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HOMOCYSTEINE AND LIPOPROTEIN A ROLE IN NON-DIABETIC OBESE AND NON OBESE SUBJECTS WITH CARDIOVASCULAR DISEASE

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ABSTRACT

Obesity has become one of the major health problems in advanced society. The Prevalence rate of overweight and obese in India is 12.8percent and 10.3 percent respectively obese. For this intensive study, initially 200 Volunteers those who were undergoing treatment for cardiovascular disease were selected. Glucose was estimated by the Glucose Oxidase and Peroxidase method. Human Analyzer kits to analyze Total cholesterol, triglycerides and HDL, VLDL and LDL. Homocysteine was estimated enzymatically by Block and kredich method. The result shows Lipoprotein α of non obese subject showed a significant (p<0.001) increase than in the obese subject. 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 and significant increase (P<0.001). out of 30 subjects 16 subjects showed the presence of Homocysteine in the blood plasma shows P<0.001 significant. 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.

KEYWORDS: Homocysteine, Lipoprotein A, Lipid Profile, Obese And Cardiovascular Disease.

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 Obstruction, kidney disease, Sleep apnea Syndrome, hernia and arthritis.[1] Cardiovascular disease is the leading cause of death and disability in the developed world. When atherosclerosis occurs in the coronary arteries it results in cardiovascular disease. Atherosclerosis means a form of arteriosclerosis in which atheromas containing cholesterol lipid material and lipophages are formed within the intima and inner media of large and medium sized arteries.[2] Obesity is due to sedentary life and nutritional Imbalance, influenced by genetic factors this is the worldwide phenomenon. It is continuing to rise at an alarming rate even in developing countries like India where hunger is also at the other side.[3] The Prevalence rate of overweight and obese in India is 12.8percent and 10.3 percent respectively obese. Recent perspective studies have demonstrated that a predominant accumulation of adipose tissue in the abdominal site confers an increased risk of cardiovascular disease and premature death.[4] Much research work has been cardiovascular disorders. Yet may questions are yet to be answered, regarding the route cause for the cardiovascular disorders and also in the treatment without side effect. Because the treatment for cardiovascular diseases will also lead to diabetic mellitus or to some other diseases. Due to the sedentary life and irregular food habits like consuming more fat rich foods, very dangerous fast foods and tin food items will human beings to obese. In this study, a light is thrown on cardiovascular disease subjects with obese and non-obese.[5] 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 the lipid profile.[6] Keeping in view of the points this study was designed in the following manner: To identify the level of blood glucose in the cardiovascular patients to exclude the diabetic patients. To characterize obese and non-obese subjects, body mass index (BMI) was calculated by taking the height and weight of the cardiovascular disease subjects.[7] The level of cholesterol in the serum of obese and non-obese is calculated. The levels of triglycerides in the serum of obese and non-obese are

calculated. The level of homocysteine in the plasma of obese and non-obese calculated. The level of lipoprotein 'a' in the plasma of obese and non-obese are calculated.

Myocardial infarction usually occurs in elderly people due to formation of atheroma in the blood vessel in long run. But in some cases there is an exception (ie) stroke in the early stage of life will occur. This kind of stroke can be possible due to two reasons.[8] Hyperhomocysteinemia. and Presence of Lipo protein ‘a’. Homocysteine, a sulfur-containing amino acid, is a key intermediate methionine metabolism. It is produced of methyl transfer reactions, which are important for the synthesis of nucleic acids, methylated proteins, neurotransmitters, and phospholipids.[9] Hyperhomocysteinemia can be caused by genetic defects, nutrional deficiencies, renal dysfunction, alcoholism, hypothyroidism, or certain medications. cystathionine beta synthase deficiency This enzyme deficiency will block the catabolism of homocysteine through our body. This lead to the accumulation of homocysteine methionine and alpha keto butyrate in blood and excreated in urine.[10]

Cobalamine deficiency The enzyme N^5 Methyl THF - Homocysteine methyl transferase is depended on vitamin $B_{12.}$ Therefore vitamin B_{12} deficiency or the mutation of the enzyme may produce alternation in methionine metabolism and hence the presence of homocysteine in urine, characteristically blood contain increased level of homocysteine while blood methionine level is low.[11] N^5 N^{10} Methylene THFA Reductase Deficiency This enzyme 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 increase in homocystine level in urine. Folate supplementation will relieve the symptoms.[12]

