JJBS

Volume 13, Supplementary Issue, December 2020
ISSN 1995-6673
Pages 581 - 588

Jordan Journal of Biological Sciences

Evaluation of *SIRT1* Gene Expression and rs3758391 C/T Polymorphism in Coronary Atherosclerosis

Rabab F. Salim[1,*], Mamdouh Z. Abadir[1], Azza M. Elbermawy[1], Heba A. Mansour[2] and Lina A. Mohamed[1]

[1] *Department of Medical Biochemistry,* [2] *Department of Cardiovascular Medicine, Faculty of Medicine, Benha University, Benha -13512, Egypt.*

Received: December 21, 2019; Revised: February 19, 2020; Accepted: February 21, 2020

Abstract

Recognition of disease-causing genes and susceptibility genes for coronary artery disease (CAD) will provide valuable data for prevention and control of CAD. The purpose of the present work was to investigate the association of *SIRT1* expression and its promoter single nucleotide polymorphism rs3758391 T/C with coronary atherosclerosis and its severity. Moreover, the current work aimed to explore the effectiveness of this polymorphism on *SIRT1* expression. The participants were categorized according to their angiographic results into CAD group with significant coronary artery atherosclerosis and non-CAD group with normal coronary artery. DNA was extracted followed by genotyping of *SIRT1* rs3758391 C/T. Extraction of total RNA was carried out, followed by PCR amplification of SIRT1 gene. CC genotype was prevalent in CAD group rather than control group (p=0.002) and conferred a greater risk of coronary atherosclerosis compared to those carrying CT+TT genotypes (OR: 3.41, CI: 1.81-6.42, $p < 0.001$). At allelic level, C allele was significantly prevalent in CAD patients rather than control group ($p < 0.001$). Additionally, *SIRT1* expression was significantly lower in CAD patients than in controls (p=0.001) and its expression reduced significantly in the CC genotype of both CAD and control groups compared to CT and TT genotypes (p=0.001). Moreover, there was an inverse correlation (rho = -0.23, p=0.02) between *SIRT1* expression of CAD patients and Gensini scores. In conclusion, *SIRT1* rs3758391 CC genotype might carry an increased risk of coronary atherosclerosis. However, *SIRT1* gene expression was inversely correlated with atherosclerosis severity suggesting its protective effects against atherosclerosis development.

Keywords:SIRT1 expression; SIRT1 rs3758391 C/T; genotyping; coronary atherosclerosis; Gensini score

1. Introduction

Coronary artery disease (CAD) remains typically the chief cause of mortality and morbidity in the whole world population (Simon and Vijayakumar, 2013). Despite persistent efforts in the management and prevention of this complex disease, it remains a big challenge to the scientists and health managers. It is expected that by the year 2020, this disease would remain as the most common and major threat to human life (Yusuf et al., 1998). In Egypt, mortality secondary to CAD is rapidly increasing. According to the latest WHO data, CAD deaths reached 21.73% of total deaths in Egypt (Ali et al., 2014) and reported a prevalence of 8.3% (Almahmeed et al., 2012).

CAD is considered as one of the complex diseases that are polygenic or multifactorial, produced by several genetic variants together with numerous environmental and lifestyle factors. It is characterized by long-term formation of atheromatous plaque, which culminates into atherothrombotic lesions resulting in tissue damage. Thrombosis and atherosclerosis are the major manifestations underlying CAD (Fawzy et al., 2017).

A huge number of laboratory researches, conducted within the past decade, have led to many achievements describing the genetic foundation of atherosclerosis (Libby et al., 2011). It has been recommended that more than a dozen of genes and genetic loci are related to CVD (Kathiresan and Srivastava, 2012).

Sirtuins (*SIRT1–SIRT7*) are family members of NAD^+-dependent protein deacetylases. Mammalian *SIRT1*, the best-recognized member of the sirtuin family, is an essential regulator of metabolism, cellular differentiation and senescence, cancer, and stress response (Poulose and Raju, 2015). Being a NAD^+-dependent enzyme, *SIRT1* regulates gene expression plans relating to cellular metabolic status (Zhang and Kraus, 2010). *SIRT1* is broadly expressed in various tissues with high expression in the vascular endothelium (Edirisinghe and Rahman, 2010).

SIRT1 plays an important role in decreasing atherosclerosis by intermediating several pathways. In addition to its function in enhancing NO production, *SIRT1* has anti-inflammatory capabilities in endothelial cells and even macrophages through decreasing the expression of numerous proinflammatory cytokines by meddling with the NF-κB signaling pathway (Stein et al., 2010 a). Furthermore, SIRT1 hinders the expression of lectin-like oxidized low-density lipoprotein (oxLDL) receptor 1 (Lox-1), a scavenger receptor for oxLDL,

* Corresponding author e-mail: rabab.salim@fmed.bu.edu.eg.

stopping the formation of macrophage foam cell, consequently delaying atherosclerosis (Stein et al., 2010 b). Besides, *SIRT1* activates ATP-binding cassette transporter A1 (ABCA1) transcription, which in turn mediates the HDL synthesis, reverses cholesterol transport and hence reduces the hazards regarding atherosclerosis and cardiovascular events (Li et al., 2007). The present body of evidence suggests that *SIRT1* dysregulation is involved in vascular dysfunction and the evolution of coronary artery disease. Therefore, *SIRT1* is considered as a hopeful target for new drugs for the management of CVD and related diseases (D'Onofrio et al., 2015).

