Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) levels in Egyptian type 2 diabetic patients

Soliman G Z A

ABSTRACT

Introduction: The existence of hyperglycaemia produces increased oxidative stress. The depletion of antioxidants as a defensive body mechanism may increase the risk of diabetic complications. Diabetes mellitus is associated with derangements in the serum levels of several biochemical parameters, and type 2 diabetes mellitus (or non-insulin-dependent diabetes mellitus) is a risk factor for cardiovascular diseases.

Methods: Data of 80 control subjects (male:female, 40:40) and 80 diabetic patients (male:female, 40:40), of similar age, gender, body mass index and duration of diabetes mellitus (for diabetic group), were collected from government and non-government organisations during the period, 2001-2004. Blood samples were also collected. Glucose, glucosylated haemoglobin, total cholesterol(TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), TC/HDL-C, HDL-C/LDL-C, triacylglycerol, malondialdehyde, glutathione and superoxide dismutase levels were determined.

Results: Mean cholesterol, LDL-C, VLDL-C and triacylglycerol levels were significantly higher in patients with type 2 diabetes mellitus in comparison to the control subjects, while the mean value of HDL-C was significantly lower. A significant elevation in malondialdehyde level and decrease in glutathione content were observed in both male and female diabetic patients in comparison to the control subjects.

Conclusion: The results suggest that the increase in lipid peroxidation, and the decline in antioxidant defences, may appear early in type 2 diabetic patients, before the development of secondary complications. This phenomenon might play an important role in the initiation and progression of diabetic complications. Our results also suggest that there seems to be an imbalance between plasma oxidant and antioxidant systems in patients with type 2 diabetes mellitus.

Department of Biochemistry, National Nutrition Institute, 16 Kasr El Aini Street, Cairo, Egypt

Soliman GZA, BSc, MSc, PhD
Assistant Professor

Correspondence to:
Dr Ghada Zaghloul Abbas Soliman
Tel: (20) 12 004 1787
Email: amr_soliman2005@yahoo.com

Keywords: diabetes mellitus, glutathione, lipoproteins, malondialdehyde, non-insulin-dependent diabetes mellitus, superoxide dismutase

Singapore Med J 2008; 49(2): 129-136

INTRODUCTION

The microvascular and neuropathic complications of diabetes mellitus are a major clinical and public health problem in Egypt.[1] During the past 20 years, major sociodemographic changes have occurred in the Eastern Mediterranean Region,[2] and changes in physical activity and dietary patterns have promoted the development of non-communicable diseases, such as diabetes mellitus. Diabetes mellitus is now considered an emerging clinical and public health problem in Egypt.[1,3] Atherosclerotic cardiovascular diseases are the major causes of morbidity and mortality in diabetic patients.[4,5]

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation.[6] Diabetes mellitus is considered to be one of a rank of free radical diseases which propagates complications with increased free radical formation.[7-9] Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and a deficiency in antioxidant defence mechanisms.[10,11] Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus.[12] Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus.[13,14] The status of antioxidant defence mechanisms in diabetes mellitus is very contradictory, as both increases and decreases of antioxidant status have been reported in diabetes mellitus.[15,16] This study aims to evaluate serum lipid peroxidation markers, malondialdehyde (an oxidant), glutathione (an antioxidant) and superoxide dismutase, in non-insulin-dependent diabetic patients.

METHODS

This study was conducted on 80 (40 male and 40 female) non-insulin-dependent diabetes mellitus (NIDDM, or type 2 diabetes mellitus) patients, who were attending government and Non-Government Organisation (NGO)

Table Ia. Clinical characteristics of NIDDM and controls.

Clinical characteristics*	Age (years)	Duration (years)	BMI (kg/m^2)	Glucose (mg/dL)	$HbA1_C$ (%)
All					
G1 control	38.2 ± 0.6		24.7 ± 0.2	95.1 ± 1.5	5. 2 ± 0.05
G2 diabetic	39.3 ± 0.5	10.0 ± 0.5	25.0 ± 0.1	228.7 ± 1.0	8.0 ± 0.04
p-value (G2 vs. G1)	NS		NS	< 0.0001	< 0.0007
Male					
G3 control	38.3 ± 0.9		24.9 ± 0.4	94.8 ± 2.0	5.4 ± 0.06
G4 diabetic	39.3 ± 0.7	10.4 ± 0.8	25.1 ± 0.1	229.3 ± 1.1	8.1 ± 0.02
p-value (G4 vs. G3)	NS		NS	< 0.0001	< 0.0006
Female					
G5 control	38.1 ± 0.8		24.6 ± 0.2	95.5 ± 2.2	5.0 ± 0.06
G6 diabetic	39.3 ± 0.7	9.7 ± 0.6	24.9 ± 0.21	228.1 ± 1.57	7.8 ± 0.06
p-value (G6 vs. G5)	NS		NS	< 0.0003	< 0.0001
Female vs. male					
p-value (G5 vs. G3)	NS		NS	NS	< 0.017
p-value (G6 vs. G4)	NS	NS	NS	NS	< 0.0001

*Values are expressed as mean ± SE; NS: not significant

Table Ib. Clinical characteristics of NIDDM and controls.

