; $p=0.014$).

Correlation of serum levels of IL-37 with inflammatory cytokines (IL-6, TNF- α , and IL-32) in patients with CAD

In another research, we find out that serum levels of TNF- α , IL-6, and IL-32 significantly elevated in CAD group subject compared with control group.¹⁸ As shown in figure 5A–C, our results demonstrated that IL-37 had a significant negative correlation with IL-6 ($r=-0.429$; $p=0.011$), TNF- α ($r=-0.386$; $p=0.021$), and IL-32 ($r=-0.408$; $p=0.016$).

Correlation of serum levels of IL-37 with ABCA1 and ABCG1 gene expression in PBMCs of the patients with CAD

The expression levels of the ABCA1 and ABCG1 genes involved in reverse cholesterol transport significantly decreased in patients with CAD.¹⁷ Our present findings indicated that serum levels of IL-37 were positively correlated with expression levels of ABCA1 ($r=0.484$; $p=0.002$) and ABCG1 ($r=0.339$; $p=0.04$) genes in PBMCs of the patients with CAD (figure 6A,B).

DISCUSSION

In this study, our results showed that IL-37 concentration in the CAD group were significantly lower than in the control group. Furthermore, serum levels of IL-37 were significantly decreased in the cardiac arterial stenosis in three main vessel (CS3) groups compared with the cardiac arterial stenosis in one main vessel (CS1) and the cardiac arterial stenosis in two main vessel (CS2) groups. Also, serum levels of IL-37 were significantly decreased in the CS2 group compared with

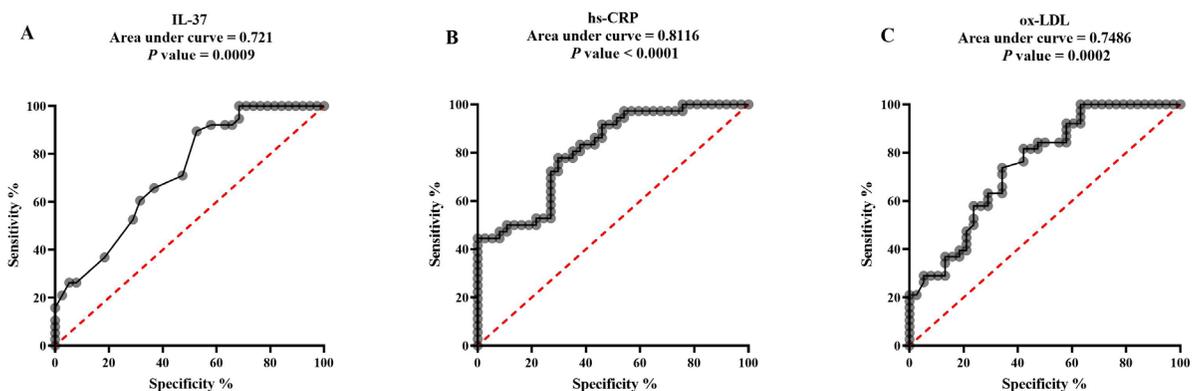


Figure 2 Receiver operating characteristic (ROC) curves for prediction of CAD according to serum levels of IL-37, hs-CRP, and ox-LDL. (A) Area under curve (95% CI) for IL-37=0.721 (0.608 to 0.834), ($p=0.0009$). (B) Area under curve (95% CI) for hs-CRP=0.8116 (0.715 to 0.907), ($p<0.0001$). (C) Area under curve (95% CI) for ox-LDL=0.7486 (0.640 to 0.857), ($p=0.0002$). CAD, coronary artery disease; hs-CRP, high-sensitivity C reactive protein; IL, interleukin; ox-LDL, oxidized low-density lipoprotein.

