

*Full Length Research Paper*

## **New insight on premature atherosclerosis in Egyptian children with $\beta$ -thalassemia major**

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**Beta-thalassemia major ( $\beta$ -TM) in children is associated with an increase in the risk of premature cardiovascular complications caused by accelerated atherosclerosis which significantly contributes to morbidity and mortality. The molecular mechanisms underlying  $\beta$ -TM associated atherosclerosis and its relation to biochemical risk factors still remain obscure. We aimed to investigate the association between SIRT1 single nucleotide polymorphism (SNP), lipid profile, ferritin, malondialdehyde (MDA), 8-hydroxydeoxy guanosine (8-OHdG) and total antioxidant capacity (TAC) in relation to carotid intima media thickness (CIMT) trying to explain some mechanisms of cardiovascular complication in beta-Thalassemia major ( $\beta$ -TM) children. This study was carried out on 100 Egyptian children with  $\beta$ -TM (Group II) subdivided equally according to CIMT into Group IIa, with CIMT<0.5 mm and Group IIb with CIMT  $\geq$  0.5 mm, in addition to 50 healthy children as controls (Group I). All groups were subjected to measurement of plasma Ferritin, lipid profile, MDA and TAC colorimetrically. 8-OHdG levels were estimated by enzyme-linked immunosorbent assay (ELISA) in addition genotyping pattern for the SIRT1 (rs7069102) SNP was evaluated using PCR-CTPP technique. Values for AIP, ferritin, MDA and 8-OHdG levels, were significantly higher in  $\beta$ -TM groups compared to control with higher levels in patients with CIMT more than 0.5 mm but values of TAC and (HDL-C) showed significant decrease. CIMT was significantly correlated with age, atherogenic index of plasma (AIP), ferritin, MDA and 8-OHdG, HDL-C and TAC. There was significant difference in C allele distribution of SIRT1 rs7096102 was 57% in control subjects, 29% in Group IIa and 23% in Group IIb. However, the allele G distribution was 43% in control subjects, 71% in Group IIa and 77% Group IIb. Significant association of SIRT1 (rs7069102) polymorphism with dyslipidemia, ferritin and oxidative stress may conduct atherosclerotic potential in  $\beta$ -TM by affecting the severity of CIMT.**

**Key words:** Beta-thalassemia major, carotid intima media thickness.

### **INTRODUCTION**

Beta-thalassemia ( $\beta$ -TM) represents the commonest cause of hemolytic anemia in Egypt with carrier rate

ranges from 9-10% (Li, 2017). Early vascular alteration, atherosclerosis and coronary artery diseases have

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emerged as important cardiovascular complications among  $\beta$ -TM patients (Sullivan, 2009). The mechanisms underlying the accelerated atherosclerosis in  $\beta$ -TM are not clear because the traditional risk factors fail to account fully for the excess of cardiovascular events in  $\beta$ -TM patients. Therefore, it has been suggested that patients with  $\beta$ -TM possess additional risks in addition to the traditional risk factors for the development of accelerated atherosclerosis (Tantiworawit et al., 2016). In atherogenesis, arterial wall morphological changes occur during a presumably long subclinical lag phase, and characterized by gradual thickening of the intima. Beside the traditional diagnostic methods such as angiography and stress-testing, measurement of the intima-media (IMT) thickness of the large arteries, especially the carotids, is a non-invasive predictor of early arterial wall alteration, which identifies and quantifies early structural vascular abnormalities and is currently considered as a marker of premature atherosclerosis and also is predictive of future cardiovascular events (Plasencia and García, 2017).

Studies have suggested a link between iron load in transfusion-dependent thalassemia patients and risk of atherosclerosis. The excess iron in  $\beta$ -thalassemia patients saturates the ability of the transferrin iron transport system. This leads to non-transferrin bound iron and labile plasma iron which start to circulate in the plasma and subsequently become deposited inside susceptible cells including the cells within the atherogenic plaque, as endothelial cells, macrophages and VSMCs (Porter et al., 2016).

