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Evaluating SORT1 and SESN1 genes expression in peripheral blood mononuclear cells and oxidative stress status in patients with coronary artery disease

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Abstract

Background Coronary artery disease (CAD) significantly contributes to global fatalities. Recent studies have demonstrated the crucial roles of sortilin1 (SORT1) and sestrin1 (SESN1) in lipid metabolism, as well as their involvement in the development of CAD. The aberrant expression or activity of SORT1 can consequently lead to metabolic and vascular diseases. Sestrins, including SESN1, play a crucial role in helping cells survive by maintaining metabolic balance while also reducing oxidative stress (OS). OS contributes to the progression of atherosclerosis-related diseases, such as CAD. The study aimed to compare the gene expression of SORT1 and SESN1 in peripheral blood mononuclear cells (PBMCs), alongside serum OS markers, in CAD patients and controls.

Materials The case-control study included 49 CAD patients and 40 controls. The expression of the SORT1 and SESN1 genes was quantified using qRT-PCR, and the expression of the SORT1 protein was evaluated by western blotting. OS markers, including total oxidation status (TOS), total antioxidant capacity (TAC), and malondialdehyde (MDA), were measured using spectrophotometric and fluorometric methods.

Results SORT1 gene and protein expressions were similar between groups. CAD patients had a non-significant decrease in SESN1 gene expression. MDA levels were significantly higher in CAD patients, whereas TOS and TAC levels did not differ significantly.

Conclusion For atherosclerosis-related disorders like CAD, MDA shows potential as a non-invasive, easy-to-use, affordable, and stable biomarker. Further research is needed to elucidate the precise roles of SORT1 and SESN1 in CAD pathogenesis.

Keywords Coronary artery disease, Arteriosclerosis, Sortilin1, Sesterin1, Peripheral blood mononuclear cells, Oxidative stress

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Introduction

Cardiovascular disease (CVD) has emerged as a highly prevalent ailment in recent decades, and is responsible for approximately 30% of all deaths worldwide [1]. Coronary artery disease (CAD), also known as atherosclerosis, is a prevalent CVD characterized by the development of plaques, known as atheroma, inside the inner layer of the coronary arteries [2, 3]. Plaque accumulation can result in stenosis, occlusion, and ruptures over time, which can lead to various symptoms, including stable angina, unstable angina, myocardial infarction (MI), or sudden cardiac death [3, 4]. New insights into the cellular and molecular causes of atherosclerosis have emerged in recent years, but the precise process behind the formation of atherosclerosis remains uncertain [3, 5]. Sortilin (SORT1) is a type I transmembrane multiligand receptor from the Vacuolar protein sorting10 (Vps10p) protein family [6]. SORT1 is present in a variety of cells, particularly in neurons, hepatocytes, adipocytes, leukocytes and macrophages. It is involved in regulating the cellular levels and activities of various substrates through intracellular sorting and trafficking mechanisms [6, 7]. However, the precise regulatory mechanisms governing SORT1 expression and trafficking activity remain largely unknown [6]. Approximately 90% of SORT1 protein is found in the late Golgi apparatus, where it binds with cargo proteins to regulate their localization and secretion. The remaining 10% is located on the cell membrane and functions as a surface receptor for external ligands, facilitating lysosomal recycling or destruction of internalized ligands [7]. Soluble SORT1 is also produced by SORT1 cleavage and its subsequent extracellular release [6]. Due to its critical biological roles, SORT1 has lately attracted a great deal of interest. Aberrant SORT1 expression and function are linked to diseases, including atherosclerosis [7–9]. Animal studies demonstrate that SORT1 plays an important role in the pathophysiology of vascular and metabolic diseases, most notably atherosclerosis, by regulating lipoprotein metabolism, arterial wall inflammation, and vascular calcification [10]. SORT1 is involved in the modulation of lipoprotein metabolism, particularly low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in hepatocytes. For instance, hepatic SORT1 enhances VLDL secretion from the liver, contributing to the development of atherosclerosis [7], and regulates hepatic cell trafficking of LDL-C and helps LDL enter macrophages, which helps foamy cells form [11]. Curiously, it also enhances the elimination of plasma LDL, ultimately lowering the advancement of atherosclerotic diseases [7]. However, the specific involvement of SORT1 in LDL metabolism and atherosclerosis is uncertain [11].

Sestrin (SESN1) belongs to the Sestrin family. Sestrins maintain metabolic balance in different kinds of cells and are involved in a wide variety of physiological and pathological functions [12]. Sestrins, as stress-inducible proteins and protective, are activated in reaction to different types of cellular stress, including hypoxia, metabolic stress, and DNA damage, and aid in cell survival [12, 13]. Sestrins function as cysteine sulfinyl reductases, playing a crucial role in regulating peroxide signaling and enhancing oxidant defense through their activity as cysteine sulfinyl reductases. Their principal role involves modulating AMPK-mTORC1 signaling, which helps to minimize oxidative damage and aging [13]. Sestrins, consisting of three types—SESN1, SESN2, and SESN3—play a crucial role in protecting against cardiovascular disorders, including atherosclerosis [14]. The expression pattern and functions of SESN1, in comparison to other SESNs, remain not fully elucidated. SESN1 has emerged as a novel candidate associated with cholesterol in both mice and humans. Under in vitro conditions, the knockout of the SESN1 gene leads to an accumulation of cellular cholesterol [15].

Free radicals are natural byproducts of cellular function, produced by both endogenous and exogenous sources. Antioxidants neutralize free radicals [16, 17]. An imbalance between the formation and destruction of free radicals can cause excessive accumulation and oxidative stress (OS). This can lead to cellular damage due to their harmful reactions to proteins, lipids, and DNA. Such damage can lead to various OS-related diseases, resulting in abnormalities in gene expression. Recent studies have highlighted the significance of OS in the development and progression of CAD [16]. Malondialdehyde (MDA) is an end-product of lipid peroxidation caused by an enzymatic reaction in oxidized LDL [18]. It is highly reactive and can produce toxic effects. MDA plays a role in atherosclerosis pathogenesis, facilitates LDL oxidation, and is involved in inflammation and vascular dysfunction. The quantity of MDA in the bloodstream is a reliable biomarker for lipid peroxidation and OS [19]. Smoking as a risk factor for CAD is closely associated with increased OS, and the number of cigarettes smoked plays an important role in increasing the level of oxidative damage and reducing antioxidant defence [20].

Liver aminotransferases are ubiquitous enzymes that play a key role in the metabolism of amino acids. An elevation in liver enzyme levels has been indicated to correlate with a heightened risk of CVD. The relationship between higher aminotransferases and heightened risk of cardiovascular disease (CVD) remains inadequately elucidated. Non-alcoholic fatty liver disease (NAFLD) is proposed as a plausible reason for this correlation, as it is associated with hepatic insulin resistance and metabolic syndrome. Studies indicate that increased aminotransferase levels, even within the normal spectrum, correlate with inflammation, endothelial dysfunction, and impaired haemostasis, which are associated with an

enhanced risk of CVD. Monitoring the concentration of liver enzymes could serve as a valuable indicator for predicting CVD in its early stages [21].

