Apolipoprotein A and Apolipoprotein B Levels Along With Standard Lipid Profile in Type II Diabetes Mellitus Patients in a Tertiary Care Centre in Sub Himalayan Region.

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ABSTRACT

Background: Apolipoprotein A (Apo A) and apolipoprotein B (Apo B) are the key structural components of high density lipoprotein (HDL) particles and atherogenic lipoproteins respectively. The present study was designed to estimate and investigate the role of Apo A and Apo B levels along with standard lipid profile in patients with type 2 diabetes mellitus (T2DM). **Methods:** The study was carried out on 200 subjects viz. 100 healthy controls and 100 T2DM cases. Whole blood sample was utilized for glycosylated hemoglobin (HbA1c) and serum obtained was analyzed for quantitative estimation of total cholesterol, triglycerides, low density lipoprotein (LDL), HDL, Apo A and Apo B by automated chemistry analyzer. **Results:** A significant increase (p < 0.005) was observed in the mean HbA1c, cholesterol, triglycerides, LDL and Apo B levels in patients with T2DM as compared to healthy controls. On the contrary, a significant decrease (p < 0.005) was witnessed in the mean HDL and Apo A values of T2DM patients as compared to healthy controls. A positive correlation was seen between HbA1c and total cholesterol (r = 0.427); HbA1c and triglyceride levels (r = 0.281); HbA1c and LDL (r = 0.399); HbA1c and Apo B (r = 0.403). However, a negative correlation was observed between HbA1c and HDL (r = -0.337); HbA1c and Apo A (r = -0.426). **Conclusion:** There is a high prevalence of dyslipidemia with poorly controlled HbA1c in T2DM patients. Apo A and Apo B assays should be incorporated in the standard lipid profile, for better risk prediction in diabetics and to curtail the risk of future cardiovascular mortality.

Keywords: Apolipoprotein A, Apolipoprotein B, Lipid profile, Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders associated with chronic hyperglycemia and a major health problem worldwide.[1] Type 2 diabetes mellitus (T2DM) occurs as a result of inadequacy of pancreatic β cells to secrete insulin, or insulin resistance in tissues that are sensitive to insulin such as liver, skeletal muscles and adipose tissue.[2] Epidemiological studies relating to the incidence of DM suggest that 1 in 11 adults is suffering from DM, of whom 90 % have T2DM. Asia (China and India) has emerged as the epicenter of this T2DM epidemic.[3] According to World Health Organization in the year 2015, India alone had an estimated diabetic population of 70 million, out of which more than 50 % (36 million) of people with diabetes went undiagnosed.[4]

Sedentary lifestyle and consumption of unhealthy diets have emerged as the main driving forces for global T2DM epidemic. Additionally, intrauterine exposures during the developmental phase are also important in susceptibility to T2DM in the later stages of life.[3] DM patients often have an abnormally high level of lipids (about 70 % of diabetic population is dyslipidemic) and are more likely to be predisposed to coronary heart disease.[5] The most common findings in T2DM patients are elevated triglycerides (TG), decreased levels of high density lipoprotein (HDL) cholesterol, normal or raised low density lipoprotein (LDL) cholesterol and occurrence of small dense LDL particles that are cholesterol depleted.[6] Moreover, in diabetic population the projected risk of atherosclerotic cardiovascular disease has been shown to be augmented by 18% for each 1% rise in glycosylated hemoglobin (HbA1c).[7]

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Apolipoprotein A (Apo A) and apolipoprotein B (Apo B) are the key structural components of HDL particles and atherogenic lipoproteins respectively.[6] Estimation of Apo A and Apo B can provide supplementary information to that obtained by accessing HDL and LDL. In these lipoproteins the protein concentration are linked to particle numbers and are an indication of metabolic status, specifically if used in combination with plasma Triglycerides.[8] Additionally, the assays for Apo A and Apo B are precise, consistent, usually available and the apolipoprotein concentrations are minimally affected from biological variables as compared to lipid estimations.[9]

However, there are inconsistent reports on the role of Apo A and Apo B in in the development of T2DM.[10,11] Better understanding and introduction of these parameters in the lipid profile can be beneficial in prediction of risk factors, along with the improvement in lipid lowering treatment and can pave way for new therapeutic strategies. Therefore, keeping in mind the above mentioned facts the present study was designed to estimate Apo A and Apo B levels along with standard lipid profile in patients with T2DM in a tertiary care centre in north India.

