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Original Research

The Investigation of the Oxidative Stress-Related Parameters in Type 2 Diabetes Mellitus

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ABSTRACT

Objective: Oxidative stress, defined as an imbalance between reactive oxygen species production and breakdown by endogenous antioxidants, is closely associated with diabetes mellitus. The diabetes is characterized by hyperglycemia together with biochemical alterations of glucose and lipid peroxidation. Oxidative stress has been implicated in the pathogenesis of type 2 diabetes and its complications.
Methods: This study was conducted to investigate the variation in oxidative stress-related parameters in type 2 diabetes. Blood serum samples were collected from diabetes patients and nondiabetes healthy controls. Glucose concentrations, levels of glycated hemoglobin (A1C) and serum oxidative stress markers (glucose-6-phosphate dehydrogenase [G6PDH], malondialdehyde [MDA], glutathione [GSH], glutathione reductase [GR], glutathione peroxidase [GPx] and superoxide dismutase [SOD]) were estimated.
Results: Fasting serum glucose concentration in type 2 diabetes patients of both sexes was increased significantly as compared with the healthy controls. Level of A1C was greater than standards. Significant elevation in MDA level and depletion in GSH content were observed in diabetes patients in comparison with controls. The diminution in G6PDH activity was accompanied in part by a decrease in the anti-oxidative enzymes activities (GPx and GR), and in part by an increase in SOD activity in all diabetes patients as compared with the control group. The regression analysis showed no correlation between diabetes duration and severity of oxidative stress; however, there was a significant association between A1C and severity of oxidative stress.
Conclusions: The present study shows that there is an oxidative stress state in type 2 diabetes patients compared with healthy subjects. Our data suggest that chronic hyperglycemia causes a significant change in oxidative stress markers.

Mots clés :
glutathion peroxydase
glutathion réductase
malondialdéhyde
stress oxydatif
superoxyde dismutase
diabète de type 2

RÉSUMÉ

Objectif : Le stress oxydatif, qui est défini comme étant le déséquilibre entre la production et l'élimination des espèces réactives de l'oxygène par les antioxydants endogènes, est étroitement associé au diabète sucré. Le diabète est caractérisé par l'hyperglycémie associée aux altérations biochimiques du glucose et à la peroxydation des lipides. Le stress oxydatif a été impliqué dans la pathogenèse du diabète de type 2 et de ses complications.
Méthodes : Cette étude a été réalisée pour évaluer la variation des paramètres du stress oxydatif lié au diabète de type 2. Les échantillons du sérum sanguin ont été prélevés auprès de patients diabétiques et de témoins non diabétiques en santé. Les concentrations de glucose, les taux d'hémoglobine glyquée (A1c) et les marqueurs sériques du stress oxydatif (glucose-6-phosphate déshydrogénase [G6PDH], malondialdéhyde [MDA], glutathion [GSH], glutathion réductase [GR], glutathion peroxydase [GPx] et superoxyde dismutase [SOD]) ont été évalués.
Résultats : La concentration du glucose sérique à jeun chez les patients des 2 sexes ayant le diabète de type 2 a augmenté de manière significative par rapport aux témoins en santé. Le taux d'A1c a été

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supérieur aux normes. Une élévation significative du taux du MDA et une déplétion de la teneur en GSH ont été observées chez les patients diabétiques en comparaison des témoins. La diminution de l'activité de la G6PDH est accompagnée en partie par une diminution des activités des enzymes antioxydantes (GPx et GR) et, en partie par une augmentation de l'activité de la SOD chez tous les patients diabétiques par rapport au groupe témoin. L'analyse de régression ne montre aucune corrélation entre la durée du diabète et la gravité du stress oxydatif. Cependant, il y avait un lien significatif entre l'A1c et la gravité du stress oxydatif.

Conclusions : La présente étude montre qu'il existe un état de stress oxydatif chez les patients ayant le diabète de type 2 par rapport aux sujets en santé. Nos données suggèrent que l'hyperglycémie chronique cause un changement significatif des marqueurs du stress oxydatif.

