Relationship between level of circulating modified LDL and the extent of coronary artery disease in type 2 diabetic patients

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ABSTRACT

The impact of diabetes on health is due almost entirely to a series of complications that characterise the disease. It is associated with an increased incidence of macrovascular complications including coronary artery disease (CAD). The aim of the present study is to evaluate the possible relationship between the circulating levels of the modified derivatives of low-density lipoprotein (LDL) and the development of angiopathy in type 2 diabetic patients with CAD. The status of the antioxidant defences and the role of supplementation with antioxidant combinations are also studied in these patients. The study was conducted on three groups: group I (controls); group II (type 2 diabetic patients without complications – CAD[–]); and group III (including type 2 diabetic patients with stable CAD – CAD[+]). Patients in group III received adjunct treatment of antioxidant tablets for three months. The results of the present study clearly indicated that there was excessive exposure to oxidative stress in diabetic patients. The increase in free radicals was coupled with disturbance in free radical scavengers, particularly the glutathione system. The disturbance was more prominent in CAD(+) patients. The study has shown alteration in the lipid profile in diabetic groups, where the oxidised LDL (ox-LDL) levels were significantly higher than in control subjects. Diabetics with CAD had higher levels of ox-LDL than did patients without CAD. The intima/media thickness (IMT) of the carotid artery was within clinically accepted normal values if the ox-LDL level was below 100–110 u/L. Once the ox-LDL exceeded this range, IMT increased sharply with the increase in plasma ox-LDL. It seems that the level of ox-LDL should be kept below an upper limit of the 100–110 u/L range in order to avoid the serious atherosclerotic effects of this factor. The results demonstrate that plasma levels of ox-LDL correlate with the extent of coronary artery disease in type 2 diabetic patients and suggest that elevated levels of ox-LDL, can serve as an independent and significant predictor for future cardiac events in type 2 diabetic patients with CAD.

KEY WORDS: Coronary artery disease.
Diabetes mellitus.
Lipoprotein, low density.

Introduction

Diabetes mellitus is associated with an increased incidence of macrovascular complications including coronary artery disease (CAD), cerebrovascular and peripheral vascular disease. These atherosclerotic vascular diseases constitute the principal cause of mortality among diabetic patients. Type 2 diabetes is associated with two- to four-fold excess risk of CAD.[1] The atherosclerotic process is indistinguishable from that affecting the non-diabetic population but begins earlier and may be severe.[2]

The mechanisms by which diabetes accelerates atherosclerosis are not well understood. The customary clusters of risk factors for CAD, which are more common in diabetes, are not sufficient to explain this phenomenon. Oxidative stress, as a consequence of hyperglycaemia, has been suggested as a potential mechanism for accelerated atherosclerosis.[3]

It is widely accepted that atherosclerosis is an inflammatory vascular disease.[4] Endothelial dysfunction and accumulation of modified low-density lipoprotein (LDL), particularly the oxidised form (ox-LDL) in the subendothelial space, represent the early changes of the disease. Oxidised LDL is thought to play a key role in the progression of the atherosclerosis process. The modification of LDL by oxidation alters its native properties. Oxidised LDL becomes incorporated in macrophages by scavenger receptors[5] and modulates the expression of the genes involved in the cellular function of endothelial cells and vascular smooth muscle cells in vessel walls.[6] In this context, the detection and quantification of circulating ox-LDL might reflect the severity and phases of atherosclerosis.

The aim of the present study is to evaluate the possible relationship between circulating levels of the modified derivatives of LDL and the development of angiopathy in type 2 diabetic patients with CAD (CAD[+]). The status of the antioxidant defence and the role of supplementation with a combination of antioxidants as free radical scavengers are also studied in these patients.

Subjects, materials and methods

Group I (controls) included 15 healthy individuals aged from 41 to 71 years. Group II (CAD[–]) included 15 type 2

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diabetic patients not suffering from cardiac complications, as judged from disease history and clinical criteria such as the absence of hypertension or cardiac arrhythmia or abnormalities on electrocardiograph (ECG) or echocardiography. Other hepatic or renal functions (especially microalbuminuria) were within the clinically acceptable range. Ages ranged from 39 to 72 years and the reported durations of their diabetes were between two and eight years. These patients were among those seen on a regular basis by the medical staff in the diabetes out-patient clinic of the Medical Research Institute hospital.

