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RESEARCH ARTICLE

ROLE OF METABOLISM OF HDL AND ITS SUBTYPES IN OCCURRENCE OF DIABETES IN OFFSPRINGS OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Patients with Diabetes mellitus (DM) develop low levels of High Density Lipoprotein (HDL) during the initial progression of disease. There is growing appreciation that Insulin resistance is associated with lowering of HDL. The exact prevalence of the Insulin resistance syndrome among those with low HDL in the general population has not been clearly established.
Methods: One twenty one patients and one twenty one controls were taken. Precipitation reagent (0.06 ml) containing 1071 U/ml Heparin, 500 mmol/l Magnesium Chloride and 12 mg/ml Dextran Sulfate was added to a Serum (0.3 ml). Sample was incubated and centrifuged at 10,000 rpm for 10 minutes. HDL3 was measured by a homogenous HDL assay in the supernatant and HDL2 estimated by subtracting the HDL3 from direct HDL.
Results: Mean HDL of patients and controls was 35.74 ± 8.18 and 49.27 ± 37.28 respectively. HDL3 was 23.84 ± 5.97 and 31.08 ± 4.60 respectively.HDL2 resulted18.27± 5.55 and 11.84 ± 3.61 respectively. Mean Insulin levels were 11.33 ± 8.46 and 9.14 ± 5.60 respectively.
Conclusions: HDL2 is higher than HDL3in off springs of patients with Type 2 DM.HenceHDL2 may serve as an initial marker for Type 2 DM

INTRODUCTION

This study was aimed to analyze the relation between HDL, HDL2 and HDL3 in off springs of patients with type 2 DM. There have been previous studies suggesting decrease in HDL concentration in patients with type 2 DM (**Howard, 1987**). It has been observed that there is a decrease in Lecithin cholesterol acyltransferase (LCAT) activity in such patients (**De Man *et al*., 1996**) as well as increase in the activity of Hepatic Lipase (HL) resulting in increased catabolism of HDL (**Syvanne *et al*., 1995**). HDL is a heterogeneous group of particles, their core comprising of Cholesteryl Ester and TG, which is surrounded by amphipathic layer of Phospholipids, Apo lipoproteins and free cholesterol (**Evans *et al*., 2009**).Apo lipoproteins are specialized group of proteins that associated with lipids and mediate many biochemical steps in plasma lipid metabolism. The amount of cholesterol transported in HDL particles is measured as HDL Cholesterol (HDL). HDL particles transport cholesterol from peripheral cells to Liver (**Bruce *et al*., 1998**) thus exhibiting anti-atherogenic property. HDL particles also express anti-inflammatory action because of their anti-oxidative properties. Other functions of HDL include improvement of endothelial function and anti-platelet aggregation (**Rashid *et al*., 2009**). HDL species are identified on the following basis (**Shah, 2009**).

1. On the basis of their major Apolipoprotein components- apoA-I or apoA-II
2. On the basis of their density- HDL2 and HDL3.
3. On the basis of their electrophoretic mobility- α and pre β

HDL is associated with low cardiovascular risks in epidemiological trials (**Gordon, Rifkind, 1989**). A large number of pathologies such as type 2 DM, obesity, Insulin resistance and metabolic syndrome are associated with low and dysfunctional HDL (**Ford *et al*., 2002; Hoang *et al*., 2007**). On the other hand high HDL levels are associated with aerobically trained individuals (**Kraus *et al*., 2002**). There are two possible sources of HDL that have been identified, one is the secretion of the nascent HDL and another possible synthetic route is by lipolysis of VLDL and Chylomicrons in the plasma compartment (**Patsch *et al*., 1978**). There is

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evidence suggesting that concentration of HDL2 is a better indicator of the protective effect of HDL than the concentration of total HDL or HDL3 (**Miller *et al*., 1981; Calabresi *et al*., 1990; Syvanne *et al*., 1995**). There are several factors involved affecting HDL-C levels in the body:

1) Exercise favorably affects a number of conditions like obesity, insulin resistance, lipoprotein profile and blood pressure (**Durstine *et al*., 2001**). Regular exercises increase the HDL concentrations in the blood.
2) Alcohol increases HDL and HDL3 mass, but not HDL2 (**Elizabeth *et al*., 2000**).
3) Monounsaturated fatty acids, Omega-3 fatty acids and fibers increase HDL levels without increasing the total cholesterol (**Schaefer *et al*., 1981**).
4) Although the prevalence of Insulin resistance syndrome among the general population with low HDL has not yet been clearly established, many studies suggest that lowering of HDL causes insulin resistance (**Haskell *et al*., 1984; Despres *et al*., 2000).** In other studies Insulin resistant states have been associated with low plasma concentration of HDL-C, hyper triglyceridemia and qualitative changes in LDL (**Haffner *et al*., 1990**).

