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Original Article

Biochemistry Section

Evaluation of Oxidative DNA Damage among Type 2 Diabetes Mellitus Patients and Healthy Individuals in Duhok, Iraq: A Case-control Study

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ABSTRACT

Introduction: The association between oxidative Deoxy Ribonucleic Acid (DNA) damage and diabetes is well established. Increased glucose levels can stimulate free radical production. However, data regarding DNA damage in type 2 Diabetes Mellitus (DM) patients and healthy individuals are controversial and scarce in Iraq.

Aim: To assess the DNA damage among patients with type 2 Diabetes Mellitus (DM) and healthy individuals and to analyse its relationship with oxidative stress biomarkers.

Materials and Methods: This case-control study was conducted at the Duhok Diabetes Center, Duhok, Kurdistan Region, Iraq, from September 2016 to March 2018. In this study, biomarkers of both oxidative stress and DNA damage including Total Antioxidant Capacity (TAC), Malondialdehyde (MDA) and serum 8-Hydroxy-2-deoxiguanosine (8-OHdG) were measured in 297 patients with type 2 diabetes and 188 healthy individuals. Selection of cases and healthy individuals was done using random sampling technique. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 18.0 and a p-value <0.05 was set as a cut off value of statistical significance.

Results: The mean age, sex and Body Mass Index (BMI) were 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. In diabetic patients, a positive correlation of 8-OHdG was observed with MDA ($r=0.220$), and a negative correlation was observed with TAC ($r= -0.47$). Based on the estimated cut-off point of DNA damage (8-OHdG of 4.0 ng/mL), 84.51% of patients had high levels of DNA damage compared with healthy individuals (28.7%).

Conclusion: Oxidative DNA damage increased in diabetic patients, and was associated with lower antioxidant capacity. Antioxidant supplementation may be an effective public health intervention to reduce DNA damage and oxidative stress.

Keywords: Deoxyribonucliec acid, 8-hydroxy-2-deoxiguanosine, Diabetes mellitus, Malondialdehyde, Oxidative stress, Total antioxidant capacity

INTRODUCTION

Cellular dysfunction occurs as a result of proteins and lipids oxidation as well as DNA modifications [1]. When DNA repair system and endogenous antioxidant network are overwhelmed, oxidative DNA damage occurs [2]. Association between oxidative stress and DNA damage has been reported [3]. Some studies have shown that oxidative stress plays an important role in cellular injury through persistent increased blood sugar [4,5]. The data regarding association of increased glucose and amount of DNA damage are controversial [6,7]. Numerous studies showed an increase in DNA damage in patients with type 2 Diabetes Mellitus (DM) when compared with apparently healthy subjects [8,9].

Persistent hyperglycemia can produce large amount of free radicals [10]. Because the body's defenses are inadequate to deal with the increased production of Reactive Oxygen Species (ROS), an imbalance between ROS production and their defenses develops. This results in oxidative stress [10]. These substances that are produced can cause breaks in the strands of DNA molecule as well as cause base modification, including guanine residues oxidation which is most potential base in DNA molecule that is oxidized more than other bases because of imidazole ring in its structure and leads to 8-OHdG production that is an oxidized nucleoside of DNA. The DNA damage by oxidation has been demonstrated by measuring the 8-OHdG levels, a known marker of oxidant induced DNA damage, in diabetic subjects [3,11]. The most frequent studied and detected biomarker of oxidative DNA damage is 8-OHdG [8,12].

Recently, numerous clinical studies have studied levels of 8-OHdG in human organs, leukocyte DNA and urine concerning oxidative stress and diabetes mellitus [8,13]. Since 8-OHdG is an established biomarker of oxidative DNA damage [14], it is pivotal to study the association of oxidative DNA with other related factors such as lipid oxidation by-product (such as MDA) and total antioxidant capacity in patients with type 2 DM and healthy individuals.

To the author's knowledge little data are available in this area investigating oxidative stress and DNA damage in patients with diabetes [8]. This study is a part of previously published study [8]. Thus, this study was conducted to assess oxidative DNA damage, as well as antioxidant capacity in patients with type 2 DM and healthy individuals in Duhok, Kurdistan Region (Iraq).

MATERIALS AND METHODS

This case-control study was conducted at the Duhok Diabetes Center, Duhok, Kurdistan Region, Iraq, from September 2016 to March 2018. Medical Ethics Committee approval (Reference No: 07062016-4) and informed written consent was obtained for all participants.