Group III (CAD[+]) included 15 type 2 diabetic patients with stable coronary artery disease. They were among the patients diagnosed, treated and followed-up in the cardiology unit of the Medical Research Institute hospital. Their ages ranged from 46 to 72 years. After establishing the baseline values for different studied parameters, patients in this group received adjunct treatment of antioxidant tablets for three months and the assessed parameters were re-evaluated after three months of supplementation.

At the time of the study, all diabetic patients in groups II and III were treated with diet control and oral antidiabetic agents. Most (22 out of 30 patients) were being treated with a combination of a 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), while seven were on rosiglitazone (2–4 mg, three times a day). Prescribed dosages were individualised according to patient requirements.

Supplementation in group III patients consisted of antioxidant tablets containing 30 mg vitamin E, 100 mg ascorbic acid, 5.54 mg vitamin A acetate, 50 µg selenium and 105 mg medical yeast.

Criteria for exclusion from the study included a history of ketoacidosis, renal or liver dysfunction, smoking, use of vitamins or antioxidant supplements, treatment with lipid-lowering agents, or other drugs known to affect serum lipids.

Biochemical assays

Fasting plasma glucose level was determined by the glucose oxidase method.[7] Glycated haemoglobin (HbA1c) was determined using a turbidimetric inhibition immunoassay for haemolysed whole blood.[8]

Determination of the lipid pattern included assays for triglycerides (TG),[9] total cholesterol (TC)[10] and high-density lipoprotein cholesterol (HDL-C).[11] Serum apolipoprotein B (apo-B) was assayed by immunonephelometry[12] using a kit (Dade Behring, Germany).

The quantitative measurement of ox-LDL in human plasma was carried out using the Mercodia ox-LDL enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden).[13] Serum autoantibodies against ox-LDL (ox-LDL-Ab) were determined using an enzyme immunoassay (EIA) kit (Biomedica, USA).[14] Also, the susceptibility of LDL to *in vitro* oxidation was assayed by its ability to form peroxides following incubation with copper and hydrogen peroxide, measuring the thiobarbituric acid reactive substances (TBARS) produced according to the method described by Scoccia *et al*.[15] The lipid peroxidation was measured as TBARS.[16]

The enzymatic method described by Griffith[17] was used to measure the plasma total glutathione (tGSH) and the reduced (rGSH) and oxidised (GSSG) fractions. The Nernst equation was used to calculate the plasma redox potential.[18] The total antioxidant status (TAS) was measured in serum using a commercially available kit (Randox, UK).

Statistical analysis

All data are presented as mean±SD. A one-way analysis of variance (ANOVA) was performed on each variable and the Bonferroni statistics employed to compare the mean values from the groups of diabetic patients and the control group. Paired *t*-test was used to assess the effect of antioxidant treatment in the CAD(+) diabetic group at one and three months. The Kolmogorov-Smirnov test was used to study the normal distribution of the studied parameters. 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 glycaemia and the lipid profiles in the different studied groups are summarised in Table 1. As expected, diabetic patients showed higher fasting glucose levels than did the controls. At the start of the study, CAD(+) patients had slightly higher glucose levels than did CAD(–) patients. Compliance with treatment throughout the study period was good, as judged by HbA1c levels.

The lipid pattern of type 2 diabetics showed deviation from non-diabetic controls in all lipid fractions assessed. Triglycerides showed a definite increase in the CAD(–) and CAD(+) groups, and the concentrations of TC in diabetics

Table 1. Clinical and biochemical characteristics of the study groups.

	Control	CAD(–)	CAD(+)
	n=15	*n*=15	*n*=15
Age (years)	44.6±8.0	48.3±6.8	47.6±7.5
Gender (M/F)	8/7	8/7	8/7
Fasting glucose (mmol/L)	4.9±0.37	10.6±2.31*	11.7±2.68*
HbA1c (%)	4.7±0.43	7.6±0.84*	7.94±0.80*
TG (mmol/L)	1.44±0.39	2.41±0.53*	2.56±0.45*
TC (mmol/L)	4.6±0.47	4.9±0.44*	5.0±0.49*
LDL-C (mmol/L)	2.58±0.44	2.79±0.32	2.87±0.53
HDL-C (mmol/L)	1.33±0.15	1.03±0.13*	1.02±0.1*
LDL/HDL	1.98±0.44	2.77±0.52*	2.83±0.26*
TC/HDL	3.48±0.48	4.91±0.76*	4.94±0.66*
Apo-B (g/L)	0.96±0.19	1.21±0.33*	1.27±0.35*

Data presented as mean±SD.

