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SPECIFIC LIPID COMPONENTS IN INSULIN DEPENDENT DIABETES MELLITUS AMONG DIFFERENT AGE GROUPS IN LOCAL FEMALE POPULATION

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Abstract: Circulatory serum lipids have been estimated in already diagnosed insulin-dependent diabetes mellitus (IDDM) subjects and were compared with healthy control female subjects. Clinical facility of diabetes was available at different hospitals of Lahore. Healthy volunteers were sampled out from cities of Lahore, Sialkot and Narowal. Comparisons have been made between all of the samples of diabetic population and healthy controls and also among different age groups i.e., 20-29, 30-39 and 40-49 years of both the categories. Total cholesterol, LDL-cholesterol and VLDL-cholesterol concentrations were significantly 64.44%, 61.85% and 247.56% greater, respectively, in diabetics compared to normal subjects and were also significantly increased with advancing age. Serum lipids were non-significantly lowered in diabetics than respective controls but increased significantly with advancing age. HDL-cholesterol showed no significant variations in diabetics than normal and also among different age groups of both the categories. Total cholesterol/total lipid ratio was significantly 39.44% higher in diabetics, however, HDL-cholesterol/total cholesterol and LDL-cholesterol/total cholesterol ratios were found to be significantly lower in diabetics compared to respective controls.

Key words: Serum lipids, diabetes mellitus

INTRODUCTION

The clinical indisposition of underutilization of glucose in the body is generally referred as diabetes. Diabetes is not a single disease but referred as a syndrome and is dominantly a metabolic disorder characterized by hyperglycemia, glycosuria and excess fat metabolism, therefore, is clinically heterogeneous disorder with multi-factorial etiology (Fajans *et al.,* 1978; Cahill, 1985). Diabetes mellitus is of four main types, type I or insulin dependent diabetes mellitus (IDDM), type II or non-insulin dependent diabetes mellitus (NIDDM), impaired glucose tolerance (IGT) and gestational diabetes mellitus (GDM) (Harris and Cahill, 1979). IGT, NIDDM and to certain extent GDM are associated with insulin resistance or ineffectiveness of the hormone (Groop, 1997). Both principal types of diabetes, IDDM and NIDDM differ considerably in their pathogenesis. IDDM involves progressive loss of pancreatic islets β-cells and its hormone, insulin production (Dahlquist, 1993). In the development of IDDM, genetic and environmental factors have been found to be specifically involved, however, the genetic predisposition, the

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environmental factors and the life style which may be contributory in genetic expression of the diabetes, vary at different parts of the world Genetic predisposition is although necessary but not sufficient for IDDM development. May be genes interact with other environmental factors e.g., IDDM susceptibility gene which is regarded to be environmental response gene is free radial scavenger gene (Pociot and Mandrup-Poulsen, 1997). It is now well accepted that autoimmune mechanisms that include islet specific and glutamic acid decarboxylase (GAD) autoantibodies are responsible for β-cell destruction or insulin production mechanism (Gleichman and Bottazzo, 1987; Landin-Olsson *et al.,* 1989). Several factors have been reported to be contributory in immunogenic development of diabetes e.g., cow's milk (an environmental factor) showed an increased risk of child diabetes before 4 months of age (Gerstein, 1994; Verge *et al.,* 1994). Hypoinsulinemia adversely affects glucose utilization and enhance glucagon medicated glucose synthesis resulting in osmotic changes which cause dehydration, thirst, weight loss etc. IDDM results in ketoacidosis at any time of the disease (Foster and McGarry, 1983). IDDM manifests various lipids and lipoprotein alterations leading to severe cardiovascular complications. In families of IDDM patients, higher frequency of risk factors for cardiovascular diseases have been found (Decosmo *et al.,* 1997) and this high prevalence of disease is due to their nephropathy development. Abnormalities in composition and confirmation of lipoproteins occur in diabetes which may be one factor according to increased atherosclerosis despite the existence of normal lipid profile (Ziegler *et al.,* 1997). Keeping in view specific genetic predisposition, environmental factor and life style of population in northern Punjab, this study has been carried out to observe the pertinent lipid target in this population.

