Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels

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ABSTRACT

Introduction: Patients with type 2 diabetes mellitus (DM) are subject to chronic oxidative stress. Lipid peroxidation of cellular structures is an important process in atherosclerosis and late complications of DM. Malondialdehyde (MDA) plays a major role in low-density lipoprotein modification. This study aimed to evaluate whether DM duration is an independent predictor of serum MDA levels.

Methods: A total of 120 patients with type 2 DM (60 with DM duration of 120 months or less and 60, with more than 120 months) and 45 gender- and body mass index (BMI)-matched healthy adults were studied. Fasting blood samples were obtained and the fasting plasma glucose (FPG), cholesterol, high- and low-density lipoprotein cholesterol, triglycerides, creatinine, haemoglobin A1c (HbA1c), extracellular superoxide dismutase (EC-SOD) and MDA levels were measured.

Results: The MDA level was significantly higher in DM patients than in controls (p is less than 0.001), and in those with DM duration more than 120 months than those with DM duration of 120 months or less (p is less than 0.001). The level of MDA was significantly correlated with DM duration (correlation coefficient 0.254, p is less than 0.01) and the EC-SOD level (correlation coefficient 0.299, p is less than 0.001). In multivariate regression analysis, the association between MDA and DM duration remained significant after adjustments were made for age, gender, BMI, FPG, HbA1c, EC-SOD, plasma creatinine and anti-diabetic medications (p is less than 0.05).

Conclusion: The results of this study suggest that in type 2 DM patients, DM duration is independently associated with increased levels of lipid peroxidation. Longitudinal studies are required to confirm these results.

Keywords: diabetes mellitus, lipid peroxidation, low-density lipoproteins, malondialdehyde, superoxide dismutase

INTRODUCTION

Diabetes mellitus (DM) is associated with endothelial dysfunction and oxidative stress.[1-6] Chronic exposure to elevated glucose and fatty acid concentrations can cause damage in different types of cells by a variety of mechanisms collectively known as glucolipotoxicity, and oxidative stress may be a common link.[7,8] The oxidative stress in DM is greatly increased due to prolonged exposure to glycaemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of oxidative stress.[9] Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of DM.[10,11]

Malondialdehyde (MDA) is a major player in low-density lipoprotein (LDL) modification and is a product of the peroxidation of arachidonic, eicosapentaenoic and docosahexaenoic acids.[12,13] Oxidised-LDL(ox-LDL) results from the interactions between aldehydes such as MDA and lysine residues in apoB-100 of LDL.[14,15] The pathologic effects of ox-LDL include the induction of atherosclerosis (by stimulating monocyte infiltration and smooth muscle cell migration and proliferation),[16,17] atherothrombosis (by inducing endothelial cell apoptosis),[18,19] and plaque erosion (by impairing the endothelial anticoagulant balance).[20] Although microvascular and macrovascular complications of DM are known to increase with DM duration,[21-23] the association between DM duration and MDA levels in type 2 DM remains controversial. The present study evaluated the independent correlation between MDA levels and DM duration in a sample of Iranian patients with type 2 DM.

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Table I. Characteristics of the patients and controls.