In order to prevent, predict, and cure atherosclerosis, innovative mechanisms and targets must be identified. Overall, this can lower death rates. This study examined the expression levels of SORT1 and SESN1 in the peripheral blood mononuclear cells (PBMCs) of CAD patients compared to controls due to recent findings on their role in lipid metabolism and CVD, particularly atherosclerosis and conflicting results. Given the importance of the OS statement, a complex risk factor in CAD pathogenesis, OS indices in CAD patients and controls are measured, and their association with SORT1 and SESN1 expression is examined.

Materials and methods

Population and angiographic features

From February to October of 2022, 89 Iranian patients ranging in age from 40 to 70 participated in this case-control study. An interventional cardiologist from Hamadan University of Medical Sciences' Farshchian Hospital conducted coronary angiography on the patients. The pattern of CAD was identified, and the presence of obstructive stenosis in the main coronary arteries was assessed using coronary angiography according to conventional criteria.

Subjects in the control group did not exhibit considerable stenosis, and the degree of stenosis in the vessels was below 25%. In contrast, patients with one of the main arteries of the heart exhibited a lesion with more than 50% diameter stenosis, known as SVD (single-vessel disease). Those with involvement of two main arteries exhibited a condition known as DVD (double-vessel disease), and patients with lesions in three main arteries were diagnosed with TVD (triple-vessel disease). The presence of SVDs, DVDs, and TVDs with lesions in the left main (LM) artery were categorized as LM+SVD, LM+DVD, and LM+TVD, respectively.

Furthermore, the magnitude and extent of coronary artery involvement were assessed by an interventional cardiologist using the Gensini score (GS). This score considers the percentage of vessel diameter narrowing as well as the location of lesions in the coronary circulation [22, 23]. The study included 49 individuals with CAD (GS>20.5) and 40 controls (GS≤20.5). The research did not include anyone who was already receiving steroids, immunosuppressants, or anti-inflammatory drugs or had a history of diabetes, cancer, kidney failure, liver disease, or any other chronic illness. Under the guidelines laid out by the Helsinki Declaration, the study was authorized by the Research Ethics Committee of Hamadan University of Medical Sciences (Approval code: IR.UMSHA.REC.1400.616), and all participants were required to sign an informed consent document.

Sample size and sampling techniques

According to a study conducted by Oh TJ et al. [24], the sample size was determined using the formula for serum SORT1 levels. The number of participants required for each group was estimated to be 40, with a statistical power of 80% and a confidence level of 95%.

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

$$\alpha = 5\%\ Z_{1-\alpha/2} = 1.96 \qquad \beta = 20\%\ Z_{1-\beta} = 0.84$$

$$\mu 1 = 1.4 \quad \mu 2 = 1.8 \quad \sigma 1 = 0.8 \quad \sigma 2 = 0.4 \quad \mathrm{n} = 40$$

Meanwhile, all eligible patients were included in two groups using a census method until the required sample size was achieved, thereby ensuring non-biased sampling.

Clinical and demographic information

Patients' age, gender, weight, height, body mass index (BMI), smoking habits, opium and alcohol consumption, drug usage history, family history of premature CAD (in males under 55 years or females under 65 years in first-degree relatives), medical history of ischemic heart disease, MI, stroke, hypertension, hypercholesterolemia, diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), and left ventricular ejection fraction (LVEF) as assessed by echocardiography were all recorded using a checklist.

Determination of hematological parameter

The hematological parameters were examined using a Sysmex KX-21 N automated hematology analyzer from Germany. These parameters include the following: total white blood cell (WBC) count, total red blood cell (RBC) count, hemoglobin content (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT). This analyzer is well-known for its high-quality technology, which allows it to produce extremely accurate and precise findings.

Measurement of biochemical parameter

Each subject's blood was obtained after an eight-hour fast the night before. In order to extract serum, the samples were centrifuged at 4 °C and 1000 g for 10 min. The serum was then stored at -80 °C until the empirical investigations began. The concentrations of total cholesterol (TC), triglycerides (TG), High-density lipoprotein cholesterol (HDL-C), LDL-C, fasting blood glucose (FBS), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline Phosphatase (ALP) calcium magnesium, Phosphorous and high-sensitivity C-reactive protein

(hs-CRP) were determined using a biochemical auto-analyzer (Dirui CF400, China) and reagent kits (Biorexfars, Iran) using the manufacturer's instructions.

Assessment of serum stress oxidative markers

Serum total oxidant status (TOS) levels were assessed using the FOX technique, which involves detecting the ion oxidation of ferrous iron to ferric iron ions in an acidic environment with oxidant agents [25]. The serum total antioxidant capacity using the commercial TAC kit (Kiazist, Iran), which operates by the CUPRAC (Cupric Reducing Antioxidant Capacity) method and converting cupric ions (Cu2+) to cupro ions (Cu1+) in the presence of an anti-oxidant. Finally, the Yagi method was employed to quantify MDA, with the fluorescence intensity of the resulting reaction product quantified using fluorometry (Jasco FP-6200, Tokyo, Japan) at an excitation wavelength of 515 nm and an emission wavelength of 553 nm [26].

Quantification of SORT1 and SESN1 mRNA in PBMCs

First, using Ficoll-paque (Pharmacia Biotech, Sweden), 10 ml of whole blood (with EDTA) was used to extract PBMCs [27]. According to the instructions of GeneAll Biotechnology of Korea, RiboEx reagent was used to extract total RNA. A Revert Primer cDNA Synthesis Kit (SMOBIO, Taiwan) was used to convert 1000 ng RNA samples into cDNA, which was then used to examine the gene expression of SORT1 and SESN1. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using Real Q Plus 2x Master Mix Green-No Rox on a LightCycler 96 (Roche, Germany) from Amplicon, Denmark. The following primers were used for the amplification of specific genes: SORT1 5'- GAGACTATGTTG TGACCAAGC-3' (forward) and 5'-ACCCATTTGTTG TTAGGTGTTC-3' (reverse) with NCBI accession number NM_002959.7 and NM_002959.7 and SESN1 5'-AG ATTCTTTTGCTGCTTTGGG-3' (forward) and 5'-AA GTAGGAGCACTGATGTCTTG-3 (reverse) with NCBI accession number NM-001199934.2, NM_001199933.2, and NM_014454.3. Additionally, β-actin was used as a housekeeping gene with the primers 5'-GAGCCTCGC CTTTGCCGATCC-3' (forward) and 5'-ACATGCCGG AGCCGTTGTCG-3' (reverse) with NCBI accession ID NM_001101.5. Next, the 2-ΔΔCt (Livak) Method [28], which uses the expression level of β-actin as a reference, was used to calculate the relative gene expression levels (fold change).