MATERIALS AND METHODS

Experimental Planning

Serum samples of adult patients, registered at diabetic clinics of National Health Research Complex (NHRC), Sheikh Zayed Hospital, Fatima Memorial Hospital, Punjab Institute of Cardiology (PIC) and Jinnah Hospital, Lahore were obtained for the study. The criteria for inclusion was the diagnosed female diabetic patients (age 20-50 years) based on clinical history and oral glucose tolerance test. The criteria for exclusion was the patients with other complications along with diabetes. Blood samples of healthy volunteers comprised Master Degree students at the University of the Punjab, female employees of Government College for Women, Gulberg College, Lahore, Garison College for Women, Lahore Cantt and residents of different areas in Lahore, Sialkot and Narowal city. The investigation of control volunteers to assess their healthy and non-diabetic status was initially done by their glycemic levels according to standard procedures in the clinical practice.

A total of 82 samples, 45 of diabetic patients (IDDM) and 37 of normal subjects were selected from a collected lot of 150 samples of varying age from 20-50 years. These were categorized into different groups on the basis of age. One group was of entire

diabetic subjects with respective controls including all the samples. These were further categorized into groups of 20-29, 30-39 and 40-49 years of age.

Glycemia and Lipid Fraction's Analysis

Total plasma glucose was estimated by commercially available glucose oxidase kit (Randox Laboratories Ltd., U.K.) employing the method of Raabo and Terkildsen (1960). Total serum lipids were estimated with sulphophosphovanillin method (Bachorik and Wood, 1977). Estimation of serum cholesterol was carried out by commercially available cholesterol kit (Randox Laboratories Ltd., U.K.) employing the enzymatic end point method. HDL-cholesterol component was estimated by CHOD-PAP method. LDL-cholesterol component was measured by fully enzymatic calorimetric test. VLDL-cholesterol component was calculated by subtracting the HDL-cholesterol and LDL-cholesterol fractions from total cholesterol. Different parameters were obtained by calculating the % ratios of estimated lipids and fraction's concentrations i.e., % total cholesterol/total lipid ratio, % HDL-cholesterol/total cholesterol ratio and % LDL-cholesterol/total cholesterol ratio. Intergroup as well as intragroup comparisons were carried out in each age group category of diabetics as well as the controls. The results were expressed as Mean $\pm$ SE. Analysis of Variance (ANOVA) followed by Student's "t" test was done by computer software programme, Minitab. The 5% probability level was used to detect the significance. The magnitude of differences in between the groups was expressed as decrease/increase in comparisons.

RESULTS

Glycemia (Fig. 1A)

Glycemia in all the control groups was almost in a narrow range with no significant difference statistically. The intensity of glycemia increased with the advancing age in diabetic subjects. Glycemia was 284% greater in all the diabetic subjects compared to the respective controls. In an analysis, it has been found that in 20-29, 30-39 and 40-49 years age groups, the glycemia was 123%, 233.2% and 294.4%, respectively, greater in diabetics compared to their respective controls. Hyperglycemia in IDDM subjects has been found to be intensified with advancing age, however, no significant difference has been observed in different age groups of diabetics.

Total Lipids (Fig. 1B)

Total lipid content was 19% lower in all diabetic subjects compared to the respective controls, however, non-significant statistically. In age group comparisons, similar pattern has been found i.e., lower concentration of total lipids (non-significant statistically) in all diabetic age groups than respective controls. In normals, total lipid concentrations were significantly greater in older groups than younger group of 20-29