Iron overload state  $\beta$ -TM patient is associated with generation of reactive oxygen species (ROS) (Cihan et al., 2017). In physiological conditions, ROS are scavenged by the antioxidant system, but when their concentration is too high, an oxidative damage to proteins, lipids, and DNA occurs (Braeckman et al., 2017). Free radicals can cause DNA-protein cross-links, damage to the deoxyribose-phosphate backbone, and specific modifications of purine and pyrimidine bases (Martins et al., 2017). Among more than 20 different products known to be formed by exposure of DNA bases to the -OH, 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the major oxidized DNA bases accordingly, it was proposed to be an excellent marker for estimating oxidative damage to DNA (Jin et al., 2016). Studies investigated the oxidative DNA damage that occurs in human atherosclerotic plaques and DNA repair mechanisms which are upregulated in response to DNA damage (Vakonaki et al., 2016; Shah et al., 2017).

One of the candidate molecules in the pathogenesis of atherosclerosis in  $\beta$ -TM is the SIRT1 protein which belongs to Sirtuin family consisting of seven members in humans (*SIRT1-7*) (Chen et al., 2017). *SIRT1* gene is located on chromosome 10q21.3 and consists of 11 exons and 10 introns. The *SIRT1* gene has different single nucleotide polymorphisms including, (rs7895833

A.G in the promoter region, rs7069102 C.G in intron 4 and rs2273773 C.T in exon 5 silent mutation) (Kilic et al., 2014). SIRT1 protein, a NAD-dependent histone deacetylase, is expressed in brain, heart, kidney, liver, pancreas, spleen, skeletal muscle and endothelial tissue. Any alteration in endothelial SIRT1 will affect normal endothelial function and thereby vascular physiology (Winnik et al., 2015). Although its roles in initiation and progression of atherosclerosis were already reported (Kilic et al., 2014), there is almost no study related SIRT1 gene single-nucleotide polymorphisms (SNPs) and the development of carotid atherosclerosis in Egyptian children with  $\beta$ -TM.

The aim of this study was to investigate, the relationship between alteration in SIRT1 rs7069102 (SNP) and lipid profile, ferritin, MDA, 8-OHdG and TAC in Egyptian children with  $\beta$ -TM. And, their associated alteration in relevance to CAD development based on their correlation to CIMT which may ultimately help us to present early detection of cardiac phenotype. The study protocol was approved by the research and ethical committee of Faculty of Medicine, Tanta University and written informed consent was taken from parents or guardian of each participant.

## MATERIALS AND METHODS

This study was carried out on 100  $\beta$ -thalassemia major children aged 6-14 years presented to Outpatient Clinic of hematology unit of Pediatric Department, Tanta University Hospital. As well as 50 apparently healthy children of matched age and sex represented as control group (Group I). Group II ( $\beta$ -thalassemia major group) were subdivided according to CIMT as two groups: Group IIa, with CIMT < 0.5 mm (n = 50) and Group IIb, with CIMT  $\geq$  0.5 mm (n = 50).

### Exclusion criteria

Those with familiar hypercholesterolemia (confirmed by history), cardiovascular symptoms suggesting the presence of heart failure or chronic systemic illness, Renal failure, Hepatitis c virus infection, Liver failure, Diabetes mellitus, Chronic hepatitis and HIV infection. Informed written consent was obtained from all children's parents enrolled in the study. Approval was obtained from the Local Research Ethics Committee, Tanta University.

### Carotid intima media thickness (CIMT)

By Duplex Ultrasound B-mode and color-coded duplex sonography. All studies were performed using Tissue Doppler using G.E. Vivid 7 echocardiogram. All ultrasound examinations were performed by pediatric cardiologist who was unaware of the clinical and laboratory details of the examined children. Examination started by locating the common carotid artery (CCA) in the lower neck in the transverse plane. The vessels were evaluated meticulously for the presence of subintimal lucency, and atherosclerotic plaques that bulge into the lumen, followed by measuring the intimal plus medial thickness (IMT). For each subject, three measurements on both sides were obtained on the anterior, lateral, and posterior projection of the far wall. Values for the different projections and for right and left arteries were then averaged. The average of the two sides was

considered the patient's overall mean CIMT (Stein et al., 2008). In this study thalassemia patients were classified into patients with CIMT <0.5 mm (n= 50) and patients with CIMT  $\geq$  0.5 mm (n= 50).

