LDL Particles Size Index and Lipid Peroxidation in Type 2 Diabetic Male Patients


ABSTRACT:
BACKGROUND:
Diabetes mellitus is a heterogeneous condition reflecting different metabolic disorders accompanied by a variety of complications. In DM, there is a change in oxidative stress (elevation in free radical generation with increase lipid peroxidation and derived oxidized products, and serum lipid profile abnormalities.

OBJECTIVE:
Several free radical species are normally produced in the body to perform specific functions. Increased free radicals in diabetes may cause the pathogenesis of atherosclerosis, and the degenerative disorders. In the present study, the oxidative stress in type 2 diabetic male patients was evaluated by estimating the lipid peroxidation. Malondialdehyde (MDA) is one of the major aldehyde derived from lipid peroxidation.

PATIENTS AND METHODS:
Serum MDA, Oxidized HDL (ox.HDL), and lipid profile were measured after 12 hr fasting in 30 diabetic patients, their age range was (40-55) years and compared with 30 healthy controls.

RESULTS:
Serum MDA and ox.HDL were significantly increased in the diabetic group (P<0.05). All patients had significant elevation in serum levels of glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and LDL particles size index (TG/HDL-C ratio).

CONCLUSION:
This may be due to different glycemic control in these patients. There was a significant positive correlation between serum MDA and LDLs size index, while serum MDA was negatively correlated with serum HDL-C in diabetic patients. Our results indicate that oxidative stress status increases during type 2 diabetes mellitus in parallel to glucose and lipid changes.

KEY WORDS: oxidative stress, type 2 diabetes mellitus, lipid peroxidation.

INTRODUCTION:
Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic process and its treatment more than the nature of the process itself.

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Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control. Oxidative stress plays a role in inflammation, accelerates aging and contributes in variety of degenerative conditions e.g., cardiovascular diseases, atherosclerosis. Cardiovascular complications, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes. Malondialdehyde (MDA) with its formula CH\(_2\)(CHO)\(_2\) is an end product of polyunsaturated fatty acids peroxidation. The products of lipid peroxidation are easily detected in the blood plasma and have been used as a marker of oxidative stress. Lipid peroxides are disintegrated quickly forming reactive carbon compounds. Among these, MDA is an important...
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reactive carbon compound used commonly as an indicator of lipid peroxidation (3). High density lipoprotein (HDL) is thought to protect against coronary heart disease (CHD) by promoting reverse cholesterol transport and by antioxidant, antiinflammatory and antithrombotic effects. HDL are the most complicated and diverse of the lipoproteins, as they contain many different protein constituents, whose main purpose is to enable secretion of cholesterol from cells, esterification of cholesterol in plasma, transfer of cholesterol to other lipoproteins, and the return of cholesterol to the liver for excretion – a process that has been termed ‘reverse cholesterol transport’ (6). HDL is also subject to oxidation. In vitro, HDL lipids are oxidized in preference to those of LDL. In human atherosclerotic lesions, HDL lipids are oxidized to an extent comparable with that of LDL. Such oxidized HDL could conceivably re-enter the circulation, as HDL is smaller than LDL and is comparatively less retained via interaction with extracellular matrix in the vessel wall. Indeed, there is evidence for the presence of oxidized HDL in the circulation of subjects with atherosclerotic disease (7).

Dyslipidemia characterized by elevated TC, LDL-C and lowered HDL-C, is a conventional risk factor observed in cardiovascular patients, and is the major cause of atherosclerosis are suggested to act synergistically with non-lipid risk factors to increase atherogenesis. Low-density lipoprotein cholesterol (LDL-C) is the main therapeutic target in the prevention of CVD. Increased triglycerides (TG) and decreased high-density lipoprotein (HDL-C) are considered to be a major risk factor for the development of Insulin resistance and metabolic syndrome. Although the TG/HDL-C ratio has been used as a clinical indicator for Insulin resistance, results were inconsistent (8).

The aim of this study is to evaluate the role of LDL size index and oxidative stress in type2 diabetic male patients on basis of lipid peroxidation.

Experimental:

SUBJECTS AND METHODS:

This study was conducted during the period from the June 2011 until the end of January 2012. A total of male (30 NIDDM patients + 30 normal healthy). The patients were collected from the medical city. Their age range was (40-55) years. All patients had high levels of blood glucose and HbA1c. They were treated with oral antidiabetic agents, mainly glibenclamide and metformin only, and they had no other medications. and thirty healthy individuals as control group. Ten ml of venous blood was obtained after a 12 hour fast from type 2 diabetic patients. Two milliliters of blood were transferred into EDTA tube for HbA1c assay. Blood samples were transferred into plain tube, allowed to stand for 15 minutes at room temperature, centrifuged at 3500 rpm for 10 minutes.

Measurements:

The resulting serum was separated using the following measurements:

a. Glucose, by using the enzymatic colorimetric method (9).

b. HbA1c has been measured by using the variant hemoglobin A1c program developed by Bio–Rad (10).

c. MDA as an index of lipid peroxidation by using TBARS method (11).

d. Lipid hydroperoxide. All serum lipoproteins except HDL-C were precipitated by phosophotengestic acid-MgCL2,Supematant was used for measurement of oxidized HDL-C using the thiobarbituric method. The results were corrected for dilution of the serum (12).

e. Total cholesterol (TC), triglyceride (TG), high and low density lipoprotein cholesterol (HDL-C and LDL-C respectively) determined by the enzymatic colorimetric method (13,14,15).

Statistical Analysis:

Statistical Analysis System- SAS (2004) (16), was used to analyze of data. The T-test was used to compare between means in this study (control and NIDDM group).