MATERIALS AND METHODS

The study was carried out at the Department of Biochemistry, Dr. Rajendra Prasad Government Medical College, Kangra at Tanda, District Kangra, Himachal Pradesh, India. The study commenced after obtaining approval from the Protocol Review and Institutional Ethics Committee (No. HFW-H-DRPGMC/Ethics/2016, Protocol No. 62/2016).

Patients

Sampling population comprised of all adult subjects (both male and female) coming to the centralized sample collection centre of the hospital. Total 200 subjects viz. 100 healthy controls and 100 T2DM cases were taken for the study. After obtaining the written consent, demographic features, personal history, general physical examination of subjects was carried out. Patients with chronic illness like tuberculosis, cancer or immunocompromised states, pregnant or lactating mothers and subject's who had donated or received blood two weeks prior to obtaining the blood sample were excluded from the study.

Methods

Whole blood sample approximately 10 ml was collected from the median cubital or cephalic vein after venipuncture; 2 ml in the ethylene diamine tetra acetic acid (EDTA) tube for HbA1c estimation and 8 ml in plain vial. Whole blood sample was utilized for HbA1c using Nyco card (Alere Technologies AS, Oslo, Norway) reader. The serum obtained was analyzed for quantitative estimation of total cholesterol, triglycerides, LDL and HDL by XL 300 (Erba, Mannheim, Germany) automated chemistry analyzer using commercially available kits (Transasia Bio-Medicals Ltd., Baddi, India). Serum Apo A and Apo B levels were estimated by XL 300 (Erba, Mannheim, Germany) using kits procured from Agappe Diagnostics Ltd., Ernakulam, India.

Statistical Analysis

The data was analyzed using independent samples t-test between the groups using Statistical Package for Social Sciences (SPSS) software version 20. Data was expressed as mean ± standard deviation (SD) for continuous variables. Value with $p < 0.005$ calculated at 95 % confidence limit, was considered statistically significant. Correlation studies were carried out using Pearson's correlation coefficient (r).

RESULTS

The mean HbA1c of controls was 5.55 ± 0.41 and that of cases was 7.93 ± 1.68 [Table 1]. A statistically significant difference was obtained in the mean HbA1c values of controls and cases ($p < 0.005$). A significant increase ($p < 0.005$) was observed in the total cholesterol levels in T2DM patients (211.20 ± 36.96) as compared to controls (169.71 ± 30.79) [Table 1]. Serum triglyceride levels of controls were 143.74 ± 57.90 and that of T2DM patients were measured to be 196.75 ± 99.94. The difference in mean triglycerides levels of controls and T2DM patients was statistically significant ($p < 0.005$) [Table 1]. However, a significant decrease ($p < 0.005$) was observed in the mean HDL values of T2DM patients (42.16 ± 12.25) as compared to healthy controls (53.31 ±10.97) [Table 1]. Serum LDL levels of controls was 84.61 ± 26.35 and that of cases was 128.29 ± 39.15 exhibiting a statistically significant difference ($p < 0.005$) between the two groups [Table 1].

The serum Apo A value in controls (176.38 ± 40.30) was found to be higher as compared to that of T2DM patients (136.40 ± 16.70). A statistically significant difference in the serum Apo A values of controls and cases was observed ($p < 0.005$) [Table 2]. However, mean serum Apo B value in controls was observed to be 98.33 ± 9.74 whereas the mean value of serum Apo B was 110.32 ± 10.51. There was a statistically significant difference in the serum Apo B values of controls and cases ($p < 0.005$) [Table 2].

A positive correlation was observed between HbA1c and total cholesterol (r = 0.427). It was observed that with higher HbA1c values there was a subsequent rise in the values of total cholesterol [Figure 1A]. Similarly, a positive correlation was also observed between HbA1c and triglyceride levels (r = 0.281) [Figure 1B]. However, a negative correlation was witnessed between HbA1c and HDL (r = -0.337) and with increase in the HbA1c values there was a decrease in the values of HDL [Figure 1C]. In line

with the cholesterol and triglyceride levels a positive correlation was observed between HbA1c and LDL (r = 0.399) [Figure 1D]. A negative correlation was observed between HbA1c and Apo A (r = -0.426) [Figure 2A]. On the contrary, a positive correlation was noticed between HbA1c and Apo B (r = 0.403) [Figure 2B].

