

**Original Article:****Low-dose captopril and antioxidant combination as adjunct therapy in type-2 diabetic patients with coronary artery disease: A preliminary study**H. Elewa<sup>1</sup>, Z.A. Zalat<sup>1</sup>, G. Oriquat<sup>2</sup>, R. Rifaat<sup>3</sup>, W. El-Hadidy<sup>3</sup>, S. Yacoub<sup>4</sup>**Abstract:**

Atherosclerosis constitutes a major cause of complications of diabetes. The mechanisms involved include, oxidative modification of low density lipoproteins (LDL) through a multitude of endogenous mediators including homocysteine and angiotensin II. Assessment of supplementation with either low-dose ACE inhibitor or a combination of antioxidants to the treatment regimens of type 2 diabetics with CAD on some markers of atherosclerosis. Thirty subjects, of the same socio-economic background, were recruited and divided into 3 groups. Group I included 10 healthy individuals. The 20 diabetic subjects with CAD were randomly distributed into of group II and group III. Treatment of patients in group II was supplemented by a once daily tablet containing antioxidant combination. Supplementation in group III patients consisted of 12.5 mg daily dose of the ACE inhibitor, captopril. Compared to the healthy individuals, diabetic patients showed hyperglycemia, elevated glycated hemoglobin, increased oxidative stress, depressed antioxidant defense, hyperhomocysteinemia and elevated levels of oxidized LDL autoantibodies. The results of a three month follow-up of type 2 diabetic patients indicated that both adjunct treatments improved all parameters tested, including glycemic control, oxidative stress, and hyperhomocysteinemia. However, the titer of circulating ox-LDL autoantibodies, which declined very rapidly, was not a good prognostic indicator for atherosclerosis.

Conclusion: The observed shift in the disease indices towards normal levels make the use of both adjuvant therapies in type 2 diabetics with atherosclerotic CAD worth pursuing in a larger clinical study.

**Key words:** Type 2 diabetes, atherosclerosis, antioxidants, low dose captopril

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**Introduction:**

The pathology of diabetes mellitus is associated with an increased incidence of macrovascular complications including coronary artery disease (CAD) [1]. It is well established that diabetes is one of the major risk factors for atherosclerosis and diabetic patients have a two- to four- fold higher risk of coronary heart disease than non-diabetic individuals [2]. These atherosclerotic complications constitute the main cause of mortality among diabetic patients, in general, and in type-2 diabetics, in particular [3]. Although the atherosclerotic process is indistinguishable from that affecting non-diabetic population, it begins earlier and may be severer [4].

The accelerated atherosclerosis in diabetes involves a multitude of mechanisms. Oxidative modification of low density lipoprotein (LDL) has been implicated as a major factor in the pathogenesis of coronary atherosclerosis [5]. A

correlation has also been established between the level of circulating ox-LDL and the extent of CAD in type 2 diabetic patients [6]. Once formed, the native properties of oxidized LDL are altered and may contribute to rapid progression of atherosclerosis, by a multitude of mechanisms [7,8]. Homocysteine causes autoxidation of LDL through generation of the superoxide radical, reduction of the antioxidant status, and affecting nitric oxide production, which could also injure vascular endothelial cells [9, 10]. Another important factor responsible for accelerated atherosclerosis in diabetes is the non-enzymatic reaction between glucose and proteins or lipoproteins leading to glycated products in arterial walls [1].

Reactive oxygen species (ROS) provide a link between angiotensin II and atherosclerosis. Angiotensin II is a potent stimulator of ROS production in endothelial cells and vascular smooth muscle cells [11]. Angiotensin converting enzyme inhibitors (ACEIs) therapy was found to reduce oxidant stress in the blood vessel wall, as measured by decreased ox-LDL or improved endothelial non-dependent vasorelaxation. [12].

The aim of the present study is to compare the possible role of supplementation with either a low dose of captopril or a combination of antioxidants to the regular treatment regimens of type 2 diabetic patients with CAD on some markers of atherosclerosis.