Diabetes mellitus is one of the major risk factors for cardiovascular disease (**Ueshima *et al*., 2008**), which in turn is the most common cause of morbidity and mortality worldwide (**Bonow *et al*., 2002**). Type 2 DM along with insulin resistance is generally accompanied by low HDL levels and high plasma triglycerides (**Diabetologia 28,1985; Ganda, 1980); Singleton *et al*., 2003; Shaw *et al*., 1999; Pontiroli *et al*., 2000; Eckel *et al*., 2005**) Similar findings mainly attributed to glucose intolerance, hyper-insulinemia and obesity have been found in the off springs of individuals with type 2 DM (**Shaw *et al*., 1999; Haffner *et al*., 1990; Chathurvedi *et al*., 2009; Jouret *et al*., 2007**). The relation between type 2 diabetes mellitus and lipoproteins has been demonstrated in a number of studies. It has been observed that there is an overproduction of triglycerides- rich VLDL particles and Apo lipoprotein B-100 in patients with type 2 DM (**Evans *et al*., 1999**).

There is an increase in activity of Cholesteryl ester transfer protein (CETP) which in turn causes the increased lipid exchange between triglyceride-rich VLDL and both HDL and LDL (**Howard, 1987**). This results in decrease in concentration of HDL due to formation of triglyceride-rich HDL and LDL particles. Small HDL particles have been observed in patients with type 2 DM. Small HDL particle size and low level of HDL2 Is also associated with hyperinsulinemia and hyper triglyceridemia (**Ginsberg, 2000**). Hepatic lipase has also been observed to induce catabolism of HDL due to its over activity which results in smaller, denser, abnormal lipoproteins (**Syvanne *et al*., 1995**). There is now substantial evidence that progression to T2DM can be delayed or prevented through lifestyle and pharmacologic interventions. (**Chiasson, 2007**). A number of studies have documented beneficial effects of lifestyle intervention, including weight-reducing diets and moderate-intensity exercise, in preventing the development of T2DM in high-risk subjects (**Buchanan, 2007**). Thus the present study was designed to analyze the relationship if any between HDL, HDL2 and HDL3.

MATERIALS AND METHODS

Sample Collection

Blood samples were collected in a fasting state from healthy subjects in EDTA vials kept in ice, centrifuged immediately and plasma was stored at -20°C until assayed. All basal parameters like Insulin, HDL, Cholesterol, LDL, VLDL, TG, Glycosylated Hemoglobin (HbA1c) and Cortisol were estimated in the sample. The subclasses of HDL as HDL2 and HDL3 were also included in the study. Blood samples for plasma glucose were collected in potassium oxalate/ sodium fluoride vials.

Ethical Approval

The study was approved by the institutional ethics committee as per ICMR guidelines. Written informed consent was obtained from all subjects who wished to participate in the study. In case of children less than 18 years, consent was obtained from one of the parents and verbal assessment of the child was also done.

Inclusion and Exclusion Criteria

All non-diabetic children and grandchildren of the index cases were included in the study. Subjects diagnosed with diabetes or other systemic disorders and pregnant women were excluded. Controls comprised normal subjects without a family history of diabetes in two generations. The controls were normal in all aspects except for minor ailments. Details of medical history were collected and physical examination including anthropometry was performed. Height was measured with a Stadiometer to the nearest centimeter and weight was also measured. BMI was calculated as weight (kg) divided by the square of the height in meters. A value of 25 or more was considered as overweight for the adult population for BMI. For children and adolescents up to 18 years cut-offs recommended by the International Obesity Task Force (IOTF) were used (**Cole *et al*., 2000**).

Size

One hundred and twenty one subjects with family history of DM volunteered for the study and were enrolled as cases and one hundred twenty one matched subjects without family history of DM were enrolled as controls.