Genetic variations such as single-nucleotide polymorphisms (SNPs) within the *SIRT1* gene have been linked with obesity (Zheng et al., 2012), type 2 diabetes (Han et al., 2015), hypertension (Zhong et al., 2015), and myocardial infarction (Cui et al., 2012; Yamac et al., 2019). Promoter region variants might be the cause of differential *SIRT1* expression, making people liable to certain pathologies (Nasiri et al., 2018; Mohtavinejad et al., 2015). SNPs within the *SIRT1* gene have been proposed to affect the messenger ribonucleic acid (mRNA) expression by modifying the binding ability of transcription factors or microRNAs (miRNAs) (Hu et al., 2015).

The management and evaluation of subjects with suspected CAD have been established on the noninvasive recognition of ischemia followed by using invasive coronary angiography (ICA) to approve the presence of luminal stenosis produced by atherosclerotic plaque (Schuijf et al., 2008). Coronary angiography is an essential tool for the quantification of CAD plaque burden in clinical practice (Roger et al., 2011). Despite being the golden standard for CAD diagnosis, ICA has numerous limitations (Yamashitaet al., 2010). Consequently, it has become a vital need to get an alternative noninvasive tool for precise diagnosis of CAD.

Identification of disease-causing genes and susceptibility genes for CAD as well as determining blood genetic markers for accurate noninvasive diagnosis of CAD are still a subject for research. Thus, the purpose of the current work was to investigate the association of *SIRT1* gene expression and its promoter single nucleotide polymorphism (SNP) rs3758391 T/C with coronary atherosclerosis and to explore the effectiveness of this polymorphism on *SIRT1* gene expression. Moreover, the current work aimed to evaluate the correlation between *SIRT1* expression and coronary atherosclerosis severity. Finally, the current work aimed to explore the value of *SIRT1* gene expression as a potential genomic predictor for CAD, validated by angiographic Gensini score.

2. Methods

2.1. Subjects and study design

The research ethics committee of Benha Faculty of Medicine approved the current clinical study. The current study was conducted according to World Medical Association (WMA) Declaration of Helniski (2008) and all study participants gave their written informed consents. Two hundred and fourteen subjects were invited to participate in the study, while fourteen refused to participate. The study subjects were different genders undergoing elective coronary angiography in the Cardiology Catheter Unit at Benha University Hospitals. According to coronary angiography, the subjects were categorized into CAD group (100 patients) with significant coronary artery atherosclerosis and non-CAD group (100 patients) with normal coronary angiograms to be a control group. Noteworthy, Significant coronary artery atherosclerosis was described as at least one particular major coronary artery having ≥70% or left main coronary artery having ≥ 50% luminal diameter stenosis (Reiber et al., 1989). Patients with heart failure, myocarditis, cardiomyopathies or those with history of coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) were excluded from the study. Exclusion criteria also included diabetes mellitus, severe renal or liver function defect, malignant tumor, inflammatory diseases or acute or chronic infections, and hematologic disorders.

2.2. Blood sampling

After an overnight fasting, 7.5 ml venous blood samples were withdrawn under complete aseptic conditions before undergoing coronary angiography. The particular collected blood samples were split up into three parts. First part (3 ml) was picked up in sterile tubes and left to clot for serum separation to be used for biochemical laboratory investigations, including total cholesterol, high-density lipoprotein (HDL), triglycerides (TG) and low-density lipoprotein (LDL). The second part (1.5 ml) was picked up in sterile glass vacutainer tubes containing ethylene- diaminetetraacetic acid (EDTA) then transferred into sterile Eppendorf tubes and stored at -20 ℃ to be used later for genotyping. The third part (3 ml) was collected in sterile EDTA- vacutainer tubes and the buffy coat layer containing peripheral blood mononuclear cells (PBMCs) was separated by Ficoll density-gradient centrifugation method using Ficoll Histopaque®-1077 (Sigma-Aldrich, U.S.A.) (Amos and Pool , 1976) following manufacturer's instructions. PBMCs were kept at -80 °C until later relative quantification (RQ) of *SIRT1* mRNA expression.

2.3. Coronary angiography

Elective coronary angiography was carried out for all patients included in the study via femoral artery using the Judkins technique or a radial approach. The angiographic characteristics were obtained from multiple views for both the left and the right coronary arteries. Two experienced cardiologists who were blinded to laboratory and clinical findings of the patients evaluated all the coronary angiograms. Gensini score assessed severity of CAD, which is grounded on the stage of the luminal stenosis and its regional importance (Gensini, 1983). Narrowing of the lumen was graded by Gensini score as follows: grade 1 (≤25% occlusion), grade 2 (26%–50% occlusion), grade 4 (51%–75% occlusion), grade 8 (76%–90A% occlusion), grade 16 (91%–99% occlusion) and grade 32 (total occlusion). Afterward, this primary score was multiplied by a component, which brings into consideration the significance of the location of the lesion within the coronary arterial tree. Gensini score was demonstrated as the summation of the scores for all 3 coronary arteries to estimate the total extent regarding CAD. Gensini score of 20 or more was considered severe CAD that was more or less equivalent to single stenosed

lesion of 70% or more within the proximal left anterior descending artery (Gensini et al., 1983).

2.4. Genotyping and SNP rs3758391 C/T analysis of SIRT1 gene:

DNA was extracted from peripheral blood using QIAmp DNA Blood Mini Kit supplied by (Qiagen, Germany) (Hedberg et al., 2015) in line with the manufacturer's instructions. Genotype of the samples was determined by TaqMan SNP Genotyping Assay (Applied Biosystems, U.S.A) specific for SIRT1 rs3758391 C/T according to the manufacturer's instructions. Briefly, the reaction contained 10 µL of 2X TaqMan® Universal Master Mix II (Applied Biosystems, U.S.A.), 1 µL of 20X TaqMan®Assay, 20 ηg/µL genomic DNA and RNase-free water up to 20 µL final volumes. The reactions were performed on StepOnePlus™ Real-Time PCR system (Applied Biosystems, U.S.A.). Genotypes were analyzed using StepOne Software v2.3 (Applied Biosystems, U.S.A.).

