Oxidative Stress and DNA Damage in Patients with Type 2 diabetes

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ABSTRACT

Background: The association between DNA damage and diabetes mellitus promote us to study the extent of this health problem in the community.

Objectives: To test the hypothesis that patients with diabetes have a greater extent of DNA damage than healthy individuals and to analyze its relationship with oxidative stress biomarkers.

Materials and Methods: The study was carried out between September 2016 and March 2018 at Duhok Diabetes Center, Azadi General Teaching Hospital, Duhok, Kurdistan Region (Iraq).In this study, we measured biomarkers of both DNA damage and oxidative stress including serum 8-hydroxy-2-' deoxiguanosine (8-OHdG), malondialdehyde (MDA) and total antioxidant capacity (TAC) in 297 patients with type 2 diabetes and 188 healthy individuals

Results: Significantly higher 8-OHdG and MDA levels (p<0.001 and p<0.010 respectively) together with lower TAC levels (p=0.010) were found in diabetics compared to healthy individuals. In diabetic patients, a positive correlation of 8-OHdG was observed with MDA (p<0.010), and a negative correlation was observed with TAC (p<0.001). Based on the estimated cutoff point of DNA damage (8-OHdG of 4.0 ng/ml), 84.6% of patients had high levels of DNA damage compared with healthy individuals (28.7%).

Conclusions: DNA damage was observed in approximately two-thirds of the population of this study, particularly diabetic patients appears to be associated with lower antioxidant capacity and a high degree of oxidative stress. Antioxidant supplementation may be an effective public health intervention to reduce DNA damage and oxidative stress.

Keywords: diabetes mellitus, oxidative stress, MDA, TAC, 8-OHdG

INTRODUCTION: Oxidative stress leads to protein, lipid, and DNA modifications that cause cellular dysfunction[1]. DNA damage most likely occurs when the endogenous antioxidant network and DNA repair systems are overwhelmed[2]. Oxidative stress plays a pivotal role in cellular injury

from hyperglycemia. High glucose levels can stimulate free radical production[3]. The weak defense system of the body becomes unable to counteract the enhanced reactive oxygen species (ROS) generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress[3]. Reactive oxygen species (ROS) can cause strand breaks in DNA and base modifications, including oxidation of guanine residues which is most potential base in DNA molecule that is oxidized than other bases because of imidazole ring in its structure and lead to 8-hydroxy-2'deoxyguanosine (8-OHdG) - an oxidized nucleoside of DNA. Oxidative damage to DNA has been demonstrated by measuring levels of 8-OHdG, a recognized biomarker of oxidant-induced DNA damage, in both mononuclear cells and sperm from diabetic subjects[4,5]. 8-OHdG is the most frequently detected and studied DNA lesion[6]. In recent years, several clinical studies have analyzed levels of 8-OHdG in human organs, leukocyte DNA and urine concerning oxidative stress and diabetes mellitus[7].

To our knowledge little data are available in our area investigating oxidative stress and DNA damage in patients with diabetes, for that our aim in this study was to determine the oxidative DNA damage, oxidative stress as well as antioxidant capacity in patients with type 2 diabetes mellitus and healthy individuals in Duhok, Kurdistan Region (Iraq).

Materials and Methods:

Study population

Four hundred-eighty five subjects, 297 patients with type 2 diabetes and 188 healthy individuals (ages 35-65 years) were enrolled in the study. Inclusion

criteria were patients: with fasting glucose (>125 mg/dl and HbA1c >7.0%). Exclusion criteria for diabetic patients include cardiovascular, respiratory, rheumatoid, renal and hepatic diseases, history of malignancy, recent infections, pregnancy, smoking, and alcoholics. Inclusion criteria for healthy individuals: no history of chronic diseases or history of diabetes mellitus among first-degree relatives. Individuals who had CRP>6 mg/dl, HbA1c>5.5% and glucose levels >100mg/dl were excluded from the study. All the participants provided with written informed consent. The study protocol was approved by the Ethics committee of the Medical College, University of Duhok. The sample size for this study was calculated with 80% power at a 5% level of statistical significance.

