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Oxidative Stress Status in Type 2 Diabetic Patients in Eastern Algeria

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Abstract: The present study was carried out to evaluate the oxidative status in Urban Algerian patients suffering from type 2 diabetes mellitus (T2DM) and treated with hypoglycaemic agents. Investigations had been conducted on 29 adult T2DM patients as compared with 11 healthy adult subjects (as control). Plasma glucose level and haemoglobin A1C (HbA1C) were determined, as well as total antioxidant capacity (TAC), catalase (CAT), glutathione reduced (GSH) and lipid peroxides (MDA). Kidney function tests were also assessed depending upon blood urea and creatinine values. The result revealed that fasting plasma glucose level was higher ($P<0.01$) and HbA_1C was markedly increased ($P<0.05$) in T2DM group as compared to healthy subjects.. Patients revealed higher concentration of MDA ($P<0.01$) and low activity of CAT ($P<0.05$) as compared to the control group. GSH and TAC concentrations decreased ($P<0.001$) in the tested group than the healthy control group. A negative correlation was found between fasting plasma glucose levels, HbA_1C on one hand and TAC, CAT, GSH, MDA values in T2DM patients on the other hand. no alteration in kidney functions was found as indicated by urea and creatinine values. It was concluded that T2DM patients are undergo an important oxidative stress, even under hypoglycæmic control, they were considered to be poorly controlled.

Key words: Type 2 diabetes mellitus · Oxidative stress · Total antioxidant capacity · Catalase · GSH · MDA · Algeria

INTRODUCTION

Investigations on diabetes mellitus had showed the involvement of reactive oxygen species (ROS) including free radicals in the genesis of chronic complications related to the disease, as cardiovascular affections, renal failure and neurodegenerative changes [1-3]. Oxidative stress is more obvious in type 2 diabetes and this appears to underlie the development of diabetic complications [4]. Hyperglycemia leads to metabolic disorders, characterized by alterations in the metabolism of carbohydrate, protein and lipid. Diabetes induced disturbance in lipid profiles, especially an increased susceptibility to lipid peroxidation [5], which is responsible for increased incidence of atherosclerosis [6]; a major complication of diabetes mellitus. Macrovascular complications, which manifest in about 80 percent of patients with type 2 diabetes, are a leading cause of morbidity and mortality worldwide [7].

In non-insulin-dependent (type 2 diabetes mellitus), oral hypoglycaemic agents are used to stimulate the pancreatic beta cells to secrete insulin and/or increase the sensitivity of peripheral insulin receptors to the action of endogenous insulin [8] with the hope of achieving better glycaemic control and attenuating related complications.

Among hypoglycaemic agents; Rosiglitazone used currently by type 2 diabetic patients due to its efficacy for improving the sensitivity of tissues to insulin and reduces insulin resistance [9,10], which in turn improves markers; HbA1c is the most representative marker of hyperglycemia for assessing glycemic control and glycated albumin) of cardiovascular complication [11,12,13]. Metformin, is another hypoglycaemic drug used in clinical treatment of type-2 diabetes for over 35 to 40 years, it enhances the sensitivity of both hepatic and peripheral tissues to insulin, it inhibits gluconeogensis in the liver and lowering plasma triglyceride and low-density

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lipoprotein (LDL) cholesterol and total lipid levels. This drug is most often associated with sulphonylurea to exert an evident lowering effect, on blood glucose level [14,15].

Previous studies have shown that diabetes mellitus can worsen antioxidant status, hense deficiencies in some vitamins and trace elements related to the extrinsic antioxidants, such as vitamin C [16, 17] vitamin E [18] and zinc [19] can aggravate the several complications of diabetes.

This study was conducted to evaluate the oxidative status in a group of Urban Algerian with type 2 diabetes mellitus (T2DM) treated with hypoglycaemic agents (non-insulin-dependent diabetes mellitus) compared to a normoglycemic group.

MATERIALS AND METHODS

Subjects: Twenty-nine patients suffering from T2DM including 20 males and 9 females with a mean age of 57.57±2.39 (range 35-82) years were randomly selected from the Center of Diabetology Al-Kantara, Constantine, Algeria. Patients were informed of the purposes of the study. Eleven age and gender-matched healthy subjects including 5 males and 6 females with a mean age of 49.80±4.49 (range 27-73) years, who came to the center for check up, with no known family history of T2DM, were also enrolled to the study as the control group.