Introduction

Diabetes mellitus has become a major health problem worldwide in recent times (1). It is widely recognized as one of the leading causes of death and disability (2). There were approximately 194 million adults aged 20 to 79 years with diagnosed diabetes in 2003 (with type 2 diabetes accounting for 90% to 95% of all diagnosed cases) around the world, and that number is expected to increase to 333 million over the next 20 years (3). Diabetes is a chronic disorder of carbohydrate, lipid and protein metabolism (4), and it is characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin (1,3). The resulting high blood sugar (glycemia) produces the classic symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (5). Although the etiology of this disease is not well defined, viral infection, autoimmune disease (3) and numerous genetic and environmental factors have been implicated (3,6).

In 1997, experts from the American Diabetes Association introduced the new classification system used today, abandoning the terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus and retaining the terms type 1 diabetes mellitus and type 2 diabetes mellitus (4). Type 2 diabetes is commonly manifested in middle (40 years)-to-late-aged adults; however, its prevalence is increasing in younger populations (5). This disease is a well-known endocrine and metabolic disorder that has reached epidemic proportions worldwide and represents a serious public health concern (7).

Type 2 diabetes is a heterogeneous metabolic disorder defined by the presence of hyperglycemia, which results from a combination of resistance to insulin action and an inadequate compensatory insulin secretion response (8). With type 2 diabetes, patients cannot metabolize carbohydrates, proteins or lipids owing to improper production of insulin, a blood glucose regulator or resistance to insulin. Insulin helps cells use glucose as a main energy source (5). Increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia. In addition to genetic risk factors for type 2 diabetes, acquired or environmental factors play a major role; foremost among these is obesity (9).

There is a growing scientific and public interest in connecting oxidative stress with a variety of pathological conditions, including diabetes mellitus as well as other human diseases. Previous experimental and clinical studies report that oxidative stress plays a major role in the pathogenesis and development of complications of both types of diabetes (2). Over the past decade, there has been substantial interest in oxidative stress and its potential role in diabetogenesis and the development of diabetes complications (10).

Oxidative stress develops from an imbalance between free radical production, often increased through dysfunctional mitochondria, and reduced antioxidant defences (11,12). The mitochondria electron transport chain is a major source of reactive oxygen species (ROS) in insulin secretion cells, insulin peripheral sensitive cells and endothelial cells (13). Oxidative stress is produced under diabetes conditions and is likely involved in the progression of pancreatic beta-cell dysfunction found in diabetes (14). Hyperglycemia causes tissue damage through 5 major mechanisms: 1) increased flux of glucose and other sugars through the polyol pathway; 2) increased intracellular formation of advanced glycation end products; 3) increased expression of the receptor for advanced glycation endproducts and its activating ligands; 4) activation of protein kinase C isoforms, and 5) overactivity of the hexosamine pathway. Several lines of evidence indicate that all 5 mechanisms are activated by a single upstream event: mitochondrial overproduction of ROS (15).

Oxidative stress has been implicated in the pathogenesis of type 2 diabetes and its complications (16–18). The metabolic disturbances contribute to oxidative stress and compromise the antioxidant defence system in type 2 diabetes patients (19). There seems to be imbalance between oxidant and antioxidant systems in type 2 diabetes patients. These patients are considered to be under oxidative stress because of prolonged exposure to hyperglycemia (20). In view of the above considerations, the present study aimed to evaluate the oxidative status in a group of male and female Algerian patients with type 2 diabetes treated with hypoglycemic agents. Values of glucose, malondialdehyde (MDA) and oxidative stress markers (glucose-6-phosphate dehydrogenase [G6PDH], glutathione [GSH], glutathione reductase [GR], glutathione peroxidase [GPx] and superoxide dismutase [SOD]) in the serum were estimated in comparison with healthy nondiabetes volunteers as controls. Blood percentage of glycated hemoglobin (A1C) was measured, and the body mass index was calculated.

Subjects and Methods

Subjects

This study was conducted with 59 patients with type 2 diabetes who were attending a Diabetes Centre (Annaba, Algeria) for their monthly routine medical examination. There were 34 men (mean age, 47.2±11.4 years) and 25 women (mean age, 48.7±12.0 years). Forty-eight apparently healthy volunteers were also recruited as a control group, 27 men (mean age, 45.1±9.2 years) and 21 women (mean age, 44.2±10.9 years). All subjects were randomly selected and had not been taking any medications other than antidiabetes drugs for the past year (receiving only diabetes treatment). None of the subjects was receiving antioxidant supplementation.

