Study of Serum Lipid Profile in Type 2 Diabetes Mellitus in Male and Female: More Apparent in Postmenopausal and Premenopausal Female

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ABSTRACT

There is increasing evidence from epidemiological studies that females were prone at higher level to Type 2 Diabetes Mellitus (T2DM) then T2DM male. Moreover, postmenopausal diabetic female have highest elevated risk of cardiovascular disease. However, it is still uncertain whether the estrogen itself contributes significantly to this increase in risk. Consequent of the cross-sectional analysis of 30 T2DM patients and 20 control, age and sex matched healthy individuals (group) these patients and controls subject were classified as male (15 patients, 10 controls), females (15 patients, 10 controls). Females then subdivided into premenopausal females (7 patients, 5 controls) and postmenopausal females (9 patients, 5 controls) were reported. The data revealed significant elevation of serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, except HDL-cholesterol which is decreased significantly, in all diabetic patients when compared with those of control groups. Female patients had higher level, of total cholesterol triglycerides, LDL-cholesterol, VLDL-cholesterol and low HDL-cholesterol, compared to age matched male patients. However, there was no significant difference in the triglycerides between the diabetic premenopausal, and postmenopausal females, and a significant reduction in the level of HDL-cholesterol in the postmenopausal group, and significant increase in the value of the total cholesterol and LDL-cholesterol. The results also conclude that the diabetic postmenopausal females are more prone for hyperlipidemia than diabetic premenopausal females.

1. Introduction

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Its prevalence has increased in an exponential manner over the last century. T2DM is associated with long-term micro- and macro-vascular complications, which account for the overall increased morbidity and mortality, the most common causes of death being cardiovascular diseases [National Diabetes Data Group 1995, A. Norhammar & K. Schenck-Gustafsson 2012]. The metabolic disturbances in T2DM include hyperinsulinemia, insulin resistance, hyperglycemia, dyslipidemia and obesity, all of which contribute to the accelerated atherogenesis in diabetes [National Institutes of Health 1985, National Institutes of Health, 1987]. Obesity and dyslipidemia are integral parts of the insulin resistance syndrome [DeFronzo RA, Ferrannini E. 1991], and insulin resistance with or without compensatory hyperinsulinemia provides a possible cause for the metabolic abnormalities. T2DM is one of the most common secondary causes of hyperlipidemia. The relationship between hyperlipidemia and vascular complication of both tend to occur with greater frequency in T2DM. Insulin resistance and obesity combine to cause dyslipidemia, hyperglycemia and hyperlipidemia have additive cardiovascular risk. The most common lipid abnormality noted in diabetes is hypertriglyceridemia. In obese diabetics the hepatic clearance of lipids and insulin are decreased due to increased levels of portal free fatty acids levels (FFA). The interaction of fibroblast from normal individuals with LDL isolated from diabetics was studied, a significant impairment was observed in diabetic LDL internalization and degradation. It was suggested...
that chemical modification of the LDL particles itself might result in its increased incorporation in the arterial wall via a receptor independent pathway resulting in high incidence of cardiovascular and cerebrovascular disease in T2DM individuals [Gopalan C. 2006].

Materials and Methods

After adequate education on the purpose of the study, a total of 50 diabetic subjects of age range 38-65 years who gave their consent and are not on insulin were recruited. These patients and control subjects were classified as male (15 patients, 10 controls), females (15 patients, 10 controls). Females then subdivided into premenopausal females (7 patients, 5 controls) and postmenopausal females (8 patients, 5 controls). These T2DM patients were diagnosed on the basis of history, biochemical investigations and urine examination.

Under all aseptic conditions, about 5 ml of blood was obtained by venipuncture from each male and female diabetic patient and control. The blood was put into centrifuge tubes. This was allowed to clot and then centrifuged at 3000 rpm for 15 min at room temperature. The serum obtained was pipetted into a clean blood sample vials. Care was taken to exclude the haemolysed serum. The obtained serum is analyzed for serum lipid and lipoprotein assay using systronic visible-spectro 105 and biocraft centrifuge.

Estimation of Blood Glucose

Glucose oxidase (GOD) oxidizes glucose to gluconic acid and hydrogen peroxide in presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to form coloured quinoneimine dye. Absorbance of colored dye is measured at 505 nm and is directly proportional to glucose concentration in the sample (Trinder P 1969).

Estimation of Total Cholesterol (TC)

TC was determined by an enzymatic method. The cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase. The free cholesterol is then oxidized by Cholesterol oxidase to cholesten-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced couples with 4-AAP and phenol, in the presence of peroxidase, to yield a chromogenic with maximum absorbance at 505 nm (Lorenzo Gordon et al 2010).