Biochemical Analysis

Fasting glucose was estimated on a fully–automated Cobas Integra 400 plus (Roche) by using standard commercially available kits or a standard methodology was adopted wherever necessary. HbA1c was estimated by D-10 dual HbA1c program. Total cholesterol, HDL, LDL, VLDL and TG were determined directly in the serum using a fully–automated cobas integra 400 plus (Roche). (Cobas integra 400 plus (Roche) employs four different techniques namely Absorption

photometry, Fluorescence Polarization Immune-assay (FPIA), Immuno-turbidimetry and Potentiometry. Its analyzer uses a unique reagent system using unique patented cassettes, which require no preparation. (These cassettes for the estimation of Cholesterol, TG and Glucose were procured from New Golden Enterprises in New Delhi, India) Plasma insulin and Plasma Cortisol were measured by electro-chemiluminescence assay employing ELECSYS 2010 (Roche Diagnostics, Indianapolis, USA). This assay uses monoclonal antibodies against insulin. (The Insulin and Cortisol kit used in ELECSYS 2010 was procured from Vikas Enterprises in New Delhi, India)

Single precipitation method with Heparin/Magnesium Chloride and Dextran Sulphate (Mn/Ds) for estimation of HDL2 and HDL3

A single precipitation method was standardized and results were obtained in the below explained condition. A precipitation reagent (0.06 ml) containing 1,071 U/ml heparin, 500 mmol/l MnCl2, and 12-mg/ml-dextran sulfate was added to a serum (0.3 ml). The sample was incubated and centrifuged at 10,000 rpm for 10 min. HDL3 was measured by a homogenous HDL assay in the supernatant and HDL2 was estimated by subtracting the HDL3 from the direct HDL (**Tsutomu Hirano *et al*., 2008**).

Statistical Analysis

The statistical analysis was carried out using SPSS version 16 software and the technique applied was student t-test to compare continuous data in two groups. Log transformation was applied to the skewed data.A chi square test was done to evaluate the difference in frequency between the two groups. Pearson correlation was applied to find the relationship between continuous-numeric parameters. A forward stepwise multiple linear regression was performed to determine the effect of possible predictors on serum HDL and LDL cholesterol levels. $P<0.05$ was considered as significant.

RESULTS

One hundred and twenty one cases and controls completed the study. There were 60 males and 61 females in each group and their age ranged from 10 years to 45 years. The parameters upon which the study was based included Fasting Blood Glucose (FBG), Glycosylated Hemoglobin (HbA1c), Cholesterol, HDL, HDL3. HDL2, LDL, Triglycerides (TG), VLDL, HDL: LDL Ratio and Insulin FBG was statistically insignificant (p=0.980), mean FBG value of cases and controls were 88.03±9.53 and 88.06± 10.63 respectively.

Similarly HbA1c comparison between cases and controls was insignificant (p=0.690); mean HbA1c value of cases and controls were 5.11 ± 0.43 and 5.20 ± 0.44 respectively. Mean cholesterol comparison between cases and controls was insignificant too (p=0.353), mean cholesterol value of cases and controls were 163.94±38.70 and 168.35 ± 42.39. Mean HDL was significantly lower in cases as compared to controls. (p= 0.000), Mean HDL of patients and controls was 35.74 ± 8.18 and 49.27 ± 37.28 respectively. Similarly, Mean HDL3 was lower in cases in comparison to controls (p=0.000).The comparison between patients and controls showed mean HDL3 values of 23.84 ± 5.97 and 31.08 ± 4.60 respectively. Mean HDL2 on the other hand was significantly higher in cases than controls. (p=0.000) Mean HDL2 values in cases and controls were 18.27± 5.55 and 11.84 ± 3.61 respectively. There was no statistical significance in LDL comparison between patients and controls, which resulted 100.55 ± 31.29and 104.14 ± 30.63 respectively (p= 0.327).

However a significant difference in plasma TG comparison between patients and controls was observed. TG was significantly higher in cases as compared to controls. (p=0.030). Mean TG values obtained were 126.53 ± 67.92 and 108.12 ± 55.04 in cases and controls respectively. VLDL comparison between patients and controls was statistically insignificant (p=0.072), mean VLDL results were 26.53 ± 16.92 and 23.47 ± 12.33 respectively. Plasma Insulin was significantly higher in patients as compared to controls(p=0.007), mean insulin results being 11.33 ± 8.46 and 9.14 ± 5.60 in patients and controls respectively.(0.007).A summary of the comparison of above mentioned parameters between cases and controls is given below in Table 1. A detailed analysis on the prevalence of HDL, HDL2 and HDL3 was done apart from mean values and p value,odd ratio and 95% cumulative indexwere calculated as well.