The selection criteria for the subjects were based on a questionnaire. Inclusion criteria were patients with diabetes for at least 5 years, fasting glucose ≥126 mg/dL and levels of glycosylated hemoglobin (A1C) ≥6.5%, in accordance with the World Health Organization (WHO) diagnostic criteria for type 2 diabetes. In our study, normal weight (body mass index <25 kg/m^2) based on the current WHO guidelines is an inclusion criterion to ensure that

hyperglycemia could be the main causal factor of the type 2 diabetes-related oxidative stress. Key exclusion criteria included smoking, pregnant or lactating women, persons receiving gastric or diuretic treatment, patients with high blood pressure, kidney or heart or liver disease, and acute infection.

All of the subjects enrolled in the present study were of Algerian ethnicity. Determining ethnicity of the cohort is important in assessing prevalence of diabetes and its complications. In Algeria, prevalence of diabetes among adults aged 20 to 79 years for the years 2011 and 2030 ranged from 6.3% to 7.6% in the national population and 6.9% to 7.7% in the world population. The data suggest that the prevalence of diabetes in all countries studied (110 countries), including Algeria, is increasing as a consequence of increasing incidence due to demographic changes such as ageing and as a result of risk factors such as obesity and sedentary lifestyle becoming more common, and also as a result of better healthcare that improves the longevity of people with diabetes (21). Approval was obtained from the institutional review board, and all participants were informed of the purposes of the research and provided written consent before enrolment in the study.

Methods

The fasting venous blood was drawn from diabetes patients and healthy volunteers. Blood was collected in dry tubes and centrifuged at 4000 rpm for 5 minutes. The serum collected was used to assay biochemical parameters, namely, glucose, G6PDH, MDA, GSH, GR, GPx and SOD. To determine A1C levels, blood was anticoagulated by ethylenediamine tetraacetic acid. Glucose was estimated enzymatically using enzymatic kit (BioSystems, Barcelona, Spain). Serum glucose concentration was determined using the standard enzymatic method, glucose oxidase-peroxidase. Lipid peroxidation end product MDA was measured by the method as outlined by Esterbauer et al (22). This method is based on MDA reaction with 2 molecules of thiobarbutiric acid (TBA), and the resulting MDA-TBA2 complex was quantified by photometric reading at 535 nm. The reduced GSH level was measured by adopting the method described by Weckbecker and Cory (23). The principle of this assay is based on measuring the optical absorbance of 2-nitro-5-mercapturic acid. That results from the reduction of 5,5 ′-dithio-bis-2-nitrobenzoïc (Ellman's reagent) by groups (-SH) of glutathione. The SOD assay measured all 3 types of SOD (Cu/Zn-, Mn-, and Fe-SOD), and its activity was estimated according the spectrophotometric method, as described by Masnini (24), which is based on the dismutation of superoxide anion into oxygen and hydrogen peroxide. The GR and GPx activities were determined using Randox Laboratories kits (Antrim, UK). The GPx was measured by following the decrease in absorbance when oxidized glutathione is converted to GSH by GR; the enzyme GR catalyzes the reduction of GSSG in the presence of nicotine amide adenine dinucleotide phosphate (NADPH), which is oxidized to $NADP^+$. The decrease in absorbance at 340 nm was measured. The G6PDH activity was estimated by measuring the rate of absorbance due to the reduction of $NADP^+$ using a standard Sigma Diagnostics kit (St. Quentin Fallavier, France). Assessment of A1C% was carried out by quantitative chromatographic spectrophotometric determination of glycohemoglobin in whole blood using a A1C kit (Bio-Systems). The body mass index is calculated as weight in kilograms divided by the square of height in metres (kg/m^2).

Statistical analysis

All values were expressed as the mean ± SE obtained from the number of experiments. Student's *t* test was used for comparing diabetes patients with normal subjects, and correlation coefficients were determined by Pearson's simple linear regression analysis using Minitab software, version 13.31 (State College, PA, USA). All p values <0.05 were considered to be significant.

