Detection of Biochemical Causes the Diabetic Retinopathy in Diabetes (Type II) Patients in Al-Muthanna Province

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Abstract

**Background:** Accumulating evidence indicates that oxidative stress, imbalance between reactive oxygen species production and antioxidant scavenging that may play a role in the etiology of type 2 diabetes mellitus and occurrence diabetic retinopathy as a result from increasing activity of free radicals and accumulation of lipid peroxidation products.

**Aim:** To investigate the association between glycemic control, lipid peroxidation and antioxidant status.

**Methodology:** A descriptive cross-sectional study on non-probability was conducted (sample collection) of (232) type 2 diabetes mellitus patients. Plain tubes, the tools and instruments that were used to collect data.

**Results:** The results of the study revealed that dyslipidemia and hypertension were found to be more prevalent in the diabetic retinopathy subjects than the regular diabetic subjects. Plasma antioxidant levels were higher in the diabetic subjects than the diabetic retinopathy subjects while malondialdehyde levels were found to be higher in the diabetic retinopathy subjects.

**Conclusion:** Duration of diabetes has very important effect on both MDA and uric acid levels for diabetic patients with/without retinopathy. Oxidative parameters value not influence by residence as well as body mass index not has any effect on uric acid level.

**Keywords:** Antioxidant Status, Oxidative Stress, Diabetic Retinopathy, Lipid Profile.

Introduction

Diabetic retinopathy (DR) is the most common disease that caused by micro-vascular complication of diabetes, occurs in both T1, T2 DM and is secondary to prolonged uncontrolled hyperglycemia and other risk factors. In adolescents and young adults with type 1 diabetes, over 80% have some form of DR when the duration of diabetes is over 15 years. It is estimated that 90% of the blindness due to DR is preventable (¹) (²). Long term of high blood glucose levels in uncontrolled diabetes can be the cause of glucose auto-oxidation, nonenzymatic protein glycation and activation of the polyol pathway with increase oxidative stress.

The global number of individuals with diabetes in 2000 was estimated to be 171 million (2.8% of the world's population). The number of people with DM worldwide is projected to increase to 366 million by 2030, 298 million of whom will live in developing countries (³) (⁴). There is a relationship between chronic hyperglycemia and long-term complications in diabetes. The long-term effects of diabetes include progressive evolution of micro-vascular complications, particularly in the eye and the kidney, and an increased frequency of macro-vascular disease such as peripheral vascular and coronary heart disease (³) (⁵). Complications of micro-vascular and macro-vascular are the leading cause of

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morbidity and mortality in diabetics. However, diabetic patients often die from macro-vascular disease and main mortality is the coronary heart disease (CHD). Long-term complications of DM include retinopathy with potential vision loss (6) (7) (8) (9).

Diabetic retinopathy (DR) is the most common disease that caused by micro-vascular complication of diabetes, occurs in both T1,T2 DM and is secondary to prolonged uncontrolled hyperglycemia and other risk factors. In adolescents and young adults with type 1 diabetes, over 80% have some form of DR when the duration of diabetes is over 15 years. It is estimated that 90% of the blindness due to DR is preventable (1) (2). Long term of high blood glucose levels in uncontrolled diabetes can be the cause of glucose auto-oxidation, nonenzymatic protein glycation and activation of the polyol pathway with increase oxidative stress.

Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant ratio in favor of the former, potentially leading to form macromolecules and dysfunction in the cell Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant ratio in favor of the former, potentially leading to form macromolecules and dysfunction in the cell. Improved oxidative stress contributes to the damage of pancreatic β-cell progressively due to glucose toxicity, which leads to severe weakness of glucose-stimulated insulin secretion (11).

