Serum superoxide dismutase and malondialdehyde as oxidative stress biomarkers in coronary artery disease

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Background
Oxidative stress is caused by increased oxidant generation with impairment of endogenous antioxidant mechanisms. It plays an important role in the pathogenesis of atherosclerosis, and it is increased by traditional cardiovascular risk factors. Carotid intima-media thickness (CIMT) is a noninvasive technique that is used for the assessment of subclinical cardiovascular disease.

Aim
To investigate the relation between serum superoxide dismutase (SOD) and malondialdehyde (MDA) and CIMT in patients at risk or with established coronary artery disease (CAD).

Patients and methods
The study included 30 patients with CAD diagnosed by coronary angiography (group I) and 30 patients without CAD (group II) with cardiovascular risk factor (diabetes or hypertension or both). Moreover, 20 apparently healthy individuals were included as a control group (group III). Serum SOD and MDA were measured in all participants.

Results
CIMT was more in patients with CAD than in patients in the high-risk group (hypertension and/or diabetes) and healthy individuals. Patients with CAD had higher serum MDA levels than patients in the high-risk group and healthy individuals. Patients in the high-risk group without established CAD had higher MDA levels than healthy individuals. Patients with CAD and patients in the high-risk group had lower SOD activity than healthy individuals.

Conclusion
MDA is a good predictor of CIMT and can be used as an early marker of atherosclerotic CAD in diabetic and hypertensive patients.

Keywords:
carotid intima-media thickness, coronary artery disease, malondialdehyde, oxidative stress, superoxide dismutase

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Introduction

Coronary artery disease (CAD) is an atherosclerotic disease, manifested by stable angina, unstable angina, myocardial infarction (MI), or sudden cardiac death. It is the leading cause of death in both developed and developing countries [1]. Atherosclerosis is a chronic inflammatory disease characterized by accumulation of lipids and inflammatory cells in the walls of medium-sized and large-sized arteries. The pathogenesis of atherosclerosis involves activation of pro-inflammatory signaling pathways, expression of cytokine/chemokine, and increased oxidative stress [2].

Oxidative stress is caused by increased oxidant generation with impairment of endogenous antioxidant mechanisms. It plays an important role in the pathogenesis of atherosclerosis, and it is increased by traditional cardiovascular risk factors (e.g. diabetes mellitus, hypertension, dyslipidemia, smoking, sex, and age) [3].

In the field of modern biology to assess oxidative stress, malondialdehyde (MDA) is an extensively utilized biomarker to predict the pattern of various diseases, such as diabetes, hypertension, cancer, heart failure, and atherosclerosis. MDA has been used as a potent biomarker in both *in vivo* as well as *in vitro* studies [4].

Superoxide dismutase (SOD) forms the front line of defense against reactive oxygen species-mediated injury. SOD constitutes a very important antioxidant defense against oxidative stress in the body [5].

Carotid artery disease is used as a surrogate marker of subclinical coronary atherosclerosis. The association between carotid intima-media thickness (CIMT) and cardiovascular disease (CVD) has been well established

in the general population [6]. Increased CIMT in the common carotid segment is accompanied by yearly risk of 0.7–2.2% for coronary heart disease, 0.4–1.8% for stroke, and from 1.8 to 3.2% for total CVD [7].

This study aimed to investigate the relation between serum SOD and MDA and CIMT among patients with stablished CAD or at risk to develop CAD, as compared with healthy controls, and to study the relation between these markers and severity of CAD.

Patients and methods

This was a case–control study that was conducted in the clinical pathology and cardiovascular medicine departments at Assiut University Hospitals in the period from March 2019 to December 2019. Patients with malignancy, stoke, or autoimmune disease were excluded. The included patients were divided as follows:

(1) Group I: 30 patients with established CAD diagnosed by coronary angiography (27 males and three females).
(2) Group II: 30 patients without CAD as per coronary angiography, but with cardiovascular risk factor (diabetes and/or hypertension) (eight males and 22 females).
(3) Group III: 20 apparently healthy individuals as a control group (11 males and nine females).

All patients and controls were subjected to the following:

Ethical consideration

A written consent was taken from each patient or control and the study was presented to the Institutional Ethics Research Committee was accepted before starting the work. No risk on the subjects included in the study.

Clinical evaluation

It was done as follows:

(1) Full medical history and clinical examination.
(2) Calculation of BMI; the weight (kg) was divided by height squared (m^2).
(3) ECG and echocardiography.
(4) CIMT measurement by Doppler ultrasound of carotid artery.
(5) Coronary angiography for all patients.

