Original article

Evaluation of oxidative stress among coronary diabetics patients

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Abstract. *Objectives:* Determination of the superoxide dismutase (SOD), glutathione peroxidase (GPX) and the total antioxidant status (TAS) and evaluation of inflammation by the use of high sensitivity C reactive protein (hs-CRP) among Tunisian coronary diabetic patients. *Materials and methods:* We measured the erythrocyte GPX activity and the plasmatic TAS concentration by colorimetric methods, the apolipoproteins [ApoA1, ApoB], hs-CRP and the fibrinogen by immunonephelometry assays. *Results:* TAS and GPX were significantly decreased among patients compared to the controls [TAS: 1,14 ± 0,28 mmol/l vs 1,55 ± 0,35 mmol/l, GPX: 59,32 ± 10,72 U/gHb vs 149,19 ± 30,95 U/gHb]. For the coronary diabetic patients, the TAS is correlated positively with hs-CRP [r= 0,01, $p<10^{-3}$]. Pearson's correlation shows a significantly positive correlation between GPX and TAS among all patients. *Conclusions:* Determination of antioxidative defense markers contributes to understanding the effect of stress oxidative on the development, prevention and therapy of cardiovascular disease. (www.actabiomedica.it)

Key words: glutathione peroxidase [GPX], total antioxidant status [TAS], high sensitivity C reactive protein (hs-CRP), cardiovascular diseases, diabetes

Introduction

Although mortality from cardiovascular diseases (CVD) has shown a tendency to decrease in developed countries, and remains the main cause of death.

The origin of these diseases is multifactorial, but cardiovascular risk factors (CVRF) represent some of most important causes. Age and gender, are unmodifiable CVRF whereas smoking, high blood pressure, hypercholesterolemia ...are modifiable CVRF (1).

Diabetes mellitus (DM) have also been shown to play an important role in CVD (1). Patients with diabetes have a two to threefold increased incidence of CVD and those who present in the fourth and fifth decade of life have a twofold increase in mortality (2). Actually, Interest has developed in alternative markers, such as plasma markers of oxidative stress which have a role to predict CVD risk (3).

However, oxidative stress plays a big role in the development of many pathologies.

In fact hyperglycaemia can increase oxidative stress through several pathways and induce intracellular reactive oxygen species (ROS) produced by the proton electrochemical gradient generated by mitochondrial electron transport chain and resulting in increased production of superoxide (4). The other mechanism involves the transition metal catalysed auto-oxidation of free glucose yielding superoxide anion and hydrogen peroxide (5).

There is also evidence that hyperglycaemia may compromise natural antioxidant defense. Under normal circumstances free radicals are rapidly eliminated

by antioxidants such as reduced glutathione, vitamin C and vitamin E. Reduced glutathione content, as well as reduced vitamin E, have been reported among diabetics patients (6).

But now days, we know that there were additional participants in diabetic development that involves inflammation, endothelial injury, lipoproteins retention in the arterial wall, and lipoproteins modifications (7). Hyperglycaemia can promote vascular complications by multiple postulated mechanisms. Increased glucose concentrations can activate nuclear factor-κB (8), a key mediator that regulates multiple pro-inflammatory and pro-atherosclerotic target genes in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and macrophages (7). Inflammation has been strongly implicated in both atherosclerosis and type 2 DM (9, 10). Despite this, no single mechanism yet explains why this pattern is found in diabetic patients (11). C-reactive protein (CRP), a hepatically derived marker of systemic inflammation, is the prototypic inflammatory biomarker. A direct role for CRP in the pathogenesis of vascular damage has been proposed and is supported by some, but not all, experimental data (12). CRP is independently associated with incident CVD and is the only biomarker to be endorsed in guidelines for primary prevention (13, 14).

Accordingly, its exploration seems to be interesting to study the implications of the CVD and the diabetes. So, the purpose in this study is to explore the antioxidant status by the determination of the SOD, GPX and the total antioxidant status (TAS) among coronary diabetic patients and to evaluate inflammation by the use of high sensitivity C reactive protein (hs-CRP).

Patients and methods

Study population

The study population consisted on two groups. The control subjects made of 120 healthy volunteers (80 men and 40 women) with no history of CVD, diabetes or cerebrovascular diseases. Their mean age is 40±7 years.

150 consecutive patients (115 men and 35 women) with angiographically documented CVD were enrolled from Cardiovascular Department of University Hospital Fattouma Bourguiba of Monastir, Tunisia. The mean age of this group is 61,15±10,64 years.

This patients group consisted on 70 diabetic patients and 80 non diabetic subjects. All participants were interviewed and data in dyslipidemia, DM, hypertension, smoking habits were recorded.

Determination of antioxidant parameters

SOD, GPX activities and TAS concentration were performed using commercials tests manufactured by Randox Laboratories (UK, Antrium) in a Daytona - analyser.

Laboratory analysis:

Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and glucose were measured with colorimetric assays using an automated system (Cx 9 Pro- Bechman Coulter - Fuller -Ton, CA).

In Addition, blood samples were collected for the determination of serum apolipoproteins (ApoA1 and ApoB), high sensitive C-reactive proteins (hs-CRP) and fibrinogen according to the instructions of the manufacture using particle-enhancer immunonephelometric assays (BNII Dade Behring, Marburg, Germany).

