RESEARCH ARTICLE

Oxidative Stress Status in Sera of Type 2 Diabetic Iraqi Patients with Coronary Artery Diseases

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ABSTRACT

The leading cause of morbidity among patients with diabetes is cardiovascular complications, and coronary artery disease is the most common cause of mortality. This study was carried out to look at the differences in the oxidative stress status in sera of Iraqi patients with Coronary Artery Disease (CAD) in the presence and absence of Type 2 Diabetes Mellitus (T2DM). Thus, to achieve that, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI), as well as the levels of malondialdehyde (MDA), Uric acid (UA), and oxidized low-density lipoprotein (OxLDL) were measured in two patients groups and the healthy individuals. The study was included a total of 90 subjects divided into three groups: 30 CAD patients with T2DM (G1), 30 CAD patients without T2DM (G2), and 30 healthy as a control group (C). The results showed that the total oxidant status (TOS), oxidative stress index (OSI), oxLDL, and MDA levels were highly significant increase ($p < 0.001$) in (G1 & G2) groups as compared to the C group as well as a highly significant increase in G1 compared to G2. A highly significant decrease was observed in TAS and UA levels in G1 & G2 groups in comparison with the C group, while there was no significant difference between G1 and G2 groups in levels of TAS and UA. Therefore, the variation in the oxidative stress status in sera of Iraqi CAD patients with and without T2DM, supports the possibility that OS plays a vital role in developing CAD and the pathogenesis of T2DM.

Keywords: Coronary artery disease, Diabetes Mellitus, Lipid peroxidation, Oxidative stress, oxLDL.

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INTRODUCTION

Coronary artery disease (CAD) is one of the major causes of mortality and morbidity in the world. The risk factors for CAD include hyperlipidemia, hypertension, family history, diabetes, smoking, gender, age, obesity, and physical inactivity.[1] Diabetes mellitus (DM) is a disease associated with abnormalities of carbohydrate metabolism and can be identified by evaluating blood glucose. It can be classified into several kinds: type 1, type 2, gestational, and monogenic diabetes.[2] Many diseases are associated with chronic hyperglycemia with long-term dysfunction damage and lead to failure of various organs, especially the kidneys, eyes, nerves, heart, and blood vessels. Persons with untreated Type 2 diabetes (T2DM) are also at significantly higher risk for stroke, CAD, and peripheral vascular disease (PVD) than the non-diabetic population.[3] Cardiovascular complications are the leading cause of morbidity among patients with diabetes, and CAD is the most common cause of death.[4]

Besides DM, oxidative stress (OS) is also a strong contender in the development of CAD. There are two reactive species: reactive oxygen species (ROS) and reactive nitrogen species (RNS).[5] All the aerobic cells can produce the reactive oxygen and nitrogen species (RONS).[6] Therefore, if an imbalance occurs between production and removal of RONS, it leads to OS because of overproductions and/or an impaired ability to neutralize them or repair the resulting damage to a wide range of macromolecules *i.e*., lipids, proteins, and nucleic acids.[5]

The total antioxidant status (TAS) provides a measure of plasma defenses against RONS; a significant relationship between plasma TAS levels and the extent of CAD was reported.[7] Antioxidants had been hypothesized to inhibit free radicals and therefore play a protective role in the development of CAD.[8] The oxidative stress index (OSI) is the most accurate method for calculating the degree of OS.[9] It was calculated as the ratio between TOS and TAS levels in serum samples. Oxidized Low-Density Lipoprotein (oxLDL) is the oxidative modification of LDL under the OS and plays an important role in the initiation and development of atherosclerosis.[10,11]

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Malondialdehyde (MDA) is a breakdown product of the peroxidation of long-chain fatty acids, and it is considered a vital OS biomarker.[12] MDA is the most well-studied marker of lipid peroxidation and OS, generated by the peroxidation of polyunsaturated fatty acids.[13]

UA is the final catabolic product of purines formed from the breakdown of adenosine and guanine. It exists in blood plasma at the maximum level of solubility.[14] Several studies have been reported a relation between UA levels in serum and a wide variety of cardiovascular conditions, such as hypertension,[15] metabolic syndromes,[16] CAD,[17] cerebrovascular diseases, vascular dementia,[18] and preeclampsia.[19] Thus, the current project aims to study the role of some OS parameters in Iraqi CAD patients with and without T2DM.