### **Subjects, Materials and Methods:**

The Participants in the study were normotensive subjects of the same socio-economic class and were divided into three different groups. Group I (controls) included 10 healthy non-obese individuals aged 41-71 years. Group II and group III subjects (10 patients in each group) were type 2 diabetics with atherosclerotic CAD. Their ages ranged from 46 to 72 years. These patients were diagnosed, treated and followed-up in the outpatient clinic of the cardiology unit of the Medical Research Institute of Alexandria University. The reported duration of their diabetes ranged between 2 and 8 years. Their hepatic and renal functions (particularly microalbuminuria) were within the clinically acceptable ranges. Criteria for exclusion from these two groups included a

history of ketoacidosis, severe renal or liver dysfunction, malignancy and endocrine problems other than diabetes, smoking and the use of antioxidant or multivitamin supplements.

Type 2 diabetic patients, randomly distributed in groups II and III, were treated with diet and oral antidiabetic agents. Most (16 out of 20 patients) were treated with a combination of sulphonylurea; either chlorpropamide (100-250 mg daily) or glyburide (2.5-5 mg daily), and metformin (500 mg 2-3 times daily). One patient was on glyburide (5 mg daily) as monotherapy, while 3 were on rosiglitazone (2-4 mg three times daily). Prescribed dosages were individualized according to the patient requirement. The study was approved by the ethics committee of the Medical Research Institute and informed written consent was obtained from each participant, before enrollment.

At the beginning of the study, treatment of each of the 10 patients in group II was supplemented by once daily antioxidant tablet (Antox ®, Pharco Pharmaceuticals and Alexandria), containing 30 mg vitamin E, 100 mg ascorbic acid, 5.54 mg vitamin A acetate, 50µg selenium and 105 mg medical yeast. Supplementation in group III patients consisted of a 12.5 mg daily dose of the angiotensin converting enzyme inhibitor, captopril (Capoten™, Squibb Egypt and Cairo).

### **Biochemical Assays:**

Fasting plasma glucose level was assayed by the glucose oxidase method [13]. Glycated hemoglobin (HbA1c) was determined using a turbidimetric inhibition immunoassay for hemolyzed whole blood [14]. The enzymatic method described by Griffith [15] was used to measure the plasma total glutathione (tGSH) and the reduced (rGSH) and oxidized (GSSG) fractions. The Nernst equation was used to calculate the plasma redox potential [16].

The lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) and calculated as malondialdehyde (MDA) [17]. Serum level of autoantibodies against oxidized low density lipoproteins was estimated by using an enzyme immunoassay kit (Biomedica, USA) [18]. Serum homocysteine

was determined by an immunochemical assay using a commercial kit (Axis Biochemicals).

### Statistical Analysis:

All data have been presented as mean + SD. One-way analysis of variance (ANOVA) was performed on each variable and the Bonferroni statistics employed to compare the mean values from the different groups. Paired t-test was used to assess the effect of adjunct therapies used at one and three months. Differences were considered significant at  $P < 0.05$ . All statistical analyses were performed using SPSS statistical software (version 10).

### Results:

The results of the control of glycemia and other clinical chemistry parameters in the different studied groups are summarized in Table 1. As may be expected, diabetic patients showed higher fasting blood glucose levels than did the controls. Compliance with antidiabetic treatment was reasonably good, as judged by HbA<sub>1c</sub> levels. However, in diabetic patients with CAD, the plasma homocysteine levels, at the beginning of the study, were 60% to 70% higher, as compared with the control group. Besides, the antibodies against oxidized LDL (oxLDL-Abs) were also elevated by 50-60%.

In general, addition of the antioxidant combination or captopril to the treatment regimens of type 2 diabetic patients with CAD improved their glycemic status. Better control in the form of a stepwise decline in fasting blood glucose was observed, which could be detected even after the first month of the study period. With the antioxidant combination, the average fasting blood glucose level showed statistically significant decreases to  $10.2 \pm 1.39$  mmol/L, after one month and to  $8.7 \pm 1.83$  mmol/L at 3 months; the end of the study period of. However, the changes in the glycated hemoglobin were not as prominent, as was expected. It showed decline to  $6.50 \pm 0.80\%$  and to  $6.00 \pm 0.73\%$  after 1 and 3 months of initiating adjunct antioxidant therapy, respectively. Such changes in HbA<sub>1c</sub> level were without statistical significance. The low dose of captopril had a relatively milder effect on glycemic control. The fasting blood glucose declined by 10.3% to  $9.83 \pm 1.01$  mmol/L and by 22.1% to  $8.54 \pm 1.27$  mmol/L, while the HbA<sub>1c</sub>

showed slight declines down to  $6.74 \pm 1.08\%$  and to  $6.30 \pm 1.09\%$  after one and three months respectively.

