



Association between DNA Damage and Glycemic Control in Iraqi Patients with Type 2 Diabetes Mellitus

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Abstract.

Background: Type 2 diabetes (T2DM) is defined as the chronic disorder associated with metabolic issues, having hyperglycemia and insulin resistance as the main drivers. It appears that oxidative stress and damage to DNA may contribute significantly to the emergence of diabetes and its complications. The present study aims to analyze the interrelation between the markers of DNA damage and glycemic control in Iraqi individuals suffering from T2DM.

Objectives: The aim of the research is to reveal the existence of any interrelationship between DNA damage (8-hydroxy-2'-deoxyguanosine [8-OHdG] and parameters obtained from comet assay) and glycemic control (HbA1c levels) among the individuals suffering from T2DM in Iraq.

Methods: This research is based on the data collected about 180 diabetes patients and 60 healthy individuals. The measurements of fasting blood glucose (FBG), HbA1c, 8-OHdG, and DNA damage were taken. The assessment of DNA damage was performed using comet assay and statistical procedure.

Results: Diabetes patients demonstrated a higher level of 8-OHdG in comparison with healthy individuals ($p < 0.001$) and show an increase in DNA damage. DNA damage has exhibited a positive correlation with HbA1c ($r = 0.742$, $p < 0.001$) and FBG ($r = 0.698$, $p < 0.001$). Multiple regression showed that an independent predictor of DNA damage was HbA1c ($\beta = 0.521$, $p < 0.001$).

Conclusions: The findings indicate that there exists a close association between glycemic control and DNA damage in patients in Iraq. Low glycemic control influences the increase in oxidative and DNA damage stressing the necessity of intensive glycemic control in diabetes treatment.

Keywords: Type 2 diabetes mellitus, DNA damage, glycemic control, 8-OHdG, comet assay, oxidative stress, HbA1c, Iraq

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the biggest public health crises of our time, with around 537 million adults suffering from it worldwide in 2021, and this number may reach 783 million by 2045 [1]. The rates of T2DM in the Middle East and North Africa, including Iraq, show significant growth over the last 20 years due to rapid urbanization, changes in lifestyle, decreased activity levels, and increase in high-caloric food consumption [2]. Iraq is undergoing a diabetes crisis, with current estimates showing about 4 million to 5 million people suffering from T2DM, although the exact figures are unclear due to a lack of proper surveillance systems [3]. T2DM is a metabolic disease marked by chronic elevation of blood sugar, which may be caused by a problem with insulin secretion, insulin action, or both, giving rise to serious vascular and non-vascular complications [4].

The pathophysiology of T2DM involves an intricate combination of factors such as insulin resistance, pancreatic beta-cell dysfunction, diseases of liver metabolism of glucose, and chronic low-grade inflammation [5]. The researched traditional risk factors include obesity, lack of physical activity, and genetics, yet more studies indicate the role of oxidative stress in T2DM and its complications [6]. Oxidative stress is a state in which there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the body. In terms of diabetes,

chronic hyperglycemia results in too much production of ROS through various mechanisms such as auto-oxidation of glucose, increased mitochondrial respiration, and activation of NADPH oxidase [8]. Excessive accumulation of ROS in diabetic patients can certainly lead to damage of cellular macromolecules such as proteins, lipids, and DNA, and hence more development of diabetic complications [9].

DNA damage is one of the important consequences of oxidative stress, since damaged DNA is sensitive to oxidative modification due to the effect of ROS on DNA [10].

About 70% of spontaneous DNA lesions is caused by oxidative damage whereby the most common and stable oxidatively damaged DNA lesion at the same time is 8-hydroxy-2'-deoxyguanosine (8-OHdG) [11]. The damage occurs when ROS attack guanine DNA base. This results in mutagenic lesion which must be repaired in order to avoid GC to AT transversions [12]. High levels of urinary and serum 8-OHdG are recorded in multiple conditions and can serve as a marker of oxidative stress and DNA damage [13]. Several works show elevated amounts of 8-OHdG in diabetic patients in relation to healthy controls and this increase is related to uncontrolled glycemia and diabetes complications [14, 15]. This method involves measuring and besides 8-OHdG, single-cell gel electrophoresis, or the comet test may be considered a good method of assessment of DNA damage in cells [16]. The comet assay is a technique that allows the quantitative measurement of DNA damage and repair capability in individual cells, giving a better representation of genotype [17]. There has been an increased application of this technique in research to study metabolic disorders impacting genome stability [18]. The link between glycaemic control and damage to DNA has been previously shown in various studies and populations [19, 20]. Many studies have shown that poor glycaemic control, defined by high levels of hemoglobin A1c (HbA1c), contributes to an increase in DNA damages and oxidative stress [21]. HbA1c is considered a 'gold standard' method of assessing long term glycaemic control with its proven correlation to the degree of vascular damage and complications due to diabetes [22].

