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Changes in Lipid Profile during different stages and complications of type 2 diabetes mellitus

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ABSTRACT

Aims: This study was undertaken with the aim to evaluate the changes in lipid profile emerging at various stages of type 2 diabetes mellitus and also ensuing several complications due to type 2 diabetes mellitus.

Methods: Serum lipid profile of type 2 diabetes mellitus patients was compared with healthy volunteers. Further the impact of the duration of diabetes on serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was elucidated. Correlation of body mass index with serum levels of total cholesterol, HDLc, LDLc and triglycerides was also undertaken.

Results: Serum levels of total cholesterol, LDLc, and triglycerides were significantly raised, whereas, serum level of HDLc was significantly decreased in type 2 diabetes mellitus and in patients with hypertension and liver diseases compared to controls. There was a significant strong positive correlation between the duration of diabetes (in years) and the serum levels of total cholesterol, LDLc and triglycerides, whereas there was a significant strong negative correlation between the duration of diabetes (in years) and the serum levels of HDLc. A significant strong positive correlation between the body mass index (BMI) of diabetic patients and the serum levels of total cholesterol, LDLc and triglycerides was noticed, whereas there was a significant strong negative correlation between the BMI of diabetic patients and the serum levels of HDLc.

Conclusion: Efforts to keep serum lipids levels within normal range are required by diabetes patients minimize cardiovascular complications, by reducing the intake of saturated fats and cholesterol and increased intake of unsaturated fats.

Key words: Diabetes Mellitus type 2; hypertension; liver disorders; serum lipid profile

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INTRODUCTION

Diabetes mellitus not a single disease, but is rather the pathological and metabolic state caused by inadequate insulin action, feature common to all types is glucose intolerance [1]. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [2]. Numerous clinical and epidemiological studies revealed that increased serum triglyceride levels are closely related to atherosclerosis, independently of serum levels of HDLc and LDLc [3]. Hypercholesterolemia causes focal activation of endothelium by infiltration and retention of LDLc in arteries causing inflammatory response and activation of reactive oxygen species (ROS) [4]. The central role of dyslipidemia in causing progression of atherosclerosis in adults with diabetes has been elucidated. There are a few researchers who have reported higher levels of total cholesterol, LDLc and triglyceride with higher HbA1c concentrations in diabetic patients [5,6]. This study was undertaken with the aim to evaluate the changes in lipid profile emerging at various stages and complications of type 2 diabetes. Further the impact of the duration of diabetes on serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was elucidated. Correlation of body mass index with serum levels of total cholesterol, HDLc, LDLc and triglycerides was also undertaken.

METHODS

A quantitative, cross-sectional, hospital-based study was conducted at Diabetes Care Center. A total of 100 patients with type 2 diabetes were enrolled for this study, who regularly visited the diabetes center for routine follow up. Fifty healthy volunteers (age and sex matched) were included as control group. An informed consent, aims and benefits of this study were explained to all the participants. Clinical data was recorded for each participant using a standard questionnaire for diabetes. After informed consent, a local antiseptic (70% ethanol) was used to clean the skin. Venous blood (5 ml) was taken from each participant by standard procedures, divided into two containers, 2 ml in fluoride oxalate anticoagulant container for plasma glucose, and 2 ml in heparin container and then centrifuged at 3000 RPM for three minutes and obtained plasma for cholesterol, triglycerides, HDLc and LDLc. Serum and plasma were separated in plain containers and kept at -20°C until use.

Measurement of serum total cholesterol

Principle [7,8]

The cholesterol present in the sample originates a colored complex, according to the following reaction:

\[ \text{Cholesterol esters} + \text{H}_2\text{O} \rightarrow \text{Cholesterol} + \text{fatty acids} \]

\[ \text{Cholesterol} + \text{O}_2 \rightarrow \text{Cholestenona} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{-Aminophenazone} \rightarrow \text{Quinonimine} + 4\text{H}_2\text{O} \]

The intensity of the color formed is proportional to the cholesterol concentration in the sample. The procedure used for the estimation of serum total cholesterol was as

**Measurement of serum HDLc**

**Principle [11]**

Very low density (VLDLc) and low density (LDLc) lipoproteins from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. The supernatant contains HDLc. The HDLc is then estimated spectrophotometrically by means of coupled reaction described below:

\[
\text{Cholesterol esters} \xrightarrow{\text{Chol.esterase}} \text{Cholesterol + Fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Chol.oxidase}} 4\text{-Cholestenone} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{- Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + \text{H}_2\text{O}
\]

The intensity of the color formed is proportional to the LDLc concentration in the sample. Estimation of VLDLc and LDLc was done as per the procedure available in NCEP, 2001 [10].

