Evaluation of antioxidants capacity of non-enzymatic antioxidants and its effect in glucose level in diabetic patients

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Abstract

Background: Oxidative stress might participate in the pathophysiology of diabetes type 2**.** Systems of non-enzymatic antioxidants are made up of scavenging molecules that are formed endogenously**.**

The Study Objective: This study's aims were evaluation of the non-enzymatic antioxidants and their effect in levels of glucose among diabetic patients.

Methodology: A total of eighty patients with DM type 2 and forty healthy persons (control) were enrolled within this study. Serum and plasma were obtained from collected blood samples. The Ferric Reducing Ability of Plasma (FRAP) method was used for detection of the total antioxidant capacity (TAC). Ellman's method was used to determine the level of reduced glutathione. The serum CAT activity was detected by the technique explained by Sinha.

Results: The findings revealed that, among diabetic groups, there was a significant elevation in glycosylated hemoglobin and glucose levels; HbA1c and FBG levels were significantly higher; MDA, NO, LPI, TAC and GSH showed significant greater values. The activity of SOD showed significantly greater mean value among the control group.

Conclusion: The results suggest that antioxidants defense might be decreased in T2DM, as TAC levels were decreased. The increased levels of MDA, NO, LPI, TAC and GSH were associated with oxidative stress. Assessment of GSH could help in recognition of the extent of oxidative stress in diabetes as well as prevention and control of diabetic complications.

Keywords: *Antioxidant, total antioxidant capacity, non-enzymatic antioxidants, diabetes*

Introduction

Hyperglycemia that results from insulin dysfunction leads to interruption in the glucose homeostasis, one of these disorders is type 2 diabetes mellitus (T2DM)**.** Diabetes is a chronic disorder that is, today, considered a common disease globally, and it is also widely spread among Arab countries [1]. Recently, there are evidences suggesting that oxidative stress might participate in the pathophysiology of DM type 2 via rising the insulin resistance or making impairments in the secretion of insulin hormone [2]. Regarding the fact that DM type 2 has an association with cardiovascular complications, oxidative stress was found to likely have a role in the diabetes pathogenesis as well as cardiovascular disease [3]. Systems of non-enzymatic antioxidants are made up of scavenging molecules that are formed endogenously like glutathione, vitamin E, vitamin C, selenium, carotenoids, etc. [4]. The imbalance amongst radical-generating and radical-scavenging systems is what causes oxidative stress which means more production of free radical or decrease in the antioxidant defense activity or both [5]. It was reported that diabetes mellitus disease is along with an increase in the free radicals' formation in addition to a decrease in the antioxidant capacity, resulting in an oxidative damage for cell elements [6]. On the other hand, antioxidants are the line of defense that protects the body cells from the free radicals' attack. The function of antioxidants is adding a large number of electrons into the blood vessels to be given to the monovalent free oxygen radicals that search for the lost electron,

provided by antioxidants, so they become divalent, and hence, they settle and make no damage to the body cells [5]. In diabetes, it was found that the levels of certain antioxidants like vitamin E as well as vitamin C were lowered [7]. Additionally, among diabetic patients, the actions of antioxidant enzymes catalase, superoxide dismutase, as well as glutathione peroxidase was found to be diminished [8]. Previously, numerous researches have been performed for studying the biomarkers of oxidative stress [9]. In the early 1990s, a new total antioxidant capacity assay has been developed by Miller et al. [10], and it was termed "total antioxidant capacity" (TAC).

Methodology

A total of 80 patients with DM type 2 and 40 healthy persons (control) were enrolled within the present study. Basic data such as age, gender, habits, lifestyle and previous medications were collected.

Samples collection:

Venous blood samples were collected from every fasted participant, within 2 various tubes, a plain tube contains sodium fluoride and another one EDTA coated. Samples that were collected in the plain tubes were centrifuged for obtaining the serum samples which were used for determination of antioxidant markers. Samples which were collected in EDTA coated tubes were utilized for determination of the glycated hemoglobin (HbA1c ≥ 6.2 0%) as well as levels of glucose in plasma.

