**Determination of Lipid Peroxidation, Lipid profile and Vitamins A, E in Type II Diabetes Mellitus**

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**ARTICLE INFO**

**Article History**
Received: 30/6/2015
Accepted: 22/8/2015

**Keywords**:
Vitamins A, E
Type II Diabetes Mellitus

**ABSTRACT**

**Background**: Type 2 diabetes is the commonest form of diabetes and associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species (ROS) and oxidative stress, the aim of present study was to Determined of Lipid Peroxidation, Lipid profile and Vitamins A, E In Sudanese with Type II Diabetes Mellitus.

**Methodology**: 100 healthy subject were control group with mean FBS= 5.61 m. mol/L. The age ranged from (25-78) years old. The mean age average was 50.2 (years). Results: shows significant raised of the means of the plasma levels of malondialdehyde, fasting plasma glucose, cholesterol, LDL, triglyceride, and HbA1c of the test group when compared with healthy control group subjects, whereas the means of the plasma levels of antioxidant vitamins (A, E), and HDL showed significant reduction when compared with that of control groupsignificant difference when compared with healthy subjects.

**Conclusion**: The present study shows that significant lipoprotein abnormalities in type 2 diabetic patients when compared with healthy subjects. The increased level of serum MDA and lower level of serum vitamin E clearly shows that diabetic patients was exposed to an increased oxidative stress via lipid peroxidation.

**INTRODUCTION**

Type 2 diabetes is the commonest form of diabetes and associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species (ROS) and oxidative stress(Kumawat M, et al. 2012). In diabetes, there are more mechanisms that induce oxidative stress than in normal individuals; glucose auto-oxidation, non-enzymatic glycation of protein, and polyol pathway.

These pathways enhance generation of reactive oxygen species (ROS) that leading to the tissue damage and cause several complex syndromes in diabetic patients such as cataracts, renal dysfunction, nerve damage, and atherosclerosis.

Especially, atherosclerosis leading to the coronary heart disease (CHD) is the major cause of death among diabetics (Thavatchai P, et al. 2006). Atherosclerosis is the thickening and rigidity of the artery and arterioles. Its pathogenesis begins with oxidation of low density lipoproteins (LDL-c) by ROS. Increased lipid peroxidation (measured as levels of malondialdehyde or MDA) caused crosslink formation between single molecules of proteins and oxidation of LDL particles and led to the oxidized LDL formation. The oxidized LDLs cannot be recognized by LDL receptors and be scavenged by macrophages Lipid to generate foam cells. After these foam cells accumulation, fatty streak will form and progress to a fibrous plaque and finally to the atherosclerosis. Restriction of blood supply in the coronary arteries causes myocardial infarction and sudden death (Giugliano D, et al. 1996).

Type 2 diabetes mellitus is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. There is increasing evidence that, in certain pathological states, the increase production and / or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury. Endothelial dysfunction is considered an intrinsic element in the pathogenesis of diabetic angiopathies. A variety of potential mechanisms diabetes for the initiation of endothelial dysfunction of type 2 diabetes have been described including the effects of hyperglycemia, advanced glycation end products (AGE) and dyslipidemia (Lee BL, et al. 1992). In addition, hyperglycemia has been showed to induce free radical release and reduce anti-oxidant defense, both which are associated with endothelial dysfunction (Yi-Cheng, et al. 2010).

Vitamins A, C and E are diet – derived and detoxify free radicals directly. They also interact in recycling processes to generate reduced forms of the vitamins, α-Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated and recycled by glutathione. These vitamins also foster toxicity by producing prooxidants under some conditions. Vitamin E, a component of the total peroxyl radical-trapping antioxidant system (Weber P, et al. 1997), reacts directly with peroxyl and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation. The deficiency of vitamin E is concurrent with increasing peroxides and aldehydes in many tissues. There have been conflicting reports about vitamin E levels in diabetic animals and human subjects. plasma and / or tissue levels of vitamin E are reported to be unaltered (Feroozrai M., et al. 2006). Increased, or decreased by diabetes. Discrepancies among studies in terms of preventive or deleterious effects of vitamin E on diabetes induced vascular aberrations may arise from the variety of examined blood vessels or the administered dose of vitamin E (OnyesomL., et al.2011 and Sarita N, et al. 2011).

MATERIALS AND METHODS

100 healthy subject were control group with mean FBS= 5.61m. mol/L., The age ranged from (25-78) years old. The mean age average was 50.2 (years). Type 2 diabetic patients were 200. The
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Ages ranged from (30-80) years old. The mean age average was 51.1 (years). All samples were in a state of fasting for 12 hours before drawing blood were obtained on these samples Advance diagnostic center in Khartoum, Sudan form the period between May 2013 until August 2015.