Table 1
Clinical characteristics of type 2 diabetes patients and controls

Clinical characteristics	Male		Female	
	Nondiabetes (n=27)	Diabetes (n=34)	Nondiabetes (n=21)	Diabetes (n=25)
Mean age, years	45.1±9.2	47.2±11.4	44.2±10.9	48.7±12.0
Body mass index, kg/m^2	20.9±2.2	21.6±3.1	24.2±3.5	24.2±3.9
Diabetes duration, years	—	10.4±2.4	—	9.6±2.8
A1C, %	—	8.01±1.59	—	7.89±0.28
Glucose, mmol/L	5.08±0.50	11.86±0.45*	4.90±0.40	10.20±0.40*

A1C, glycated hemoglobin.
Values are mean ± SE. Body mass index is calculated as weight in kilograms divided by the square of height in meters.
* $p<0.001$, significantly different from control.

Results

Individual subject characteristics of age, sex, duration of the disease, body mass index, levels of blood glucose in type 2 diabetes patients and healthy subjects (controls) and A1C percentage are shown in Table 1. Fasting serum glucose level in type 2 diabetes patients of both sexes was increased significantly whereas the healthy nondiabetes control subjects had a normal blood glucose level. The percentage of A1C was greater than standard in male and female patients with diabetes. Serum MDA level increased significantly in diabetes patients with respect to controls.

The GSH content decreased significantly in subjects with diabetes of both sexes in comparison with nondiabetes subjects, as shown in Table 2. Table 3 indicates a significant decrease in antioxidant enzymes activities (G6PDH, GPx and GR) with a significant increase in SOD activity in type 2 diabetes patients as compared with the control group.

Data in Tables 4 and 5 report the correlation coefficients between duration of diabetes, A1C and the oxidative stress parameters studied in type 2 diabetes patients. As revealed in Table 4, there is a positive and nonsignificant correlation between diabetes duration and concentrations of MDA and SOD in male and female diabetes patients. However, GSH, G6PDH, GPx and GR levels in diabetes patients of both sexes were correlated negatively and nonsignificantly with duration of diabetes. Results in Table 5 present a positive and significant correlation between A1C and MDA and SOD levels in male and female diabetes patients. Conversely, GSH, G6PDH, GPx and GR in patients of both sexes correlated negatively and significantly with A1C.

Discussion

In this study, we investigated the oxidative stress-related parameters in type 2 diabetes. Our findings suggest that diabetes patients have more severe oxidative stress than healthy persons. Elevated oxidative stress was reported in diabetes patients and in

Table 2
Malondialdehyde and glutathione levels in type 2 diabetes patients and controls

	Male		Female	
	Nondiabetes (n=27)	Diabetes (n=34)	Nondiabetes (n=21)	Diabetes (n=25)
Malondialdehyde, nM/mL	0.10±0.02	0.27±0.04*	0.11±0.01	0.24±0.02*
Glutathione, nM/mL	4.50±0.15	3.79±0.63†	4.45±0.12	3.09±0.34*

Values are mean ± SE.
* $p<0.001$, significantly different from control.
† $p<0.01$, significantly different from control.

Table 3
Oxidative stress enzyme activities in type 2 diabetes patients and controls

Oxidative stress enzymes	Male		Female	
	Nondiabetes (n=27)	Diabetes (n=34)	Nondiabetes (n=21)	Diabetes (n=25)
G6PDH, UI/mL	11.15±1.28	8.05±0.97*	9.65±1.3	7.36±1.43†
Glutathione peroxidase, UI/mL	3.66±0.48	2.70±0.34*	3.45±0.45	2.50±0.33†
Glutathione reductase, UI/mL	3.86±0.38	2.43±0.41*	3.50±0.31	2.60±0.37*
Superoxide dismutase, UI/mL	87.47±17.12	123.70±12.06†	96.12±18.96	141.82±15.31*

G6PDH, glucose-6-phosphate dehydrogenase.
Values are mean ± SE.
* $p<0.001$, significantly different from control.
† $p<0.01$, significantly different from control.

animal models of diabetes (25). The possible sources of increased oxidative stress might include increased generation of free radicals or an impaired antioxidant defence system. Enhanced levels of free radicals were found in diabetes (16). Oxygen-free radicals are toxic to tissue because of their high reactivity and ability to form covalent bonds nonenzymatically (26). Extensive studies with biological materials have shown clearly that the reactive free radicals are able to produce chemical modifications in the cells and damage the proteins, lipids, carbohydrates and nucleotides. To overcome these consequences, cells have antioxidant defence systems, which scavenge the free oxygen radicals and suppress free radical chain and lipid peroxidation (27).