**Table 1: Mean values of HbA1c, Total cholesterol, Triglycerides, HDL and LDL in Control and Case groups.**

Test Parameter	Control (mean ± SD)	Case (mean ± SD)	p value
HbA1c (%)	5.55 ± 0.41	7.93 ± 1.68	< 0.005
Total Cholesterol (mg/dl)	169.71 ± 30.79	211.20 ± 36.96	< 0.005
Triglycerides (mg/dl)	143.74 ± 57.90	196.75 ± 99.94	< 0.005
HDL (mg/dl)	53.31 ± 10.97	42.16 ± 12.25	< 0.005
LDL (mg/dl)	84.61 ± 26.35	128.29 ± 39.15	< 0.005

Table 2: Mean values of Apo A and Apo B in Control and Case groups.

Test Parameter	Control (mean ± SD)	Case (mean ± SD)	p value
Apo A (mg/dl)	176.38 ± 40.30	136.40 ± 16.70	< 0.005
Apo B (mg/dl)	98.33 ± 9.74	110.32 ± 10.51	< 0.005
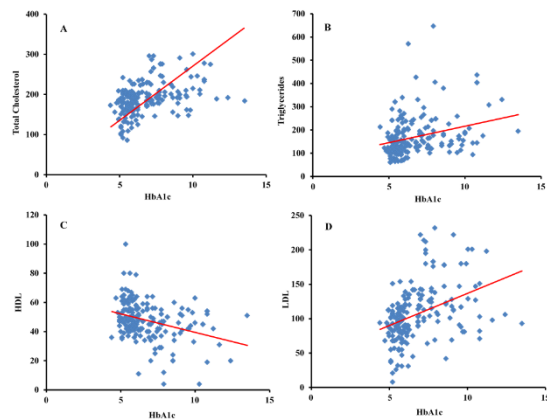
Figure 1: Scatter plot showing positive correlation between HbA1c and Total Cholesterol (r = 0.427) (A), positive correlation between HbA1c and Triglycerides (r = 0.281) (B), negative correlation between HbA1c and HDL (r = -0.337) (C) and positive correlation between HbA1c and LDL (r = 0.399) (D).
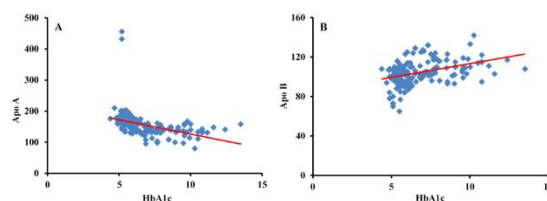
Figure 2: Scatter plot showing negative correlation between HbA1c and Apo A (r = -0.426) (A) and positive correlation between HbA1c and Apo B (r = 0.403) (B).

DISCUSSION

In the present study T2DM patients (cases) showed poorly controlled HbA1c values as compared to controls and the difference between the two groups was statistically significant. The mean total cholesterol levels of T2DM patients were significantly higher than the control group. Moreover, the present study also exhibited a significant positive correlation between HbA1c and total cholesterol. These results are in line with previous studies where a significant correlation was observed between HbA1c and total cholesterol in T2DM patients from Punjab and Kathmandu region.[12,13] In a prospective study carried out by Sheth et al. (2015) on western Indian population comprising of 430 T2DM patients and 501 control subjects a significant correlation was observed between HbA1c and total cholesterol.[14]

A statistically significant difference was observed in the mean value of triglycerides in controls (143.74 ± 57.90) as compared to cases (196.75 ± 99.94) in the present study. These findings are in agreement with previous studies where a significant increase in the triglycerides levels was observed in diabetic patients.[13,15] Moreover, the elevated triglycerides in diabetic patients has also been associated with diminution in adipose and muscle lipoprotein lipase activity.[16] The present study also exhibited a positive correlation between HbA1c and triglycerides, thus implying that poor glycemic control led to elevated triglycerides. Similar correlation between HbA1c and triglycerides has also been observed in diabetic patients of south Indian origin in a study carried out by Arivarasan et al. (2012).[17]

HDL is known to play a role in reverse cholesterol transport by removing cholesterol from peripheral tissues and transporting it to liver for breakdown.[18] A reduction in the plasma HDL cholesterol is an important feature of metabolic syndrome and a predisposing factor for T2DM and coronary heart disease, suggesting that HDL has anti-atherogenic properties.[19] The present study exhibited that T2DM patients had lower levels of HDL as compared to controls. Similarly, a negative correlation was observed between HbA1c and HDL suggesting that subjects with higher value of HbA1c had a lower value of HDL. The decrease in the HDL levels observed in diabetic patients can be attributed to increased concentration of very low density lipoproteins (VLDL) that initiates the exchange of triglycerides from VLDL for cholesteryl esters present in HDL.[20] In a study carried out by Bardini et al. (2013) the decrease in HDL cholesterol levels was accompanied with beta-cell dysfunction in subjects with reduced glucose tolerance.[21] Moreover, Ramona et al. (2011) exhibited a reverse correlation between HbA1c and HDL levels in T2DM patients.[22]