**Measurement of serum triglycerides**

**Principle [12]:**

Sample triglycerides when incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPD) to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H\text{2}O\text{2}).

In the last reaction, hydrogen peroxide (H\text{2}O\text{2}) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye.

\[
\text{Triglycerides} + \text{H}_2\text{O} \xrightarrow{\text{LPL}} \text{Glycerol + free fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol, kinase}} \text{G3P+ ADP}
\]

\[
\text{G3P + O}_2 \xrightarrow{\text{GPO}} 2\text{ DAP} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-AP} + \text{p-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + \text{H}_2\text{O}
\]

The intensity of the color formed is proportional to the triglycerides concentration in the sample and the same has been estimated by the method described by Young, et al. 1975 [9].
The amount of LDLc was obtained by using the following formulae.
LDL cholesterol = cholesterol total-(Triglyceride/5+HDL)

Quality Control
The precision and accuracy of all methods used in this study were checked each time by analyzing a batch of commercially prepared control sera.
Statistical analysis: Statistical Package for Social Science (SPSS version 11.5) computer software was used for data analysis. The means and standard deviations, t-test and correlations were used.

RESULTS
Hundred patients (56 males: 44 females) with type 2 diabetes agreed to serve as a test group in this study. Additionally fifty (29 males: 21 females) healthy volunteers took part as control group. Age and gender were matched. The 36% patients were hypertensive whereas only 11% of patients had liver disease. At P ≤ 0.05 this study showed there is no significant difference in age and body height (P=0.489), (P=0.068) between study group and control with mean ± SD values of 60.72±9.18, 59.75±12.36 years and 164.86±13.18, 162.20±14.50 cm respectively. While, a significant difference in body weight and body mass index (BMI) was found (P=0.013), (P=0.045) with mean ± SD values of 74.30±14.85, 69.89±14.33 Kg, and 28.92±5.29, 25.86±4.91 Kg/m² for diabetic patients and healthy individuals respectively (Table 1).
Comparison of lipids profiles

At P ≤ 0.05 significant difference was detected (P=0.000), (P=0.021), (P=0.013), (P=0.020), in serum levels of total cholesterol, HDLc, LDLc and triglycerides respectively with mean ± SD values of (190.24±30.53, 164.63±13.37) mg/dL, (40.30±10.10, 53.95±8.17) mg/dL, (122.37±39.38, 95.06±13.14) mg/dL, (138.68±40.85, 100.28±4.87) mg/dL for diabetic patients and healthy individuals respectively (Table 2).

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Patients with hypertension n=36</th>
<th>Patients without hypertension n=64</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>229.52 ± 9.45</td>
<td>170.44 ± 13.51</td>
<td>0.000</td>
</tr>
<tr>
<td>HDLc</td>
<td>28.82 ± 0.98</td>
<td>46.08 ± 7.27</td>
<td>0.006</td>
</tr>
<tr>
<td>LDLc</td>
<td>159.81 ± 6.19</td>
<td>103.50 ± 14.59</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>183.43 ± 15.39</td>
<td>116.13 ± 29.47</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P≤ 0.05 Significant

Table 3: Comparison of mean serum levels of Total cholesterol, HDLc, LDLc, and triglyceride of the diabetic patients with and without hypertension

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Patients with liver disease n=11</th>
<th>Patients without liver disease n=89</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>223.50 ± 4.95</td>
<td>189.90 ± 30.49</td>
<td>0.017</td>
</tr>
<tr>
<td>HDLc</td>
<td>30.00 ± 0.00</td>
<td>40.40 ± 10.09</td>
<td>0.024</td>
</tr>
<tr>
<td>LDLc</td>
<td>153.00 ± 4.24</td>
<td>122.06 ± 29.37</td>
<td>0.010</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>173.00 ± 1.41</td>
<td>138.33 ± 40.91</td>
<td>0.022</td>
</tr>
</tbody>
</table>