### Blood sample collection

After 12 h of overnight fasting, 7 ml of venous blood samples were taken from every investigated subject, they were divided into two parts, the first in DNA extraction specific (EDTA) containing blood collecting tubes for PCR analysis. The rest of the sample was collected in disposable plastic tube containing EDTA and centrifuged for plasma separation and frozen at  $-80^{\circ}\text{C}$  for future analysis.

### Laboratory investigations included

(i) Total cholesterol (TC), triglyceride (TG) and the high density lipoprotein-cholesterol (HDL-C) concentration were determined using commercial kits (Biomed Diagnostics). Low-density lipoprotein -cholesterol (LDL-C) concentrations were calculated using Friedewald's equation. ( $\text{LDL-C}=\text{TC}-\text{HDL-C}-\text{TG}/5$ ) (Friedewald et al., 1972).

(ii) Plasma ferritin levels as index of iron overload by colorimetric method using commercial kits (Biomed Diagnostics) (Finch et al., 1986).

(iii) Plasma malondialdehyde (MDA) levels by colorimetric method using commercial kits (Biodiagnostics) (Ohkawa et al., 1979).

(iv) Plasma 8-hydroxy 2-deoxyguanosine (8-OHdG) levels by ELISA using Bioseps® 8-OHdG ELISA kit supplied by (Vivantis International) according to Öngöz et al. (2013).

(v) Plasma Total antioxidant capacity (TAC) levels by colorimetric method using commercial kits (Biodiagnostics) (Koracevic et al., 2001).

(vi) Genotyping of the SIRT1(rs7069102) gene polymorphism by PCR-CTPP technique according to Atsuta and Hamajima (2003) and Kilic et al. (2015). Briefly, Genomic DNA was extracted from 200  $\mu\text{l}$  of EDTA-anticoagulated blood using the Qiagen mini blood DNA purification kits (Qiagen, Canada) according to the manufacturer's instructions. SIRT1 genomic variants were detected by polymerase chain reaction using confronting two-pair primers (PCR-CTPP) technique. The primers used for amplification were: (forward1) 5'-GTAGCAGGAACCTACAGGCCTG -3' (forward2) 5'-GAGAAGAAAAGAAAGGCATAATCTCTGTC-3', (reverse1) 5'-CTATCTGCAGAAATAATGGCTTTTCTC-3', (reverse2) 5'-GATCGAGACCATCCTGGCTAAG-3'.

### Preparation of working primer mix (5 $\mu\text{M}$ each primer)

After reconstitution of each lyophilized primer which yield 100  $\mu\text{M}$  stocks, the working primer was prepared by mixing 15  $\mu\text{l}$  from each primer at equimolar concentration (5  $\mu\text{M}$  each primer) was added to 240  $\mu\text{l}$  PCR grade water (1:20 dilution). The working primer mix was stored at  $-20^{\circ}\text{C}$ . PCR was performed in 50  $\mu\text{l}$  volumes containing 25  $\mu\text{l}$  2X Taq master mix, 8  $\mu\text{l}$  Primer mix (Vivantis International, Egypt), 12  $\mu\text{l}$  extracted DNA, 2.5  $\mu\text{l}$  nuclease free water and 2.5  $\mu\text{l}$   $\text{MgCl}_2$ . Cycle conditions used were  $95^{\circ}\text{C}$  for 15 min, followed by 35 cycles of  $95^{\circ}\text{C}$  for 30 s,  $62^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 40 s, with a final extension of  $72^{\circ}\text{C}$  for 10 min. The PCR product was analyzed by visualization in agarose gels containing 2% ethidium bromide. Samples showing two bands of 391 and 277 bp were considered C/C, samples showing two bands of 391 and 167 bp were assigned

G/G and samples showing three bands of 391, 277 and 167 bp were typed as C/G.

### Statistical analysis

Statistical analysis in this study was performed using SPSS version 23. Basic and clinical variables are mentioned as mean  $\pm$  SD for the quantitative variables, and are summarized using frequency (percentage) for the categorical variables. ANOVA test was applied to compare variables between 3 groups, whereas the frequencies of various alleles and genotypes were compared between the groups by Chi-square test ( $\chi^2$ ). Pearson's correlation analysis was used to examine the relationships between IMT and tested atherosclerotic risk factors and between age, ferritin, lipid profile, AIP, MDA, plasma 8-OHdG and TAC. Associations between CIMT, and other risk factors were evaluated by multiple linear regressions. All statistical tests were two-tailed and only a P value  $\leq 0.05$  was considered statistically significant.