MATERIALS AND METHODS

Study Subjects

A total of 90 individuals participated in the current study divided into three groups: 30 CAD patients with T2DM (G1), 30 CAD patients without T2DM (G2), and 30 healthy as a control group (C). Each group consists of 16 males and 14 females with ages ranging between 40 to 74 years and a body mass index (BMI) ranging between 25 to 30 kg/m^2. The exclusion criteria included: alcoholism, smoking, intravenous drug abuse, pregnancy, the persons who used antioxidant supplements, kidney and liver diseases, rheumatoid arthritis, patient after surgery, and other diseases that may be interfering with this study. The patients have attended the Ibn Al-Bitar Specialized Cardiac Surgery Centre in Baghdad, Iraq. Patients with CAD were diagnosed according to ECG/ECHO by expert physicians of the cardiac unit. The Ethics Committee approved this study protocol of the College of Sciences/University of Baghdad.

Blood Samples

After overnight fasting for 8–12 hs, approximately 8 mL of blood were drawn in a plane tube and left for 15 minutes at room temperature for clotting. Then, the serum samples were separated by centrifugation at (4000 rpm) for 10 minutes. The serum samples were aliquoted and stored at −20°C until use.

Experimental

Fasting blood glucose (FBG) was measured by a glucose oxidase method and UA was determined by using Sanders' method [Sanders *et al*, 1980] (Linear Chemicals, Spain).

The Erel's methods were used to determine both TOS (μmol H_2O_2 Eq./L)[20] and TAS (mmol glutathione Eq./L)[21] in serum samples (patients and control). The OSI which is an indicator of degree of OS[22] was calculated according to the formula:

$$OSI = TOS\ (\mu mol\ H_2O_2\ Eq./L) / TAS\ (\mu mol\ glutathione\ Eq./L).$$ [22]

The MDA level in the serum was measured by precipitation method using thiobarbituric acid (TBA) as active substance.[23] The absorbance of the supernatant was measured at 532 nm. The result of MDA expressed by nmol/mL and using a molar extinction coefficient for MDA of $1.52 \times 10^5 M^{-1} cm^{-1}$.

The determination of serum oxLDL level was done using My biosource (USA) oxLDL Enzyme Linked Immunosorbent assay (ELISA) Kit.

Statistical Analysis

The data were analyzed using the program Statistical Package for the Social Science (SPSS for windows, version 20) and the data was presented as Mean±Standard deviation (Mean± SD). The differences were tested between groups by using one-way analysis of variance (ANOVA) to compute the mean. A value of ($p < 0.05$) was considered statistically significant and ($p < 0.001$) statistically highly significant, while a value of ($p > 0.05$) considered as statistically non-significant. The correlation between variables were determined by Person correlation coefficients (r).

RESULTS

The FBG levels showed highly significant ($p < 0.001$) increases in group G1 as compared to G1 and C groups. In addition, no significant difference ($p > 0.05$) between G2 and C, as shown in Figure 1.

TOS has been identified as: *in vivo* marker of a shift developing in an oxidant/antioxidant ratio in favor of the oxidative side.[24] Therefore, TOS concentration was measured in the serum of all studied groups. In serum of patients (G1&G2) and control (C) groups, the results showed that there was highly significant increase ($p < 0.001$) in TOS of (G1 and G2) groups in comparison to that of the C group. Also, there was a highly significant increased ($p < 0.001$) in G1 compared to G2 groups, as shown in Table 1.