Measurement of the antioxidant markers:

The Ferric Reducing Ability of Plasma (FRAP) method was used for determination of the total antioxidant capacity (TAC), where a ferric tripyridyltriazine complex of no color is reduced into a ferrous complex of blue color by the antioxidants present in serum. The findings were described in μmol per mg of protein. The Ellman's method [11] used to determine the level of reduced glutathione, via developing of a yellowish color which was read in the spectrophotometer at 412 nm. The results were expressed as μmol/mg protein. The serum CAT activity was detected by the technique explained by Sinha [12]. The findings were described as U/mg of protein.

Statistical Analysis

Statistical analysis was done using SPSS (Statistical Packages for Social Sciences- version18). Data were analyzed via using One Way Analysis of Variance (ANOVA) for calculation of the p-value for healthy and other patients' groups. Software Graph Pad Instat 3 was applied for testing the relation amongst the groups of the study. The results were presented as mean ± standard error. Statistical significant difference was considered at the level of ($p \leq 0.05$).

Results

The basic data of the study participants were shown in Table 1. Among the study groups, males appeared to be more than females, where the sex ratio was [1.09]. The patients had an age ranged from thirty to ninety years with mean of [54 ± 3] years. The age represented was mostly from forty to sixty among healthy people as well as the diabetic patients, resembling (75%) and (60%) respectively.

Table 1: Basic data of the sample population:

		Diabetic patients N= 80(%)	Control N= 40 (%)
Gender	Female	47.5	40
	Male	52.5	60
Age	30< age <40	10	5
	40< age <50	27.5	40
	50< age <60	32.5	35
	60< age <70	20	10
	>70	10	10
Family history		25	50
Hypertension		20	10
Smoking		5	0

Variation of clinical parameters:

Fasting blood glucose (FBG) and Glycated hemoglobin levels were detected for evaluation of the glycemic state among diabetic cases, they were shown in Table 2.

The study findings revealed a significant elevation in glycosylated hemoglobin as well as levels of glucose among the group of diabetics in comparison with healthy group. HbA1c and FBG levels of patients with diabetes were significantly higher than healthy people as HbA1c mean ± SD= (8.21 ± 0.45, and 4.57 ± 0.11, respectively), and FBG mean ± SD=(2.12 ± 0.14, and 1.14 ± 0.04, respectively). The body mass index (BMI) values had no significant change among patients and control groups.

Table 2: Clinical parameters variation between control and diabetic groups:

	Diabetic patients (mean ± SD) N = 80	Control (mean ± SD) N = 40	P value
BMI (kg m2)	27.21 ± 5.27	29.03 ± 5.32	P > 0.05
HbA1C (%)	8.21 ± 0.45	4.57 ± 0.11	P < 0.05
FBG (g/l)	2.12 ± 0.14	1.14 ± 0.04	P < 0.05

Variation of markers of oxidative stress:

The current study also reveals that there were modifications within both oxidant and antioxidant conditions of patients compared to controls.

The activity of superoxide dismutase (SOD) showed significantly greater mean value among the control group (0.42 ± 0.04) compared to the diabetic patients (0.37 ± 0.03) (P < 0.05). Malondialdehyde (MDA) is an aldehyde of low molecular weight which could be resulted from the attack of free radical on polyunsaturated fatty acids of biological membranes and its detection is useful in examining lipid peroxidation. The levels of serum MDA, nitrite oxide (NO) and lipid peroxidation index (LPI) showed greater values among diabetic patients (0.28 ± 0.02, 216.06 ± 8.95, and 0.182, respectively) than control group (0.29 ± 0.03, 191.40 ± 11.54, and 0.181, respectively) which indicate the oxidative damage of lipids mediated by free radicals.