HDL Concentrations in cases

In Table 2 are listed concentrations (mean ± SD) of HDL and HDL Subclasses for normal males and females from different age group categorized as below. HDL subclasses were analyzed at 0.09 g/dl DS. In cases there were no significant differences among different age groups in both males and females.

HDL Concentrations in controls

In Table 3 are listed concentrations (mean ± SD) of HDL and HDL subclasses for normal males and females from different

Table 1. Comparison of parameters between Cases and Controls

	Cases (n = 121)		Controls (n = 121)		P value
	Means ± SD	min – max	Means ± SD	min – max	
FBG	88.03 ± 9.53	63 – 153	88.06 ± 10.63	70 - 158	0.980
HbA1c	5.11 ± 0.43	4.1 – 6.5	5.20 ± 0.44	4.1 – 6.1	0.690
Cholesterol	163.94±38.70	80 – 296	168.35 ± 42.39	89 - 294	0.353
HDL	35.74 ± 8.18	19 – 54	49.27 ± 37.28	20 - 70	0.000
HDL3	23.84 ± 5.97	9 – 38	31.08 ± 4.60	15 - 50	0.000
HDL2	18.27 ± 5.55	5 – 33	11.84 ± 3.61	4 - 20	0.000
LDL	100.55 ±31.29	25 – 209	104.14 ± 30.63	19 - 210	0.327
TG	126.53 ± 67.92	23 – 401	108.12 ± 55.04	41 - 425	0.030
VLDL	26.53 ± 16.92	9 – 122	23.47 ± 12.33	8 - 87	0.072
RATIO	2.97 ± 1.13	0.51 – 6.45	2.13 ± 0.64	0.37 – 4.85	0.000
INSULIN	11.33 ± 8.46	1.67 – 74.29	9.14 ± 5.60	3 – 39.60	0.007

Table 2. Concentrations of HDL, HDL2 and HDL3 in cases

Age	N	HDL	HDL2	HDL3
		MALES		
<25	55	34.56 ± 8.55	16.73 ± 4.97	23.12 ± 6.20
25-34	23	34.65 ± 8.31	19.53 ± 4.50	23.12 ± 6.20
35-45	9	33.56 ± 4.92	18.87 ± 6.01	21.92 ± 2.84
Total	87	34.48 ± 8.12	18.28 ± 5.18	23.00 ± 5.96
		FEMALES		
<25	35	38.51 ± 8.78	16.90 ± 4.50	26.74 ± 6.20
25-34	43	35.98 ± 8.32	18.74 ± 7.65	23.31 ± 5.77
35-45	14	35.93 ± 4.77	19.29 ± 4.71	23.39 ± 4.15
Total	92	36.93 ± 8.10	18.26 ± 5.93	36.93 ± 8.10

age group categorized as below. HDL subclasses were analyzed at 0.09 g/dl DS. In females there were no significant differences among different age groups. Among males in the concentration in the 25-34 year group was greater than that in the ‹ 25 and 35-45.

Table 3. Concentrations of HDL, HDL2 and HDL3 in controls

Age	N	HDL	HDL2	HDL3
		MALES		
<25	23	46.96 ± 5.24	11.42 ± 3.68	30.22 ± 3.11
25-34	21	52.86 ± 5.16	11.46 ± 3.26	33.33 ± 3.92
35-45	16	49.50 ± 7.41	11.52 ± 2.51	30.62 ± 3.36
Total	60	49.70 ± 6.30	11.44 ± 3.44	31.42 ± 6.0
		FEMALES		
<25	21	48.24 ± 7.04	11.77 ± 4.10	31.81 ± 4.99
25-34	23	49.43 ± 11.08	12.47 ± 3.77	30.70 ± 6.97
35-45	17	48.82 ± 4.31	12.54 ± 2.79	29.47 ± 2.47
Total	61	48.85 ± 8.17	12.22 ± 3.75	30.74 ± 5.35

Prevalence of HDL in cases and controls

To study the prevalence of HDL, both cases and controls were tested for HDL levels. Only 4.9% of controls had normal HDL levels (<34). Almost half of both cases and controls had HDL levels between 35-45.

