Assessment of Lipoprotein abnormalities in Diabetic and non-diabetic patients with hyperlipidemia

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ABSTRACT:
Background: Hyperlipoproteinaemia is a metabolic abnormal condition and is largely regulated by Apolipoproteins types and subtypes. Moreover, lipoprotein abnormalities contribute significantly to the risk of developing cardiovascular disease and diabetes. Additionally, abnormal glycemic state, lipid and lipoprotein abnormalities have also been shown to contribute in early atherosclerosis. Objectives: Our present study evaluates the status of apo-lipoprotein A and B in hyperlipidemic patients with both diabetic and non-diabetic conditions. Methods: Study period was May 2006 to Dec 2007. 63 patients of both gender (males = 36, females = 27) sub-grouped as n = 46 non-diabetic hyperlipidemic (NDHL) and n = 17 diabetic hyperlipidemic (DHL) patients were included in the study. All parameters were determined with standard methods on IVD instruments with recommended pathological and normal controls. Results: Total cholesterol and Apo B was noted to be significantly higher in DHL than NDHL patients. Moreover, levels of Apo B was higher than Apo A in both DHL and NDHL groups when compared with Healthy group. Elevated levels of triglyceride and total cholesterol in both DHL and NDHL groups depicts a strong hyperlipidemic state. Conclusion: Conclusion were drawn from present study that diabetes and hyperlipidemia are important risk factors, in addition to the fact that higher levels of Apo B and A and that of higher Apo B than Apo A are indicative of dyslipidemic state and thus significant parameters for assessing the prevailing conditions and extent of risk for developing coronary heart disease (CHD) and atherosclerosis.

KEY WORDS: Apolipoprotein A, B, hypertriglyceridemia, hyperlipoproteinaemia

INTRODUCTION:
Hyperlipoproteinaemia is a metabolic condition and is mainly regulated by apolipoproteins. Moreover, concentration of apolipoproteins, particularly Apo B and Apo A1, are more viable indicators of abnormalities in patients and health people than cholesterol and lipoprotein concentrations. It is well documented that main apolipoproteins in the plasma lipoprotein groups are Apo A (major protein of the high density lipoprotein or HDL) and Apo B (major protein in the low density lipoprotein or LDL). Apo B is also the major protein in the intermediate density lipoprotein (IDL) and very low density lipoprotein (VLDL) fractions. It has been reported that hyperlipoproteinaemia may result from an abnormality of plasma lipoprotein metabolism due to some factors such as diabetes mellitus or perhaps a defect in the function of plasma apolipoprotein or in lipoprotein receptor especially in LDL receptor. Traditionally, it is postulated that diabetes mellitus is an endocrine disease, however, biochemically, its major manifestations are that of metabolic origin. It was proven by research that a correlation exist between increased levels of glucose, cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and decreased high density lipoprotein cholesterol (HDL-C) and diabetes mellitus. Because of this modality, the assessment of the patients with diabetes and comparing them with non-diabetics, has gained great importance for detection of early changes related to carbohydrate and lipid metabolism (lipoprotein abnormalities) and possible measures to evaluate some of the complications such as cardio-vascular disease (CVD).

Past studies shown that lipoprotein anomalies contribute significantly to the risk of development of CVD and diabetes, whereas lipid and lipoprotein
abnormalities have been shown to be important contributors to early atherosclerosis\textsuperscript{1,13-16}. Important in this aspect is the result reported by the SEARCH for Diabetes in Youth study which reported higher prevalence of having two or more traditional CVD risk factors among youth with T1 diabetes, compared with national estimates for youth without diabetes, whereas the prevalence of adverse CVD risk profile in youth with T2 diabetes was more than 90%\textsuperscript{17}.

The present study, therefore, was undertaken to evaluate the status of apolipoproteins concentrations of diabetic and non-diabetic hyperlipidemic patients.