Increased production of TBARS and perturbed glutathione system representing excessive oxidative stress was clear in the diabetic patients (Table 2). The blood levels of TBARS were about twice as high as the average of controls. This was coupled with lower levels of total and reduced GSH. The decrease was more pronounced in reduced GSH, reaching 83.7% and 73.6% below control in groups I and II respectively. In contrast to the reduced GSH, plasma concentration of GSSG was higher in diabetics, being more than three-fold higher than the control value. The large decrease in GSH/GSSG ratio was indicative of the oxidative stress in diabetic patients. From the obtained glutathione results, the calculated redox potential confirmed the shift in the redox environment of the plasma of the diabetic patients towards a more oxidative state.

Adjunct treatment with antioxidant combination was effective in alleviating the stressful condition in diabetic plasma (Table 3).

Even in the short follow-up period of three months, TBARS slowly but steadily declined towards the normal control level. Although the mean blood concentration declined by 31.1% from that of base line at the end of the follow-up period, it was still 51.6% higher than control. Improvement in the redox environment was also manifested in the changes of the glutathione values. The increase in total GSH was coupled with a relative large elevation in reduced GSH and a decrease in GSSG, but the GSH/GSSG ratio was still far remote from that of control. A gradual improvement in the blood redox potential was evident; showing only 17.3% difference from control value. Adjunct treatment with captopril gave a similar qualitative pattern, but the quantitative improvement was lesser. This could be seen in the results of the different fractions of glutathione and the calculated redox potential at the end of the study period (Table 4), which was less prominent than those of the antioxidant treatment.

The changes in the serum levels of the ox-LDL Abs following the adjunct therapy are illustrated in Figure 1. The oxidized LDL

antibodies showed a strong response to the administration of the antioxidant combination represented by a 23.1% decline after one month and practically reaching mean control value at the end of 3 months. Again, the effect of captopril was qualitatively similar with somewhat less quantitative response. After one month of treatment, the serum level of the ox-LDL Ab decreased by 12.6% and after 3 months by 26.4% being only 10.8% higher than the control value.

The results of adjunct therapy on the serum concentration of homocysteine are presented in Figure 2. The decline in the elevated homocysteine values in diabetic patients paralleled with what was seen with ox-LDL Abs.

However, the rate of decline was slower, reaching values at the end of the study, still significantly higher than the control value of 11.58  $\mu\text{mol/ml}$ . With the antioxidant combination, the mean homocysteine concentration in plasma, which was 18.88 + 2.08  $\mu\text{mol/ml}$  at baseline, decrease by 23.4% after one month and by 32.0% after 3 months of therapy to reach 12.86 + 1.37  $\mu\text{mol/ml}$ . This was 11.1% higher than the control value. The decline with captopril was somewhat slower. It decreased from a baseline value of 19.69 + 3.09  $\mu\text{mol/ml}$  to 15.89 + 1.98  $\mu\text{mol/ml}$  after one month and to 13.83 + 2.96  $\mu\text{mol/ml}$  at the end of the study. This was still 19.4% higher than control.

**Table 1. Characteristics of study groups.**

Variables	Diabetics with CAD		
	Group I (Control)	Group II (Antioxidants)	Group III (Captopril)
	n = 10	n = 10	n = 10
<b>Age range (years)</b>	41-70	44-71	42-69
<b>Gender (M/F)</b>	6/4	7/3	7/3
<b>FBG (mmol/L)</b>	4.8 + 0.54	13.7 + 2.94*	11.0 + 1.46*
<b>HbA1c (%)</b>	5.0 + 0.51	6.9 + 0.89*	7.0 + 1.08*
<b>Homocysteine (<math>\mu\text{mol/ml}</math>)</b>	11.6 + 2.08	18.9 + 2.08*	19.7 + 3.09*
<b>Ox-LDL Ab (mU/ml)</b>	304.1 + 61.5	476.2 + 172.3*	457.9 + 94.5*

Data presented as mean + SD.