The values of TAC showed a significant increase (P < 0.05) among diabetic patients (160.45 ± 7.64) than control group (210.47). In addition, the reduced glutathione (GSH) had significantly higher values (P < 0.05) in diabetic patients (9.79 ± 0.59) than control group (6.76 ± 0.58), Figure 1.

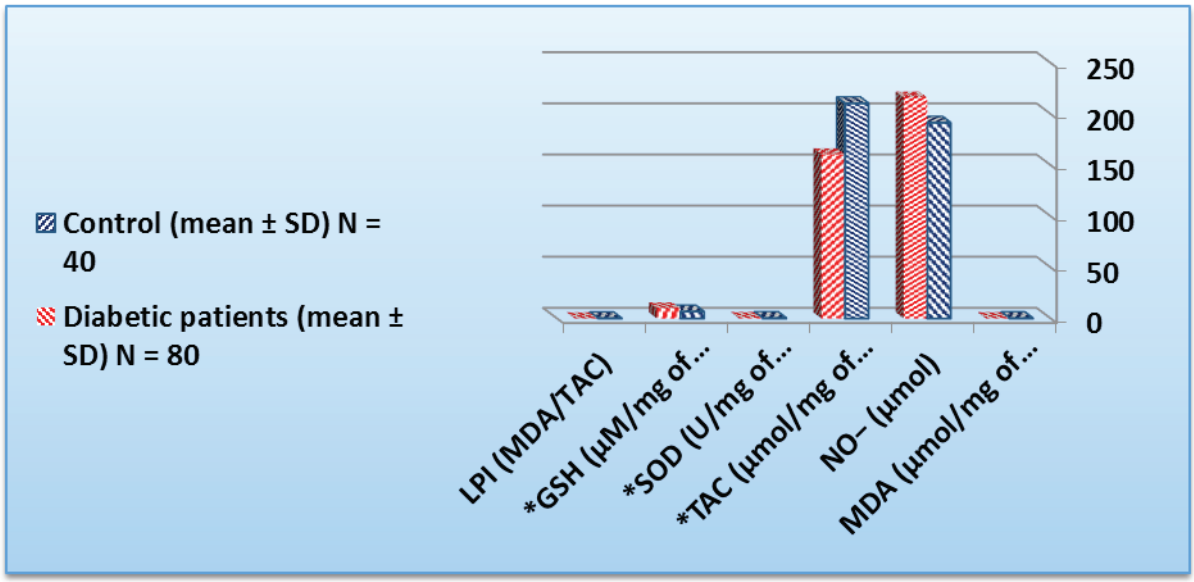

Figure 1: Levels of oxidant and antioxidant markers.

(MDA malondialdehyde, NO nitrite oxide, TAC total antioxidant capacity, SOD superoxide dismutase, GSH reduced glutathione, LPI lipid peroxidation index. * significant at $P < 0.05$)

Also, a significant negative relation between FBG and glutathione were detected in diabetic patients ($P < 0.05$), while HbA1 showed a significant positive correlation with NO among diabetic patients ($P < 0.05$) indicating that oxidative stress might influence HbA1c, Table 3.

Table 3: Correlation between clinical parameters and antioxidant in control and patients' group:

Diabetic patients (mean ± SD) N = 80			Control (mean ± SD) N = 40	
	r2	P value	r2	P value
HbA1 and NO	0.74	0.003	0.391	0.04
FBG and CAT	−0.152	0.340	0.2521	0.285
FBG and glutathione	−0.541	0.001	0.133	0.573
TAC and glutathione	0.123	0.440	0.562	0.010

Discussion

Oxidative stress might participate in the pathophysiology of diabetes type 2 via rising the insulin resistance or making impairments in the secretion of insulin hormone **[2]**. In T2DM, the oxidative stress had an association with massive alterations on the systems of antioxidant enzyme (SOD, CAT, GPx, GSH) as well as total antioxidant capacity (TAC) that result in peroxidative damage for proteins, lipids, carbohydrates, and the nucleic acids, as well that could be used as biomarkers for diabetes [13].