A pre-tested questionnaire was designed to obtain information on gender, age, weight, and height. BMI was measured for each subject. Participants were instructed to avoid any heavy physical activity for more than 2 hours before the examinations.

Biochemical Measurements: Morning blood samples after overnight fasting for 12-14 hours were collected between 9:00-11:30 am at the Lab-Department of Clinical Biochemistry at Azadi General Teaching Hospital. About 10ml of blood was withdrawn by venipuncture, using **VACUTAINER** from the antecubital vein and collected in BD Vacutainer System CAT- plain tubes. 2ml of blood was collected in an EDTA tube for measurement of HbA1c%. The sera were separated by centrifugation using a (HITACHI centrifuge, Model O5P-21) at 5000 rpm for 10 min at room temperature and collected into two tubes, one processed immediately for measuring serum FBG, MDA and TAC levels. MDA and TAC were estimated by colorimetric methods at 532 nm wavelength for MDA and at

570 nm for TAC. The latest liquid sera are stored at -80^{0}C for later measurement of serum 8-OHdG levels by using enzyme-linked immunosorbent assay (ELISA) kit (Catalog number: E-EL-0028, ELABSCIENCE. USA). Assessment of DNA damage was based on the levels of 8-OHdG, subjects with ≥4.0 ng/ml were considered to have a high level of DNA damage.

Statistical analysis:

All data were analyzed using the Statistical Package for Social Science SPSS version 18.0 computer software. Descriptive statistics were adapted to present data in means± standard deviation. Differences between groups were evaluated with Student's-t-test. A correlation analysis by Pearson's® correlation coefficient was used to determine the relationship between 8-OHdG levels and other variables. The level of statistical significance (*P*-value) was set at ≤ 0.05.

RESULTS:

Table 1 illustrates the demographic and laboratory characteristics of the studied subjects. Age, Sex distribution and BMI were nearly similar between patients and healthy individuals. Significantly higher 8-OHdG and MDA levels ($p<0.001$ and $p<0.010$ respectively) together with lower TAC levels ($p=0.010$) were found in diabetics compared to healthy individuals. DNA damage quantitated by the 8-OHdG was detected in 251(84.6%) of patients with type 2 diabetes as compared to 54(28.7%) in healthy individuals, $p<0.010$.

Table 1.Baseline characteristics of patients with type 2 diabetes and healthy individuals

Characteristics	Patients (n=297) mean±SD	Healthy individuals(n=188) mean±SD	*P-value*
Age(Years)	52.9±8.5	47.0±8.5	0.07
Male sex [n (%)]	106(35.7)	59(31.4)	0.55
BMI(Kg/m^2)	31.36±4.5	29.4±6.4	0.05
8-OHdG (ng/ml)	6.04 ±2.7	3.59±2.9	<0.001
HbA1c %	10.2±2.9	5.4±0.6	<0.01
FBS (mg/dl)	219±95.9	96±19.2	<0.01
MDA(n mol/L)	1.54±0.6	1.38±0.4	<0.010
TAC (mmo/l)	1.25±0.09	1.79±0.14	0.010
DNA damage, n(%) 8-OHdG≥4.0 ng/ml	251(84.6)	54(28.7)	<0.010

Table 2 illustrates the mean ±SD of 8-OHdG levels for age, sex, BMI and oxidative stress biomarkers of patients with type 2 diabetes. A significantly higher mean 8-OHdG level was found in the overweight and obese group compared with the normal weight group (p<0.050), also the mean 8-OHdG level was significantly higher (p=0.020) for patients with low antioxidant capacity as compared to that of high antioxidant capacity patients. No

statistically significant differences were found in the mean values of 8-OHdG with respect to age and gender.

Table 2. Serum 8-OHdG levels according to demographic and oxidative stress biomarkers in patients with type 2 diabetes.