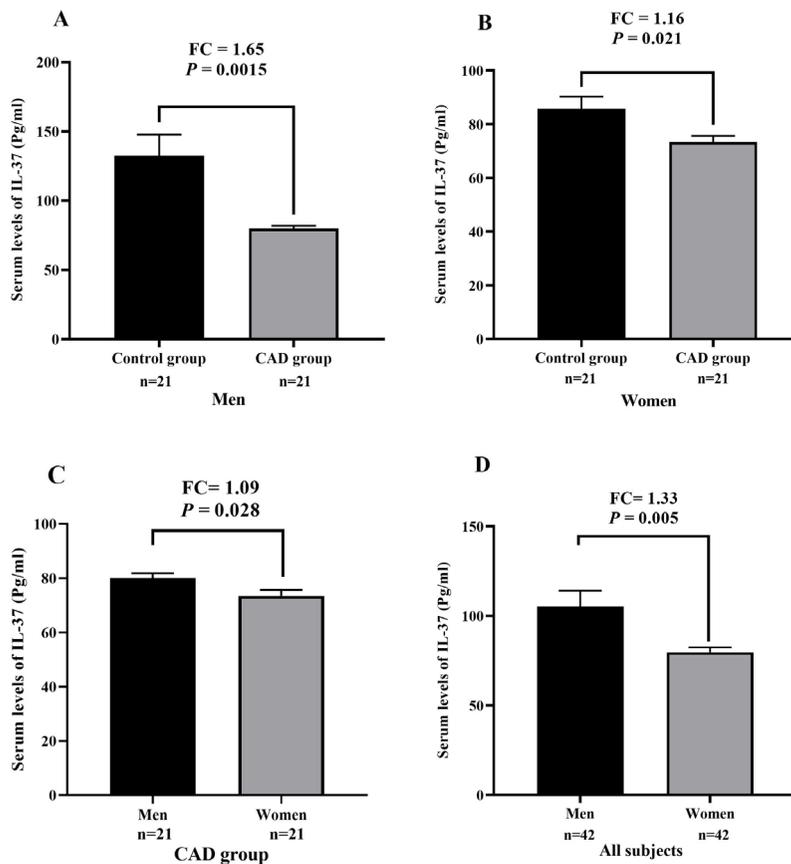


Figure 3 The comparison of serum levels of IL-37 between CAD and control groups individually in men and women. (A) The serum levels of IL-37 were significantly increased in the men of the control group than in the men of the CAD group (FC=1.65). (B) The serum levels of IL-37 were significantly increased in the women of the control group than in the women of CAD group (FC=1.16). (C) The serum levels of IL-37 were significantly increased in men with CAD than in women with CAD group (FC=1.09). (D) Serum levels of IL-37 were significantly higher in men than women (FC=1.33). P value ≤ 0.05 was considered as a significant value. Results are expressed as the mean \pm SEM. CAD, coronary artery disease; IL, interleukin.

the CS1 group. In addition, our findings indicated that the serum levels of IL-37 were significantly increased in men with CAD than in women with CAD.

Recent studies have reported the significant role of IL-37 in human inflammatory diseases.^{10–13, 19} Yang *et al* demonstrated that IL-37 increases in patients with rheumatoid arthritis and is positively associated with disease progression.²⁰ Yin *et al* have found that single nucleotide polymorphism (rs3811047) in the *IL-37* gene increases the risk of CAD by reducing its mRNA expression.²¹ Another study indicated that IL-37 increases in calcified arterial tissue of patients with diabetes mellitus.²²

Previous studies have presented different results regarding the relationship of IL-37 with inflammatory cytokines. For example, Nold *et al* showed IL-37 recruit IL-1R8 to exert its anti-inflammatory effect through PTEN, Mer, and STAT3 signaling pathways to inhibit NF- κ B activity.⁴ Ye *et al* reported that IL-37 protein and mRNA levels markedly reduced in the PBMC of patients with Behçet's disease than healthy subjects. Also, recombinant IL-37 in DCs decreased the production of an inflammatory cytokine such as IL-1 β , IL-6,

and TNF- α and oxidative stress marker (reactive oxygen species (ROS)) through inactivation of JNK, ERK1/2, P38 MAPK pathway and inhibition of Th17 and Th1 cell responses.²³ In contrast, Chen *et al* indicated that patients with active ankylosing spondylitis had a higher IL-37 in serum and PBMCs compared with healthy subjects, which positively correlated with related proinflammatory cytokines.²⁴ Furthermore, the result of our recent study showed an increase in the serum concentrations of TNF- α , IL-6, and IL-32 in the CAD group compared with the control group and there was a significant association between the serum TNF- α and IL-32 concentration in the CAD group with the grades of arterial stenosis.¹⁸ The results of this studies showed that serum levels of IL-37 were negatively correlated with the serum levels of IL-6, TNF- α , and IL-32 in patients with CAD.

