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Evaluation of some biochemical changes in diabetic patients

Mosaad A. Abou-Seif[a,*], Abd-Allah Youssef[b]

[a] Biochemistry Department, Faculty of Science, Mansoura University, El-Mansoura, Egypt
[b] Internal Medicine I, Mansoura University Hospital, Mansoura University, El-Mansoura, Egypt

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Abstract

Objective: Hyperglycemia is considered a primary cause of diabetic vascular complications and is associated with oxidative stress, impaired trace element and lipid metabolism as well as pancreatic enzyme abnormalities. The role of trace elements in some of the metabolic dysfunctions and their contributions in the development of vascular complications is not clear. Therefore, the present study investigates the relationship among diabetes mellitus, trace elements status, advanced glycation end products (AGEs), advanced oxidation protein products (AOPP), lipid profiles, antioxidant status, nitric oxide and pancreatic amylase activity in the sera of 55 non-insulin-dependent diabetes mellitus (NIDDM; 35 with microvascular complications and 20 without vascular complications), 40 insulin-dependent diabetes mellitus (IDDM; 25 with microvascular and 15 without microvascular complications), and 20 nondiabetic healthy control subjects. The mean age of the diabetic patients was similar to that of control. The mean duration of the disease was 11.8 ± 6.8 years (3–27 years) in IDDM and 7.1 ± 4.7 years (1–15 years) in NIDDM. *Methods*: Plasma Cu, Zn, Mg, Ca, thiobarbituric acid-reactive substance (i.e. malondialdehyde; MDA), nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), ceruloplasmin (Cp) and amylase activities as well as AOPP were assessed spectrophotometrically whereas AGEs were estimated spectrofluorometrically in two types of diabetes mellitus (DM) as well as control subjects of matched sex and ages. *Results*: SOD, CAT and Cp activities were decreased whereas serum α-amylase activity was increased in two types of DM in comparison to the corresponding activities of the control subjects. The plasma levels of MDA, NO and Cu were increased but GSH, Zn, Mg and Ca levels were significantly diminished in diabetic patients as compared to the controls. The averages of total cholesterol (CHOL), triglyceride (TG) and low-density lipoprotein-cholesterol (LDLc) were higher in both types of diabetes mellitus in comparison to the control subjects. The mean value of high-density lipoprotein-cholesterol (HDLc) was lower in both types of diabetes mellitus. Further, the mean values of AGEs and AOPP were elevated in diabetic patients vs. control. These parameters are significantly higher in NIDDM patients when compared to the IDDM subjects. Slight but not significant differences in these parameters were observed in patients with diabetic complications when compared to that of without diabetic complications. *Conclusion*: These findings may explain the role of impaired trace element status, defect of antioxidants and increased of AGE and AOPP in the pathogenesis of pancreas and the vascular complications of diabetes mellitus. Oxidative stress is increased in both types of DM, but it is more in NIDDM

Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; MDA, malondialdehyde; Cp, ceruloplasmin; AGEs, advanced glycation end products; AOPP, advanced oxidation protein products; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; NO, nitric oxide; CHOL, cholesterol; TG, triglyceride; HDLc, high-density lipoprotein-cholesterol; LDLc, low-density lipoprotein cholesterol; DM, diabetes mellitus.

* Corresponding author. Fax: +20-50-2246781.
E-mail address: Aseif12@hotmail.com (M.A. Abou-Seif).

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patients than in IDDM subjects. In addition, oxidative stress also plays an important role in the formation of AGEs and AOPP in DM.

Keywords: Diabetes mellitus; Trace elements; Antioxidants; Amylase and nitric oxide; Lipid profiles; AGEs; AOPP

1. Introduction

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Long-term vascular complications represent a major cause of morbidity and mortality in patients with diabetes mellitus. In addition, various biochemical disorders associated with vascular complications, such as hyperlipidemia and oxidative stress which frequently co-exist with diabetes mellitus [1], appear inadequate to explain the increased risk of vascular diseases. The observations suggest that additional factors may be involved in the acceleration of diabetic vascular disease.

Metal ions are known to play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients. It has been reported that the urinary excretion of calcium, zinc and magnesium is increased in two types of diabetes mellitus causing a decrease in blood levels of these elements from these patients [2,3]. Another study reported that the levels of zinc and magnesium were significantly lower while the level of copper was significantly higher in serum of patients with IDDM [4].