Clinical characteristics*	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	TC: HDL-C	HDL-C: LDL-C	TG (mg/dL)
All							
G1 control	193.9 ± 2.1	56.0 ± 0.5	111.6 ± 1.4	26.3 ± 0.5	3.5 ± 0.02	0.5 ± 0.005	101.6 ± 1.5
G2 diabetic	232.0 ± 1.8	47.8 ± 0.4	139.3 ± 1.2	44.8 ± 0.5	4.9 ± 0.03	0.3 ± 0.002	131.4 ± 1.6
p-value (G2 vs. G1)	< 0.0003	< 0.0004	< 0.0001	< 0.0004	< 0.0001	< 0.0001	< 0.0004
Male							
G3 control	196.6 ± 2.8	56.1 ± 0.7	113.5 ± 1.6	27.0 ± 0.8	3.5 ± 0.03	0.5 ± 0.01	104.0 ± 2.2
G4 diabetic	237.3 ± 2.1	48.5 ± 0.6	143.1 ± 1.3	45.8 ± 0.5	4.9 ± 0.04	0.4 ±0.001	133.9 ± 2.0
p-value (G4 vs. G3)	< 0.004	< 0.0002	< 0.001	< 0.0004	< 0.0004	< 0.0001	< 0.0004
Female							
G5 control	191.2 ± 3.0	55.9 ± 0.7	109.7 ± 2.2	25.6 ± 0.5	3.4 ± 0.03	0.5 ± 0.01	99.3 ± 2.1
G6 diabetic	226.6 ± 2.4	47.2 ± 0.6	135.6 ± 1.7	43.8 ± 0.7	4.8 ± 0.04	0.3 ± 0.003	128.9 ± 2.5
p-value (G6 vs. G5)	< 0.0004	< 0.001	< 0.003	< 0.0004	< 0.0001	< 0.0004	< 0.0004
Female vs. male							
p-value (G5 vs. G3)	NS	NS	NS	< 0.0004	< 0.01	< 0.02	< 0.03
p-value (G6 vs. G4)	< 0.0004	< 0.027	< 0.0004	< 0.0025	< 0.03	< 0.005	< 0.03

*Values are expressed as mean ± SE; NS: not significant

hospitals and clinics (during the period 2001–2004), and 80 healthy (40 male and 40 female) controls of similar ages (30–45 years), body mass index (BMI; mean 25.0 kg/m^2; range 22.41–27.89 kg/m^2), duration of NIDDM (mean 10.0 years; range 4.5–17 years) and gender distribution. The selected subjects were non-smokers. The NIDDM patients were not taking any medicines other than oral anti-diabetic pills for the past four years. The diagnosis of NIDDM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2000).[17] Diabetic patients with complications, such as nephropathy and neuropathy, were excluded.

For the study, six groups were formed, categorised as follows: Group 1 (G1): Control, comprising 80 male and female control subjects; Group 2 (G2): Diabetic, comprising 80 male and female NIDDM patients; Group 3 (G3): Control, comprising 40 male control subjects; Group 4 (G4): Diabetic, comprising 40 male NIDDM patients; Group 5 (G5): Control, comprising 40 female control subjects; and Group 6 (G6): Diabetic, comprising

Table Ic. Clinical characteristics of NIDDM and controls.

Clinical characteristics	MDA (μmol/L)	Glutathione (μmol/L)	SOD
All			
G1 control	0.3 ± 0.009	7.8 ± 0.1	277.2 ± 3.9
G2 diabetic	0.4 ± 0.007	6.7 ± 0.06	330.0 ± 2.8
p-value (G2 vs. G1)	< 0.0002	< 0.004	< 0.0004
Male			
G3 control	0.34 ± 0.01	7.6 ± 0.2	285.4 ± 4.6
G4 diabetic	0.45 ± 0.01	6.6 ± 0.08	339.2 ± 1.8
p-value (G4 vs. G3)	< 0.0001	< 0.001	< 0.004
Female			
G5 control	0.3 ± 0.01	7.9 ± 0.1	269.0 ± 5.8
G6 diabetic	0.4 ± 0.01	6.9 ± 0.1	320.7 ± 4.4
p-value (G6 vs. G5)	< 0.002	< 0.001	< 0.004
Female vs. Male			
p-value (G5 vs. G3)	< 0.02	< 0.03	< 0.02
p-value (G6 vs. G4)	< 0.001	< 0.001	< 0.0004

*Values are expressed as mean ± SE; NS: not significant

Table II. Distribution of BMI among control and diabetic patients.