Inclusion criteria: For patients it was fasting glucose (>125 mg/dL and HbA1c >7.0%). For healthy individuals inclusion criteria were: no history of chronic diseases or history of diabetes mellitus among first-degree relatives , they were included as controls.

Exclusion criteria: Exclusion criteria for cases were: cardiovascular, respiratory, rheumatoid, renal and hepatic diseases, history of malignancy, recent infections, pregnancy, smoking, and alcoholics. Individuals who had CRP>6 mg/dL, HbA1c>5.5% and glucose levels >100mg/dL were excluded from the study.

Sample size calculation: The sample size for this study was calculated with 95% confidence interval with 80% power at a 5% level of statistical significance [15]. Four hundred-eighty five subjects, 297 patients with type 2 DM and 188 apparently healthy individuals (aged 35-65 years) were enrolled in the study. During the sampling period of this study all patients (n=5783) who attended Duhok diabetes center were interviewed and asked to participated in the study. At the beginning, a total of 337 participate in the study. After exclusion of 40 responders who were unfit according to the inclusion criteria, the remaining participants were enrolled in this study.

A pre tested proforma was designed to obtain information on gender, age, weight, and height. The BMI was measured for each subject. Participants were instructed to avoid strenuous physical activity for more than two hours before the examinations.

Study Procedure

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 10 mL of blood was withdrawn by venipuncture, using vacutainer from the antecubital vein and collected in Becton Dickinson (BD) Vacutainer System CAT- plain tubes. Total 2 mL of blood was collected in an Ethylenediamine Tetraacetic Acid (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 was collected into two tubes- one was processed immediately for measuring serum Fasting Blood Glucose (FBG), MDA and TAC levels. The MDA and TAC were estimated by colorimetric methods at 532 nm wavelength for MDA and at 570 nm for TAC. The second tube with liquid sera was stored at -800°C for measurement of serum 8-OHdG levels by using Enzyme Linked Immunosorbent Assay (ELISA) kit (Catalog number: E-EL-0028, ELABSCIENCE. USA). Assessment of BMI was done. Subjects with BMI less than 25 Kg/m^2 were considered normal, while those with BMI between 25 Kg/m^2 and 2 9.9 Kg/m^2 were considered overweight and those with BMI ≥30 Kg/m^2 were considered obese. [16]. Assessment of MDA and TAC was done. A cut-off point of MDA<1.4 nmol/L and of TAC>1.77 mmol/L (as obtained from the laboratory) were used to classify patients as having low oxidative stress [17]. Assessment of DNA damage was based on the levels of 8-OHdG. Subjects with 8-OHdG >4.0 ng/mL were considered to have a high level of DNA damage [8].

STATISTICAL ANALYSIS

Analysis of data was done using version 18.0 SPSS computer software. Means±Standard Deviation (SD) adopted to present data. Student's t-test was used to evaluate the differences between groups. Pearson's® correlation coefficient was used to estimate the correlation between 8-OHdG levels and other variables. A p-value at ≤ 0.05 was used as statistical significance cut off value.

RESULTS

[Table/Fig-1] illustrates the demographic and laboratory characteristics of the studied subjects. Age, sex distribution were statistically similar between patients and healthy individuals. The mean+SD of serum 8-OHdG of patients and healthy individuals was 6.04±2.7 ng/mL and 3.59±2.9 ng/mL, p<0.001. Significantly higher 8-OHdG and MDA levels (p<0.001 and p<0.01 respectively) together with lower TAC levels (p=0.01) were found in diabetics compared to healthy individuals. The DNA damage quantitated by the 8-OHdG was detected in 251 (84.5%) of patients with type 2 DM as compared to 54 (28.7%) in healthy individuals, p<0.01.

Characteristics	Cases (n=297) (mean±SD)	Control (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 (nmol/L)	1.54±0.6	1.38±0.4	<0.01
TAC (mmo/L)	1.25±0.09	1.79±0.14	0.01
DNA damage, n(%) 8-OHdG>4.0 ng/mL	251 (84.51)	54 (28.7)	<0.01

[Table/Fig-1]: Baseline characteristics of patients with type 2 diabetes and healthy individuals.
*Student t-test used to set statistical cut-off value with significance p<0.05

[Table/Fig-2] illustrates the mean±SD of 8-OHdG levels for age, sex, BMI and oxidative stress biomarkers of patients with type 2 DM. No statistically significant differences were found in the mean values of 8-OHdG with respect to age and gender, p=0.250 and p=0.680. A significantly higher mean 8-OHdG level was found in the overweight and obese group compared with the normal weight group (p=0.045), 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.