**MATERIAL AND METHOD:**

This study included 63 patients of both gender, subgrouped as 46 non-diabetic hyperlipidemic (NDHL) and 17 diabetic hyperlipidemic (DHL) patients. 20 healthy control subjects with no known family history of diabetes mellitus were included as control. Protocols of Akanji (2002)\textsuperscript{16} was followed for all research design of anthropometric and chemical analysis. The study period was May 2006 to December 2007 at the department of Biochemistry lab (LNH) and department of Pathology (GLGH). Both control and diabetic related subjects were in the age group of 31-56 years, from lower and middle socio-economic group. Among control group there were 10 males and 10 females while in the NDHL group, 26 males, 20 females; and DHL group, 10 males and 7 females. Fasting blood samples of controls subjects, NDHL and DHL patients were collected. Measurements of plasma glucose, cholesterol (TC), triglyceride (TG), HDL cholesterol, low density lipoprotein (LDL), Apo A, Apo B, and uric acid were determined on a Hitachi 912 autoanalyzer (Roche Diagnostics, Basel). Lp(a) was determined using a double-monoclonal antibody-based ELISA\textsuperscript{18}.

Statistical significance of the results was evaluated by SPSS version 13 and considered significant only when $P < 0.05$.

**RESULT:**

Results are summarized in Table I. There were a total of 63 patients and 20 healthy controls. Out of 63, 46 were grouped in NDHL with males 26, female 20 and 17 in DHL group with males 10 and females 7. Average ages were 44.7 y, 47.2 year and 39.1 years, respectively in NDHL, DHL and Healthy control groups. BMI was more or less similar in NDHL and DHL groups where as 26.2 kg/m\textsuperscript{2} in healthy group. Waist hip ratio was also found to be similar in all three groups. When healthy group was compared with patients groups of NDHL and DHL; total cholesterol (TC), triglyceride (TG), LDL, Apo A and B and Lp (a) was noted to be normal in healthy group ($P < 0.001$) than in DHL and NDHL groups. However glucose was significantly different ($P < 0.001$) than DHL, NDHL and Healthy group ($P < 0.001$). LDL in NDHL group was significantly higher ($P<0.05$) than healthy group but within the range with DHL group. TC and Apo B in DHL was noted to be significantly higher than those in NDHL group ($P < 0.01$) and healthy group ($P<0.001$). It was also noted that Apo B was higher in both NDHL and DHL group than Apo A. Elevated concentrations of TC and TG depicts a hyperlipidemic state.

**DISCUSSION:**

It is documented that the known lipoprotein abnormalities in type 2 diabetes are increased serum triglycerides, decreased HDL concentrations, presence of increased amounts of small, dense LDL in the circulation and increased postprandial lipaemia\textsuperscript{12,16,19}.

Generally stating the condition as hyperlipoproteinemia, it is regarded as one of the significant risk factors for the development of artherosclerotic diseases. Studies indicate routine determinations of triglycerides and cholesterol, in addition to quantitative determination of the corresponding apolipoproteins is also very vital. One of the specific components, that need to be assessed, is the Apo B, which is a carrier protein for low density lipoprotein (LDL b-lipoprotein). Apo B determination noted to be is useful in the differential diagnosis of hyperlipoproteinaemia. When evaluated in hyperlipemic subjects (especially type IV), the results is also affected by the Apo B content of the pre-b-lipoproteins or VLDL\textsuperscript{16}. Another component Lp(a) and its role in diabetes is a matter of debate and still needed to be explored thoroughly\textsuperscript{20}. Analysis of Lp(a) in a large group of type 2 diabetic patients suggest that Lp(a) levels and apo(a) polymorphism may be reliable predictors of CAD severity in type 2 diabetic patients\textsuperscript{21}. 
It has been stated that these lipid abnormalities are often present before the clinical onset of diabetes and are known to become worse with the development of diabetic long-term complications such as nephropathy. Interestingly non diabetic but hyperlipidemic group (NDHL) also demonstrated similar pattern, but in a lower range as compared to DHL. It is also been noted that there were important differences in the statistical relationships between LDL and HDL and their respective apolipoproteins, apo B and apo A-I, in diabetic, non-diabetic and healthy subjects. Similar pattern was reported in selected diabetic and non-diabetic Kuwaiti population. Moreover, Lp(a) levels were somewhat similar in our study in both NDHL and DHL group but significantly higher than the healthy group. As regard Apo B and A, a recent research reported that serum levels of apolipoprotein B (Apo B) and Apo B to apolipoprotein A-I (Apo A-I) ratio were better predictors of atherosclerotic vascular disease compared with LDL-C.

