HIGH DENSITY LIPOPROTEINS ARE SIGNIFICANTLY REDUCED IN EARLY STAGES OF UNTREATED TYPE 2 DIABETES MELLITUS

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ABSTRACT

Introduction: *The patients of type 2 diabetes are prone to develop dyslipidemia which is characterised as triad of hypertriglyceridemia, low HDL-C and dense LDL. Most of the research is concentrated towards the patients with well-established diabetes of long duration. We aimed to evaluate the lipid profile during early stages of the disease in patients of type2 diabetes.*
Material and Methods: *Blood samples from patients (n=40) with a history of <6 months, diagnosed for the first time in our hospital and healthy controls (n=40) were processed for plasma glucose (fasting and post prandial), lipid profile and other routine biochemical parameters before any treatment was started. Statistical analysis was carried out by using students"t' test and Pearson correlation analysis.*
Results: *There was no significant difference in the lipid profile of patients and controls except HDL-C. Patients of type 2 diabetes had significantly low (p<0.03) level of HDL-C. Though triglycerides (TGs) levels were not significantly different in the two groups, a negative correlation was observed between HDL-C and TGs in patient group.*
Discussion: *In early stage of the disease it is the HDL-C level that is altered rather than other lipid profile parameters. Low HDL-C is probably an early indicator of setting in of dyslipidemia. Since there is a negative correlation between HDL-C and TGs in patients, it appears that reducing the TG levels may also raise HDL-C in these patients.*

Key words: *Dyslipidemia, lipid profile, early type 2 diabetes*

INTRODUCTION

Diabetes is one of the leading causes of morbidity and mortality in the world and also major health problem in India. In 2013, there were 65.1 million people between 20 and 79 years of age with diabetes and this number is predicted to rise to 109 million by 2035 (1). Vascular complications associated with type 2 diabetes are responsible for most of the life threatening clinical conditions faced by diabetic patients. Mortality due to coronary heart disease (CHD) is about two- to four-fold higher in diabetic individuals as compared to non-diabetic ones (2). About 80% of diabetes-related death is due to cardiovascular complications (2). Atherosclerosis is a major component of the increased cardiovascular risk associated with type 2 diabetes. Although the pathophysiology of these processes has not yet been fully elucidated, changes in the endothelium of the blood vessels, deposition of fatty plaques and reduction in blood flow are all implicated. The atherosclerotic lesions may appear similar in patients with and without diabetes; however, those with type 2 diabetes show more accelerated pathogenesis.

Dyslipidemia, a prominent feature of type 2 diabetes plays a major role in this accelerated a thermogenesis. It is characterized by an increase in triglyceride rich lipoproteins (very low-density lipoproteins [VLDL] and their remnants), a decrease in plasma levels of high-density lipoprotein cholesterol (HDL-C) and an increase in small dense low-density lipoprotein (sdLDL) particles(3). Plasma LDL-C levels, however, may be normal or only slightly elevated. Abnormal serum lipids are likely to contribute to the risk of coronary artery disease in diabetic patients [4] and the determination of the serum lipid levels in people with diabetes is now considered as a standard of the diabetes care [5]. It is well established that, irrespective of the plasma (LDL-C) concentration, a low level of high density lipoprotein cholesterol (HDL-C) represents a significant independent risk factor for cardiovascular

events [6].Altered metabolism of triglyceride-rich lipoproteins is crucial in the pathophysiology of the atherogenic dyslipidemia of diabetes. Alterations include both increased hepatic secretion of triglyceride rich VLDL and impaired clearance of VLDL. This results in higher levels of triglycerides and enrichment of LDL and HDL with TAGs owing to increased activity of Cholesterol ester transfer protein (7).However, it is difficult to recognize the early changes in lipid profile associated with the onset of diabetes as most of the patients are evaluated when the disease process has already set in and progressed to later stages (8,9,10). In this study we evaluated the lipid profile of newly diagnosed diabetic patients and compared with age and sex matched controls to identify the lipid fractions which are deranged in the initial stages of pathogenesis of diabetes.

MATERIAL AND METHODS

The study was conducted in the department of Biochemistry and Medicine in University college of Medical Sciences and GTB Hospital, Delhi, India after obtaining ethical clearance from the institutional ethical committee for human studies. The study was a case control study in which patients (n=40) with a history of <6 months, diagnosed for the first time in our hospital were recruited from the diabetic clinic before initiating the treatment. Non diabetic controls (n=40) were recruited from the staff members and relatives of patients. All the participants were more than 35 years of age and were diagnosed for the first time in our hospital on the basis of ADA criteria[11]. Exclusion criteria included presence of thyroid disorders, renal dysfunction, liver dysfunction, previous history of DM and previous or present history of cardiovascular disease as assessed from history of chest pain, stroke and ECG.

