SERUM LIPIDS, APOLIPOPROTEINS AND TOTAL ANTIOXIDANT ACTIVITY LEVELS OF OBESE, DIABETIC AND HYPERTENSIVE SUBJECTS IN AN INDUSTRIAL SET UP IN BARODA, GUJARAT, INDIA

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ABSTRACT

The alarmingly increasing rates of chronic degenerative diseases in the less developed countries and economies in transition is well documented and the World Health Organization has warned of an epidemic of cardiovascular diseases in the developing nations. There are various risk factors, which act synergistically leading to the development of cardiovascular diseases. In order to predict the risk of development of cardiovascular disease, various conventional biochemical parameters have been used such as the total cholesterol and its content in various lipoproteins. However, in the recent years, newer biochemical markers such as the apolipoproteins and the antioxidants have gained a lot of importance. Therefore, the present study was planned with an objective to see the efficacy of these parameters in the assessment of coronary risk. The clinical profile of the subjects revealed the body mass index of 23.5± 3.62 for normal subjects and 27.43±4.27, 25.01±4.5 and 24.16±3.88 for overweight and obese, diabetics and hypertensive subjects respectively. The waist to hip ratio of the subjects was found to be 0.85± 0.06, 0.90±0.05, 0.87± .06 and 0.86±0.07 for the normal, overweight and obese, diabetic and hypertensive subjects respectively. The dietary analysis showed the fat intake to be higher and vitamin C intake to be lower amongst those suffering from any kind of chronic degenerative diseases. On comparison of the lipid levels of the normal and overweight and obese subjects it was observed that more aberrations were seen among the overweight and obese subjects. The fasting blood glucose and lipid levels of the diabetic subjects showed higher values of fasting blood glucose, total cholesterol, high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (LDL-C) and the triglycerides (TG). On comparison with normals, hypertensives also elicited hyperlipidemia. When the apolipoproteins were analysed, the data showed higher levels of Apo B and lower levels of Apo A1 among the overweight and obese, diabetic and hypertensive subjects. Similarly the ratio of Apo A1:Apo B was found to be significantly lower among the overweight and obese, diabetic and hypertensive subjects. The total antioxidant activity of the subjects revealed that the normal subjects had higher levels of antioxidant activity in comparison to those suffering from various chronic degenerative diseases. These results thus indicate that aberrations in lipid levels are seen as one develops various chronic degenerative diseases. In addition the apolipoproteins and the total antioxidant activity showed adverse trend in subjects suffering from various chronic degenerative diseases, thereby suggesting that they can be used as additional tools along with conventional lipoprotein analysis for mapping the risk of cardiovascular diseases.

KEY WORDS : Lipids; Apolipoproteins; Antioxidants; Type 2 diabetes; Hypertension.

INTRODUCTION

The health research community is now well aware of the impending global pandemic of cardiovascular disease and the alarmingly increasing rates of these disorders in less developed countries and economies in transition. Globalization brings about not only changes in the prosperity of these countries but also ill health. The population quickly adapts to fast food (rich in fat and salt), a sedentary lifestyle, and very little relaxation. All these factors contribute directly to increased prevalence of non-communicable diseases and cardiovascular disease (CVD) in particular.

In profiling the risk of these non-communicable diseases it has been customary to utilize the measurement of fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), high density

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lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). However, LDL-C may not be an accurate predictor of heart attack or other cardiac events in healthy adults. Instead, the investigators suggest that blood levels of two proteins found in cholesterol – Apo B and Apo A1 may be better 'markers' for heart risk in persons without the signs and symptoms of heart attack.

The oxidative modification of LDL-C is thought to play a crucial role in the initiation of atherogenesis. Antioxidant vitamins can protect the LDL-C from oxidation, and high intakes or blood concentrations of these vitamins have been linked with a reduced risk of cardiovascular disease. Few data are available on the importance of antioxidant vitamins in earlier stages of atherogenesis (1). Hence, the current study was undertaken to see the effectiveness of these parameters in day-to-day routine analysis in the Indian context, where less data is available on these parameters.