MDA in serum performed as described by Muslih et al. in brief, serum was mixed with 20% TCA and allowed to stand for 10 minutes. After that 0.05m H2So4 and TBA were added. The mixture was mixed and place in 70 °C water bath for 30 min. The resulting chromogen was extracted with n-butanol and centrifuged at 2000 rpm / min, and measured against butonol blank at 532 nm excitation and 553 nm emission by spectrophotometer. These antioxidant vitamins were assay by HPLC. In brief, serum, α-tocopheryl acetate Lipid Peroxidation, Lipid profile and Vitamins A, E in Type II Diabetes Mellitus as internal standard and ethanol was mixed for 15 sec. The hexane was added and mixed vigorously for 2 min. The tube was centrifuged at 5198xg, 4°C for 5 min. The hexane layer was transferred and evaporated under a stream of nitrogen gas. The lipid residue was dissolved in ethanol and injected into the sphere clone 5 μ ODS, 250 × 4.60 mm of HPLC. The mobile phase was methanol: acetonitrile: chloroform (25:60:15) at a flow rate 1.5 ml/min. Vitamin A and E were detected at 290 nm (Muslih R.K, et al. 2005).

Statistical analysis:

Statistical Package for Social Science SPSS (version 13) computer software was used for data analysis. The means and standard deviations of variable calculated and T-test (paired samples) was used for comparison (significant level was set at P ≤ 0.05).

Ethical consent:

The study was approved by College of Medical Laboratory Science, University of Science and technology in Khartoum Region, Sudan. All study subjects consented for participation by completing the self-administered questionnaire.

RESULTS

In this study 300 participants from Advance diagnostic center in Bahri were investigated for the MDA, Lipid Peroxidation, Lipid profile and Vitamins A, E in Type II Diabetes Mellitus. The ages ranged from (30-80) years old. The mean age average was 51.1 (years). Table 1The results of this study showed the baseline characteristics of the test group and control group. and there is no significant difference in age between the two groups Age mean ±SD was 46.2±14.0 for the control group versus 53.1±11.1 for the test group (P = 0.06). Weight, height and BMI showed significant differences between the test and control group. Weight mean ±SD was 74.5±12.2 kg for control group and 79.7±22.8 kg for the test group (P = 0.032). Height mean ±SD was 171±10.0 cm for the control group and was 164±10.0 for the test group (P = 0.0006). Body Mass Index (BMI) mean ±SD was 25.2±3.2 for control group and 29.5±8.1 for test group (P = 0.0004), (Table 1).

Table 1: Baseline characteristics of the respondents :

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (non-diabetics) (mean ± SD) (n=100)</th>
<th>Test Group (diabetics) (mean ± SD) (n=300)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.2±12.0 (25.0-78.0)</td>
<td>51.1±11.5 (30.0-80.0)</td>
<td>0.06*</td>
</tr>
<tr>
<td>(Max-Min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.5±12.2 (52.0-105.0)</td>
<td>79.7±22.8 (50.0-180.0)</td>
<td>0.032*</td>
</tr>
<tr>
<td>(Max-Min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71±0.1 (1.52-1.96)</td>
<td>1.16±0.1 (1.35-1.90)</td>
<td>0.0006*</td>
</tr>
<tr>
<td>(Max-Min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (w/h²)</td>
<td>25.2±3.2 (19.1-34.6)</td>
<td>29.5±8.1 (19.6-63.0)</td>
<td>0.0004*</td>
</tr>
<tr>
<td>(Max-Min)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 The study revealed significant raised of the means of the plasma levels of malondialdehyde, fasting plasma glucose, cholesterol, LDL, triglyceride, and HbA1c of the test group when compared with healthy control group subjects, whereas the means of the plasma levels of antioxidant vitamins (A, E) and HDL showed significant reduction when compared with that of control group significant difference when compared with healthy subjects, (Table 2).

Table 2: Comparison of the means of Blood Parameters between diabetics and none diabetics:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (non-diabetics) (mean ± SD) (n=100)</th>
<th>Test Group (diabetics) (mean ± SD) (n=300)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>81.2±21.8 (10.0-133.0)</td>
<td>50.3±20.0 (14.0-94.0)</td>
<td>0.0009*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>15.6±4.8 (3.5-24.0)</td>
<td>5.2±1.8 (1.0-9.0)</td>
<td>0.0005*</td>
</tr>
<tr>
<td>MDA</td>
<td>2.4±1.1 (1.0-12.0)</td>
<td>6.7±6.2 (1.0-35.0)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>HbAc1%</td>
<td>4.9±0.3 (4.2-5.5)</td>
<td>7.5±1.4 (6.0-13.3)</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>107.1±20.1 (60.0-150.0)</td>
<td>124.2±79.4 (18.0-497.0)</td>
<td>0.033*</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>117.3±20.9 (80.0-165.0)</td>
<td>164.8±46.0 (37.0-526.0)</td>
<td>0.0006*</td>
</tr>
<tr>
<td>LDL</td>
<td>86.6±20.6 (47.0-133.0)</td>
<td>104.4±405 (20.0-290.0)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>HDL</td>
<td>51.9±6.2 (41.0-65.0)</td>
<td>41.8±11.9 (20.0-88.0)</td>
<td>0.0008*</td>
</tr>
<tr>
<td>FBS</td>
<td>101.5±11.9 (70.0-120.0)</td>
<td>160.4±65.5 (75.0-480.0)</td>
<td>0.0002*</td>
</tr>
</tbody>
</table>

* Significant differences in all blood parameters between control and test group (P value < 0.05).