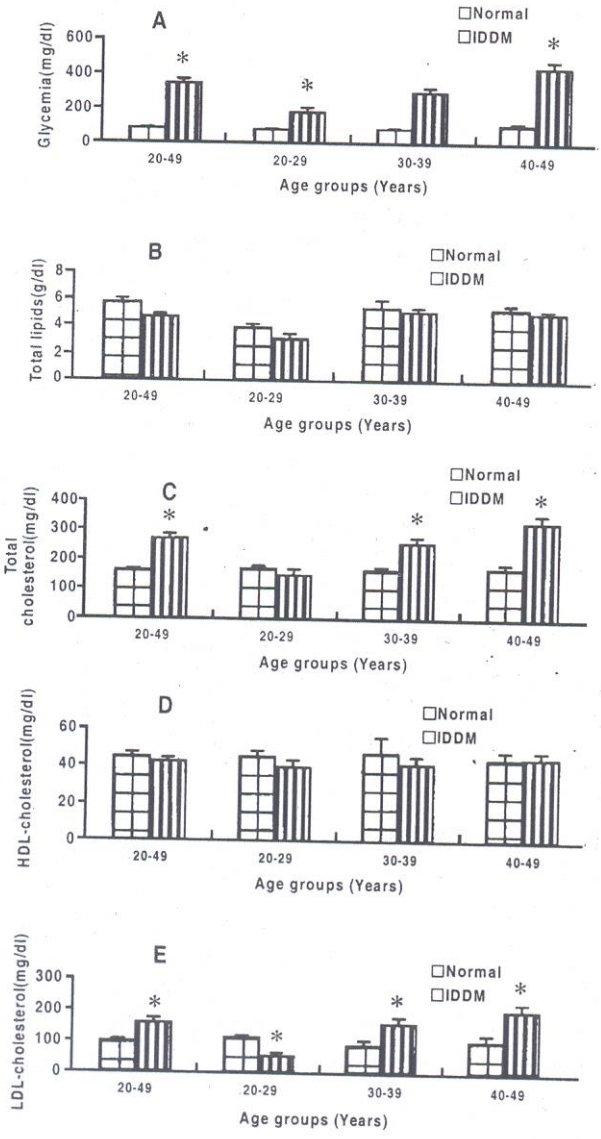

FIG. 1. Glycemia (A), Total lipids (B), total cholesterol (C), HDL cholesterol (E) and LDL cholesterol (F) in normal and IDDM subjects and also in different age groups of each category. *Significance at $P<0.05$.