MATERIALS AND METHODS

Two hundred and five subjects (75 females and 130 males) in the age group of 20-60 years were enrolled from an industrial set up in Baroda, Gujarat. The clinical profile of the subjects is given in Table 1. The dietary information of the subjects was collected using the 24-hour dietary recall method and the nutritive value of the foods consumed was calculated using the values given in the 'Nutritive Value of Indian Foods' (2). Their medical history was obtained through a structured questionnaire. Standing height, weight and circumferences of the waist and hip were measured without shoes. Body mass Index (BMI) and the waist to hip ratio (WHR) were calculated as a measure of fat distribution.

Venous blood sample was collected after an overnight fast of 12 hours and the serum was used for the estimation of fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) using the Boehringer Mannheim enzymatic kits on a Boehringer Mannheim autotek instrument. LDL-C and VLDL-C were calculated using the Friedwald's formula (3). Apolipoproteins (Apo A1 and Apo B) were measured on the Array Protein Systems (Beckman Instruments, Brea, California) following the principle of antigen-antibody reaction by rate nephelometry. The ferrymyoglobin/ABTS assay for measuring the total antioxidant activity was carried out using the Raxdox (Canada) kit.

Statistical Analysis: Students 't' test was used for finding out the significant difference between the two means.

RESULTS

The clinical profile of the subjects revealed that the average age of the normal subjects was 44±8 years followed by 40±4, 42±7 and 43±7 years of the

Table 1: Dietary Analysis of the Subjects (Mean ± SD)

	NORMALS	OVER -WEIGHT + OBESE	DIABETICS	HYPERTENSIVES
CALORIES (Kcal)	1900 ± 200	2400 ± 345	2250 ± 400	2215 ± 275
CHO (g)	260.34 ± 55.12	265.56 ± 45.36	250.78 ± 34.58	245.95 ± 40.67
PROTEINS (g)	67.7 ± 10.55	60.84 ± 8.65	64.54 ± 11.23	66.56 ± 15.2
FATS (g)	65.12 ± 20	98.87 ± 25.33	93.57 ± 30.25	90.22 ± 28.88
FIBER (g)	9.2 ± 2.42	7.56 ± 3.05	8.01 ± 2.55	8.5 ± 4.25
VIT.C (mg)	78.40 ± 12.7	68.5 ± 13.91	59.55 ± 13.24	66.50 ± 15.23
b - CAROTENE (mg)	1590.1 ± 985.2	1409.3 ± 785.5	1560.8 ± 684.9	1115.7 ± 1000.5
PERCENTAGE OF CALORIES COMING FROM				
CHO	54.8	44.2	44.5	44.4
PROTEIN	14.2	10.1	11.4	11.8
FATS	30.8	37.0	37.4	36.0

overweight and obese, diabetic and hypertensive subjects. The clinical profile of the subjects revealed the body mass index of 23.5± 3.62 Kg/m^2 for normal subjects and 27.43±4.27Kg/m^2, 25.01±4.5Kg/m^2 and 24.16±3.88Kg/m^2 for overweight and obese, diabetics and hypertensive subjects respectively. The waist to hip ratio of the subjects was found to be 0.85± 0.06, 0.90±0.05, 0.87± .06 and 0.86±0.07 for the normal, overweight and obese, diabetic and hypertensive subjects respectively.

The dietary intake of the subjects is depicted in Table 1. The caloric intake of the overweight and obese, diabetic and hypertensive subjects was significantly higher than the normal subjects. Significantly higher values of fat and lower values of vitamin C were observed amongst the diabetics, overweight and obese and hypertensive subjects in comparison to normal subjects.

When the lipid profile of the normal and overweight and obese subjects (Table 2) was compared it was noticed that the TC, LDL-C, VLDL-C and TG values were significantly higher among the overweight and obese subjects.