Advanced glycation end products (AGEs) are complex components formed from the nonenzymatic glycosylation of proteins, i.e. binding of monosaccharides to amino groups of proteins, which alter protein structure and functions. In addition, advance oxidation protein products (AOPP) are formed during oxidative stress by the action of chloramines (produced by myeloperoxidase in activated neutrophils). These compounds (AGEs and AOPP) accumulate in biological systems and thus take part in the diabetic long-term complications by causing damage to biological membranes and endothelium [5]. A recent study reported that AGEs were elevated in NIDDM only whereas AOPP were elevated significantly in both IDDM and NIDDM [6].

It has been reported that diabetic patients have significant defects of antioxidant protections and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications [7]. Decrease of superoxide dismutase (SOD), catalase (CAT), peroxidase (Px), ceruloplasmin (Cp) and glutathione peroxidase (GSH-Px) activities as well as a decrease in the GSH level and an increase in the concentration of glutathione disulfide (GSSG) were observed in erythrocytes of diabetic patients and in tissues from diabetic animals [8].

In diabetes mellitus (DM), the disorders of carbohydrates, lipids and proteins metabolism play predominant role in diabetic complications. Hypercholesterolemia (CHOL) and hypertriglyceridemia (TG) are mostly observed and related largely to the degree of diabetic control [9]. Serum HDLc was reported to be low in diabetic patients of both types of DM [10]. Hyperglycemia may alter lipoproteins to a form that promotes atherogenesis. Low-density lipoprotein-cholesterol (LDLc) levels are frequently altered in diabetic patients.

Lipid peroxidation products, which increase in clinical and experimental diabetes, are important results of oxygen-derived free radicals stress. These products may be important in the pathogenesis of vascular complications in DM [11].

Nitric oxide (NO) is considered as a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature. The previous studies have demonstrated that the development of diabetic complications in diabetes is closely related to the increased generation of superoxide anion ($O_2^{\bullet -}$) and nitric oxide (NO) [12].

Acute pancreatitis was confirmed by elevation of serum amylase activities. Previous study has reported that the exocrine pancreatic function and secretion of amylase in particular are altered in diabetes [13]. It has been reported that the activity of amylase was elevated in poorly controlled diabetes [14].

Alterations in the plasma concentrations of several trace elements have been suspected in diabetic patients and may be involved in some of the metabolic dysfunctions in diabetes mellitus. Interconnecting systems of antioxidant micronutrients (minerals) and enzymes also accomplish the body's defense against oxidative stress. In addition, diabetes mellitus is frequently associated with pancreatic enzyme abnormalities and oxidative stress. Therefore, the present study aimed to show the role of trace element status including Cu^{2+}, Zn^{2+}, $Mg^{2+}$and Ca^{2+}, NO levels, antioxidants, AGEs and AOPP formation, lipid profiles and serum amylase activity in the pathogenesis and progression of two types of diabetes mellitus (DM). In addition, it investigated the relationship between these parameters and vascular complications in both types of diabetic patients.

2. Materials and methods

2.1. Materials

Hydrogen peroxide (30% H_2O_2), Na_2HPO_4, KH_2PO_4, *p*-phenylenediamine and HCl were purchased from Merck (Darmstadt, Germany). Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), NADH, sodium pyrophosphate, thiobarbituric acid (TBA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were from Sigma (St. Louis, MO, USA).

2.2. Subjects

Fasting blood was withdrawn from 55 patients with non-insulin-dependent diabetes mellitus (NIDDM) and from 40 patients with insulin-dependent diabetes mellitus (IDDM) as well as from 20 healthy volunteers. Thirty-five subjects of NIDDM group and twenty-five subjects of IDDM group had microvascular complications. The mean duration of the disease was 11.8 ± 6.8 years (3–27 years) in IDDM and 7.1 ± 4.7 years (1–15 years) in NIDDM. The blood samples were obtained from patients of the Department of Internal Medicine I, Mansoura University Hospital, El-Mansoura, Egypt.

Patients of NIDDM had been treated with an oral antidiabetic agent (Amaryl; glimepiride tablets, Hoechst-Roussel Pharmaceuticals, Division of Aventis Pharmaceuticals, Kansas City, MO 64137, USA) but patients of IDDM had been treated with injected insulin (Mixtard 30HA, Novo Nordisk, 2880 Bagsvaerd, Denmark). Both types of diabetic patients were free from other diseases. The clinical characteristics of the study subjects are shown in Table 1.