Determination of protein levels by western blotting analysis

The expression of the SORT1 protein was evaluated by western blotting. The total protein was extracted using RIPA lysis buffer with a protease inhibitor from PBMCs, quantified using a bicinchoninic acid kit, and 40 µg of protein was loaded per lane onto a 10% SDS-PAGE gel and electroblotted onto 0.45 µm nitrocellulose membrane. Membranes were incubated with primary antibodies, anti-SORT1 (1:1,000 dilution; cat. no. ab263864), and anti-β-actin (1:2,000 dilution; cat. no. ab8227), and subsequently incubated with a secondary antibody (1:1,500 dilution; cat. no. 7074 S; Cell Signaling Technology, Inc.). Finally, the antibody-bound proteins were detected on the X-ray film using enhanced chemiluminescence (Thermo Fisher Scientific, Inc.) and then analyzed using gel analyzer software.

Data analysis

Statistical analyses were conducted using Stata version 14. The mean and standard deviation were used to present quantitative results. Qualitative variables between the two groups were compared using a chi-square test and Fisher's exact test. Additionally, quantitative variables were compared using a t-test. A significance level of 0.05 or lower was considered significant. Gene expression changes between the two groups were analysed and compared using GraphPad Prism software (version 10.2.1) to calculate fold change, perform statistical analysis, and create graphical representations. Pearson's correlation and simple linear regression analysis were conducted to examine the correlation between gene expression of SORT1 and SESN1 with oxidative indices in the groups studied.

Results

Demographic, clinical, and angiographic data

There were no significant differences in age, gender, BMI, DBP, hypertension, family history, opium use, and alcohol consumption between groups. However, SBP, GS, LVEF, and smoking were significantly higher in the CAD group compared to the control group ($p<0.05$). Demographic characteristics, clinical data, and coronary angiography data are summarized in Table 1.

Biochemical parameters

The comparison of biochemical parameters between the two groups indicates that elevated levels of liver enzymes such as AST, ALT and ALP were present in the patient group. However, only the increase in ALT was insignificant. The serum concentrations of TC and LDL-C in patients were higher than in control subjects, but the increase was insignificant. The other biochemical parameters did not change. The results of the biochemical parameters are provided in Table 2.

Hematological parameters

Table 3 displays the results of the hematological parameters. The results showed that the CAD group had significantly higher levels of HB compared to the control group

Table 1 Demographic, clinical, and angiographic data of the studied groups

Parameter		Control Group ($n=40$)	CAD Goup ($n=49$)	*P*-Value
Age (years), mean ± SD		53.32 ± 8.51	55.24 ± 8.75	0.3*
BMI (kg/m2), mean ± SD		27.2 ± 4.07	27.43 ± 4.3	0.793*
SBP (mmHg), mean ± SD		122.65 ± 11.56	128.71 ± 14.09	**0.031***
DBP (mmHg), mean ± SD		80.87 ± 8.04	84.1 ± 10.59	0.115*
LVEF (%), mean ± SD		50 ± 9.54	45 ± 8.73	0.003*
Hypertension, n (%)		14 (35)	26 (53.06)	0.088**
CVD history, n (%)		0	21 (42.86)	**<0.001****
Family history, n (%)		16 (40)	23 (48.94)	0.404**
Coronary angiograms (n, %)		Non-significant CAD (40,100)	SVD (22, 44.9) DVD (10, 20.41) TVD (12, 24.49) LM + TVD (5, 10.2)	**<0.001****
Gender:	Male	27 (67.5)	39 (79.59)	0.195**
	Female	13 (32.5)	10 (20.41)	
Smoking, n (%)		10 (25)	25 (48.98)	0.012**
Opium, n (%)		13 (35.5)	19 (38.78)	0.539**
Alcohol use, n (%)		2 (5)	7 (14.29)	0.148**
Fruit & vegetable intake, n (%)	Low	4 (10)	4 (8.7)	0.646**
	Moderate	26 (65)	34 (73.91)	
	High	10 (25)	8 (17.39)	
Exercise, n (%)	No	17 (42.5)	28 (57.14)	0.102**
	Low	8 (20)	12 (24.49)	
	Moderate	15 (37.5)	8 (16.33)	
	High	0 (0)	1 (2.04)	
Medication:				
Aspirin, n (%)		15 (37.5)	35 (71.43)	**0.001****
Beta blockers, n (%)		9 (22.5)	29 (59.18)	**0.001****
P2Y12 inhibitors, n (%)		4 (10)	26 (53.06)	**<0.001****
ACEIs/ARBs, n (%)		10 (25)	22 (44.9)	0.052**
Nitrates, n (%)		3 (7.5)	11 (22.45)	0.054**
Statins, n (%)		16 (40)	40 (81.63)	**<0.001****

Data are presented as means ± S.D. BMI is body mass index; SBP is systolic blood pressure; DBP is diastolic blood pressure; SVD is a single-vessel disease; DVD is a double-vessel disease; TVD is a triple-vessel disease; LM, Left Main; LVEF is left ventricular ejection fraction; P2Y12, Purinergic receptor type Y, subtype 12; ACEI/ARB, Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers. * indicate Student's t-test, ** Chi-Square test. A p-value < 0.05 was considered significant

($p<0.05$) and a lower platelet count ($p<0.05$). However, there were no notable differences in other hematology parameters between the two groups.

Measurement of OS markers

According to the findings presented in Table 4, the serum concentration of TOS was higher than in patients with CAD compared to control subjects ($p=0.628$), but this increase was not statistically significant. There were no significant differences in the serum concentration of TAC between the two groups ($p=0.407$). However, there was a significant rise in the serum concentration of MDA in patients with CAD compared to controls ($p=0.0004$).

Table 2 Comparison of biochemical parameters between CAD and controls

Parameter	Control group ($n=40$)	CAD group ($n=49$)	*P*-Value *
FBS (mg/dl)	94.67 ± 10.17	98.91 ± 12.53	0.087
TC (mg/dl)	153.23 ± 49.16	165.71 ± 37.71	0.181
TG (mg/dl)	181.2 ± 76.53	181.41 ± 79.14	0.981
HDL-C (mg/dl)	34.3 ± 9.43	34.22 ± 7.74	0.967
LDL-C (mg/dl)	88.32 ± 40.26	100/18 ± 32.92	0.131
VLDL-C	36.2 ± 15.3	36.283 ± 15.82	0.981
TC/HDL-C	4.64 ± 5.03	5.03 ± 1.45	0.213
TG/HDL-C	5.87 ± 3.51	5.89 ± 3.64	0.982
LDL-C /HDL-C	2.67 ± 1.15	3.04 ± 1.16	0.134
AST/GOT (UI/L)	33.35 ± 11.66	39.42 ± 19.65	0.0884
ALT/GPT (UI/L)	23.12 ± 18.72	35.79 ± 30.02	**0.022**
ALP (UI/L)	192.35 ± 45.46	213.34 ± 53.41	0.052
Calcium (mg/dl)	9.14 ± 0.39	9.17 ± 1.29	0.910
Phosphorous (mg/dl)	3.67 ± 0.54	3.68 ± 0.43	0.926
Magnesium (mg/dl)	2.23 ± 0.137	2.28 ± 0.194	0.121
Hs-CRP (mg/dl)	4.08 ± 8.72	10.51 ± 22.03	0.086