IL-37 can regulate cholesterol homeostasis within macrophages present in the subendothelium through the AMPK pathway. Ballak *et al* confirmed that the AMPK signaling pathway activated in adipocytes treated with recombinant IL-37.²⁵ In addition, Ma *et*

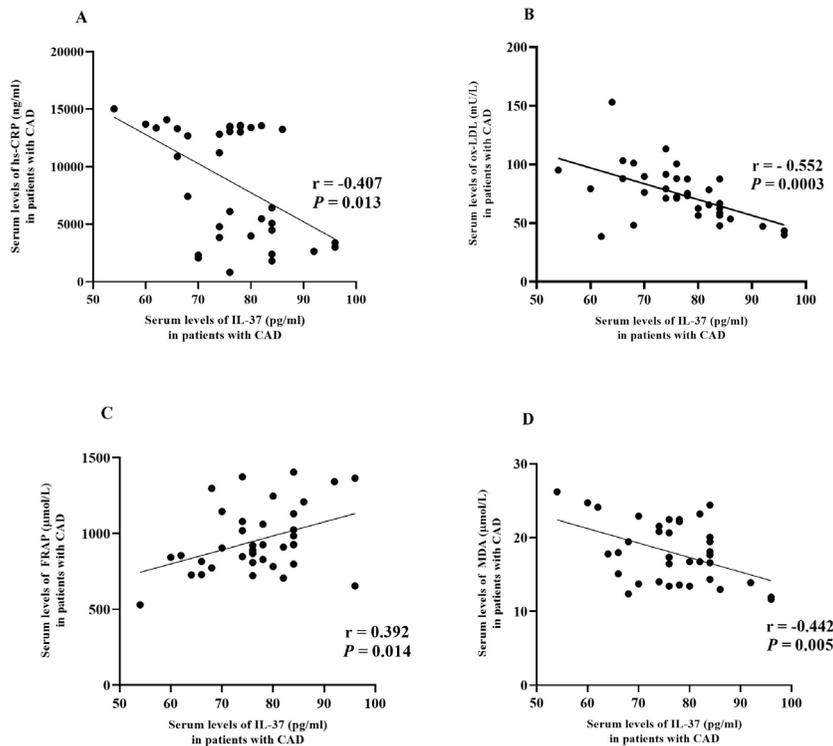


Figure 4 Correlation between serum levels of IL-37 with hs-CRP, ox-LDL, FRAP, and MDA in the patients with CAD. (A) Serum levels of IL-37 was negatively correlated with hs-CRP ($r=-0.407$; $p=0.013$). (B) Serum levels of IL-37 was negatively correlated with ox-LDL ($r=-0.552$; $p=0.0003$). (C) Serum levels of IL-37 was positively correlated with FRAP ($r=0.392$; $p=0.014$). (D) The negative correlation between serum levels of IL-37 and MDA was not significant ($r=-0.442$; $p=0.005$). P values ≤ 0.05 were considered as significant using Pearson's rank correlation. CAD, coronary artery disease; FRAP, ferric-reducing antioxidant power assay; hs-CRP, high-sensitivity C reactive protein; IL, interleukin; MDA, malondialdehyde; ox-LDL, oxidized low-density lipoprotein.

al demonstrated that AMPK activation improved the reverse cholesterol transport pathway by increased expression of *ABCA1* and *ABCG1* genes in macrophages.²⁶ Furthermore, regarding the effects of IL-37 on cholesterol efflux, overexpression of IL-37 upregulates the peroxisome proliferator-activated receptor- γ and *ABCA1* gene expression in macrophages, which results in a considerable decrease in atherosclerosis.²⁷ Previous studies have also reported that *ABCA1* and *ABCG1* gene expression and serum concentration of FRAP were significantly lower in the CAD group compared with the control group. Also, the serum levels of hs-CRP, ox-LDL, and MDA were significantly higher in the CAD group compared with the control group.¹⁷ In line with previous studies, our results suggested that the positive relationship of serum IL-37 with the *ABCA1* and *ABCG1* genes expression is probably due to its essential role in regulating cholesterol homeostasis. Our results showed that decreasing IL-37 concentration in patients with CAD is correlated with increasing the serum levels of total cholesterol, TG, LDL-C, hs-CRP, ox-LDL, and MDA. Also increasing the serum levels of IL-37 in patients is correlated with increasing the serum levels of reducing antioxidant capacity (FRAP value). In this regard, Ballak *et al* illustrated that IL-37 transgenic mice had diminished plasma

cholesterol levels compared with wild-type mice fed a high-fat diet.²⁵ Zhang *et al* showed that IL-37 transfection inhibits inflammation by increasing the expression of antioxidant enzyme (superoxide dismutase) and decreasing MDA in glucose-treated podocyte cells.²⁸ In addition, recombinant IL-37 in mice hepatocyte inhibits the neutrophil activation and protection against liver damage by reducing hepatic ROS and serum TNF- α levels.²⁹ Therefore, it is inferred that IL-37 probably prevents inflammation by increasing antioxidant levels and decreasing cholesterol oxidation.