Estimation of Total Triglycerides (TG)

In this direct colorimetric procedure, Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of ADPS and 4-aminophenazine in the presence of peroxidase (POD) and hydrogen peroxide produces a rose colored dye which is measured at 550 nm. The intensity of the color formed is directly proportional to the triglycerides concentration in the sample. (Fossati and Colleagues)

Estimation of High-Density Lipoprotein Cholesterol (HDL)

HDL cholesterol was measured by an enzymatic method on the supernatant obtained after selective precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid, in the presence of magnesium ions and centrifugation. (Lorenzo Gordon et al 2010)

Estimation of Low-Density Lipoprotein Cholesterol (LDL)

Estimates LDL cholesterol using the Friedewald equation by subtracting the amount of cholesterol associated with other particles, such as HDL and VLDL, assuming a prolonged fasting state (G. Russell Warnick, 1990).

\[ \text{LDL-C} = \text{TC} - \text{HDL} - \left( \frac{\text{TRIGLYCERIDE}}{5} \right) \]

Estimation of Very Low-Density Lipoprotein Cholesterol (VLDL)

In the absence of chylomicrons, only three forms of lipoproteins are present in the sera-VLDL, LDL and HDL. Since VLDL is the primary triglyceride carrying form in the fasting state; its concentration can be approximated by dividing the amount of plasma triglycerides by described by Friedwald formula in 1972 (G. Russell Warnick, 1990).

\[ \text{VLDL}=\frac{\text{TRIGLYCERIDE}}{5} \]

Statistical analysis

The results were expressed as (mean ±SD) and analyzed statistically, the difference between the results of patients and control group were assessed by students t test. Significant variation was considered when the P value was less than 0.05 (Zar, J. H. 1999).
Result

Table 1: Status of blood sugar and serum lipid profile in control & diabetic male and females (then subdivided into premenopausal and postmenopausal females).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Blood glucose (mg/dl)</th>
<th>Lipid Profile (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPBS</td>
<td>FBS</td>
</tr>
<tr>
<td>Control Males</td>
<td>157.3 ±13.5</td>
<td>107 ±5.5</td>
</tr>
<tr>
<td>Diabetic Male</td>
<td>262.4 ±58.8</td>
<td>190.53 ±63.9</td>
</tr>
<tr>
<td>Control Females</td>
<td>156.7 ±18.2</td>
<td>104.5 ±10.7</td>
</tr>
<tr>
<td>Diabetic Female</td>
<td>266.31 ±44.6</td>
<td>178.18 ±33.8</td>
</tr>
<tr>
<td>Control Premenopause Females</td>
<td>160.4 ±22.3</td>
<td>100.6 ±12.5</td>
</tr>
<tr>
<td>Diabetic Premenopause Females</td>
<td>253.17 ±49.4</td>
<td>185.33 ±47</td>
</tr>
<tr>
<td>Control Postmenopause Females</td>
<td>153 ±11.8</td>
<td>108.4 ±6.6</td>
</tr>
<tr>
<td>Diabetic Postmenopause Females</td>
<td>281.33 ±37.1</td>
<td>178.77 ±20.3</td>
</tr>
</tbody>
</table>