## RESULTS

Table 1 shows significant positive correlation between CIMT and age, ferritin, AIP, MDA and 8-OHdG. However, there was statistically significant negative correlation between CIMT and HDL-C and TAC in thalassemic patients with CIMT < 0.5 mm. Also, significant positive correlation between CIMT and age, ferritin, TG, AIP, MDA and 8-OHdG. However, there was statistically significant negative correlation between CIMT and HDL-C and TAC in thalassemic patients with CIMT < 0.5 mm ( $P < 0.05$ ).

Table 2 shows multiple linear regression analysis of factors that might independently be associated with CIMT. It was performed on a number of predictors including plasma levels of ferritin, TAG, HDL-C, AIP, MDA, 8-OHdG and TAC as independent variables, and CIMT as the dependent variable. It was found that ferritin followed by 8-OHdG levels were the most important predictors of CIMT. SIRT1 rs7069102 gene polymorphism distribution among the studied groups. The frequency of the CC (reference gene) was 12% in patients vs 28% in the control children and the frequency of the CG was 28% in patients vs 58% in the control children with odds ratio (OR= 1.126). However, that of GG was 60% in patients vs 14% in the control children (OR=8.878), with ( $\chi^2=29.303$ ;  $P=0.001$ ) as shown in Table 3. Table 4 shows comparison between studied genotypes of SIRT1 rs7069102 as regards to Atherosclerotic risk factors in  $\beta$ -TM patients (Group IIa and Group IIb). There were significant differences in age, TG, LDL-C AIP, CIMT and plasma ferritin MDA, 8-OHdG and TAC values among different genotypes (CC, CG and GG),  $P < 0.05$ . PCR-CTPP products of (rs7069102) SIRT1 gene polymorphism run on ethidium bromide stained 2% agarose gel were represented in (Figure 1). The figure shows the possible three genotypes for (rs7069102) SIRT1 gene; homozygous (CC), homozygous for (GG) and heterozygous (CG).

**Table 1.** Correlation between CIMT and age, ferritin, lipid profile, AIP, MDA, plasma 8-OHdG and TAC in  $\beta$ -TM (Group IIa and IIb).

Parameter	Correlation CIMT (mm)					
	$\beta$ -TM CIMT < 0.5 mm (Group IIa) (n=50)			$\beta$ -TM CIMT $\geq$ 0.5 mm (Group IIb) (n=50)		
	Mean $\pm$ SD	r	P-value	Mean $\pm$ SD	r	P-value
Age	8.840 $\pm$ 1.777	0.551	<0.001**	8.850 $\pm$ 1.911	0.540	<0.001**
Ferritin (ng/dl)	3872.52 $\pm$ 1007.12	0.548	<0.001**	4701.62 $\pm$ 1541.67	0.567	<0.001**
Total-cholesterol (mg/dl)	125.04 $\pm$ 4.553	0.065	0.513	127.88 $\pm$ 4.805	0.071	0.623
TG (mg/dl)	102.02 $\pm$ 1.202	0.023	0.876	136.14 $\pm$ 0.726	0.414	0.003*
HDL-C (mg/dl)	53.88 $\pm$ 4.446	-0.405	0.003*	38.28 $\pm$ 3.399	-0.644	<0.001**
LDL-C (mg/dl)	57.16 $\pm$ 5.567	0.164	0.254	58.96 $\pm$ 7.165	0.158	0.273
AIP	0.277 $\pm$ 0.047	0.283	0.046*	0.552 $\pm$ 0.043	0.720	<0.001**
MDA (nmol/ml)	6.076 $\pm$ 1.094	0.264	0.048*	7.421 $\pm$ 0.783	0.312	0.027*
8-OHdG (ng/L)	47.501 $\pm$ 15.836	0.292	0.040*	63.351 $\pm$ 14.809	0.521	<0.001**
TAC (mM/L)	0.377 $\pm$ 0.083	-0.297	0.036*	0.309 $\pm$ 0.080	-0.393	0.005*