*Significantly different from control group by one-way ANOVA ($P<0.05$).

were, in general, somewhat higher than in the normal controls (Table 1); however, no detectable differences was seen between the CAD(–) and CAD (+) patients. The percentage difference in TC among different studied groups were almost matched by the differences in LDL-C. This was accompanied by a small decrease in high-density lipoprotein cholesterol (HDL-C) in diabetic patients. Treatment with adjunct antioxidants did not have any effect on the lipid profiles in CAD(+) patients during the follow-up period (Table 2).

It should be noted that despite the absence of change in LDL-C and HDL-C, ratios of LDL-C:HDL-C and TC:HDL-C showed some interesting changes. LDL-C:HDL-C ratio in the diabetics (CAD[–] and CAD[+]) were about 1.4 times that of the control group. In CAD(+), the ratio declined slowly with antioxidant therapy (Table 2). TC:HDL-C ratio showed the same pattern with the same ratios among different groups (Table 1).

Apo-B was higher in type 2 diabetic patients compared with controls. As with all other lipid parameters, apo-B was refractory to change following treatment with antioxidants.

Increased production of TBARS as a reflection of oxidative stress was clear in type 2 diabetics (Table 3), and CAD(+) patients suffered a stronger oxidative stress than did CAD(–) patients. Treatment with antioxidants was effective in alleviating the stressful condition. Even in the short follow-up period of three months, the level of TBARS slowly but steadily declined towards the normal control value (Table 4).

Levels of total and reduced GSH were much lower in diabetes, especially in the CAD(+) patients compared to controls (Table 3). The decrease was more pronounced in reduced GSH. The antioxidant treatment quickly improved this situation as the level of total and reduced GSH showed a steady increase during the follow-up period. (Table 4).

In contrast to reduced GSH, plasma concentration of GSSG was higher in diabetics than in control subjects, being three-fold higher than the control value in the CAD(–) group, while in CAD(+) patients it was 3.25-fold higher. The antioxidant adjunct treatment for one month did not correct the GSSG level, but the level was decreased by the end of the third month (Table 4).

From the obtained glutathione values, the calculated redox potential of plasma clearly indicated a more oxidative environment in diabetics than control subjects (Table 3). The GSH:GSSG ratio also decreased in the

Table 2. Effect of antioxidant treatment on biochemical characteristics in the CAD(+) group.

	CAD(+)		
	Baseline	1 month	3 months
Fasting glucose (mmol/L)	11.7±2.68	11.4±2.54	11.1±2.45
HbA1c (%)	7.94±0.80	7.89±0.78	7.33±0.74
TG (mmol/L)	2.56±0.45	2.54±0.51	2.49±0.45
TC (mmol/L)	5.0±0.49	5.0±0.39	4.9±0.41
LDL-C (mmol/L)	2.87±0.53	2.79±0.46	2.77±0.46
HDL-C (mmol/L)	1.02±0.11	1.06±0.13	1.08±0.11
LDL/HDL	2.83±0.26	2.68±0.64	2.57±0.63
TC/HDL	4.94±0.66	4.78±0.78	4.65±0.65
Apo-B (g/L)	1.27±0.35	1.22±0.35	1.22±0.29

Data presented as mean±SD.

Table 3. Oxidative stress and antioxidant parameters in the plasma of the groups studied.

	Control	CAD(–)	CAD(+)
TBARS (nmol/mL)	3.14±0.39	4.51±0.41*	5.08±0.55*,†
tGSH (nmol/mL)	2.82±0.51	1.72±0.34*	1.28±0.07*,†
rGSH (nmol/mL)	2.57±0.52	1.02±0.34*	0.50±0.14*,†
GSSG (nmol/mL)	0.12±0.02	0.35±0.11*	0.39±0.07*
GSH/GSSG	21.75±6.45	3.31±1.78*	1.39±0.72*,†
Redox potential (mV)	138±6.2	100±12.2*	81±8.8*,†
TAS (mmol/L)	1.33±0.17	1.02±0.11*	0.97±0.10*

Data presented as mean±SD.

