Evaluation of DNA damage and Environmental Antioxidants defense systems in type 2 diabetes mellitus Patients

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Abstract. The presents study was conducted to evaluate the DNA damage markers, antioxidants makers and lipid peroxidation in type 2 diabetes mellitus patients, the results of presents study was showed the DNA damage markers such as comet length, tail length, %DNA in tail and tail moments were significantly increased at p<0.05 in diabetic patients as compared with healthy control group. Also all biochemical markers such as superoxide dismutase SOD, catalase CAT, Glutathione peroxidase GPx, Glutathione GSH and Malondialdehyde MDA were significantly elevated with diabetic patients as compared with healthy control

Keywords: DNA damage, Comet assay, Antioxidants defense, Oxidative stress and Diabetic mellitus

Introduction

World Health Organization demonstrated that diabetes affects more than 170 million people worldwide and in 2030, this number will rise to 370 million (World Health Organization, 2004). Microvascular and macrovascular complications of diabetes mellitus are the leading cause of mortality and morbidity (American Diabetes Association, 2002). Many biological pathways, such as glucose autoxidation, polyol pathway, prostanoid synthesis and protein glycation are triggered in hyperglycaemia state leading to increased production of free radicals (Guigliano et al., 1996). Oxidative stress is being considered as a common pathogenic factor in diabetes mellitus and its complications (Lehmann and Schleicher, 2000). The most important free radicals that cause oxidative stress are superoxide, hydroxyl radical and hydrogen peroxide. In human, there are antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione (GSH) and its enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx) & glutathione-s-transferase, which scavenges the action of free radicals in order to protect the body (Dincer et al., 2002). A number of studies have estimated the status of oxidative stress in diabetics. Some researchers have reported oxidative damage in Diabetic mellitus patients (Kedziora-Kornatowska et al., 1998 and Dincer et al., 2002).

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 (Yjford et al., 2005 and Chandie et al., 2006).
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 Östling and Johansson in 1984 (Östling 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, 2003 and Haines et al., 2003).

Material and methods

Collection of blood samples

Five milliliters of venous blood was withdrawn from both healthy individuals (No. 10 individual) and patients fasting (30 individual) for 10 hours into two sterile vacutainers, one containing EDTA and the other without additives to separate serum.

DNA damage measurements

Taken a 100µl of venous blood is placed in a 2 ml microcentrifuge tube containing 1.5 ml of phosphate buffer solution, then added 40µl of proteinase K to remove protein and Centrifuged at 13000 r.p.m for 15min, 4°C, taken 2-5 µl of suspension cell to a clean 1.5 ml tube and mix on Comet slide with 40µl of low melting agarose, then prepare lysis solution that consist of 2.5M NaCl, 100 mM EDTA, 10 mM Tris-base and 8g NaOH, all dissolved and complete to 700 ml deionized water, then added 110 ml from 55 ml 1% triton X and 55 ml 10% dimethyl Sulfoxide after that complete the volume to 100 ml by deionized water and chill at 4C or on ice for at least 20 min before use, and combine 7.5µl with 75µl low melting agarose and immediately spread the mix onto the clear part of a comet slide, then warm comet slide on a heating plate at 42-50 C before application to prevent permit evenly spreading of the agarose and prevent the formation of air bubbles, slides may be stored in lysis solution at 4C for 60 minutes, after that, remove the lysis solution and replace by alkaline solution contain 6 g NaOH and 500 µl 0.5% Na2EDTA for 5-60 minutes at room temperature in dark, then remove slide from alkaline solution gently tap excess buffer from slide and wash by immersing in 1X TBE buffer for 5 minutes, after that, transferred slide from 1X TBE buffer to an horizontal electrophoresis apparatus and place slides on flat of gel tray and pour 1X TBE buffer until to cover the slides for 60 min, vol 70, then very gently tap off excess TBE and added some drops of 70% ethanol on slides to remove the water, and stain the slides by ethidium bromide then leave the slides for 24 hour and view slide by fluorescence microscope (Singh et al, 1988).

Environmental Antioxidants defense measurements

Superoxide dismutase activity SOD was determined by autooxidation of Pyrogallol according to Marklund and Marklund, (1974). While Catalase activities was measured according to procedure of Clariborn, (1985) and Aebi, (1974) but Glutathione activity was determined according to the method of Moron et al, (1979), the acid soluble sulfhydryl groups form a yellow colored complex with dithionitrobenzene (DTNB). The activity of glutathione peroxidase was investigated according to procedure of Rotruck et al.,(1973).

Lipid peroxidation

Lipid peroxidation was estimated by the Thiobarbituric acid assay for Malondialdehyde (MDA) concentration according to Aust, (1985) and Burtis, (1999).

Statistical analysis

Data of present study was analyzed according to the system of statistical package for social science (SPSS) to found means, Standard deviation, Least Significant differences by ANOVA.
Results

DNA damage Markers

According to the picture was taken figure (1), the DNA damage markers were showed significant differences in control and patient according to statistical analysis at (p<0.05). the Comet length record highest Mean±SD in patients was reached to (107.6±5.6) µm as compared with control (22±2.1) µm figure (2). The tail length was recorded in patients (6.64±1.9) µm While in control (1.88±0.23) µm figure(3).While the % DNA in tail in control of serum was (0.77±0.02) % While in diabetic patients was reached to (3.27±0.54) % figure (4). Whereas the tail moments in patients (1.38±0.34) µm and (0.55±0.02) µm in control figure (5).