2.3. Methods

Fasting blood samples were freshly withdrawn from both patients and healthy volunteers and immediately transferred from Mansoura University Hospital to our laboratory in an icebox. Each sample was centrifuged at 4000 rpm and the serum was separated and stored at −20 °C until analysis.

2.4. Biochemical analysis

2.4.1. Determination of copper, zinc, magnesium and calcium levels in sera

The serum contents of copper (Cu^{2+}), zinc (Zn^{2+}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were measured by using atomic absorption spectrophotometer (Perkin-Elmer 2380, Norwalk, CT 06859-0012, USA) following acidification of the samples.

2.4.2. AGEs and AOPP assays

The AGEs concentrations were estimated spectrofluorometrically according to the method described by Kalousva et al. [6]. The fluorescence intensity was recorded at 440 nm emission and at 350 nm excitation. AOPP levels were determined spectrophotometrically according to the method of Kalousva et al. [6].

Table 1
Clinical characterization of the study subjects

	Control ($n = 20$)	IDDM ($n = 40$)	NIDDM ($n = 55$)
Sex (M/F)	12/8	24/16	35/20
Age (year)	52 ± 6.1	53.24 ± 5.87	52 ± 8.9
Weight (kg)	73.5 ± 6.74	89.55 ± 11.75	84.8 ± 10.56
Height (cm)	160.63 ± 18.6	163.76 ± 10.06	166 ± 12.46
Fasting blood glucose (mmol/l)	4.26 ± 0.18	16.79 ± 0.94	15.41 ± 0.79

2.4.3. Determination of lipid profiles and fasting blood sugar (FBS) level

Serum glucose, total cholesterol (CHOL) and triglyceride (TG) levels were determined by glucose oxidase, CHOD-PAP and GPO-PAP enzymatic test kits supplied by Spinreact (SpinReact, Ctra. Santa Coloma, 7E-17176 Santa Esteve, de Bas, Spain). HDLc was determined by precipitation of LDL and VLDL in the presence of phosphotungstic acid and magnesium chloride (HDL-cholesterol precipitating reagent from Biotrol, Paris, France). LDLc was calculated according to the Friedewald formula [15].

2.4.4. Estimation of reduced glutathione (GSH) content

GSH content in sera of diabetics and healthy subjects was estimated spectrophotometrically at 412 nm using DTNB [16]. Results are expressed as mg/g protein.

2.4.5. Determination of malondialdehyde (MDA)

Malondialdehyde (MDA), an end product of unsaturated fatty acid peroxidation, can react with thiobarbituric acid (TBA) to form a colored complex called thiobarbituric acid-reactive substance (TBARS). TBA reactivity was assayed by the method of Erdincler et al. [17]. Results were expressed as nmol/l.

2.4.6. Estimation of nitric oxide (NO) level

Nitric oxide (NO) release can be determined spectrophotometrically by measuring the accumulation of its stable degradation products, nitrite and nitrate. Serum nitrite levels was determined according to Hortelano et al. [18]. Results were expressed as nM.

2.5. Enzyme assays

2.5.1. Superoxide dismutase (SOD) assay

The assay of total SOD activity was performed in serum by quantifying the inhibition of NBT transformation to formazan, using the method of Kakker et al. [19]. The results were expressed as percentage of inhibition per gram serum protein.

2.5.2. Catalase (CAT) assay

The activity of serum CAT was determined according to the method described by Bock et al. [20].The results were expressed as U/g serum protein.

2.5.3. Ceruloplasmin (Cp) assay

Serum Cp concentration was estimated by measuring spectrophotometrically the purple colour produced from the oxidation of *p*-phenylenediamine according to the method of Henry et al. [21]. Results are expressed as mg/g protein.

2.5.4. α-Amylase assay

The activity of α-amylase was analyzed according to the procedure described by Henry and Chiamori [22]. The concentration of reducing products yielding from the action of α-amylase on starch expressed as glucose is taken as the units of amylase activity (mg/dl of sample). Results are expressed as mg/g protein.

2.5.5. Determination of total serum proteins

Serum proteins were estimated according to the method mentioned by Wootton [23].