Table 2: Lipid Profile of Normal and Overweight + Obese Subjects (mg/dl) (Mean ± SD)

VARIABLE	NORMALS	OVERWEIGHT & OBESE
n	52	54
FBS	86.42 ± 12.04	88.17 ± 20.17
TC	181.16 ± 31.46	207.29 ± 23.22 ***
HDL- C	37.84 ± 7.87	36.70 ± 7.10
LDL-C	119.75 ± 31.05	131.90 ± 22.91*
VLDL-C	20.55 ± 11.61	28.55 ± 12.09***
TG	111.28 ± 54.6	141.26 ± 60.44**
TC/H	5.19 ± 1.58	5.96± 1.19
L/H	3.82 ± 1.45	3.80 ± 1.05
TC/L	1.52 ± 0.24	1.53 ± 0.20

** Significantly different from normals at P < 0.05. ** Significantly different from normals at P < 0.01. *** Significantly different from normals at P < 0.001.*

When FBG values of the normal and diabetic subjects were compared (Table 3), a significantly higher value of FBG was seen in the diabetic subjects (168.12 ± 36.94 mg/dl) in comparison to normal subjects (86.42 ± 9.26 mg/dl). The lipid profile of the diabetic subjects showed significant increases in the TC, LDL-C, VLDL-C and TG values.

Table 3: Fasting Blood Glucose (FBG) and Lipid Profile of Normal and Diabetic Subjects (mg/dl) (Mean ± SD)

VARIABLE	NORMALS	OVERWEIGHT & OBESE
n	52	50
FBG	86.42 ± 12.04	168.32 ± 36.94 ***
TC	181.16 ± 31.46	213.72 ± 33.57 ***
HDL- C	37.84 ± 7.87	37.40 ± 6.31
LDL-C	119.75 ± 31.05	141.52 ± 32.25 ***
VLDL-C	20.55 ± 11.61	33.88 ± 12.33 **
TG	111.28 ± 54.6	171.26 ± 60.10 ***
TC/H	5.19 ± 1.58	5.75 ± 1.15
L/H	3.82 ± 1.45	3.83 ± 1.08
TC/L	1.52 ± 0.24	1.54 ± 0.18

** Significantly different from normals at P < 0.05. ** Significantly different from normals at P < 0.01. *** Significantly different from normals at P < 0.001.*

When a similar comparison was made between the normal and hypertensive subjects (Table 4) it indicated the TC, LDL-C, VLDL-C and TG levels and the TC / H ratio to be significantly higher among the hypertensive subjects.

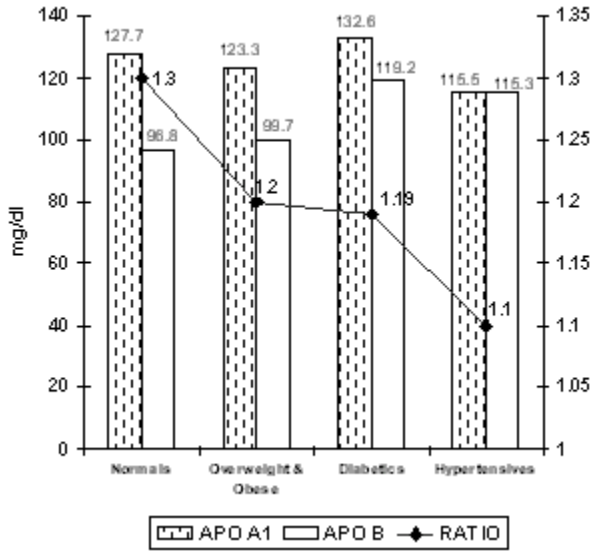
Table 4: Fasting Blood Glucose (FBG) Lipid Profile of Normal and Hypertensive Subjects (mg/dl) (Mean ± SD)

VARIABLE	NORMALS OBESE	OVERWEIGHT &
n	52	49
FBG	86.42 ± 12.04	90.02 ± 15.02
TC	174.69 ± 27.40	212.04 ± 24.87 ***
HDL-C	34.56 ± 7.31	35.55 ± 5.05
LDL-C	117.58 ± 22.46	133.34 ± 31.62 **
VLDL-C	22.50 ± 9.98	35.80 ± 16.46 ***
TG	111.29 ± 49.91	179.02 ± 82.3 ***
TC/H	5.19 ± 0.90	6.44 ± 1.87 **
L/H	3.53 ± 0.84	4.22 ± 1.56
TC/L	1.50 ± 0.17	1.58 ± 0.26

** Significantly different from normals at P < 0.05. ** Significantly different from normals at P < 0.01. *** Significantly different from normals at P < 0.001.*