After obtaining informed consent from all participants, anthropometric measurements were carefully carried out and recorded. Weight was measured using a digital scale whose accuracy and precision was checked periodically using standard weights, height was measured using wall mounted scale. Body mass index (BMI) was calculated as weight in kg divided by squared height (m^2). Waist hip ratio (WHR) was calculated as ratio of waist circumference measured at the level of umbilicus after expiration to hip circumference (measured as maximal horizontal circumference at the level of the buttocks). To remove subjective errors, all measurements were carried out by two individuals and mean reading was recorded.

The blood samples were collected after an overnight fast and after 2 hours of 75 gm glucose load by standard protocol to assess glycemic status (11). Fasting blood samples were processed to separate serum. The serum was analysed for lipid profile parameters, renal functions (S. urea, S. creatinine, S. electrolytes), liver functions (S. bilirubin, ALT, AST, ALP). For all biochemical parameters, control sera covering reference intervals were measured simultaneously with patient samples to ensure accuracy. The data was compared with the age and sex matched healthy controls (n=40) whose blood samples were also analysed as described above.

Plasma glucose was estimated using glucose oxidase method (12). Lipid profile was done by using cholesterol esterase/ oxidase method for serum cholesterol(13) and glycerol kinase method was used for serum triglycerides (14). HDL-C was measured after precipitation (15) and LDL-C calculated by Freidwald formula (16). Dyslipidaemia was diagnosed on the basis of NCEP–ATP III criteria (7). HbA1c was analysed in whole blood by HPLC method using Bio-Rad D-10 Haemoglobin Testing System. Serum Insulin was analysed by commercially available ELISA kit (Diametra, Italy) following manufacturer's protocol. HOMA–IR was calculated by the formula; serum fasting insulin (μU/ml) X Fasting glucose (mmol/l)/22.5 (17). Statistical analysis was done by using students "t' test. Value of $p<0.05$ was considered significant. Correlation between different parameters was carried out by Pearson correlation analysis in both groups.

RESULTS

The comparisons of various clinical and biochemical parameters are shown in table 1. Though BMI was significantly higher

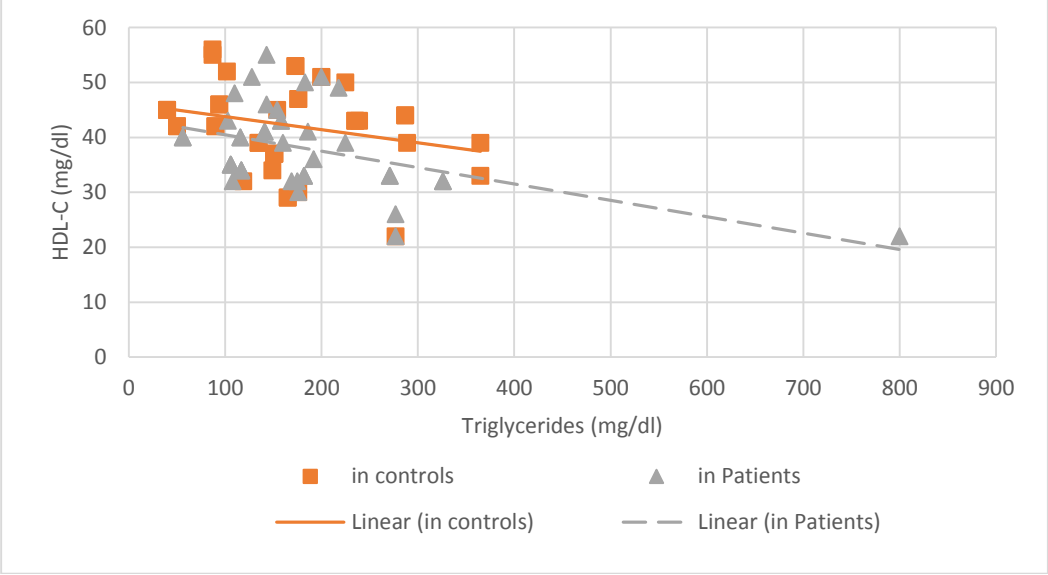
in patients, both waist circumference and waist hip ratio was similar in both groups. In patient group, 90% were dyslipidemic while in controls 75% were having dyslipidaemia. Considering waist circumference as a measure of obesity (18), 65% of patients and 60% of controls were found to be obese. All lipid profile parameters were similar in both groups except HDL-cholesterol levels which were found to be significantly reduced in patients. Correlation analysis was also carried out to analyse the association between different parameters in both groups. It was observed that HDL- C levels correlated negatively with triglycerides in both groups but the association was significant only in patients (r= -0.478, p<0.01 *vs* r= -0.257, p>0.05) (fig 1). Significant correlation of HDL-C was not observed with HOMA-IR (r=0.060, p>0.05), waist circumference (r=-0.11, p>0.05), BMI (r=0.12, p>0.05) and WHR (r=-0.25, p>0.05).