Characteristics		N	mean ±SD	Low (≤4.0 ng/mL)	High (≥4.0 ng/mL)	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	0.045
	Overweight and obese	276	6.13±2.77	0.1	18.0	
MDA (n mol/L)	≤1.4	250	6.09±2.7	0.1	14.9	0.11
	>1.4	47	6.20±2.7	0.1	18.0	
TAC (m mol/L)	≤1.77	266	6.15±2.8	0.1	11.0	0.020
	>1.77	31	5.07+2.2	0.1	15.5	

[Table/Fig-2]: Serum 8-OHdG levels according to demographic and oxidative stress biomarkers in patients with type 2 diabetes.
*Student t-test used to set statistical cut-off value with significance p<0.05

In the patient group, 8-OHdG negatively correlated with TAC (r=-0.47, p<0.001) and it correlated positively with MDA (r=0.22, p=0.015). No significant correlation was found with respect to age and BMI in cases group [Table/Fig-3].

DISCUSSION

The results of this study confirm that DNA damage in type2 DM patients characterised by the low antioxidant levels which may play an important role in the disease progression and disease

Variable	Cases		Controls	
	r	p-value	r	p-value
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

[Table/Fig-3]: Pearson's correlation coefficients (r) between 8-OHdG and studied parameters in diabetic patients and healthy controls.
*statistically significant p<0.05

related complications. A significantly higher serum 8-OHdG levels were seen in overweight and obese diabetic subjects when compared to those with normal weight group. Further, high MDA level was seen in diabetic subjects when compared to healthy individuals. These results are in accordance with a previous study [18]. In this study, 84.51% of the patients and 28.7% of the healthy individuals had high degree of DNA damage (8-OHdG>4.0ng/mL). Such a high prevalence of DNA damage is worthy of discussion. It is favorably comparable to values from the developing countries [18,19], but it is markedly higher than values of diabetic and normal populations in other studies [14,20,21]. The discrepancy may be related partly to the low antioxidant status. In a study done in Iraq, significantly higher levels of 8-OHdG was found in patients with type I diabetes than in healthy controls. [14] Significantly higher levels of oxidative stress markers (like MDA, glutathione peroxidase) and 8-OHdG were reported in type 2 diabetic patients when compared to healthy individuals in a study in Saudi Arabia [22]. Similar findings were also reported from another study from Baghdad [23]. In a review from India, authors have stated that hyperglycemia leads to overproduction of reactive oxygen species thus leading to microvasculature damage [20]. However, in a study from United Kingdom, authors did not find any association between increased DNA damage markers in diabetic patients [24]. The difference in results possibly could be because of the difference in the duration of diabetes and glycemic control [25].

A study by Ali AF and Altimim DJ reported a lower vitamin D status may increase DNA damage [26]. A recent research by Mahmoud HM et al., also reported that micronutrients including zinc element act as a powerful antioxidant and may reduce DNA damage in diabetic patients [8]. Several factors are known to increase DNA damage in diabetic patients, such as glycemic controls, duration of diabetes, repair capacity, diet, lifestyle and environment [5,6]. Recently some studies reported a variety of anti DNA damaging effect by dietary antioxidants, including the effects of antioxidant supplements [8,27]. So it is permissible to speculate that the impact of zinc supplement and/or other antioxidants and supplements may have a protective 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 Coronavirus Disease-2019 (COVID-2019), although the mechanism of action is still not well understood, particularly in diabetic patients who are at a high risk of COVD-19 disease complications [28]. Further studies to elucidate this hypothesis could be of concern.

The results of the present study is in accordance with previously published results and suggests that oxidative DNA damage through oxidative stress process is mediated mainly by hyperglycemia [4,5], and supported that serum level of 8-OHdG is a potentially a valuable marker in estimating the severity of DNA damage, especially in diabetic patients.

Limitation(s)

The major limitation of this study is the relatively small sample size and data from a single population as the duration for the sampling was limited. Hence, the control were less than the cases. Further, lack of using more than one marker of oxidative stress, DNA damage as well as for antioxidant capacity to detect more association between oxidant-antioxidant balance and DNA damage was a drawback of this study.

CONCLUSION(S)

Elevated levels of serum 8-OHDG with abnormal oxidative stress biomarkers may be associated with reduced antioxidant status in diabetic patients. In this study, a negative correlation between 8-OHdG and TAC in diabetic patients was seen. More than half of the population of this study, particularly diabetic patients had lower antioxidant capacity and a high degree of oxidative DNA damage. Antioxidant supplementation may be an effective public health intervention to reduce DNA damage and oxidative stress.

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