Key exclusion criteria included smokers, pregnant women, persons receiving trace element or antioxidants supplements in the previous three months, persons with gastric or diuretic treatment, patients with acute renal failure (creatinine>1.20 mg/dl) and patients with a recent surgery or acute infection. Only patients with fasting blood glucose ≥150 mg/dl and HbA1c ≥7.5% were included.

Blood Samples and Biochemical Assays: Blood samples were drawn from each T2DM patient and control healthy subject after an overnight fast at the Center of Diabetology Al-Kantara, Constantine. Three blood samples were collected from each subject. The first sample was collected in a tube containing sodium fluoride for plasma glucose determination. The second sample was collected in a tube containing heparin for estimation of plasma antioxidants, creatinine and urea. The third sample was anticoagulated by ethylenediamine tetraacetic acid (EDTA) and was used for determination of HbA1c. Fasting plasma glucose was estimated enzymatically using glucose oxidase [20], creatinine value using Semi-micromethod [21] and urea using urease enzyme [22] were determined using an Auto-analyser ; ADVIA 1650 *Chemistry System Bayer-Diagnostic* Laboratory of biochemistry CHU IBN BADIS Constantine. Glycated haemoglobin (HbA1c) was measured using *BIO-RAD* D-10™ *UNITED STATES, Bio-Rad* Laboratories, Inc Hercules CA 94547. The D-10 Hemoglobine A_1C program utilises principles of ion-exchange high performence liquid chromatography (HPLC).

Antioxidant Markers Assessment: Plasma lipid peroxidation product (MDA) and antioxidants status were measured using specific kits purchased from *Bio-diagnostic*, Dokki, Egypt.

Plasma MDA was determined according to the method of [23]. This technique is based on thiobarbituric acid reacts with MDA in acidic media at temperature of 95°C for 30min. to form thiobarbituric reactive product the absorbance of the resultant pink product can be measured at 534 nm.

Plasma glutathione reduced (GSH) was estimated according to the method of [24]. The method depends on the reduction of 5,5′dithiobis (2-nitrobenzoïc acid) (DTNB) with glutathione to produce a yellow compound. The reduced chromogen directly proportional to (GSH) concentration and its absorbance can be measured at 405nm.

Plasma total antioxidant capacity (TAC) was determined according to the method of [25]. This determination is performed by the reaction of antioxidants in the plasma with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided H_2O_2. The residual H_2O_2 is determined by an enzymatic reaction which involves the conversion of 3,5, dichloro-2-hydroxybenzensulphonate to a coloured product and read at 505nm.

Plasma catalase (CAT) was estimated by the method of [26]. The method depends on catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. The presence of peroxidase, remaining H_2O_2 reacts with 3,5 Dichloro-2 hydroxybenzene sulfonic acid and 4-Aminophenazone to form a chromophore with a colour intensity inversely proportional to the amount of CAT in the sample and read at 510nm.

Statistical Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error (mean±SE). Student *t*-test was used for the evaluation of data. Differences were considered

significant at $P<0.05$ level [27], using SPSS version 10 computer programme.

RESULTS

The individual characteristics; age, gender and duration of the disease (in diabetic patients) and healthy subjects are shown in Table 1.

Fasting blood glucose level of T2DM patients was higher *(P<0.01)* than that in healthy adult subjects. Also, the HbA1c exhibited clear increase *(P<0.05)*. There was no significant difference in the level of plasma creatinine and urea in T2DM patients and healthy adult subjects during the period study (Table 2, Fig. 1).

The plasma lipid peroxidation end product, malondialdehyde (MDA) increased in T2DM patients

Table 1: Age, gender and duration of diabetes in healthy subjects and type 2 diabetes mellitus patients. (Mean±SE)

	Groups	
Parameters	Healthy subjects (Control, n=11)	Type 2 Diabetes (n=29)
Age (years)	49.80±4.49 (27.00-73.00)	57.57±2.30 (35.00-82.00)
Gender (Male/Female)	5/6	20/9
Duration of diabetes (years)	Non	1-20

SE = Standard error.

