Evaluation of lipid peroxidation, lipid profile and antioxidant status in patients with non-insulin dependent diabetes mellitus in Najaf / Iraq.

Abstract:

Background: Diabetes mellitus arises when insufficient insulin is produced, or when the available insulin does not function correctly. Without insulin, the amount of glucose in the blood stream is abnormally high, causing unquenchable thirst and frequent urination. The body’s inability to store or use glucose causes hunger and weight loss (1). Type 2 diabetes – occurs when there is a severe lack of insulin due to the destruction of most or all of the beta (β - cells) in the islets of Langerhans. Diabetes mellitus is considered to be one of a rank of free radical diseases which propagates complications with increased free radical formation. Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms. Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus. Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus. The study was designed to find out the relationship between lipid peroxidation, and complication of diabetes mellitus and to estimate the mutual relationship between serum lipoproteins levels and diabetes severity (2).

Keywords: Diabetes mellitus, Lipid profile, Glutathion, Oxidative stress (Malondialdehyde), Catalase and Uric acid
INTRODUCTION

Diabetes Mellitus is caused by an absolute or relative insulin deficiency. It has been defined by the World Health Organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration more than or equal to 7.0 mmol/L (on more than one occasion or once in the presence of diabetes symptom) or random venous plasma glucose concentration more than or equal to 11.1 mmol/L. Non-insulin-dependent type 2 diabetes occurs when the body does not produce enough insulin, and the insulin that is produced becomes less effective. This type of diabetes usually appears in people over the age of 40, and tends to have a more gradual onset. In most cases, glucose levels in the blood can be controlled by diet, or diet and tablets, although sometimes insulin injections may be needed. About 90 percent of diabetics are non-insulin dependent. There are probably 100 million people in the world with diabetes mellitus and incidences of diabetes are on the rise. As diabetes progresses patients are at increased risk of developing coronary disease. Insulin deficiency causes excessive metabolism of free fatty acids, this may lead to a disorder in lipid metabolism. Insulin is a hypoglycemic hormone secreted from β-cell of the islet of pancreas. Insulin also has an effect on lipid metabolism.

Type 2 diabetes (non-insulin-dependent diabetes) is a multi-causal disease which develops slowly and in a stepwise order. Initially it commences with insulin resistance, which progresses gradually with time until the body fails to maintain glucose homeostasis resulting in glucose intolerance. Systemically these perturbations are accompanied with changes in a variety of biochemical processes such as obesity, an altered lipid profile and lipid peroxidation.

Oxidative damage to unsaturated lipids is a well-established general mechanism for oxidative stress-mediated cellular injury, in addition to increased lipid peroxidation. The occurrence of free-radical-induced lipid peroxidation causes considerable changes in the cell membrane. Peroxidation of the lipid membrane has been related to the pathogenesis of many degenerative diseases, such as atherosclerosis, aging, carcinogenesis and diabetes mellitus. Evidence suggests that oxidative stress is increased in diabetes, because of excessive production of reactive oxygen species (ROS) and an impaired antioxidant defence mechanism. It has been suggested that ROS induce membrane lipid peroxidation and that the toxicity of the generated fatty acids peroxides are important causes of cell malfunction. The most widely used assay for lipid peroxidation involves the measurement of malondialdehyde (MDA) due to its simplicity. Thus, the lipid peroxide in the blood provides useful information for the prognosis of diabetes in which secondary disorders are often fatal. Antioxidants can be defined as substances whose presence in relatively high concentration significantly inhibits the rate of oxidation of lipids, proteins, carbohydrates and DNA. Antioxidants such as uric acid (UA) and glutathione (GSH) act as potent electron donors; they donate hydrogen atoms to pair up with unpaired electrons on free radicals. Thus, they convert reactive free radicals into inactive substances. The determination of the oxidative stress and antioxidants require sometimes invasive techniques such as taking blood samples.
The aim of the present study was to determine the levels of serum glucose, lipid peroxidation marker (MDA), lipid profile; total cholesterol (TC), high density lipoprotein (HDL), Triglyceride (TG), Low density Lipoprotein (LDL) and very Low Lipoprotein (VLDL) and make a statistical study on these parameters were done by the methods based on enzymatic determination and antioxidant parameters Glutathione peroxidase (GPx), Reduced Glutathione (GSH), Glutathione reductase (GR), Catalase (CAT) and Uric acid (UA) in patient with Diabetes mellitus.