Fig. 1 The results of this study showed strong positive correlations between the levels of plasma MDA and serum vitamin E of the test group (r=0.36, P = 0.01).
DISCUSSION
The present study showed that MDA was increased significantly in serum of all diabetic patients comparing to healthy subjects. The rise in the MDA indicated that any oxidative stress incurred sufficiently cause of free radical – mediated peroxidation of lipid components in cell membrane (Ahmed R., et al. 2005 and Varashree B., et al. 2011).

Therefore, MDA is a good indicator for evaluating oxidative stress in degenerative diseases like diabetes mellitus. MDA levels were not different among all patients , these may be due to the enhancement of the serum lipid peroxide removal by aldehyde dehydrogenase enzyme in liver mitochondria, This enzyme has function to destroy toxic aldehyde and protects tissue aldehyde accumulation (Ramchandra K., et al. 2012). In addition serum MDA can be moderated by enhancement of the degradation of excretion (Suryawanshi N., et al. 2006). These results demonstrated that diabetic patients were prone to accumulation of potentially harmful oxidative stress .These finding are consistent with the reports of the others (Maharjan B., et al. 2008).All patients of diabetes are showed hyperglycemia which can directly cause increased reactive oxygen species (ROS) generation. Glucose can undergo autooxidation and generate hydroxyl .OH- radicalicals (Goycheva P., et al. 2006).In addition, glucose reacts with proteins in a non-enzymatic manner leading to the formation of advanced glycation end products (AGEs) .ROS is generated at multiple steps during this process. In hyperglycemia , there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also result in enhanced production of superoxides O_2^- . Type 2 diabetes mellitus we found that HDL cholesterol levels were significantly low and other parameters of lipoproteins were significantly high as compare to healthy subjects. The low levels of HDL cholesterol which exerts anti-atherogenic and anti-oxidative effects when present in sufficient amounts is key feature for oxidative stress status (Sophie V., et al. 2010).

Protein identified as a key component of the VLDL assembly process leads to increase level of TG and reduce levels HDL-c in addition the elevation of free fatty acid and glucose in diabetes mellitus can decrease activity of lipoprotein lipase a pivotal enzyme in the removal of these lipoproteins from circulation that control the TG rich lipoproteins and HDL protein (Maritim A., et al. 2003).

The determination of antioxidant vitamins to prevent lipid peroxidation were also performed in this study. Many researchers reported the role of antioxidant vitamins including vitamin C, E, A, and β-carotene to defense damage by ROS in human disease such as cancer, inflammation, and arthritis. Diabetes mellitus is another interesting one and it is currently under study. (Horwitt H., et al.1972), suggested that total lipid content has an influence on the plasma vitamin E level since vitamin E is mainly found in LDL particles. There is evidence that vitamin E: cholesterol ratio is a more reliable criterion for vitamin E status then plasma vitamin E alone (SrivatsanR., et al. 2009). This is because the use of this ratio can correct for conditions that result in increased plasma lipid levels (Hisalkar P., et al. 2012). In our study the lower levels of vitamin E status and high levels of serum MDA in type 2 patients may be supported by these evidence. In the present study antioxidant vitamin A in type 2 diabetic patients showed significantly reduction compared to healthy subjects. This
indicate that oxidative stress induced by high level of glucose may increase superoxide radical production with diabetic patients.

REFERENCES
Sophie V, Frank S and Heinz-peter S. (2010). High-density lipoprotein at the interface of type 2 diabetes mellitus and cardiovascular
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ARABIC SUMMARY

قياس مستوى الدهون المؤكسدة وغير المؤكسدة في مرضى السكر من النوع الثاني السودانيين

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المقدمة: أجريت هذه الدراسة لقياس مستوى الدهون المؤكسدة وغير المؤكسدة وفايتمينات أ، عند مرضى السكر من النوع الثاني السودانيين، حيث اشترك في الدراسة ٢٠٠ مريض م_IE0 الصدر في جنوب السودان مع الدهون المؤكسدة بالاعتماد على مجموعات البيانات التي جمعت من ٩٠ مريض مصاب بالإنسولين و٨٠ مريض مصاب بالإنسولين. ونلاحظ زيادة كبيرة في مستوى الدهون المؤكسدة عند مرضى السكر. كما يحدث أيضا انخفاض كبير في مضادات الأكسدة عند مرضى السكر. وقد يكون ذلك نتيجة لزيادة استهلاكهم أثناء عملية مكافحة الجذور الحرة المفرطة والتي تنتج من مرض السكر.