P≤ 0.05 - Significant

Table 4: Comparison of mean serum levels of Total cholesterol, HDLc, LDLc and triglycerides of the diabetic patients with and without liver disease

Lipids profiles in diabetics patients with and without hypertension

At P ≤ 0.05 a significant difference was found (P=0.000), (P=0.006), (P=0.000), (P=0.000), in serum levels of total cholesterol, HDLc, LDLc and triglycerides respectively, with Mean ± SD values of (229.52±9.45, 170.44±13.51) mg/dL, (28.82±0.98, 46.08±7.27) mg/dL, (159.81±6.19, 103.50±14.59) mg/dL, (183.43±15.40, 116.13±29.47) mg/dL for diabetic patients with hypertension when compared with those without hypertension respectively (Table 3).

Lipids profiles in diabetics patients with and without liver disease

At ≤0.05 a significant difference was assessed (P=0.017), (P=0.024), (P=0.010), (P=0.022), in serum levels of total cholesterol, HDLc, LDLc, and triglycerides with Mean ± SD values of (223.50±4.95, 189.90±30.50) mg/dL, (30.00±0.00, 40.40±10.10) mg/dL, (153.00±4.24, 122.06±29.37) mg/dL, (173.00±1.41, 138.33±40.91) mg/dL for the diabetic patients with liver disease when compared with those without liver disease.
Figure 1: A scatter plot shows the relationship between the duration of diabetes (years) and the serum levels of total cholesterol (mg/dL) ($r=0.72$, $P=0.000$).

Figure 2: A scatter plot shows the relationship between the duration of diabetes (years) and the serum levels of HDLc (mg/dL) ($r=-0.63$, $P=0.000$).
Figure 3: A scatter plot shows the relationship between the duration of diabetes (year) and the serum levels of LDLc (mg/dL) ($r=0.72$, $P=0.000$).

Figure 4: A scatter plot shows the relationship between the duration of diabetes (year) and the serum levels of triglycerides (mg/dL) ($r=0.73$, $P=0.000$).
Figure 5: A scatter plot shows the relationship between the BMI (Kg/m2) and the serum levels of total Cholesterol (mg/dL) (r=0.79, P=0.000).

Figure 6: A scatter plot shows the relationship between the BMI (Kg/m2) and the serum levels of HDLc (mg/dL) (r=-0.70, P=0.000).
Figure 7: A scatter plot shows the relationship between the BMI (Kg/m²) and the serum levels of LDLc (mg/dL) \((r=0.77, P=0.000)\).

Figure 8: A scatter plot shows the relationship between the BMI (Kg/m²) and the serum levels of Triglycerides (mg/dL) \((r=0.90, P=0.000)\)
respectively (Table 4).

Figure-1 depicts a significant and moderate reverse correlation between the duration of type 2 diabetes (years) and the serum levels of HDLc (mg/dL) ($r = -0.634$, $P = 0.000$). Figure-2 shows a significant and strong reverse correlation between the BMI (kg/m²) and the serum levels of HDLc (mg/dL) ($r = -0.70$, $P = 0.000$). Figure-3 portrays a significant and strong positive correlation between the duration of type 2 diabetes (years) and the serum levels of LDLc (mg/dL) ($r = 0.73$, $P = 0.000$). Figure-4 illustrates a significant and strong positive correlation between the BMI (kg/m²) and the serum levels of LDLc (mg/dL) ($r = 0.77$, $P = 0.000$) and the serum levels of LDLc (mg/dL) ($r = 0.18$, $P = 0.067$).

Figure-5 represents a significant strong positive correlation between the duration of type 2 diabetes (years) and the serum levels of triglycerides (mg/dL) ($r = 0.73$, $P = 0.000$). Figure-6 describes a significant strong positive correlation between the BMI (kg/m²) and the serum levels of triglycerides (mg/dL) ($r = 0.91$, $P = 0.000$). Figure-7 points out a significant strong positive correlation between the duration of type 2 diabetes (years) and the serum levels of total cholesterol (mg/dL) ($r=0.729$, $P=0.000$). Figure-8 reveals a significant strong positive correlation between the BMI (kg/m²) and the serum levels of total cholesterol in mg/dL and ($r=0.79$, $P=0.000$).

DISCUSSION

Diabetes mellitus type 2 (formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is characterized by hyperglycemia as a result of an individual’s resistance to insulin with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. However, these patients are more likely to go into a hyperosmolar coma and are at an increased risk of developing macro vascular and micro vascular complications [13].