**MATERIAL AND METHOD**

**Chemical and Apparatus**

All laboratory chemical and reagents were of analar grade. Trichlroacetic acid, was obtained from Hopking Williams, Thiobarbituric acid from Merek Germany Co. Ltd, reduced glutathione from Biochemical’s Co. Ltd, Hydrogen peroxide from Merek CO. Ltd, (di-potassium hydrogen phosphate, potassium dihydrogen phosphate and di-sodium hydrogen phosphate from Merek Germany Co. ltd) were used during our study, lipid profile and uric acid from BIOLABO SA laboratories Ltd. France.

**Patients and control subjects**

Sera of 100 subject were collected, out of which 40 apparently healthy individuals of age group (35-70 years) were taken as control with normal plasma glucose include (20) male and (20) female. (60) elderly with non insulin dependent (NIDDM) subjects of age (35-70 years) were taken as cases include (30) males and (30) females were obtained from Al-Najaf Center for diabetes and Endocrine Department in Al - Sader Medical Teaching City / Najaf/ Iraq and the results were compared with healthy individuals with comparable age. The (NIDDM) Patients were not taking any medicines other than oral anti-diabetic pills for the past four years. Patients suffered from other disease interferes with data excluded in the current study. The study was carried out at the Department of Biochemistry, College of Medicine, University of Kufa.

**Blood specimens**

Disposable syringes and needles were used for blood collection. Blood samples were obtained from patients and control group by vein puncture. Sample were allowed to clot at 37°C then centrifuged at 3000 Xg for 10 minutes. Sera were removed and stored at -20°C until analysis time.
Methods

Both cases and controls were subjected to estimation of biochemical parameters like Fasting plasma Glucose (FPG) \(^{(19)}\), Total cholesterol (TC), Triglyceride (Tg), HDL-Cholesterol, LDL-Cholesterol and VLDL-Cholesterol\(^{(20,21)}\). GSH-Px was assayed according to the procedure of Rotruck et al. with some modification\(^{(22)}\) while reduced Glutathione was assayed as described by Burits et al.\(^{(23)}\) (by spectrophotometric assay based on 5,5'-dithiobis - nitrobenzoic acid) on the other hand Glutathion reductase (GR) was assayed by enzymatic method by Horn \(^{(24)}\). Catalase CAT activity was assayed as described by Aebi H\(^{(25)}\) and uric acid also was assayed by enzymatic method\(^{(26)}\). Malondialdehyde was assayed using thiobarbituric reactive substances method described by Guidet B and Shah \(^{(27)}\).

Type 2 diabetes mellitus patients were diagnosed on the basis of history, physical examination and biochemical investigations and according to the biochemical criteria laid down by the National Diabetes Data Group (NDDG) of the National Institute of health in 1985/WHO criteria \(^{(28)}\). The diagnosis of NIDDM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes mellitus (2000) \(^{(29)}\).

Biostatistics analysis

The result were expressed as mean ± SD. Students t-test was used for comparison of results of patients and the control group. Significant variation was considered when p value was less than < 0.05. The correlate between the values of oxidative stress parameters and various factor were performed by the liner regression analysis Table(3) and Figures (1- 5).

RESULTS

In the current study we observed a significant increase \(p < 0.001\) in the lipid profile was observed except HDL cholesterol, which was decreased Table(1) and Figures(1,2) , Also significant decrease \(p < 0.001\) in antioxidant enzymes such as glutathione peroxidas, reduced glutathione, glutathione reductase, except uric acid and catalase were seen as compared to the control subjects Table(2). Other findings observed was that the level of lipid peroxide (MDA) increased as per the increase in concentration of blood glucose. Our findings indicate that the increase in the lipid peroxidation product MDA together with uric acid and catalase and decline in glutathione-dependent antioxidant defenses may appear early in non insulin dependent type 2 diabetes mellitus patients (NIDDM). Serum MDA had a week positive relation with serum UA and serum CAT.
fig (1): Correlation between Total cholesterol (TC) and Triglyceride (TG)