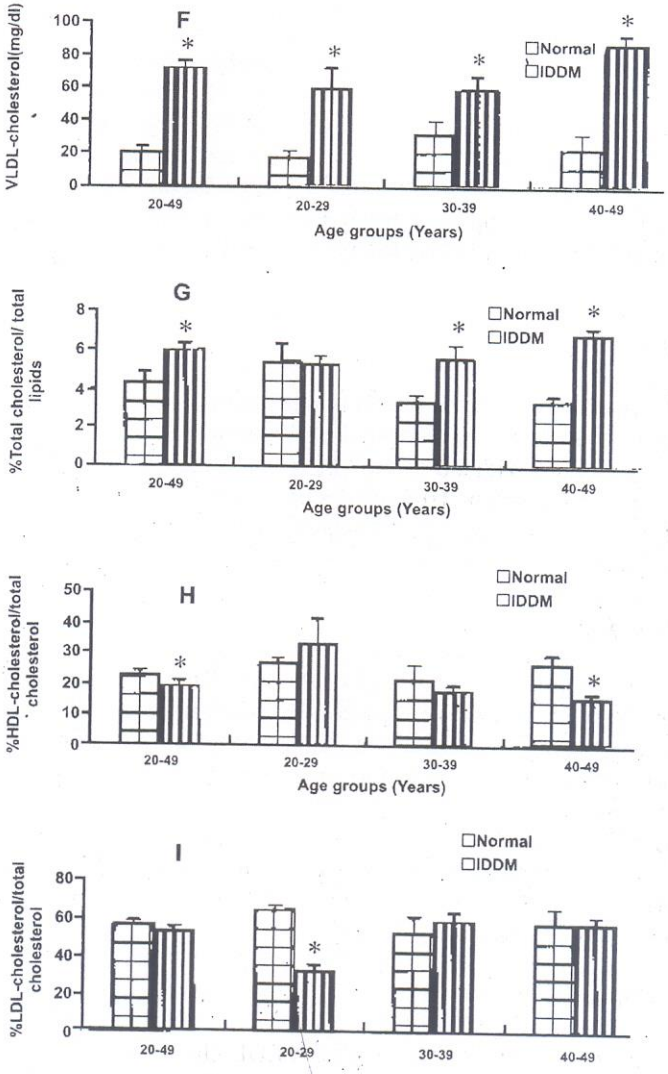

FIG. 2. VLDL cholesterol (F), total cholesterol/total lipids ratio (G), HDL cholesterol/ total cholesterol ratio (H) and LDL cholesterol ratio (I) in normal and IDDM subjects and also in different age groups of each category. * Significance at $P<0.05$.

years. There was a negligible difference between 30-39 and 40-49 years age groups. A similar pattern has been found in case of diabetic age groups.

Total Cholesterol (Fig. 1C)

Total cholesterol concentration was found significantly 64.5% greater in all diabetic subjects in comparison to respective controls. In comparisons, there was significantly higher concentration of total cholesterol in 30-39 and 40-49 years age groups of diabetics compared to the respective controls, however, lower in 20-29 years of diabetics than respective control which is statistically non-significant. No significant difference was found in all age groups of normal subjects. In age group comparisons, it has been found that total cholesterol concentration was intensified with advancing age in diabetics.

HDL-Cholesterol (Fig. 1D)

A trend of lower concentration of HDL-cholesterol, however, non-significant statistically was noticeable in diabetics compared to the normal subjects. This trend was appreciable in younger groups with no difference in older age group. There was no difference in concentration of serum HDL-cholesterol among different age groups of normal subjects, however, it increased slightly with advancing age in diabetic subjects but non-significant, statistically.

LDL-Cholesterol (Fig. 1E)

A significantly higher concentration of 61.85% in LDL-cholesterol was found in diabetics when compared to the respective controls. With an exception of 20-29 years age group where LDL-cholesterol concentration was significantly lower, in older age groups LDL-cholesterol concentration had been found to be tremendously greater in diabetics compared to the normal subjects. It was found to be 81% and 96.7% greater in 30-39 and 40-49 years age groups of diabetics than respective controls. A negligible difference had been found in different age groups of normal subjects, however, in diabetics there was an increase in LDL-cholesterol concentration with advancing age i.e., lower most in 20-29 years and highest in 40-49 years age group.

VLDL-Cholesterol (Fig. 2F)

Diabetic subjects showed 247.56% higher VLDL-cholesterol level compared to respective controls and the difference was highly significant, statistically. Among different age groups, VLDL-cholesterol had been found to be tremendously greater in diabetics compared to the normal subjects. In normal subjects a higher concentration of VLDL-cholesterol was found in older compared to youngest age group. An exception had been noticed that in 30-39 years, their values were greater than 40-49 years age group.

VLDL-cholesterol concentrations in 40-49 years age group were greater when compared to 20-29 and 30-39 years age group of IDDM subjects.

% Total Cholesterol/Total Lipid Ratio (Fig. 2G)

A general trend of total cholesterol / total lipid ratio was found similar to the total lipid and total cholesterol concentrations in diabetics when compared to the respective controls as wells as among different age groups of diabetics. An unexpected result of significantly lower value of the ratio had been observed in older compared to the youngest age group of normal subjects.

% HDL-Cholesterol/Total Cholesterol Ratio (Fig. 2H)

Diabetic subjects exhibited significantly lower ratio when compared to normal subjects. The older age groups showed a pattern of lowering of the ratio in diabetics compared to controls and this difference was specifically larger in oldest age group due to the major contribution of total cholesterol. Among different age groups of normal as well as diabetics the ratio reflected the pattern of HDL-cholesterol and total cholesterol concentrations.