**Group I:** healthy children (control group); **Group IIa:** thalassemia patients with CIMT < 0.5 mm; **Group IIb:** thalassemia patients with CIMT  $\geq$  0.5 mm; **CIMT:** carotid intima media thickness; **TG:** triglycerides; **HDL-C:** high density lipoprotein cholesterol; **LDL-C:** low density lipoprotein cholesterol; **AIP:** atherogenic index of plasma; **MDA:** malondialdehyde; **8-OHdG:** 8 hydroxy 2 deoxy guanosine; **TAC:** total antioxidant capacity.

\* means significant at  $P \leq 0.05$ .

**Table 2.** Multiple regression analysis of ferritin, TG, HDL-C, AIP, MDA, 8-OHdG and TAC  $\beta$ -TM patients as regards CIMT.

Variable	B	S.E.	t	P-value	Beta	95.0% C.I. for odd	
						Lower	Upper
Ferritin (ng/dl)	2.071	0.000	3.519	0.001*	0.107	0.000	0.001
TG (mg/dl)	0.001	0.004	0.049	0.951	0.015	0.002	0.009
HDL-C (mg/dl)	0.002	0.010	0.178	0.859	0.055	0.021	0.038
AIP	1.195	1.082	1.105	0.272	0.654	0.955	3.346
MDA (nmol/ml)	0.005	0.009	0.525	0.601	0.027	0.022	0.33
8-OHdG (ng/L)	0.001	0.001	2.404	0.018*	0.083	3.954	15.429
TAC (mM/L)	-0.031	0.114	-0.274	0.785	-0.009	-0.258	0.195
Constant	-0.124	0.516	-0.240	0.811			

**Table 3.** SIRT1 rs7069102 gene polymorphism distribution among the studied groups.

Polymorphism		Group		OR	95%CI
		Control (Group I) (n=50)	$\beta$ -TM (Group II) (n=100)		
CC	N	14	12	--	--
	%	28	12		
CG	N	29	28	1.1	0.445-2.854
	%	58	28		
GG	N	7	60	8.8	3.470-22.713
	%	14	60		
Total	N	50	100		
	%	100.00	100.00		
Chi-square	$X^2$		29.303		
	P-value		0.001*		