2.6. Statistics

All data were expressed as mean ± S.D. Statistical comparisons were performed by analysis of variance (ANOVA) test. The comparison between two items was performed by Student's *t*-test.

3. Results

The mean levels of Zn^{2+}, Ca^{2+} and Mg^{2+} were highly significantly lower ($a = p < 0.001$) in the sera of both types of DM when compared to the control group. On the other hand, the mean levels of Cu^{2+} were significantly ($b = p < 0.01$) and highly significantly higher ($a = p < 0.001$) in the sera of IDMM and NIDDM groups, respectively, in comparison to the control subjects (Table 2). The mean level of Cu^{2+} was highly significantly increased ($d = p < 0.001$), but the mean levels of other metals were highly significantly decreased in the sera of NIDDM patients in comparison to that of IDDM patients (Table 2).

As illustrated from Table 3, the mean fluorescence intensity of AGEs was significantly ($b = p < 0.01$) and highly significantly ($a = p < 0.001$) elevated in patients with IDDM and NIDDM groups, respectively when compared with the control group. The average values of AGEs were significantly ($e = p < 0.01$) elevated in NIDDM patients in comparison to IDDM patients.

Table 2
Levels of trace elements (μg/dl), copper (Cu^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}) and zinc (Zn^{2+}) in plasma of diabetic patients

Groups	Cu^{2+}	Mg^{2+}	Ca^{2+}	Zn^{2+}
Control Mean ± S.D. (*n*)	101.53 ± 3.34 (20)	22.13 ± 1.65 (20)	167.11 ± 8.33 (20)	87.91 ± 4.78 (20)
IDDM Mean ± S.D. (*n*) *P*	105.46 ± 6.59 (40) (b)	20.38 ± 0.52 (40) (a)	132.65 ± 5.67 (40) (a)	79.53 ± 2.36 (40) (a)
NIDDM Mean ± S.D. (*n*) *P*	114.12 ± 4.87 (55) (a and d)	17.76 ± 0.66 (55) (a and d)	114.72 ± 4.32 (55) (a and d)	49.93 ± 3.84 (55) (a and d)

a = $p < 0.001$ = highly significant vs. control subjects.
b = $p < 0.01$ = significant vs. control subjects.
d = $p < 0.001$ = highly significant vs. IDDM subjects.

The average values of AOPP were highly significantly (a = $p < 0.001$) elevated in the patients with two types of diabetes mellitus than that of the control subjects. The average levels of AOPP were highly significantly (d = $p < 0.001$) increased in type II (NIDDM) in comparison to that of type I (IDDM) (Table 3).

As shown in Table 4, the mean activity values of SOD, CAT and Cp were highly significantly (a = $p < 0.001$) decreased while a highly significant (a = $p < 0.001$) increase in serum α-amylase activity was observed in both types of diabetes mellitus in comparison to the corresponding activities of the control group (Table 4). In addition, the mean activities of SOD, CAT and Cp were highly significantly (d = $p < 0.001$) decreased whereas the mean activity value of α-amylase was highly significantly (d = $p < 0.001$) increased in the sera of NIDDM patients when compared to the corresponding activities of IDDM patients (Table 4).

Table 5 summarizes the concentrations of lipid profiles, GSH, NO and MDA in diabetic patients. The average levels of total cholesterol (CHOL), low-density lipoprotein-cholesterol (LDLc) and triglyceride (TG) were higher, while the average concentration of high-density lipoprotein-cholesterol (HDLc) was highly significantly (a = $p < 0.001$) diminished in both types of DM in comparison to the control subjects. The average concentrations of NO and MDA were highly significantly (a = $p < 0.001$) increased whereas the average level of GSH was highly significantly (a = $p < 0.001$) decreased in both types of DM when compared with the control group (Table 5). On the other hand, the average concentrations of both CHOL and LDLc were highly significantly (d = $p < 0.001$) increased and the average concentration of TG was significantly (e = $p < 0.01$) increased, while the average concentration of HDLc was highly significantly (d = $p < 0.001$) diminished in NIDDM group when compared to the corresponding mean value of the IDDM subjects (Table 5). The average levels of NO and MDA were highly significantly (d = $p < 0.001$) increased, whereas the average level of GSH was highly significantly (d = $p < 0.001$) decreased in the sera of NIDDM subjects in comparison to the mean level of the IDDM group (Table 5).