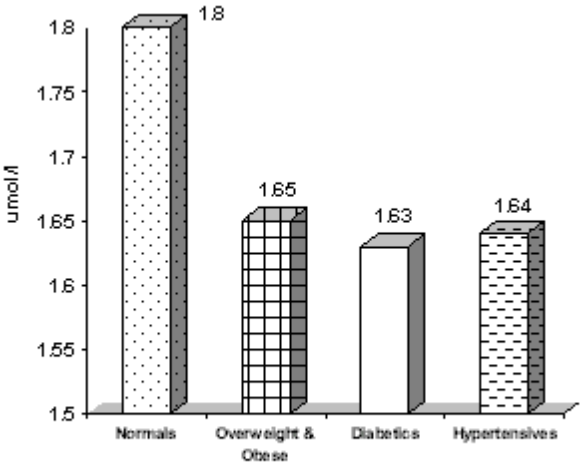
The apolipoproteins, which are said to be better predictors of cardiovascular risk, are shown in Figure 1. No significant difference was observed among the normal and overweight and obese subjects in relation to the apolipoproteins. However, the ratio of Apo A1 / Apo B was found to be lower among the overweight and obese subjects. The Apo A1 and Apo B levels were found to be 132.6 ± 31.7 mg / dl and 119.2 ± 24.1 mg / dl among the diabetic subjects, whereas they were 127.7 ± 21.5 mg / dl and 96.8 ± 21.3 mg / dl respectively in the normal subjects. Also, the ratio of Apo A1:Apo B was significantly lower in the diabetic subjects. Similarly, the Apo A1 and Apo B levels were found to be 115.5 ± 21.63 mg/dl and Apo B to be 115.33 ± 19.68 mg/dl among the hypertensives, which again showed that they were significantly different from normal subjects. The ratio of Apo A1:Apo B ratio was also found to be significantly lower among the hypertensive subjects.

Figure 1: Apolipoprotein Levels of Normal, Overweight and Obese, Diabetic and Hypertensive Subjects (mg/dl)



The scientific community is now well aware of the role of antioxidants in protecting us from the damage caused by free radicals and thereby helping in preventing disease and extending life. When the total antioxidant activity (TAA) of the subjects was compared, it showed that the normal subjects had significantly higher levels of antioxidants (1.80 ± 0.20 µmol/l) in comparison to overweight and obese (1.65 ± 0.17 µmol/l), diabetic (1.63 ± 0.15 mmol/l) and hypertensive (1.64 ± 0.17 µmol/l) subjects respectively (Figure 2).

Figure 2: Total Antioxidant Activity Levels of Normal, Overweight and Obese, Diabetic and Hypertensive Subjects (µmol/l)



DISCUSSION

Coronary artery disease (CAD) is the single most important disease entity in terms of both mortality and morbidity and is a leading cause of death (4). Epidemiological studies have demonstrated that major risk factors such as dyslipidemia, hypertension, diabetes mellitus and the use of tobacco products act in a synergistic manner (5). Other risk factors include physical inactivity, obesity, age, gender, alcohol consumption etc. Raised serum concentration of cholesterol, LDL-C and low serum concentration of HDL-C is all associated with an increased risk of coronary atherosclerosis (6,7). However, recent studies suggests that we would do better to monitor levels of the apolipoproteins Apo B and Apo A1 as predictors of possible coronary artery disease. It is said that higher levels of Apo B may indicate increased risk of cardiovascular disease even when the total LDL-C is not high (8). On the other hand recent research has also shown that antioxidants play a powerful role in the prevention of cardiovascular disease.

Thus, the estimation of apolipoproteins and total antioxidant activity in addition to the routine parameters of lipids would help one to predict the risk of CAD more strongly. Therefore, the present study tried to see the efficacy of these parameters in addition to the routine analysis of lipid profile among Indian people where data available is scanty on these parameters.

The dietary analysis of the subjects in the present study revealed that the intake of calories was more among the overweight and obese, diabetic and hypertensive subjects. A significant increase in the fat intake was also observed among these subjects. This could be attributable to the consumption of fried foods available in the industrial canteen by the employees.

Aberrations in the lipid profile were observed in case of obesity, diabetes and hypertension. Obesity has been established as an independent risk factor for the development of CAD (9). And it has been aptly said to be the mother of important degenerative diseases, which predisposes an individual to the risk of development of diabetes, hypertension and CHD (10). The lipid profile of the overweight and obese subjects showed significantly higher levels of TC, LDL-C, VLDL-C and TG when compared with normal subjects. These results thus validate the fact that the overweight and obese subjects have an altered lipid profile and that they are at a higher risk of developing other degenerative diseases.

Diabetes mellitus has been shown to be associated with lipid abnormalities. A two to three time higher risk for coronary heart disease rate is seen in diabetic patients (11,12). Impairment in insulin secretion leads to an excessive and prolonged rise in glucose concentration. Similar results were seen when the FBG of the normal subjects were compared with diabetic subjects. The frequency of raised plasma lipid levels in diabetic subjects is between 20-90% depending on the degree of diabetic control and the type of diabetes (13). Similarly in the present study raised TC, LDL-C, VLDL-C and TG were observed.

Hypertension has been accepted as the most important risk factor for the development of cardiovascular morbidity. Elevated lipid levels were observed among the hypertensive subjects and significant differences were noted when compared to normal subjects indicating their being at a greater risk of developing other secondary complications.

Plasma levels of Apo B and Apo A1 are important determinants of the risk of premature coronary artery disease. In fact, Apo B and Apo A1 are thought to be better predictors of acute myocardial infarction than the TC and LDL-C (8). Apo B might be a good indicator because it reflects the number of lipoproteins that are associated with the development of atherosclerosis. Figure 1 shows the levels of Apo A1 and Apo B among the normal, overweight and obese, diabetic and hypertensive subjects. The Apo A1 and Apo B levels did not show any significant differences among the normal and overweight and obese subjects. However, the ratio of Apo A1:Apo B was found to be lower among the overweight and obese subjects thus indicating that they might be at a higher risk of developing other degenerative diseases.

The Apo A1 and Apo B were found to be significantly different among the diabetic subjects i.e. the Apo A1 values were lower and the Apo B was found to be higher in comparison to the normal subjects. Among the hypertensive subjects, it was observed that levels of Apo A1 were significantly lower and the Apo B were significantly higher. It is clearly evident from the figure that the ratios of Apo A1:Apo B show a decreasing trend among the overweight and obese, diabetic and hypertensive subjects thus indicating their being at a greater risk of developing CVD because the ratio is considered to be a sensitive index for predicting the risk.

The evidence that antioxidants may play a role in the prevalence of atherogenesis has been increasing rapidly in recent years (14,15,16). Several studies have suggested that antioxidants such as alpha tocopherol, retinol, albumin and selenium may reduce cardiovascular mortality (17). They have also suggested that variations in serum antioxidant levels may explain the cross-cultural differences in the incidence of ischemic heart disease better than the classic risk factors such as raised serum cholesterol, high blood pressure and smoking (17). Therefore, in the present study ferrylmyoglobin/ABTS assay for measuring total antioxidant activity (i.e. the total potential of the constituent antioxidant) was carried out. This is a measure of the collective hydrogen donating ability of the antioxidant in the sample (18). The results showed that the total antioxidant activity of the normal subjects was significantly higher than the overweight and obese, diabetic and hypertensive subjects. These results strengthen the fact that low levels of antioxidants may play a role in the development of ischemic heart disease. One of the reasons attributable for the low levels of antioxidants among the subjects could be that their dietary intake of antioxidant was low, which is evident from the significant difference observed in the vitamin C intake of the subjects (Table 2). However, the beta carotene intake did not show any significant difference. Therefore, we cannot attribute it solely to the dietary intake. Moreover, in the current study the 24-hour dietary recall was recorded which might under or over estimate the nutrient intake. Therefore, this fact suggests that along with 24-hour dietary intake, the

frequency of consumption various vitamin rich foods as well as maintenance of a diet diary should be recommended in order to arrive at any concrete conclusion regarding the dietary habits and the antioxidant status.

Hence, from the present study it is clear that aberrations in the lipid levels are seen as one develops various chronic degenerative diseases (CDD). The apolipoproteins and the total antioxidant activity showed an adverse trend in the subjects suffering from various CDD, thereby suggesting that these parameters can be used as additional tools for mapping the risk of development of cardiovascular disease.

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