Experimental evidence also supports the theory that high homocysteine cause vascular problems: induced hyperhomocysteinemia in animals produces vascular abnormalities, including endothelial dysfunction and vascular hypertrophy, that are very similar to those seen with other risk such as hypertension, diabetes, and hypercholesterolemia.[13]

Hyperhomocysteinemia also accelerates atherosclerosis progression in apoliporotein – E deficient mice. In human subjects, acute Hyper homocysteinemia induced by oral methionine loading causes endothelial dysfunction.[14]

Possible mechanisms of hyperhomocysteinemia's vascular effects include impaired release of endothelium derived nitric oxide, oxidative injury, accelerated atherosclerosis, altered haemostatic balance, endoplasmic reticulum stress, and activation of inflammatory pathways.

Lipoprotein a or LP a or alpha should not be confused with apo A. Apo A is a constituent of HDL and is present in all persons, while LP a is seen as a constituent of LDL in certain persons only. LP a is very strongly associated with Myocardial Infarction and is some times called the "Little rascal".[15] LP a has two alleles, eleven Phenotypes and nineteen genotypes, Depending on the number of repetitive domains. The smaller iso form are associated with higher LP a concentration. LP a consists of particle of low density lipoprotein cholesterol (LDL –C) linked by a disulfide bond to a large hepatically derived glycoprotein, apolipoprotein a which is structurally similar to plasminogen. Theoretically speaking LP a could promote cardiovascular disease in two ways: its apolipoprotein a moiety could promote thrombogenesis and its LDL-C moiety could promote atherogenesis.[16]

Some studies suggested Lp a is a strong, independent predictor of coronary heart disease, particularly in women and young men. While other found no such association. One recent prospective trail found that while Lp a did not independently increases coronary heart disease risk, it seemed to increase the risk of elevated total cholesterol, LDL – C, and apolipoprotein B (the major lipoprotein of the atherogenic lipids) and cardio protective effect of high level of HDL – C.. Obese person usually contain elevated leptin levels which may be due to an increased Leptin gene expression and partly also for excessive productive by larger fat cells. It is hypothesized that obesity is due to resistance to leptin. In overweight women resistance to leptin explains the greater prevalence of obesity in women than men.

Different methods to assess obesity: It is very important to measure obesity, but there was not a clear direct method to measure of adiposity. There are various indirect methods to assess obesity which includes Body mass index (BMI) or Qutelet's index, Skin fold thickness.Waist hip ration and Underwater weighing (densitometry).

MATERIALS AND METHODS

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. The sample collection was done at Pranav Hospitals private limited located at salem.

Sample selection: For this intensive study, initially 200 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 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 history was carefully examined and studied. Since, the Study was mainly on non-diabetics, 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 measurements of 30 human subjects fell into category of obese since their BMI value is greater than or equal to 25. Form the remaining human subjects, 30 human subjects was selected has non-obese with the BMI is 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 factor 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 subject to find out cardiovascular risk factors on obese and non-obese.

COLLECTION OF BLOOD: The blood was drawn from the vein of the subjects. The blood was mixed with anticoagulant consisting of either heparin or EDTA. Blood sample containing non anticoagulant was used as a plasma. The blood was centrifuged at 3000 rmp for 15 min In a clinical centrifuge at room temperature. Thus blood serum was obtained and collected in Aseptic condition, frozen and preserved at 4^0 C still further use. In this study, whole blood without anticoagulant (plasma) or blood serum was used for biochemical analysis.

Read the BMI in kg/m^2 where the straight line crosses the middle lines when the height and weight are connected. Height and weight are without shoes. Obese greater than or equal 25: Non-obese less than or equal to 25. Glucose was estimated by the Glucose Oxidase and Peroxidase method. Human Analyzer kits to analyze Total cholesterol, triglycerides and HDL, VLDL and LDL. Homocysteine was estimated enzymatically by Block and kredich method.