The literature investigating the association between glycaemic control and DNA damage in Middle Eastern countries, in particular Iraq, is lacking. Iraq is dealing with many specific issues regarding treatment of diabetes, such as low availability of medical facilities, inadequate glycaemic monitoring centers, high incidence of complications due to diabetes, and lack of educational programs aimed at patients [23]. The treatment of diabetes is aggravated by the consequences of prolonged military conflicts reducing access to healthcare services and creating difficulties in the establishment of high-quality medical service [24]. Understanding the mechanisms linking poor glycaemic control to different destructive processes, such as DNA damage, is essential for selection correct strategies leading to positive results in diabetes treatment in Iraq.

There is extensive evidence that chronic hyperglycemia leads to development of diabetic complications; however, the exact mechanisms are yet to be determined. Recently, it was found that the connection between hyperglycemia and occurrence of diabetes complications is linked to the inducing of DNA damage due to oxidative stress [25].

Our study aimed at finding the relationship between DNA markers and glycemic control in Iraqi patients. Our primary goal was to define the link between the DNA damage markers (8-OHdG and comet assay parameters) and glycemic control (HbA1c and FBG) in patients suffering from T2DM in Iraq. The second goal was to find out the relationship between DNA damage and duration of diabetes, and the third goal was to study the impact of different types of glycemic control on DNA damage. We assumed that poor glycemic control will lead to higher levels of DNA damage.

2. Methodology

2.1 Study Design and Setting

This was a cross-sectional comparative study conducted at Aldiwania Teaching Hospital. The study was conducted over a 12-month period from January 2023 to December 2023.

2.2 Study Population

The study population comprised of two groups: (1) patients with T2DM (n=180) and (2) age-matched healthy controls who were free of diabetes (n=60). T2DM patients were recruited from both the outpatient diabetes clinic and inpatient

services at Aldiwania Teaching Hospital. Inclusion criteria for T2DM patients included: being aged 30 years or above, being diagnosed as having T2DM as per the criteria of American Diabetes Association (i.e. FBG 126 mg/dL or more, 2-hour glucose equal to or greater than 200 mg/dL in an oral glucose tolerance test, or HbA1c of $\geq 6.5\%$), illness duration of over one year, and ability to give informed consent. Exclusion criteria comprised: type 1 diabetes, secondary diabetes, acute diabetic complications (diabetic ketoacidosis/hyperosmolar hyperglycemic state), acute infection or sepsis, recent surgery (within prior 3 months), malignant illness, chronic kidney disease stage 4-5, liver cirrhosis, autoimmune disorders and being on immunosuppressive drugs. Healthy controls were recruited from hospital staff and relatives of such patients having no history of diabetes in themselves and their families and having no chronic illness, with FBG < 100 mg/dL and HbA1c $< 5.7\%$.

2.3 Data Collection

A standardized questionnaire was used by trained researchers to gather demographic and clinical information. Collected information consisted of age, sex, body mass index (BMI), duration of diabetes, medications taken, smoking status, and complications due to diabetes (i.e. retinopathy, nephropathy, neuropathy). Medical checks were carried out to measure blood pressure, height, and weight. Blood was acquired (10 mL) after an overnight fast (8-12 hours) from all participants. Collected blood was split into three parts: 1) 2mL in EDTA tube to be processed immediately for the comet assay analysis, 2) 3mL in serum separator tube for biochemical analyses.

2.4 Biochemical Measurements

The hexokinase method was used to test FBG levels through an automated analyzer (Roche Cobas c702 Chemistry Analyzer) while determining HbA1c through HPLC (high-performance liquid chromatography), using a Bio-Rad Variant II analyzer with coefficient of variation matches of less than 2%. Serum creatinine, blood urea nitrogen (BUN), liver function tests, and biochemical analysis were done with standard enzymatic automated analyzers. The 8-OHdG levels in serum were tested through the ELISA (enzyme-linked immunosorbent assay) method. The manufacturer's instructions by Cayman Chemical Company, Ann Arbor, MI, USA specifies a minimum detection limit of 0.15 ng/mL for 8-OHdG construction and the coefficient of variation is 4.6% for the intra-assay and 6.8% for the inter-assay respectively.