Variable	Healthy controls (n = 45)	DM patients		Total DM patients (n = 120)
		Duration ≤120 mths (n = 60)	Duration >120 mths (n = 60)	
Gender				
Female	20	35	21	56
Male	25	25	39	64
Age (yrs)	50.02 ± 6.89	56.06 ± 7.86**	60.70 ± 8.81***†††	58.38 ± 8.64
Duration of diabetes (mths)	-	80 ± 30.61***	187.73 ± 69.76***†††	133.87 ± 76.18
Body mass index (kg/m²)	27.16 ± 2.77	25.31 ± 4.20	26.85 ± 5.62	26.09 ± 5.00
Systolic blood pressure (mmHg)	125.22 ± 10.44	130.9 ± 15.5	141.46 ± 16.26***†††	136.18 ± 16.73
Diastolic blood pressure (mmHg)	78.44 ± 7.82	82.05 ± 8.77	86.56 ± 9.83**†	84.31 ± 9.55
History of coronary heart disease	0	3 (5.0)	7 (11.7)	10 (8.3)
History of hypercholesterolaemia and statin use	0	11 (18.3)	22 (36.7)	33 (27.5)
ACEI/ARBs use	0	15 (25.0)	38 (63.3)	53 (44.2)
Anti-diabetic agents				
Glibenclamide	-	14 (23.3)	16 (26.7)	30 (25.0)
Metformin	-	10 (16.7)	12 (20.0)	22 (18.3)
Insulin	-	25 (41.7)	23 (38.3)	48 (40.0)
Fasting plasma glucose (mg/dL)	88.62 ± 7.08	171.04 ± 63.2***	173 ± 65.56***	172.82 ± 64.13
HbA1C (%)	4.88 ± 0.41	8.79 ± 2.01***	8.85 ± 1.48***	8.82 ± 1.76
Plasma creatinine (mg/dL)	0.95 ± 0.15	0.99 ± 0.23	1.09 ± 0.36*	1.05 ± 0.31
Triglycerides (mg/dL)	105.68 ± 37.01	209.03 ± 85.79***	185 ± 73.35***	197.18 ± 80.40
Total cholesterol (mg/dL)	205.42 ± 22.62	196.43 ± 50.99	199.38 ± 47.49	197.91 ± 49.09
LDL (mg/dL)	113.22 ± 11.46	88.13 ± 28.2 7***	92.46 ± 27.63***	90.30 ± 27.92
HDL (mg/dL)	52.01 ± 11.05	31.81 ± 7.59***	32.25 ± 5.84***	32.03 ± 6.75
EC-SOD (μ/ml)	-	85.03 ± 19.7	84.01 ± 19.37	84.53 ± 19.48
MDA (nmol/ml)	2.91 ± 0.59	3.45 ± 0.87**	4.20 ± 0.85***†††	3.82 ± 0.93

Values are mean ± standard deviation or number of patients (%).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to controls. †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$ compared to DM patients with DM duration ≤ 120 months.
DM: diabetes mellitus; ACEI/ARB: angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; HbA1C: haemoglobin A1c; LDL: low-density lipoprotein; HDL: high-density lipoprotein; EC-SOD: extracellular superoxide dismutase; MDA: malondialdehyde

METHODS

This cross-sectional study was conducted between December 2007 and February 2008 in our outpatient type 2 DM clinic. A total of 120 patients with type 2 DM (60 with DM duration ≤ 120 months and 60 with DM duration > 120 months) and 45 gender- and body mass index (BMI)-matched healthy adults were studied. Smokers, and patients with previous antioxidant treatment, plasma creatinine > 2 mg/dl and thyroid disease were excluded from the study. The research was carried out according to the principles of the Declaration of Helsinki, and the local ethics committee of our university approved the study protocol. All participants provided written informed consent before enrollment in the study.

Demographic and anthropometric data, including age, gender, height, weight, DM duration, history of coronary heart disease and medications, were recorded.

Blood pressure (systolic and diastolic) of the patient was measured in the sitting position after ten minutes of rest, and the average of two measurements (with a 5-minute interval) was used for analysis. Blood samples were collected following overnight fasting. The fasting plasma glucose (FPG), total cholesterol, triglyceride, high-density lipoproteins (HDL), LDL, haemoglobin A1c (HbA1c), extracellular superoxide dismutase (EC-SOD) and MDA levels were measured.

Glucose measurements (intra-assay coefficient of variation [CV]: 2.1%, inter-assay CV: 2.6%) were carried out using the glucose oxidase method. Cholesterol, triglyceride, HDL and LDL levels were determined using enzymatic methods (Pars Azmoon, Karaj, Iran). HbA1c was determined by high-pressure liquid chromatography. EC-SOD was measured with a commercially available kit (Cayman Chemicals Inc, Ann Arbor, MI, USA).

Table II. Multivariate linear regression analysis for the prediction of MDA levels by DM duration after adjustment for potential confounders (n = 120).

Model	Standardised ß	p-value
Adjustment for age and gender	0.256	0.005
Additional adjustment for BMI	0.253	0.006
Additional adjustment for FPG and HbAIC	0.218	0.019
Additional adjustment for EC-SOD and creatinine	0.214	0.021
Additional adjustment for anti-diabetic agent use*	0.216	0.020
Additional adjustment for statin use	0.227	0.012

*metformin, glibenclamide or insulin
Each model includes additional adjustments compared to the previous model.
DM: diabetes mellitus; MDA: malondialdehyde; BMI: body mass index; FPG: fasting plasma glucose; HbAIC: haemoglobin Alc; EC-SOD: extracellular superoxide dismutase

The intra- and inter-assay CVs were 3.2% and 3.7%, respectively. Serum MDA levels were measured using the colourimetric method described by Satoh.[24] After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol. Separation of the organic phase was facilitated by centrifugation at 3000 rpm for ten minutes, and its absorbance was determined spectrophotometrically at 530 nm (Cayman Chemicals Inc, Ann Arbor, MI, USA).

