Assessment of serum malondialdehyde and vitamin c status among Sudanese patients with type II Diabetes mellitus

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Abstract
A cross sectional study conducted during the period from May 2013 to August 2015 to assess the serum levels of malondialdehyde (as a marker of lipid peroxidation), antioxidant vitamin (C), lipid profile in Sudanese with type 2 diabetes mellitus compared to healthy volunteers. Three hundred of diabetic patients who attended ADC centre in Bahri for routine follow up and a control group of 100 healthy subjects (non-diabetic). Blood specimens were collected from both groups, and plasma levels of Malondialdehyde, antioxidant vitamin (C), lipid profile in addition to fasting plasma glucose and glycated hemoglobin (HbA1c) were determined. Age and gender of the test group were matched with the control group. The results of the study indicate a significant raised in 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 vitamin (C) and HDL showed significant reduction when compared with that of control group. Also shows significant correlation between plasma MDA and serum vitamin C of the test group (r=0.22, P = 0.03). In conclusion, the present study indicate that the plasma levels of MDA, antioxidant vitamin (c) and, FPG, lipid profile and HbA1c are important markers for evaluation of oxidative stress, antioxidant status, and control of glycemic diabetic patients respectively.

Key words: Diabetes mellitus, Oxidative stress, Malondialdehyde, vitamin C

Introduction:
Increased free radical production and attenuation of antioxidant system is currently receiving the highest attention when discussing pathogenesis of diabetic mellitus and its complications. Hyperglycaemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus. Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress. Lipid peroxides are disintegrated quickly and form reactive carbon compounds, among these, malondialdehyde (MDA) is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation, and has become one of widely reported analytes for the purpose of estimating oxidative stress effects on lipids. Since free radical production is increased whereas capacity of antioxidant system is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compare to healthy individuals. Sundaram –Langenstroer Malondialdehyde is a organic compound with the formula CH2(CHO)2. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids present on cell membrane forming malondialdehyde. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism. Antioxidants depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of oxidative stress related diseases. Vitamin C is a water soluble free radical scavenger, can directly scavenge O2 and OH- radicals and help to neutralize physiological oxidant burden created by both exogenous and endogenous sources. Vitamin C is an important antioxidant in human, capable of scavenging oxygenderived free radicals. Several studies showed decreased basal vitamin C level in diabetic patients and also it is suggested that oxidative stress is
increased in diabetes.\textsuperscript{(10)} The present study was conducted with an objective to evaluate the oxidative status and serum vitamin antioxidant levels in diabetics and to correlate them with the disease process.\textsuperscript{(11)}

**Materials and Method:**
In this study, the test group included 300 Sudanese patients with Type 2 Diabetes, The ages ranged from 30 to 80 years old. The mean age average was 51.2 years and 100 healthy subjects with mean FBS 5.61= m mol/L. as a control group. The age ranged from 22 to 78 years old. The mean age average was 50.1 years. All samples were in a state of fasting for 12 hours before drawing blood were obtained on these samples Advanced Diagnostic Center in Bahri from the period between May 2013 until August 2015. A venous blood sample (5 ml) was collected from each participant and allowed to clot to obtain serum. MDA in serum performed as described by Muslih \textit{et al.} (2002) 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. Vitamin C was measured using HPLC technique.

Statistical analysis was performed using SPSS (version 13) computer software was used for data analysis. The means and standard deviations of variable calculated and T-test was used for comparison (significant level was set at \( P \leq 0.05 \)).

**Results**
In this study, the test group included 300 Sudanese patients with Type 2 Diabetes and 100 healthy subjects as a control group. No significant difference in age, between the Mean ±SD of the test group and the control group (51.2 ± 7.56) vs (50.1 ± 7.21), respectively, (\( P= 0.06 \)).

(Figure 1) : Shows insignificant difference between the means of the age in the diabetic and non diabetic group

(Table1) : Shows a significantly raised mean of the serum levels MDA in study group (diabetics) when compared to control group.

(Table 2) : Shows the rise in glucose level of the diabetic group were contributed by the impaired insulin secretion.