Graph 1: Comparative status of blood glucose level and serum lipid profile (mg/dl) in male and females (control & diabetic).
Diabetes mellitus (DM) is a disorder of metabolism. Lipid abnormalities represent an important cardiovascular risk factor in T2DM (Gordon, T., et al 1977) in addition increases in plasma triglycerides in some studies have been better predictors of CHD in diabetics than increased in cholesterol (Kannel, W.B. 1979) (Hulley, S.B., et al 1980). The most common lipid anomaly in diabetes is hypertriglyceridaemia, caused by increase in plasma VLDL triglyceride, but sometimes chylomicrons may accumulate. This may not be unrelated to insulin resistance which has been closely associated with diabetes dyslipidaemia and hypertension (Mgonda et al., 1998). Lipoprotein lipase (LPL) is the main enzyme for the catabolism of chylomicrons and very low density lipoprotein (VLDL) particles which enable them to form remnants that are cleared by APO E or APO B receptors in the liver. LPL is an insulin dependent enzyme and insulin resistance will lead to increased TG levels. Metabolism of HDL cholesterol in T2DM is not well understood but its nearness is thought to be mediated by VLDL receptors, which are mainly located in the liver. Insulin has been said to play a role in regulation of VLDL receptor binding and internalization leading to a decrease in HDL catabolism. After the age of 40s, the most common feature amongst females is menopause, estrogen level secretion is decreased that has cardioprotective functions. Menopause can have a profound effect on chronic illnesses such as type 2 diabetes. The changes in estrogen and other hormonal levels that occur during pre-menopause can cause major fluctuations in blood glucose levels. Women with T2DM who have gone through menopause may no longer have wild hormonal swings affecting blood glucose levels, but they are at higher risk of developing atherosclerosis. Weight gain after menopause is not unusual, but it seems to be more common among women with T2DM. This adds to the increased risk of premature menopause, which can lead to higher risk of heart disease. It seems that Diabetes mellitus may alter lipid profiles more adversely in female compared to male. Diabetes mellitus increases the risk of cardiovascular disease in woman to a great extent than in men (Lee, W.L.; et al 2000, Kanaya, A.M.; et al 2002, Barrett-Connor, E.; Wingard, D.L (1983), Barrett-Connor et al 2004, M. Nakhjavani: et al 2006). There are variations in lipid levels obtained in diabetic postmenopausal woman when compared to that of diabetic premenopausal woman. After menopause, there is less of ovarian function. This results in adverse changes in glucose and insulin metabolism, body fat distribution, coagulation, fibrinolysis and vascular endothelial dysfunction [Spencer, C. et al 1977]. There is also derangement of lipoprotein profile independent of age [Bales, A. (2000)]. A number of changes that occur in the lipid profile after menopause are associated with increased cardiovascular disease risk. Lack of estrogen is an essential factor in this mechanism. A part from maintaining friendly lipid profile, estrogen changes the vascular tone by increasing nitrous oxide production. It stabilizes the endothelial cells, enhances antioxidant effects and alters fibrinolytic protein (Taddec, S. 1996). All these are cardio protective mechanisms, which are lost in menopause. In fact it has been postulated that, most of the diabetic person suffers from Hyperlipidemia. Hyperlipidemia is more aggressive in postmenopausal diabetic females. It is thus important to note this and device means of correcting the hyperlipidemia. The values obtained in case of comparative study of mean for blood glucose and lipid profile of control and diabetic male (graph1) shows that the TC, TG, LDL value of diabetic patients increased with respect to control whereas HDL-c decreased in diabetic male also there was increase in blood sugar. Similarly on comparative study of blood glucose and serum lipid in diabetic females and control (graph 1) it was found that all lipid profile parameters, TC, TG, LDL and VLDL level was increased with the significant decrease in HDL level. The parameter of blood sugar was increased. Moreover, when comparative study of blood glucose level and lipid profile in diabetic male and female (graph 1) was performed it was illustrated that the parameter of blood sugar postprandial blood sugar (PPBS) ceases more in diabetic male whereas Fasting blood sugar ceases more in diabetic female. Also the level of TC, TG, and LDL raises high in diabetic female than in diabetic male, but the level of cardio protective HDL- cholesterol was more in diabetic male. It is very clearly indicated by these values that lipid profile is altered in diabetic patients. This difference is much more clearly observed in post-menopausal female as compared to pre-menopausal female in (graph 2). The menopause diabetic women may no longer have hormonal swings affecting blood glucose levels. The female subjects in this study had significantly higher triglyceride and LDL cholesterol but lower HDL level than their male counterpart. This is contradictory with previous studies in African Americans (Sapna et al, 2008). Race and sex differences in patterns of serum lipids have been noted in diabetics (Summerson et al, 1992; Werk et al., 1993). African-Americans with Type 2 diabetes reportedly have lower TG and higher HDL cholesterol concentrations than Caucasians, and women with diabetes have higher LDL and HDL cholesterol concentrations than their male counterparts (Summerson et al, 1992; Werk et al, 1993). However in an Indian diabetic population higher level of TC, LDL-C and TG was found among the male subjects (Sapna et al., 2008).
Conclusion:

This study demonstrated that Type 2 diabetes is associated with dyslipidemia which contribute to accelerated cardiovascular diseases (CVD). Menopause, in addition favors to the development of diabetes, is associated with dyslipidemia with lowered HDL-cholesterol compared to male patients. However, a significant reduction in the level of HDL-cholesterol in the postmenopausal group, and significant increase in the value of LDL-cholesterol was seen. Therefore type 2 diabetic postmenopausal female are more profoundly to risk of atherogenic dyslipidemia compared to type 2 diabetic premenopausal female. Therefore, lipid profiling for all persons with T2DM should be a routine test. All persons with T2DM must be started on primary prevention by encouraging healthy lifestyle diet so as to reduce the risk of CHD. Since the use of HRT and lipid lowering drug is still controversial. Further studies should be undertaken to establish pattern estrogen, proper dietary, social and physical habits in T2DM.

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Reference


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