	No. of control patients		No. of diabetic patients	
	Non-obese (%)	Obese (%)	Non-obese (%)	Obese (%)
All	52 (65)	28 (35)	56 (70)	24 (30)
Male	20 (50)	20 (50)	32 (80)	8 (20)
Female	32 (80)	8 (20)	24 (60)	16 (40)

40 female NIDDM patients.

Weight and height were measured in indoor clothing without shoes, and BMI was calculated as weight (kg) / height^2 (m^2). Fasting blood samples were drawn and collected in three tubes, two of them with anticoagulant added. They were kept at -80°C if they were not analysed immediately. Glucose,glycosylated haemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), TC/HDL, HDL/LDL, triacylglycerol (TG), superoxide dismutase (SOD, EC 1.15.1.1), malondialdehyde (MDA), glutathione levels were determined. Repeated freezing and thawing were avoided.

Glucose level was determined using Randox kit.[18] HbA1c was determined using HPLC.[19,20] TC was determined using the bioMérieux kit (bioMérieux, Marcy l'toile, France);[21,22] Total TG was determined using the Bicon kit (Bicon Diagnostik, Marienhagen, Germany).[23,24] Serum HDL[25,26] and serum LDL-C[27-29] were respectively determined using the bioMérieux kit (bioMérieux, Marcy l'Etoile, France). VLDL-C was determined by using the following equation: VLDL-C = TC – (HDL-C + LDL-C). Glutathione[30-32], MDA[33] and SOD[34-36] were respectively determined using the Randox kit (Randox Laboratories, San Francisco, CA, USA).

Data are expressed as mean ± standard error (SE). Data were assessed by the Student's *t*-test.[37,38] The correlation coefficients were determined by Pearson's simple linear regression analysis.[38] Statistical significance was accepted at $p < 0.05$. Multiple linear regressions were done using the Statistical Package for Social Sciences version 13 (SPSS Inc, Chicago, IL, USA).

RESULTS

Tables Ia–c show the clinical characteristics of NIDDM patients and controls. They were of similar age, gender distribution and BMI. Age or BMI showed no significant differences between the controls and diabetics. The age range was 30–45 years, mean BMI 25.0 kg/m^2 (range 22.41–27.89 kg/m^2 and 23.21–26.08 kg/m^2 for the control and NIDDM groups, respectively). The mean duration of diabetes mellitus in NIDDM patients was 10.0 years (range 5.5–16 years). In the control groups, 65% of G1 were non-obese (BMI < 25.0 kg/m^2) and 35% were obese (BMI ≥ 25.0 kg/m^2); in G3, the ratio of non-obese to obese was 50:50, while in G5, it was 80:20. In the diabetic groups, 70% of G2 were non-obese and 30% were obese; in G4, the ratio of non-obese: obese was 80:20 and in G6, it was 60:40 (Table II).

Data shown in Table Ia reveals that glucose and HbA1c level of G2, G4 and G6 are significantly higher than their respective control groups. Our results for glucose and HbA1c are in agreement with the findings of Chan et al.[39] Gender has no significant effect on the glucose level, and there are no significant differences between the male and female gender of the control or NIDDM groups (i.e. G3 vs. G5; G4 vs. G6). However, for HbA1c, females showed a significant increase when compared with males (i.e. G3 vs. G5; G4 vs. G6) (Table Ia). A highly significant positive direct correlation between glucose and HbA1c was found for all six groups (r 0.74–0.96, $p < 0.001$) (Table IIIa).

Data shown in Table Ib reveals that the levels of TC, LDL-C and VLDL-C of patients of the three NIDDM groups (G2, G4 and G6) are significantly higher than the controls, while the HDL-C levels are significantly lower than those of the controls. A very highly significant correlation ($p \leq 0.0001$) between HDL-C and TG was found in all groups. The results for HDL-C agree with those of Chan et al.[39]

Gender has no effect on the TC, HDL-C and LDL-C levels of the control groups, since no significant difference between the males and females of the control groups were found, except for VLDL-C level of G3, which showed significantly higher levels than G5 (Table Ib). In contrast, for the diabetic patients, a significant difference between the male and female groups was found for all parameters. Males showed a significant increase in the variable levels

Table IIIa. Correlation coefficient between age, duration of diabetes mellitus, BMI, HbA1c and other parameters.

