EVALUATION OF DNA DAMAGE AND ANTIOXIDANTS DEFENSE SYSTEMS IN TYPE 2 DIABETES MELLITUS PATIENTS

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Abstract

**Background:** World Health Organization (WHO) demonstrated that more than 170 million people have diabetes worldwide and in 2030, this number might rise to 370 million. Moreover, hyperglycemia generates ROS then oxidative stress in many tissues that generate diabetic complications like protein, lipid and DNA modifications which may lead to teratogenic or carcinogenic consequences. The present study was conducted to evaluate the DNA damage markers, antioxidants makers and lipid peroxidation in Type 2 diabetes mellitus patients. **Methods:** About 5ml of venous blood was collected from 10 healthy persons and 30 patients, fasting for 10 hours. Comet assay used to measure DNA damage and antioxidants were assessed biochemically. Lipid peroxidation was estimated by the Thiobarbituric acid assay. **Results:** The results of presents study showed that DNA damage markers such as comet length, tail length, DNA% in tail and tail moments were increased significantly at \( P <0.05 \) in diabetic patients as compared with healthy control group. Also, all biochemical markers such as SOD, CAT, GPx, GSH and MDA were significantly elevated in diabetic patients as compared with healthy control. **Conclusion:** The present study showed that in patients with Type 2 diabetes mellitus there is increased DNA damage through elevation in markers of DNA such as comet length, tail length, DNA% in tail and tail moments. In addition, there was significant increase in SOD, CAT, GSH, MDA and GPx activities in diabetic patients.

**Keywords:** DNA damage, Comet assay, Antioxidants, Diabetic mellitus, thiobarbitiric acid assay.

Introduction

World Health Organization (WHO) demonstrated that more than 170 million people have diabetes worldwide and in 2030, this number might rise to 370 million \(^{(1)}\). In addition, microvascular and macrovascular complications of diabetes mellitus (DM) are the leading cause of mortality and morbidity \(^{(2)}\). Many biological pathways are triggered in hyperglycaemia state like glucose auto-oxidation, polyol pathway, prostanoid synthesis and protein glycation leading to increased free radicals production where oxidative stress is a common pathogenic factor in diabetes mellitus and its complications \(^{(3,4)}\). In humans, there are antioxidant enzymes like superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH) and its enzymes, such as Glutathione reductase (GR), Glutathione peroxidase (GPx) and glutathione-transferase, which scavenge free radicals in order to protect the body \(^{(5)}\). Some studies have estimated the status of oxidative stress in diabetics and reported oxidative damage in DM patients \(^{(5,6)}\). Polyunsaturated fatty acids are readily oxidized by reactive oxygen species (ROS) to produce fatty acid radicals that generate new free radicals by reacting with adjacent lipid molecules. Breakdown of lipid peroxidation products form many reactive aldehydes like MDA which is highly reactive with protein and DNA molecules leading to adducts formation with these macromolecules \(^{(7)}\). Moreover, hyperglycemia generates ROS then oxidative stress in many tissues that generated diabetic complications like protein, lipid and DNA modifications which may lead to teratogenic or carcinogenic consequences \(^{(8-10)}\). Furthermore, there is an increasing concern of the effects of DNA damage in chronic diseases. Comet assay is a sensitive, simple, economic and rapid technique to detect DNA damage and repair, radiation pollution, genetic toxicology and aging \(^{(11-14)}\). Therefore, the present study was conducted to evaluate DNA damage markers, antioxidants makers and lipid peroxidation in type 2 diabetes mellitus patients.

Materials and Methods

Collection of blood samples

A sample of 5ml venous blood was collected from each of 10 healthy persons and 30 patients, fasting for 10 hours.

DNA damage measurement

A sample of 100µl venous blood was put in a 2ml microfuge tube containing 1.5ml of phosphate buffer solution. Then 40µl of proteinase K were added and Centrifuged at 10000xg for 15min at 4°C then 2.5µl of cell suspension transferred to a clean 1.5ml tube and mixed on Comet slide with 40µl of low melting agarose. Lysis solution was prepared that consisted of 2.5M NaCl, 100mM EDTA, 10mM Tris-base and 8g NaOH, all dissolved and volume completed to 700ml by deionized water. A volume of 110ml was added of 1% triton X and 55ml of 10% Dimethyl Sulphoxide (DMSO). After that, the volume completed to 100ml by deionized water and stored at 4°C or on ice for at least 20min before use. About 7.5µl of the solution was combined with 75µl of low melting agarose and immediately the mix was spread on the clear part of a comet slide, then warmed on a heating plate at 42-50°C before application. Slides were stored in lysis solution at 4°C for 60 minutes, then the lysis solution was replaced by alkaline solution containing 6g NaOH and 500µl of 0.5%
Na$_2$EDTA for 60 minutes at room temperature in the dark. The slides removed from alkaline solution and washed by immersing in 1X TBE buffer for 5 minutes. They were transferred to horizontal electrophoresis apparatus and placed on the gel tray and 1X TBE buffer was poured to cover the slides. The apparatus was run for 60 min at 70V, then gently tapped to get excess TBE off and some drops of 70% ethanol were added on slides to remove the water. Slides were stained by ethidium bromide and left for 24 hour before checking by fluorescence microscope (15).