Laboratory investigations

Overall, 10 ml of venous blood was collected from all participants. The blood sample was divided into the following:

(1) Two milliliters was withdrawn into EDTA-coated tube and mixed gently for CBC on ABX Pentra XL 80 (ABX Pentra XL 80, HORIBA, Montpellier, France).
(2) Two milliliters was collected in a citrated tube (3.2% trisodium citrate) and mixed gently. Plasma was separated by centrifugation and used to measure the prothrombin time and concentration by Sysmex CA-1500.
(3) Six milliliters was collected into two serum separator tubes:
    (a) In the first tube, after centrifugation for 15 min at 3000 rpm, serum was separated and divided in two aliquots: one was used for routine investigations and the other was stored at −80°C until assay of MDA.
    (b) In the second tube, after centrifugation at 4000 rpm for 15 min at 4°C, serum was separated and stored at −80°C until assay of SOD enzyme activity.

Routine investigations

(1) Complete blood count was done on ABX Pentra XL 80. Prothrombin time and concentration and international normalization ratio were assessed by Sysmex CA-1500, and fasting serum glucose, urea, creatinine, liver function tests, and lipid profile tests, all those biochemical investigations were done by a Cobas Integra 400 plus analyzer.

Special investigations

(1) Serum MDA was measured by the enzyme-linked immunosorbent assay (ELISA) technique using an Elabscience MDA ELISA kit with catalog number: E-EL-0060 based on competitive ELISA principle.
(2) Serum SOD activity was measured by the colorimetric method using a kit provided by Biodiagnostic with catalog number: SD 25 20. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye by the colorimetric method [8].

Carotid ultrasound

CIMT measurements were obtained with the patient lying in the supine position and with the neck rotated to the opposite side of examination. Common carotid artery images were obtained to measure CIMT by using three different angle views for each vessel. Initially, a transversal scanning view of the common carotid artery was performed in the longest extension possible from the base of the neck to the carotid bulb. At least three IMT points were measured in the near and far walls in the most thickened area of each vessel. Moreover, lateral wall measurements were taken when both thickening was

evident and accurate images were possible. Subsequently, the vessel was scanned by two longitudinal views: posterolateral, with the transducer positioned parallel to the posterior border of the sternocleidomastoid muscle, and anterolateral, with the transducer positioned parallel to the anterior border of the sternocleidomastoid muscle; at least three IMT measurements were obtained for each near and far wall. Optimal B-mode settings of gain, depth, focal zone placement, and compression were individually adjusted for each vessel to enhance arterial wall structures and image quality. The maximum IMT value was selected for each angle. CIMT greater than 0.9 mm was considered an abnormal finding [9].

Coronary angiography

Coronary angiography was performed according to standardized protocols. After access site was prepared and sterilized, patients were draped. Coronary angiography was performed via radial or femoral routes according to the operator's discretion. Patients were designated as having one-vessel, two-vessel, or three-vessel disease, according to the number of main vessels affected along with its main branches. All angiograms were revised by an experienced operator.

Statistical methodology

Date entry and data analysis were done using SPSS, version 24 (Statistical Package for Social Science, SPSS: IBM -SPSS Inc, Chicago, IL, USA). Continuous data were expressed in the form of mean ± SD or (range), whereas nominal data were expressed in the form of frequency (percentage). Student *t* test was used to compare mean of two different groups and analysis of variance test for more than two groups. Pearson correlation was used to determine the correlation between different variables. Multivariable regression analysis was used to assess the predictors of increased CIMT. *P* value was considered statistically significant when *P* value less than 0.05.

Results

Classification of patients according to carotid intima-media thickness

According to CIMT, patient groups (groups I and II) were classified into the following:

(1) Group I:
   (a) Group Ia: this group included eight patients with CIMT less than 0.9 mm.
   (b) Group Ib: this group included 22 patients with CIMT more than or equal to 0.9 mm.
(2) Group II:
   (a) Group IIa: this group included 19 patients with CIMT less than 0.9 mm.
   (b) Group IIb: this group included 11 patients with CIMT more than or equal to 0.9 mm.

Classification of patients with coronary artery disease according to severity

Group I (CAD) patients were classified according to number of vessels affected detected by coronary angiography into the following:

(1) CAD with one vessel disease: 16 patients.
(2) CAD with two vessel disease: eight patients.
(3) CAD with three-vessel disease: six patients.