In addition, the Total Antioxidant Status (TAS): which is defined as a biomarker for measuring the antioxidant potential of body fluids {the moles of oxidants neutralized by one liter of solution}. It was measured in all studied groups. The results presented in Table (1) indicate a highly significant decreased in TAS of G1 and G2 when compared with C as well as a high significant dscreased in G1 compared to G2.

It is clear from (Table 1) that there is a highly significant increase ($p < 0.001$) in serum OSI of G1 and G2 groups than

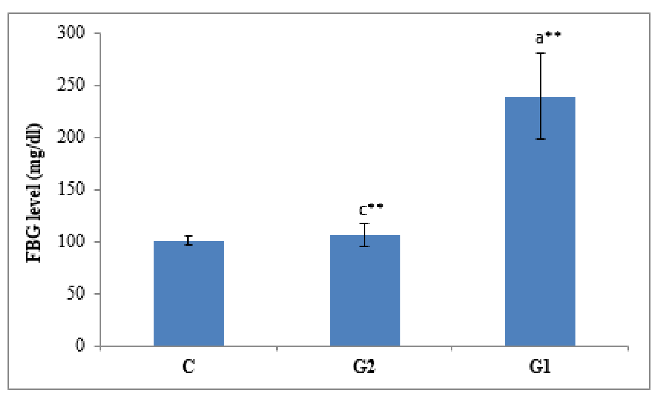

Figure 1: The FBG level for all studied groups.
Note: *$p < 0.05$, **$p < 0.001$, no asterisk $p > 0.05$.
a: difference between G1 and C
b: difference between G2 and C
c: difference between G1 and G2

C group and there is a high significant increase ($p < 0.001$) in G1 group in comparison to the G2 group.

The MDA concentration was measured in serum of all studied groups. The results as described in Table 2 show that there is a high significant increase ($p < 0.001$) in MDA level in serum of both G1 and G2 groups compared to C group, and high significant increase ($p < 0.001$) in G1 group in comparison to the G2 group.

Table 2 showed the values of oxLDL (ng/mL), which were significantly higher ($p < 0.001$) in G1 and G2 than that of C as well as higher in G1 group than G2 group ($p < 0.001$).

Table 1: The levels of TOS,TAS and OSI in patients and control groups

	Groups		
Parameters	*G1 (n=30)*	*G2 (n=30)*	*C (n=30)*
TOS (μmol/L)	27.35±4.84 ^{ac**}	22.80±5.62 ^{b**}	11.39±3.34
TAS (mmol/L)	0.37±0.18 ^{ac**}	0.42±0.17 ^{b**}	0.56±0.23
OSI	0.087±0.035 ^{ac**}	0.061±0.026 ^{b**}	0.029±0.039

Note: *$p < 0.05$, **$p < 0.001$, no asterisk $p > 0.05$.
a: difference between G1 and C
b: difference between G2 and C
c: difference between G1 and G2

Table 2: The levels of MDA, UA and oxLDL in patients and control groups.

	Groups		
Parameters	*G1 (n=30)*	*G2 (n=30)*	*C (n=30)*
MDA (nmol/mL)	7.07±1.15 ^{ac**}	6.03±1.09 ^{b**}	2.81±0.44
UA (mg/mL)	3.76±0.57^{a**}	3.99±0.62 ^{b**}	5.45±0.81
oxLDL (ng/mL)	16.19±3.64 ^{ac**}	12.20±3.33 ^{b**}	5.86±1.45

Note: *$p < 0.05$, **$p < 0.001$, no asterisk $p > 0.05$.
a: difference between G1 and C
b: difference between G2 and C
c: difference between G1 and G2

The results of UA levels in serum of patients and control groups are shown in Table 2. The levels of UA was highly significant ($p < 0.001$) decreased in patient groups G1 and G2 when compared to C group C. While there are no significant difference ($p > 0.05$) between G1 and G2.