2.5. Analysis of human SIRT1 gene expression in PBMCs

RNA extraction

Total RNA was extracted from PBMCs using Direct-zol™ RNA MiniPrep supplied by Zymo Research, U.S.A. (Zhang et al., 2013) following the manufacturer's instructions. Nanodrop 2000 (Thermo Fisher scientific, USA) was used for the assessment of the quality and the quantity of the extracted RNA. Extracted RNA was then stored at -80°C for further processing.

Reverse transcription

Extracted RNA was reverse transcribed using high-capacity cDNA reverse transcription kit, supplied by Applied Biosystems, USA (Glenn et al., 2010) in line with the manufacturer's instructions. 1 µL of extracted RNA, completed to 10 µL by Nuclease- free H2O, was added to 10 µL of 2X RT master mix. The G-Storm Thermal Cycler (Gene Technologies, U.K.) program was 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and 4°C for ∞. The cDNA product was diluted in 100 µL Nuclease-free H2O.

Real time PCR

PCR amplification of SIRT1 gene and the housekeeping gene GAPDH were carried out in separate PCR tubes using gene specific primers as follows: SIRT1: forward 5′- TGAGGCACTTCATGGGGTATGG-3′; reverse 5′- TCCTAGGTTGCCCAGCTGATGAA-3′ (Zhang et al., 2013); and GAPDH: forward 5′-GAAATCCCATCACCATCTTCCAGG-3′; reverse 5′-GAGCCCCAGCCTTCTCCATG-3(Li et al., 2014). The amplification reaction contained 12.5 µL of 2x SYBR® Green PCR master mix (Applied Biosystems, U.S.A.) (Romeiro et al., 2016), 2 µL of cDNA, 1 µL of the forward primer, 1 µL of the reverse primer and Nuclease-free H2O up to 25 µL final volumes. The PCR conditions were performed in the StepOne Real-Time PCR system (Applied Biosystems, USA) according to the following program: 95°C for 10 min, 40 cycles of 95°C for 15 Sec, anneal/extend temperature of (60°C for SIRT1 and 56°C for GAPDH) for 60 Sec.

Melting curve analysis using StepOne software (Applied Biosystems, USA) was performed to assess specificity of the amplification products. The level of SIRT1 mRNA in each sample was normalized to the mRNA level of GAPDH. Controls were chosen as the reference samples, and fold change of SIRT1 mRNA was determined by $2^{-\Delta CT}$ method (Schmittgen and Livak, 2008) using StepOne Software v2.3 (Applied Biosystems, U.S.A.).

2.6. Statistical analysis

The study data were analyzed using STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas). Quantitative variables were expressed as mean ±SD and range. Qualitative variables were shown as number and percentage. Comparison of qualitative data was achieved using chi-square (χ^2) and Fisher's exact tests. The student t-test (t) and Mann-Whitney test (z) were used to compare two groups regarding parametric and non-parametric data respectively, while, Kruskal Wallis test ($\chi 2$) was used to compare more than two groups followed by post-hoc analysis using the Bonferroni method. The Odd's Ratio (OR) and 95% Confidence Interval (95% CI) were also considered. Spearmen correlations were used to correlate different variables. Receiver Operating Characteristics (ROC) analysis was performed to assess the diagnostic performance of *SIRT1* expression for atherosclerosis. The best cutoff point and the matching sensitivity and specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Area Under the Curve (AUC) were analyzed. p value < 0.05 was considered significant.

3. Results

3.1. Baseline characteristics

A total of two hundred subjects who had undergone elective coronary angiography were included in this study. Demographic, laboratory and clinical data of the study subjects are presented in Table 1. Smoking was significantly higher in CAD patients, as compared with the control group (p=0.047). Additionally, there was a significant statistical decrease in the mean HDL-C level of the CAD group, as compared with the control group (p<0.001). Otherwise, no significant association with other studied parameters was detected.

3.2. Genotyping and allele frequency of SIRT1 rs3758391 T/C

The genotype distribution and allelic frequency of SIRT1 rs3758391 T/C among CAD and control groups are presented in Table 2. There was a statistically significant difference between both groups regarding genotype distribution of SIRT1 rs3758391 T/C (p value < 0.05). In the additive model, the frequency of rs3758391 CC vs. TT genotype was significantly higher in CAD patients than controls (OR: 3.34, CI: 1.50-7.47, p value < 0.05). In accession, in the recessive model, the frequency of rs3758391 CC vs. TT+CT genotypes was significantly higher in CAD group than controls (OR: 3.41, CI: 1.89-6.14, p value < 0.05). Moreover, the frequency of C allele was significantly higher in CAD group than controls (OR: 2.26, CI: 1.50-3.43, p value < 0.05).