Data are presented as means ± S.D. FBS, Fasting blood sugar; TC, Total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; AST, Aspartate aminotransferase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; hs-CRP, High-sensitivity C-reactive protein. * Student's t-test. A p-value < 0.05 was considered significant

Table 3 Comparison of hematological parameters between CAD and controls

Parameters	Control group ($n=40$)	CAD group ($n=49$)	*P*-Value *
WBC ($10^3/\mu L$)	6615 ± 1763	7293 ± 1752	0.073
Neut (%)	60.07 ± 10.05	59.4 ± 18.3	0.837
Lym (%)	35.95 ± 9.55	32.24 ± 12.07	0.131
PLT ($10^3/\mu L$)	245.05 ± 22	223 ± 44.53	**0.0005**
RBC ($10^6/\mu L$)	5.051 ± 0.691	5.205 ± 0.531	0.237
MCV (fL)	85.65 ± 6.4	85.46 ± 4.98	0.874
MCH (pg)	29.82 ± 3.42	30.20 ± 2.38	0.531
MCHC (g/dL)	34.71 ± 2.22	35.68 ± 3.07	0.097
Hb (g/dL)	14.92 ± 1.69	15.67 ± 1.6	0.035
HCT (%)	42.97 ± 4.12	44.32 ± 3.36	0.092

Data are presented as means ± S.D. WBC, white blood cells; Neut, neutrophils; Lym, lymphocytes; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; PLT, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. * Student's t-test. A p-value < 0.05 was considered significant

Table 4 Serum concentration of oxidative stress markers between CAD and controls

Parameters	Controls ($n=40$)	CAD ($n=40$)	*P*-Value *
TOS (μmole/L)	7.17 ± 3.56	7.57 ± 4.13	0.628
TAC (nmole/mL)	528.12 ± 94.37	545.266 ± 97.16	0.407
MDA (μmole/L)	3.25 ± 0.73	4.35 ± 1.78	**0.0004**

Data are presented as means ± S.D. TOS, total oxidation status; TAC, total antioxidant capacity. * Student's t-test. A p-value < 0.05 was considered significant

Expression of SORT1 and SESN1 levels

The results of this study show that there were no differences in the expression levels of the SORT1 gene ($p=0.789$) and protein ($p=0.966$) between the two groups. The CAD patients had a 1.061-fold increase in SORT1 gene expression and a 1.005-fold increase in SORT1 protein expression compared to the control group (Fig. 1a and c). The level of SESN1 expression was significantly reduced in the CAD patients compared to the control, indicating a decrease of 0.735-fold (Fig. 1b). Nevertheless, this disparity did not reach statistical significance ($p=0.161$). Figure 1 shows a comparison of PBMC gene expression of SORT1 and SESN1, as well as protein expression of SORT1 in the studied groups.

Correlation analysis

Pearson's correlation and simple linear regression analysis showed a significant and positive correlation between the expression of SORT1 and SESN1 genes in the two groups. This association in controls ($p=0.0005$) compared to CAD patients ($p=0.0049$) was stronger. The relationship between the gene expression levels of SORT1 and SESN1 in the control and CAD groups is illustrated in Fig. 2. The investigation assessed the correlation between the expression levels of the SORT1 and SESN1 genes with markers of OS. However, there was no significant relationship between the expression of the SORT1 gene and OS markers in the two groups. Notably, there was a negative association between the expression of the SESN1 gene and TOS in the two groups, although this correlation was only significant in the normal group. ($p=0.012$). Furthermore, no statistically significant correlation was observed between SESN1 with TAC and MDA in either group. The relationship between the SESN1 gene expression levels and OS markers is shown in Fig. 3. According to our findings, there is no connection between the SORT1 and SESN1 genes and lipid profiles such as TC, TG, LDL and HDL. The results of correlation