(Figure 2) : A scatter plot shows a moderate negative correlation between the levels of serum vitamin C and MDA of the test group

![Figure (1): The means of the ages of the diabetic and non diabetic group.](image)
Table (1) Comparison the means of plasma MDA and vitamin C between diabetics and none diabetics

<table>
<thead>
<tr>
<th>Variables</th>
<th>None-diabetics (n=100)</th>
<th>Diabetics (n=300)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin C (Max-Min)</strong></td>
<td>10.0±2.2 (4.6-18.0)</td>
<td>3.9±1.3 (1.2-6.2)</td>
<td>0.0003*</td>
</tr>
<tr>
<td><strong>MDA (Max-Min)</strong></td>
<td>2.4±1.1 (1.0-12.0)</td>
<td>6.7±6.2 (1.0-35.0)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

- The table shows the mean + SD, range in brackets ( ) and probability (P)
- T-test was used for comparison.
- * Significant differences in all blood parameters between control and test group (P value < 0.05).

Table (2) Comparison the means of Biochemical parameters between diabetics and none diabetics

<table>
<thead>
<tr>
<th>Variables</th>
<th>None-diabetics (n=100)</th>
<th>Diabetics (n=300)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HbA1c% (Max-Min)</strong></td>
<td>4.9±0.3 (4.2-5.5)</td>
<td>7.5±1.4 (6.0-13.3)</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Triglycerides (Max-Min)</strong></td>
<td>107.1±20.1 (60.0-150.0)</td>
<td>124.6±79.1 (45.0-497.0)</td>
<td>0.033*</td>
</tr>
<tr>
<td><strong>Total Cholesterol (Max-Min)</strong></td>
<td>117.3±20.9 (80.0-165.0)</td>
<td>164.8±45.6 (73.0-526.0)</td>
<td>0.0006*</td>
</tr>
<tr>
<td><strong>LDL (Max-Min)</strong></td>
<td>86.6±20.6 (47.0-133.0)</td>
<td>109.0±34.7 (70-290.0)</td>
<td>0.0003*</td>
</tr>
<tr>
<td><strong>HDL (Max-Min)</strong></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><strong>FBS (Max-Min)</strong></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>

- The table shows the mean + SD, range in brackets ( ) and probability (P)
- T-test was used for comparison.
- * Significant differences in all blood parameters between control and test group (P value < 0.05).
Figure (2): A scatter plot shows a moderate negative correlation between the levels of serum vitamin C and MDA of the diabetic group (r = 0.22, P value = 0.03*)

Discussion
Several studies showed decreased basal vitamin C level in diabetic patients and also it is suggested that oxidative stress is increased in diabetes. The present study was conducted with an objective to evaluate the oxidative status and serum vitamin antioxidant levels in diabetics and to correlate them with the disease process.

In the present study rise in glucose level of the diabetic group were contributed by the impaired insulin secretion. However the mechanism of insulin resistance is not very clear. Hyperglycemia work through different mechanisms such as activation of protein kinase C, polyol and hexosamine pathways and advanced glycation end products production. All of these pathways, in association to hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote reactive oxygen species (ROS) accumulation that, in turn, promote cellular damage and contribute to the diabetic complications development and progression. (7,12)

MDA level were significantly raised in study group (diabetics) when compared to control group. This explains the generation of free radicals during disease process. Vitamin C level also significantly decreased in study group (diabetics when compared to control group. MDA and glucose level showed a significant positive correlation thus showing contributory role of hyperglycemia towards generation of oxidative stress. This generation of ROS has been potentiated by the marked dyslipidemia and increased lipid peroxidation of the disease process. (13,14)

The results of this study showed negative correlation between MDA and vitamin C reveals about negative impact of ROS on the bioavailability on vitamin C. Oxidative stress may be a common pathway linking diverse mechanism for the pathogenesis of diseases as well as complications in DM. ROS can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen activated protein kinases and redox sensitive transcription factors causing changes in protein expression and, therefore, irreversible oxidative modifications. (8,15)
Conclusion:
The present study indicate that the plasma levels of MDA, antioxidant vitamin (c) and FPG, lipid profile and HbA1c are important markers for evaluation of oxidative stress, antioxidant status, and control of glycemic diabetic patients respectively. The increased level of serum MDA and lower level of serum vitamin C clearly shows that diabetic patients was exposed to an increased oxidative stress via lipid peroxidation.

References: