Association between serum Copper, Oxidized HDL and Glycemic control in patients with type 2 Diabetes Mellitus in relation to Microalbuminuria

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Abstract

Background: diabetes mellitus (DM) is associated with a markedly increased mortality rate from cardiovascular and renal disease, not explainable by traditional risk factors. Although data are not yet conclusive, oxidative stress, dyslipidemia, glycemic control and possibly lipid peroxidation has been increasingly implicated in the pathogenesis of diabetic micro- and macrovascular disease. Little is known, however, about the role of copper in type 2 diabetes.

Aim: The present study includes measurement of free radical activity marker (lipid peroxides expressed as malondialdehyde MDA) along with the serum and urine copper, serum lipid profile, glycated haemoglobin (HbA1c) in addition to urinary protien : creatinine ratio in 55 patients with type 2 DM (T2DM) .

Results: The patients were divided according to the spot urine albumin excretion (urinary albumin ug / mg creatinine ratio) into two groups:- microalbuminurics & normoalbuminurics.

The results were compared with those obtained from 37 age-matched apparently healthy control subjects.

There was a significant elevation in serum malondialdehyde MDA , the percentage of oxidized non high-density lipoprotien (ox. non-HDL%) and serum copper with a significant reduction in the percentage of oxidized high-density lipoprotien (ox. HDL%) in the diabetic patients (particulary in the microalbuminurics) as compared with the control subjects. Serum MDA was significantly and positively correlated with serum copper in microalbuminurics and HbA1c% in both diabetic groups.

LDL size index was significantly increased in microalbuminuric T2DM patients as compared to the controls and normoalbuminurics indicating smaller LDL size in the diabetics in general and in microalbuminuric in particular.

Conclusion: the results of present study suggest an increase in free radical activity , dyslipidaemia and serum copper level favoring atherosclerotic state more in poor glycemic control in type 2 DM particularly in microalbuminurics.

The suggested mechanisms underlying these events are discussed.

Key words: Copper, lipid peroxides, diabetes mellitus., Microalbuminuria.

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Introduction:

Approximately 150 million people worldwide suffer from type 2 diabetes and it has been predicted that this number will double within the next 15 years [1], and its incidence is rising in developed and developing countries [2, 3].

The prevalence of type 2 DM is growing at an exponential rate [4]. T2DM is characterized by insulin resistance coupled with an inability of the pancreas to sufficiently compensate by increasing insulin secretion, with onset generally in middle or old age.

Diabetic nephropathy is the most common cause of renal failure in the Western World [5].

Several risk factors have been related to the development of diabetic nephropathy (DN) in type 2 diabetic patients, such as hyperglycemia,

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arterial hypertension, dyslipidemia, and smoking [6,7,8]. Microalbuminuria is a strong predictor of diabetic nephropathy and cardiovascular disease in both type 1 and type 2 diabetes mellitus [9].

The present study will go further to relate oxidative stress events with microaluminuria of type 2 DM.

Material & methods:

A-Subjects:

The study group comprised 55 patients (24 males and 31 females) with type 2 diabetes mellitus (mean age 51.6 +/- 8.1 years) diagnosed according to the WHO definition[10] .The patients were divided into two groups: microalbuminuric (group 1), n = 31 and normoalbuminuric (group 2), n = 24. All patients were recruited from the outpatient Diabetes clinic of the AL-kadhymia Teaching Hospital during the study period from 1 September 2004 to 30 March 2005, .

The main exclusion criteria included any recent illness, impaired thyroid or renal function , diagnosis of renal disease, treatment with estrogen or glucocorticoides, or other drugs except oral hypoglycemic and /or beta blocker antihypertensive drugs & pregnant women, All patients included in the study were nonsmokers; none was taking antioxidant supplements or drugs with known antioxidant activity, The mean duration of diabetes was (7.96 +/- 3.45 years) .

The control group consisted of 37 healthy, sex- and age-matched subjects (48.92 +/- 8.9 years) They were all volunteers recruited from different places and from the staff of the medical college of AL-Nahrain University.