% LDL-Cholesterol/Total Cholesterol Ratio (Fig. 2I)

LDL-cholesterol compensated total cholesterol in LDL-cholesterol/total cholesterol ratio with an exception of youngest age group where the ratio was significantly lower in diabetics than the respective controls. No contrasting behaviour had been found in the ratio and metabolite's concentration i.e., LDL-cholesterol and total cholesterol, among different age groups of both diabetic and normal subjects.

DISCUSSION

In diabetics of local population, there is a trend of lower lipid concentration compared to healthy volunteers, however, statistically non-significant, which seems to be an unusual result. Generally it is agreed that diabetes accompanies hyperlipidemia. Zianutdinov (1992) found the accumulation of the lipids in the blood of diabetics and this accumulating capacity is regulated with bioactive substances. The lowering of fraction is specifically in the younger age group. This difference may be attributed to the dietry habits of our populations compared to the populations investigated in most of the published reports. It would be interesting to collect more data of this target.

In the present study, there is clear hypercholesterolemia in diabetics compared to the control population and has been found to be intensified with advancing age. These results are in accordance with the reports of workers on other populations. Umeki *et al.* (1989) have reported elevated levels of total cholesterol, triglycerides, uric acid and serum

proteins in subjects with diabetes mellitus than in normal subjects. Rao and Al-Ageli (1993) have observed hypercholesterolemia in diabetic patients which was correlated with systolic blood pressure levels Sobenin *et al.* (1993) observed that there is cholesterol accumulation in cells incubated with LDL and is highly correlated with that in cells exposed to type I (IDDM) patient's serum.

A noticeable difference in HDL-cholesterol concentration in diabetics and control subjects is not exhibited. HDL-cholesterol is considered to be the fraction which protects against atherosclerosis (Miller, 1980). It is also reported to be low in concentrations in poorly controlled diabetes especially in women (Gordon *et al.*, 1977). Although it is not significantly expressed to be low in the present population, however, the result of trend of lowering is similar to the other reports. HDL-cholesterol concentration was found to be lower in diabetic subjects.

LDL-cholesterol has been found to be markedly greater, in the present population, in diabetics compared to the normal subjects with an exception of youngest age group where the concentration of LDL-cholesterol was significantly lower. This result mainly of the older groups, is quite in agreement with the several reports on other populations. Manzato *et al.* (1993) have observed an increased level of intermediate density lipoprotein (IDL) and LDL-cholesterol concentration in insulin dependent diabetes mellitus. Insulin deficiency directly reduces LDL-receptors and so the catabolism of LDL via the LDL-receptor mediated pathway is decreased which in turn increased serum LDL concentration (Chait *et al.*, 1978). Insulin enhances receptor mediated LDL degradation, therefore, insulin deficiency may impair LDL catabolism leading to hypercholesterolemia (Chait *et al.*, 1979).

The tremendously greater concentration of VLDL-Cholesterol in diabetics compared to the normal subjects is in accordance with Goldstein *et al.* (1983). They reported that an increased production of VLDL in insulin deficiency probably is secondary to increased lipolysis and elevated free fatty acids level in plasma. Free fatty acids that escape oxidation to ketones are re-esterified to triglycerides, packaged and secreted by liver as nascent VLDL. VLDL overproduction may lead to increased LDL-cholesterol via sequence VLDL to IDL to LDL (Goldstein *et al.*, 1983). Nikkila and Kekki (1973) have been observed that hypertriglyceridemia associated with poor control results from increased hepatic production of very low density lipoproteins (VLDL) coupled with delayed removal of VLDL and chylomicrons in some patients.

It has been found that, lipid, cholesterol and lipoprotein's complications were more pronounced in older age than the youngest age groups. The difference in younger and older age groups is most likely that in youngest groups the early or timely diagnosis of syndrome has not taken time to develop complications. In older groups, on the other hand, late diagnosis or inadequate management has resulted in complications. It may be

pointed out that in our populations, the timely diagnosis and proper management of IDDM could prevent development of complications in older groups.

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