Table 2: Values of some plasma biochemical parameters in healthy subjects and type 2 diabetes mellitus patients (Mean±SE)

	Groups	
Parameters	Healthy subjects (Control, n=11)	Type 2 Diabetes mellitus (n=29)
Glucose (mg/dl)	95.27±7.18 (73.00-151.00)	154.52±11.13** (76.00-277.00)
HbA_1c (g %)	6.27±0.23 (4.92-7.60)	7.96±0.39* (5.00-12.60)
Urea (mg/dl)	36.70±2.54 (26.00-54.00)	39.79±2.19 (21.00-71.00)
Creatinine (mg/dl)	1.14±0.06 (0.92-1.48)	1.12±0.03 (0.90-1.61)

SE = Standard error. HbA1c = glycated hemoglobin. * = $P < 0.05$. ** = $P < 0.01$.

Table 3: Values of some serum oxidative stress markers in healthy subjects and type 2 Diabetes mellitus patients (Mean±SE)

	Groups	
Parameters	Healthy subjects (Control, n=11)	Type 2 Diabetes mellitus (n=29)
Lipid peroxides (nMol/ml)	42.87±1.37 (29.47-45.26)	51.21±1.61 ** (34.74-71.58)
Glutathione reduced (mMol/l)	7.99±0.93 (4.44-11.10)	4.45±0.25*** (2.22-6.66)
Total antioxidant capacity (mMol/l)	3.81±0.15 (2.71-4.59)	2.91±0.09*** (1.95-3.95)
Catalase activity(Unit/l)	317.81±18.34 (262.73-490.62)	283.80±5.78* (231.90-356.56)

SE = Standard error. * = $P < 0.05$. ** = $P < 0.01$. *** = $P < 0.001$

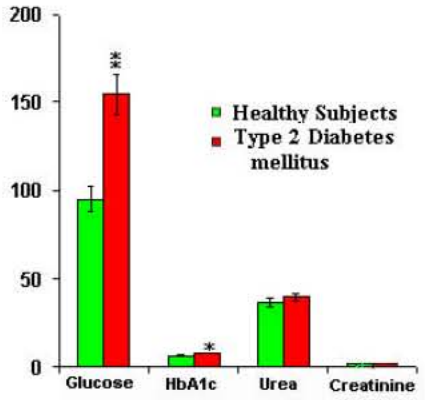

Fig. 1: Plasma glucose (mg/dl), glycated haemoglobin (HbA1c), plasma urea (mg/dl) and creatinine (mg/dl) in healthy subjects (control) (N=11) and type 2 Diabetes mellitus patients (* = *P<0.05*. ** = *P < 0.01*)

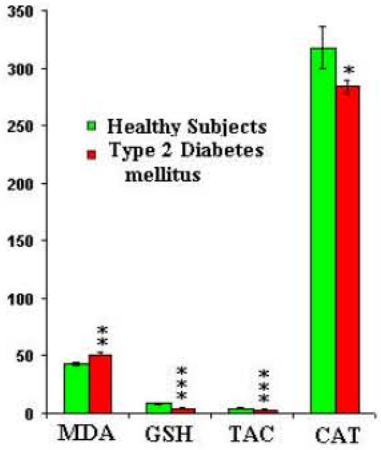

Fig. 2: Plasma lipid peroxides; MDA (nM/ml), glutathione reduced; GSH (mM/l), catalase activity; CAT (U/l) and total antioxidant capacity; TAC (mM/l) in healthy subjects (control) (N=11) and type 2 Diabetes mellitus patients (* = *P<0.05*. ** = *P < 0.01*. *** = *P < 0.001*)

(P<0.01) as compared with the control group, while, the activity of plasma CAT was decreased in T2DM patients *(P<0.05)*. GSH *(P<0.001)* and the total antioxidant capacity (TAC) ($P<0.05$) decreased in T2DM group as compared to the control group (Table 3, Fig. 2).