Statistical analysis

Statistical analysis was done with *Spss 15.0*. Testing of numerical characteristics was done by using *Student's t-test*, and testing of correlations by use of *Pearson's test*. All parameters were given as (mean ± SD)

Results

Characteristics laboratory variables and the values of antioxidative parameters patients and controls subjects are shown in table 1.

Statistical data processing revealed significantly lower TAS, GPX, SOD and hs-CRP values among

Table 1. Characteristics laboratory variables and the values of antioxidative parameters patients and controls subjects

		Patients (n=150)	Controls (n=120)	
Age	(years, X±δ)	61,23±10,81	40±7	ns
BMI	(Kg/m², X±δ)	28,51±5,02	27,8±3,4	ns
Diabetes	(%)	46,7	0	
Hypertension	(%)	48	0	
Smoking	(%)	48	35	
GPX	(U/gHb, X±δ)	59,32±10,72	140,19±30,95	P<0,001
TAS	(mmol/l, X±δ)	1,14±0,28	1,55±0,28	P<0,001
SOD	(U/gHb, X±δ)	629,58±392,033	1378,47± 360,95	P<0,001
Glucose	(mmol/l, X±δ)	8,8 ±3,92	4,50 ± 1,8	P<0,001
ApoA1	(g/l, X±δ)	1,21±0,98	1,55±0,28	P<0,001
ApoB	(g/l, X±δ)	1,45±0,49	0,86±0,22	P<0,001
ApoB/ApoA1		1,85±1,45	0,57±0,19	P<0,001
hs-CRP	(mg/l, X±δ)	7,6±8,8	2,09±0,34	P<0,001
Fibrinogen	(g/l, X±δ)	3,9±1,39	2,61±0,51	P<0,001
HDL-C	(mmol/l, X±δ)	1,3±0,45	1±0,37	P<0,001
LDL-C	(mmol/l, X±δ)	3,75±0,98	1,88±0,8	P<0,001
TG	(mmol/l, X±δ)	1,8±1,15	0,97±0,28	P<0,001
TC	(mmol/l, X±δ)	5,97±1,12	3,6±0,45	P<0,001

patients compared to the controls (TAS: 1,14±0,28 mmol/l vs 1,55±0,355 mmol/l, GPX: 59,32±10,72 U/gHb vs 149,19±30,95 U/gHb, SOD: 629,58±392,033 U/gHb vs1378,47±360,95 U/gHb, hs-CRP: 7,6±8,8 mg/l vs 2,09±0,34 mg/l) (Table 1).

Table 2 shows characteristics laboratory variables and the values of antioxidative parameters in diabetic patients and non diabetic subjects. Diabetic patients had significantly lower values of TAS, GPX, SOD and and hs-CRP in relations with coronary subjects without diabetics complications (TAS: 1,15±0,25 mmol/l vs 1,14±0,29 mmol/l, GPX: 58,37±10,63 U/gHb vs 60,28± 10,70 U/gHb, SOD: 615,42±396,97 U/gHb vs 651,23±389,76 U/gHb, hs-CRP: 7,94±10,82 mg/l vs 7,5±6,6 mg/l). Additional, coronary patients with diabetic complications had significantly higher values of ApoB/A1 ratio, fibrinogen and TG compared to non diabetic patients (Table 2).

Table 2. Characteristics laboratory variables and the values of antioxidative parameters in diabetics patients and non diabetics subjects

		Diabetics patients (n=70)	Non diabetics patients (n=80)	
Age	(years, X±δ)	62,26±9,63	60,48±11,52	ns
BMI	(Kg/m², X±δ)	29,40±4,87	27,87±5,08	ns
GPX	(U/gHb, X±δ)	58,37±10,63	60,24±10,70	P<0,001
TAS	(mmol/l, X±δ)	1,15±0,27	1,14±0,29	P<0,001
SOD	(U/gHb, X±δ)	615,42±396,97	651,23±389,76	P<0,001
Glucose	(mmol/l, X±δ) 9,6 ±4,22	5,01 ± 1,4	P<0,001	
ApoA1	(g/l, X±δ)	1,36±1,10	1,09±0,82	P<0,001
ApoB	(g/l, X±δ)	1,41±0,48	1,47±0,5	P<0,001
ApoB/ApoA1		1,55±1,09	2,08±1,65	P<0,001
hs-CRP	(mg/l, X±δ)	7,94±10,82	7,5±6,6	P<0,001
Fibrinogen	(g/l, X±δ)	4,07±1,34	3,9±1,43	P<0,001
HDL-C	(mmol/l, X±δ)	1,05±0,35	1,07±0,41	P<0,001
LDL-C	(mmol/l, X±δ)	3,53±1,9	3,44±1,09	P<0,001
TG	(mmol/l, X±δ)	2±1,23	1,67±1,04	P<0,001
TC	(mmol/l, X±δ)	4,25±1,28	4,17±0,9	P<0,001

Table 3. Correlations of TAS activity with some clinical and laboratory variables in coronary diabetics patients as well as coronary non diabetics subjects

