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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(1): 310-315. **OPEN ACCESS**

Oxidative Stress and DNA Damage in Type 2 Diabetes Mellitus Patients in Population of Baghdad

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Introduction and aim: Diabetes mellitus type 2 (DMT2) is a chronic disease effective to population and become epidemic worldwide. Diabetes is associated with excessive production of reactive oxygen species (ROS), which can damage cellular macromolecules. The aims of this study to investigate the relationship between oxidative stress and DNA damage in patients with diabetes mellitus type 2 and healthy control. The study population consisted of 40 patients with type 2 diabetes mellitus and 20 healthy controls. Blood sample were collected from patients and healthy control and Oxidative stress parameter represented by (SOD and MDA) and alkaline comet assay to detected DNA damage were done. The result of this study showed increase in DNA damage in patient with DMT2 compared with control this may be due to increase of oxidative stress which lead to increase genomic (DNA) instability and increase complication in DMT2 patient may be lead to increased risk of cancer.Type 2 diabetic patients have more oxidative DNA damage than healthy controls.

Keywords: Oxidative stress, DNA damage, Comet assay. complications

INTRODUCTION

Diabetes mellitus type 2 (DMT2) also known non-insulin dependent diabetes mellitus (NIDDM), is a metabolic disorder characterized by high blood glucose in association with insulin resistance and relative insulin deficiency. It is becoming one of the most challenging health problems in the 21st century as it has reached epidemic proportions worldwide. 285 million people are known to be suffering from T2DM globally which is expected to increase to 438million in 2030. India ranked first in the world for the prevalence of the disease, followed by China and USA. .The high prevalence of T2DM in India specifically West Bengal is a serious public health concern because it is the third highest cause of mortality (Ibarra-Costilla et al., 2009).

Several studies have given relationship between oxidative stress and diabetes by measuring many biomarker included DNA damage and products of lipid peroxidation. This studies believed that in the occur and developed of late diabetic complication, due to free radical which have major role in damage of lipids, proteins and DNA(Ayepola et al., 2014).

There is considerable evidence that hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues, and playing an important role in diabetic complications (Chandie et al., 2006; Yfjord et al., 2005). Oxidative stress leads to protein, lipid, and DNA modifications that cause cellular dysfunction and this could have teratogenic or carcinogenic consequences (Jee et al., 2005).

During the past ten years, there has been

increasing awareness of the effect of DNA damage in chronic diseases. Single cell gel electrophoresis (SCGE) or comet assay to measure DNA damage was first developed by (Ōstling and Johansson, 1984). It is a sensitive, simple, inexpensive, and rapid method that can be used to detect DNA damage to individual cells and reveal the presence of double-strand breaks, single-strand breaks and alkali-labile sites (Hartmann et al., 2003). It has been widely used in studies on DNA repair, genetic toxicology, radiation, pollution and ageing (Moller and Loft,2002; Haines et al, 2002).

Oxidative stress has been demonstrated in many studies to participate in the progression of diabetes which plays important role during diabetes, including impairment of insulin action and elevation of the complication incidence. Antioxidants have already shown to be prospective in the treatment of diabetes both type 1 and type 2. Increase in the levels of oxygen and nitrogen free radicals (ROS/RNS) has been linked with lipid peroxidation, non-enzymatic glycation of proteins and oxidation of glucose which contributes toward diabetes mellitus and its complications. Most of the studies have shown relationship between oxidative stress and diabetes along with their complications related to heart, liver kidney and eye. Thus, oxidative stress seems to be more worrying in metabolic disorders specially diabetes type 2.

MATERIALS AND METHODS

Study design:

Whole blood sample were obtained from 40 patients with DMT2 and 20 healthy control from some physicians private clinics, to confirmed patient with DMT2 by measured fasting glucose (>120 mg/dl) while healthy control have normal glucose metabolism and non-had family history with diabetes.

Collection of blood:

Five milliliter from peripheral blood were collected by vein puncture from patients DMT2 and healthy control and divided in to two tubes one with EDTA for comet assay study and another with plan tube to separate serum.

Serum parameters:

Separated of serum from whole blood without anticoagulant by centrifuge and tests done: Glucose (Spinreact, S,A Spain) kit. Superoxide dismutase SOD and Malondialdehyde MDA (Cohesion, China) kits.

Serum parameters:

1-Glucose: serum glucose were measured after fasting by glucose kit (Spinreact, Spain).

2- HbA1C:

3- Superoxide Dismutase (SOD): measured by using micro plate assay kit (cohesion Biosciences, Chinas).

4- Malondialdehyde MDA measured by using micro plate assay kit (cohesion Biosciences, Chinas).

Alkaline comet assay:

Comet assay procedure done according to: Singh et al., (1988).