In the present study, we examined oxidative stress markers in type 2 diabetes patients and in normoglycemic subjects. Values of fasting serum glucose, A1C and biomarkers of oxidative stress (G6PDH, MDA, GSH, GR, GPX and SOD) in the diabetes patients were significantly changed. From the results obtained, fasting serum glucose level was significantly higher in both male and female patients as compared with healthy subjects, and the percentage of A1C was greater than standards. These results were obtained even though patients with type 2 diabetes were receiving hypoglycemic treatment, and they were presumably due to poor control of diabetes.

Type 2 diabetes is considered to develop from a state of increased insulin resistance and development of beta-cell dysfunction (28). With diabetes or insulin resistance, failure of insulin-stimulated glucose uptake by fat and muscle causes glucose concentrations in blood to remain high. The increase in blood glucose level depends upon the degree of beta-cell destruction (29). Consequently, glucose uptake by insulin-independent tissues increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defences by multiple interacting nonenzymatic, enzymatic and mitochondrial pathways (11). The proposal has been made that high serum glucose levels, common in diabetes patients and in experimental animals with diabetes, can increase intracellular glucose levels, thereby inducing an increased rate of glycolysis. If that is correct, increased substrate delivery to the mitochondria could increase accumulation of ROS (26).

Furthermore, this study found an increase in the MDA level in both sexes of patients with type 2 diabetes compared with non-diabetes subjects, supporting findings by other studies (1,29–31). As an aldehydic product of lipid peroxidation, MDA is a biomarker of intensified lipid peroxidation and also indirect evidence of high free radical production in diabetes (1). Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (29). Increased lipid peroxide may be due to the increased glycation of proteins in diabetes, and the glycated proteins might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which also may be thought to play a role in increased lipid peroxidation in diabetes (31). The study by Bhutia et al (32) noted significant increases of MDA level and fasting plasma glucose in poorly controlled type 2 diabetes. In the present study, the increased levels of MDA clearly show that diabetes patients were exposed to an increased oxidative stress through lipid peroxidation.

The serum G6PDH activity shows a significant decrease in type 2 diabetes patients (male and female) as compared with controls. The role of G6PDH as an antioxidant enzyme has been recognized in erythrocytes for a long time. As the first and rate-limiting enzyme of the pentose phosphate pathway, G6PDH is indispensable to maintain intracellular redox balance by serving as the principal intracellular source of NADPH, which is used as a reducing equivalent (33). The NADPH is then the principal intracellular reductant, and its production is mainly dependent on G6PDH activity. Hyperglycemia inhibits G6PDH activity in diabetes patients; thus, this inhibition leads to lower intracellular NADPH levels and therefore increased oxidative stress processes. As a major source of NADPH, the role of G6PDH as an antioxidant enzyme has been well elaborated recently (34). Deficiency of G6PDH, the most common enzymopathy worldwide, is associated with increased cellular oxidant stress. Hyperglycemia, which is associated with increased oxidant stress in aortic endothelial cells, has recently been shown to exert its deleterious effects, in part, by inhibiting G6PDH activity (35). Xu et al (34) suggest that chronic hyperglycemia leads to a decrease of G6PDH in kidney cortex of experimental diabetes animals and leads to increased oxidative stress. This acquired G6PDH inhibition in diabetes kidneys partly may be due to decreased G6PDH expression and increased phosphorylation of G6PDH apoenzyme caused by protein kinase A activation. G6PDH occurs at least in part from high glucose–stimulated increases in cyclic adenosine monophosphate levels in various cell types, such as pancreatic islets (34,36).