	Diabetics patients		Non diabetics patients	
	r	p	r	p
Age	-0,033	0,0001	0,017	0,0001
BMI	0,37	0,0001	0,045	0,0001
GPX	0,081	0,0001	0,026	0,0001
SOD	-0,321	0,0001	0,006	0,0001
Glucose	-0,102	0,0001	0,224	0,0001
ApoA1	0,266	0,531 ns	0,179	0,105 ns
ApoB	-0,053	0,0001	0,06	0,0001
ApoB/ApoA1	-0,111	0,0001	-0,04	0,0001
hs-CRP	-0,76	0,0001	0,01	0,0001
Fibrinogen	-0,025	0,0001	0,135	0,0001
HDL-C	-0,121	0,0001	-0,003	0,0001
LDL-C	0,152	0,0001	0,201	0,0001
TG	0,011	0,0001	0,164	0,0001
TC	-0,135	0,0001	-0,105	0,0001

Table 3 shows the correlations between TAS activity and some clinical and laboratory variables in coronary diabetic patients as well as coronary non diabetic subjects.

In coronary diabetic patients, *Pearson's* correlation coefficient revealed significant positive correlation between TAS and hs-CRP (r=0,001, $p<10^{-3}$) (figure 1), TAS and ApoB(r=0,06, $p<10^{-3}$), TAS and fibrinogen

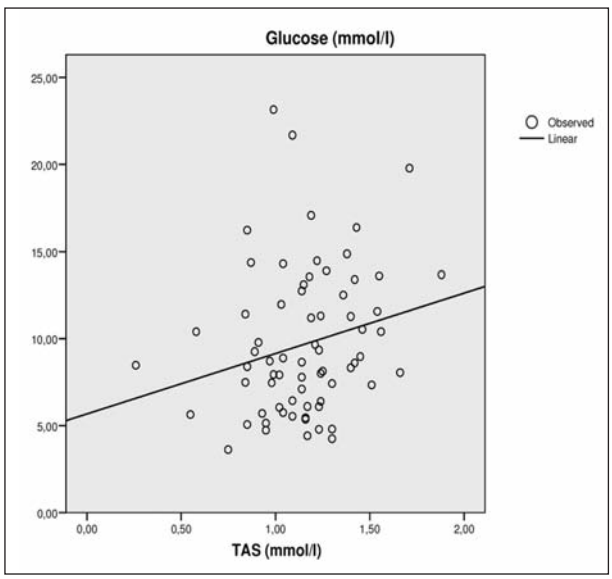

Figure 1. In coronary diabetics patients, Pearson's correlation coefficient revealed significant positive correlation between TAS and glucose (r=0,224, $p<10^{-3}$)

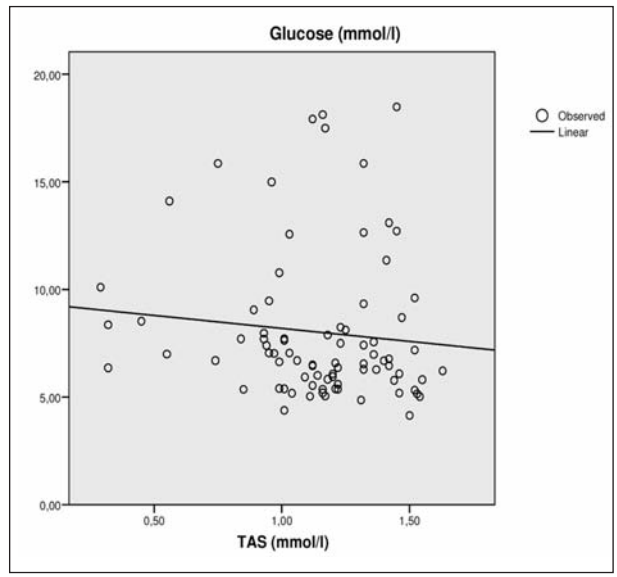

Figure 2. In the coronary not diabetic subjects, the TAS presents a significant negative correlation with glucose (r=-0,102, $p<10^{-3}$)

(r=0,135, $p<10^{-3}$) and TAS and glucose (r=0,224, $p<10^{-3}$) (figure 1). Although, at the coronary not diabetic subjects, the TAS presents a significant negative correlation with the hs-CRP (r=-0,76, $p<10^{-3}$), ApoB(r=-0,053, $p<10^{-3}$), fibrinogen (r=-0,148, $p<10^{-3}$) and glucose (r=-0,102, $p<10^{-3}$) (figure 2).

Then, table 4 shows the correlations of GPX activity with some clinical and laboratory variables in

Table 4. Correlations of GPX activity with some clinical and laboratory variables in coronary diabetics patients as well as coronary non diabetics subjects

	Diabetics patients		Non diabetics patients	
	r	p	r	p
Age	-0,031	0,916 ns	0,04	0,0001
BMI	-0,136	0,0001	0,2	0,0001
TAS	0,081	0,0001	0,026	0,0001
SOD	0,021	0,0001	0,076	0,0001
Glucose	0,129	0,0001	-0,16	0,0001
ApoA1	-0,052	0,0001	-0,161	0,0001
ApoB	0,051	0,0001	0,064	0,0001
ApoB/ApoA1	0,109	0,0001	0,198	0,0001
hs-CRP	-0,027	0,0001	-0,073	0,0001
Fibrinogen	-0,145	0,0001	-0,149	0,0001
HDL-C	0,101	0,0001	0,027	0,0001
LDL-C	0,206	0,0001	-0,071	0,0001
TG	0,072	0,0001	-0,153	0,0001
TC	0,015	0,0001	-0,024	0,0001

coronary diabetic patients as well as coronary non diabetic subjects. Coronary patients with diabetics complications had significantly negative correlation between GPX-hs-CRP (r=-0,073, $p<10^{-3}$), GPX-glucose (r=-0,160, $p<10^{-3}$) (figure 3) and GPX-fibrinogen (r=-0,149, $p<10^{-3}$). In these diabetic patients Pearson's correlation revealed significant positive correlation between GPX and ApoB (r=0,064, $p<10^{-3}$).