In normal physiological conditions, there is a balance in the generation of reactive oxygen and nitrogen species (ROS, RNS) and antioxidant defense system to protects organisms against ROS, RNS toxicity. In diabetes, imbalance in the pro-oxidant/antioxidant can damage cellular macromolecules, leading to protein modification and lipid peroxidation (12). Lipid peroxidation is an autocatalytic free radical mediated destructive process where poly unsaturated fatty acids in cell membranes degraded to form lipid hydro peroxides. Through lipid peroxide products such as conjugated dienes and malondialdehyde (MDA) are increased in patients with T2DM. MDA is produced as a relatively stable end product from the oxidative degradation of polyunsaturated fatty acids (PUFA), and this free radical-driven lipid peroxidation has been causatively implicated in the aging process atherosclerosis, Alzheimer's disease, and cancer (13) (14). Serum MDA used as a bio-marker of lipid peroxidation and served as an indicator of free radical damage (15).

The study of the oxidative stress status may be the knowledge base for understanding of the pathogenic mechanisms of complications in diabetes and may have important implications for antioxidant supplements in order to slow progress, choose optimal treatments and prevent complications and their consequences.

**Materials and Methods**

232 subjects as three groups, 58 patients (24 male and 34 female) have diabetic retinopathy, 78 patients (26 male and 52 female) have type 2 diabetes mellitus without retinopathy cases and 96 subjects (52 male and 44 female) as healthy control groups. For the purpose of this study, 232 subjects and age and gender-matched control subjects were recruited. Plain tubes, the tools and instruments that were used in the study.

Metabolic biochemical markers for investigation

1. Estimation of fasting plasma sugar by glucose oxidase/peroxidase method Trinder’s method.

2. Measurement of total cholesterol by Cholesterol esterase peroxidase or CHOD-POD method

3. Measurement of triglycerides by Lipoprotein lipase, Glycerol phosphate oxidase and Peroxidase. The method for the analysis is a modification of that of Trinder.

4. Measurement of malondialdehyde; MDA is determined as Thiobarbituric acid reactive substances.

5. Measurement of superoxide dismutase; Superoxide dismutase was measured by the method of Kuthan H, et al., (1986).

6. Measurement of total antioxidant capacity; Measures total antioxidant capacity in which Cu²⁺ is reduced by antioxidant to Cu⁺. The resulting Cu⁺ specifically forms a colored complex with a dye reagent. The color intensity at 570 nm is proportional to TAC in the sample.

**Statistical Analysis**

Completely randomized design (CRD) was used...
for this study in the analysis of variance for the means of studied parameters (sugar, lipid, oxidative stress, and antioxidants parameters) by using one-way ANOVA test, t-test, and Dennett’s test at a 5% level of significance. Moreover, data were processed and analysis by using statistical program social science (SPSS 22) and the results were expressed as Mean ± SD.

Results and Discussion

Figure (1): Comparison of HbA1c and FBS means among study groups.

The study showed that T2DM and DR subjects had significantly higher FPS and HbA1c levels compared with control subjects according to reports of the American Diabetes Association (ADA) and the World Health Organization (WHO). Furthermore, increased HbA1c level was observed in DR more than T2DM. The results of the study under hand go along with study entitled (Total Antioxidant Status in Type 2 Diabetic Patients in Palestine) that carried out in Palestine found that there is a significant relationship between HbA1c and different group at (\( \square = 0.002 \)) \(^{(16)}\). Also, in other study that carried out in African stated that the FPG and HbA1c levels were respectively 2.05 and 2.32 times higher in the group of patients with diabetes and complications compared to those of healthy persons \(^{(17)}\). HbA1c is a product of non-enzymatic addition of glucose to hemoglobin and the rate of this conjugation is directly proportional to the level of blood glucose. So, the high % of HbA1c level observed in our study denotes that the type 2 diabetic subjects are under poor glycemic control.
Figure (2): Comparison of lipid parameters means among study groups, concentration unite (mg/dl).