B-Blood samples

About 10 milliliters of venous blood were collected from each subject of the study after a 12- hour fast. Two milliliters of the blood were collected in EDTA containing tubes and sent to the hospital Laboratory for HbA1c assay. The rest was collected in plain plastic tubes; which was centrifuged at 3000 rpm for 7 min within about 30 minutes from the time of collection. The serum was used for subsequent measurement of creatinine, lipid profile (total cholesterol, HDLc, TG), total MDA Level, Oxidized HDLc and copper concentration.

Urine samples:

Random morning urine specimen was obtained from each subject in the study, to quantify albuminuria (albumin-to-creatinine ratio) & creatinine.

Methods:

Serum total MDA was measured by the thiobarbiturate method [11] while serum lipids (Tc, HDL-c and TG) were measured by enzymatic methods using kits from bioMeriux, France.

Serum LDL-c was calculated by Friedewald fomula [12].

Serum oxidized HDL was measured by precipitation of all lipoproteins, except HDL-c, which was measured by phospho-tungstic acid – MgCl2 reagent. The supernatant was used for estimation of oxidized HDL by the same method used for the measurement of total MDA.

Serum copper was measured by flame atomic absorption spectrophotometer after 1: 10 dilution with de-ionized water,

Urine albumin was measured by staining with Ponceau S dye following the method of (Pesce and Strande 1973) [13] and urine creatinine by alkaline picrate kinetic method [14],

Glycated haemoglobin was measured by Variant™ HbA1c program .[15].

Diabetic patients (n = 5٥) were divided according to urine albumin excretion measuered in ug per mg creatinine (Table 1) into:-

1. patients with albumin-creatinine ratio of 30 - 299 µg/mg were considered microalbuminurics (n = 31)
2. patients with albumin excretion less than 30 µg albumin per mg creatinine were considered normoalbuminurics (n = 24)

Lipid peroxides presented as total MDA and oxidized HDL were measured, then the value of oxidized non-HDL was obtained by subtraction (Total MDA - oxidized HDL = oxidized non-HDL.) as in table (2).

Serum MDA was significantly elevated in the microalbuminuric diabetic patients compared with the normoalbuminuric patients (P = 0.004) and control subjects ($p <10^{-10}$). MDA was also significantly higher in the normoalbuminuric patients than the control subjects ($P = 6. 10^{-7}$), as shown in table (2).

Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), atherogenic index, AI, (expressed as LDLc / HDLc) and LDL size index (expressed as TG / HDLc) were measured in all groups studied, (table 3).

As expected from the results of serum LDLc and HDLc, both diabetic groups have increased (AI) as compared with the control subjects (table 4). Furthermore, microalbuminurics had significantly higher atherogenic index than the normoalbuminuric diabetics ($P <0.05$) both diabetic groups showed a significant increase in the LDL size index when compared with controls, Indicating smaller LDL in patient's groups table (4).

Serum copper was higher in the diabetics than the controls being significantly higher in the microalbuminurics than the normoalbuminurics as shown in table (4).

There was a significant positive correlation between serum copper and each of total MDA level and the urine albumin / cratinine in the microalbuminurics only. (Fig 1 & 2)

Discussion:

Most published studies have found increased Lipid Peroxides in T2DM patients [16, 17].

Serum MDA was significantly higher in the microalbuminuric than normoalbuminuric patients. These results confirm earlier reports [18, 19]. A positive correlation between albumin excretion and plasma Thiobarbiturate reactive substance levels was also found [20]

However, not all instances of diabetes result in elevated oxidation. For example, a lower TBA reactivity in tissues of rats with alloxan - induced diabetes [21] and similar levels of MDA in micro-or normoalbuminuric type 1 DM patients were found by others [22, 23]. The suggested biochemical mechanisms for increased lipid peroxide in DM patients are [24] :

- Increased non-esterified fatty acids from increased lipolysis result in an increase in MDA.
- Peroxidative damage of membrane lipids.
- Lipids are more readily oxidized in the presence of increased glucose concentrations.
- Reactive oxygen species (ROS) can also be generated within the kidney by macrophages and polymorphonuclear leucocytes. In inflammatory cells, different sources of ROS have been suggested.
- Transition metals (copper and iron) catalytically activate the oxidation of polyunsaturated fatty acids. An enhancement in plasma transition metal concentration has been noted in diabetic animal models and in

diabetic patients showing complications [25].