At the coronary patients without diabetic complications, Pearson's correlation revealed a positive significantly correlation between GPX-glucose (r=0,129, $p<10^{-3}$) (figure 4), GPX-ApoB (r=0,051, $p<10^{-3}$), and a significantly negative correlation between GPX-hs-CRP (r=-0,027, $p<10^{-3}$).

Moreover, table 5 shows the correlations between SOD activity and some clinical and laboratory variables in coronary diabetic patients as well as coronary non diabetic subjects. In coronary diabetic patients, *Pearson's* correlation coefficient revealed significant positive correlation between SOD and GPX (r=0,021, $p<10^{-3}$). In these diabetic patients Pearson's correlation revealed significant negative correlation between SOD-glucose (r=-0,202, $p<10^{-3}$) (figure 5), SOD-ApoB(r=-0,078, $p<10^{-3}$), SOD- hs-CRP(r=-0,167, $p<10^{-3}$), and SOD-fibrinogen (r=-0,131, $p<10^{-3}$) (Table 5).

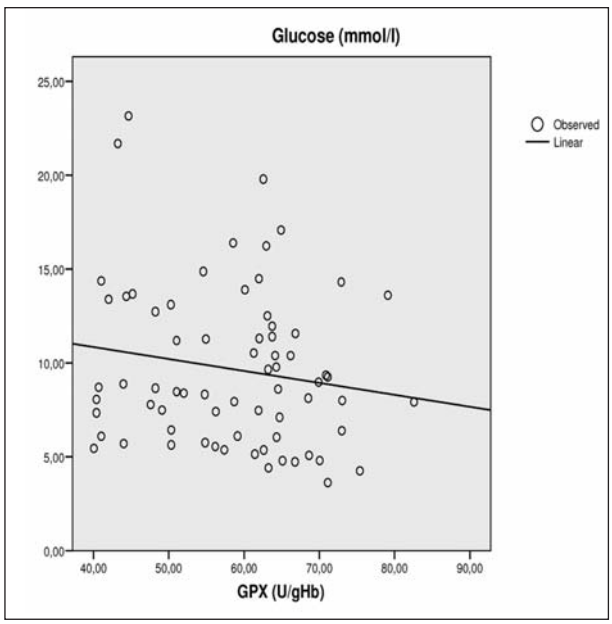

Figure 3. Coronary patients with diabetics complications had significantly negative correlation between GPX-glucose (r=-0,160, $p<10^{-3}$)

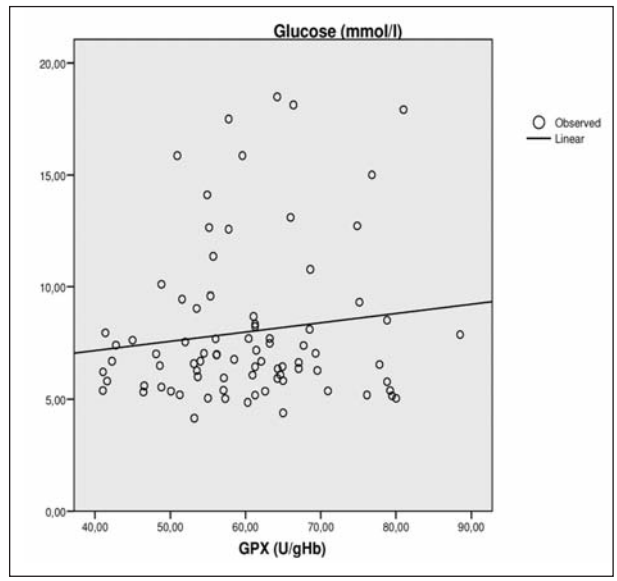

Figure 4. In coronary patients without diabetics complications, Pearson's correlation revealed a positive significantly correlation between GPX-glucose (r=0,129, $p<10^{-3}$)

Although, at the coronary not diabetic subjects, the SOD presents a significant positive correlation with the glucose (r=0,112, $p<10^{-3}$) (Figure 6) and ApoB (r=0,074, $p<10^{-3}$) and a significantly negative correlation between SOD-hs-CRP (r=-0,013, $p<10^{-3}$) and SOD-fibrinogen(r=-0,119, $p<10^{-3}$) (Table 5).