Lipoprotein abnormalities in youth with type 2 diabetes are a matter of great concern and recently it was reported that in youth with type 1 diabetes, elevated apoB and dense LDL were not highly prevalent, whereas elevated apoB and dense LDL were common. A previous finding suggests that the ratios of apoB/LDL cholesterol and apoB/non-HDL cholesterol may have a role in the risk stratification of diabetic patients with dyslipidaemia. Moreover, in two groups of hemodialysis patients, diabetic and non-diabetic, higher triglyceride and IDL cholesterol (P <0.001), and lower high-density lipoprotein (HDL) cholesterol (P <0.01) and apo A-I (P <0.001) levels were noted as compared to the control group.

In agreement with our study, a group of hypertriglyceridemic subjects had shown increased non-HDLc and apoB, whereas in the normotriglyceridemic group, several had increased non-HDLc and a large number had increased apoB. Furthermore, as seen in our study, low correlation of Apo A1 appears to be a main component of the dyslipidemic serum profile observed in diabetic patients as well as those with atherosclerotic occlusive disease of the lower extremities. Similarity with our outcome have been reported earlier where dyslipidemic profile characterized by increased triglyceride level, decreased apolipoprotein A1 level and small dense LDL associated with both NIDDM and non-diabetic subjects.

CONCLUSION:
It is concluded that diabetes and hyperlipidemia are important risk factors. Moreover, higher levels of Apo B and A and that of higher Apo B than Apo A are indicative of dyslipidemic state and thus must regard as significant parameters for assessing the prevailing conditions and extent of risk for developing coronary heart disease (CHD) and atherosclerosis.

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RESULTS:

Table 1. Anthropometric indices and biochemical parameters in non-diabetic (n = 46) and diabetic (n = 17) hyper-lipidemic patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NDHL (n = 46)</th>
<th>DHL (n = 17)</th>
<th>H (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>44.7±10.8</td>
<td>47.4±8.7</td>
<td>39.1±5.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32.3±3.5</td>
<td>31.5±3.2</td>
<td>26.2±3.4</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94±0.09</td>
<td>0.97±0.04</td>
<td>0.82±0.05</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>254±38.4</td>
<td>280±30.6^a</td>
<td>141±10.3*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>273±41.7</td>
<td>290±30.9</td>
<td>104±10.7*</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>49±5.30</td>
<td>44±6.13</td>
<td>58.1±5.16</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>163 ±12.9***</td>
<td>172±13.15</td>
<td>110±9.18*</td>
</tr>
<tr>
<td>Apo A-I, mg/dl</td>
<td>141±10.62</td>
<td>148±20.11</td>
<td>113±9.13*</td>
</tr>
<tr>
<td>Apo B, mg/dl</td>
<td>165±16.73</td>
<td>180±11.09^a</td>
<td>90±4.10*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>93±4.9</td>
<td>165±11.13*</td>
<td>86±3.1**</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>4.3 ±0.63</td>
<td>4.8±0.11</td>
<td>3.0±0.32</td>
</tr>
<tr>
<td>Lp(a), mg/dl</td>
<td>53.0± 4.5</td>
<td>47.5 ±4.12</td>
<td>32.2± 3.58*</td>
</tr>
</tbody>
</table>

*Significantly different from other members of group (p < 0.001).
** Significantly different from DHL group (p < 0.01)
*** Significantly different from Healthy group (p < 0.05)
^a Significantly differ from NDHL group (P<0.01) and HL (P < 0.001)

BMI = Body mass index; WHR = waist/hip ratio;
NDHL = non-diabetic, hyperlipidaemic; DHL = diabetic hyperlipidaemic;
H = healthy non-diabetic non-hyperlipidaemic; TC = total cholesterol; TG = triglycerides; LDL = low-density lipoproteins; HDL = high-density lipoproteins;