4. Discussion

Dysfunctional neuroendocrine–endocrine interactions contribute to the disturbances in trace element metabolism and cause severe complications in diabetes

Table 3
Advanced glycation end-products (AGEs; expressed in fluorescence intensity) and advanced oxidation protein products (AOPP) in patients with diabetes mellitus and in healthy controls

Groups	AGEs (fluorescence intensity)	AOPP (μmol/l)
Control Mean ± S.D. (*n*)	156.16 ± 31.28 (20)	65.71 ± 18.15 (20)
IDDM Mean ± S.D. (*n*) *P*	188.15 ± 54.95 (40) (b)	116.47 ± 35.42 (40) (a)
NIDDM Mean ± S.D. (*n*) *P*	223.75 ± 76.79 (55) (a and e)	181.09 ± 41.23 (55) (a and d)

a = $p < 0.001$ = highly significant vs. control subjects.
b = $p < 0.01$ = significant vs. control subjects.
d = $p < 0.001$ = highly significant vs. IDDM subjects.
e = $p < 0.01$ = significant vs. IDDM subjects.

Table 4
Plasma activities of superoxide dismutase (SOD), catalase (CAT), ceruloplasmin (Cp) and α-amylase in diabetic patients

Groups	SOD (% inhibition)/g protein	CAT ($K \times 10^{-5}$/g protein)	Cp, mg/g protein	α-Amylase, U/g protein
Control Mean ± S.D. (*n*)	10.5 ± 1.75 (20)	289.0 ± 65 (20)	32.26 ± 3.5 (20)	3.14 ± 0.63 (20)
IDDM Mean ± S.D. (*n*) *P*	6.0 ± 0.86 (40) (a)	28.1 ± 4.5 (40) (a)	21.76 ± 4.22 (40) (a)	14.32 ± 2.59 (40) (a)
NIDDM Mean ± S.D. (*n*) *P*	3.8 ± 0.37 (55) (a and d)	2.78 ± 0.61 (55) (a and d)	18.75 ± 2.87 (55) (a and d)	24.49 ± 3.14 (55) (a and d)

a = $p < 0.001$ = highly significant vs. control subjects.
d = $p < 0.001$ = highly significant vs. IDDM subjects.

mellitus [24]. The present results showed that the levels of zinc, calcium and magnesium decreased in the blood of both types of DM (Table 2). The loss of these minerals might be attributed to impaired absorption and/or the excess excretion of these metals in urine (glycosuria) in these patients, which may induce a deficiency or marginal state of these minerals in blood of diabetic patients [2,4]. The increase in the Cu^{2+} ion levels in patients with DM might be attributed to hyperglycemia that may stimulate glycation and release of copper ions from copper-containing enzymes. This argument has been supported by Lin [25] who found the elevation of the concentrations of both lenticular copper ions and the protein-unconjugated copper ions than that of protein-conjugated copper ions, resulting in the decrease of the reactivity of copper-containing enzymes such as SOD and Cp in the lens of diabetic patients which is the actual case (Table 4).

Our results show a significant elevation of AGEs in patients with two types of DM which may be due to the following reasons: (1) The increase of oxidative stress in diabetic patients (as shown in Table 4) may accelerate the formation of AGEs [6]. (2) The elevation of copper ions in the sera of diabetic patients may accelerate the oxidative stress, which, in turn, may enhance the formation of AGEs which is the actual case (Tables 2 and 3). This is in harmony with the finding that glucose autoxidation is a transition metal-catalyzed process that generates oxygen free radicals ($O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$) and ketoaldehydes [26]. This argument is strengthened by the finding that the incubation of collagen with high concentration of glucose in the presence of copper increased the rate of accumulation of AGEs but incubation of collagen with copper ions alone did not show any increase in AGEs [27]. The elevation of advanced oxidation protein products (AOPP) in diabetic patients of the present work may be due to the generation of reactive oxygen species and oxidative modification of LDL thus leading to diabetic complications [6]. This argument has been supported by the finding that two types of modified LDLc (glycated and oxidized) were prominent in diabetics [28].