Table 5. Correlations of SOD activity with some clinical and laboratory variables in coronary diabetics patients as well as coronary non diabetics subjects

	Diabetics patients		Non diabetics patients	
	r	p	r	p
Age	-0,200	0,0001	0,001	0,0001
BMI	-0,095	0,0001	0,040	0,0001
GPX	0,021	0,0001	0,076	0,0001
TAS	-0,321	0,0001	0,006	0,0001
Glucose	-0,202	0,0001	0,112	0,0001
ApoA1	-0,015	0,0001	-0,153	0,0001
ApoB	-0,078	0,0001	0,074	0,0001
ApoB/ApoA1	-0,004	0,0001	0,125	0,0001
hs-CRP	-0,167	0,0001	-0,013	0,0001
Fibrinogen	-0,131	0,0001	-0,119	0,0001
HDL-C	-0,108	0,0001	-0,016	0,0001
LDL-C	-0,187	0,0001	0,181	0,0001
TG	0,064	0,0001	0,089	0,0001
TC	-0,097	0,0001	0,064	0,0001

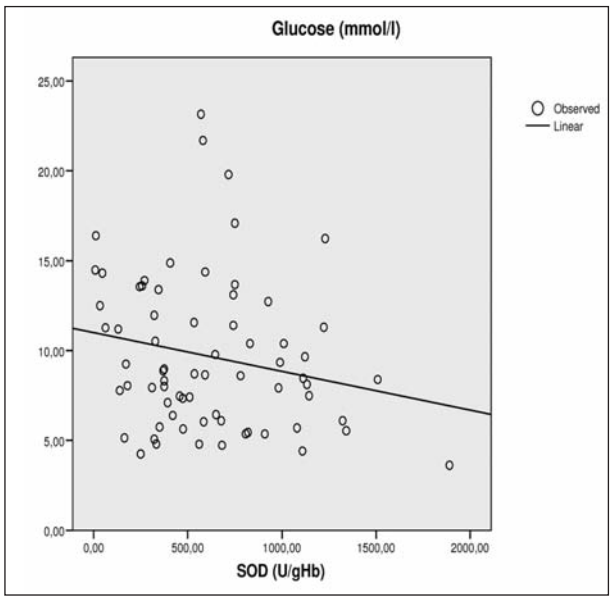

Figure 5. Coronary patients with diabetics complications had significantly negative correlation between SOD-glucose (r= -0,202, $p<10^{-3}$)

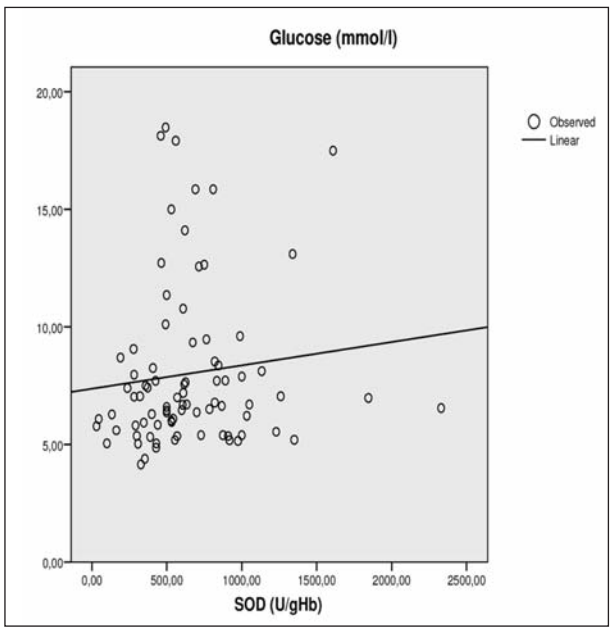

Figure 6. In coronary patients without diabetics complications, Pearson's correlation revealed a positive significantly correlation between SOD-glucose (r=0,112, $p<10^{-3}$)

While, in these two groups, Pearson's correlation shows a significantly positive correlation between GPX- TAS and GPX- SOD (Table 5).

Table 6. Correlations of hs-CRP activity with some clinical and laboratory variables in coronary diabetics patients as well as coronary non diabetics subjects

	Diabetics patients		Non diabetics patients	
	r	p	r	p
Age	0,036	0,0001	0,002	0,0001
BMI	0,194	0,0001	-0,141	0,0001
GPX	-0,073	0,0001	-0,027	0,0001
TAS	0,010	0,0001	-0,076	0,0001
SOD	-0,167	0,0001	-0,013	0,0001
Glucose	-0,058	ns	-0,013	ns
ApoA1	0,012	0,0001	0,067	0,0001
ApoB	0,056	0,0001	-0,134	0,0001
ApoB/ApoA1	-0,053	0,0001	-0,170	0,0001
Fibrinogen	0,415	,003	0,410	0,0001
HDL-C	0,064	0,0001	-0,048	0,0001
LDL-C	-0,073	0,001	-0,149	0,0001
TG	0,141	0,0001	-0,031	0,0001
TC	0,074	0,006	0,091	0,0001

Finally, table 6 shows the correlations of hs-CRP activity with some clinical and laboratory variables in coronary diabetic patients as well as coronary non diabetic subjects.

Coronary patients with diabetics complications had significantly positive correlation between hs-CRP-ApoB (r=0,056, $p<10^{-3}$), hs-CRP-fibrinogen (r=0,415, $p<10^{-3}$) and hs-CRP-TG (r=0,141, $p<10^{-3}$) (Table 6).

Although, at the coronary not diabetic subjects, the hs-CRP presents a significant negative correlation with the ApoB(r=-0,134, $p<10^{-3}$) and the TG (r=-0,031, $p<10^{-3}$) (Table 6).