Separation of lymphocyte:

Whole blood with EDTA (3ml) mixed with 5 ml PBS, add 5 ml from mixed and drain to 10ml test tube contain 3ml ficoll (histopaque-1077 sigma chem, USA) and centrifuged 30 mins at 400g 20ºC this procedure separation buffy coat layer of lymphocyte by gradient density technique, separated the lymphocyte from buffy coat and wash twice with PBS and finally suspended with RPMI-1640 medium.

Slide preparation:

frosted microscopic slide coated with agarose low melting , mixed 100µl from low melting agarose with 20µl from cells at concentration 10^6/ml, take 100 µl on forested slide with cove slipped gently, Remove the cove slipped after agarose solidification at 4ºC.

Lysis of cells, gel electrophoresis, DNA staining:

After incubation at 4°C to allow the formation of agarose gel, lysis was carried out by submerging the slides in lysis buffer (2.5% sodium dodecylsulfate, 1% sodium sarcosinate, and 25 mM ethylene-diaminetetraacetic acid, pH 9.5) for 15 min at 25°C to 30°C. Slides were washed for 5 min in distilled water at 10°C and electrophoresed (90 mM Trizma base, 90 mM boric acid, and 2.5 mM ethylene-diaminetetra-acetic acid, pH 8.3) at 2 V/cm for 5 min at 10°C. After a brief rinse in distilled water, slides were air dried at 45°C on a hot plate and stored in a cool humid box until use. Comets were stained with SYBER green after rehydration of slides in distilled water for 5 min and observed under an Olympus fluorescence microscope (Olympus Optical Co., Tokyo, Japan).

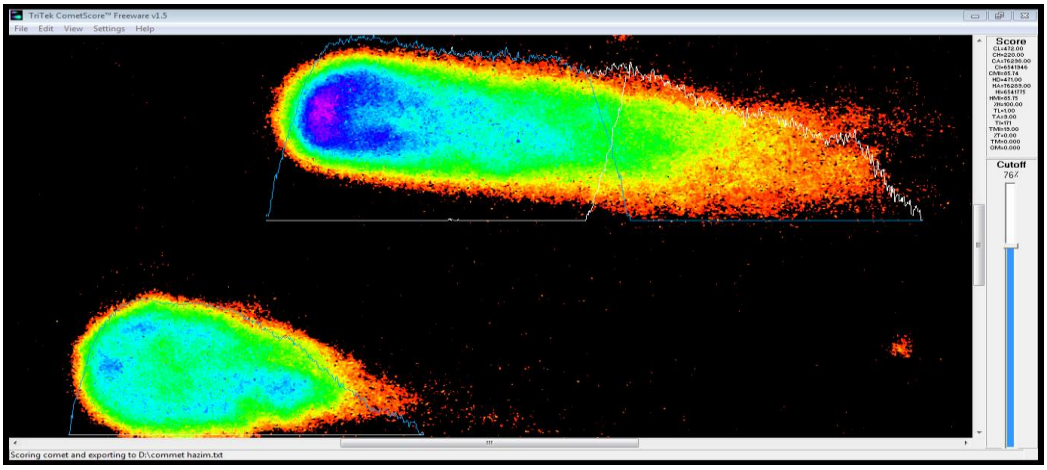

Figure 1. Example of scoring and analysis for comet assay by comet score program (AL-Ahmed, 2015)(11)

Comet scoring:
Chosen 100 cells from each slide randomly under fluorescent microscope, capture image and analyzed by using comet score program Comet tail DNA %, Tail length (µm) and Olive tail moment. figure1

Statistical analysis:
Comparisons quantitative data were handled by means of ANOVA tests. Statistical analysis was performed using the statistical package SPSS for windows (version 13, SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was adopted to indicate statistical significance for each test.

RESULTS AND DISCUSSION

The results of the present study showed significant increase in glucose and HbA1C in patients with DMT2 compared with healthy control, Table (1).

Table (1): Serum Fasting glucose and HbA1C levels in DMT2 patients and healthy control.

Parameters groups	Fasting glucose Mg/dl (mean+SD)	HbA1C (%) (mean+SD)
Control	A 98.47+11.09	A 4.93+1.06
DM2	B 143.62+12.84	B 8.06+2.11

Table (2):Serum antioxidant level in DMT2 patients and healthy control.

Parameters groups	SOD Ug/ml (mean+SD)	MDA nmol/ml (mean+SD)
Control	A 13.41+3.49	A 7.44+1.52
DM2	B 5.52+1.84	B 12.28+2.85

Table (2) showed the significant decrease in SOD while significant increase in MDA in patient with DMT2 (5.52+1.84, 12.28+2.85 respectively) compared with control group (13.41+3.49, 7.44+1.52).

Superoxide dismutase provides first line defense against ROS mediated cell injury by catalyzing the proportion of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide. We can say that superoxide is dismutated to other compounds that are less toxic by SODs (Tiwari et al., 2013).