*Significantly different from control subjects by one-way ANOVA ($P<0.05$).

†Significantly different from group II diabetic patients by one-way ANOVA ($P<0.05$).

Table 4. Effect of antioxidant treatment on oxidative stress and antioxidant parameters in the plasma of CAD(+) group.

	CAD(+)		
	Baseline	1 month	3 months
TBARS (nmol/mL)	5.08±0.55	4.75±0.54	3.94±0.49*,†
tGSH (nmol/mL)	1.28±0.07	1.82±0.31*	2.34±0.39*,†
rGSH (nmol/mL)	0.50±0.14	1.05±0.30*	1.71±0.32*,†
GSSG (nmol/mL)	0.39±0.07	0.39±0.13	0.31±0.07
GSH/GSSG	1.39±0.72	3.07±1.61*	5.68±1.29*,†
Redox potential (mV)	81±8.8	100±9.1*	115±4.5*
TAS (mmol/L)	0.97±0.10	1.12±0.11*	1.3±0.10*,†

Data presented as mean±SD.

*Significantly different from baseline of group III diabetic patients by paired *t*-test ($P<0.05$).

†Significantly different from one month treatment of group III diabetic patients by paired *t*-test ($P<0.05$).

plasma of diabetics (Table 3). As might be expected, adjunct antioxidant therapy greatly improved the GSH:GSSG ratio. Also, the redox environment of the plasma was improved by 19% in the first month and by 41.9% at the end of the third month (Table 4).

Total antioxidant status was below normal in both CAD(–) and CAD(+) patients (Table 3). With adjunct antioxidant therapy, the TAS value increased quickly to reach levels comparable to those of the non-diabetic controls by the end of the three-month follow-up period (Table 4).

Despite the absence of change in LDL values and refractoriness of LDL to change by antioxidant adjunct therapy, ox-LDL showed different results (Fig. 1). A clear distinct increase in ox-LDL level compared to controls was present in the CAD(–) patients (26.1%) and in the CAD(+) patients (81.6%). However, it responded favourably to antioxidant therapy. The change in mean plasma ox-LDL level in CAD(+) patients closely correlated with the average TAS over the follow-up period (Fig. 2).

Differences were also detected in ox-LDL-Ab among the groups (Fig. 3). It showed a higher level in diabetics compared to controls, but no significant change was detected between CAD(+) and CAD(–) patients. Adjunct treatment resulted in a rapid decrease in ox-LDL-Ab. A strong correlation was obtained between the change in the mean ox-LDL level and its antibodies in CAD(+) patients throughout the follow-up period (Fig. 4). It was also noted that *in vitro* susceptibility of LDL to oxidation was significantly higher in type 2 diabetics (Fig 5). Moreover, LDL in CAD(+) diabetics was more prone to oxidation than it was in CAD(–) diabetics. There was also a stepwise decrease in the susceptibility of LDL to oxidation with duration of antioxidant therapy.

The intima/media thickness (IMT) of the carotid artery was measured to give a reflection of the degree of atherosclerosis in CAD(+) type 2 diabetic patients. The average IMT at the beginning of the study was 0.98±0.18 mm, and the relationship between IMT and plasma ox-LDL is presented in Figure 6. It can be seen that IMT did not exceed the upper normal limit of 0.8 mm until the ox-LDL level exceed 100–110 u/L. Above this value, IMT increased sharply with the increase in ox-LDL.

Discussion

As hyperglycaemia is the hallmark of diabetes mellitus, it is logical to find higher fasting plasma glucose levels in diabetics. However, no significant difference was observed between CAD(–) and CAD(+) patients in this study. The addition of antioxidant to the treatment regimen in the CAD(+) group resulted in improvement in glycaemic status, represented by decreases in the fasting glucose and HbA1c levels. The changes may reflect a tendency towards overall improvement in general health and tissue metabolic status and alleviation of oxidative stress.