Table 4. Prevalence of HDL in cases and controls

Cut offs for HDL	Cases (n = 121) n(%)	Controls (n = 121) n(%)	P value	Odds ratio	95% CI
<34 (normal)	29 (24.0)	5 (4.9)	-	-	-
35- 45	57 (47.4)	53 (43.8)	0.000	0.065	0.015-0.279
>45	35 (29.6)	63 (52.1)	0.000	0.010	0.002-0.043

High HDL levels (>45) were found in 52.1% of controls whereas only 29.6% of cases showed high levels of HDL. A detailed report of this analysis as well as P-value, Odds Ratio and 95% Cumulative Index is given below in Table 4. A graph comparing the prevalence of HDL in cases and controls is depicted in Figure 1.

Prevalence of HDL2 in cases and controls

Both cases and controls were tested for HDL2 levels to study its prevalence. Only 2.5% of cases showed normal HDL2 levels (<10). On the other hand 63.6% of controls showed normal HDL2 levels whereas a huge percentage of cases, 66.2%, showed >15 HDL2 whereas only a 2.5% controls showed >15 HDL2.A detailed report of this analysis as well as P-value, Odds Ratio and 95% Cumulative Index is given below in Table 5. A graph comparing the prevalence of HDL2 in cases and controls is depicted in Figure 2

Table 5. Prevalence of HDL2 in cases and controls

Cut offs for FBG	Cases (n = 121) n (%)	Controls (n = 121) n (%)	P value	Odds ratio	95% CI
< 10 (normal)	3 (2.5)	77 (63.6)	0.000	-	-
10-15	38 (31.3)	41 (33.9)	0.001	0.134	0.039-0.457
> 15	80 (66.2)	3 (2.5)	0.000	0.024	0.007-0.082

Prevalence of HDL3 in cases and controls

HDL3 was studied in both cases and controls. In cases, 54.2% cases showed normal HDL3 levels (<19) whereas in Controls a mere 5% showed normal HDL3 levels. On the other hand, 45.8% of cases showed higher HDL3 levels of >19 whereas a huge 95% of controls showed higher HDL3 levels of >19.A detailed report of this analysis as well as P-value, Odds Ratio and 95% Cumulative Index is given below in Table 6. A graph comparing the prevalence of HDL3 in cases and controls is depicted in Figure 3.

Table 6. Prevalence of HDL3 in cases and controls

Cut off for HDL3	Cases (n = 121) n (%)	Controls (n = 121) n (%)	P value	Odds ratio	95% CI
< 19(normal)	65 (54.2)	6 (5.0)	0.000	0.050	0.012-0.211
> 19	55 (45.8)	115 (95.0)	0.000	-	-

DISCUSSION

The purpose of this study was to estimate and analyze HDL and its subclasses in off springs of patients with Type 2 Diabetes Mellitus. Previous relevant studies have revealed that patients with Diabetes have usually low HDL as well has higher TG as compared to non-diabetics (**Syvanne *et al*., 1995**). These low HDL levels in Diabetics have been successfully linked with a high risk of Coronary Heart Disease CHD (**Laakso, 1997**). Several studies reveal a diminished HDL2 levels in patients with Type 2 DM (**Bakogianni *et al*., 2001; Pérez-Méndez *et al*., 2007**). A study done on healthy Japanese individuals suggested higher levels of HDL2 than