**Table 4.** Atherosclerotic risk factors of  $\beta$ -TM patients regarding SIRT 1 gene polymorphism.

$\beta$ -TM CIMT < 0.5mm Group IIa (n=50)	ANOVA			ANOVA	P value	$\beta$ -TM CIMT $\geq$ 0.5mm Group IIa (n=50)	ANOVA			ANOVA	P value
	CC (n=7)	CG (n=15)	GG (n=28)				CC (n=5)	CG (n=13)	GG (n=32)		
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	F			Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	F	
Age	8.140 $\pm$ 1.574	9.130 $\pm$ 1.922	10.36 $\pm$ 1.592	3.429	0.041*	Age	8.20 $\pm$ 1.924	9.31 $\pm$ 2.016	9.88 $\pm$ 1.913	0.254	0.777
Ferritin (ng/dl)	3348.6 $\pm$ 466.634	3348.67 $\pm$ 640.22	4438.5 $\pm$ 870.455	18.97	<0.001*	Ferritin (ng/dl)	2248 $\pm$ 346.357	4233.62 $\pm$ 1538.44	5233 $\pm$ 1297.89	12.15	<0.001*
Total-cholesterol (mg/dl)	125.71 $\pm$ 3.684	122.667 $\pm$ 4.859	126.12 $\pm$ 4.231	3.199	0.05	Total-cholesterol (mg/dl)	121.5 $\pm$ 7.295	127.08 $\pm$ 6.103	128.68 $\pm$ 3.458	1.365	0.265
TG (mg/dl)	94.714 $\pm$ 1.254	99.733 $\pm$ 7.950	105.07 $\pm$ 8.463	5.922	0.005*	TG (mg/dl)	131.25 $\pm$ 2.50	132.25 $\pm$ 2.41	138.867 $\pm$ 4.64	18.02	<0.001*
HDL-C (mg/dl)	55.857 $\pm$ 5.490	54.067 $\pm$ 2.463	53.285 $\pm$ 4.198	1.197	0.311	HDL-C (mg/dl)	42.667 $\pm$ 2.582	40.692 $\pm$ 2.897	36.419 $\pm$ 2.110	26.20	<0.001*
LDL-C (mg/dl)	54.427 $\pm$ 2.637	55.20 $\pm$ 5.281	58.893 $\pm$ 5.750	3.440	0.04*	LDL-C (mg/dl)	57.32 $\pm$ 8.0	61.77 $\pm$ 5.96	61.387 $\pm$ 7.22	2.076	0.137
AIP	0.231 $\pm$ 0.04	0.265 $\pm$ 0.043	0.295 $\pm$ 0.043	7.151	0.002*	AIP	0.50 $\pm$ 0.034	0.52 $\pm$ 0.030	0.576 $\pm$ 0.031	25.11	<0.001*
CIMT (mm)	0.20 $\pm$ 0.01	0.223 $\pm$ 0.04	0.30 $\pm$ 0.07	13.20	<0.001*	CIMT (mm)	0.59 $\pm$ 0.052	0.64 $\pm$ 0.077	0.81 $\pm$ 0.134	17.01	<0.001*
MDA (nmol/ml)	3.486 $\pm$ 0.807	4.580 $\pm$ 0.484	5.757 $\pm$ 0.793	37.680	<0.001*	MDA (nmol/ml)	5.980 $\pm$ 0.477	6.992 $\pm$ 0.582	7.878 $\pm$ 0.525	33.686	<0.001*
8-OHdG (ng/L)	29.752 $\pm$ 2.723	36.316 $\pm$ 5.079	58.717 $\pm$ 12.545	39.621	<0.001*	8-OHdG (ng/L)	44.130 $\pm$ 1.375	52.315 $\pm$ 7.272	71.706 $\pm$ 12.706	23.626	<0.001*
TAC (mM/L)	0.426 $\pm$ 0.022	0.31 $\pm$ 0.0318	0.320 $\pm$ 0.0891	6.867	0.002*	TAC (mM/L)	0.346 $\pm$ 0.012	0.290 $\pm$ 0.0485	0.240 $\pm$ 0.0464	12.601	<0.001*



**Figure 1.** PCR-CTPP products of (rs7069102) SIRT1 gene polymorphism of representative samples run on ethidium bromide stained 2% agarose gel. **Lane M** represents a 100 bp marker ladder (Ultra Low Range DNA Ladder) (100-3000 bp) served as reference for DNA fragment size. **Lane 1, 3 and 8** show two bands at 391 bp and 277 bp. It is homozygous for (CC). **Lane 4, 6, 10, 11, 13, 14 and 15** show two bands at 391 bp and 167 bp. It is homozygous for (GG). **Lane 2, 5, 7, 9 and 12** show 3 bands at 391 bp, 277 bp and 167 bp they are heterozygous (CG).

## DISCUSSION

The  $\beta$ -TM is considered the commonest cause of hemolytic anemia in Egypt with carrier rate (El-Beshlawy and Youssry, 2009) Cardiac complications attributable to the development of accelerated atherosclerosis represent the primary cause of mortality and one of the major causes of morbidity in children with thalassemia it may be related to gene-environment interaction due to epigenetic factors such as hyperlipidemia, hypertension, age, family history, diabetes mellitus, and obesity (Nafady et al., 2017). Iron overload noted in patients with  $\beta$ -TM could ultimately lead to alterations in the arterial structures and potentiate the development of premature atherosclerosis with increased arterial stiffness and endothelial dysfunction (Farmakis, 2017).

In the current study, there was significant increase in plasma ferritin levels, the marker of iron overload in both groups of  $\beta$ -TM as compared to controls. In fact, the excess iron saturates the ability of the transferrin iron transport system. When the magnitude of the cellular labile iron pool exceeds the capacity of the cell to synthesize new ferritin molecules, a critical concentration are reached that can generate reactive oxygen species (ROS) (Bresgen and Eckl, 2015). This finding came in alignment with reports by Lathanatudom et al. (2011) who suggested that high ferritin levels in  $\beta$ -TM coordinated with development of autophagy in erythroblasts.