DISCUSSION

Fasting plasma glucose level was a high *(P<0.01)* in T2DM group, even they took hypoglycaemic agents as

compared with healthy subjects. Although this condition is consequently accompanied with a marked elevation of HbA_1C ($P<0.05$), indicating that glycaemic in our diabetic population was moderately well-balanced, which explains the low rate of non-enzymatic glycation of haemoglobin A_1C observed essentially in diabetes.

Oxidative stress status of patients with T2DM was evaluated by measuring plasma lipid peroxidation end product MDA, an impotant index marker of the extend lipid peroxidation and evaluation of oxidative stress, as well as glutathione reduced (GSH) concentration, total anti-oxidant capacity (TAC) and the catalse enzyme activity. In this study, oxidative stress status was disturbed in diabetic patients with T2DM as compared to healthy subjects.

Plasma MDA concentration was higher in T2DM patients as compared with healthy control subjects, indicating higher lipid peroxidation [28]. The elevation of lipid peroxidation is related to the duration of diabetes [29,30]. This was consistent with the previous studies [31,32] especially in patients with vascular complications [33,34]. Thus, moderate, as in the current case, or poor diabetes control may enhance lipid peroxidation and diminishes the body's anti-oxidant capacity, hence a negative correlation between hyperglycaemic and oxidative stress was observed and agree with [35]. This increase of lipid peroxidation is frequently observed in diabetes due to mobilization of lipids for a further use as an energy source rather than glucose.

Plasma GSH, decreased in T2DM patients as compared to healthy control subjects which indicates the extend degree of oxidative stress in diabetes [36,37], it accompanies the decrease in the activity of catalase, which plays a co-enzyme role for scavening H_2O_2. On the other hand, the decrease of plasma glutathione reduce may be due also, to a decrease in ascorbic acid concentration which plays a synergic role with GSH in the regeneration of vitamin E, during the elimination of free radicals [38]. The depletion of GSH is due in great part to a deficiency of $NADPH_2$ used in some oxido-reduction reactions like polyol pathway over and above its reduction formation through pentose phosphate and malic acid pathways owing to chronic hyperglycaemic as showed by [39] and [40], leading to impairement of GSH regeneration and depletion of an important free radical scavenger [41], this condition disturbs the antioxidant defenses and accelerate the oxidative damage [42] and hence reconfirming the negative effect of hyperglycaemic on GSH levels.

The TAC, represents the extrinsic (micronutriments) trace elements, vitamins (A, E, β-caroten and ascorbic acid) and intrinsic factors including group of organic anti-oxidants such as enzyme catalase glutathion peroxidase, superoxide dismutase and non-enzymatic anti-oxidants (GSH) and others like flavonoids, bilirubin and uric acid [43]. In this study, a very drastic decrease in plasma TAC in T2DM patients vs the control group was observed and agree with [37, 44], but less than that reported by [32]. In this respect, [45] reported that this diminution is due to poor control diabetes, which concords trully with the disturbance in HbA_1C [46].

Treatment of T2DM patients with hypoglycaemic agents is often followed by a strict regimen, which in turn must be well adapted to each diabetic patient, to avoid depletion in extrinsic antioxidants, that were not be influenced by auto-glycation as it's the case with antioxidant proteins .

Catalase (CAT) is a hemeprotein catalysing the reduction of hydrogen peroxides and protects against highly reactive hydroxyl radicals; decreased CAT activity during diabetes disease could result from inactivation by glycation of enzyme [47]. The present decrease in CAT activity in T2DM group was 10% ($P<0.05$), however, [46] observed a diminution by 30 % in type 2 diabetics. Negative correlations between serum glucose level and HbA_1C and CAT were noticed in this study. The diminution in the catalase activity is also observed in diabetics with poor glucose control and vascular complications. Previous studies have shown that plant hypoglycaemic extracts like *Syzigium cumini* seeds increases catalase activity by diminishing blood glucose level [48] and vitamin C, as a potential antioxidant, also increases catalase activity [49], this may be due to the alleviate action of hypoglycaemic agents on enzyme glycation.

In conclusion, results from this study suggest that hypoglycaemic treatment has no favorable effect on antioxidant system in T2DM patients compared with healthy subjects. This condition suggests that under hypoglycaemic treatment a supplementation with micronutriments is necessary to improve the intrinsic antioxidant system.

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