	Gender/group	BMI (kg/m^2)	Glucose (mg/dL)	$HbA1_C$ (%)
Age	Both/G1	0.17	0.04	0.02
	Both/G2	0.09	0.002	0.15
	Male/G3	0.40 [4]	0.48 [4]	0.54 [4]
	Male/G4	0.07	0.15	0.04
	Female/G5	0.44 [4]	0.42 [4]	0.52 [4]
	Female/G6	0.20	0.10	0.23
Duration	Both/G2	0.24 [1]	0.17	0.19
	Male/G4	0.11	0.29	0.27
	Female/G6	0.38 [2]	0.06	0.16
BMI	Both/G1		-0.15	-0.12
	Both/G2		-0.42 [4]	-0.40 [4]
	Male/G3		-0.29	-0.10
	Male/G4		-0.14	-0.22
	Female/G5		-0.08	-0.14
	Female/G6		-0.57 [4]	-0.50 [4]
Glucose	Both/G1			0.81[4]
	Both/G2			0.89 [4]
	Male/G3			0.85 [4]
	Male/G4			0.74 [4]
	Female/G5			0.78 [4]
	Female/G6			0.96 [4]

[1]$p < 0.05$; [2]$p < 0.02$; [3]$p < 0.01$; [4]$p < 0.001$

when compared with the respective female group (Tables Ib & IIIb).

The data shown in Table Ib reveals that atherogenic indices of NIDDM patients (as TC/HDL-C and HDL-C/LDL-C fractions) showed a significant increase when compared with the control group. The atherogenic indices were also significantly higher in males compared to females for both control and NIDDM groups; this finding agrees with al-Muhtaseb et al.[40] The data in Table Ib also reveals that plasma TG levels were significantly higher in the NIDDM patients than normal controls, which corroborates with other studies.[40-42] A gender difference in TG concentration was found, where male groups showed a significantly higher TG level than female groups for both control and diabetic groups (104.01 ± 2.16 vs. 99.25 ± 2.06; 133.94 ± 1.95 vs. 128.87 ± 2.45; $p < 0.03$ for control and NIDDM groups, respectively) (Tables Ib & IIIb).

Data of Table Ic shows the level of lipid peroxidation marker. In the present study, serum MDA (an oxidant) and the SOD level (a marker of oxidative stress) increased significantly, whereas glutathione (an antioxidant) content decreased significantly in male and female NIDDM patients, in comparison to non-diabetic healthy controls. Our results are in agreement with most other studies for SOD[43-45] and MDA.[46,47] However, the results did not corroborate with findings from studies by Kesavulu et al and Sekeroğlu et al,[48,49] where no significant changes were observed in SOD activity in NIDDM patients. Our results were also in agreement with studies conducted on diabetic rats.[50,51]

Gender had an effect on MDA, glutathione and SOD concentrations (Table IIIc). Males showed significantly higher levels of SOD and MDA than females, while the opposite was true for glutathione, where females showed higher levels than males in both control and NIDDM groups ($p < 0.02$, 0.03, 0.002 for controls; $p < 0.01$, 0.001, 0.0004 for diabetics; for MDA, glutathione, SOD, respectively).

Data in Tables III a–c show the correlation coefficient between age, duration of diabetes mellitus, BMI, glucose, $HbA1_C$ and the studied parameters. BMI, glucose and $HbA1_C$ of males and females in the control groups were significantly and positively correlated with age. A significant, direct correlation between TC, HDL-C, LDL-C and age was found in NIDDM males and females. A significant, direct correlation between MDA, glutathione and age was found in all NIDDM groups (i.e. G2, G4 & G6). The significance is borderline for MDA and glutathione levels of male diabetics. SOD levels of all NIDDM groups correlated negatively and significantly with age. No correlation was found between atherogenic indices and age in all groups, nor was any correlation found between any studied parameters and duration of diabetes mellitus for all groups, except for BMI, where a significant, positive correlation was found in G2 and G6. A significant, negative correlation was found between glucose, $HbA1_C$, HDL-C, glutathione and BMI, in G2 and G6.

No correlation between SOD and BMI was found in this study, contrary to findings by Adachi et al.[52] On the other hand, very highly significant, positive, direct correlation between glucose and $HbA1_C$ was found for all groups (r 0.74–0.96, $p \leq 0.00001$) which agree with findings by Akanji et al.[41] HDL-C levels in all groups correlated positively and significantly with glucose. Atherogenic indices of diabetic male or female groups correlated positively and significantly with glucose, contrary to results reported by al-Muhtaseb et al.[40] MDA of control groups (G1, G3 and G5) correlated positively with glucose. Glutathione of almost all groups except G2 correlated positively and significantly with glucose. SOD of control groups, G1 and G5, correlated negatively and significantly with glucose. No correlation between MDA, SOD and glucose for all NIDDM groups was found. The results for SOD corroborate with findings by Mizobuchi et al,[53] but not with Adachi et al.[52] HDL-C of groups G1–G4 and G6, correlated significantly and positively with $HbA1_C$. Atherogenic indices of female diabetic patients correlated positively and significantly with $HbA1_C$. TG