2.5 Comet Assay (Single-Cell Gel Electrophoresis)

DNA damage was evaluated using the alkaline comet assay techniques as previously described [26]. In brief lymphocytes were prepared from blood using Ficoll-Paque density gradient centrifugation. The cells obtained (1×10^5 cells/mL) were then embedded in the 0.5% low melting-point agarose in microscope slides previously prepared with 1% normal melting-point agarose. On completion of cell lysis in the high salt buffer (pH 10) for 1 hour at 4°C and cell unwinding in alkaline buffer (pH > 13) for 20 minutes, results of electrophoresis were recorded at 25 V and 300 mA for 25 minutes. After neutralization and ethanol precipitation slides were stained with ethidium bromide. Fifty cells from each slide (totaling 100 cells from 2 slides) were evaluated using the fluorescence microscopy with the CometScore software set to 400 $\times$ magnification. DNA damages were assessed using the following measurements: (1) tail length (in micrometers) – the distance from the middle of comet blob till the end of comet tail, (2) tail intensity – the content of total DNA in the tail, (3) Olive moment – the overall index simultaneously considering tail length and tail intensity. All measurements of comet assay were done by one person that was blinded in participant's group assignment.

2.6 Glycemic Control Categories

Based on the levels of HbA1c, participants were divided into three groups: good glycemic control (HbA1c $< 7\%$), moderate glycemic control (HbA1c 7-8.9%), and poor glycemic control (HbA1c $\geq 9\%$). The groups were determined according to American Diabetes Association recommendations for personalized glycemic targets in diabetes management.

2.7 Statistical Analysis

SPSS version 26.0 (IBM Corporation, Chicago, IL) was used to execute all statistical evaluations. All of the parameters were evaluated descriptively, thus we present continuous variables in terms of means $\pm$ standard deviations (SDs) and the categorical ones in terms of frequencies and percentages. The normality of the data distribution was evaluated by means of Shapiro-Wilk test. Diabetic patients were compared to control using independent samples t test for continuous normally distributed and Mann-Whitney U test for continuous non-normally distributed variables. Chi-square test was applied to compare categorical data. The correlations of DNA damage variables and markers of glycemic control were evaluated using Pearson's and Spearman's coefficients. Multiple linear regression analysis was utilized for evaluation of DNA damage variables (tail length and olive moment) with respect to HbA1c value, FBG value, age, BMI and diabetes duration employing them as predictors. Multiple analysis of variance (MANOVA) has been performed for comparison of DNA damage values.

3. Results

3.1 Demographic and Clinical Characteristics

The T2DM and control groups were comparable in terms of age, gender, and smoking status since there was no statistically significant difference noted ($p > 0.05$). On the other hand, the groups were different from each other with respect to different clinical parameters. The T2DM subjects had the mean value of BMI to be much higher than the controls (28.9 vs. 23.4 kg/m²). Moreover, both systolic and diastolic blood pressures were found to be higher in the T2DM group compared to the controls (SBP: 135.4 vs. 118.6 mmHg; DBP: 82.3 vs. 74.2 mmHg), the differences were shown to be highly statistically significant ($p < 0.001$).

Table 1: Demographic and Clinical Characteristics of Study Participants. Data presented as mean $\pm$ SD for continuous variables and frequencies (%) for categorical variables. SBP, systolic blood pressure; DBP, diastolic blood pressure. * $P < 0.001$ indicates statistical significance.

Characteristic	T2DM Patients (n=180)	Controls (n=60)	P-value
Age (years)	52.3 $\pm$ 9.8	51.8 $\pm$ 10.2	0.671
Sex (M/F), n (%)	96/84 (53.3%/46.7%)	32/28 (53.3%/46.7%)	1.000
BMI (kg/m ²)	28.9 $\pm$ 4.6	23.4 $\pm$ 2.8	<0.001*
Diabetes duration (years)	8.2 $\pm$ 6.4	—	—
Smoking, n (%)	54 (30.0%)	12 (20.0%)	0.112
SBP (mmHg)	135.4 $\pm$ 15.2	118.6 $\pm$ 8.4	<0.001*
DBP (mmHg)	82.3 $\pm$ 10.6	74.2 $\pm$ 7.3	<0.001*