Clinical data of the control and patient groups

Clinical evaluation of the studied groups included medical history regarding age, with a mean ± SD value of 54.53 ± 10.89 years for group I, 50.13 ± 9.04 years for group II, and 46.65 ± 13.96 years for group III. Regarding sex, 90% were males in group I, 73.3% were females in group II, and 55% were males in group III. Overall, 63.3% were smokers in group I, 23.3% in group II, and 30% in group III. Overall, 80% had a history of diabetes in group I, 53.3% in group II, and none in group III. Moreover, 50% had a history of hypertension in group I, 90% in group II, and none in group III. Moreover, BMI was calculated, with a mean ± SD value of 26.23 ± 2.56 in group I, 27.97 ± 4.90 in group II, and 25.08 ± 2.77 in group III.

The clinical data in the studied groups regarding serum cholesterol revealed no statistically significant difference when comparing the three groups. There was statistically significant elevation of serum triglyceride level in both groups I and II compared with control group ($P < 0.001$ and 0.001, respectively). However, no statistically significant difference was found when comparing groups I and II. There was a statistically significant reduction of serum high-density lipoprotein cholesterol and elevation of serum low-density lipoprotein cholesterol levels in group I compared with control group ($P < 0.001$). No statistically significant difference was found in other group comparisons. The oxidative stress biomarkers were compared in the studied groups. Regarding CIMT, in group I, CIMT ranged from 0.6 to 1.6, with a mean ± SD value of 1.03 ± 0.27 mm; in group II, the range was 0.4–1.5, with a mean ± SD value of 0.73 ± 0.26 mm; and in the control group, CIMT ranged from 0.3 to 0.8, with a mean ± SD value of 0.5 ± 0.15 mm. A statistically significant elevation of CIMT was observed in group I compared with both group II and the control group ($P < 0.000$ for both). Moreover, a significant elevation of CIMT was observed in group II compared with the control group ($P < 0.05$). Tables 1 and 2 show oxidative stress biomarkers in groups I and II patients,

Table 1 Oxidative stress biomarkers in group I patients classified according to carotid intima-media thickness

Items	Groups		*P*
	Group Ia CIMT <0.9 (*n*=8)	Group Ib CIMT ≥0.9 (*n*=22)	
Serum malondialdehyde (ng/ml)	278.68±42.9	384.22±79.25	<0.001
Serum superoxide dismutase (U/ml)	2.65±1.01	2.69±1.15	Non significant

Data were expressed as mean±SD. CIMT, carotid intima-media thickness. *P*<0.05.

Table 2 Oxidative stress biomarkers in group II patients classified according to carotid intima-media thickness

Items	Groups		*P*
	Group IIa CIMT <0.9 (*n*=11)	Group IIb CIMT ≥0.9 (*n*=19)	
Serum malondialdehyde (ng/ml)	263.13±57.17	338.11±66.08	<0.05
Serum superoxide dismutase (U/ml)	2.9±1.37	2.33±0.94	Non significant

CIMT, carotid intima-media thickness.

Table 3 Lipid profile in the studied groups

Groups	Items			
	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Group I (*n*=30)	164.7±32.3	151.3±56.9	36.5±9.2	97.5±32.9
Group II (*n*=30)	157.0±33.9	179.4±64.4	42.0±11.5	79.1±26.1
Group III (*n*=20)	148.3±26.0	107.9±36.3	48.1±8.6	82.2±24.0
P				
Group I vs. III	>0.05	<0.001	<0.001	<0.001
Group II vs. III	>0.05	<0.000	>0.05	>0.05
Group I vs. II	>0.05	>0.05	>0.05	>0.05

Data were expressed as mean±SD. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *P* value was significant if less than 0.05.

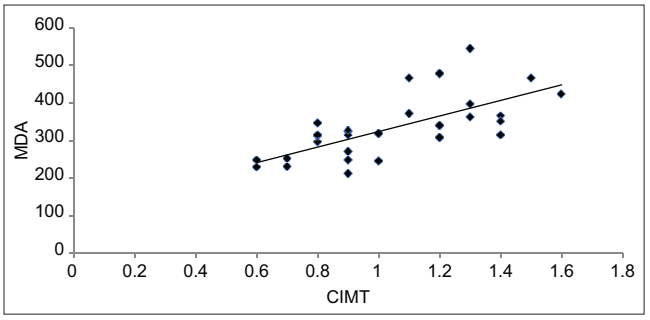
Table 4 Oxidative stress biomarkers in the studied groups