Table 1: Comparison of different parameters in patients and controls

	Control (n=40)	Patients (n=40)
Age(years)	45.4±7.96	48.03±9.70
Waist circumference (cm)	94.03± 10.63	93.03±13.44
Body mass index	24.98±3.33	27.70±4.50*
Waist hip ratio	0.898±0.068	0.928±0.082
Total Cholesterol (mg/dl)	197±36.46	189.43±30.56
HDL-Cholesterol (mg/dl)	42.03±7.81	37.73±8.06*
LDL-Cholesterol (mg/dl)	119.5±34.8	118.78±33.23
Triglyceriges (mg/dl) (geometric mean by log transformation)	143.54	161.77
Fasting plasma glucose(mg/dl)	80.73±13.17	176.28±53.04**
Post prandial plasma glucose(mg/dl)	105.35±20.67	292.85±81.8**
HbA1c (%)	5.34±0.62	10.81±2.59**
S.Insulin(µIU/ml)	9.0192±2.82	12.08±5.76
HOMA –IR	2.03±0.09	4.38±2.22**

All values are expressed as mean± SD, p values -* = p <0.05, ** = p < 0.001
Body mass index, fasting and post prandial plasma glucose, HbA1c and insulin resistance were significantly higher in patients. HDL cholesterol was significantly lower in patients

Figure1 Correlation between HDL-C and Triglycerides in patients and controls



HDL- Cholesterol levels correlated negatively with triglycerides in both groups but the association was significant only in patients (r= -0.478, p<0.01 *vs* r= -0.257, p>0.05)

DISCUSSION

The present study has evaluated the lipid profile in early stages of type 2 diabetes. It is evident in our study that in early stage of the disease, it is the HDL-C level that is altered rather than other lipid profile parameters. Low HDL-C is probably an early indicator of setting in of dyslipidemia in these treatment naïve patients. Our findings are in contrast to most studies which have indicated that there is triad of high cholesterol, low HDL-C and high LDL-C in patients of type 2 diabetes (5,8,9). However, these observations were deduced from the studies which recruited patients who had diabetes of longer duration with/ without complications and already were receiving treatment. The uniqueness of our study is that we recruited patients in early stages of the disease. Additionally, our participants were not on any type of treatment which may be a confounding variable.

HDL constitutes a dynamic polydisperse group of particles which are central to lipid metabolism. Its protein component is exceedingly diverse, comprising structural apolipoproteins, enzymes, co-factors for enzymes and numerous other proteins (19). HDL-C exerts its cardio-protective effect primarily through its role in reverse cholesterol transport and its anti-inflammatory, anti-thrombotic and antioxidative properties (20). It is well documented that during the acute phase response (APR) to infection, inflammation or trauma, cytokine mediated changes in the composition of plasma proteins occur which results in dramatic reduction of serum HDL levels (21). Diabetes is a chronic inflammatory condition (22). It is well established that inflammation and inflammatory cytokines are intricately linked with the pathogenesis of diabetes (23). Therefore, we hypothesize that lowering of HDL fraction may be one of the early dyslipidemic changes to appear in diabetic patients. Our study also reveals a negative correlation between HDL-C and TAGs in diabetes patients. Since TAGs alter the composition and concentration of HDL-C through CETP, it appears that reducing the TG levels may help in maintaining HDL-C in these patients and strategies focussing on managing HDL-C and TAGs levels, if introduced early in diabetes patients, may help to reduce the future risk of CVD. Moreover, lipid profile in healthy controls is not very different from early diabetes patients, which points out to the fact that reference ranges established for other population cannot be followed for Indian population. Thus there is a need to establish reference range for lipid profile in Indian population so as to identify lipid derangements earlier in diabetes patients.

CONCLUSION

The earliest derangement to appear during progression of diabetes is reduced HDL-C. Its negative correlation with TGs indicate that management of HDL-C in such individuals should not be carried out without due consideration to TGs. However, the findings of this study warrants a large-scale study for validation.

Table 2: Comparison of lipid profile in patients and controls of present study with Dudhane et al 2013

	Present Study (Garg et al)			**Dudhane et al 2013 (19)**		
	Cases (newly diagnosed Type II diabetes)	**Control**	**Difference between means**	**Cases (long standing complicated type II diabetes)**	**Control**	**Difference between means**
Total cholesterol	189.43±30.56	197±36.46	7.57	290.7±57.2	172.9±30.1	117.8 *
LDL	118.78±33.23	119.5±34.8	0.72	209.3±55.8	98.6±32.4	110.7 *
HDL	37.73±8.06*	42.03±7.81	4.3*	42.9±7.8	49.4±11.01	6.5 *
TG	161.77	143.54	18.23	192.8±93.5	125.2±27.8	67.6 *
Duration of diabetes	Newly diagnosed			Mean duration: 7.5 years		
Treatment status	Samples taken before commencement of treatment			On anti-diabetic treatment		

p value *<0.05

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