The higher atherogenic index (AI) and the presence of smaller LDL particle size in the diabetics were reported to associate the increase in their oxidation susceptibility which is consistent with the present results and agree with previous reports [26-28]. This is further aggravated by the poor glycemic control indicated by the higher HbA1C level.

In another study poor glycemic control correlated significantly with micro- and macroalbuminuria in type 2 DM patients [29]. It may be concluded that poor glycemic control may be considered as a risk factor for the progression from normo- to microprotienuria in type 2 DM [30].

The higher serum copper in diabetics, particularly in the microabuminurics, is thought to be another event of oxidative stress (fig.1).

Serum Cu level was reported to be affected by renal excretion and kidney disease which is one of the major complications of diabetes [31]. In the present study Serum copper correlated positively and significantly with urinary protein excretion in microalbuminurics (fig. 2)

Hypothetically glycated proteins bind transition metals such as copper and iron, and that such `glycochelates' accumulate within the vasculature in diabetes and catalytically inactivate endothelial derived relaxation factor (EDRF) [32].

In the presence of available cellular reductants, copper in low molecular weight forms may play a catalytic role in the initiation of free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates, resulting in wide-ranging impairment in cellular function and integrity [33].

Table (1) displays the clinical characteristics of the study subjects:

	Microalbuminuric	Normoalbuminuric	Controls
Number	31	24	37
Male/female	11 / 20	13 / 11	15 / 22
Age (years) (NS)	49.5 ± 7.6	52.2 ± 8.2	48.9 ± 8.9
Hemoglobin A1C %	8.11 ± 1.16*****	7.68 ± 0.9*****	4.87 ± 1.0
FBG(mmol/L)	8.9 ± 2..4 *	6.3 ± 1.1	5.1 ± 0.3

* P < 0.001, versus the control subjects
Mean values are shown, with standard deviations (S.D.)

Table (2): Lipid peroxidation and its fractions percentages in the two diabetic groups (1: microalbuminuric, 2: normoalbuminuric) and control group as Mean ± SD

Groups	**S.MDA µmo/L**	**OX. HDL %**	**OX. non-HDL %**
Group (1) T2DM	0.963 *† ± 0.1	53.9%**‡ ± 15.3	46.1%**‡ ± 15.3
Group (2) T2DM	0.833* ± 0.18	72.0%** ± 11.6	27.97*% ± 11.6
test P-value	0.03	0.0175	0.013
Controls	0.580 ± 0.124	75.5% ± 16.0	24.5% ± 16.0

Student t-test was done between each diabetic group and control (for p < 0.05, ** for p <0.01) †Student t-test was done between microalbuminuric and Normoalbuminuric diabetes patients († for p < 0.05, ‡ for p <0.01). F test was done between macroalbuminurics and Normoalbuminurics

Table (3): Serum Lipid Profile (mean ± SD) in mmol / L in the diabetic and Control groups.

Groups	***Microalbuminurics***	***normoalbuminurics***	***F test***	***Controls***
T.C	*5.61 ± 1.0 ***	*5.29 ± 0.78*	*0.04*	*4.40±0.58*
TG .	*1.93 ± 0.2 ***	*1.63 ± 0.4 ***	*12 x10^{-5}*	*1.28 ± 0.4*
HDLc	*1.1 ± 0.2 ***	*1.12 ± 0.18 ***	*0.81*	*1.48 ± 0.2*
LDLc	*3.6 ± 1.1 **	*3.46 ± 0.8 ***	*0.037*	*2.34±0.55*

- **Student t-test was done between each diabetic group and control p < 0.001
- F test (one way ANOVA) was done between Microalbuminuric and Normoalbuminuric diabetic patients