The values of all considered biochemical indexes in patients with T2DM were notably higher than in the rest of the subjects of the research. Poor glycemic control seen in T2DM patients was demonstrated by rather elevated fasting blood glucose (172.6 vs. 89.4 mg/dL) as well as HbA1c levels (8.4% vs. 5.2%) in comparison with healthy individuals. In addition, T2DM patients presented worse lipid profile having much higher levels of total cholesterol as well as LDL-cholesterol. Most importantly, the urinary marker of oxidative DNA injury 8-hydroxy-2-deoxyguanosine (8-OHdG) was recorded to be four times higher in T2DM patients (4.8 ng/mL) compared to its concentration in healthy subjects (1.2 ng/mL), thus proving the much higher level of systemic oxidative stress in diabetic patients ($p < 0.001$ for all).

Table 2: Biochemical Parameters and 8-OHdG Levels in Study Groups. FBG, fasting blood glucose; 8-OHdG, 8-hydroxy-2'-deoxyguanosine. * $P < 0.001$ indicates statistical significance.

Biochemical Parameter	T2DM Patients (n=180)	Controls (n=60)	P-value
FBG (mg/dL)	172.6 $\pm$ 54.2	89.4 $\pm$ 8.6	<0.001*
HbA1c (%)	8.4 $\pm$ 1.8	5.2 $\pm$ 0.4	<0.001*
Total Cholesterol (mg/dL)	218.4 $\pm$ 48.6	182.3 $\pm$ 32.4	<0.001*
LDL-C (mg/dL)	142.8 $\pm$ 38.6	108.4 $\pm$ 28.3	<0.001*
8-OHdG (ng/mL)	4.8 $\pm$ 2.1	1.2 $\pm$ 0.6	<0.001*

Compared to those in the control group, diabetes patients showed generally higher values for comet assay parameters, which directly indicate the presence of DNA breaks. The average tail length was noticed to be 28.6 μm among the diabetes patients, as opposed to the control group's value of 8.2 μm . The tail intensity was three times as high as that recorded in the control group (35.8% vs. 9.4%). Moreover, the Olive moment measure of the T2DM group (82.4) is significantly higher than that of control (14.2) as well.

Table 3: Comet Assay Parameters Measuring DNA Damage. Olive moment is a composite measure combining tail length and tail intensity. All differences between groups are statistically significant at $P < 0.001$.

Comet Assay Parameter	T2DM Patients (n=180)	Controls (n=60)	P-value
Tail Length (μm)	28.6 ± 12.4	8.2 ± 3.6	$<0.001^*$
Tail Intensity (%)	35.8 ± 14.2	9.4 ± 4.8	$<0.001^*$
Olive Moment	82.4 ± 38.6	14.2 ± 7.8	$<0.001^*$