The correlation among all the studied groups and parameters in serum was done as illustrated in Tables 3 and 4. The results indicated a significant negative correlation between TAS and OSI as well as a significant positive correlation between OSI and MDA (in G1 group). While the results showed a significant negative correlation between TAS and OSI, as well as a significant positive correlation between FBG with (TOS, OSI and MDA) and between TOS and MDA (in G2 group).

DISSCUTION

Diabetes mellitus (DM) is a disease that associated with abnormalities of carbohydrate metabolism, and can be identified by evaluation of blood glucose.[2] Many diseases are associated with the chronic hyperglycemia with long-term dysfunction includes the heart and blood vessels. The CVD is a major cause of death and disability among people with diabetes.[25] Figure (1) showed an individuals significant increase in FBG in group G1 in comparison with G2 and C group. Our results in a line with Hao *et al*., suggested that the patients with T2DM is more likely to develop CAD when compared to healthy subjects.[26] Adults with diabetes, historically, have a higher prevalence rate of CVD than adults without diabetes,[27] and the risk of CVD increases continuously with rising fasting plasma glucose levels, even before reaching levels sufficient for a diabetes diagnosis.[28] T2DM increased the risk of recurrent CVD. Older patients with T2DM, in particular, may require more intensive and comprehensive monitoring.[29] Good control of evaluation glucose levels remains the main foundation for managing T2DM.[30]

Table 3: The Pearson correlation among the studied parameters in serum of G1 group.

Serum G1 group	*TOS*	*TAS*	*OSI*	*UA*	*MDA*	*oxLDL*
FBG	-0.230	-0.052	0.006	-0.065	-0.096	0.057
TOS		-0.002	0.265	0.112	0.017	-0.045
TAS			-0.862*	0.122	-0.371	0.077
OSI				-0.131	0.423*	-0.229
UA					-0.241	0.207
MDA						-0.250

Note: *$p < 0.05$

Table 4: The Pearson correlation among the studied parameters in serum of G2 group.

Serum G2 group	*TOS*	*TAS*	*OSI*	*UA*	*MDA*	*oxLDL*
FBG	0.468*	-0.197	0.392*	0.223	0.400*	0.116
TOS		-0.071	0.652*	0.029	-0.006	0.079
TAS			-0.731*	0.067	-0.079	0.197
OSI				0.042	0.057	-0.161
UA					0.210	0.119
MDA						-0.186

Note: *$p < 0.05$

Oxidative stress (OS) is a pervasive aspect of CVD and occurs whenever the release of reactive oxygen species (ROS) exceeds endogenous antioxidant capacity.[31] OS and inflammation play a key role in the pathogenesis of CVD.[32] Our study showed elevation of TOS in CAD patients compared to healthy subjects as well as CAD patients with T2DM greater than without T2DM. Interestingly, T2DM is one of the risk factors of atherosclerosis and constitute a major risk factor for CAD. Which is induce arterial endothelial dysfunction and atherosclerosis is the activation of a pathway that leads to increasing OS.[33] The patients with T2DM are 2 to 4 times more likely to develop CVD, and have 3 times higher overall mortality rate when compared to the patients without T2DM.[34]

The current results were in agreement with Bhat & Gandhi, who studied serum TOS in Indian patients with CAD and reported a significantly increased of TOS in CAD patient groups compared to that of the control group.[35] Also, Kilic *et al*., observed an increased in TOS level in patients with CAD compared with control.[36] Free radical formation is naturally controlled by antioxidants. Antioxidants are capable of deactivating or stabilizing free radicals before they attack the different components of the cells.[37] The results in the above Table (1), were in line with some studies such as: the study of Bastani *et al.*, who studied the TAS in Iranian patients with CAD and found a significant decreased in patient group when compared to that of healthy subjects,[38] as well as a study by Bhat & Gandhi., who determined the decreased in TAS in Indian patients.[35] Furthermore, other study by Khaki-khatibi *et al*., also showed that the TAS was significantly reduced in patients with CAD as compared with healthy subjects.[39] The OSI is the most accurate method for calculating the degree of OS.[9] The present results (Table 1) are in agreement with the study of Bhat *et al*., who showed there were in an increase in (OSI) in serum of Indian patients with CAD.[35] Moreover, Turan *et al*., pointed out high OSI values in CAD patients compared with healthy subjects.[40]