RESULTS:

Table (1) shows a significant increase in blood glucose in the diabetic group with a significant increase in HbA1c as compared with their controls. Diabetic patients showed a significant increase in serum MDA and ox.HDL in diabetic patients as compared with controls, table (2). There was a significant positive correlation between serum MDA and serum HDL-C with a significant correlation between serum MDA and LDL-C in diabetic patients. A significant positive correlation was found between serum MDA, and ox.HDL with LDLs size index (expressed as TG/HDL-C ratio) in diabetic patients, as shown in Figures (1), (2).
Table 1: Serum glucose, and HbA1c in the diabetic group and controls (means ± SD)

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>NIDDM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.55</td>
<td>40.55</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>165.73 ± 4.18*</td>
<td>86.36 ± 1.36</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.39 ± 0.14*</td>
<td>4.36 ± 0.09</td>
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</table>

*P < 0.05.

Table 2: Biochemical characteristic of diabetic patients and controls (mean ± SD)

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>NIDDM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/l)</td>
<td>1.78 ±0.10*</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>Ox.HDL (µmol/l)</td>
<td>1.35 ± 0.09*</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>Ox.n.HDL (µmol/l)</td>
<td>0.44 ± 0.04*</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>% Ox.HDL</td>
<td>76</td>
<td>67</td>
</tr>
<tr>
<td>S.T.C (mg/dl)</td>
<td>190.6 ± 7.73*</td>
<td>152.91 ± 2.17</td>
</tr>
<tr>
<td>S.TG (mg/dl)</td>
<td>192.44 ± 15.27*</td>
<td>105.03 ± 6.02</td>
</tr>
<tr>
<td>S.HDL-C (mg/dl)</td>
<td>38.03 ± 1.28*</td>
<td>65.17 ± 0.96</td>
</tr>
<tr>
<td>S.LDL-C (mg/dl)</td>
<td>114.21 ± 6.84*</td>
<td>66.10 ± 3.05</td>
</tr>
<tr>
<td>S.VLDL-C (mg/dl)</td>
<td>38.54 ± 3.05*</td>
<td>29.51 ± 1.20</td>
</tr>
<tr>
<td>LDLs size index (TG/HDL-C ratio)</td>
<td>5.46 ± 0.56*</td>
<td>1.62 ± 0.09</td>
</tr>
</tbody>
</table>

*P < 0.05.

Figure 1: Relationship between TG/HDL-C ratio and serum MDA (µmole/l) in NIDDM patients, r= 0.78, P= 0.001

Figure 2: Relationship between TG/HDL-C ratio and serum oxidized HDL-C (µmole/l) in NIDDM patients, r= 0.68, P= 0.001
**DISCUSSION:**
Type 2 diabetes mellitus (DM) is a major global health problem that affects over 200 million individuals worldwide. It is characterized by insulin resistance (IR) in peripheral tissue and an insulin secretory defect of the beta cells of the pancreas. IR is a major contributor to the pathogenesis of type 2 DM and plays a key role in associated metabolic abnormalities, such as dyslipidemia and hypertension. At the high levels found in DM, glucose reacts with and forms adducts (advanced glycation end products) on macromolecules including proteins and DNA, eliciting cellular dysfunction and leading to vascular disease (17).

Free oxygen radicals in DM cause peroxidative breakdown of phospholipids that lead to accumulation of malondialdehyde (MDA). MDA plays a key role in modifying low density lipoprotein (LDL), which mediates the pathophysiological changes by non-enzymatic and auto-oxidative glycosylation (18).

The main findings of the present study are evaluation of serum MDA level and lipid hydroperoxide (oxHDL), and its relationship with TG/HDL-C ratio in type 2 diabetic male patients, (P<0.001).

The values were compared between diabetic groups, and control group. This study reveals:
1- increased levels of total cholesterol, triacylglycerols, LDL-C, and decreased levels of HDL-C which are well known risk factors of cardiovascular diseases.
2-the main disorders of lipid metabolism in this study are hypercholesterolemia, and hypertriglyceridemia. This findings is in concord with a previous study on hypertriglyceridemia (19).

Hydrolysis of triglyceride in LDLs and HDLs by hepatic lipase reduces their particle size, and generates small, dense LDLs and small dense HDLs. Small dense LDLs are especially prone to oxidation and thus more likely to be taken up by macrophages in the artery wall, leading to further progression of the atherosclerotic plaque (20). There exists increasing evidence of a positive correlation between plasma TG concentrations and increased risk of (CHD). HDL-C concentrations and predominant LDL sizes show an inverse relationship with CHD. Relatively, high TG concentrations generally mean reduced HDL-C concentrations and a predominance of small LDL particles implying that the existing concentrations are closely related. Although most studies suggest that small dense LDL particles are especially atherogenic, there are additional possibilities to be considered. Small dense LDL often coexists with hypertriglyceridemia and low level of HDL. This triad has been nominated as the atherogenic lipoprotein phenotype or atherogenic lipoprotein profile (21). These relationships are difficult to interpret in terms of cause. One hypothesis is that prolonged presence of the TG-rich lipoproteins in the circulation leads to increased exchange of their TG for cholesterol ester in HDL as well as LDL by cholesterol ester transfer protein. These neutral lipid exchanges decrease the HDL-C concentration. In addition, TG-enriched LDL may be removed by hepatic lipase, leading to small dense LDL. This study confirmed associations between concentrations of both TG and HDL-C. As described in other reports, the TG concentration correlated more closely with the predominant LDL size than with the HDL-C concentration (22). However, an inverse correlation between the TG-to-HDL cholesterol molar ratio and LDL size was even stronger. It may be suitable for the selection of patients needing an earlier and aggressive treatment of lipid abnormalities.

**CONCLUSION:**
Diabetic patients experienced oxidative stress and lipid abnormalities. This may be due to different glycemic control in these patients.

**REFERENCES:**