Discussion

Diabetes mellitus is a complex and multifactorial disease indulging severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis, lipid and protein metabolism. The metabolic dysregulation associated with diabetes causes secondary pathophysiologic changes in multiple organ systems that impose a heavy burden of morbidity and mortality from macrovascular and microvascular complications. Oxidative stress plays an important role in chronic complications of diabetes and is postulated to

be associated with increased lipid peroxidation (15). The present study has examined the changes in both extra and intracellular antioxidants status in diabetic patients. Diabetes has shown to be associated with numerous thrombotic, atherosclerotic, and cardiovascular diseases. Cholesterol has been singled out as the cause of atherosclerosis. However, other lipids, such as triglycerides and phospholipids, also have shown similar correlations (16). In our study, the levels of serum lipids were found to be elevated in diabetic patients. The abnormally high concentration of serum lipids in diabetes is mainly a result of the increase in mobilization of free fatty acids from peripheral depots, because insulin inhibits the hormone-sensitive lipase. On the other hand, glucagons, catecholamines, and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots. The increase and fall in the individual lipoprotein levels is a reflection of the total serum cholesterol levels; that is, the levels of VLDL-C, LDL-C, and HDL-C increase or decrease with the level of total serum cholesterol, and it is their ratio that determines the pathophysiology of lipoprotein metabolism (17,18).

Moreover, many studies have demonstrated the presence of oxidative stress in CVD as an expression of increased free radical production and decreased antioxidant defense (1, 19, 20, 21). Also, there are many different markers that can be used to prove the presence of oxidative stress in CVD or in DM (22). Free radicals are very unstable due to their high reactivity (23, 24).

The involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defense system have been studied. Lipid peroxide-mediated damage has been observed in the development of type 1 and type 2 diabetes mellitus. Insulin secretion is also closely associated with lipoxygenase-derived peroxides (25). Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. The levels of these defense mechanisms are altered in diabetes and, therefore, the ineffective scavenging of free radicals plays a crucial role in determining the extent of tissue injury (26).

Because of their nature, they have a short lifetime and are difficult to measure and accurately determine *in vivo* as well as in biological material such as plasma or other body fluids. So, on the basis of the obtained results, it may be concluded that the values of studied antioxidative parameters (SOD, GPX and TAS) were significantly lower in coronary patients with or without diabetic complications comparatively to the controls.

It's important to highlight that in diabetic group, the increase of glucose concentration is followed by higher activity of TAS and GPX, which means that among these patients hyperglycaemia induce a positive response from the antioxidative defense system (27).

On the contrary, in coronary non diabetic patients, negative response of antioxidative defense system may be related to the effect of protein glycosylation and the impact of oxidative stress on reduced catalytic SOD, TAS and GPX activity all contributing to impaired total antioxidative defense of diabetics patients with cardiovascular complications (28).

Therefore, it is belived that these enzymes are significant in conditions of oxidative stress in atherosclerotic lesions. GPX is an essential enzyme for the elimination of organic and inorganic peroxides, and it is a crucial intracellular antioxidative enzyme (29).

Accordingly, deficiency of GPX and redox glutathione cycle in atherosclerotic tissue may considerably weaken their antioxidative potential and therefore favour the pro-oxidative and atherosclerotic processes, even if is a normal concentration of low molecular ìscavengingî antioxidant exists (30). SOD is considered one of the primary enzymes since they are involved in the direct elimination of ROS. SOD scavenges the superoxide radical by converting it to H_2O_2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. The activity of SOD was found to be lower in diabetic subjects. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes (31). Oda et al. (32) proved, by *in vitro* experiment, that incubation of Cu,Zn-SOD with increasing glucose concentration ranging from 10-100 mmol/l in a time period of 2-120 hours produces increased gly-

cosylation of this enzyme and reduces its activity by 40%. The same authors confirmed this *in vitro* experiment by an *in vivo* one, where the activity of erythrocytic Cu,Zn-SOD in insulin-independent diabetics correlated negatively with glucose concentration, suggesting that hyperglycaemia brings about the glycosylation and inactivity of this enzyme. Therefore, increased oxidative stress may not only result from hyperglycaemia associated with diabetes, but may also have an important causal role in β-cell failure and the development of insulin resistance and type 2 DM (3). Several studies have demonstrated that plasma markers of oxidative stress are elevated in CVD or in the presence of its classical risk factors (33-35). The oxidative of vulnerable cell membrane unsaturated lipids (36) may modulate diverse signal transduction pathways (33, 37) leading to numerous adverse effects implicated in the pathogenesis of atherosclerosis (38).

On the other hand, several studies have suggested that atherosclerosis is a chronic inflammatory disease (39, 40). CRP, an acute phase protein produced mainly in the liver, is an indicator of inflammation. Therefore, patients with type 2 diabetes who are, usually obese, could potentially have high CRP. It is shown also that patients with an elevated CRP have up to 8.5-fold increase in morbidity and mortality (41, 42). In diabetic patients, clustering of traditional and non-traditional CVD risk factors in the same individual may explain their associations with CVD via the common pathway of chronic inflammation. We have studied the associations of hs-CRP concentrations in coronary patients with and without type 2 diabetes. Concentrations of hs-CRP increased when compared with age and sex-matched diabetic controls without CVD suggesting that hs-CRP is a stronger discriminator for detection of CVD in the patients. The elevated hs-CRP concentration may be a reflection of the low level, chronic inflammatory state caused by tissue damage in atheromatous lesions or a consequence of infection with atherogenic organisms such as Helicobacter pylori, Chlamydia pneumoniae, cytomegalovirus or herpes simplex virus (39, 43- 45).