\* Significantly different from control group by one-way ANOVA

FBG, Fasting blood glucose, HbA1c, glycosylated hemoglobin, ox-LDL Ab, oxidized low-density lipoprotein antibodies

**Table 2: Oxidative stress and antioxidant parameters in the plasma of the studied groups.**

Variables	Control	CAD + antioxidants	CAD + captopril
	Group I	Group II	Group III
<b>TBARS (nmol/mL)</b>	2.52 + 0.46	5.08 + 1.68 <sup>a</sup>	4.87 + 1.30 <sup>a</sup>
<b>tGSH (nmol/mL)</b>	2.99 + 0.48	1.25 + 0.06 <sup>a</sup>	1.57 + 0.21 <sup>a</sup>
<b>rGSH (nmol/mL)</b>	2.76 + 0.49	0.45 + 0.26 <sup>a</sup>	0.73 + 0.13 <sup>a</sup>
<b>GSSG (nmol/mL)</b>	0.12 + 0.01	0.40 + 0.06 <sup>a</sup>	0.42 + 0.05 <sup>a</sup>
<b>GSH/GSSG</b>	23.9 + 5.4	1.2 + 0.6 <sup>a</sup>	1.7 + 0.3 <sup>a</sup>
<b>Redox potential (mV)</b>	-139 + 4.7	-78 + 8.2 <sup>a</sup>	-66 + 4.0 <sup>a</sup>

Data presented as mean + SD

<sup>a</sup> Significantly different from control value by one-way ANOVA

TBARS: thiobarbituric acid reactive substances calculated as malondialdehyde (MDA)

tGSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione

**Table 3: Effect of adjunct treatment with antioxidant combination on oxidative stress and antioxidant parameters in type 2 diabetic patients with CAD (Group II)**

Variables	Baseline	1 month	3 months
<b>TBARS (nmol/mL)</b>	5.08 + 1.6	4.40 + 1.4 (↓12.6%)	3.50 + 0.9 <sup>a</sup> (↓31.1%)
<b>tGSH (nmol/mL)</b>	1.52 + 0.06	1.79 + 0.3 (↑ 17.8%)	2.39 + 0.39 <sup>ab</sup> (↑ 57.2%)
<b>rGSH (nmol/ml)</b>	0.45 + 0.26	1.04 + 0.26 <sup>a</sup> (↑ 131.1%)	1.75 + 0.30 <sup>ab</sup> (↑ 288.9%)
<b>GSSG (nmol/mL)</b>	0.40 + 0.06	0.38 + 0.11 (↓ 5.0%)	0.32 + 0.07 <sup>ab</sup> (↓ .20.0%)
<b>GSH/GSSG</b>	1.2 + 0.6	3.0 + 1.2 <sup>a</sup>	5.4 + 1.3 <sup>a</sup>
<b>Redox potential (mV)</b>	-78 + 8.2	-100 + 7.3 <sup>a</sup>	-115 + 3.8 <sup>ab</sup>

Data presented as mean + SD

<sup>a</sup> Significantly different from baseline value by paired t-test ( $p < 0.05$ )

<sup>b</sup> Significantly different from one month treatment by paired t-test ( $p < 0.05$ )

Number in parentheses represents percentage deviation from baseline value

TBARS: thiobarbituric acid reactive substances calculated as MDA

tGSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione

**Table 4: Effect of adjunct treatment with low-dose captopril on oxidative stress and antioxidant parameters in type 2 diabetic patients with CAD (Group III)**