Table 1. Demographic, laboratory and clinical characteristics of the study subjects

Qualitative variables		CAD patients (no.=100) %	Controls (no.=100) %	p
Sex	females	38	46	0.25
	males	62	54	
Smoking	Non smokers	46	60	0.047*
	Smokers	54	40	
HTN	Negative	35	45	0.15
	Positive	65	55	
Family history	Negative	80	89	0.08
	Positive	20	11	
Statin use	Negative	36	49	0.06
	Positive	64	51	

Quantitative variables	CAD patients (no.=100)		Controls (no.=100)		p
	Mean ±SD	Range	Mean ±SD	Range	
Age (years)	57.28±5.86	47-71	56.3±5.91	46-68	0.24
BMI (kg/m^2)	31.3±4.37	25.2-44.6	30.92±4.76	24.2-44.2	0.56
Cholesterol [mg/dl]	197.89±34.27	141-282	199.04±34.34	141-268	0.81
HDL-C [mg/dl]	36.12±3.72	27-43	39.66±4.98	30-51	<0.001*
TG [mg/dl]	165.16±54.35	70-287	159.02±32.44	90-235	0.33
LDL-C [mg/dl]	128.05±30.57	78-187.6	127.58±31.71	72-192	0.91

Variable	CAD patients (no.=100)	
	Mean ±SD	Range
Gensini score	36.05±27.68	6-122

*p<0.05 is significant

3.3. Genotyping and allele frequency of SIRT1 rs3758391 T/C

The genotype distribution and allelic frequency of SIRT1 rs3758391 T/C among CAD and control groups are presented in Table 2. There was a statistically significant difference between both groups regarding genotype distribution of SIRT1 rs3758391 T/C (p value < 0.05). In the additive model, the frequency of rs3758391 CC vs. TT genotype was significantly higher in CAD patients than controls (OR: 3.34, CI: 1.50-7.47, p value < 0.05). In accession, in the recessive model, the frequency of rs3758391 CC vs. TT+CT genotypes was significantly higher in CAD group than controls (OR: 3.41, CI: 1.89-6.14, p value < 0.05). Moreover, the frequency of C allele was significantly higher in CAD group than controls (OR: 2.26, CI: 1.50-3.43, p value < 0.05).

Table 2. The genotype distribution and allele frequency of *SIRT1* rs3758391 T/C among the study groups.

Genotype		**CAD (no.=100)**		**Controls (no.=100)**		χ^2	**p**	**OR (95% CI)**
		No.	**%**	**No.**	**%**			
Additive	TT	14	14.0	23	23	17.21	<0.001*	1.00
	CT	29	29.0	49	49	0.004	0.94	0.97 (0.43-2.81)
	CC	57	57.0	28	28	9.05	0.002*	3.34 (1.50-7.47)
Dominant	TT	14	14.0	23	23	2.69	0.10	1.83 (0.88-3.81)
	CT+CC	86	86.0	77	77			
Recessive	TT+CT	43	43.0	72	72	17.21	<0.001*	3.41 (1.89-6.14)
	CC	57	57.0	28	28			
Alleles	T	57/200	28.5	95/200	47.5	15.32	<0.001*	2.26 (1.50-3.43)
	C	143/200	71.5	105/200	52.5			

*p<0.05 is significant
OR: odd ratio
CI: confidence interval

3.4. Demographic and clinical characteristics according to different SIRT1 rs3758391 genotypes of the CAD and the control groups.

There were non-significant statistical differences regarding gender, age, smoking habit, hypertension, family history, statin use and BMI between different *SIRT1* rs3758391 genotypes among both CAD and control groups (P values > 0.05).

3.5. Laboratory parameters according to different SIRT1 rs3758391 genotypes of the CAD and the control groups.

There were non-significant statistical differences regarding mean cholesterol level, HDL-C level, TG level and LDL-C level between different *SIRT1* rs3758391 genotypes among both CAD and control groups (P values > 0.05).

3.6. Gene expression analysis

SIRT1 gene expression levels were analyzed and calculated as 2^-ΔCT. *SIRT1* normalized gene expression levels in the studied groups are presented in Figure 1. *SIRT1* expression levels were significantly (p=0.001) reduced in CAD patients with mean±SD of 0.53±0.41 compared to the control with mean±SD of 1.05±0.86. Moreover, *SIRT1* gene expression levels were significantly lower in CC genotype compared to TT and CT genotypes of both CAD and control groups Table 3.

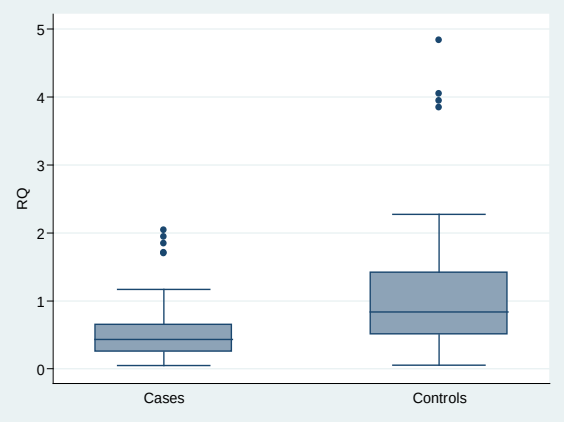

Figure 1. Normalized *SIRT1* gene expression levels among CAD and control groups.

Table 3. *SIRT1* expression levels according to different *SIRT1* rs3758391 genotypes of CAD and control groups.

Group	Genotype	*SIRT1* expression levels			Kruskal Wallis test (χ^2)	p
		No.	Mean ±SD	Range		
CAD group	TT	14	0.85±0.6	0.15-1.94	20.99	<0.001*
	CT	29	0.67±0.39	0.07-2.04		
	CC	57	†‡0.38±0.28	0.05-1.7		
control group	TT	23	1.39±0.89	0.05-4.04	17	<0.001*
	CT	49	1.14±0.92	0.08-4.83		
	CC	28	†‡0.63±0.47	0.09-1.68		

*p<0.05 is significant

†: Significant difference compared to genotype TT

‡: Significant difference compared to genotype CT

3.7. Gensini score analysis

Gensini score showed a significant inverse correlation with HDL-cholesterol (rho = -0.28, p=0.004) and a significant positive correlation with age (rho = 0.25, p=0.01). Moreover, Gensini score was significantly higher in males than in females (p=0.02) and even significantly higher in smokers than in non-tobacco users (p =0.04). Furthermore, Gensini scores were significantly higher in CC genotype, as compared with TT and CT genotypes of CAD group Table 4. On the other hand, non-significant associations or correlations with the other demographic, clinical, or laboratory data were detected.

Table 4. Gensini scores according to different *SIRT1* rs3758391 genotypes of CAD group.

Genotype	Gensini score			Kruskal Wallis test (χ^2)	p
	No.	Mean ±SD	Range		
TT	14	22.28±19.14	7-80	11.51	0.003*
CT	29	28.07±21.67	7-72		
CC	57	†‡43.49±29.96	6-122		

*p<0.05 is significant

†: Significant difference compared to genotype TT

‡: Significant difference compared to genotype CT

3.8. Correlation of SIRT1 gene expression with quantitative demographic and clinical data of patients and control

SIRT1 gene expression levels were correlated with quantitative demographic and clinical data as indicated in Table 5. *SIRT1* expression levels revealed a significant positive correlation with HDL-cholesterol in both CAD patients and even control group. On the other hand, it exhibited a significant inverse correlation with Gensini score in the CAD group. Otherwise, no other significant correlations were established.

3.9. Relation of SIRT1 expression levels with qualitative demographic and clinical data of CAD patients and control.

There were non-significant statistical differences in *SIRT1* expression levels regarding gender, smoking habit, hypertension, family history and statin use of both CAD and control groups (P values > 0.05).

3.10. Performance characteristics of SIRT1 expression (2^-ΔCT) as a predictor for atherosclerosis:

The best cut off values for 2^-ΔCT of the expressed *SIRT1* gene with the highest specificity, sensitivity, PPV and NPV for diagnosis of coronary atherosclerosis were determined and analyzed as shown in Table 6 and Figure 2. Using 0.0665 as a cut off value for 2^-ΔCt of *SIRT1* expression, the sensitivity and specificity of *SIRT1* to rule out coronary atherosclerosis was 76 % & 65%, respectively.

Table 5. Spearman's correlation of *SIRT1* expression levels with the quantitative demographic and laboratory data of CAD patients and control.

Group	Variable (no.=100)	Spearman correlation coefficient (rho; ρ)	p
CAD group	Age (years)	-0.02	0.80
	BMI (kg/m^2)	-0.09	0.38
	Cholesterol	-0.12	0.24
	HDL-C	0.30	0.003*
	TG	-0.08	0.45
	LDL-C	-0.16	0.11
	Gensini score	-0.23	0.02*
Control group	Age (years)	-0.14	0.17
	BMI (kg/m^2)	-0.11	0.27
	Cholesterol	-0.08	0.41
	HDL-C	0.22	0.03*
	TG	-0.09	0.38
	LDL-C	-0.11	0.28

*p<0.05 is significant

Table 6. Performance characteristics of *SIRT1* expression (2^-ΔCT) in predicting coronary atherosclerosis

Cut-off	0.665
Sensitivity (%)	76.00%
Specificity (%)	65.00%
PPV (%)	67.9%
NPV (%)	72.7%
Correctly diagnosed (%)	70.5%
AUC	0.7354

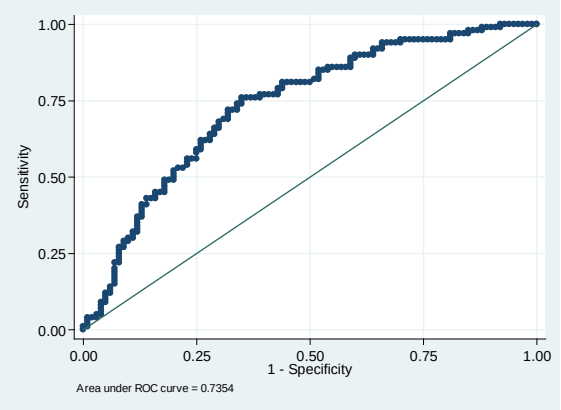

Figure 2. ROC analysis for *SIRT1* expression as a predictor for atherosclerosis

4. Discussion

CAD is a complex disease driven by numerous interactions of environmental and genetic factors. Identification of these interactions will offer valuable data for prevention and control of CAD. Important improvements have been made in identifying disease-causing genes and susceptibility genes for CAD (Abraham et al., 2014).

The International HapMap Project has recognized the presence of a single-nucleotide T/C polymorphism (rs3758391) in the identified p53-binding sequence of the *SIRT1* gene. The C allele of this polymorphism disturbs the mirror-image symmetry in the preserved nucleotides of the second half-site of the p53 binding sequence, raising the probability that this polymorphism may affect p53 binding affinity and hence impair *SIRT1* expression (Naqvi et al., 2010).