**Antioxidant measurements**

SOD activity was determined by auto-oxidation of Pyrogallol according to (16), while CAT activity was measured according to procedure of (17,18). GSH activity was measured according to (19); the acid soluble sulfhydryl groups form a yellow colored complex with dithionitrobenzene (DTNB). The activity of GPx was investigated according to procedure of (20).

**Lipid peroxidation**

Lipid peroxidation was estimated using Thiobarbituric acid assay for Malondialdehyde (MDA) concentration according to (21,22).

**Statistical analysis**

Data of present study were analyzed using SPSS (Version 20) to find mean, standard deviation and least significant differences by ANOVA. Values where $P<0.05$ were considered significant.

**Results**

**DNA damage Markers**

According to Figure (1), DNA damage markers showed significant differences between control and patient groups according to statistical analysis at ($P<0.05$). The Comet length record highest Mean±SD in patients reached to 107.6±5.6µm as compared with the controls (22±2.1µm; Figure 2). The tail length was recorded in patients (6.64±1.9µm), while in controls (1.88±0.23µm; Figure 3). In addition, DNA% in serum of controls was (0.77±0.02%) while in diabetic patients it reached to (3.27±0.54%; Figure 4). On the other hand, the tail moment in patients was (1.38±0.34µm) and (0.55±0.02µm) in controls (Figure 5).
Figure 1: DNA damage makers in diabetic mellitus patient (B) and healthy controls (A).

Figure 2: Comet length in controls and diabetic patients.
Figure 3: Tail length in controls and diabetic patients.

Figure 4: DNA% in tail in controls and diabetic patients.
Biochemical markers

All biochemical markers showed significant differences between controls and diabetic patients. SOD activity (Figure 6) in serum of controls was (22±2.4U/mg), in patient reached to (201±21.9U/mg) while the activity of CAT in serum of controls was (15±2.8U/mg) and in diabetic patients was elevated to reach (180±22.4U/mg) (Figure 6). The GSH activity (Figure 6) showed significant differences between controls and diabetic patients. Its activity in serum of controls was (45±3.5µmol/ml) while its activity in patients rose to (280±34.5µmol/ml). Significant differences were seen between controls and patients in GPx activity (Figure 6). Its activity in serum of controls was (33±3.5µmol/ml); whereas it was elevated to 177±34.5µmol/ml in diabetic patients. The concentration of MDA was significantly different between controls and patients (Figure 6). Its activity in serum of controls was (15±1.07µmol/ml); whereas its activity was increased in diabetic patients to reach (190±6.7µmol/ml).
Figure 6: The biochemical markers in controls and patients with Type 2 diabetes mellitus.

Discussion

The association of diabetes mellitus with oxidative stress results in oxidation of proteins and lipids peroxidation. Also, effects on antioxidants status in initial effects of most toxic molecules that enter the body of human and organism can cause increasing activities of all antioxidants defense mechanisms. In the final stage of its effects, the antioxidant activity will be decreasing due to oxidative stress that occurs by free radicals and DNA damage (23). The oxidized base 8-OHdG in blood or urine can also be used as a DNA damage marker (24). Comet assay is used for measuring of DNA damage, as less susceptible method to artifacts than measuring 8-OHdG that detects very low levels of DNA damage (25). On the other hand, high levels of ROS in diabetes cause DNA strand breaks and base modifications (oxidation of guanine to 8-OHdG) (26,27). Other studies reported that there was no relation between diabetes and increased levels of DNA damage (28). This is possibly due to the difference in glycemic control, diabetes duration or the cell type used in the assay (27). The SOD and CAT activities were increased significantly in the serum of diabetic mellitus patients due to oxidative stress generated by free radicals that cause lipid peroxidation, imbalances in antioxidants defense and increased ROS formation (29). GSH regulates the intracellular redox status and acts as a cofactor in many metabolic reactions (30). A significant elevation was observed of GSH in serum of diabetic mellitus patients as compared with controls may be due to an imbalance in the redox state that induced compensatory response because of excessive H2O2 production, or as a result of overproduction for cell protection against oxidative stress (31) and increased demands on GSH utilization (32). The GPx is catalyzing the reduction of H2O2 to oxygen and water by using GSH as a substrate. The result of present study reported that GPx activity was also significantly increased in the serum of diabetic mellitus patients as compared with controls due to the response of antioxidant systems and to reflect the adaptation to oxidative condition (33). Lipid peroxidation plays key role in pathogenesis of diseases that occur by oxidative stress through imbalance between low production of antioxidant defense and high production of ROS that lead to increased lipid peroxide levels (34). The results of present study showed that the concentration of MDA was
significantly increased in serum of diabetic mellitus patients as compared with controls because oxidative stress that occurred by free radicals caused increased lipid peroxidation \(^\text{(34)}\).

**Conclusion**

The present study showed in patients with Type 2 diabetes mellitus, there is increased DNA damage through elevation in markers of DNA such as comet length, tail length, DNA\% in tail and tail moments the leukocytes of diabetic patients. In addition, there is significant increase in SOD, CAT, GSH, MDA and GPx activities.

**Ethical Clearance**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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**References**