CONCLUSIONS

Our results suggest that IL-37 regulates inflammatory cytokines and reverse cholesterol transport pathways in line with more previous studies. It can be a valuable target for the treatment and prevention of atherosclerosis. Therefore, decreased serum levels of IL-37 and its negative relationship with inflammatory cytokines and oxidative stress in patients with CAD probably is one of the reasons for the progression of atherosclerosis. However, further studies such as genetic polymorphism studies are required to evaluate the exact role of IL-37 in CAD pathogenesis.

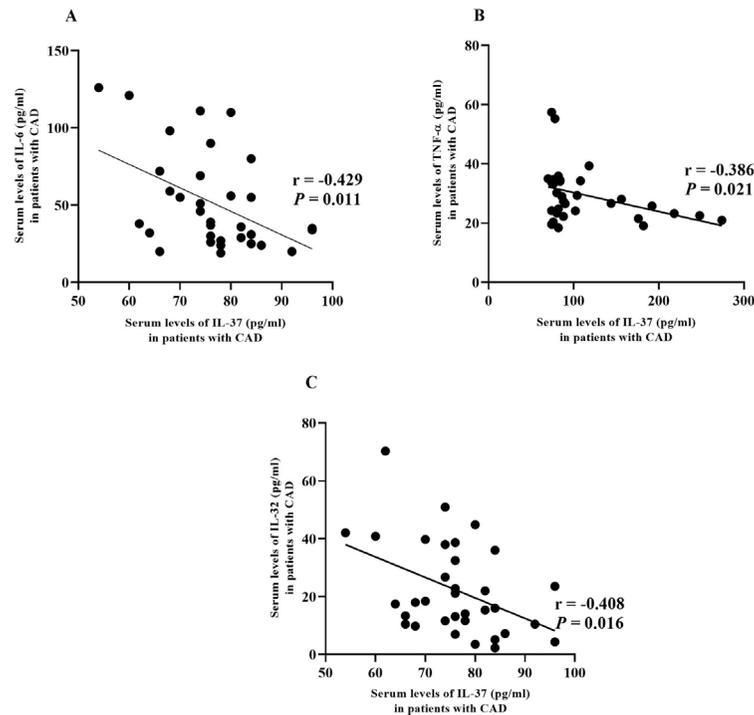


Figure 5 Correlation between serum levels of IL-37 with IL-6, TNF- α , and IL-32 in the patients with CAD. (A) Serum levels of IL-37 was negatively correlated with IL-6 ($r=-0.429$; $p=0.011$). (B) Serum levels of IL-37 was negatively correlated with TNF- α ($r=-0.386$; $p=0.021$). (C) Serum levels of IL-37 was not correlated with IL-32 ($r=-0.408$; $p=0.016$). P values ≤ 0.05 were considered as significant using Pearson's rank correlation. CAD, coronary artery disease; IL, interleukin; TNF- α , tumor necrosis factor- α .

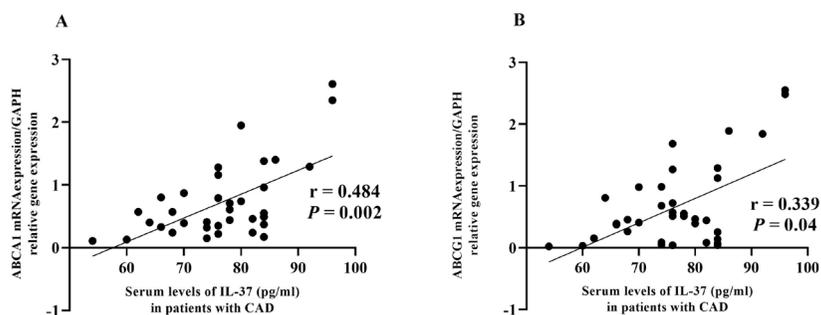


Figure 6 Correlation of serum levels of IL-37 with *ABCA1* and *ABCG1* genes expression in PBMCs of the patients with CAD. Serum levels of IL-37 had a significant positive correlation with expression levels of *ABCA1* ($r=0.484$; $p=0.002$) and *ABCG1* ($r=0.339$; $p=0.04$) genes in PBMCs of patients with CAD. P values ≤ 0.05 were considered as significant using Pearson's rank correlation. *ABCA1*, ATP-binding cassette transporter A1; *ABCG1*, ATP-binding cassette transporter G1; CAD, coronary artery disease; IL, interleukin; PBMCs, peripheral blood mononuclear cells.

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Data availability statement No data are available.

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