Regarding *SIRT1* rs3758391 T/C polymorphism, the current study revealed that CC genotype was more frequent in the CAD group (57%) than the control group (28%). Moreover, CC genotype conferred an increased risk of coronary atherosclerosis compared to those carrying CT+TT genotypes (OR: 3.41, CI: 1.81-6.42, p < 0.001). At the allelic level, C allele was significantly prevalent in CAD patients than the control group (71.5 % vs. 52.5 %) (OR: 2.27, CI:1.47-3.51, p < 0.001). These findings were consistent with a previous work on Iranian population, which revealed that rs3758391 CC genotype was more frequent in CAD patients in both additive (CC vs. TT) and recessive models (CC vs. TT +CT genotype) (Mohtavinejad et al., 2015). Furthermore, Mohtavinejad et al. (2015) concluded that the increase in the frequency of rs3758391 CC genotype might motivate the susceptibility of patients to CAD. In line with our results, they also reported that C allele was more prevalent in CAD patients than controls; however; the difference did not reach the borderline of statistical significance.

The present study revealed that there were no associations between rs3758391 SNP and total cholesterol, LDLc, HDLc and triglyceride levels. These results might not be representative of actual lipid profiles of patients involved in this study because most patients were under statin treatment. Nevertheless, these outcomes were consistent with a study conducted in Ashkenazi Jews (Han et al., 2015).

The current study demonstrated that Gensini scores were significantly higher in rs3758391 CC genotype carriers than in CT and TT carriers. In agreement with this finding, a previous study reported that carriers of the rs3758391 T allele had lower cardiovascular mortality risk among 1245 participants who were 85 years old or older (Kuningas et al., 2007).

The results of the current study revealed a statistically significant decrease in the expression level of *SIRT1* in PBMCs of CAD group, as compared with the control group. Several previous studies were in concordance with our results and reported the downregulation of *SIRT1* in PBMCs of CAD patients (Breitenstein et al., 2013; Li et al., 2016). Likewise, *SIRT1* expression was reported to be reduced in human atherosclerotic plaques in a study carried out by Gorenne et al. (2013). This finding was supported by a recent study, which reported that *SIRT1* inhibition induced the development of atherosclerotic plaque in ApoE–/– mice by increasing the expression of monocyte chemoattractant protein-1 (MCP-1) in addition to macrophage accumulation (Yang et al., 2017). Noteworthy, Chan et al. (2017) reported that LOX-1/oxidative stress signaling was induced and antioxidant enzyme activities were suppressed within CAD monocytes, suggesting the particular *SIRT1* role in protecting the cardiovascular system simply by means of its antioxidant, anti-inflammatory activities.

Based on the observations of the current study as well as the previous studies, the downregulation of *SIRT1* expression in CAD patients could be assigned to different factors. One of these factors may be the genetic variants of *SIRT1* gene. Increasing evidence suggested that *SIRT1* rs3758391 SNP impacts gene expression by decreasing p53 binding affinity, resulting in reduction of *SIRT1*

promoter activation and hence, down-regulation of *SIRT1* expression (Hu et al., 2015; Naqvi et al., 2010). Thus, rs3758391 in *SIRT1* gene promoter could be a functional SNP that might affect the transcription level of *SIRT1* gene.

The outcomes of the present work revealed that *SIRT1* expression correlated positively with HDL levels in both CAD patients and control groups. This finding was consistent with a study by Breitenstein et al. (2013) who suggested another contributing factor in the down-regulation of *SIRT1* expression in CAD patients. Breitenstein et al. (2013) proposed a regulatory role of HDL on *SIRT1* expression. They found that HDL of healthy subjects activated monocytes *SIRT1* expression considerably more prominently than HDL of patients with cardiac disorders, suggesting a signal mediating interaction between HDL and *SIRT1*. In addition, they suggested that Paraoxonase 1 (PON1), an HDL-associated antioxidant enzyme, is essential to stimulate *SIRT1* expression. Thus, reduced *SIRT1* expression levels in CAD patients appeared to be linked to reduced HDL levels and HDL dysfunction.

The current study revealed that *SIRT1* expression significantly reduced in CC genotype (the predominant genotype among the CAD group) as compared with CT and TT genotypes. This observation was supported by Hu et al. (2015) who demonstrated a significant association between the rs3758391 TT genotype and a higher *SIRT1* expression.

This study revealed that *SIRT1* expression levels in PBMCs of CAD patients showed a significant inverse correlation with Gensini score suggesting its role as an anti-atherogenic gene. Consistent with our results, de Kreutzenberg et al. (2010) reported that *SIRT1* expression was negatively correlated with subclinical atherosclerosis evaluated by carotid intima - media thickness. Conversely, our results were in contrast with Li et al. (2016) who did not detect a relationship between *SIRT1* expression, and the severity of coronary lesions evaluated by the Syntax score in patients with CAD and T2DM, recommending that *SIRT1* role in preventing atherosclerosis in diabetic patients be clarified. This inconsistency could be assigned to different measures of patient selection and to different amplification techniques used. Their study included diabetic patients and used semi quantitative PCR in evaluating *SIRT1* expression.

To estimate the diagnostic performance of *SIRT1*gene expression for atherosclerosis, the best cut off point for 2-ΔCt of the expressed gene and the matching sensitivity and specificity, PPV, NPV for prediction of coronary atherosclerosis were analyzed. The results showed that *SIRT1* expression had a good capability (Šimundić, 2009) in predicting CAD, suggesting that *SIRT1* expression might be of significant value in the prediction of CAD. Consistent with our results, He et al., 2019 reported that serum SIRT1 might play a predictive role in screening high- risk coronary plaques. The limitations of the current study were the relatively small sample size and the inability to study the racial and the ethnic effects because the study participants were all Egyptians.