Characteristics		n	mean ±SD	Low	High	*p*-value
Age (years)	**<40**	59	6.71±2.92	1.1	13.9	0.250
	≥40	238	5.97±2.7	0.1	18.0	
Gender	**Male**	106	6.14±2.40	1.3	11.0	0.680
	Female	191	5.98±2.91	0.1	18.0	
BMI	**Normal**	21	4.76±1.80	0.1	7.7	<0.05
	Over wt and obese	276	6.13±2.77	0.1	18.0	
MDA (n mol/L)	**<1.2**	42	5.24±3.0	0.1	14.9	0.11
	>1.4	47	6.20±2.7	0.1	18.0	
TAC (m mol/L)	**<1.13**	59	6.20±2.1	0.1	11.0	0.020
	>1.77	31	5.07+2.2	0.1	15.5	

In the patient group, 8-OHdG negatively correlated with TAC(r=-0.47, p<0.001) and was correlated positively with MDA (r=0.22, *p*=0.015).No significant correlations were found with respect to age and BMI (**Table 3).**

Table 3: Pearson's correlation coefficients (r) between 8-OHdG and studied parameters in diabetic patients and healthy controls

Variable	patients		Healthy individuals	
	r	***p***	**R**	***P***
Age(years)	-0.038	0.590	0.090	0.299
BMI (kg/m^2)	0.040	0.540	0.220	0.015
MDA	0.220	0.015	0.093	0.280

TAC	-0.47	<0.001	-0.17	0.100

DISCUSSION: The results of this study provided evidence that patients with diabetes mellitus had high levels of DNA damage. High DNA damage was associated with low antioxidant capacity represented by low TAC and high oxidative stress status represented by a high MDA level. The results confirm a significant higher serum 8-OHdG levels in over-weight and obese diabetic subjects when compared to those with normal weight group.

Moreover, the results of this study showed that DNA damage was highly prevalent in diabetic patients 84.6% compared to healthy individuals, although 28.7% of the healthy individuals also showed a high degree of DNA damage. Our results in combination with previously published results[8,9], suggests that oxidative DNA damage through oxidative stress process mediated mainly by hyperglycemia[10], and serum 8-OHdG level is a potentially useful biomarker for evaluating the severity of DNA damage in human, particularly in patients with diabetes mellitus. The oxidative stress plays an important role in the initiation and progression of the disease and development of its complications. Increasing evidence from both experimental and clinical studies suggest that there is a close link between hyperglycemia, oxidative stress and diabetic complications. Hyperglycemia is the major factor in the development of micro and macro-vascular complications, although the mechanisms by which increased glucose levels contribute to these changes have not been fully understood[11]. Numerous studies have demonstrate that free radicals formation through oxidative stress process mediated mainly by hyperglycemia, as well as these free

radicals thought to be mediated in the process of oxidative DNA damage, but there is controversy about which one from these free radicals as a marker of oxidative stress or markers of DNA damage is the most reliable and suitable marker for clinical practice[11]. Free radicals formed due to many environmental processes beside physiological oxidations as air pollutants[12], and industrialized lifestyle may play a role in this process[13]. Many endogenous enzymes can form free radicals at physiological concentrations: xanthine oxidase, cyclo-oxygenases, and lipo-oxygenases, NADPH oxidase, nitric oxide synthases, P450 cytochrome, and mitochondrial chain[14]. These free radicals which formed will then be counteracted (neutralized) by many endogenous antioxidants like superoxide dismutase[15,16]. It has been reported that micronutrients may act as a powerful antioxidant and may reduce DNA damage. A recent research by mahmoud *et al* 2020 also reported that micronutrients including zinc element act as a powerful antioxidant and may reduce DNA damage in diabetic patients[17]. So it is permissible to speculate that the impact of zinc supplement may have a protection effect against DNA damage by reducing oxidative stress, a finding supported by the current uses of zinc for protection of healthy subjects from the initiation and progression of COVD-19, although the mechanism of action still not well understood, particularly in diabetic patients who are at a high risk of COVD-19 disease. Further studies to elucidate this hypothesis could be of concern.

CONCLUSION: Diabetic patients have more severe oxidative stress and oxidative DNA damage than normal persons, suggesting that increased oxidative stress appears to be associated with lower antioxidant capacity.

Antioxidant supplementation may be an effective public health intervention to reduce DNA damage and oxidative stress.

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