Variables	Baseline	1 month	3 months
<b>TBARS (nmol/mL)</b>	4.87±1.21	4.69±1.30 (↓3.70%)	3.99±1.02 (↓18.1%)
<b>tGSH (nmol/mL)</b>	1.57±0.21	1.82±0.38 (↑15.9%)	2.33±0.28 <sup>ab</sup> (↑48.4%)
<b>rGSH (nmol/ml)</b>	0.73±0.13	0.88±0.30 <sup>a</sup> (↑20.5%)	1.61±0.28 <sup>ab</sup> (↑120.5%)
<b>GSSG (nmol/mL)</b>	0.42±0.05	0.40±0.05 (↓4.76%)	0.36±0.04 <sup>a</sup> (↓14.3%)
<b>GSH/GSSG</b>	1.7 + 0.3	2.6 + 0.4	4.5 + 1.1 <sup>a</sup>
<b>Redox potential (mV)</b>	-66±4.0	-75 + 4.2 <sup>a</sup>	- 88 + 4.9 <sup>ab</sup>

Data presented as mean + SD

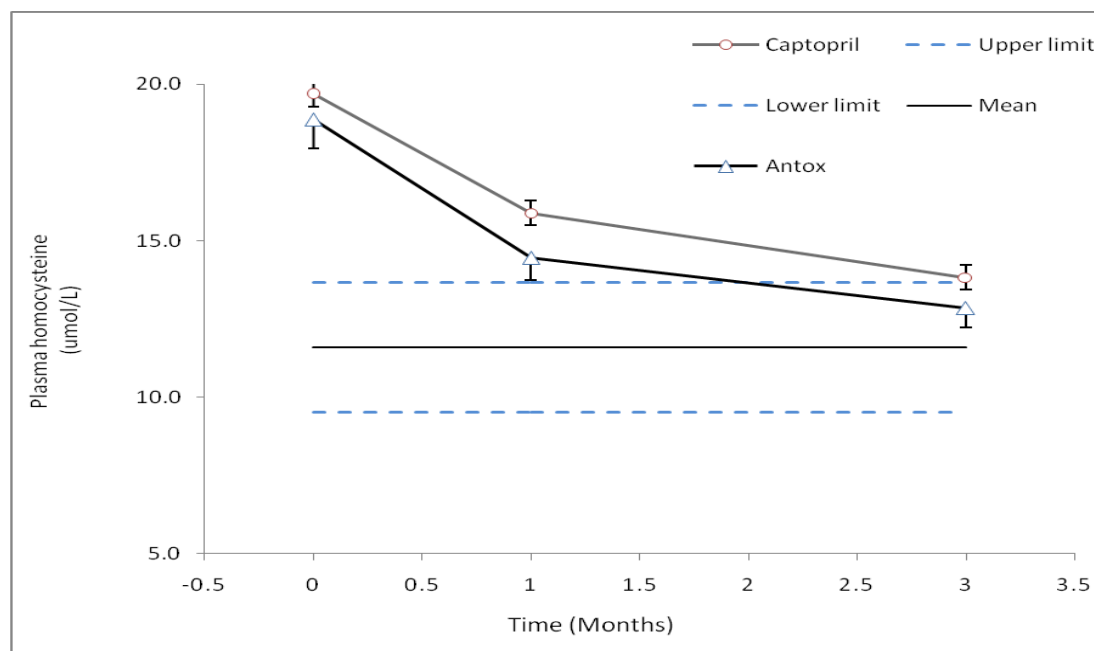
<sup>a</sup> Significantly different from baseline value by paired t-test (p<0.05)

<sup>b</sup> Significantly different from one month treatment by paired t-test (p<0.05)