5. Conclusion

SIRT1 rs3758391 CC genotype might confer an increased risk of coronary atherosclerosis. Additionally, *SIRT1* gene was down regulated in PBMCs of CAD patients and its expression was inversely correlated with atherosclerosis severity suggesting its protective effects against atherosclerosis development. Moreover, the variation in *SIRT1* expression was related to the presence of promoter SNP rs3758391 providing awareness to the mechanisms by which common non-coding genetic variants affects the target gene expression. Furthermore, *SIRT1* expression might be a valuable adjunct in diagnosing CAD.

Acknowledgements

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Abraham G., Bhalala O, de Bakker P, Ripatti S, Inouye M. 2014. Towards a molecular systems model of coronary artery disease. *Curr Cardiol Rep.*, 16: 488.

Ali O, Abdelgawad H, Mohammed M, El-Awady R. 2014. Ischemic heart diseases in Egypt: role of xanthine oxidase system and ischemia-modified albumin. *Heart Vessels*., **29:** 629- 637.

Almahmeed W, Arnaout M, Chettaoui R, Ibrahim M, Kurdi M, Taher M, Mancia G. 2012. Coronary artery disease in Africa and the Middle East. *Ther Clin Risk Manag*., **8**: 65- 72.

Amos DB and Pool P. 1676. "HLA typing" in Manual of Clinical Immunology, Rose, N.R., and Friedman,H., eds., *American Society for Microbiology*., 797-804.

Breitenstein A1, Wyss CA, Spescha RD, Franzeck FC, Hof D, Riwanto M, Hasun M, Akhmedov A, von Eckardstein A, Maier W, Landmesser U. 2013. Peripheral blood monocyte Sirt1 expression is reduced in patients with coronary artery disease. *PLoS One.*, **8(1)**: e53106.

Chan S, Hung C, Shih J, Chu P, Cheng Y, Lin H and Tsai K. 2017. SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease. *Redox Biol.*, **13**: 301-309.

Cui Y, Wang H, Chen H, Pang S, Liu D, Yan B. 2012. Genetic analysis of the SIRT1 gene promoter in myocardial infarction. *Biochem Biophys Res Commun*., **426**: 232–236.

de Kreutzenberg SV, Ceolotto G, Papparella I et al. 2010. "Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms," *Diabetes.*, **59 (4)** 1006–1015.

Edirisinghe I and Rahman I 2010. Cigarette smoke-mediated oxidative stress, shear stress, and endothelial dysfunction: role of VEGFR2. *Ann. N. Y. Acad. Sci*., **1203**:66–72.

Fawzy M, Toraih E, Aly N, Fakhr-Eldeen A, Badran D, Hussein M. 2017. Atherosclerotic and thrombotic genetic and environmental determinants in Egyptian coronary artery disease patients: a pilot study. *BMC Cardiovasc Disord*., 13;**17(1):**26.

Gensini GG. 1983. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol*., 51: 606.

Glenn S, Head K, Teh B, Gross K, Kim H. 2010. Maximizing RNA yield from archival renal tumors and optimizing gene expression analysis. *J Biomol Screen.*, **15**: 80- 85.

Gorenne I, Kumar S, Gray K, Figg N, Yu H, Mercer J, Bennett M. 2013. Vascular smooth muscle cell sirtuin 1 protects against DNA damage and inhibits atherosclerosis. *Circulation.*, **127**: 386-396.

Han J, Wei M, Wang Q, Li X, Zhu C, Mao Y, Wei L, Sun Y, Jia W. 2015. Association of Genetic Variants of SIRT1 With Type 2 Diabetes Mellitus. *Gene Expr.*, **16**: 177- 185.

He X, Zheng J, Liu C. 2019. Low serum level of sirtuin 1 predicts coronary atherosclerosis plaques during computed tomography angiography among an asymptomatic cohort. *Coron Artery Dis.* **30(8):**621-625.

Hedberg S, Eriksson L, Malm K, Mölling P, Sundqvist M, Andersson S. 2015. Droplet Digital PCR for absolute quantification and determination of proviral load of HTLV-1 and 2. *Retrovirology*., 12: 71.

Hu Y, Wang L, Chen S, Liu X, Li H, Lu X, Yang X, Huang J, Gu D. 2015. Association between the SIRT1 mRNA expression and acute coronary syndrome. *J Atheroscler Thromb.*, **22**: 165-182.

Kathiresan S and Srivastava D. 2012. Genetics of human cardiovascular disease. *Cell.*, **148**: 1242–1257.

Kuningas M, Putters M, Westendorp R, Slagboom P, van Heemst D. 2007. SIRT1 gene, age-related diseases, and mortality: the Leiden 85-plus study. *J Gerontol A Biol Sci Med Sci.*, **62**: 960–965.

Li P1, Liang ML1, Zhu Y1, Gong YY1, Wang Y1, Heng D1, Lin L1. 2014. Resveratrol inhibits collagen I synthesis by suppressing IGF1R act ivation in intestinal fibroblasts. *World J Gastroenterol.*, 28;**20(16):**4648-61.

Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. 2007. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol Cell*., **28:**91-106.

Li Y, Ni J, Guo R, Li W. 2016. In Patients with Coronary Artery Disease and Type 2 Diabetes, SI RT1 Expression in CirculatingMononuclear Cells Is Associated w ith Levels of Inflammatory Cytokines but Not with Coronary Lesions. *Biomed Res Int.*, 8734827.

Libby P, Ridker P, Hansson G. 2011. Progress and challenges in translating the biology of atherosclerosis. *Nature*., **473**: 317- 325.

Mohtavinejad N, Nakhaee A, Harati H, Poodineh J, Afzali M. 2015. SIRT1 gene is associated with cardiovascular disease in the Iranian population. *The Egyptian Journal of Medical Human Genetics.*, **16**: 117- 122.