The reports about the status of antioxidants and antioxidant enzymes in diabetes patients are very contradictory, and both increase and decrease of antioxidant activity have been reported (31,37). We observed in blood serum of type 2 diabetes patients a significant decrease of reduced GSH level, GPx and GR activities, with the exception of an increase in activity of SOD as compared with the control subjects. The depletion of GSH levels is in agreement with other studies (5,29–31,38). Moussa (29) has found a link between hyperglycemia and GSH depletion. In hyperglycemia conditions, glucose is preferentially used in the polyol pathway that

Table 4
Correlation between diabetes duration and oxidative stress parameters in type 2 diabetes patients*

Duration, years	Sex	Oxidative stress parameters											
		MDA (nM/mL)		GSH (nM/mL)		G6PDH (UI/mL)		GPx (UI/mL)		GR (UI/mL)		SOD (UI/mL)	
		r	p	r	p	r	p	r	p	r	p	r	p
	Male	0.155	0.381	−0.056	0.755	−0.040	0.645	−0.073	0.682	−0.073	0.682	0.047	0.794
	Female	0.231	0.266	−0.185	0.376	−0.097	0.644	−0.234	0.260	−0.145	0.490	0.093	0.658

G6PDH, glucose-6-phosphate dehydrogenase; *GPx*, glutathione peroxidase; *GR*, glutathione dismutase; *GSH*, glutathione; *MDA*, malondialdehyde; *SOD*, superoxide dismutase.
* Male, n=34; female, n=25.

Table 5
Correlation between glycated hemoglobin and oxidative stress parameters in type 2 diabetes patients*

A1C %	Sex	Oxidative stress parameters											
		MDA (nM/mL)		GSH (nM/mL)		G6PDH (UI/mL)		GPx (UI/mL)		GR (UI/mL)		SOD (UI/mL)	
		r	p	r	p	r	p	r	p	r	p	r	p
	Male	0.722	0.000†	−0.476	0.004†	−0.629	0.000†	−0.624	0.000†	−0.601	0.000†	0.386	0.024†
	Female	0.660	0.000†	−0.512	0.009†	−0.482	0.015†	−0.462	0.020†	−0.637	0.001†	0.661	0.000†

A1C, glycated hemoglobin; *G6PDH*, glucose-6-phosphate dehydrogenase; *GPx*, glutathione peroxidase; *GR*, glutathione dismutase; *GSH*, glutathione; *MDA*, malondialdehyde; *SOD*, superoxide dismutase.

* Male, n=34; female, n=25.

† Significant correlation.

consumes NADPH necessary for GSH regeneration by the GR enzyme. Hyperglycemia is, therefore, indirectly the cause of GSH depletion. Thus, increases in flux through the polyol pathway may deplete NADPH, because the first step in the polyol pathway is the reduction of glucose to sorbitol by NADPH. The depletion of NADPH maintains the level of GSH in the reduced state (26).

The main function of antioxidant enzymes is to protect cells against different hydroperoxides resulting from reactive ROS through scavenging reactions (39). The activities of GPx and GR were significantly decreased. This decrease may be due to protein glycation, which is a mechanism that damages the protein within antioxidant enzymes (30). Gillery (40) explains that hyperglycemia generates an increase of the intensity of the reactions of nonenzymatic glycation proteins that are associated with oxidative stress, well described in patients with diabetes. Thus, increased glycation in diabetes patients and subsequent reactions of proteins may affect amino acids close to the active sites of the enzyme or disturb the stereochemical configuration and cause structural and functional changes in the molecule.

The reduction of the GPx activity in type 2 diabetes patients has been proved by Niedowicz and Daleke (38) and Rahbani-Nobar et al (41). The low activity of GPx could be directly explained by the low content of GSH found in diabetes patients, because GSH is a substrate and cofactor of GPx. However, Hisalkar et al (42) have linked the decrease in GPx activity with increasing MDA and explain that there is possible significance in the occurrence of increased MDA together with reduced levels of GPx in diabetes. It is possible that the observed reduction in GPx in these diabetes samples may indirectly lead to increased lipid peroxidation, as lipid hydroperoxides are destroyed by GPx. Another possibility is that erythrocyte GPx activity may in some way be inhibited by the presence of higher levels of MDA.

The GR was found to be decreased in type 2 diabetes patients. It is another mechanism that may explain the GSH reduction in diabetes. The GSH is regenerated by the GR, using reducing equivalents from NADPH. The NADPH is generated through the pentose phosphate pathway, which is stimulated by insulin. Production of NADPH in diabetes may be sluggish, probably resulting in lowered GR activity and reduced GSH recycle (31).