Table (4): Serum MDA,copper (Cu), AI(LDL.c/HDL.c), LDL.c size index (TG/HDL.c) and glycated Hb % in the diabetic groups and their controls (mean +/- SD)

Type 2 DM	Hba1c %	MDA µmol/L	S. Cu µmol/L	AI *(LDLc/HDLc)*	LDL.c size index *(TG/HDLc)*
Microalbuminurics (n =31)	8.1* +/- (1.2)	0.96*† +/- (0.1)	27.7**†† +/- (6.2)	3.39** +/- (1.3)	1.79**† +/- (0.42)
Normoalbuminurics (n =24)	7.68* +/- (0.9)	0.833* +/- (0.2)	20.3 +/- (2.9)	3.178** +/- (1.1)	1.49** +/- (0.5)
Controls (n = 37)	4.9 +/- (1.0)	0.580 +/- (0.1)	18.97 +/- (4.4)	1.625 +/- (0.560)	0.9 +/- (0.3)

*p<0.05 versus controls †P<0.05 group (1) versus group (2)
**P<0.001 versus controls ††P<0.001 group (1) versus group (2)

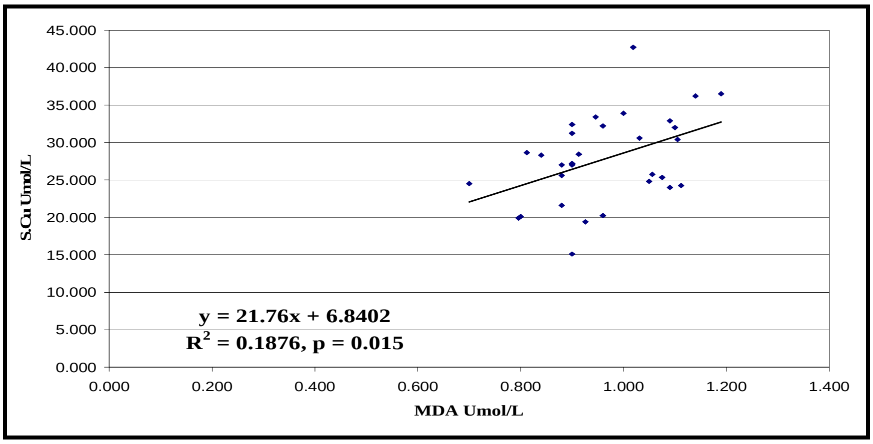

Fig. (1) Correlation between serum copper and total MDA in microalbuminuric type 2 diabetic patients

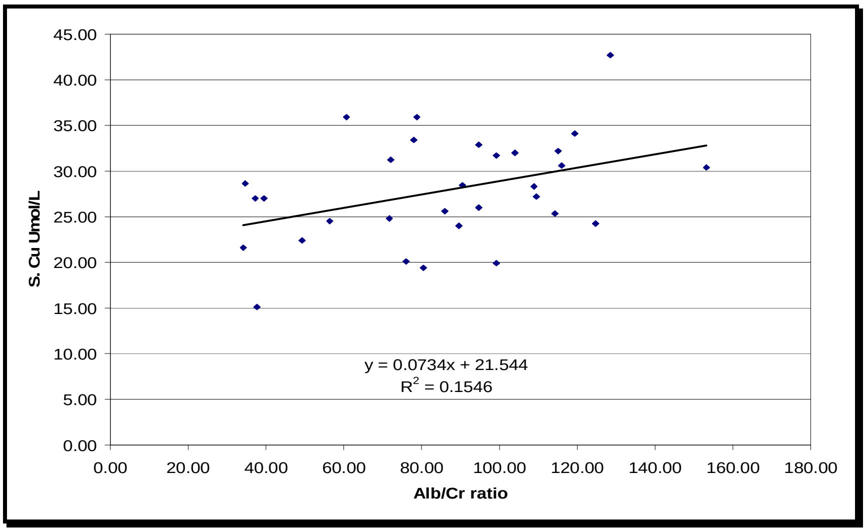

Fig (2) Correlation between serum copper and urine albumin / creatinine ratio in microalbuminuric Type2 diabetic patients.

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