This figure shows that significantly high levels of total cholesterol, triglyceride, LDL and VLDL in patients with T2DM than control. However, no significant difference in the level of HDL were identified between the three groups. These findings not harmonizing with study that conducted in Turkey stated that mean total cholesterol, triglyceride, LDL,HDL and VLDL levels were not significantly different between the groups ( =0.693, =0.774, =0.644, = 0.910 and =0.967 respectively (18). However, it is going along with study that carried out in South Africa found that serum triglyceride (TG) ( = 0.004) and high-density lipoprotein cholesterol (HDL-C) ( = 0.007) showed significant differences among group (19). Moreover, study the proportions of lipid profile disorders were higher in DM patients compared to apparently healthy controls Moreover, study the proportions of lipid profile disorders were higher in DM patients compared to apparently healthy controls (20) (21).

Table (1) : Comparison of oxidative stress parameters means

<table>
<thead>
<tr>
<th>Oxidative Parameters</th>
<th>Control Mean±SD</th>
<th>DM Mean±SD</th>
<th>Retinopathy Mean±SD</th>
<th>P value</th>
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<tbody>
<tr>
<td>Uric acid</td>
<td></td>
<td></td>
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<tr>
<td>mg/dl</td>
<td>4.5±1.5</td>
<td>4.9±1.3</td>
<td>4.7±1.2</td>
<td>a- &lt;0.0001*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>b- 0.064</td>
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<td></td>
<td></td>
<td></td>
<td>c- &lt;0.0001*</td>
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<td></td>
<td>d- 0.0001*</td>
</tr>
<tr>
<td>MDA µM</td>
<td>2.3±0.7</td>
<td>4.9±0.5</td>
<td>5.2±0.6</td>
<td>a- &lt;0.0001*</td>
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<td>b- &lt;0.0001*</td>
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<td>c- &lt;0.0001*</td>
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<td>d- &lt;0.0001*</td>
</tr>
<tr>
<td>SOD U/ml</td>
<td>7.8±1.3</td>
<td>4.3±0.9</td>
<td>3.5±1.1</td>
<td>a- &lt;0.0001*</td>
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<td>b- &lt;0.0001*</td>
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<td>c- &lt;0.0001*</td>
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<td>d- &lt;0.0001*</td>
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<tr>
<td>TAC mM</td>
<td>1.3±0.6</td>
<td>1±0.3</td>
<td>0.7±0.3</td>
<td>a- &lt;0.0001*</td>
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<td>d- &lt;0.0001*</td>
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The result of current study goes along with study that conducted in Nigeria to investigate some oxidative stress related parameters in T2DM. Who found that significant differences of oxidative stress between patients and control groups (22). While, in regarding to uric acid the current study shows no significant difference in the level of uric acid between T2DP and healthy controls, and that inconsistent with study that carried out in India found that there is a significant difference between uric acid and three different group of study at P<0.001(23). Glucose intake may affect both the production and excretion of uric acid by several mechanisms. An increased flux of glucose is thought to increase purine generation, while increased anaerobic glycolysis generates increased circulating lactate which leads to a reduction in renal excretion of uric acid (24).

Moreover, in other study that carried out in Africa reported that a statistically higher level of malondialdehyde (MDA) was observed in a group of patients with diabetes and complications compared to those without complications this finding compatible with study under hand (17). The high concentration of serum lipids in the type 2 diabetic subjects is mainly as a results of increased mobilization of free fatty acids from peripheral depots, due to loss of the inhibitory action of hormone sensitive lipase. Peroxidation of polyunsaturated fatty acids in blood produces malondialdehyde (MDA) that leads to oxidative damage. T2DP has reported an increased levels of lipid peroxidation (MDA) and reduced antioxidant status may play a major role in diabetic complications (25). Pavithra et al. reported higher levels of MDA in patients with DM compared with controls and in patients with DR versus those without DR, also suggesting that oxidative stress may play a significant role in the development of DR. As a result, levels of MDA are used as an index of LPO and in consequence of oxidative stress (2). Hyperglycaemia leads to overproduction of ROS through several pathways that may lead to destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress (26).

Financial disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Chemistry and all experiments were carried out in accordance with approved guidelines.

References