The reports about the SOD activity in diabetes are controversial, with some researchers reporting no change in SOD activity whereas others report increased or decreased SOD activity (31,37). According to the literature, SOD activity response to diabetes has been conflicting, but in the present study, increased SOD activity in type 2 diabetes patients, both male and female, was reported as compared with the nondiabetes subjects. The same result was noted by Moussa (29), Soliman (37), Seghrouchni et al (43), Kimura et al (44) and Padalkar et al (45). SOD is considered a primary enzyme because it is involved in the direct elimination of ROS. It is an important defence enzyme that catalyzes the dismutation of superoxide radicals (29). The increased activity of antioxidant enzymes stimulates cellular capacity to scavenge free radicals, thereby limiting the damage caused by ROS (39). Thus, the increase in total SOD activity suggests a possible adaptive response, probably due to the increased production of the superoxide anion ($O_2\bullet^-$), which would lead to an augmentation in the production of H_2O_2 (46). The increase in SOD activity tries to fight against oxidative stress and may be a compensatory mechanism in response to increased oxidative stress in diabetes patients (45). However, the increase in the activity of this antioxidant enzyme in diabetes is not sufficient to protect cells during oxidant exposure, because increased MDA, depleted GSH and decreased antioxidant enzymes activities (G6PDH, GPx and GR) indicate that oxidative cell damage has already occurred.

In our study, the regression analysis indicates the absence of correlation between duration of diabetes and severity of oxidative stress. This finding is consistent with the results of Nakhjavani et al (47), who have reported no significant correlation between diabetes duration and MDA and SOD concentrations. In contrast, significant correlation between the degree of glycemic control (A1C) and severity of oxidative stress was observed. The findings are compatible with those of Soliman (37) that correlated A1C with GSH and SOD levels, and with those of Kassim (48) that associated A1C with MDA and SOD levels. Goodarzi et al (49) support the correlation between the degree of hyperglycemia and oxidative stress. In contrast to the findings of the present study, no correlation was found by Rahbani-Nobar et al (41), who have correlated A1C with SOD and GPx levels in type 2 diabetes patients. Others have not observed a correlation between A1C and MDA (50). Finally, we believe that glycemic control has an influence on the oxidative stress in diabetes patients.

Conclusion

We examined in this study, along with oxidative stress markers, the values of G6PDH, MDA, GSH, GR, GPx and SOD in serum of type 2 diabetes subjects, comparing them with normoglycemic control subjects. Our results confirm that hyperglycemia in type 2 diabetes patients leads to an inhibition of antioxidant enzyme activities (G6PDH, GPx and GR), elevation in SOD activity and depletion of GSH level. By using these different markers, the existence of oxidative stress has been demonstrated. Hypoglycemia treatment has probably no favourable effect on the antioxidant system in patients because the system has been altered by hyperglycemia, presumably by poor control of diabetes. Patients with type 2 diabetes are exposed to increased oxidative stress, which can promote the development of complications. The significant correlation found between A1C and parameters of oxidative stress indicate that good glycemia control can alleviate the long-term complications of diabetes by decreasing oxidative stress.

There is a need to continue to explore the relationship between free radicals, diabetes and the complications of diabetes, and to elucidate the mechanisms by which increased oxidative stress accelerates the development of diabetes complications, in an effort

to expand treatment options. Improvement of glycemia control seems to be a beneficial factor to decrease oxidative stress in type 2 diabetes patients. For a better investigation of oxidative stress in diabetes patients, it would be wise to complete this study by determination of total antioxidant status, hydroxydeoxyguanosine, a marker of DNA oxidation, serum-advanced glycation end-products, lipid peroxidation products and advanced oxidation protein products. However, owing to the limited number of cases in our study, future study should be planned with more cases to substantiate the results and arrive at a definite conclusion.

Our findings suggest that hypoglycemia treatment has no favourable effect on the antioxidant system in type 2 diabetes patients compared with healthy subjects. With hypoglycemia treatment, supplementation with micronutrients (antioxidants, vitamins, minerals, trace elements and essential fatty acids) is necessary to improve the intrinsic antioxidant system. Diabetes patients should eat more fruits and vegetables, preferably those with higher antioxidant content, but they must also rely on unrefined cereals, nuts and legumes to provide a variety of antioxidants that is as complete as possible.

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Author Disclosures

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