Groups	Items	
	Serum malondialdehyde (ng/ml)	Serum superoxide dismutase (U/ml)
Group I (*n*=30)	331.45±82.49	2.67±1.06
Group II (*n*=30)	278.13±65.39	2.78±1.30
Group III (*n*=20)	236.29±31.54	3.52±0.92
P		
Group I vs. III	<0.001	<0.01
Group II vs. III	<0.05	<0.05
Group I vs. II	<0.01	Nonsignificant

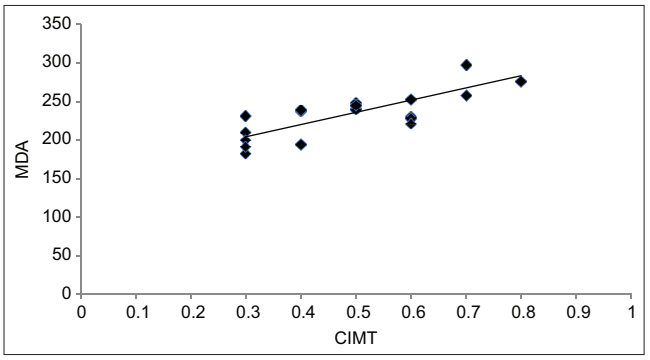
respectively, classified according to CIMT. Regarding CAD severity, no statistically significant difference was found in both MDA and SOD levels among the three groups ($P > 0.05$) (Tables 2-5).

Correlation studies

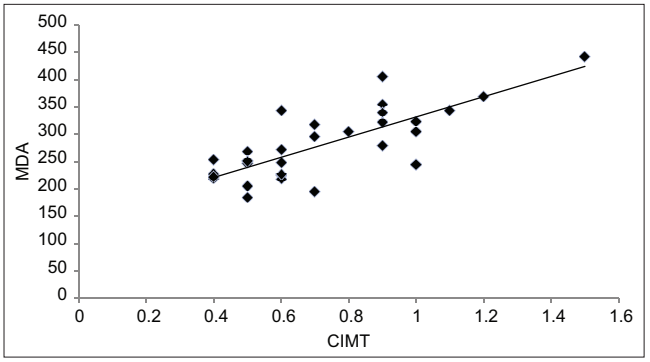
A statistically significant positive correlation was found between CIMT and serum MDA in group I ($r = 0.68$, $P < 0.001$), group II ($r = 0.777$, $P < 0.001$), and in control group ($r = 0.811$, $P < 0.001$). There was a significant negative correlation between serum SOD and platelet count in group I (r=–0.551, $P < 0.01$). There was a significant positive correlation between serum SOD activity and hemoglobin concentration in the control group ($r = 0.456$, $P < 0.05$). No other statistically significant correlations were found between other variables.



Correlation between MDA and CIMT in group I.



Correlation between MDA and CIMT in group II.



Correlation between MDA and CIMT in control group.

Multivariate regression analysis between CIMT and other variables (MDA, SOD, cholesterol, and low-density lipoprotein) was done to assess the predictors of increased CIMT as an early diagnostic tool for atherosclerosis. Only serum MDA was statistically significant ($P < 0.001$).

Table 5 Multivariate regression analysis between carotid intima-media thickness and other variables coefficients

Model	Unstandardized coefficients		Standardized coefficients	*t*	Significance
	B	SE	Beta		
Co (Constant)	−0.023	0.740		−0.031	0.976
MDA	0.004	0.001	0.649	4.705	0.000
SOD	−0.033	0.065	−0.069	−0.505	0.618
Cholesterol	0.000	0.007	−0.007	−0.017	0.987
LDL	−0.006	0.006	−0.369	−0.894	0.380

LDL, low-density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase. [a]Dependent variable: CMT.

Results of sensitivity and specificity of oxidative stress biomarkers

At cutoff value of 1.72 U/ml, serum SOD activity had a sensitivity of 83.3% in group I and 73.3% in group II. The specificity was 18% for both groups. At cutoff value of 299.37 ng/ml, serum MDA had a sensitivity of 66.7% in group I and 36.7% in group II. The specificity was 15 and 14% in groups I and II, respectively.

Discussion

Consistent with the literature, the results of the present study showed a significant increase in serum triglycerides and low-density lipoprotein cholesterol and reduction of high-density lipoprotein cholesterol in patients with CAD (group I) compared with the control group. In addition, a significant increase in triglycerides was detected in patients with risk factors (group II) compared with the control group. However, hyperlipidemia is considered the second most common risk factor for ischemic heart disease [10].