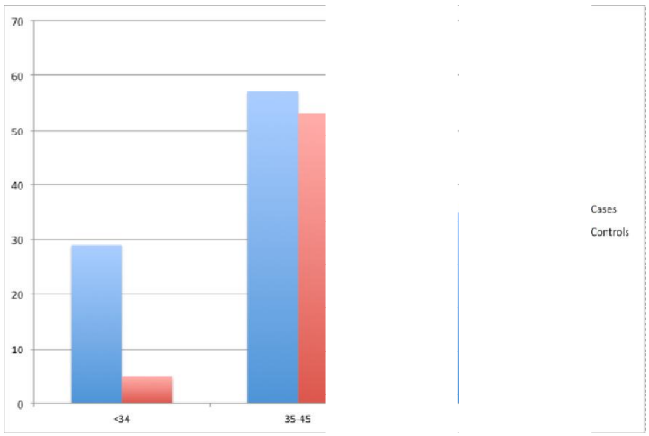

Figure 1. Graph showing percentage prevalence of HDL in cases and controls

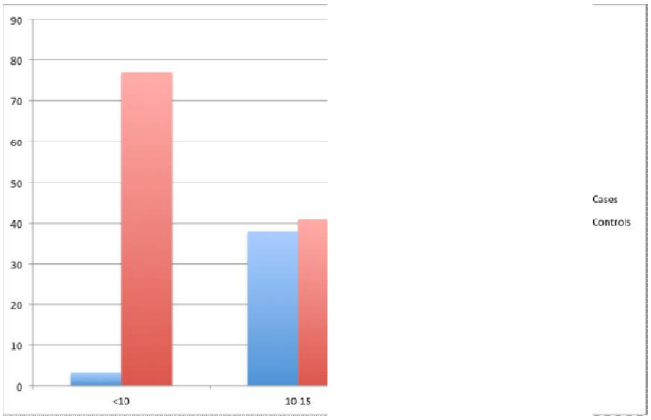

Figure 2. Graph showing percentage prevalence of HDL2 in cases and controls

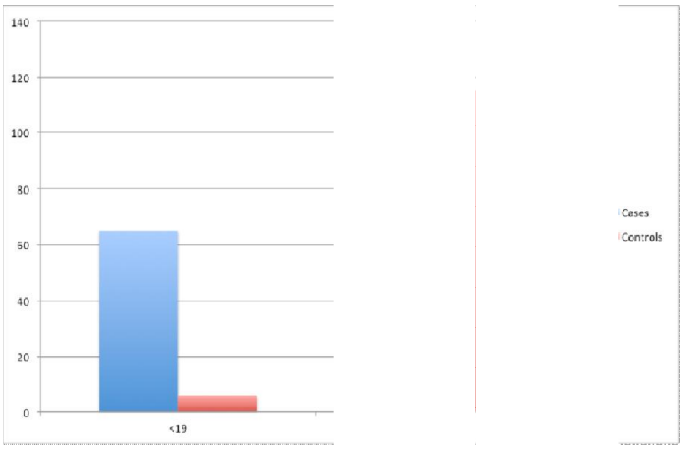

Figure 3. Graph showing percentage prevalence of HDL3 in cases and controls

HDL3 (**Hirano *et al*., 2008**) whereas a study done on Western population suggested that HDL3 is more than HDL2-C (**Bakogianni *et al*., 2001; Pérez-Méndez *et al*., 2007).** These varied results in the concentration of HDL and its subclasses press upon the importance of further estimation of this lipid in population prone to development of Type 2 DM, hence our basis of doing this study based on the off springs of population having Type 2 DM. The present study has shown that HDL concentration is low in off springs of patients with type 2 DM as compared to general population. A similar pattern is found in case of HDL3. However in case of HDL2, there is contrasting evidence suggesting higher levels in the off springs of Type-2 Diabetics as compared to controls.

To the best of our knowledge, no such study has been done on the off springs of Type 2 Diabetics in which HDL and its subclasses have been studied. A relevant study suggests that there is low HDL cholesterol among normal weight, normoglycaemic off springs of Type 2 Diabetics (**Edavan P. Praveen *et al*., 2011**). Our study, based upon 121 cases and controls, did not show much difference in terms of FBG between cases and controls. HbA1c was slightly lower in cases (5.11 ± 0.43) as compared to controls (5.20 ± 0.44). Cholesterol was slightly higher in off springs of patients with Type 2DM as compared to normal population. Tan *et al* study done on the South East Asian population too revealed that total Cholesterol level was not significantly different (Tan *et al*., 2008). However on the other had, TG levels were high in cases (126.53 ± 67.92) as compared to Controls (108.12 ± 55.04) suggesting that off springs of Type-2 Diabetics may have higher TG levels than the general population. According to a study on Caucasian Greek subjects, total Cholesterol, TG, LDL, LP (ct) were significantly higher in off springs of Diabetic parents as compared to off springs whose parents did not have Type 2 DM (**Psyrogiannis *et al*., 2003**). The same study suggested lower HDL levels in off springs of patients with Type 2 DM as compared to the off springs of non-diabetic parents, which is similar to our findings. VLDL and Insulin were observed to be higher in the off springs of patients with Type 2 DM as compared to normal population.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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