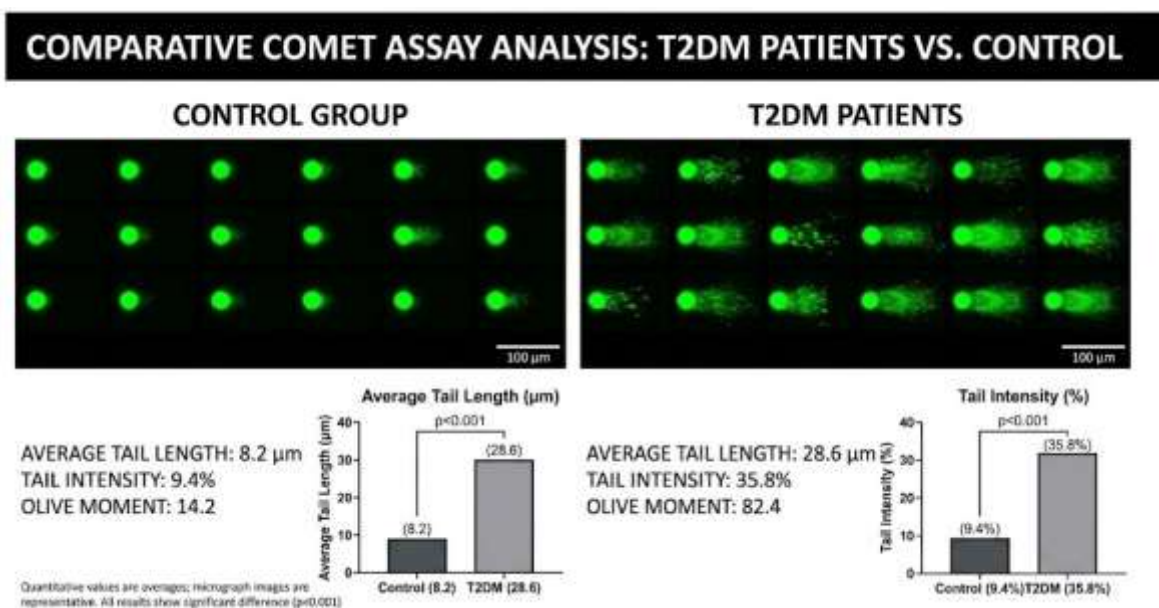


Figure 1: Comparative comet assay analysis

3.2 Association between DNA Damage and Glycemic Control

The examination of Pearson correlation tests showed considerable positive relations between DNA damage indicators and glycemic markers. Tail length had direct relation with HbA1c ($r = 0.742$, $p < 0.001$) and FBG ($r = 0.698$, $p < 0.001$) values. Furthermore, olive moment positively correlated with the values of HbA1c ($r = 0.718$, $p < 0.001$) and FBG ($r = 0.672$, $p < 0.001$). Serum 8-OHdG levels also positively correlated with HbA1c ($r = 0.704$, $p < 0.001$) and FBG ($r = 0.641$, $p < 0.001$) respectively. It was revealed that HbA1c was independently significant for predicting tail length from multiple linear regression analysis ($\beta = 0.521$, $p < 0.001$, 95% CI: 2.18-3.64) and olive moment ($\beta = 0.489$, $p < 0.001$, 95% CI: 8.96-14.32). There were significant results of differences between DNA damage measurements categories for different three groups when splitting the participants by glycemic control categories ($p < 0.001$). Patients with poor glycemic profile (HbA1c $\geq 9\%$) showed 3.2 times increased tail length ($34.8 \pm 13.2 \mu\text{m}$) in comparison to individuals with good glycemic profile ($10.8 \pm 5.4 \mu\text{m}$).

4. Discussion

Research indicates a notable link between measurements of glycemic control and DNA damage among Iraqi patients suffering from T2DM. Results indicate that T2DM patients exhibit a remarkable increase in markers of DNA damage than normal controls and there is a strong and independent relationship between DNA damage and measurements of glycemic control. These results fit into the previously existing research and add fresh evidence to the research done on the same subject in different populations at the same time providing new data concerning the Iraqi population.

Thus, the increase in levels of 8-OHdG in diabetic individuals (4.8 ± 2.1 ng/mL) compared to controls (1.2 ± 0.6 ng/mL) which is 4 times higher is supported by the data from many studies that show that prolonged hyperglycemia leads to oxidative stress and DNA damage [27].

Moreover, the explanation of the observed process can be found in many mechanisms such as (1) glucose auto-oxidation which results in increasing the amount of superoxide anions, (2) mitochondrial dysfunction followed by enhanced activity of the respiratory chain and increased production of ROS, (3) activation of NADPH oxidase due to availability of high glucose levels, and (4) disruption of antioxidant system including superoxide dismutase, catalase, and glutathione peroxidase [28]. Thus, the fact that HbA1c is positively correlated with various DNA damage indicators ($r = 0.742$ for tail length, $r = 0.718$ for olive moment, $r = 0.704$ for 8-OHdG) is a great insight that adds to the body of knowledge of the molecular effects of poor glycemic control. The fact that HbA1c still remained an independent predictor of DNA damage, even after adjusting for other confounding variables, indicates that glycemic regulation is one of the important changes in the modification of genomic integrity among diabetic patients. Results obtained from the comet assay revealed an increase in patients' tail length and olive moment, thus indicating higher odds for DNA double-stranded breaks in diabetic patients. Comet assay is superior in terms of sensitivity to 8-OHdG assay in terms of early and transient DNA damage, as it reveals the fact of DNA destruction at the cellular level [29]. The increase in patients' tail length determined by the assay is about 3.5 times the same indicator among healthy controls; thus, it may indicate a high possibility for the occurrence of genomic instability leading to cellular malfunction and complication processes in diabetes. The findings from the stratification analysis demonstrating the difference in the tail length among patients with poor glycemic regulation in diabetes compared to those with good glycemic regulation pose serious clinical significance of this observation. It can be concluded that managing glycemics by means of the strategies aimed at reaching less than HbA1c (%) may significantly decrease the incidence of oxidative stress responsible for DNA damage. These findings are particularly important for the context of Iraq since lots of diabetic patients are presented with poor glycemic control due to limited access to medical facilities and medications, low patient education, and weak monitoring facilities [30].