Finally, determination of markers of antioxidative defense as very sensitive parameters not only contributes to a better understanding of oxidative stress effect but on CVD development, diabetes and the treatment of these two diseases. In addition, it is particularly important, determination of markers of antioxidative defense in the prevention of atherosclerosis and diabetes. Also, as the pathogenesis of both diabetes and cardiovascular disease involves oxidative stress, the use of antioxidants is an appealing therapy. However, the use of traditional antioxidants such as vitamin E or C in large clinical trials has failed to demonstrate any beneficial effect on cardiovascular disease or all-cause mortality, even when only diabetic patients were analyzed (46, 47). The use of antioxidant vitamins may have been initiated too late or for too a short time in the course of atherogenesis to demonstrate an effect (48). There is evidence that both vitamins C and E not only are ineffective at lowering biological markers of oxidative stress (49, 50), but can act as pro-oxidants (44, 45). These findings have prompted a search for new antioxidants which may be able to impact atherosclerosis. One of these, AGI-1067 (the monosuccinic acid ester of probucol), has shown promising results and is currently undergoing phase III trials (51, 52). Importantly, pharmacologic agents currently in use that have been shown to be effective in reducing cardiovascular mortality are known to have antioxidant properties. An important issue in the use of either antioxidants or specific inhibitors of ROS signaling is that of tissue- or cell specific delivery, as oxidative molecules are also active in tissues and cells that are not participating in a pathologic process. An approach that is currently being tested is the use of nanoparticle or liposome-enclosed pharmacologic agents (53, 54). These ligand-conjugated vehicles can deliver a therapeutic agent to cells expressing a specific receptor.

Conclusion

CVD is the major cause of mortality in patients with diabetes, and hense the cost and clinical implications of the condition are significant. Oxidative cellular damage provides a possible explanation for the increase risk seen in diabetes and perhaps for unexplained cardiovascular events in subjects with little in the way of other risk factors. The early initiation of therapy aimed at reducing oxidative stress and/or

modulating ROS-sensitive signalling pathways may be of benefit for reducing cardiovascular disease in diabetes. Further insights into the molecular mechanisms of the metabolic basis of diabetes will prove invaluable in the treatment and prevention of this debilitating condition.

References

1. Godin DV, Wohaeib SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol Cell Biochem* 1988; 84: 223-33.
2. Panzram G. Mortality and survival in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1987; 30: 123-31.
3. Bucala R, Mitchell R, Arnold K, Innerarity T, Vlassara H, Cerami A. Identification of the major site of apolipoprotein B modification by advanced glycosylation end products blocking uptake by the low density lipoprotein receptor. *J Biol Chem* 1995; 270: 10828-32.
4. Touyz RM. Reactive oxygen species, vascular oxidative stress and redox signaling in hypertension: what is the clinical significance? *Hypertension* 2004; 44: 248-52.
5. Stephens WJ, Khanolkar MP, Bain SC. The biological relevance and measurement of plasma markers of oxidative stress in diabetes and cardiovascular disease. *Atherosclerosis* 2009; 202 (2): 321-9.
6. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; 404: 787-90.
7. Wolf SP. Diabetes mellitus and free radicals. Free radical transition metals and oxidative stress in the aethiology of diabetes mellitus and complications. *Br Med Bull* 1993; 49: 642-52.
8. Orasanu G, Plutzky J. The Pathologic Continuum of Diabetic Vascular Disease. *J Am Coll Cardiol* 2009; 53 (5 Suppl): S35-42.
9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352: 1685-95.
10. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; 116: 1793-801.
11. Dasu MR, Devaraj S, Jialal I. High glucose induces IL-1 beta expression in human monocytes: mechanistic insights. *Am J Physiol Endocrinol Metab* 2007; 293: E337-46.
12. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, Creactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. *J Am Med Assoc* 2005; 293: 1609-16.
13. Pasupathi P, Chandrasekar V, Kumar US. Evaluation of oxidative stress, enzymatic and non-enzymatic antioxidants and metabolic thyroid hormone status in patients with diabetes mellitus. *Diabetes & Metabolic Syndrome: Diabetes Metab Syndr* 2009; 3: 160-5.
14. Venkateswaran S, Pari L, Saravanan G. Effect of Phaseolus vulgaris on circulatory antioxidants and lipids in rats with streptozotocin-induced diabetes. *J Med Food* 2002; 5: 97-103.
15. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian J Clin Biochem* 2006; 21: 126-30.
16. Giugliano D, Acampora R, D'Onofrio F. Medical hypothesis: cardiovascular complications of diabetes Mellitus - from glucose to insulin and back. *Diabete Metab* 1994; 20: 445-53.
17. Noda Y, Mori A, Packer L. Gliclazide scavangers hydroxyl, superoxide and nitric oxide radicals: an ESR study. *Mol Pathol Pharmacol* 1997; 96: 115-24.
18. Richard C, OÆBrien T, Ming L. The effect of gliclazide and other sulfonylureas on low-density lipoprotein oxidation invitro. *Metabolism* 1997; 46: 22-5.
19. Banga JD. Lower extremity arterial disease in diabetes mellitus. Diabetes Rev Int 1994; 3: 6-11.
20. Paolisso G, Giugliano D. Oxidative stress and insulin action: is there a relationship? *Diabetologia* 1996; 39: 357-63.
21. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Glarendon Press 1989.
22. Kuyvenhoven JP, Meinders AE. Oxidative stress and diabetes mellitus pathogenesis of long-term complications. *EJIM* 1999; 10: 9-19.
23. Ramachandran B, Ravi K, Narayanan V, Kandaswamy M, Subramanian S. Effect of macrocyclic binuclear oxovanadium complex on tissue defense system in streptozotocin-induced diabetic rats. *Clin Chim Acta* 2004; 345: 141-50.
24. Granic P, Laboratorijski pokazatelji okidacijskog stresa. In: Bradamente V, Lackovic Z, eds. Oksidativni stres i djelotvornost antioksidansa. *Zangreb: Medicinska naklada* 2001: 49-65
25. Borcea V, Nourooz-Zadeh J, Wolf SP, et al. Alfa lipoic acid decreases oxidative stress even in diabetic patients with poor glycemic control and albuminuria. *Free Radic Biol Med* 1999; 22: 1495-500.
26. Jandric-Balen M, Bozikov V, Bozikov J, Metelko Z, Jandric I, Romic Z. Impact of glycemic control on antioxidant enzyme activity in patients with type 2 diabetes mellitus. *Diabetologia Croatica* 2004; 33 (4):131-5
27. Blankenberg S, Rupprecht HJ, Bickel C, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* 2003; 349 (17): 1605-13.
28. Suarna C, Dean RT, May J, Stocker R. Human atherosclerotic plaques contain both oxidized lipids and relatively large amounts of a-tocopherol and ascorbate. *Arterioscler Thromb Vasc Biol* 1995; 15: 1616-24.
29. Sozmen EY, Sozmen B, Delen Y, Onat T. Catalase/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. *Arch Med Res 2001*; 32: 283-7.
30. Colak E, Majkiμ-Singh N, Stankoviμ S, et al. Parameters of antioxidative defense in type 2 diabetic patients with cardiovascular complications. *Ann Med* 2005; 37: 613-20.

31. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol* 2003; 91: 7A-11A.
32. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000; 87: 840-4.
33. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med* 2000; 28: 1815-26.
34. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; 23: 599-622.
35. Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 1997; 22: 269-85.
36. Ceconi C, Boraso A, Cargnoni A, Ferrari R. Oxidative stress in cardiovascular disease: myth or fact? *Arch Biochem Biophys* 2003; 420: 217-21.
37. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. *New Engl J Med* 1992; 326: 242-50.
38. Alexander RW. Inflammation and coronary artery disease. *New Engl J Med* 1994; 331: 468-9.
39. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. *New Engl J Med* 1994; 331: 417-24.
40. Ridker PM, Hennekens CH, Buring JE, Rifai N. C Reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New Engl J Med* 2000; 342 (12): 836-43.
41. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; 362 (6423): 801-9.
42. Patel P, Mendall MA, Carrington D, et al. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. *BMJ* 1995; 311 (7007): 711-4.
43. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ* 1996; 312 (7038): 1061-5.
44. Heart Protection Study Collaborative. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; 360: 23-33.
45. Lonn E, Yusuf S, Hoogwerf B, et al. Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes: results of the HOPE study and MICRO-HOPE substudy. *Diabetes Care* 2002; 25 (11): 1919-27.
46. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury. Part II. Animal and human studies. *Circulation* 2003; 108 (17): 2034-40.
47. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 1996; 94 (1): 19-25.
48. Meagher EA, Barry OP, Burke A, et al. Alcohol-induced generation of lipid peroxidation products in humans. *J Clin Invest* 1999; 104 (6): 805-13.
49. McGregor G.Vitamin C contributes to inflammation via radical generating mechanisms: a cautionary note. *Med Hypotheses* 2004; 62 (4): 641-2.
50. Upston JM, Terentis AC, Morris K, Keaney Jr JF, Stocker R. Oxidized lipid accumulates in the presence of alpha-tocopherol in atherosclerosis. *Biochem J* 2002; 363 (Pt 3): 753-60.
51. Tardif JC, Grogoire J, Lavoie MA, L'Allier PL. Pharmacologic prevention of both restenosis and atherosclerosis progression: AGI-1067, probucol, statins, folic acid and other therapies. *Curr Opin Lipidol* 2003; 14 (6): 615-20.
52. Tardif JC, Gregoire J, Schwartz L, et al. Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* 2003; 107 (4): 552-8.
53. Carlsson J, Kullberg EB, Capala J, Sjoberg S, Edwards K, Gedda L. Ligand liposomes and boron neutron capture therapy. *J Neuro-Oncol* 2003; 62 (1-2): 47-59.
54. Hattori Y, Maitani Y. Enhanced in vitro DNA transfection efficiency by novel folate-linked nanoparticles in human prostate cancer and oral cancer. *J Controlled Release* 2004; 97 (1): 173-83.

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