Table IIIb. Correlation coefficient between age, duration of diabetes mellitus, BMI, glucose, HbA1C and other parameters

	Gender/group	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	C: HDL-C	HDL-C: LDL-C	TG (mg/dL)
Age	Both/G1	0.18	0.15	0.13	0.27 [2]	0.1 [1]	-0.04	0.22 [1]
	Both/G2	0.35 [4]	0.36 [4]	0.34 [4]	0.12	-0.07	0.01	0.25 [1]
	Male/G3	0.49 [4]	0.40 [4]	0.50 [4]	0.38 [2]	0.25	-0.23	0.45 [4]
	Male/G4	0.26	0.30 [1]	0.24	0.09	-0.16	0.12	0.18
	Female/G5	0.16	-0.13	-0.18	0.05	-0.09	0.11	0.05
	Female/G6	0.52 [4]	0.43 [4]	0.53 [4]	0.16	0.03	-0.14	0.33 [1]
Duration	Both/G2	-0.08	-0.11	-0.04	-0.09	0.06	-0.09	-0.05
	Male/G4	-0.28	-0.23	-0.23	-0.25	0.03	-0.05	-0.29
	Female/G6	-0.01	-0.02	0.01	-0.03	0.03	-0.05	0.14
BMI	Both/G1	-0.03	-0.23 [1]	-0.06	0.31 [4]	0.28 [2]	-0.12	0.05
	Both/G2	-0.08	-0.25 [1]	-0.09	0.15	0.26 [2]	-0.21 [1]	0.16
	Male/G3	0.07	-0.36 [2]	0.08	0.39 [2]	0.60 [4]	-0.55 [4]	0.29
	Male/G4	-0.10	-0.21	0.00	0.14	0.20	-0.24	0.12
	Female/G5	-0.24	-0.06	-0.31 [1]	0.001	0.35 [1]	0.35 [1]	0.44 [4]
	Female/G6	-0.18	-0.35 [1]	-0.25	0.25	0.29	-0.16	0.28
Glucose	Both/G1	0.42 [4]	0.51 [4]	0.33 [4]	0.36 [4]	0.021	0.03	0.40 [4]
	Both/G2	0.02	0.34 [4]	0.11	0.33 [4]	0.17	0.05	0.01
	Male/G3	0.48 [4]	0.50 [4]	0.43 [4]	0.39 [2]	0.11	0.04	0.56 [4]
	Male/G4	0.30 [1]	0.50 [4]	0.19	0.15	0.38 [2]	0.39 [2]	0.36 [1]
	Female/G5	0.40 [2]	0.52 [4]	0.29	0.40 [3]	0.04	0.06	0.31
	Female/G6	0.14	0.54 [4]	0.23	0.50 [4]	0.63 [4]	0.50 [4]	0.15
HbA1c	Both/G1	0.29 [3]	0.33 [4]	0.20	0.33 [4]	0.05	0.04	0.35 [4]
	Both/G2	0.41 [4]	0.41 [4]	0.47 [4]	0.01	0.08	0.06	0.18
	Male/G3	0.31 [1]	0.24	0.30 [1]	0.31 [1]	0.20	0.16	0.46 [4]
	Male/G4	0.07	0.11	0.17	0.03	0.21	0.28	0.44 [4]
	Female/G5	0.17	0.42 [4]	0.05	0.27	0.31 [1]	0.31 [1]	0.11
	Female/G6	0.24	0.57 [4]	0.30	0.36 [1]	0.55 [4]	0.43 [3]	0.21

[1]$p < 0.05$; [2]$p < 0.02$; [3]$p < 0.01$; [4]$p < 0.001$

of diabetic males correlated positively and significantly with HbA1c, contrary to results reported by findings by Chan et al,[39] but not with findings by al-Muhtaseb et al.[40] Glutathione levels of female diabetic patients correlated positively and significantly with HbA1c, while SOD levels correlated negatively and significantly with HbA1c. A significant negative correlation between SOD and MDA for all six groups was found (r -0.78,-0.96 and -0.51,-0.97 for control and NIDDM groups, respectively; $p < 0.0001$).

In multiple linear regression analysis using stepwise analysis, the independent variables were: glucose, gender, diabetes mellitus, glutathione, SOD, MDA, BMI, age and HbA1c, and the dependent variable was lipid profile, mainly TC. Table IV shows that HbA1c was the most influential variable among the tested variables (62 %).