The increased levels of TBARS in diabetic patients may be due to the following reasons: (1) oxidative stress in diabetic patients. (2) Compositional changes in LDL may lead to conformational changes, possibly resulting in a different exposure of fatty acids to oxygen free radicals that enhance a faster rate of lipid peroxidation [29]. This argument is supported by the finding that oxidized LDL is thought to promote atherogenesis by increased levels of thiobarbituric acid-reactive substances (TBARS) [30]. (3) The increased copper levels, a transition metal that is a redox-active and catalyzes lipid peroxidation, may enhance oxidation of LDL causing increased level of TBARS in diabetic patients as reported by Heinecke et al. [31]. (4) Hypertriglyceridemia [32], hypercholesterolemia [33], and both AGEs and AOPP [6] were associated with oxidative modification of LDL [31], thus leading to excess production of lipid peroxidation products and vascular complications.

The present data revealed that hyperglycemia produced marked oxidant impact as evidenced by the significant increase in lipid peroxidation products as well as a significant decrease in antioxidants including SOD, CAT, Cp and GSH content. The decrease of antioxidants may be due to the deficiency of blood zinc and magnesium in diabetic patients. These observations are supported by the findings that zinc, copper and magnesium have antioxidant activities because not only do they constitute the active sites and/or stabilize the conformation of several antioxidant enzymes, but they also compete for iron- and copper-binding sites and can provide protection against transition metal-mediated and free radical-induced

Table 5
The concentrations of cholesterol (CHOL), triglycerides (TG), high-density lipoproteins (HDL) low-density lipoproteins (LDL), nitric oxide (NO), reduced glutathione (GSH) and malondialdehyde (MDA) in plasma of diabetic patients and healthy group

Groups	CHOL (mmol/l)	TG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	NO (μmol/l)	GSH (mg/g protein)	MDA (nmol/l)
Control Mean ± SD. (*n*)	3.15 ± 0.20 (20)	1.03 ± 0.11 (20)	1.68 ± 0.14 (20)	1.14 ± 0.01 (20)	1.75 ± 0.32 (20)	4.19 ± 0.58 (20)	1.71 ± 0.22 (20)
IDDM Mean ± SD. (*n*) *P*	3.92 ± 0.45 (40) (a)	1.44 ± 0.19 (40) (a)	1.05 ± 0.13 (40) (a)	2.29 ± 0.234 (40) (a)	3.42 ± 0.49 (40) (a)	3.04 ± 0.38 (40) (a)	7.12 ± 0.45 (40) (a)
NIDDM Mean ± SD. (*n*) *P*	4.45 ± 0.36 (55) (a and d)	1.55 ± 0.18 (55) (a and e)	0.84 ± 0.14 (55) (a and d)	2.90 ± 0.138 (55) (b and d)	5.19 ± 0.77 (55) (a and d)	2.35 ± 0.39 (55) (a and d)	12.69 ± 1.26 (55) (a and d)

a = $p < 0.001$ = highly significant vs. control subjects.
d = $p < 0.001$ = highly significant vs. IDDM subjects.
e = $p < 0.01$ = significant vs. IDDM subjects.

injury [34]. The decrease of SOD activity might be attributed to the following reasons: (1) Hyperglycemia activates various biochemical pathways such as glucose autoxidation, nonenzymatic glycation of proteins (glycoxidation process of AGEs) and activation of protein kinase C, which, in turn, overproduce oxidants like superoxide and hydroxyl radicals as well as hydrogen peroxide [35]. (2) The increase of glycosylated SOD that leads to the inactivation of this enzyme [36]. (3) Loss of its two factors, Zn^{2+} and Cu^{2+}. This is in harmony with the finding that in diabetic patients, as in healthy people, there is a close correlation between decreased SOD activity and loss of its two factors, Zn^{2+} and Cu^{2+} [25,37]. The decrease of CAT as an inducible enzyme may be due to the decreased level of H_2O_2 generated by SOD [38]. Glutathione (GSH) participates in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates. Thus, the decrease in GSH level might reflect a direct reaction between GSH and free radicals generated by hyperglycemia in DM. This is consistent with GSH function to scavenge oxidants by binding them covalently [39].

The decreased activity of ceruloplasmin (Cp) may be attributed to that diabetes mellitus is associated with aceruloplasminemia. The latter disease is characterized by mutation in the ceruloplasmin gene, resulting in elevation of copper ions in the blood. [40]. This observation was supported by the finding that a novel splicing mutation in the Cp gene, which would result in the skipping of exon 3 during transcription, was observed in diabetes [41]. In addition, the inhibition of Cp activity may be attributed to the fact that hyperglycemia may induce the release of copper ions from copper-containing enzyme such as SOD and Cp [25].