Fig (2): Correlation between Total cholesterol (TC) and high density lipoprotein (HDL)

Fig (3): Correlation between Triglyceride (TG) and high density lipoprotein (HDL)

Fig (4): Correlation between triglyceride (TG) and very low density lipoprotein (VLDL)

Fig (5): Correlation between high density lipoprotein (HDL) and low density lipoprotein (LDL)

### Table (1) . Serum value of lipid profile in type 2 diabetes mellitus and the control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Range</th>
<th>Patients</th>
<th>Range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>3.91± 0.66</td>
<td>1.6 – 9.4</td>
<td>6.138 ± 1.2</td>
<td>2.45 – 14.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.68± 0.3</td>
<td>0.53 – 4.72</td>
<td>2.05 ± 0.51</td>
<td>0.98 – 5.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL–C (mmol/L)</td>
<td>1.89± 0.36</td>
<td>0.32 – 2.29</td>
<td>1.28 ± 0.26</td>
<td>0.78 – 2.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL–C (mmol/L)</td>
<td>2.42± 0.36</td>
<td>1.3 - 5.5</td>
<td>3.84± 1.15</td>
<td>1.34 – 8.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VLDL–C (mmol/L)</td>
<td>0.36± 0.1</td>
<td>0.47 – 1.79</td>
<td>0.93± 0.26</td>
<td>0.34 – 2.7</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein.

Table (2). Serum value of the status of antioxidant enzymes and Malodialdehyde (MAD) in type 2 diabetes mellitus and control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>284 ± 58</td>
<td>113 – 553</td>
<td>139 ± 34</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>3.92 ± 1.16</td>
<td>1.4 – 8.8</td>
<td>2.48 ± 0.74</td>
</tr>
<tr>
<td>GR-(U/ml)</td>
<td>1.68 ± 0.33</td>
<td>0.65 – 4.02</td>
<td>1.3 8 ± 0.27</td>
</tr>
<tr>
<td>CAT (K/ml)</td>
<td>60 ± 6.6</td>
<td>26.7 – 133.2</td>
<td>65.6 ± 19</td>
</tr>
<tr>
<td>UA–(mmol/L)</td>
<td>0.29 ± 0.039</td>
<td>0.126 –0.66</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>5.64 ± 0.58</td>
<td>2.53 – 12.4</td>
<td>10.66 ± 3.2</td>
</tr>
</tbody>
</table>

GSH, Glutathione; GPx, Glutathione peroxidase; GR, Glutathione reductase; CAT, Catalase; UA, Uric acid; MDA, Malondialdehyde.

Table (3). Correlation among serum estimation of lipid profile in patients with diabetes mellitus

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC with TG</td>
<td>0.768</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC with HDL-C</td>
<td>-0.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC with LDL-C</td>
<td>0.71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC with VLDL-C</td>
<td>0.548</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TG with HDL-C</td>
<td>-0.55</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TG with VLDL-C</td>
<td>0.932</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL with LDL-C</td>
<td>-0.565</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HDL with VLDL-C</td>
<td>-0.422</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides cholesterol; VLDL-C, very low-density lipoprotein.

DISCUSSION

In diabetes mellitus, abnormally increased levels of lipids, lipoproteins and lipid peroxides in plasma may be due to the abnormal lipid metabolism (13). Patients with type 2 diabetes frequently have an abnormal blood lipid profile consisting of moderately elevated LDL-C, moderately decreased HDL-C, and high TC and triglycerides (Table 1). Thus, inadequate levels of HDL-C, in conjunction with more atherogenic forms of LDL-C may contribute to atherogenesis (30). The results of the present study showed approximately a two-fold increase in serum levels of all lipid fractions (except for HDL-C) for diabetic group when compared with control group Table (1).
Hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL-C, protein glycation and glucose auto oxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects \(^{(31)}\) Table (2). Enhanced oxidative stress was indicated by increased free radicals production \(^{(32)}\) lipid peroxidation and reduced antioxidant status \(^{(33)}\). Several studies have reported an increased susceptibility to lipid peroxidation in patients with diabetes mellitus \(^{(34)}\). The generation of free radicals may lead to lipid peroxidation and the formation of several types of damage in diabetes mellitus. In the present study, we have observed that MDA levels, a lipid peroxidation product and a marker of oxidative stress, were elevated significantly in diabetic patients \(^{(35)}\).