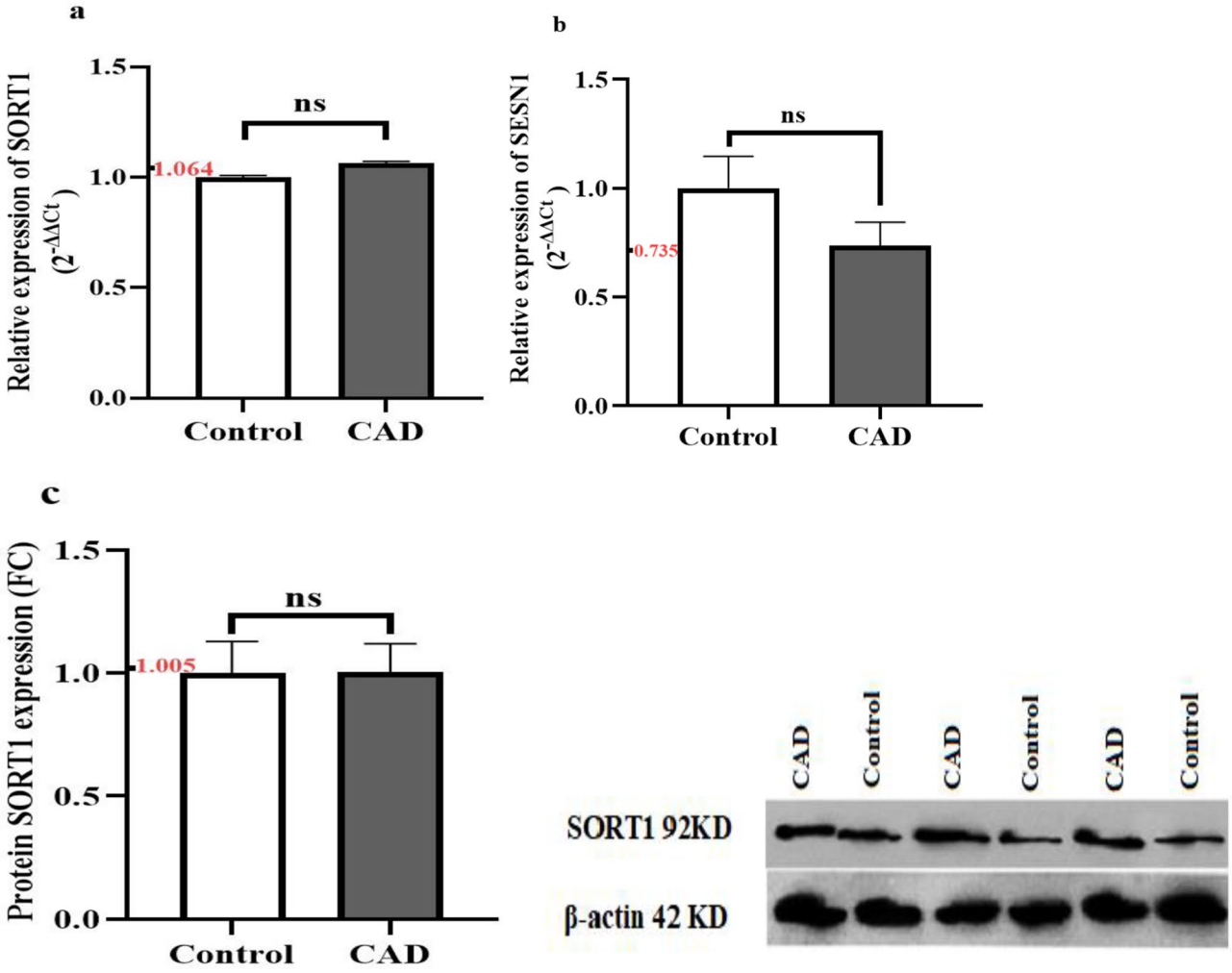

Fig. 1 Comparison of PBMC gene expression of SORT1 (**a**) and SESN1 (**b**), as well as protein expression of SORT1 (**c**) in the studied groups. A p-value < 0.05 was considered significant. ns indicates nonsignificant

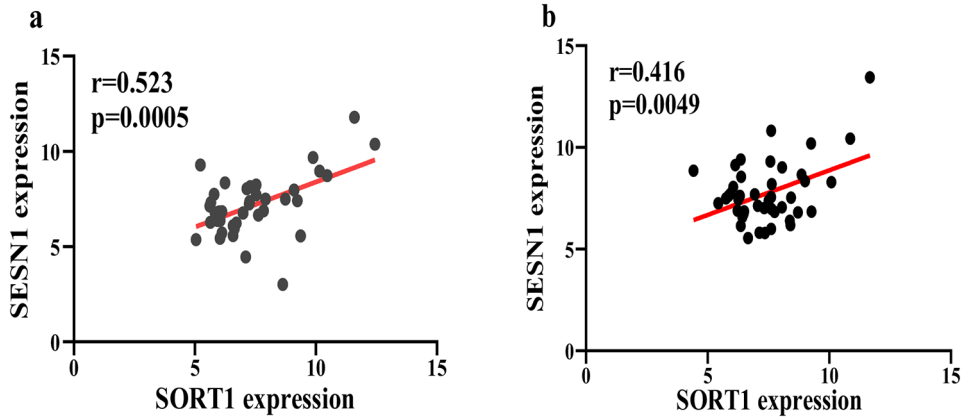

Fig. 2 (**a**) and (**b**) display the relationship between the SORT1 and SESN1 gene expression levels in the control and CAD groups, respectively. A p-value < 0.05 was considered significant

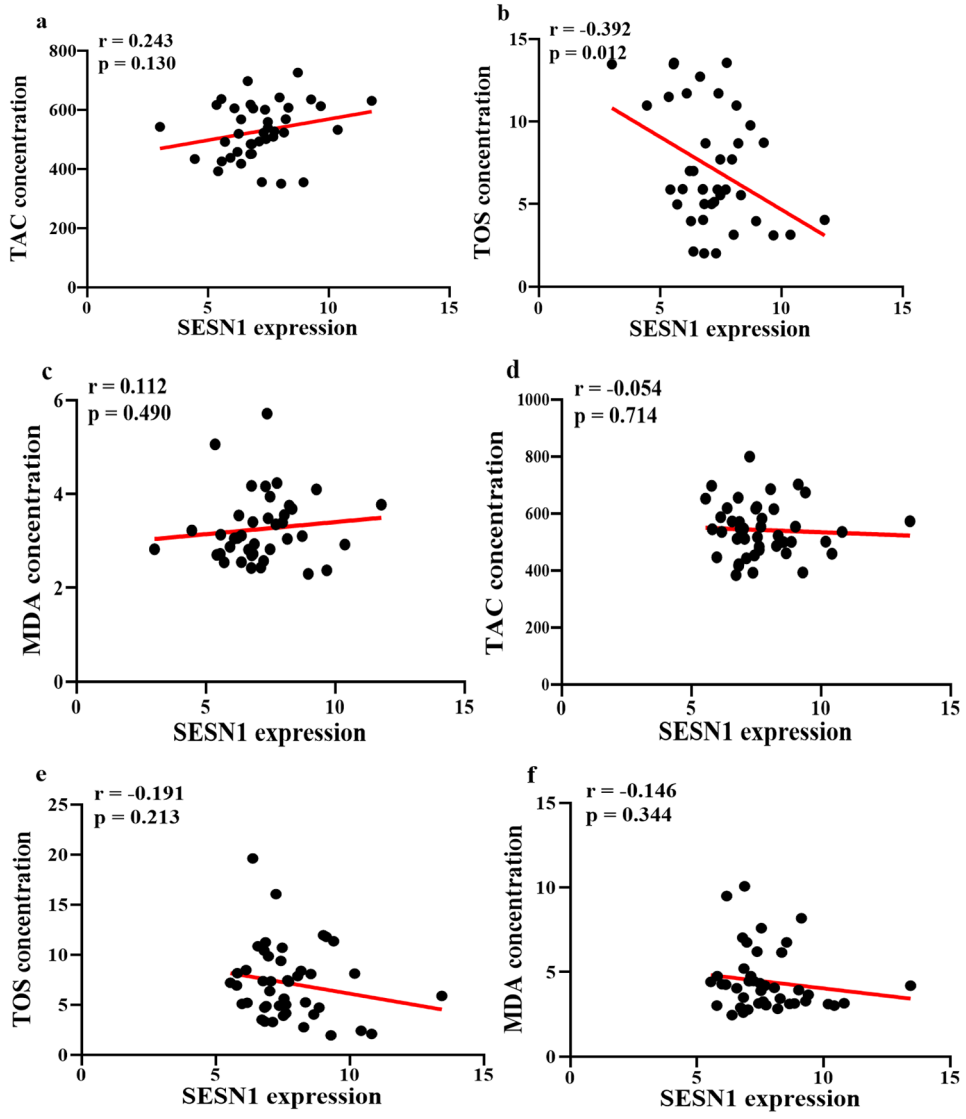

Fig. 3 (**a** – **c**) displays the relationship between the SESN1 and oxidative stress markers in the control group, while (**d** and **f**) displays the CAD group. A p-value < 0.05 was considered significant

analysis between the SORT1 and SESN1 genes and aminotransferaseswere also not significant.

Discussion

The current investigation elucidates the expression of SORT1 and SESN1 genes in patients with CAD in comparison to the controls. The analysis indicates that there was no significant disparity between the two groups. Furthermore, the assessment of OS markers in the two groups reveals a notable elevation solely in MDA among the patients.

Atherosclerosis is a precursor and underlying CVD, such as CAD, and is a major contributor to health problems and deaths worldwide in both developed and underdeveloped nations [29]. Few investigations have been conducted on the correlation between biochemical parameters and coronary disease as determined by angiography. The analysis of biochemical parameters in the two groups revealed an increase in liver enzymes ALT, AST, and ALP levels in the patient group. However, only the rise in ALT was statistically significant. Studies have shown that elevated serum levels of liver enzymes, including ALT, can predict cardiovascular events independently of traditional risk factors [30–33]. According to the study by Madan et al., increased serum liver transaminase levels are positively correlated with metabolic syndrome and NAFLD, which are also associated with CAD. Also, elevated ALT/AST levels in NAFLD may indicate the presence of CAD [34]. The findings by Hasan et al. in 2024 demonstrate that elevated levels of AST and ALT remain associated with the incidence of cardiovascular disease, even after accounting for traditional risk factors [21]. We propose that liver enzymes, particularly ALT, serve as a simple, accessible, and inexpensive biomarker for screening, diagnosing, and monitoring treatment in cardiovascular diseases, including CAD, alongside hepatic conditions.

This study indicates that patients with CAD exhibited elevated serum concentrations of TC and LDL-C in comparison to control subjects. Still, the rise did not reach a level of statistical significance. It's worth noting that almost double the number of CAD patients were taking statins compared to the control group (81.63% vs. 40% in controls). Statins are known to impact lipid profiles positively [35, 36], but even with their use, the serum concentrations of TC and LDL-C were still higher in patients. Notably, TG and HDL-C levels did not differ between the two groups. The analysis of other biochemical parameters in the two groups revealed no discernible differences.

Based on the results, individuals diagnosed with CAD exhibited notably elevated levels of HB in comparison to the control group. Furthermore, there was a significant reduction in platelet count within the CAD group, which is caused by the use of antiplatelet drugs, aspirin, and P2Y12 inhibitors. The patient group received nearly double the amount of antiplatelet medications compared to the control group (73.47% vs. 35% in controls). Notably, the remaining hematology parameters did not indicate any significant differences between the two groups.

A surge in OS and weakened antioxidant defense contributes to the initiation and progression of CAD [37]. In the conducted study, it was observed that the TOS levels were elevated in comparison to the control subjects. However, the disparity was not found to be statistically significant. Also, the TAC of patients had no discernible differences. To investigate the reasons behind this unexpected result, we analyzed the demographic data of patients and controls and compared their intake of fruits and vegetables as sources of antioxidants. Interestingly, no significant differences were found between the two groups. Further analysis revealed that the drugs used by the participants in the study had antioxidant properties. A higher percentage of patients had taken these drugs, including statins, beta-blockers, antiplatelet drugs including aspirin, Purinergic receptor type Y, subtype 12 (P2Y12) inhibitors, and Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARB). Beyond their role in lowering cholesterol, statins have a number of other protective and preventative effects on CVD, including antioxidant and anti-inflammatory actions, enhancement of vascular endothelial function, stabilization of plaque, and inhibition of platelet aggregation. Multiple studies have demonstrated that statins have antioxidant capabilities that can effectively alleviate OS by decreasing the generation of free radicals and enhancing antioxidant systems [38–40]. According to Yaribeygi's findings, the use of atorvastatin medication can effectively reduce oxidative and nitrosative stress in the heart muscle of diabetic rats [41].

The subsequent medicine with the highest prescription rate, especially among the patient group, was aspirin, with its usage in the patient group about double that of the control group (71.43% vs. 37.5% in controls). Aspirin, in addition to its anti-platelet and anti-coagulant properties, has significant and diverse antioxidant properties. It protects endothelium by reducing H2O2-.

induced toxicity and promoting ferritin synthesis. It enhances heme-oxygenase-1 (HO-1) expression and activity, protecting against oxidative tissue damage. Aspirin also enhances the Nrf2-ARE pathway, increasing antioxidant and detoxifying enzymes. It inhibits NF-kB, a key regulator of inflammatory responses and, regulates the inflammasome and triggers the expression of proinflammatory genes, including cytokines and chemokines [42].

The patient group also had a higher prescription rate for beta-blockers (71.43% vs. 37.5% in the control group). Multiple clinical trials have validated the efficacy of beta blockers, including carvedilol, metoprolol,

and bisoprolol, in mitigating OS via various mechanisms. The primary and most significant mechanism of action involves the inhibition of the ß1 receptor, as catecholamines such as isoproterenol and norepinephrine induce OS in the myocardium, subsequently, by their anti-ischemic characteristics, negative chronotropic effect, and antihypertensive action. Moreover, carvedilol has unique effects, including direct antioxidant capabilities, inhibition of reactive oxygen species (ROS) formation by leukocytes, and α-adrenergic blockage, all contributing to the reduction of OS.

Nakamura et al. indicated that beta-blocker therapy serves as an antioxidant treatment that is both advantageous and secure for individuals with heart failure, with beta-blockers including metoprolol, bisoprolol, and carvedilol capable of reversing the detrimental cycles of OS in these patients. to prevent heart failure via indirect or direct antioxidant mechanisms [43]. Toyoda et al. reported that the beta-blockers bisoprolol and carvedilol have reducing effects on inflammation and OS by lowering reactive oxygen metabolites, indicators of OS, and hs-CRP, indicators of inflammation in CHF patients [44].

Additionally, P2Y12 inhibitors were prescribed at a much higher rate to the patient group (53.06% vs. 10% in the control group). Platelets play a crucial role in inflammation and immune responses. P2Y12 inhibitors decrease the release of pro-inflammatory α-granule contents by platelets and the formation of pro-inflammatory platelet-leukocyte aggregates. Clinical research indicates that clopidogrel-induced P2Y12 inhibition is linked to a decrease in platelet-related inflammatory mediators, including soluble P-selectin and CD40L, following atherothrombosis [45]. The potential antioxidative property of P2Y12 inhibitors has primarily been assessed using clopidogrel. Clopidogrel has demonstrated the capacity not only to mitigate ROS toxicity but also to prevent a decline in glutathione (GSH) levels, thereby augmenting natural and endogenous antioxidant systems [42]. The usage of other medicines, such as nitrates and ACEI/ARB, did not show any meaningful difference.

MDA is generated through the peroxidation of unsaturated fatty acids caused by an enzymatic reaction, and its levels rise considerably during OS. Consequently, the amount of MDA in the bloodstream is a credible biomarker for lipid peroxidation and OS, which can be measured non-invasively [19, 46]. In this investigation, the MDA measurement results demonstrated a statistically significant elevation in patients compared to the control. In contrast to TAC and TOS, MDA remained consistent with previous research and was predictable and unaffected by patients' treatment circumstances. Hence, it can be utilized as a non-invasive, stable, affordable, and readily available marker for screening, prediction, diagnosis, and monitoring of atherosclerosis patients. Several research studies in this direction include the following. Mutlu-Türkoğlu et al., in 2020, a study was done to compare the levels of serum MDA in individuals diagnosed with CAD by angiography with those in control subjects. The findings indicated a rise in MDA levels, which was neither influenced by the quantity of damaged coronary arteries nor associated with the intensity of vascular lesions [47]. The study conducted by Bastani et al. in 2018 revealed that patients with acute coronary syndrome and CAD have notable elevation in MDA levels and diminished levels of plasma TAC in comparison to healthy subjects [48]. In 2015, Yaghoubi and Ghojazadeh conducted a study that showed that serum MDA levels were notably elevated in patients with CAD compared to the control group. According to their research, serum MDA can be used as a diagnostic indicator in CAD and can also help evaluate the seriousness of the disease [49]. Varadhan et al., in 2022, conducted an assessment of OS markers in patients with CAD in comparison to a control group. The patients exhibited a noteworthy rise in the levels of MDA and TOS [37]. In 2014, Uppal et al. conducted a study that revealed that individuals diagnosed with CAD exhibited markedly elevated levels of MDA compared to the control group. Elevated levels of MDA can result from OS and contribute to lipid peroxidation. Additionally, the study revealed that people suffering from unstable angina and MI exhibited elevated levels of MDA in comparison to those with stable angina [50].

Smoking is a major risk factor that can initiate and advance atherosclerosis. Cigarettes alter the endothelium's permeability to LDL, leading to decreased blood oxygen levels and increased carboxyhemoglobin [2]. Numerous research studies have demonstrated that smoking tobacco causes OS, inflammation of blood vessels, coagulation of platelets, and dysfunction of blood vessels. Additionally, it negatively affects the lipid profile and leads to harmful consequences on the cardiovascular system [51].

In this investigation, it was observed that the incidence of smoking among the CAD patients surpassed that of the control group by nearly twofold (48.98% vs. 25% in controls). This discrepancy serves to underscore the pivotal role and significance of smoking in the pathogenesis of atherosclerosis. Additionally, the concurrent escalation in TOS and MDA levels, recognized as indicative of OS, provides further evidence of smoking's involvement in OS. These findings corroborate the results of previous studies.

Our study showed no difference in the levels of SORT1 gene and protein expression between the two groups. The regulatory involvement of SORT1 in signalling molecules, metabolites, and cell surface receptors involved in the development of diseases like CVD, obesity, and

neurodegenerative disorders via protein sorting was confirmed by Carlo et al. in 2014 [52].

In 2019, the findings of Chen et al.'s study showed that deletion of the SORT1 gene in hepatocytes reduced hepatic steatosis and plasma cholesterol levels; in addition, pharmacological inhibition of Sort1 reduced plasma cholesterol in hyperlipidemic mice, and SORT1 introduced as a potential therapeutic target for the treatment of hyperlipidemia [53].

There are contradictory studies concerning the influence of SORT1 on atherosclerosis. For instance, according to several cohort studies, SORT1 expression is inversely related to lower levels of LDL and lower risk of CAD, while Several genome-wide association studies (GWAS) have linked SORT1 to lipid metabolism and atherogenesis risk [6]. In 2019, Biscetti et al. found that mice with dyslipidemia and atherosclerosis had either abnormally high or low SORT1 expression; however, the exact function of SORT1 in these conditions is still up for debate. SORT1's aberrant function and traffic are likely to be blamed for these conflicting results [54]. Han and colleagues in 2020 found that patients with CHD had a higher level of the SORT1 compared to control people. They proposed SORT1 as a potential biomarker for coronary heart disease (CHD) [10]. Nozue et al., in 2016, conducted a study on the impact of statins on SORT1 levels in patients with CAD. They found that using statins reduces the level of SORT1 [55]. In 2021, Werida et al. revealed that both rosuvastatin and atorvastatin improved the lipid profile, atherogenic index, serum SORT1, and CRP in dyslipidemic T2D patients [56]. The study conducted by Oh et al. revealed that patients with CAD and type 2 diabetes exhibit elevated plasma SORT1 levels compared to those without these conditions. The study inferred that heightened circulating SORT1 levels are linked to CAD and diabetes, suggesting its potential utility as a biomarker for both diseases in individuals not taking statins [24]. Ai et al. found that stimulation of mTORC1 reduces the expression of SORT1 in the livers of obese mice [57]. In a study conducted in 2022, Di et al. reported that SORT1 induces endothelial dysfunction by increasing OS. This effect is attributed to the activation of the NADPH oxidase 2 (NOX2) isoform [58].

So far, gene and protein expression levels have remained unstudied in SORT1 in vivo research, and the majority of studies concentrate on serum SORT1 protein levels. Given that, serum SORT1 is a fraction of the overall SORT1 that is released into the bloodstream due to breakdown. Hence, it may not accurately reflect the total amount of SORT1. Also, certain investigations have shown that the usage of statins decreases the concentration of serum SORT1, so serum SORT1 does not accurately reflect the precise value of total SORT1. Suppose we regard serum SORT1 as an indicator of overall SORT1 levels. Statin use may influence the SORT1 gene and protein expression, which in turn reduces serum SORT1. Since our study did not observe disparity in the expression of the SORT1 gene and protein. This lack of difference may be attributed to the higher statin usage in the patient group and its consequent impact on the expression of the SORT1 gene and protein. The genetic variation of the SORT1 gene, which affects the function of SORT1 and the risk of cardiovascular diseases in different populations, may contribute to the results observed in the research.

There was a non-significant decrease in the gene expression levels of SESN1 in patients compared to controls. Ricoult et al. in 2013 found that SESN1 inhibits mTORC1 (mammalian targets of rapamycin complex), an important regulator of lipid homeostasis. mTORC1 blocks lipolysis, β-oxidation, and ketogenesis, which in turn enhances lipid synthesis and storage while suppressing lipid breakdown and consumption [59]. Li and colleagues conducted in vitro and in vivo studies from 2018 to 2020 and discovered that mice lacking the SESN1 gene had elevated levels of plasma VLDL, cholesterol, and TG, as well as decreased phosphorylation of AMPK and acetyl CoA carboxylase, two proteins known to regulate lipid biosynthesis. This finding established SESN1 as a regulator of hepatic lipid synthesis and blood circulation lipid concentration, and it established a new component of the cholesterol biosynthesis pathway. Li confirmed that in vitro knockdown of the SESN1 gene leads to the accumulation of cellular cholesterol and that SESN1 suppresses the production of cholesterol that SREBP-2 triggers [15, 60].