The serum TAS and TOS levels and the OSI reflect the redox balance between oxidant and antioxidant status.[9] Therefore, our present study investigated these OS markers in CAD patients, and showed a significant association between increased levels of OSI, lower TAS and developed CAD. Likewise, an increase in OSI and decrease TAS were observed in all studied groups. Similar to our results, a study by Bhat A *et al*., shows increases in OSI and/or decrease in TAS in the pathogenesis of CAD.[41]

Malondialdehyde (MDA) is a useful biomarker of lipid peroxidation and OS. Therefore, the observation of MDA levels in different biological systems can be an important indicator of lipid peroxidation both in vitro and in vivo for various chronic diseases in humans.[13] The current study agrees with several relevant studies, who reported an increase in serum MDA levels in CAD.[42-44] Remarkably, free radicals are reactive chemical species that are attacked all the major class of biomolecules lipids are probably the most susceptible. Cell membranes are a rich source of polyunsaturated fatty acids and are readily attacked by oxidizing radicals. The oxidative destruction of polyunsaturated fatty acids is known as lipid peroxidation,[45] and the damage inflicted by the free radical is referred to as OS. Increasing levels of OS play vital roles in the pathogenesis and progression of CAD.[5]

Notably, the findings in this study indicated an elevated level of MDA in group G1 in CAD patients with T2DM than group G2 of CAD patients without T2DM. Hence, the raised MDA levels may increase in the free radical and OS in DM.[46] In general, T2DM has about twice the risk of developing CAD as do non-DM's.[47] A study by Kitano *et al*. showed increased levels of oxygen free radicals in DM compared to non-DM.[48] Thus, increasing in OS means increasing in ROS that attacked cell membrane, as a result, increase the levels of MDA.[42] Gundapaneni *et al.,* who suggested that measurement of MDA is a good marker of radical stress during reperfusion of the CAD, and also showed significantly increased in MDA concentrations in a group of CAD patients who had T2DM when compared with healthy subjects.[49]

Oxidized LDL plays an important role in the initiation and development of atherosclerosis.[10] Table 2 showed that the oxLDL level was higher in the G1 group than in that G2 group. Thus, our results suggested uncontrolled blood glucose could associate with the high oxLDL level. This could be explained by increasing OS in T2DM patients,[33] enhancing LDL oxidation and atherosclerosis acceleration.[50] In line with our results, it was reported that serum oxLDL levels are significantly higher in T2DM than in healthy individuals.[51] In other studies that show the level of oxLDL in T2DM was higher than in individuals without T2DM.[52,53]

Uric acid (UA) is the final catabolic product of purines formed from the breakdown of adenine and guanine.[54] Several studies have reported a relation between UA levels in serum and a wide variety of cardiovascular conditions, such as hypertension,[55] metabolic syndromes,[16] and CAD.[56] In the current study, we observed decreased UA levels in CAD patients than the control group. In a previous study, low serum UA levels induced more susceptibility to oxidative damage and account for the excessive free radical production. Therefore, UA confers protection against the development of atherosclerosis,[57] which supports the low UA level in CAD patients in the current study. Mouhamed *et al*., suggested that the probability of CVD increases with lower UA levels due to excessive free radical and indicating damage to OS.[58] The UA is the primary quantitative determinant of total antioxidant capacity of plasma (TAC) and is hence expected to protect against the progression of atherosclerosis.[59] In the study by Kazemi *et al.*, who found that UA was an independent risk factor for the development of CAD.[60]

CONCLUSION

In conclusion, we found that CAD patients with T2DM have significantly higher TOS, OSI, MDA, oxLDL with lower TAS and UA than CAD patients without T2DM. Thus, these results indicate that increases in T2DM contribute to complications and mortality of Iraqi patients with CAD.

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