The elevated cardiovascular risk of diabetic patients is only partially explained by the presence of conventional cardiovascular risk factors, such as glycemic control, lipids abnormalities and hypertension and visceral obesity. This has suggested that additional risk factors, such as genetic risk factors, may favor the increased cardiovascular morbidity and mortality observed in diabetic patients [14].

The current study shows a significant elevation of the serum levels of the total cholesterol of the diabetic group, diabetic patients with hypertension and diabetic patients with liver disease when compared with the control group. This occurs as a result of the disturbance in lipids metabolism by the diabetic liver, this finding was similar to a study done by Taskinem (1992) [15] also agreed with those reported by Emile, et al. (1993) [16] and disagreed with that reported by Yaron, et al. (1992) [17].

The current study revealed a significant moderate positive correlation between the duration of diabetes and the serum levels of total cholesterol as reported by Jones, et al. (1989) [18] who found a significant moderate positive correlation between the duration of diabetes and the serum levels of the total cholesterol. While, significant strong positive correlation between the BMI and the serum levels of the total cholesterol were assessed. These results were similar to that reported by Facchini, et al. (2001) [19] who found a significant strong correlation between the BMI and the serum levels of the total cholesterol.

Patients with type 2 diabetes mellitus and diabetic patients with hypertension and liver disease have a significant reduction in the mean levels of serum HDLc when compared with the control group subjects. This occurs as a result of the disturbance in lipids metabolism due to which HDLc takes large amounts of cholesterol from cells to the liver thus resulting in its low concentration in the blood. These findings are similar to that reported by Reaven, (1987) [20] and also in agreement with a study done in America by Yaron, et al. (1992) [17], who found that serum levels of HDLc was
significantly lower in diabetic patients compared to control subjects. A significant strong negative correlation was found between duration of diabetes and the serum levels of HDLc that could be due to bad glycemic control in diabetic patient. These observations agreed with those reported by Garfagnini, et al. (1995) [2,18], who found a significant moderate negative correlation between the duration of diabetes the serum levels of HDLc. Also there was a significant strong negative correlation between BMI and serum level of HDLc. Whereas, a study done by Facchini, et al. (2001) [19] showed a significant strong reverse correlation between BMI and the serum levels of HDLc.

This study expressed a significant elevation of serum levels of LDLc in diabetic patients and diabetic patients with hypertension and liver disease when compared with the control group which occurs as a result of disturbance in lipid metabolism and due to release of high amount of LDLc from injured liver cells. A significant strong positive correlation between the duration of diabetes and serum levels of LDLc that could be due to bad glycemic control in diabetic patient. This disagreed with those reported by Garfagnini, et al. (1995) [21], who found a significant moderate positive correlation between the duration of diabetes and the serum levels of LDLc. Also there was a significant (strong) positive correlation between the BMI and the serum levels of LDLc. These findings were similar to those reported by Facchini, et al. (2001) [19] and McLaughlin, et al. (2001) [22], who found a significant strong positive correlation between BMI and the serum levels of LDLc, this could be due to consumption of diet with high fat content.

The current study showed a significant elevation of the mean levels of the serum triglycerides in diabetic group, diabetic patients with hypertension and diabetic patients with liver disease when compared with the control group. This is similar to a study done by Taskinem (1992) [15] and also agrees with the result of a study done by Emile, et al. (1993) [16] but, disagrees with that reported by Yaron, et al. (1992) [17] this could be due to over production of VLDL-TG.

The present study also indicates a significant moderate positive correlation between the duration of diabetes and the serum levels of triglycerides but Garfagnini, et al. (1995) [21] found a significant strong positive correlation between the duration of diabetes and the serum levels of triglycerides this could occur due to bad glycemic control in diabetic patient which make disturbance in lipid metabolism. Further, in this study there was a significant strong positive correlation between the BMI and the serum levels of triglycerides. This agreed with those reported by McLaughlin, et al. (2001) [22] who found a significant strong positive correlation between BMI and the serum levels of triglycerides.

CONCLUSIONS

Efforts to keep the serum levels of lipids within the normal range are required by diabetes patients in order to delay or minimize development of atherosclerosis and its related cardiovascular complications, by reducing the intake of saturated fats and cholesterol and increased intake of unsaturated fats.

REFERENCES

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