DISCUSSION

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation.[6] The low level of HDL-C, which exerts anti-atherogenic and antioxidative effects when present in sufficient amounts, is a key feature of NIDDM (also known as type 2 diabetes mellitus). The reduced HDL-C levels are often accompanied by elevations in plasma TG levels,[54] a process mediated by cholesterol ester transfer protein (CETP),[55] and is associated with increased cardiovascular disease risk.[56,57] Abnormal lipid metabolism often presents in patients with NIDDM.[58] Resistance to insulin likely underlies the changes that occur in lipid parameters of NIDDM, and usually it is associated with higher concentrations of TC and TG, and lower concentrations of HDL-C.[59] NIDDM is characterised by increased secretion of apolipoprotein (apo) B as a result of increased free fatty acid (FFA) flux to the liver,[60,61] and an inhibition of microsomal triglyceride transfer protein activity, a protein identified as a key component of the VLDL assembly process, and this leads

Table IIIc. Correlation coefficient between age, duration of diabetes mellitus, BMI, glucose, HbA1c and other parameters.

	Gender/group	MDA (μmol/L)	Glutathione (μmol/L)	SOD
Age	Both/G1	0.19	0.26 [1]	-0.10
	Both/G2	0.30 [4]	0.34 [4]	-0.24 [1]
	Male/G3	0.45 [4]	0.38 [2]	-0.35 [1]
	Male/G4	0.30*	0.30*	-0.33 [1]
	Female/G5	0.11	0.05	-0.10
	Female/G6	0.36 [1]	0.43 [4]	-0.31[1]
Duration	Both/G2	0.16	-0.17	0.02
	Male/G4	0.15	-0.23	0.21
	Female/G6	0.10	-0.02	0.03
BMI	Both/G1	0.17	-0.26 [2]	-0.13
	Both/G2	0.20	-0.31[4]	0.02
	Male/G3	0.40 [4]	-0.39 [2]	-0.46 [4]
	Male/G4	0.20	-0.21	-0.28
	Female/G5	0.26	-0.01	0.18
	Female/G6	0.16	-0.35 [1]	0.02
Glucose	Both/G1	0.38 [4]	0.38 [4]	-0.44 [4]
	Both/G2	0.09	0.09	-0.03
	Male/G3	0.38 [2]	0.39 [2]	-0.03
	Male/G4	0.08	0.50 [4]	0.001
	Female/G5	0.42 [3]	0.40 [3]	-0.56 [4]
	Female/G6	0.06	0.54 [4]	-0.12
HbA1c	Both/G1	0.40 [4]	0.24	-0.25 [1]
	Both/G2	0.23 [1]	0.05	0.15
	Male/G3	0.60 [4]	0.31	-0.57 [4]
	Male/G4	0.18	0.11	0.29
	Female/G5	0.09	0.27	-0.19
	Female/G6	0.24	0.57 [4]	-0.33 [1]

[1]$p < 0.05$; [2]$p < 0.02$; [3]$p < 0.01$; [4]$p < 0.001$

to increased plasma levels of TG and reduced levels of HDL-C.

The mechanisms responsible for hypertriglyceridaemia may be an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids, and glucose. The latter could be secondary to decreased activity of lipoprotein lipase (LPL), a key enzyme for lipoprotein-TG.[62-65] Hypertriglyceridaemia usually accompanies decreased HDL-C, which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects.[62,63,65]

De Zwart et al has proposed that oxidative stress may be associated with the pathogenesis of NIDDM complications,[66] particularly cardiovascular diseases (CVD).[67,68] The reports about the status of antioxidants and antioxidant enzymes in diabetic patients are very contradictory, both increases and decreases of antioxidant activity have been reported.[15,16] The reports about the SOD activity in diabetes mellitus are controversial, with some authors reporting no change in SOD activity,[48,49] while others reported increased activity.[46,47] There are also reports of decreased SOD activity in diabetic patients.[69]

In the present study, the increased levels of MDA and SOD clearly show that diabetic patients, irrespective of the gender, were exposed to an increased oxidative stress via lipid peroxidation,[70] while a decreased level of glutathione indicates decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid peroxidation processes in these patients. Antioxidant enzyme-dependent defences play an important role in scavenging free radicals produced under oxidative stress.[70,71]

Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals, such as super oxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and

Table IV. Predictors of lipid profile using cholesterol as the dependent variable.

Variables	R^2	R change	Beta	*t*-value	p-value	F	p-value
Significant determinants							
1-HbA1c	0.619		1.034	14.896	0.000		
2-Glutathione			1.421	11.841	0.000		
3-Gender			-0.269	-6.325	0.000		
4-Duration			-0.268	-4.298	0.000		
5-BMI			-0.112	-3.70	0.000		
6-MDA			0.136	3.678	0.000		
7-Diabetes mellitus			0.149	3.11	0.002		
Constant (81.231)							
Determinants 1–7	0.876	0.257				129.679	0.000

hydroxide ($OH^{\bullet}$) radicals and deficiency in antioxidant defence mechanisms. Increased non-enzymatic and auto-oxidative glycosylation is one of the possible mechanisms that contribute to the formation of free radicals and free radical-induced lipid peroxidation in diabetes mellitus.[10,11] Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and formation of severe damage in diabetes mellitus patients.[66-68,70]

The results suggest that the increase in lipid peroxidation and the decline in antioxidant defences may appear early in NIDDM patients, before the development of secondary complications, and might play an important role in the initiation and progression of diabetic complications. Our results also suggest that there seems to be an imbalance between plasma oxidant and antioxidant systems in patients with NIDDM.