The present study clearly indicates a definite overproduction of free radicals and oxidative stress in diabetes, as reflected by increased levels of TBARS and GSSG. Moreover, diabetics with CAD were exposed to a higher oxidative stress state than were patients without such disease. These findings are compatible with many reports in the literature.[19] Hyperglycaemia can increase oxidative stress through several pathways. It appears that many reactions associated with hyperglycaemia may acutely and chronically increase the production of free radicals, resulting in an oxidant/antioxidant imbalance.[20]

The increase in oxidative stress was coupled with a disturbance in free radical scavengers, particularly the glutathione system. As the dominant non-protein thiol, GSH represents the single largest source of reducing equivalents in the cell, and accounts for about 90% of reducing equivalents.[21] Therefore, the depletion of GSH could affect the overall redox potential significantly. The present study found decreased

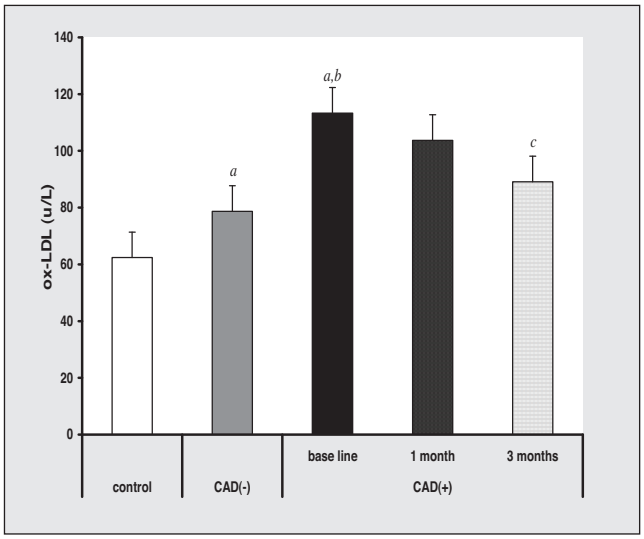

Fig. 1. The plasma level of oxidised-LDL (ox-LDL, u/L) in the non-diabetic control subjects and in the type 2 diabetic groups (CAD[–] and CAD[+]). Data presented as mean±SD. [a]Significantly different from control subjects by ANOVA ($P<0.05$); [b]Significantly different from group II diabetic patients by ANOVA ($P<0.05$); [c]Significantly different from baseline of group III diabetic patients by paired *t*-test ($P<0.05$).

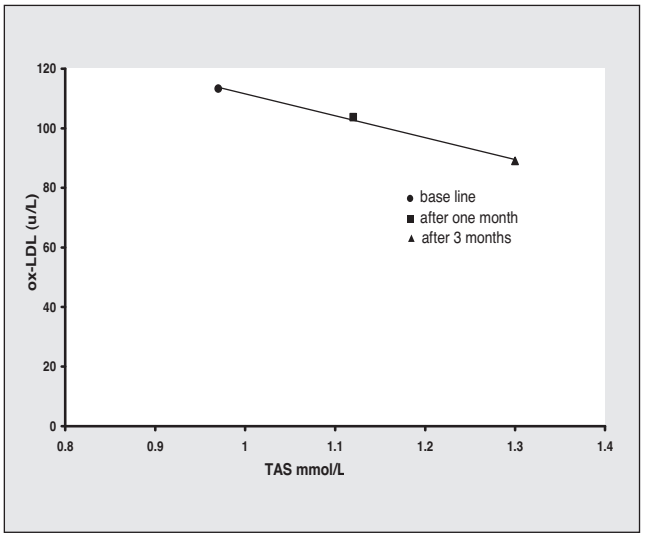

Fig. 2. Relationship between the change in the mean ox-LDL level with the change in TSA in the plasma of CAD(+) patients during the three months' adjunct antioxidant therapy.

levels of total and reduced GSH, while GSSG was increased in diabetics, especially in the CAD(+) group.

Many investigations have reported a lower concentration of GSH in the plasma of diabetic patients.[22] By inspecting the calculated redox potential in the present study, it became clear that the redox potential for the diabetic groups was shifted towards the oxidising side, and the supplementation of CAD(+) patients with a antioxidant combination for three months partially correct the balance of GSH/GSSG to restore the reducing potentials.