N. D'Onofrio, M. Vitiello, R. Casale, L. Servillo, A. Giovane, and M. L. Balestrieri. 2015. "Sirtuins in vascular diseases: emerging roles and therapeutic potential," *Biochimica et Biophysica Acta—Molecular Basis of Disease*., **1852 (7)** 1311–1322.

Naqvi A, Hoffman T, DeRicco J, Kumar A, Kim C, Jung S, Yamamori T, Kim Y, Mehdi F, Kumar S, Rankinen T, Ravussin E, Irani K. 2010. A single-nucleotide variation in a p53-binding site affects nutrient-sensitive human SIRT1 expression. *Hum Mol Genet.*, **19**: 4123- 4133.

Nasiri M, Rauf M, Kamfiroozie H, Zibaeenezhad MJ, Jamali Z. 2018. SIRT1 gene polymorphisms associated with decreased risk of atherosclerotic coronary artery disease. *Gene.*, **25;672**:16-20.

Poulose N and Raju R. 2015. Sirtuin regulation in aging and injury. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*., **1852;11:** 2442-2455.

Reiber J, van der Zwet P, van Land C, Koning G, Loois G, Zorn I, van den Brand M, Gerbrands J. 1989. "On-line quantification of coronary angiograms with the DCI system". *Medica mundi.*, **34**: 89- 98.

Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ et al. 2011. Heart Disease and Stroke Statistics Update: A Report from the American Heart Association. *Circulation*., **123**: e18–e209.

Romeiro M, de Souza W, Tolardo A, Vieira L, Colombo T, Aquino V, Nogueira M, Figueiredo L. 2016. Evaluation and optimization of SYBR Green real-time reverse transcription polymerase chain reaction as a tool for diagnosis of the Flavivirus genus in Brazil. *Rev Soc Bras Med Trop*., 49: 279- 285.

Schmittgen T and Livak K. 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc.*, **3**: 1101- 1108.

Schuijf JD, van Werkhoven JM, Pundziute G, Jukema JW, Decramer I, Stokkel MP, Dibbets-Schneider P, Schalij MJ, Reiber JHC, Van Der Wall EE, Wijns W, Bax JJ. 2008. Invasive Versus Noninvasive Evaluation of Coronary Artery Disease. 2008. *J Am Coll Cardiol Img*., **1(2):**190-199.

Simon A and Vijayakumar T. 2013. Molecular Studies on Coronary Artery Disease—A Review. *Indian J Clin Biochem.*, **28**: 215-226.

Šimundić A. 2009. Measures of Diagnostic Accuracy: Basic Definitions. *EJICC.*, **19:** 203- 211.

Stein S, Lohmann C, Schäfer N, Hofmann J, Rohrer L, Besler C, Rothgiesser KM, Becher B, Hottiger MO, Borén J, McBurney MW, Landmesser U, Lüscher TF, Matter CM. 2010 a. SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis. *Eur. Heart J.*, **31**:2301–2309.

Stein S, Schäfer N, Breitenstein A, Besler C, Winnik S, Lohmann C, Heinrich K, Brokopp CE, Handschin C, Landmesser U, Tanner FC, Lüscher TF, Matter CM.2010 b. SIRT1 reduces endothelial activation without affecting vascular fu nction in ApoE-/- mice. Aging (Albany NY)., **2(6):**353-60.

Yamac AH, Uysal O, Ismailoglu Z, Ertürk M, Celikten M, Bacaksiz A, Kilic U. 2019. Premature Myocardial Infarction: Genetic Variations in SIRT1 Affect Disease Susceptibility. *Cardiology Research and Practice*., Article ID 8921806, 10 pages.

Yamashita S, Tsubakio-Yamamoto K, Ohama T, Nakagawa-Toyama Y, Nishida M. 2010. Molecular mechanisms of HDL-cholesterol elevation by statins and its effects on HDL functions. *J Atheroscler Thromb*., **17(5):**436-51.

Yang X, Wei J, He Y Jing T, Li Y, Xiao Y, Wang B, Wang W, Zhang J, Lin R. 2017. SIRT1 inhibition promotes atherosclerosis through impaired autophagy. *Oncotarget.*, **8:** 51447- 51461.

Yusuf S, Ounpuu S, Anand S. 1998. Global burden of cardiovascular disease: A review of evidence. In: Sethi KK, ed. Coronary Artery Disease in Indians: A Global Perspective. *Mumbai: Cardiological Society of India*.,11-25.

Zhang H, Finiguerra M, Dam HG, Huang Y, Xu D, Liu G, Lin S. 2013. An improved method for achieving high-quality RNA for copepod transcriptomic studies. *J. Exp. Mar. Biol. Ecol.*, **446:**57-66.

Zhang T and Kraus WL. 2010. SIRT1-dependent Regulation of Chromatin and Transcription: Linking NAD+ Metabolism and Signaling to the Control of Cellular Functions. *Biochim Biophys Acta*., **1804(8):** 1666–1675.

Zheng J, Chen L Xiao F, Hu X, Deng X, Li H. 2012. Three single nucleotide variants of the SIRT1 gene are associated with overweight in a Chinese population: a case control study. *Endocr J*., **59:** 229- 237.

Zhong X, Miao H, Fang Z, Kuken B, Song H, Zhong H, Lu Y, Liu S. 2015. The effect of SIRT1 gene polymorphisms on ambulatory blood pressure of hypertensive patients in the Kazakh population. *Genet Test Mol Biomarkers.*, **19:** 561- 565.