REFERENCES

1. Herman WH, Aubert RE, Engelgau MM, et al. Diabetes mellitus in Egypt: glycæmic control and microvascular and neuropathic complications. Diabet Med 1998; 15:1045-51.
2. Diabetes prevention and control. A call for action. Alexandria, Egypt, World Health Organization, Regional Office for the Eastern Mediterranean, 1993.
3. Herman WH, Aubert RE, Ali MA, Sous ES, Badran A. Diabetes mellitus in Egypt: risk factors, prevalence and future burden. Eastern Med Health J 1997; 3:144-8.
4. Buse JB, Tan MH, Prince MJ, Erickson PP. The effects of oral anti-hyperglycaemic medications on serum lipid profiles in patients with type 2 diabetes. Diabetes Obes Metab 2004; 6:133-56.
5. Bustos P, Radojkovic C, Ulloa N, et al. Lipoprotein composition in children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2005; 18:257-64.
6. Pari L, Latha M. Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J 2002; 43:617-21.
7. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40:405-12.
8. Wolff SP. The potential role of oxidative stress in diabetes and its complications: novel implications for theory and therapy. In: Crabbe MJC, ed. Diabetic Complications: Scientific and Clinical Aspects. London: Churchill Livingstone, 1987: 167-221.
9. Varvarovská J, Racek J, Stetina R, et al. Aspects of oxidative stress in children with type 1 diabetes mellitus. Biomed Pharmacother 2004; 58:539-45.
10. Gillery P, Monboisse JC, Maquart FX, Borel JP. Does oxygen free radical increased formation explain long term complications of diabetes mellitus? Med Hypotheses 1989; 29:47-50.
11. Hunt JV, Smith CC, Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 1990; 39:1420-4.
12. Velázquez E, Winocour PH, Kesteven P, Alberti KG, Laker MF. Relation of lipid peroxides to macrovascular disease in type 2 diabetes. Diabet Med 1991; 8:752-8.
13. Nacítarhan S, Ozben T, Tuncer N. Serum and urine malondialdehyde levels in NIDDM patients with and without hyperlipidaemia. Free Radic Biol Med 1995; 19:893-6.
14. Losada M, Alio JL. Malondialdehyde serum concentration in type 1 diabetic with and without retinopathy. Doc Ophthalmol 1996-1997; 93:223-9.
15. Kaji H, Jurasaki M, Ito K, et al. Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin-dependent) diabetic women. Klin Wochenschr 1985; 63:765-8.
16. Matkovics B, Varga SI, Szabó L, Witas H. The effect of diabetes on the activities of the peroxide metabolism enzymes. Horm Metab Res 1982; 14:77-9.
17. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000; 23 Suppl 1: S4-19.
18. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972; 97:142-5.
19. Pérez A, Carreras G, Caixàs A, et.al. Plasma lipoprotein(a) levels are not influenced by glycemic control in type 1diabetes. Diabetes Care 1998; 21:1517–20.
20. John WG. Haemoglobin A1c: analysis and standardisation. Clin Chem Lab Med 2003; 41:1199-212.
21. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 1973; 19:1350-6.
22. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20:470-5.
23. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973; 19:476-82.
24. Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993; 26:39-42.
25. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970; 11:585-95.
26. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977; 23:882-4.
27. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502.
28. Levy RI. Cholesterol, lipoproteins, apoproteins, and heart disease: present status and future prospects. Clin Chem 1981; 27:653-62.
29. Siekmeier R, März W, Gross W. Precipitation of LDL with sulfated polyanions: three methods compared. Clin Chim Acta 1988; 177:221-30.
30. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70-7.
31. Beutler E, Yeh MK. Erythrocyte glutathione reductase. Blood 1963; 21:573-85.
32. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-8.
33. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186:421-31.
34. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46:849–54.
35. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975; 85:337-41.
36. Concetti A, Massei P, Rotilio G, Brunori M, Rachmilewitz EA. Superoxide dismutase in red blood cells: method of assay and enzyme content in normal subjects and in patients with beta-thalassemia (major and intermedia). J Lab Clin Med 1976; 87:1057-64.
37. Quantitative data. In: Goldstein A. Biostatistics: An Introductory Text. New York: Macmillian, 1964: 53-63.
38. Steel RG, Torrie JH. Principles and Procedures of Statistics. New York: McGraw Hill, 1960.
39. Chan WB, Tong PC, Chow CC, et al. Triglyceride predicts cardiovascular mortality and its relationship with glycaemia and obesity in Chinese type 2 diabetic patients. Diabetes Metab Res Rev 2005; 21:183-8.
40. al-Muhtaseb N, al-Yuosuf AR, Bajaj JS. Plasma lipoproteins and apolipoproteins in insulin-dependent and young non-insulin-dependent Arab women. Acta Diabetol Lat 1991; 28:61-9.
41. Akanji AO, Agbedana EO, Ugbode C. Plasma lipid profiles in relation to diabetic control in Nigerians. Afr J Med Med Sci 1989; 18:229-34.
42. Owens D, Stinson J, Collins P, Johnson A, Tomkin GH. Hypertriglyceridaemia and its influence on low density lipoprotein