In the present study, not only a significant increase in CIMT in Group IIb as compared with Group IIa and controls, but also its significant correlation with plasma ferritin and triglycerides was detected. It thereby reflected our main concern to investigate the associated mechanisms identifying the relationship between lipid profile, serum ferritin increments and development of CIMT in  $\beta$ -TM. Moreover, there was a colink to assess changes in 8-OHdG and SIRT1 SNP in response to oxidative stress was investigated in relevance to changes in TAC and consequent lipid peroxidation represented by MDA. On these bases, the assessed increments in the above mentioned parameters except for total cholesterol, LDL-C, HDL-C and TAC decrements were evident in children with  $\beta$ -TM who were classified according to assessed CIMT into two subgroups (Group IIa with CIMT < than 0.5 mm and Group IIb with CIMT  $\geq$  than 0.5 mm). The attained results were in agreement with scientific evidences that interrelated the adverse effects of abnormal blood lipid levels to consequent development of atherosclerosis (Ragab et al., 2014). The monitored significantly low levels of serum total cholesterol, HDL-C and LDL-C versus increments of triglycerides compared to controls was in agreement to previous report (Mori et al., 2017). In fact, the existence of hypertriglyceridemia may be due to increase the activity of hepatic lipase which subsequently results in the HDL-C degradation (Cure et al., 2017).

Evidently, in the current study, there was a significant

increase in the atherogenic index of plasma (AIP), the marker of atherogenicity monitored in Group IIb (CIMT more than 0.5 mm) when compared to Group IIa (CIMT less than 0.5 mm) and control group. This reflects the association of high risk of atherosclerosis in our cases of  $\beta$ -TM with CIMT more than 0.5 mm which came in agreement with results obtained by others (Cure et al., 2017). In addition, AIP and the triglyceride level showed positive correlation with the serum ferritin levels in thalassemic children and these results might support the hypothesis that both serum iron and triglycerides are important risk factors (Cure et al., 2017).

Confirmatively, the impact of ROS in  $\beta$ -TM cases herein was verified by the assessed increments of MDA and decrements in TAC in thalassemic patients and the illustrated higher increase in patients with CIMT more than 0.5 mm (Group IIb) as compared to controls. That delineated their roles in both the pathogenesis of the disease and the development of atherosclerosis in  $\beta$ -TM. The present study confirmed previous reports indicating the role of increased MDA values which represent a diagnostic marker of coronary artery disease, coronary endothelial dysfunction, and future cardiac events (Ito et al., 2017).

Furthermore, a significant correlation between MDA incremental levels and CIMT in both groups of thalassemic patients was confirmed herein. It was in agreement with reports reflecting the relationship between mean concentrations of lipoperoxides (evaluated as malondialdehyde/thiobarbituric acid adducts) and an increased two fold value of MDA levels observed in  $\beta$ -TM patients with respect to controls (Ito et al., 2017). From another outlook, it was also tempting herein to identify the relationship between both lipid and atherogenic profile with the mediators associating the role of ROS induced oxidative DNA modification via the role of highly reactive .OH, those are represented by 2 oxidized derivatives of guanine, namely, the 8-OHdG, the most commonly used markers for assessing oxidative DNA damage (Vakonaki et al., 2016).

In the current study there was a significant increase of 8-OHdG levels in both  $\beta$ -TM groups with more increase in Group IIb as compared to Group IIa and controls. Furthermore, it was considered that 8-OHdG was an important biomarker of generalized, cellular oxidative stress as well as risk factor for cancer, atherosclerosis and diabetes (Wu et al., 2017).

Consistently, the assessed 8-OHdG increments versus TAC decrements represented herein the impact of both parameters signifying magnitude of oxidative stress which were correlated with ferritin increments. This confirmed the role of excess iron in generating free radicals conveying tissue injury and DNA damage (Wu et al., 2017). In parallel, the attained data illustrated that there was a positive correlation between 8-OHdG and CIMT in group IIb (CIMT more than 0.5mm) as compared to group IIa (CIMT less than 0.5mm) and controls.

Conceivably, the role of SIRT1 retrospective to the oxidative stress-mediated posttranslational modifications observed in SIRT1 may represent the adaptive response to environmental stress under acute conditions that propel the deleterious atherogenic magnitude influencing CIMT and CAD (Nasiri M et al., 2018). We performed our experiments on (rs7069102) C>G in intron regions of SIRT1 gene as reported by Kilic et al. (2014). The concurrent results illustrated that the GG genotype which was predominant in cases with  $\beta$ -TM (group IIb CIMT more than 0.5 mm). This is in agreement with previous report by Kilic et al. (2014) who observed that the frequencies of mutant GG genotype and mutant G allele for rs7069102 C.G in intron 4 were significantly higher in CAD patients as compared to controls with apparently increased risk for atherosclerosis and CAD by 2.4 times in carriers of mutant G allele compared with carriers of wild-type C allele for rs7069102 C→G transition. This data was also colinked to the levels of triglycerides and MDA, 8-OHdG. Hence, these parameters were found to be significantly increased versus the associated decrements in total cholesterol, LDL-C, HDL-C and TAC in thalassemic patients carrying homozygote mutant GG genotypes as compared to CC and CG genotypes.

Consistently, earlier report illustrated that there was a significant increments in the levels of triglycerides in the GG genotype of rs7069102 which may appear at high risk for CAD. Notably, the attained data herein illustrated decrements in HDL-C levels in GG genotype when compared to CG and CC genotypes which furthermore reflect the cardio protective effect of C allele (Kilic et al., 2014).

Indeed, the assessed increments in the levels of MDA and 8-OHdG in  $\beta$ -TM cases with GG phenotype of SIRT1 gene was associated elsewhere with low levels of SIRT1 protein. Confirmatively, earlier report by Tamaki et al. (2014) illustrated that there was improvement in the systemic levels of 8-OHdG, nitric oxide metabolism, lipid peroxidation product MDA and proinflammatory cytokines that were found to be associated with SIRT1. Concordantly, the SIRT1 is known to help in elimination of ROS which is produced due to chronic oxidative stress as result of iron overload monitored by increments in plasma ferritin levels. Hence, SIRT1 activation can occur via exercise training and resveratrol treatment that was reported to augment the cardioprotective effect of SIRT1 (Zhang et al., 2016).

Evidently, the attained results also indicated that homozygote GG genotype (rs7069102) may represent high risk to develop atherosclerosis and subsequent CAD. This is associated with decrements in the TAC levels in the homozygote GG when compared to CC and CG which may suggest the cardio protective effect of C allele. Furthermore, the increase in the SIRT1 level may also reflect a compensatory mechanism involving an increased production of MnSOD and catalase activity (Gu et al., 2017). In alignment the data herein coordinates

with previous report which illustrated that increments of SIRT1 protein levels in the heterozygote CG genotypes (rs7069102) exhibited a protective effect against CAD. As SIRT1 is highly expressed in endothelial cells, thereby it regulates numerous functions, including nitric oxide synthase, cell senescence, and autophagy (Kilic et al., 2014).

Concordantly, in the present study we found that there was an association between genetic variation and phenotype. In reference, the recent discoveries denoted the importance of epigenetics in several human diseases which were clearly identified by previous report by Wegermann and Moylan (2017). Therefore, on demonstrating the attained data herein it verified their influence on CIMT via the interlink between the genetic variations reflected by SIRT1 gene polymorphism and the assessed biochemical parameters including lipid profile, ferritin, MDA, 8-OHdG and TAC. This would explain the association between genetic variation of SIRT1 (rs7069102) and oxidative stress.

## Conclusion

On the basis of the present results it could be depicted that patients with  $\beta$ -TM developed oxidative stress due to iron overload which play an important role in development of vascular complication. Measurement of CIMT may help in early detection of cardiovascular complications. Elevated levels of triglycerides, MDA and 8-OHdG for all studied SNPs of SIRT1 were significantly increased in thalassemic patients carrying homozygote mutant genotypes GG as compared to CC and CG genotypes. This indicates that the mutant G allele is associated with increased risk of CAD. The wild C allele in heterozygote CG genotype (rs7069102) may be protective against CAD. Hence, it is associated with better levels of the assessed TAC values than G allele. This may suggest that a compensatory mechanism exists which could protect the people from the detrimental effects of CAD.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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