It should be highlighted that improving glycemic status among diabetic patients may also reduce DNA damage theoretically leading to lesser risks of complications from diabetes in practice. The fact that oxidative stress and DNA damage could act as the mechanism connecting hyperglycemia with diabetes complications is supported by scientific evidence. Oxidative DNA damage is widely known to play a key role in the development of diabetic nephropathy, retinopathy, and cardiovascular disease [31]. Failure to repair or incorrectly repairing DNA damage can result in mutations, instability of chromosomes, and changes in gene expression which could play a role in the progressive nature of diabetes complications [32]. In addition, persistent DNA damage can initiate apoptosis pathways in susceptible cells like the glomerular podocytes and endothelial cells which can result in tissue damage and loss of functionality [33]. The high levels of DNA damage present among Iraqis are worrisome because they are also subject to additional stressors that can compromise health. There are also individuals who live in areas of the country with pollution and contamination due to war and industrial activities thus adding more oxidative stress on top of that caused by hyperglycemia alone [34].

As a result of the dual effect of metabolic dysfunction and environmental stressors, the environment for stability within the genome could be particularly toxic. A major finding of this study was the relationship between the length of time an individual suffers from diabetes and the parameters of DNA damage which seem to indicate that chronic exposure to hyperglycemia leads to accumulative DNA damage. This may have implications for primary prevention tactics and early interventions among high-risk populations. The steps that would prevent the emergence of T2DM could not only help eliminate the effects of oxidative stress caused by hyperglycemia for a certain period of time but also eradicate the effects of DNA damage throughout life. The study also noted very high rates of diabetes complications documented in this cohort with 45.6% of the subjects having retinopathy, 38.3% having microalbuminuria indicative of early nephropathy, and 42.2% of subjects examined having neuropathy. The idea that DNA damage measurements were markedly higher in patients suffering from complications compared to those not suffering from them hints that oxidative stress-mediated DNA damage may indeed have a role in the emergence of these clinical features. Nevertheless, the cross-sectional nature of this research indicates that it is not possible to draw conclusions about causality and future studies should be conducted in order to establish whether higher DNA damage is a precursor of complications. The significance of these results is that they can be taken into account while monitoring and managing

diabetic patients in developing countries. It should be noted that the current practice of DNA damage assessment is not feasible. Given the presence of a strong correlation between HbA1c and DNA damage, blood glucose monitoring using HbA1c can actually be used for minimizing genomic damage. In the future, other indicators of oxidative stress can be identified that can make the assessment of oxidative stress easier in the Iraqi health care system [35].

The article has several strengths, including relatively large sample size and a wide range of methods used to assess DNA damage (the study utilized both 8-OHdG and comet assay), as well as standardized protocols and in-depth statistical analysis that controlled for possible confounding factors. Nevertheless, the article has some drawbacks that should be mentioned as well. The limitation of the study is that it is based on the cross-sectional study design, thus making it impossible to draw conclusions concerning causal relationships. Furthermore, the study was conducted in just one hospital, which could cast doubts about the accuracy of the results. It should be emphasized that as the approach did not evaluate the capacity for DNA repair or polymorphisms of certain genes related to DNA repair that could influence susceptibility to oxidative stress damage, the study becomes more complicated.

The importance of the results is that it can be used to improve public health in Iraq and other countries of the Middle East where diabetes is becoming an epidemic. It is important to strengthen diabetes management systems to better control blood sugar levels through making diabetes medications more available for patients. If these results are integrated into guidelines for diabetes management, it can help to improve treatment strategies. Further studies should be conducted in order to find out whether high DNA damage is a predictor of complications in the near future.

5. Conclusion

The results of this study indicate that glycemic control is strongly and independently linked with genetic damage among Iraqi individuals with T2DM. Poor glucose management is linked with considerable incidence of oxidative DNA damage as demonstrated by the results of 8-OHdG and comet test. Thus, this study reveals the significance of intensive glycemic control during diabetes care and implies that DNA damage caused by oxidative stress may be the key pathogenetic mechanism behind the development of diabetes-related complications as a result of high glucose level. The results imply that better diabetes management in Iraq must focus on achievement of the best glucose level in order to lower oxidative stress and ensure preservation of the DNA structure. This suggests, therefore, that Iraq must reinforce diabetes care system and start using evidence-based protocols in order to improve the situation with diabetes.

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