The Statistical Package for the Social Sciences version 15 for Windows (SPSS Inc, Chicago, IL, USA) was used for analysis. The continuous variables were expressed as mean ± standard deviation. Comparisons between the groups were performed using the chi-square test and t-test, as appropriate. A multivariate regression model was built to investigate the independent relationship between MDA and DM duration after controlling for other variables. A p-value < 0.05 was considered to be statistically significant.

RESULTS

The demographic and biochemical characteristics of the groups are shown in Table I. Gender, BMI and cholesterol levels were not significantly different between the DM and healthy subjects. The group with DM was older, and had higher levels of FPG, HbA1C, triglyceride and MDA, and lower levels of HDL. The majority of the patients had plasma creatinine levels below 1.5 mg/dl. Age and blood pressure (both systolic and diastolic) were higher in patients with DM duration > 120 months than in those with DM duration ≤ 120 months. There was no significant difference between the two groups of DM patients with

regard to the use of antihypertensive, antilipidaemic and glucose-lowering agents. A history of coronary heart disease, short- and long-term glycaemic control (FPG, HbA1c) and EC-SOD levels were not significantly different between the two DM groups.

A significant elevation in MDA levels was observed in patients with DM duration > 120 months compared to those with DM duration ≤ 120 months (4.20 ± 0.85 vs. 3.45 ± 0.87; p < 0.001). The MDA levels were significantly correlated to DM duration (r = 0.254, p = 0.005) and EC-SOD (r = 0.298, p < 0.001). In multivariate regression analysis, the association between MDA and DM duration remained significant after adjustment was made for age, gender, BMI, FPG, HbA1c, EC-SOD and medications (ß = 0.227, p = 0.012) (Table II).

DISCUSSION

This study showed an increase in MDA levels in DM patients, which is in keeping with the results of previous studies.[9,11,25,26] Increased non-enzymatic and auto-oxidative glycosylation is one possible mechanism for free radical-induced lipid peroxidation in DM.[27,28] Two important consequences of hyperglycaemia in DM are oxidative stress and the formation of advanced glycation end products (AGE), but the relationship between AGE formation and oxidative stress has yet to be established. A rise in plasma MDA levels in the later stage of DM (DM duration ≥ 120 months) reflects oxidative damage to lipids. Previous studies on the association between lipid peroxides and glycaemic control have produced different results. Some authors have found a positive correlation between the MDA level and indices of glycaemic control,[29,30] while several others have failed to do so.[31,32] Our results are in support of the latter. In particular, although the FPG and HbA1c levels were not significantly different between patients with short and long DM duration in our study, we observed significantly higher levels of MDA in those with DM duration over 120 months. This result suggests that the increase in MDA with DM duration might not be due to poor short- and/or long-term glycaemic control.

Does time, *per se*, have any effect on lipid peroxidation in DM? Here lies the novelty of our findings. No significant correlation was found between the MDA levels and DM duration in a study on 80 diabetic patients with a mean DM duration of ten years.[11] One possible reason for this observation in the above study is the lack of sufficient variation in DM duration (4.5–17 years vs. 2–37 years in our study). In another study on 20 patients with newly diagnosed DM and 20 patients with a mean DM duration of seven years, patients in the latter group

had higher serum and erythrocyte MDA concentrations and lower levels of glutathione.[25] Our results, coming from a larger sample (n = 120), show a significant positive correlation between MDA levels and DM duration. We demonstrated, for the first time, to our knowledge, that this relationship is independent of age, gender, BMI, HbA1c and EC-SOD.

In our study, antioxidant activity (EC-SOD concentration) did not differ between patients with short and long durations of DM, and we suggest that the chronicity of DM promotes lipid peroxidation and MDA production, independent of glycaemic control and antioxidant activity. It is important to note that although longer durations of DM tend to be associated with older age, adjustment in our analysis for age, a factor that may intensify oxidative stress,[21] did not alter the association between MDA and DM duration. The main limitation of our study concerns the use of cross-sectional data, which prevented us from drawing causal relationships. Longitudinal studies are thus required to confirm our results.

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