In addition, the study found a clear deficiency in TAS in diabetic patients. Studies have consistently demonstrated a deficiency in individual antioxidants in type 2 diabetic patients. The lower concentrations of glutathione, vitamins E and C and in the reduced activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase, as well as a decrease in total radical trapping antioxidant parameter (TRAP), all suggest a reduced total antioxidant defence.[23] Supplementation with known free radical scavengers such as vitamins E and C have a potential role in boosting antioxidant defence.[24,25]

Scavenging antioxidants act synergistically,[26] so supplementation with two or more may enhance their individual effects. Jain *et al*.[27] reported that vitamin E supplementation can increase cellular glutathione concentrations, probably as a result of sparing by vitamin E of glutathione utilisation for the scavenging of lipid peroxidation reactions.

The present study shows alteration in the lipid profile in diabetic groups as compared to the healthy control subjects. The two diabetic groups had significant increases in triglyceride levels, moderately raised cholesterol levels, and lower levels of HDL-C compared to the control group. On the other hand, LDL-C concentrations were very similar in the control and diabetic groups. The lipid abnormalities found in the diabetic groups are in agreement with results reported elsewhere.[28]

The abnormalities in insulin action, not hyperglycaemia *per se*, are associated with this lipid abnormality,[29] which would explain the lack of correlation observed in the present study between hyperglycaemia and the changes in the pattern of different lipid fractions. Although LDL is not usually increased in diabetes, as qualitative changes in LDL-C may be present. In part, this may represent a balance of factors that affect LDL production and catabolism.

Apolipoprotein B is the protein moiety of LDL. The clinical interest in this protein lies in the fact that it provides a relatively accurate estimate of circulating LDL particle numbers.[30] The present study showed that apo-B serum concentration was significantly elevated in the diabetic groups. This parallels the results from several studies showing that apo-B levels are elevated in type 2 diabetes.[31,32]

Low-density lipoprotein may be modified by oxidation, which would make it more atherogenic, and LDL oxidation plays a key role in atherogenesis.[33]

Following the transport of LDL into the artery wall, its oxidation can occur in the microenvironment of the subendothelial space or it can be cell-mediated, and the process is known to be dependent on superoxide generated in endothelial cells during mitochondrial respiration.[34] In the present study, it was found that circulating ox-LDL levels in patients with type 2 diabetes were significantly higher than in control subjects. In addition, CAD(+) diabetic patients had higher levels of ox-LDL than did CAD(–) patients. These results agree with other published data.

The work of Holvoet *et al*.,[5] who used immunological techniques to measure circulating levels of ox-LDL and MDA-LDL, supports the fact that LDL containing oxidation-specific epitopes seems to differentiate normal patients from patients with CAD, transplant atherosclerosis and acute coronary syndrome (ACS). Other studies have demonstrated that ox-LDL levels are significantly higher in patients with diabetes mellitus than in control subjects, and that high levels of circulating ox-LDL can serve as an

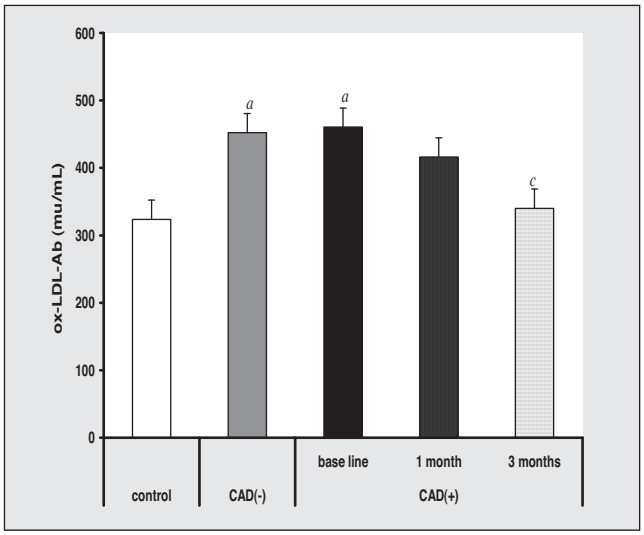

Fig. 3. The plasma level of oxidised-LDL antibodies (ox-LDL-Ab, mu/mL) in the non-diabetic control subjects and in the type 2 diabetic groups (CAD[–] and CAD[+]). Data presented as mean±SD. [a]Significantly different from control subjects by ANOVA ($P<0.05$); [c]Significantly different from baseline of Group III diabetic patients by paired *t*-test ($P<0.05$).

