Research Article

A comparative biochemical study on the non diabetic obese and non obese subjects with cardiovascular disease

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ABSTRACT

Cardiovascular disease is now a major public health problem in India and is emerging as a major killer. The non diabetic obese and non obese subjects with cardio vascular disease" were carried out with the objective of studying or investing the (effect) cause of cardiovascular diseases in obese and Non-obese subjects. The level of lipoprotein a of non obese subject showed a significant ($P < 0.001$) increase than in the obese subject. The level of lipid ratio (total cholesterol / HDL cholesterol) found to be significantly ($P < 0.001$) high in obese subjects than in non-obese subjects. The present study has been designed to evaluate or investigate the risk of cardiovascular disease due to obesity in both male and female middle age group.

Keywords: Obesity, Lipoprotein, Homocysteine, Cholesterol, Diabetes mellitus

INTRODUCTION

Obesity has become one of the major health problems in advanced society. It has been proved that obesity is a risk factor for a number of chronic disorders such as cardiovascular diseases, Non-insulin dependent diabetic mellitus, gout, gallstones, intestinal blockage, kidney disease, sleep apnea, hernia and arthritis. Obesity is due to sedentary life and nutritional abundance, influenced by genetic factors. It is continuing to rise at an alarming rate even in developing countries like India where hunger is also at the other side. According to known Prevalence rate of overweight and obese in India is 12.8 percent and 10.3 percent respectively.[1] Florentine had also estimated that more than 300 million people in the world are obese.[2] Recent perspective studies have demonstrated that a predominant accumulation of adipose tissue in the abdominal region confers an increased risk of cardiovascular disease and premature death. There is an ample of data, in published reports suggesting that increased cardiovascular risk accounts partly by the metabolic alterations associated with abdominal obesity. It has been reported that the atherosclerotic risk factors like disturbances in lipoprotein metabolism and plasma insulin glucose homeostasis are present in subject with an excessive deposition of adipose tissue at the abdominal level from a clinical point of view estimation of regional adipose tissue distribution must therefore be considered as important in the evaluation of the patient's cardiovascular risk profile. Hence, obesity has become an important risk (or) cause for cardiovascular disease. Now-a-days people are more susceptible to infectious and non-infectious diseases. Infectious disease includes the disease caused by virus (or) bacteria etc., Example for this is jaundice, typhoid, malaria, dengue. Non-infectious disease is diabetic mellitus, cardiovascular disorders,

kidney diseases among the non-infectious disease diabetic mellitus and cardiovascular disorders are more common among the middle age people. When people reached their age 35 to 40 they are affected by diabetes mellitus or by any one cardiovascular disorder. Much research work has been done on cardiovascular disorders. Yet many questions are to be answered, regarding the route cause for the cardiovascular disorders and also in the treatment without side effects. The treatment for cardiovascular diseases may also lead to diabetes mellitus or some other diseases. Due to the sedentary life style and wrong food habits like consuming more fat rich foods, very dangerous fast foods and tin food items, human beings tend to become obese.

In this study, a light is thrown on cardiovascular disease subjects with obese and non-obese. Here diabetic patients are excluded. Since the diabetic patients are more prone to cardiovascular disease with an insulin deficiency which will alter the lipid profile, a route cause for cardiovascular disease, it is the first step to identify the reasons for the cause of cardiovascular disease in non-obese and their altered lipid profile. Keeping in view the above said points, this study was designed in the following manner:

a. To identify the level of blood glucose in the cardiovascular patients to exclude the diabetic patients
b. To characterize obese and non-obese subjects, body mass index (BMI) was calculated by taking the height and weight of the cardiovascular disease subjects.
c. The level of cholesterol in the serum of obese and non-obese were calculated.
d. The level of triglycerides in the serum of obese and non-obese were calculated.
e. The level of HDL-C, LDL-C, and VLDL-C, of the lipoprotein fraction in the serum of obese and non-obese were calculated.
f. The level of homocysteine in the plasma of obese and non-obese calculated.
g. The level of lipoprotein 'a' in the plasma of obese and non-obese were calculated.

The biochemical basis in obese leading to cardiovascular disease was mainly due to the insulin resistance. Insulin is the one of the key hormone in the carbohydrate, lipid and protein metabolism. In insulin resistance, the triglycerides level in the blood increase. This is due to the increased lipolysis occurring in the adipose tissues. The double bond present in free fatty acids is oxidized by the free radical, after oxidation of double bonds. Fatty acids are taken up by the lipoprotein i.e. phagocyted by the macrophages this macrophages enter the intima of the blood vessel and get deposited leading to the formation of a fatty streak. In a long run the streak grows into atheroma and gradually encroaches the intravascular lumen. This is route of formation of atherosclerosis in obese. Insulin is the hypoglycaemic hormone and plays a

vital role in the glucose metabolism. It decreases the blood glucose level by facilitating the uptake of the glucose. Glucose is converted to glucose-6 phosphate by the enzyme glucokinase or Hexokinase. It is the rate limiting step in glucose metabolism and it is promoted by insulin. Myocardial infarction is usually occurring in elderly people due to formation of atheroma in the blood vessel in long run. But in some cases there is an exception i.e. stroke in the early stage of life will occur. This kind of stroke can be possible due to two reasons.

1. Hyperhomocysteinemia.
2. Presence of lipoprotein(a)

Homocyteine, a sulfur-cantaining amino acid, is a key intermediate of methionine metabolism. It is produced by methyl transfer reactions, which are important for the synthesis of nucleic acids, methylated proteins, neurotransmitters, and phospholipids. Cystathione beta synthase deficiency will block the catabolism of homocysteine throughout the body. This leads to the accumulation of homocysteine, methionine and alpha keto butyrate in blood and excreted in urine. The cobalmine N^5 Methyl THF- Homocysteine methyl transferase depends on vitamin B_{12}, N^5 N^{10} Methylene THFA catalyses the reaction N^5 N^{10} Methylene THFA to N^5 Methyl THFA. Deficiency of the enzyme produces decreased one carbon unit and reduced methionine synthesis with consequent increases in homocysteine level in urine. Folate supplementation will relieve the symptoms.[4] A number of retrospective (case-control and observational) and prospective studies done over the past 15 years indicate that homocysteine is a graded, independent risk factor for myocardial infarction, stroke, and venous thromboemoblism.[5]

METHODS

The experimental procedure adopted for the study "a comparative bio chemical study on the non diabetic obese and non obese subjects with cardio vascular disease" is presented as follows. The present study has been designed to evaluate or investigate the risk of cardiovascular disease due to obesity in both male and female middle age group.

Sample selection: for this intensive study, initially too hundred volunteers those who were undergoing treatment for cardiovascular disease were selected. The human subjects (samples) were briefed about this study they came forward to involve themselves. Initially the age group of the human subject was taken into account. For the feasibility of this experiment and study, the age group between 25 years to 55 years was considered. Based on the age factor out of 200 human subjects 145 were selected. Their medical case history was carefully examined and studied. Since this study is mainly on Non Diabetic, from the sample 40 known diabetic human subjects were eliminated. So 105 Non Diabetic human subjects BMI was measured and calculated. The height

and weight of each subject were taken for the calculation of body mass index to access the degree of obesity. On the basis of body mass index the subjects are categorized as obese and non obese. In BMI measurement 30 human subjects fell into the category of obese since their BMI value is greater than or equal to 25. From the remaining human subjects, 30 human subjects were selected as "Non Obese" with the BMI less than or equal to 25. All the 30 obese human subjects and 30 Non Obese human subjects, totally 60 were selected for this study to examine the cardiovascular risk factors. To confirm once again all the human subjects were non Diabetic, on fasting their blood were taken and subjected to "Fasting blood glucose Test" and concluded they were all Non-Diabetic. Then, the biochemical experiment was conducted on the human subjects to find out the cardiovascular risk factors on obese and the Non Obese. Obese greater than or equal to 25: Non-Obese less than or equal to 25.

Estimation of Glucose

1. Trichoroacetic acid (TCA) – 10%

2. Ortho Toluidine reagent: 12.5 gram of thiourea and 12.0 gram of boric acid were dissolved in 50 ml of distilled water by heating over a mild flame 75ml of ortho toludine (redistilled) and 375ml of glacial acetic acid were mixed separately. These two solutions were mixed and the total volume was made up to 500ml with acetic acid. The reagent was left overnight in the refrigerator and filtered.

3. Glucose Standard: 100 mg of pure glucose was dissolved in 100ml of distilled water. Containing 0.01 percent benzoic acid (Concentration – 1 mg/ml).

Procedure

To 0.1 ml of freshly blood 1.9 ml of TCA was added to precipitate the proteins and centrifuged. 1.0 ml of the supernatant was mixed with 4.0 ml of ortho toludine reagent and kept in a boiling water bath for 15 minutes. The greenish blue colour developed was read at 640 nm in a ultraviolet spectrophotometer. Blank containing 2.0 ml of water and strandard containing 20 to 40 mg of glucose were also treated similarly. The values were expressed as mg/ml blood.

Estimation of Cholesterol

Cholesterol was estimated by the method of Zak's 1957.

Principle Cholesterol in acetic acid medium gives a pinkish brown coloured complex with ferric chloride and sulphuric acid and the intensity of colour developed is read colorimetrically at 550 nm.

Procedure

To 0.1 ml of serum add 4.9 ml ferric chloride reagent mixed well and centrifuged for about 15 minutes from this 2.5ml of filtrate was taken and added 2.5 ml of ferric chloride diluting agent and then added 4 ml of concentrated sulphuric acid and mix thoroughly for standard curve various concentration (0.5 to 2.5 ml) of working standard solution was taken and diluted with 5 ml of ferric chloride diluting reagent then added 4 ml of concentrated sulphuric acid in all the tubes. A blank was also maintained and mixed well. The colour developed was readed at 560 nm. The values were expressed as mg/dl of blood.

Isolation of Lipoprotein

Lipoproteins were fractioned by a dual precipitations technique.

Fractional of HDL

HDL was separated from plasma by the method of Wilson and spiger (1973) using heparin – $MnCl_2$ reagent.

Procedure

2 ml of serum was taken to which 0.18 ml of heparin - $MnCl_2$ reagent was added. The solution was mixed well and allowed to stand for 30 minutes at 4 degree centigrade and then centrifuged at 2,500 rpm at 10 degree centigrade for 3 minutes. The level was expressed as mg/dl of serum.

Fractionation of VLDL

The VLDL fraction was separated from plasma using SDS by the method of Burstein and Scholink.

Procedure

2 ml of plasma was added to 0.15 ml of SDS. The contents were mixed well and incubated at 37 degree centigrade of 2 hours. The contents were centrifuged in a refrigerated centrifuge at 10,000 rpm for 5 minutes. VLDL aggregated as a peptide at the top. The supernatant contained the HDL and LDL fractions. The levels are expressed as mg/dl.

Estimation of Triglycerides

Triglycerides were estimated by enzymatic method using kit developed by Monozyme India Limited. Triglyceride was estimated using the kit in an auto analyzer (TECHNICONRA -100). Care was taken to avoid contamination by soap or glycerol, since it will affect this assay. No hemolysed samples were used. The values were expressed as mg/dl of serum

Estimation of LDL Cholesterol

Low density lipoprotein (LDL) cholesterol was calculated using the friedewald formula,

i.e. LDL – cholesterol = Total cholesterol – [(Triglycerides / 5 + HDL)].

Estimation of Homocysteine

Homocysteine was estimated enzymatically by Block and Kredich method.

Isolation of Lipoproteins: Lipoprotein a was isolated by Iodixanol solution for lipoprotein separation using a rapid single-step centrifugation method (Liposep) formulated by Lipoteck, Ltd [Merseyside, UK].

Identification of Lipoprotein(a) by Agarose Gel Electrophoresis

Lipoprotein(a) was identified by preformed agarose gels for lipoprotein identifications (Hydragel-HP-a) which resolve lipoprotein a. These are supplied with all materials for electrophoresis and staining of the gels. The kit was purchased from YSL [Clandon, UK]. To identify the lipoproteins in each fractions, aliquotos (2 micro mole) were separated by flat-bed electrophoresis using Hydragel (Lp a) agarose gels according to the manufacturer's instructions. Lipoprotein gradient fractions were negatively stained with 1% uranyl acetate applied to coated grids and examined in a Phillips CM 12 electron microscope. Separation of plasma lipoprotein after centrifugation in self generating gradients of lodixanol. After centrifugation, three bands were visible in the centrifuge tube. The lipoprotein class in each band identified by agarose gel electrophoresis. An opalescent wide band of VLDL formed at the top of the tube. A red band of LDL was visible in the middle of the tube. At the bottom of the tube was a viscous red layer of plasma proteins. Immediately above this was a reddish band of HDL. In the electron microscope, the peak fraction from each band was seen to consist of particles of different diameter. Band one (VLDL) contained particles with diameters 30 – 80 nm; band two (LDL) contained particles of diameters 20 – 25 nm; and band three (HDL) consisted of small particles, diameters 6 – 15 nm, that tended to aggregate in chains. This may be an artefact of electron microscopy. The diameters ported are for the main lipoprotein classes. In the gradient, LP (a) distributes at the junction of HDL and LDL. Usually in another tube commercially available know fraction are run along with the samples the band obtained for LDL-C and HDL-C was correlated with those obtained using commercially available kits for assay for LDL (sigma) and HDL (g enzyme) and for LDL subtractions (sigma). The sample which shows the presence of Lipoprotein (a) quantitatively estimated.

Estimation of lipoprotein(a): Lipoprotein(a) was estimated by non competitive ELISA kit purchased from sigma private limited.

Statistical Procedures

The mean and the standard deviations were calculated from the determined values by using the standard procedures.[16]

RESULTS

The present study "a comparative biochemical study on the non diabetic obese and non obese subjects with cardio vascular disease" was carried out with the objective of studying or investigating the (effect) cause of cardiovascular diseases in obese and Non-obese subjects.

Cholesterol and Triglycerides

The level of cholesterol and triglycerides in the serum of non diabetic non obese subjects with cardiovascular disease are presented in (table 1 and figure 1). The cholesterol level is significantly ($P<0.0010$ high in obese subjects than non obese subjects. Who showed a clear and consistent association existing between obesity and a high production of total body cholesterol in obese subject. The level of triglycerides is found to be significantly ($P<0.001$) high in obese subject than in non obese subjects. (Shown in Table 1 and Figure1) Results were showing an increasing level of triglycerides among the male subjects due to insulin insensitivity in obese.

Table 1: The level of cholesterol and triglycerides in the serum of (Group 1) non diabetic (Group) 2 non obese and obese subjects with cardiovascular disease.

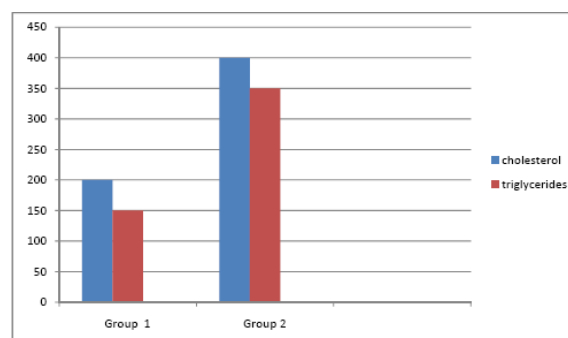| Serial No.	Subjects	Total Cholesterol (mgs/dl)	Triglycerides (mgs/dl)
1	Non-Obese subject with CVD	198 ±1.527	120 ±1.291
2	Obese subjects with CVD	380 ±1.291**	360 ±1.290**

Figure 1: The level of cholesterol and triglycerides in the serum of (Group 1) non diabetic (Group 2) non obese and obese subjects with cardiovascular disease.

Lipoprotein Fraction

The level of HDL – C, LDL – C and VLDL – C in the serum of non-diabetic non obese and obese subjects with cardiovascular disease are presented in Table II and Fig 21. The level of HDL – C showed a significant decrease (P < 0.0010 in obese when compare to non obese subjects. The LDL – C and VLDL – C fractions showed a significant increase (P < 0.001) in obese than in non obese subjects which showed a clear cut relationship between obesity and abnormalities in lipoprotein fractions. These include increase in VLDL and reduction in HDL which were obtained in both men and women. A high production of total body cholesterol in obese subjects resulting a greater production of VLDL, which inturn induces an increase in hepatic lipase in women. In women low estrogen levels would have contributed to the low HDL concentration which is a risk factor for coronary heart diseases in the middle age women. He reported that over mild to moderate overweight is associated with 40% of the coronary disease risk. As much as 70% of the coronary heart disease was observed among obese women. Multivariate analysis indicated that, although a major portion of the excess coronary risk is attributable to the influence of adiposity on blood pressure, glucose tolerance and lipid levels, a moderate residual effect persists that may be due to other mechanism.[6] Abdominal obesity and more particularly visceral obesity have been associated with disturbances in lipoprotein metabolism and plasma insulin glucose homeostasis related to a increased risk of CHD.[7,8] Klein investigated the lipid levels in younger subjects. They found a slight lowering serum cholesterol levels, lower total HDL cholesterol ratios and lower uric acid levels were less likely to have CVD and were less likely to have hypertension. In addition, increased risk can be observed as soon as 5 years later.[9] Gordon and Rifking had reported that a low HDL Cholesterol level is an important coronary risk predictor. He has also done a clinical trial addressing the efficacy and safety of intervention that specifically or atleast predominantly raise HDL levels through pharmacological treatment.[10] (Shown Table 2, figure 2).

Table 2: Level of Lipoproteins fractions of HDL – C, LDL – C and VLDL – C in serum of (Group 1) non diabetic non obese and (Group 2) obese subjects with cardiovascular disease.

Serial No.	Subjects	HDL-C (mgs/dl)	LDL-C (mgs/dl)	VLDL-C (mgs/dl)
1	Non-Obese subject with CVD	50.67± 1.5986	1233.67± 2.925	24.33± 2.1344
2	Obese subjects with CVD	28.0± 1.291**	260.17± 1.4625**	72.0± 1.291**

A low level of HDL cholesterol is a strong and independent risk factor for recurrent cardiac events among patients with established coronary artery disease[11].observational studies indicated that low HDL cholesterol level are strongly and independently associated with a high risk of coronary heart disease. The presence of high TG, low HDL – C, high LDL –C concentration is associated with such a high risk of IHD. The individuals with this lipid profile should be encouraged to make life style change and even treatment with drugs should be considered.[12] It might also be beneficial in the weight controlling diet to replace saturated fats by unsaturated fats the weight loss and physical activity[13] will have a positive effect of LDL – C level also will lower TG levels and increase HDL – C level.[14] Serum total lipid (cholesterol, and triglycerides), Lipoproteins VLDL, VDL and HDL fraction in 25 coronary artery disease patients and compared artery disease patients and compared with 25 normal healthy individuals. Patients with negative family history did not show a significant change in LDL, VLDL and triglycerides, But HDL showed a significant low level irrespective of the fact that family history was positive or negative. Lipid levels is independent of total cholesterol may contribute to the reduction in CVD events.
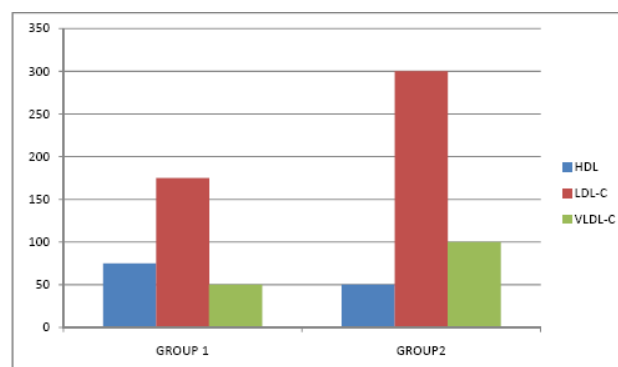

Figure 2: Level of Lipoproteins fractions of HDL – C, LDL – C and VLDL –C in serum of (Group 1) non diabetic non obese and (Group 2) obese subjects with cardiovascular disease.

Lipid Ratio (Total Cholesterol / HDL)

The level of lipid ratio of total cholesterol and HDL cholesterol in the serum of non-diabetic non obese and obese subjects with cardiovascular disease are presented is table III. The level of lipid ration (total cholesterol / HDL cholesterol) found to be significantly (P < 0.001) high in obese subjects than in non-obese subjects. Total cholesterol, low HDL cholesterol or elevated lipid ratio is risk for cardiovascular disease.

Homocysteine

Homocysteine was not normally found in the blood plasma of healthy human beings. But in the patients who have vitamin B complex deficiency or with the person with cystathionine beta synthase enzyme deficiency will have homocysteine in the blood plasma. The level of homocysteine in the plasma of

the non-diabetic non obese and obese subjects with cardiovascular disease is presented in the table 4.

Table 3: Level of ration of total cholesterol / HDL – C in serum of (Group 1) non diabetic non obese and (Group 2) non diabetic obese subjects with cardiovascular disease.

Serial No.	Subjects	Total Cholesterol/ HDL-C (mgs/dl)
1	Non-Obese subject with CVD	3.92±0.1076
2	Obese subjects with CVD	1.5607**

(**) P<0.001 High significant
Significance: ** = P<0.001
* = P<0.01 or 0.05
NS = Not significant

Table 4: Level of Homocysteine in plasma of (Group I) non diabetic non obese and (Group 2) non-diabetic obese subjects with cardiovascular disease.

Serial No	Subjects	Homocysteine (micromole/I)
1	Non-Obese subject with CVD	16.50 ± 4.99
2	Obese subjects with CVD	Absent

Homocysteine was absent in obese subjects and present in some of the non-obese subjects i.e. out of 30 subjects 16 subjects showed the presence of homocysteine in the blood plasma (Table 4 and Figure 4).

The reason for the presence of homocysteine may be due to vitamin B complex deficiency which is an important for the conversion of homocysteine to cystathionine. This may also be due to in born error of methionine metabolism. They have reported that both markedly and mildly elevated circulating homocysteine concentrations are associated with increased risk of vascular occlusion. They also reported that mild homocysteine elevation occurs in 20-30% of patients with atherosclerotic disease. Numerous studies suggest that high level of homocysteine increases the risk of heart and blood vessel disease and ischemic stroke, but how homocysteine increases the risk is unclear. He also reported that some evidence indicated that it may damage blood vessels and predisposes people to forming fatty deposit (atherosclerosis) and blood clots in arteries. He also says, researchers found the risk of stroke was more than four times higher in people with the highest homocysteine levels compared to those with lowest levels.

Lipoprotein(a) level of lipoprotein a in the plasma of the non diabetic non obese and obese subjects with cardiovascular disease are presented in the table 5.

Table 5: The level of lipoprotein(a) in serum (Group 1) of the non diabetic non obese and (Group 2) non diabetic obese subjects with cardiovascular disease.

Serial No	Subjects	Lipoprotein A mg/dl
1	Non-Obese subject with CVD	22.50 ± 6.075
2	Obese subjects with CVD	9.43 ± 4.824*

The level of lipoprotein(a) of non obese subject showed a significant ($p < 0.001$) increase than in the obese subject. There was relationship between lipoprotein, haemostatic variables and atherosclerotic complications in hypertensive patients. Univariate analysis of that study showed log lipoprotein(a) concentration to be significantly corrected with age, apolipoprotein B, plasma fibrinogen, fibrin D - dimer levels but not with body mass index, blood pressure, dietary fat intake, cholesterol, triglycerides, apolipoprotein A1, prothrombin fragment 1 + 2 and anti thrombin III. They concluded from this study there is a strong relationship between lipoprotein(a) and clotting variables in hypertensive patients that may contribute to atherosclerotic damage in these patients. Malnutrition in haemodialysis patients is associated with an increased cardiovascular mortality. To identify the cause for cardiovascular disease they evaluated the relationship between atherogenic lipid profile and serum albumin in haemodialysis patients. There was a significant inverse correlation between serum LP(a) and albumin concentrations. No correlation was found between albumin, TC, TG, HDL – C, TC/ HDL – C and Apo A-I / Apo B ratios. From these results they suggest that LP(a) could be responsible for an increased cardiovascular mortality in haemodialysis patients with malnutrition. There was not significant difference in the lipid levels in TC, LDL – C, HDL – C and TG levels in the case of coronary artery disease and controls, but the levels of LP(a) were significantly higher.

Majority of studies implicate LP(a) as a risk factor.[15] Ariyo and Colleagues conducted a study to identify whether LP a is a risk for cardiovascular complications in elderly persons. Since the basic research has already indicated that LP a lipoprotein is found in atherosclerotic plaques and play a role in thrombotic complications at these plaques. The authors concluded the elevated LP(a) level is an independent predictor of cardiovascular complication in elderly men, but not in elderly women. The level of lipoprotein(a) in non obese subjects was not similar among the 30 individuals, it varies greatly from 14.6 mg/dl to 35.0 mg/dl.

The level of lipoprotein in obese also varies greatly than non obese subjects. Only 12 out of 30 patients showed the presence of lipoprotein a ranging from 3.3 mg/dl to 14.5 mg/dl. The reason for the presence of lipoprotein in these individuals may be due to the increased LDL cholesterol or may be due to chain smoking in men because among 12 subjects 10 were men. The reason for the presence of lipoprotein(a) in young individuals may be due to familial back ground also.

DISCUSSION

Cardiovascular disease is now a major public health problem in India and is emerging as a major killer. The prevalence of cardiovascular disease in Indians is up to three times higher when compared with people of similar age groups in the western world. Little is known about the pathogenesis of atherosclerosis and cardiovascular disease in Indians. Because the cardiovascular disease affects Indians in the prime if their lives and careers and has significant socio-economic consequences, there is an urgent need to define the route cause for cardiovascular disease, and prevention measures against the cause or risk factors for atherosclerosis.

The present study was carried out to assess the risk factors in cardiovascular disease affected human beings. Since the cardiovascular disease may arise due to diabetes mellitus which was already extensively studied. It was eliminated from the study and hence non-diabetic cardiovascular subjects were selected with age group between 25 years to 55 years. The major risk factor for atherosclerosis includes the alteration in the lipid levels. Hence the present study was carried out to assess the total cholesterol, triglycerides, lipoprotein fractions that are HDL-C, LDL-C, VLDL-C, lipid ratio i.e. total cholesterol/HDL-C and other factors which include homocysteine and lipoprotein. The disturbances in lipid level was common in obese individuals than in non obese subjects than in non subjects but the level of LDL-C and VLDL-C are significantly higher in obese subjects than in non obese subjects. The level of lipid ratio (Total cholesterol/HDL-C) was significantly high in obese subjects than in non obese subjects.

The level of lipoprotein(a) in non-obese subjects was high when compared to obese subjects. The level of homocysteine was absent in obese subjects and moderately present in the non obese subjects. The reasons for lipid alterations in obese subjects may be due to the insulin resistance in obesity. The reasons for the presence of lipoprotein mainly in men may be due to smoking or familial background. The presence of homocysteine may be due to the vitamin B complex deficiency or inborn error of metabolism in the methionine that is deficiency in the cystathionine beta synthetase enzyme. Hence from this study not only obese subjects but also the person with vitamin B complex deficiency which we will consider as minor things in life will also lead to serious cardiovascular disorder.

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