Oxidative stress, capable of eliciting damage to various biomolecules including DNA, is a recognized component of diabetes mellitus and its complications. Metabolic syndrome is associated with the development of DMT2, as well as other unfavorable outcomes. Type 2 diabetes mellitus (T2DM) is one of the most prevalent complications of metabolic syndrome, as well as the condition of elevated oxidative stress, resulting from both increased production of reactive oxygen species (ROS) and disturbed antioxidant defense system (Rochette et al., 2014). ROS are intrinsically generated as by-products of normal aerobic metabolism and inflammatory/immune response to various challenges (Valko et al., 2007). Exposure to the external sources of ROS, such as radiation, environmental pollutants and toxins, also contributes to oxidative stress capable of inflicting irreversible damage to biomolecules. The increased production of ROS activates various mechanisms of defense and repair of oxidative damage (Valko et al., 2007, Halliwell and Gutteridge, 2015).

Comet score analysis of DNA damage in patients with DMT2 and healthy control summarized in Table (3). DMT2 patients showed high significant increase in DNA damage

compared with healthy control (**Figure 2)**.

Table (3): Comet parameters in DMT2 patients and healthy control.

Parameters groups	Comet tail DNA %(mean+SD)	Tail length (µm) (mean+SD)	Olive tail moment (mean+SD)
Control	A 19.21+3.35	A 10.17+2.18	A 2.31+1.08
DM2	B 33.85+7.41	B 24.52+5.08	B 3.07+1.25

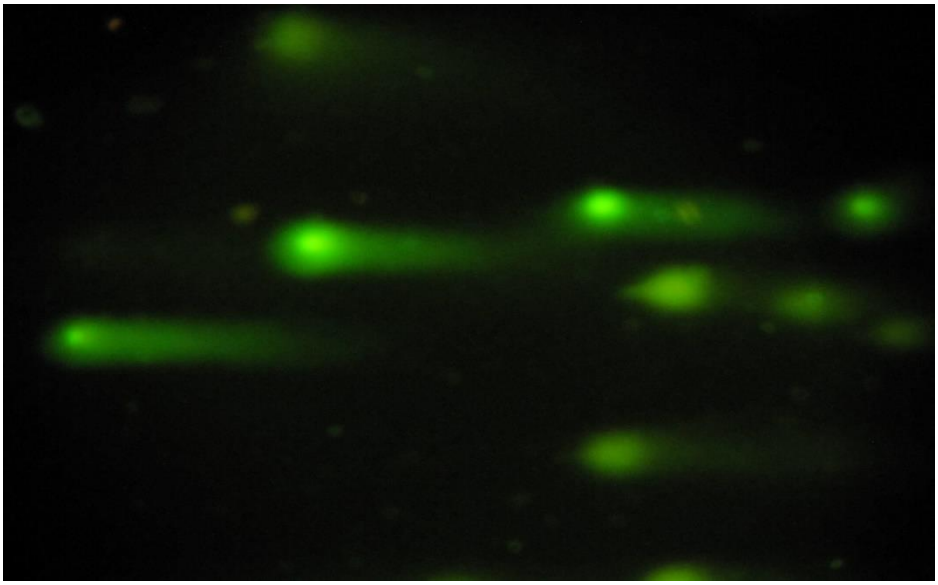

Figure (2). The examples of scoring categories for comet assay in Type 2 Diabetes Mellitus Patients.

In the present study, we used the comet assay as a measure of DNA strand-break damage, as the technique is a sensitive, simple method to detect very low levels of damage (Collins et al., 2004).
The present study revealed an increased number of DNA strand breaks in peripheral blood leukocytes of diabetic patients compared to healthy controls. The production of ROS and lipid peroxidation are increased in diabetic patients (Woods et al., 1997). Reactive oxygen species can damage cellular macromolecules, leading to DNA and protein modification and lipid peroxidation. The elevated ROS in diabetes can cause strand breaks in DNA and base modifications including oxidation of guanine residues to 8-OHdG, an oxidized nucleoside of DNA, which is the most frequently detected and studied DNA lesion (Dandona et al., 1996). Previous studies concerning DNA damage and diabetes revealed contradictory results. Several studies showed an increased extent of DNA damage in type 2 diabetic patients compared to controls (Choi et al.,2005; Pitozzi et al., 2003; Blasiak et al., 2004). On the other hand, other studies showed the lack of association between diabetes and increased DNA damage levels (Hannon-Fletcher et al., 2000; Ibarra-Costilla et al., 2009). The discrepancy between different studies is, possibly, due to difference in glycemic control, duration of diabetes or the type of cell used in the comet assay (Blasiak et al., 2004).

CONCLUSION

Type 2 diabetic patients have more oxidative DNA damage than normal controls.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We thank staff of institute of genetic engineering and workers in biotechnology research center in Al-Nahrain University for the help in the laboratory tests.

AUTHOR CONTRIBUTIONS

RAM proposing the initial idea for the study and also wrote the manuscript. MHH designed the tables and performed comet assay experiments. HIA sample collection and performed SOD and MDA tests, wrote the manuscript, data analysis. All authors read and approved the final version.

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