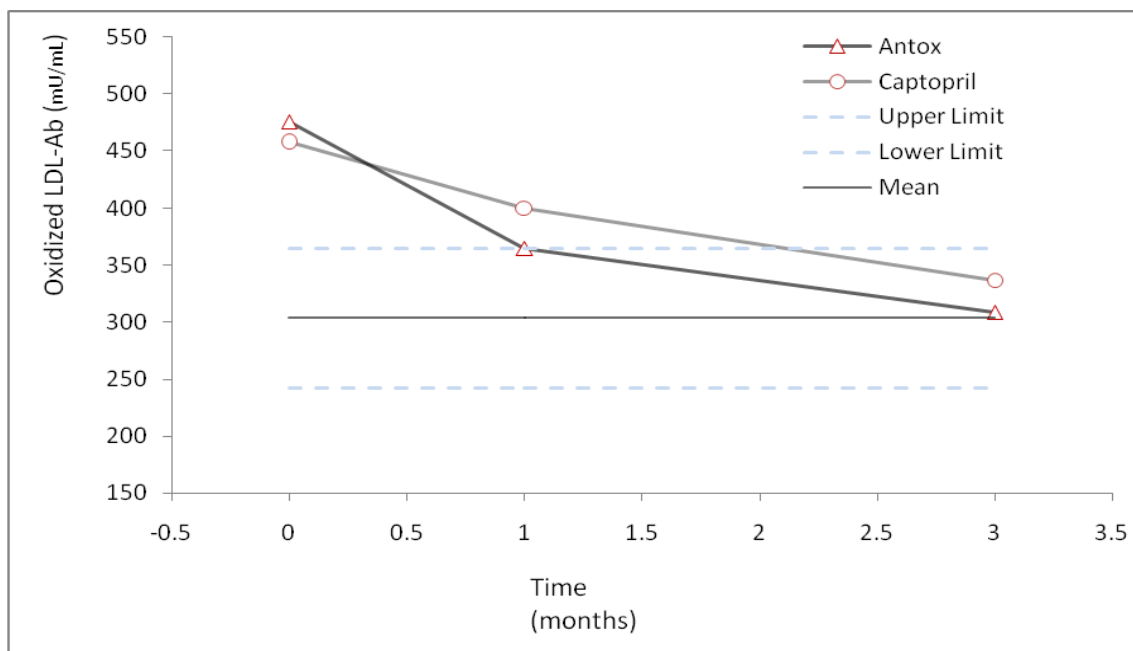
Number in parentheses represents percentage deviation from baseline value

TBARS: thiobarbituric acid reactive substances calculated as MDA

tGSH: total glutathione, rGSH: reduced glutathione, GSSG: oxidized glutathione



**Figure (1): Changes in plasma homocysteine concentration in type 2 diabetic patients with CAD following addition of antioxidant combination or low dose captopril to the basic antidiabetic treatment regimen**



**Figure (2): Changes in plasma oxidized LDL-Ab concentration in type 2 diabetic patients with CAD following addition of antioxidant combination or low dose captopril to the basic antidiabetic treatment regimen.**

#### Discussion:

Atherosclerosis has been characterized as an inflammatory disease of the blood vessel wall resulting from an initial injury that increases local oxidative stress. All the cardiovascular risk factors can initiate cellular events that lead to endothelial dysfunction by altering the redox state in the vessel wall. Complications of diabetes can be traced back to vascular origin. Hyperglycemia induces a large number of alterations in vascular tissue that potentially promote accelerated atherosclerosis. Among the sequel of hyperglycemia, oxidative stress has been suggested as a potential mechanism for atherosclerosis. A major mechanism of oxidative stress appears to be the hyperglycemia-induced intracellular ROS, generated by mitochondrial electron transport chain, leading to increased production of superoxide radicals [19]. Another mechanism involves the autoxidation of free glucose, which is catalyzed by transition metals, yielding superoxide anion and hydrogen peroxide [20].

The results of the present study clearly indicated that there was a definite overproduction of free radicals and excessive exposure to oxidative stress in diabetic patients.

The lipid peroxidation index, TBARS, was significantly higher in diabetic patients with CAD than in healthy individuals. Increased production of TBARS, which was coupled with depressed levels of total glutathione, depletion of reduced GSH, and lower levels of the redox potential in type 2 diabetics with CAD, clearly indicated that these patients suffered a strong oxidative stress, compared to non-diabetic controls. The addition of antioxidants or captopril to the treatment regimen of the diabetic patients resulted in improvement in their glycaemic status. Such improvement, represented by statistically significant decreases in the fasting blood glucose and HbA1c levels, probably reflects a tendency towards overall improvement in general health and tissue metabolic status and alleviation of oxidative stress [6]. The better control of the glycaemic status, seen by the end of the first month of the study probably indicates a very rapid action of the adjunct therapy. It has been reported that scavenging antioxidants in combination act synergistically [21], while captopril has been shown to increase

endogenous oxidant scavengers in mouse tissue [12].