In the present study, T2DM patients displayed elevated LDL levels as compared to controls and the difference was statistically significant. Additionally, a positive correlation was observed between HbA1c and LDL. Similar increase in the LDL cholesterol was observed in T2DM patients visiting a tertiary care centre.[13] In a study carried out on 128 T2DM patients in Sichuan, China a significant correlation was observed between HbA1c and LDL levels and with an increase in the HbA1c levels a significant increase was observed in LDL levels.[23] Similar correlation was observed between HbA1c and LDL levels in diabetic population from western India.[14]

In line with the previous studies, the present study suggests for lipid abnormalities including elevated total cholesterol, triglycerides, LDL and decreased levels of HDL in T2DM patients. The development of dyslipidemia may be an indication of future diabetes onset and it generally includes postprandial lipidemia, elevated triglycerides, lowered HDL cholesterol and reduced or normal LDL cholesterol.[6] Lipid anomalies in DM can be attributed to the fact that the key enzymes and pathways involved in lipid metabolism (synthesis of apoprotein, lipoprotein lipase regulation, cholesteryl ester action, peripheral and hepatic action of insulin) are affected by insulin resistance or deficiency.[15] Additionally, in diabetic dyslipidemia the composition of lipid particles is more atherogenic than in other forms of dyslipidemia suggesting that normal lipid levels in DM patients are more atherogenic than in nondiabetics.[24]

Apo A1 is the main structural protein of HDL that mediates the transfer of cholesterol from the surface of cell to lipoprotein particles and activates the enzyme required for esterification of cholesterol in the circulation.[8] The results of the present study exhibited lower value of Apo A in T2DM patients as compared to healthy controls with a negative correlation between HbA1c and Apo A. The results are in line with previous studies where a significant decline in Apo A1 levels was observed in diabetics as compared to control group.[25] In a recent study carried out by Wu et al. (2017) diminution in Apo A1 individually led to a 97% increased risk of new T2DM over 4 years among urban Chinese community based population who were healthy and without any risk factor for T2DM.[10] However, in a Turkish sample population elevated Apo A1 levels individually predicted the incidence of T2DM with top tertile of serum Apo A1 approximately doubled the possibility of T2DM when compared with low tertile.[26] The present study demonstrated a significant increase in Apo B values in T2DM patients as compared to healthy controls. Additionally, a positive correlation was observed between HbA1c and Apo B. In a study carried out in tertiary care centre in Maharashtra, India a significant increase was observed in Apo B levels in T2DM patients as compared to controls.[5] The

association of ApoB with incident of T2DM has been demonstrated with improved risk prediction as compared to HDL cholesterol or LDL cholesterol.[27] Moreover, insulin resistance is strongly associated with Apo B/Apo A1 ratio.[28]

While conventional lipid estimations have been stated to be related to the incident of T2DM, some dispute still prevails on the nature and the degree of association.[29] Moreover, the precision and comparability of HDL cholesterol and LDL cholesterol are difficult due to lack of common reference material for their measurements. Additionally, the inaccuracies of direct HDL cholesterol and LDL cholesterol estimation are not mainly concerned with the assay calibration, but as a result of inherent problems with each assay. However, estimation of Apo A and Apo B can provide supplementary information to that obtained by accessing HDL and LDL. In these lipoproteins the protein concentration are linked to particle numbers and are an indication of metabolic status, specifically if used in combination with plasma triglycerides.[8] Additionally, the assays for Apo A and Apo B are precise, consistent, usually available and the apolipoprotein concentrations are minimally affected from biological variables as compared to lipid estimations.[9] So it can be concluded that the assays for Apo A and Apo B should be included in the standard lipid profile, for better risk prediction in diabetics.

The findings of the present study are in line with most of the studies found in the literature. However few studies differ from the present results. The discrepancies between these studies can be explained by differences in the selection of subjects, their economical status, geographical variation and dietary differences. Further studies on a larger sample are needed to substantiate the present findings before firm conclusion can be drawn on the utility of these parameters for the diagnostic assessment and management of patients with T2DM.

CONCLUSION

It can be concluded from the present study that there is a high prevalence of dyslipidemia with poorly controlled HbA1c in T2DM patients. The high prevalence of lipid disorders in the present study warrants periodic screening for lipid abnormalities in diabetics. The assays for Apo A and Apo B should be included in the standard lipid profile, for better risk prediction in diabetics and to minimize the risk of future cardiovascular mortality.

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