RESULTS AND DISCUSSION

The present study "A comparative biochemical study on the non diabetic obese and non obese subjects with cardiovascular disease' was carried out with the objective of studying or investigating the (effect) cause for 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 and obese subjects with cardiovascular disease are presented in table 1. The cholesterol level is significantly (P<0.001) high in obese subjects than non obese subjects. This is in accordance with the maghapatia et al., who showed a clear and consistent association exisiting between obesity and 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.

Table: 1 The level of cholesterol and triglycerides in the serum of group1 non diabetic group 2 non obese and obese subjects with cardiovascular disease.

Sl.no	Subjects	Total cholesterol (mgs/dl)	Triglycerides (mgs/dl)
1.	Non-Obese subject with CVD	198±1.527	120±1.291
2.	Obese subject with CVD	380±1.291**	360±1.290**

Values are expressed as mean ± Sd for 30 individual in each groups.

(**) P<0.001 high significant

Significance** = P<0.001

*= P<0.01 or 0.05

NS = Not significant.

The level of cholesterol and triglycerides in the serum of group 1 non diabetic group 2 non obese and obese subjects with cardiovascular disease. Similar results was reported by Vijalakshmi and Anitha (2003) showing an increased level of triglycerides among the male subjects due to insulin insensitivity in obese.

Lipoprotein Fractions: 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 the 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 VLDL-C fractions showed and significant increase (P<0.001) in obese than on obese subjects.

This is in accordance with the observation of vijayalakshmi and Anitha (2003) showed a clear cut relationship between obesity nd abnormalitites in lipoprotein fractions. These include increase in VLDL and reduction in HDL which were obtained in both men and women.[17] A high production of total body cholesterol in obese subjects resulting a greater production of VLDL, which inturn induces and increase in hepatic lipase in women. In women low estrogen levels would have contributed to the low HDL concentration.

Manson et al (1990) studied the risk factors 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.[18] As much as 70% of the coronay 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 persist that may be due to other mechanism.

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 (Despres et al., and Bjorntorp et al., 1990).[19]

Klein et al. (2004) 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.[20]

Table –II

S. N.	SUBJECTS	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
1	Non-Obese subject with CVD	50.67 ± 1.59	123.67 ± 2.92	24.33 ± 2.13
2	Obese subject with CVD	28.1 ± 1.29	260.12 ± 1.46	72.2 ± 1.23

P<0.001 high significant

Significance** = P<0.001

*= P<0.01 or 0.05

NS = Not significant

A low level of HDL cholesterol is a strong and independent risk factor for recurrent cardiac events among patient with established coronary artery disease (Miller et al., 1992 and Pekkanen et al. 1990.[21] Jeppesen and his coworkers (2001) reported that the presence of high TG low HDL-C , High LDL-C concentration is associated with such a high risk of IHD.[22] The individuals with this lipid profile should be encouraged to make life style. Change and even treatment with drugs should be considered. It might also be beneficial in the weight controlling diet to replace saturated fats by unsaturated fats the weight loss and physical activity (HDL-C level). Serum total lipid (cholesterol, and triglycerides), Lipoproteins VLDL, LDL, HDL fraction in 25 coronary artery disease patients and 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 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 ratio (Total Cholesterol / HDL Cholesterol) found to be significantly ($p < 0.001$) high in obese subjects than in non- obese subjects. The elevated total cholesterol, low HDL cholesterol or elevated lipid ratio is risk for Cardio Vascular Disease.

Table III shows values are expressed as Mean ± SD for 30 individuals in each group

SI.NO.	Subjects	Total Cholesterol/HDL-C (mg/dl)
1	Non-Obese subject with CVD	**3.92 ± 0.10**
2	Obese subject with CVD	**13.4 ± 1.5**

P<0.001 high significant

Significance = P<0.001

= P<0.01 or 0.05

NS = Not significant.

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 are presented in the table IV. 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 P<0.001 high significant.

Table IV: shows values are expressed as Mean ± SD for 30 individuals in each group

SI.NO:	Subjects	Homocysteine (micromole/I)
1	Non-Obese subject with CVD	**16.54 ± 4.8**
2	Obese subject with CVD	**Absent**

P<0.001 high significant

Significance = P<0.001

= P<0.01 or 0.05

NS = Not significant

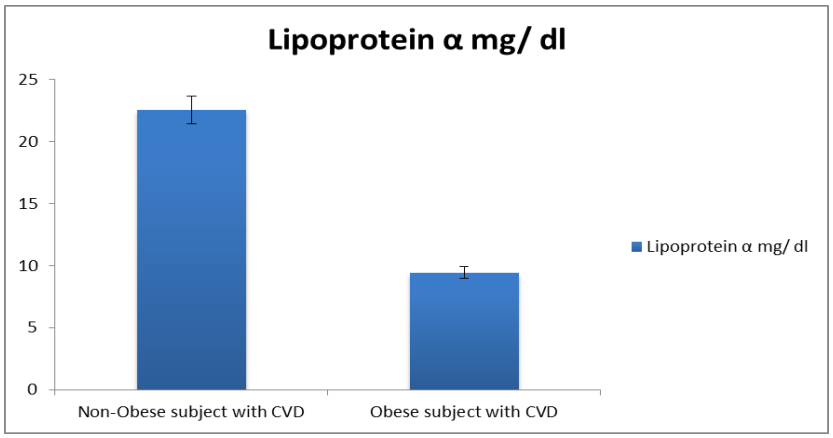

Figure 1 Lipoprotein α of none obese subject showed a significant (p<0.001) increase than in the obese subject.

Univariate analysis of that study showed lipoprotein α concentration to be significantly corrected with age, apolipoprotein B, plasma fibrinogen. Dimmer levels but not with body mass index, blood pressure, dietary fat intake, cholesterol, triglycerides, aplipoprotein A I, prothrombin fragment 1+2 and Antithrombin III. They concluded from this study there is a strong relationship between lipoprotein α and clotting variables in hypertensive patients that may contribute to atherosclerotic damage in these patients.

Yang et al (1995), found that malnutrition in hemodialysis patients is associated with an increased cardiovascular mortality.[23] To identify the cause for cardiovascular disease they evaluated the relationship between atherogenic lipid profile and serum albumin in

hemodialysis patients. There was a significant inverse correlation between albumin, total cholesterol, triglycerol, HDL_C, Total cholesterol/ HDL-C and Apo A-I / Apo B ratios. From these results they suggest that Lipoprotein α could be responsible for an increased cardiovascular mortality in hemodialysis patients with malnutrition.

Similar result was reported from the recent study done by Gupta et al., (2000). They found that there was not significant different in the lipid levels in Total Cholesterol, LDL-C, HDL-C and Triglycerol levels in the case of coronary artery disease and controls. But the levels of Lipoprotein α were significantly higher.[24]

Berglund and Ramakrishnan recently (2004) reported that lipoprotein α is present only in humans and primates. There is an interaction between lipoprotein α and other risk factors for cardiovascular disease. But the physiological role of lipoprotein α is unknown, although a majority of studies implicate lipoprotein α as a risk factor.[25]

The level of lipoprotein α in non obese subjects was not similar among the 30 individuals, it varies greatly from 14.6 mg/dl to 35.1 mg/dl. The level of lipoprotein in obese also varies greatly than non obese subjects. Only 12 out of 30 patients should the presence of lipoprotein a ranging from3.3 mg/dl 14.6 mg/dl.

The reason for the presence of lipoprotein in these individuals may be due to the increased LDL cholesterol or may due to chain smoking in men, because among 12 subjects 10 were men. The reason for the presence of lipoprotein α in young individuals may be due to familial back ground also.

CONCLUSION

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 a similar age groups in the western world. Little is known about the pathogenesis of atherosclerosis and cardiovascular disease in Indians.

Because the cardiovascular disease affect 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 of risk factors for atherosclerosis.

The present study was carried out to asses the risk factors in cardiovascular disease affected human beings. Since the cardiovascular disease may arise due to diabetic 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 is HDL-C, LDL-C, VLDL-C, lipid ratio in 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 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 subject may be due to the insulin resistance in obesity. The reasons for the presence of lipoprotein a 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 synthatase enzyme. Hence from this study not only obese subjects but also the personal 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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