The current study assessed the oxidant status, HbA1c as well as FBG in a group of patients with T2DM. The age group which was represented mostly was between

[forty to sixty] for healthy persons as well as the diabetic patients.

HbA1c and FBG levels of diabetic patients were significantly higher than healthy people. These results were in agreement with Abudawood et al. [14] who has performed a study on the association between oxidative stress, DNA damage and cancer risk in T2DM in Riyadh, KSA, and higher levels were found of HbA1c and FBG in diabetic patients compared to controls. HbA1c and FBG are the indicators of type2 diabetes mellitus where the elevated glucose levels interfere with the metabolism of the free radicals and this is associated with increasing in the lipid and protein oxidation leading to more tissue damage [15,16].

The current study showed that diabetes mellitus disease, hypertension as well as smoking increases the oxidative stress which is represented by the significantly lower mean value of the activity of SOD among the diabetic patients, and significantly higher levels of MDA, and NO that were shown among diabetic patients, ($P < 0.05$). These findings were in agreement with the findings of Pieme et al. [17] study. MDA is the most essential marker of oxidative stress [18].

Raddam et al. [18] also revealed that the levels of MDA had significant high values and lower SOD levels in all diabetic patients. Also, Evereklioughu [19] found that patients with diabetes with macular degeneration showed a lesser SOD activity and greater of MDA and NO levels compared to healthy persons.

Additionally, this study revealed a significant reduction in the total antioxidant capacity (TAC) with a higher lipid peroxidation index (LPI) (but it was non-significant) in diabetic group.

A study by Sohrab et al. **[20]** on men with T2DM showed similar findings; where it was found that higher levels of markers of oxidative stress were observed with lower TAC. McCracken et al. [21] also found that the TAC levels were reduced among diabetic patients without glycemic control, and this was partially improved with suitable glycemic control. Similarly, Čolak et al. [22] revealed that the total antioxidant capacity were significantly lower among diabetic patients. It was mentioned that the decrease in the levels of the total antioxidant capacity is correlated to a higher occurrence of diabetes disease as well as its complications [23].

Reduced GSH, is a non-enzymatic antioxidant that has a significant role via making protection of the cells from the oxidative damage and, consequently, maintain the levels of the active state of vitamins E and C within cells throughout making neutralization to the free radicals [4]. There is a possibility that the elevation in the GSH levels among diabetic patients is correlated to increasing the glutathione peroxidase activity by neutralizing free radicals that were created in the disease complications. It was found that the reduced GSH, an extracellular non-enzymatic antioxidant, makes inhibitions to the oxidative activity via several ways [27]. The increased levels of NO in addition to the positive correlation between NO and HbA1 observed in diabetic patients could be described by the theory of the effect of hyperglycemia on the endothelium of blood vessels and the related vascular tone through influencing levels of NO [28]. This study revealed that the oxidative stress markers were increased in diabetic patients with decreased SOD. In an experimental study, the kidney and heart SOD levels as well as liver GPx were increased [29].

Conclusion

This study aimed to evaluate the non-enzymatic antioxidants and its effect on levels of glucose among diabetic patients. From the findings of this study, it is suggested that there is some of evidence that antioxidants defense might be decreased in T2DM, as serum TAC was decreased. In addition, the activities of SOD were found to be reduced in diabetic patients while MDA, NO, LPI, TAC and GSH showed higher values among diabetics. The increased NO levels identifies the effect of hyperglycemia on the vascular endothelium and increasing the risk of cardiovascular complications of T2DM due to decreaseing the vascular antioxidant defense. In addition, the increased levels of GSH in T2DM support that fact that oxidative stress might participate in the pathophysiology of diabetes type 2. Therefore, it is recommended that assessment of GSH could help in recognition of the extent of oxidative stress in diabetes as well as prevention and control of diabetic complications.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

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