Many reactions associated with hyperglycemia may acutely and chronically increase the production of free radicals, resulting in an oxidant/antioxidant imbalance [20, 22]. Glutathione is the dominant intracellular non-protein thiol and the single largest source of reducing equivalents, accounting for about 90% of these equivalents [23]. Therefore, the depletion of reduced glutathione could affect the overall redox potential significantly. The present study found decreased levels of total and reduced GSH, with increased GSSG. Many investigations have reported a lower concentration of GSH in the plasma of diabetic patients [6, 24]. In addition to the oxidant/antioxidant imbalance, the decreased level of glutathione could be influenced by decreased activity of certain enzymes, such as  $\gamma$ -glutamylcysteine synthase and glutathione reductase, possibly because of their glycation by hyperglycemia [25]. In view of the observed decreased HbA1c, as an indication of protein glycation, it is possible that the recovery of the glycated glutathione metabolizing enzymes will depend more on the turn-over rate of their proteins. This could be one reason for the relative rapid increase in total and reduced glutathione.

By inspecting the calculated redox potential in the present study, it became clear that redox potential for the diabetic patients with CAD was shifted towards the oxidizing side. Supplementation with the antioxidant combination or captopril for three months partially corrected the balance of GSH/GSSG to restore the reducing potentials.

Oxidative modification of LDL has been implicated as a major factor in the pathogenesis of coronary atherosclerosis. Some studies have demonstrated that ox-LDL levels are significantly higher in patients with diabetes mellitus than in the control subjects, and that the high levels of circulating ox-LDL can serve as an independent and significant predictor for future cardiac events in type 2 diabetic patients [5, 6]. The levels of circulating ox-LDL antibodies were found to be significantly higher in patients with type 2 diabetes than in control subjects. Such higher levels were taken as

indirect indication that the levels of ox-LDL were increased. The definite and steady decline in the circulating ox-LDL antibodies was clear and is indicative of improvement in the atherosclerosis condition. Vitamin E, a component of the antioxidant mixture used and the major fat soluble antioxidant present in the LDL particle, is believed to protect LDL from oxidative damage. It does this by acting as a chain breaking antioxidant and preventing lipid peroxidation of polyunsaturated fatty acids and modification of proteins by ROS [26]. Among the endogenous plasma antioxidants, ascorbic acid is particularly active in inhibiting lipid peroxidation induced by different types of oxidative stress. Supplementation with vitamin E and vitamin C has a potential role in boosting antioxidant defense [27]. Moreover, decreasing the synthesis and/or blocking the action of Angiotensin II improve endothelial dysfunction and slow the progression of atherosclerosis in diabetic patients. However, the response of the circulating ox-LDL Abs occurred early in the study period, almost reaching control level by the end of three months. There was no equivalent reciprocal effect on oxidative stress and others parameters tested during that short period. It has been suggested that ox-LDL levels but not ox-LDL Abs titer, may serve as an independent indicator for evaluation of atherosclerosis in type 2 diabetic patients [6]. The results of the present study seem to strengthen this notion.

A strong relation has been demonstrated between homocysteine and ox-LDL. Homocysteine autoxidation has been shown to support the oxidation of LDL, not only through generation of the superoxide anion radical, but also by reducing the antioxidant status and affecting nitric oxide production, which could injure vascular endothelial cells [9, 10]. In the present study, the adjunct treatment with antioxidants or captopril was accompanied by a rapid decrease in the elevated levels of homocysteine. Such lower levels may contribute to the protection against the injury of endothelium, leading to slowing of progress of the atherogenic process.

The shift in all oxidative stress indices, observed in the present study, towards normal values and the possible slowing down of the progress or

even the regression of the process of atherosclerosis make the use of antioxidants or low-dose captopril as adjunct therapy in patients with type 2 diabetes worth pursuing in a larger clinical study. It is possible that the indices for atherosclerosis could be brought down to and maintained within normal values with sustained use of the suggested adjunct therapies.

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