Figure 1: DNA damage makers in diabetic mellitus patient and healthy control A:

Figure 2: Comet length in control and diabetic patients
Figure 3: Tail length in control and diabetic patients

Figure 4: DNA % in tail in control and diabetic patients

Figure 5: Tail moment in control and diabetic patients
Biochemical Markers

All biochemical markers was showed significant differences between control and diabetic patients, the superoxide dismutase SOD activity in serum of control were (22±2.4) U/mg, in patient was reached to (201±21.9)U/mg while the activity of catalase in control of serum was (15±2.8) U/mg and in diabetic mellitus patients which elevated to reach (180±22.4) U/mg.

The Glutathione GSH activity was showed significantly differences between control and diabetic patient, it’s activity in control of serum was (45±3.5) µmol/ml, while activity in the patient of diabetic was rised to (280±34.5) µmol/ml.

Significantly differences in control and patients in glutathione peroxidase GPx activity, it’s activity in serum control was (33±3.5) µmol/ml and the activity were elevated to (177±34.5) µmol/ml in diabetic patients. The concentration of Malondialdehyde MDA was significantly difference between control and patients in serum, it’s activity in control of serum was recorded (15±1.07) µmol/ml, its activity was increased in diabetic patients to reach (190±6.7) µmol/ml (figure 6).

![Figure 6: The biochemical markers in control and patients of diabetic mellitus type 2](image)

Discussion

The association of diabetes mellitus with oxidative stress, leading to oxidation of protein, lipid peroxidation. Also effects on antioxidants status in initial effects of most toxic molecule that inter the body of human and organism can cause increasing the activities of all antioxidants defense. While in the final stage of its effects, the antioxidants activities will be decreasing due to oxidative stress that occur by free radicals and DNA damage, oxidative DNA damage is usually evaluated using the comet assay in blood cells (Choi et al., 2005), or by measuring the oxidized base 8-OHdG (8-hydroxy-2-deoxy-guanosine) in blood cells or urine (Hinokio et al., 1999). In the current study, we have been used the comet assay for measuring of DNA strand-break damage, as the technique is less susceptible to artifacts than measuring 8-OHdG, and it is a sensitive, simple method to detect very low levels of damage (Collins et al., 2004).

The present study revealed an increased in markers of DNA such as comet length, tail length, %DNA in tail and tail moments in peripheral blood leukocytes of diabetic patients compared to healthy controls. The production of ROS and lipid peroxidation are increased in diabetic patients due to hyperglycemic (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). Several studies showed an increased extent of DNA damage in type 2 diabetic patients compared to controls (Blasiak et al., 2004). On the other hand, other studies showed the lack of association between diabetes and increased DNA damage levels (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).

The Superoxide dismutase SOD plays essential role in scavenging of superoxide free radical and converted to H$_2$O$_2$ and O$_2$ to maintain a balance between oxidants and antioxidants While catalase CAT convert the H$_2$O$_2$ to O$_2$ (Sandstro et al.,1994), the result of presents study was shown, the superoxide dismutase (SOD) and catalase (CAT) were significantly increased at (P<0.05) in serum of diabetic mellitus patients as compared with control due to oxidative stress was generated by free radicals that cause induction lipid peroxidation, imbalances in antioxidants defense and increased ROS formed (Stralin, Marklund, 1994).

Glutathione GSH is the essential compound in the regulation of intracellular redox status and its considered as an important cofactor in many metabolic reactions (Van Bladeren, 2000). The results of presents study, observed significant elevation of GSH in serum of diabetic mellitus patients as compared with control may be due to a compensatory response induced by imbalance in the redox state of the cell as the result of excessive H$_2$O$_2$ production (Campolo et al., 2006), or the GSH level increases as a results of overproduction for protection the cells from oxidative stress, therefore the capacity to increase GSH synthesis in response to increased demands on GSH utilization (maher, 2005).

The GPx was catalyzed the reduction of H$_2$O$_2$ to oxygen and water by using glutathione as a substrate (Mates et al., 1999). The result of presents study pointed the GPx activity also significantly increase in serum diabetic mellitus patients as compared with control due to the response of antioxidants systems and to reflect the adaptation to oxidative condition (Maritim et al., 2003)

Polyunsaturated fatty acids contain two or more double bonds which are readily oxidized by reactive oxygen species to produce lipid peroxy radicals and lipid hydroperoxides are called lipid peroxidation which is started by abstraction of hydrogen atom from polyunsaturated fatty acid moiety of membrane phospholipids by attacking of reactive species lead to form fatty acid radicals that would react with adjacent lipid molecules and generate new free radicals, lipid peroxidation products breakdown cause the formation of many reactive aldehydes such as malondialdehyde which is highly reactive with protein and DNA molecules lead to form adduct with these macromolecule (Niki et al., 2005), lipid peroxidation play key role in generation of pathogenesis disease that occur by oxidative stress through imbalance in low production of antioxidant defense and high production of reactive oxygen species that lead to increase in lipid peroxide level (Pasupathi et al., 2009). The results of present study showed that the concentration of malondialdihyde was significantly increased in serum of diabetic mellitus patients as compared with control because the oxidative stress that occur by free radicals that cause increase in lipid peroxidation (Pasupathi et al., 2009).

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