The highly significant elevation of serum amylase in the included patients might be attributed to the following causes: (1) hyperglycemia, where amylase activity increases according to the degree of hyperglycemia [14]. (2) The reactive oxygen species (ROS) generated in DM. These ROS resulted in activation of nuclear factor-kappa B (NF-κB) and induction of inflammatory cytokine gene expression in pancreatic acinar cells leading to amylase release from these cells [42]. This observation is supported by the finding that suppression of cerulin-induced ROS generation, NF-κB activation and cytokine gene expression by antioxidants might alleviate the inflammatory response in pancreatic acinar cells [43]. (3) The interaction of AGEs with their specific receptors (e.g. RAGE-receptor for advanced glycation end products), i.e. AGE–RAGE, generates ROS, activates NF-κB and stimulates cytokine gene expression resulting in the induction of pancreatitis which, in turn, stimulate the exocrine pancreatic secretion of α-amylase [44]. Thus, AGEs, which are elevated in the present study (Table 2), may stimulate the increase and secretion of pancreatic α-amylase. (4) The elevation of copper levels may result in the increase of serum amylase. This argument may be supported by the finding that the copper deficiency in male rats may result in pancreatic atrophy leading to diminish of pancreatic amylase activity [45]. (5) Magnesium deficiency may develop pancreatitis leading to excess excretion of pancreatic amylase as illustrated earlier by Orchard [46] who reported that magnesium deficiency is accompanied by pancreatitis. (6) The excess production of nitric oxide (NO) may cause hyperamylasemeia. This argument is strengthened by the finding of Um et al. [47] who reported that an enhanced formation of NO plays an important role in the development of acute pancreatitis leading to excess secretion of amylase.

The increase of NO level in diabetic patients of the present study might be attributed to the activation of the inducible isoforms of nitric oxide synthase in phagocytosing cells (neutrophils, eosinophils, basophils and mononuclear phagocytes) [48]. This observation is strengthened by the finding that overproduction of superoxide ($O_2^{\bullet-}$) in diabetes mellitus stimulates the activation of endothelial and inducible isoforms of nitric oxide synthase in peripheral blood granulocytes leading to increase generation of NO in diabetic patients which is the actual case (Table 5) [49]. In addition, the high glucose level may enhance cytokine-induced NO production. This argument may be supported by the finding that the pretreatment of HaCaT cells with high glucose enhanced NO production in which cytokines stimulate isoforms of nitric oxide synthase mRNA in these cells [50]. Furthermore, the AGE–RAGE interaction activates nuclear factor NF-κB, then stimulates the transcription of genes for cytokines which, in turn, activates nitric oxide synthase [44].

As shown in Table 5, the levels of total CHOL, TG and LDLc were lower in IDDM than that of NIDDM. These results may be attributed to the insulin treatment. These findings are in agreement with Morel and Chisolm [51], who stated that insulin promotes the process of lipogenesis, secondary to a satisfactory supply of energy from carbohydrate origin.

The present results observe that AGEs, AOPP, TBARS, NO α-amylase and copper are more pronounced whereas SOD, CAT and Cp activities as well as GSH, Zn^{2+}, Mg^{2+} and Ca^{2+} levels are lower in NIDDM patients than in IDDM patients. These results may indicate that oxidative stress is more in NIDDM than in IDDM.

Slightly higher levels of some of the above parameters have been observed in the patients with vascular complications when compared with the diabetic subjects without complications, but this difference was not statistically significant (data not shown).

It can be concluded that hyperglycemia in diabetes mellitus is associated with accelerated nonenzymatic glycation and oxidative stress. The impaired trace element metabolism of the present work may have a role in the pathogenesis and progression of DM where the increase of copper level and decrease of zinc, calcium and magnesium levels may disturb the antioxidants, induce the secretion of pancreatic amylase (which is considered as another diabetic complication in DM) and enhance the lipid peroxidation. Oxidative stress may play an important role in AGE formation in DM. The increase of copper levels, AGE formation, NO production and oxidative stress, but decrease of other metals, may induce the secretion of pancreatic amylase. All these parameters may contribute in the development of vascular complications in diabetic patients. Oxidative stress is increased in patients with NIDDM than of IDDM. Oxidative modification of lipoproteins, particularly LDL, may be at least one cause of vascular complications of DM.

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