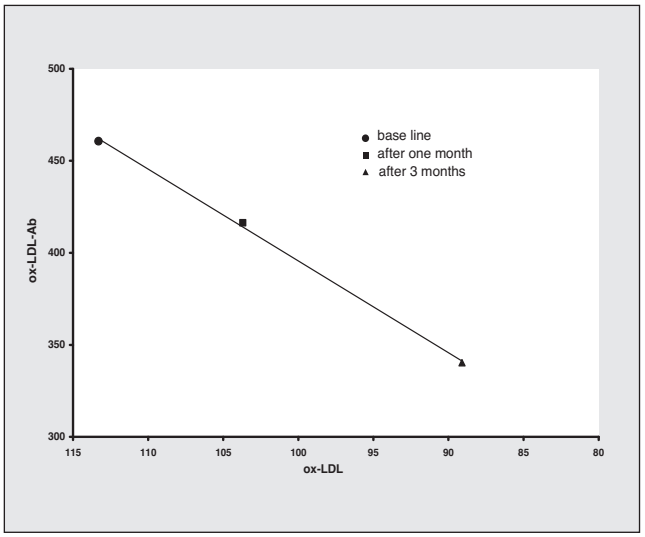

Fig. 4. Relationship between the change in the mean ox-LDL level with the change in ox-LDL-Ab in the plasma of CAD(+) patients during the 3 months adjunct antioxidant therapy.

independent and significant predictor for future cardiac events in type 2 diabetic patients with CAD.[36]

At present, the IMT is the best-studied sonographic marker for early atherosclerotic vascular wall lesions.[37] A thickening of the intima/media complex not only reflects local alterations, mostly of the common carotid artery, but also corresponds to generalised atherosclerosis. A direct correlation between IMT and the risk of myocardial infarction and stroke in a population of patients without prior history of vascular disease has been shown.[38] Using ultrasonography, the present study demonstrated that diabetic patients with CAD had increased IMT of the carotid arteries, and a relationship was found between the level of circulating ox-LDL and thickening of the carotid artery intima/media, which deserves further comment. From the results of the present study (Fig. 6) it can be seen that IMT was within the clinically accepted normal values (≤ 0.8 mm) if ox-LDL level was below 100–110 u/L. Once the ox-LDL exceeded this range, IMT increased sharply with plasma ox-LDL. Thus, ox-LDL level should be kept below the upper limit of 100–110 u/L in order to avoid the serious atherosclerotic effects of this factor.

Although adjunct therapy with an antioxidant mixture improved total antioxidant status in the treated diabetic patients, resulting in a significant decrease in ox-LDL, there was no immediate reciprocal effect on IMT. It is possible that more time is required for mobilisation of the cholesterol precipitated in the atheromatous plaques. Supplementation with exogenous antioxidants is expected to give rapid improvement in the antioxidant status, leading to suppression of free radical production and consequent decrease in ox-LDL; however, mobilisation or decrease in the size of atheromatous plaques is likely to proceed at a slower rate, but this could be expedited by the use of hypolipidimic agents.

Oxidative modification of the apolipoprotein moiety of LDL makes it antigenic, resulting in the production of autoantibodies against ox-LDL. These autoantibodies may reflect LDL oxidation *in vivo*.[39] In the present study, the antibody reactivity level against ox-LDL was significantly higher in diabetic patients than in controls, but no significant difference was seen between the two diabetic groups. The presence of antibodies against ox-LDL in patients with diabetes has been reported by several investigators,[40] and some studies suggest that the presence of high-titre autoantibodies against ox-LDL is associated with the severity of carotid atherosclerosis.[39]

A very encouraging observation in the present study is the favourable response of ox-LDL levels to antioxidant adjunct therapy. Many studies indicate that α-tocopherol supplementation decreases susceptibility of LDL to oxidation.[41] Epidemiological studies also support the hypothesis that higher dietary intake of α-tocopherol is associated with decreased risk for coronary heart disease[42] and reduced LDL oxidisability.[43] Ascorbate, another component of the antioxidant mixture used in the present study, can help to reduce lipid hydroxyl radicals or recycle the one-electron-oxidised forms of lipid-soluble antioxidants.