Uric acid UA is the end product of purine metabolism; it can act as a pro-oxidant, particularly at increased concentration Table (2) and may thus be a marker of oxidative stress \(^{(36-37)}\). But it may also have a therapeutic role as an antioxidant \(^{(38, 39)}\). Thus, it is unclear whether the increased concentration of UA in diseases associated with oxidative stress, such as diabetes mellitus, are a protective response or a primary cause. It is worth noting that hyperuricemia has been found to be associated with obesity and insulin resistance and consequently with type 2 diabetes mellitus \(^{(40-42)}\). Chen et al, (2008) \(^{(43)}\).

Sailaja et al (2003) \(^{(44)}\) reported that diabetic humans have shown increased lipid peroxidation and decreased levels of glutathione peroxidase, reduced GSH and glutathione reductase Table (2). These data suggest that the oxidative stress in these pathologies does not depend on a loss of GSH or a lack of GSH synthesis alone, but a misbalance in the oxidant/reduction cycle of GSH \(^{(45)}\).

Reznick et al (2006) \(^{(46)}\) have shown that oxidative stress exists in diabetic patients as evidenced by the increased total antioxidant capacity in the blood of patients. Indeed, there is evidence that suggests that endogenous antioxidant capacity is eroded in diabetes, due to several factors, including the impact of non-enzymatic glycation on key enzymes, the polyol pathway and its consumption of reducing power, as well as the constant demands of oxidative stress \(^{(47-52)}\). The present study showed that elevated serum endogenous antioxidant activity among diabetics is a response to the damaging effect of free radicals release due to increased oxidative stress. Indeed, the detection of increases in UA levels should therefore alert clinicians to the commensurately increased vulnerability of the diabetic patient to life-threatening cardiovascular complications.

GSH is a ubiquitous tri-peptide that presents in red cells and participates in GPx reaction. When \(\text{H}_2\text{O}_2\) is detoxified by GPx, the GSH is simultaneously converted to the oxidized form (GSSG). In the present study, found that GSH levels in type 2 DM patients were significantly lower than that in their same age-matched control subjects. These results are in good agreement with other studies \(^{(53-55)}\). As already mentioned GSH serves as an essential cofactor for the enzyme GPx and formed oxidized glutathione (GSSG) during the enzyme processes. Thus, increasing in GPx activities imply higher consumption of GSH. Other mechanisms that may explain the
GSH reduction in diabetes are that the GSH is regenerated by the enzyme glutathione reductase, using reducing equivalents from NADPH. The NADPH is generated in red blood cells through the pentose phosphate pathway, which is stimulated by insulin\(^{(56)}\). NADPH production in DM may be sluggish, probably resulting in lowered glutathione reductase activity and reduced GSH recycle.

The enzyme glutathione reductase was found to be decreased in type 2 diabetic patients as reported by Dincer et al (2002)\(^{(57)}\). Moreover in diabetes mellitus, the increased sorbitol synthesis via the polyol pathway occurred. This elevated sorbitol production caused the NADPH depletion that was required by aldose reductase enzyme in this pathway. This deficiency will also limit the GSH recycle\(^{(57)}\). There is still a controversial view regarding alteration in the activity of catalases in diabetic subjects. According to some scientist increase in level of catalase is compensatory for the removal of free radical, in the absence of glutathione peroxidase in type II diabetes mellitus\(^{(58)}\). We found in our study increased catalases level which is in agreement of other reports.

In conclusion, the assay of oxidative stress parameter has brought substantial insight into the pathogenesis and evolution of diabetes. Thus, subjects at high risk of developing hyperlipidemia may benefit from treatment with antioxidants such as vitamin E and C, which might assist endogenous antioxidant capacity and reduce peroxidation rates. Whether such supplementation might possibly delay or even prevent complications of this disease is an area of ongoing investigation.

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