The American Heart Association suggests carotid artery ultrasound, a noninvasive imaging test, for the evaluation of cerebrovascular and cardiac disease risks. It images arteriosclerosis that characterizes subclinical CVD burden, including the progressive luminal narrowing of the arteries, arterial stiffening, wall thickening, and plaque formation before a person manifests the clinical symptoms of CVD. CIMT, a noninvasive, B-mode ultrasound-based measure of the carotid artery by ultrasonography, is a well-established and commonly used early surrogate marker of subclinical atherosclerosis [11]. Oren *et al.* [12], reported that CIMT (0.9–1.0 mm) is strongly suggestive of atherosclerosis and generally referred as an intermediate phenotype for early atherosclerosis. In the present study, there was a significant increase in CIMT in patients with CAD compared with patients with risk factors and controls. Eight cases of patients with CAD (26.7%) had CIMT less than 0.9 mm and 22 (73.3%) cases had CIMT more than or equal to 0.9 mm. This finding was consistent with previous studies, which reported the relation between CIMT and CAD [13–15]. Moreover, a significant increase in CIMT was detected in patients with CAD risk factor compared with the control group. A total of 19 (63.4%) patients in the risk group had CIMT less than 0.9 mm and 11 (36.7%) cases had CIMT more than or equal to 0.9 mm. Approximately 90% patients of this group were hypertensive patients and 53.3% were diabetics. Hypertension, hyperglycemia, and dyslipidemia were confirmed to be related to CIMT [16].

A significant rise in MDA levels and lipid peroxidation with decrease in antioxidants were reported in patients with unstable angina and chronic heart failure [17]. In the present study, there was a statistically significant elevation of serum MDA level in both patient groups (groups I and II) compared with the control group. In addition, statistically significant elevation of MDA was found in patients with CAD (group I) compared with those with risk factors (group II). Previous studies also reported higher levels of MDA in patients with CAD in comparison with healthy individuals [18,19]. MDA is a breakdown product of peroxidation of long chain fatty acids, which accumulates when lipid peroxidation increases. MDA is used as an index of oxidative damage [16].

Consistent with our work, previous studies also reported elevated serum MDA levels in hypertensive patients as compared with normotensive control individuals [20]. Hypertensive effects of oxidative stress are mostly owing to endothelial dysfunction resulting from disturbances of vasodilator systems, particularly degradation of NO by oxygen-free radicals [21].

In the present study, patients with CIMT more than or equal to 0.9 showed higher serum levels of MDA than those with CIMT less than 0.9 in both patient groups. There was a significant positive correlation between serum MDA level and CIMT in all of the studied groups. Moreover, in multivariate regression analysis, serum MDA was a predictor of CIMT as an early diagnostic tool for atherosclerosis. Consistent with these results, Yoon *et al.* [22] and Tuzun *et al.* [23] found an association between high CIMT and MDA levels.

In the present study, there was a statistically significant reduction of serum SOD activity in both patient groups (groups I and II) compared with the control group. A previous study done by Shaikh and Suryakar [24] also showed reduced levels of SOD in patients with CAD.

To evaluate the predictors of increased CIMT as an early diagnostic tool for atherosclerosis, multivariate regression analysis was done. Only serum MDA gave a statistically significant association. At cutoff value 299.37 ng/ml, its sensitivity was 66.7% in CAD group

and 36.7% in the risk group, with a specificity of 15 and 14%, respectively. However, SOD activity showed higher sensitivity. At a cutoff value of 1.72 U/ml, its sensitivity was 83.3% in patients with CAD and 73.3% in the risk group, with a specificity of 18% in both groups.

Conclusion

(1) CIMT is more in patients with CAD than in patients in the high-risk group (hypertension and diabetes) and healthy individuals.
(2) Patients with CAD have higher serum MDA levels than patients in the high-risk group and healthy individuals. Patients at high risk of CAD have higher MDA levels than healthy individuals.
(3) Patients with CAD and patients in the high-risk group have lower SOD activity than healthy individuals.
(4) No significant association was found comparing serum MDA and SOD with severity of CAD.
(5) Serum MDA levels are associated with CIMT.
(6) Increase in serum MDA is associated with increase in CIMT. So, MDA is a good predictor of CIMT and can be used as an early marker of atherosclerotic CAD in diabetic and hypertensive patients.

Recommendations

(1) MDA and CIMT together can be used in screening of high-risk individuals for CAD.
(2) Further studies are needed to investigate the role of other oxidative stress biomarkers as early predictors of CAD.

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Conflicts of interest

There are no conflicts of interest.

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