regulation of cellular cholesterol synthesis: a comparison between hypertriglyceridaemic diabetic and non-diabetic patients. Diabet Med 1991; 8:745-51.
43. Seghrouchni I, Drai J, Bannier E, et al. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. Clin Chim Acta 2002; 321:89-96.
44. Palanduz S, Ademoğlu E, Gökkusu C, Tamer S. Plasma antioxidants and type 2 diabetes mellitus. Res Commun Mol Pathol Pharmacol 2001; 109:309-18.
45. Bhatia S, Shukla R, Venkata Madhu S, Kaur Gambhir J, Madhava Prabhu K. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem 2003; 36:557-62.
46. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17:24-38.
47. Sailaja YR, Baskar R, Saralakumari D. The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. Free Radic Biol Med 2003; 35:133-9.
48. Kesavulu MM, Giri R, Kameswara Rao B, Apparao C. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes Metab 2000; 26:387-92.
49. Sekeroğlu MR, Sahin H, Dülger H, Algün E. The effect of dietary treatment on erythrocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase, and serum lipid peroxidation in patients with type 2 diabetes mellitus. Clin Biochem 2000; 33:669-74.
50. Kinalski M, Sledziewski A, Telejko B, Zarzycki W, Kinalska I. Lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes. Acta Diabetol 2000; 37:179-83.
51. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biosci 2003; 28:1-5.
52. Adachi T, Inoue M, Hara H, Maehata E, Suzuki S. Relationship of plasma extracellular-superoxide dismutase level with insulin resistance in type 2 diabetic patients. J Endocrinol 2004; 181:413-7.
53. Mizobuchi N, Nakata H, Horimi T, Takahashi I. [Serum superoxide dismutase (SOD) activity in diabetes mellitus]. Rinsho Byori 1993; 41:673-8. Japanese.
54. Lamarche B, Després JP, Moorjani S, et al. Triglycerides and HDL-cholesterol as risk factors for ischemic heart disease. Results from the Québec cardiovascular study. Atherosclerosis 1996; 119:235-45.
55. Tall A. Plasma lipid transfer proteins. Annu Rev Biochem 1995; 64:235-57.
56. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Relation of high TG-low HDL cholesterol and LDL cholesterol to the incidence of ischemic heart disease. An 8-year follow-up in the Copenhagen Male Study. Arterioscler Thromb Vasc Biol 1997; 17:1114-20.
57. Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Implications for treatment. Circulation 1992; 85:37-45.
58. Rosenson RS. HDL-C and the diabetic patient: target for therapeutic intervention? Diabetes Res Clin Pract 2005; 68 Suppl 2:S36-42.
59. Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes 2003; 52:453-62.
60. Duvillard L, Pont F, Florentin E, et al. Metabolic abnormalities of apolipoprotein B-containing lipoproteins in non-insulin-dependent diabetes: a stable isotope kinetic study. Eur J Clin Invest 2000; 30:685-94.
61. Krauss RM, Siri PW. Dyslipidemia in type 2 diabetes. Med Clin North Am 2004; 88:897-909.
62. Howard BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res 1987; 28:613-28.
63. Taskinen MR. Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. Diabetes 1992; 41 Suppl 2:12-7.
64. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 1996; 37:693-707.
65. Yoshino G, Hirano T, Kazumi T. Dyslipidemia in diabetes mellitus. Diabetes Res Clin Pract 1996; 33:1-14.
66. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen, NP. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 1999; 26:202-26.
67. Jacob RA, Burri BJ. Oxidative damage and defense. Am J Clin Nutr 1996; 63:985S-90S.
68. Garewal HS. Antioxidant and Disease Prevention. New York: CRC Press, 1997.
69. Sundaram RK, Bhaskar A, Vijayalingam S, et al. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond) 1996; 90:255-60.
70. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J 2005; 46:322-4.
71. Harris ED. Regulation of antioxidant enzymes. FASEB J 1992; 6:2675-83.