Ascorbate is well known to recycle the α-tocopheroxyl radical.[44] Moreover, as a co-antioxidant, vitamin C can prevent the pro-oxidant properties of the α-tocopheroxyl radical.[45] Carr *et al*.[46] found that physiological concentrations of vitamin C can protect LDL lysine and tryptophan residues from oxidation and can partially protect LDL cysteine residues.

The antioxidant adjunct treatment used here also contained selenium, which is a major antioxidant trace element and is the co-factor of glutathione peroxidase (sGPx). Low sGPx activity observed in diabetic patients is associated with thrombosis and cardiovascular complications.[47]

From the data obtained in the present study and the

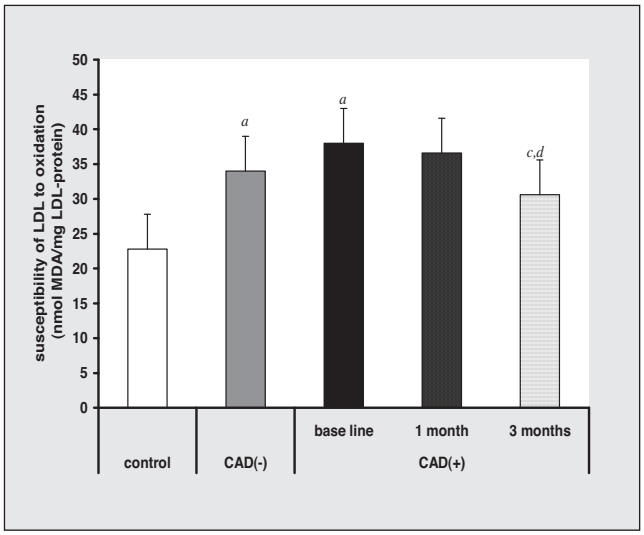

Fig. 5. The susceptibility of LDL to oxidation (nmol MDA/mg LDL-protein) in the non-diabetic control subjects and in the type 2 diabetic groups (CAD[–] and CAD[+]). Data presented as mean±SD. [a]Significantly different from control subjects by ANOVA ($P<0.05$); [c]Significantly different from baseline of Group III diabetic patients by paired *t*-test ($P<0.05$); [d]Significantly different from one month treatment of Group III diabetic patients by paired *t*-test ($P<0.05$).

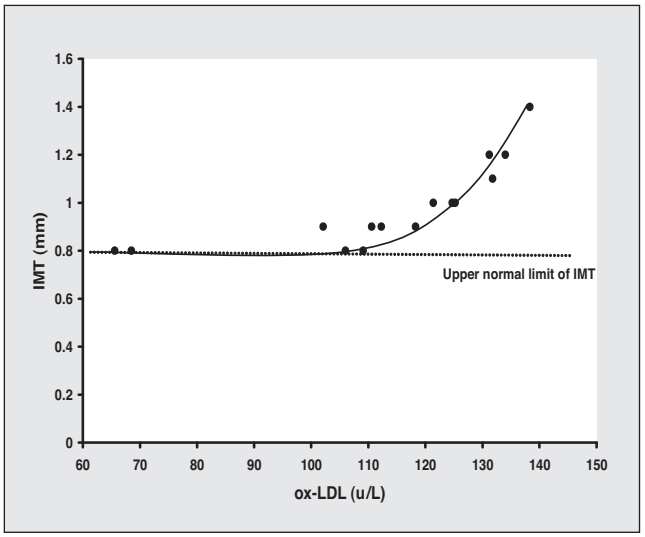

Fig. 6. Correlation curve between ox-LDL level and the IMT in CAD(+) diabetic patients (r=0.706, $P<0.05$).

subsequent discussion, it is clear that plasma levels of ox-LDL correlate with the extent of CAD in type 2 diabetic patients, and suggest that elevated levels of ox-LDL, rather than ox-LDL antibodies, can serve as an independent and significant predictor for future cardiac events in type 2 diabetic patients with CAD. □

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