In 2020, Keping and colleagues found that atherosclerosis-afflicted mice had increased SESN1 expression in their aortic macrophages. According to their research, SESN1 helps activate the NLRP3 inflammasome pathway, which in turn reduces the production of foam cells generated by cholesterol crystals and the inhibition of oxidized LDL in macrophages. In addition, their study showed that SESN1 overexpression lowered fat buildup in blood vessels and pro-inflammatory cytokine expression in invading macrophages and aortic tissue. The result was reversed when the SESN1 gene was knocked down [61]. Ye et al. in 2017 demonstrated that the severity of CAD is closely correlated with the expression level of sestrin proteins in the coronary artery. Consequently, measuring sestrins proteins can be an additional tool for diagnosing CAD severity [62].

According to Sundararajan et al., sestrin levels significantly decrease in individuals with diabetes and dyslipidemia-related diseases. Furthermore, they found a strong correlation between Sestrin2 and atherogenic risk factors and the severity of the atherogenic index. These findings

suggest that Sestrin2 could serve as a potential biomarker for evaluating atherogenesis [13].

Research has established that sestrins are a vital mediator of exercise and that several medicinal drugs can improve health and longevity [63, 64]. Sestrin isoforms provide exercise-related advantages across various organ systems and are essential for insulin sensitivity, mitochondrial function, and lipid balance. Sestrins are crucial for exercise adaption and their associated effects [64]. Upregulation of SESN1 is observed in response to physical exercise and activity [64, 65]. Given that the control group engages in higher levels of exercise and physical activity compared to the patient group, it is conceivable that this disparity could account for the elevated sestrin levels in the controls. The focus of in vivo research on SESN1 in the scientific literature is mainly on examining SESN1 protein levels in serum, while gene and protein expression levels are still poorly understood. Because patients with suspected vascular stenosis and coronary artery occlusion are promptly administered lipid-lowering medications such as statins, we could not exclude statins from our study. Our recommendation is to investigate the expression of these genes through animal study, which is feasible to enact the required limitations on the medication. Furthermore, conducting comprehensive human studies involving a large and appropriately segmented population into statin and non-statin groups. In addition, perhaps the relatively small sample size is the reason for obtaining non-significant results, and it is recommended to study these genes in a larger population.

Although lower levels of cholesterol and LDL were observed in the control group with higher SESN1 expression, no statistically significant correlation was found between SESN1 and lipid profile. In this study, SESN1 demonstrates an inverse and significant correlation with TOS among the indicators of OS. This finding validates its documented role in mitigating OS, as reported in the scientific literature. Our investigation found no evidence of an association between the SORT1 gene expression with OS markers and lipid profiles, including TC, TG, HDL, and LDL. Also, the role of liver enzymes and how they affect the main markers of this study have not been investigated in the scientific literature. Further investigation and research are warranted in this area.

Current analysis of scientific literature indicates a relationship between SESN1, SORT1, and OS in connection with mTORC1. Specifically, SESN1 inhibits mTORC1 and reduces OS, whereas SORT1 is associated with an increase in OS [58]. Additionally, mTORC1 plays a role in modulating OS [66] and also inhibits SORT1 [57]. Consequently, it can be inferred that an increase in SESN1 ultimately leads to an increase in SORT1 by inhibiting mTORC1. Our study also found a direct and significant relationship between SESN1 and SORT1, which was stronger in the control group that received less medication. Further investigations are required in this field.

Conclusion

This study examined the expression levels of SORT1 and SESN1 genes in PBMCs and OS markers in the serum of CAD patients compared to controls. The results showed no significant difference in SORT1 gene and protein expression between groups, while SESN1 gene expression showed a non-significant decrease in CAD patients. Notably, MDA levels, an indicator of OS, were significantly increased in CAD patients, highlighting the role of OS in atherosclerosis. These results suggest that MDA could serve as a cost-effective, non-invasive biomarker for predicting and monitoring atherosclerosis-related diseases such as CAD. However, the lack of significant differences in SORT1 and SESN1 expressions requires further investigation to clarify their roles in CAD development. Understanding the molecular mechanisms of these genes could provide new insights into the prevention, diagnosis, and treatment of CVD.

Limitation

Potentially, conducting sampling with a larger volume could have yielded more robust and significant results. Regrettably, this was not feasible due to financial constraints.

The exclusion of statins from the research was unfeasible because they are generally prescribed immediately upon the diagnosis of possible arterial stenosis in patients. Also, it was not possible to remove other drugs, such as antiplatelets and beta-blockers, as confounders due to the priority of treating patients.

It would be beneficial to discover noninvasive or minimally invasive methods in medical science for detecting the percentage of coronary artery occlusion instead of the invasive method of angiography. This would allow for the study of normal and healthy individuals from the general population as a control group. Such an approach could potentially lead to more robust research findings. Hopefully, this possibility will be realized in the near future.

The study's criteria for subject selection were based on the percentage of coronary artery occlusion determined through invasive angiography. Approximately 10–20% of individuals who underwent angiography had artery occlusion below 25%, leading to a shortage of control subjects. Consequently, we had to select the remaining control subjects from individuals eligible for the control group who had recently undergone angiography, which posed challenges in accessing their information and coordinating the sampling process.

Abbreviations

ACEI/ARB Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers

AMPK	Adenosine monophosphate-activated protein kinase
BMI	Body mass index
CAD	Coronary Artery Disease
CVD	Cardiovascular diseases
DVD	Double-vessel disease
DBP	Diastolic blood pressure
GS	Gensini score
GWAS	Genome-wide association study
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
LVEF	Left ventricular ejection fraction
MDA	Malondialdehyde
mTORC1	Mammalian targets of rapamycin complex
NAFLD	Non-alcoholic fatty liver disease
NRF2/ARE	Nuclear factor erythroid 2-related factor 2 /antioxidant responsive element
OS	Oxidative stress
PBMC	Peripheral blood mononuclear cells
P2Y12	Purinergic receptor type Y, subtype 12
qRT-PCR	Quantitative real-time polymerase chain reaction
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SESN1	Sestrin1
SORT1	Sortilin1
SREBP-2	Sterol regulatory element-binding protein-2
TAC	Total Antioxidant Capacity
TOS	Total Oxidant Status
VLDL-C	Very low-density lipoprotein cholesterol
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1

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Author contributions
T.G. originated and executed the project and composed the manuscript. J.K. oversaw project management, conceptualization, review, and editing. I.K. conducted the review and made revisions. A.Y. assessed the patients, while S.K. analyzed the data. Z.A.K. conducted the analysis, evaluation, and revision of bioinformatics data. K.H. oversaw the project and examined angiographic films to evaluate the extent and location of narrowing in the coronary arteries, as well as review and make revisions. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article, and further detailed ones are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
The Research Ethics Committee of Hamadan University of Medical Sciences (Approval code: IR.UMSHA.REC.1400.616) granted ethics approval for the study, and all participants gave written informed permission in accordance with the Helsinki Declaration.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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