Dissertation on

DETECTION AND COMPARISON OF LDL RECEPTOR GENE- APOB POLYMORPHISM IN OBESE AND NON OBESE PATIENTS WITH SUSPECTED CORONARY HEART DISEASE

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CERTIFICATE

This is to certify that this dissertation work entitled **"DETECTION AND COMPARISON OF LDL RECEPTOR GENE- APOB POLYMORPHISM IN OBESE AND NON OBESE PATIENTS WITH SUSPECTED CORONARY HEART DISEASE"** is a bonafide work of **Dr. PRABHA SURESH** in partial fulfillment of the requirements for the M.D. Biochemistry (Branch XIII) Examination of **The Tamilnadu Dr.M.G.R. Medical University** to be held on May 2018.

Prof. Dr. ARUNA KUMARI .R, MD,
Professor and Guide,
Department of Biochemistry
Karpaga Vinayaga Institute of Medical Sciences,
Madhuranthagam.

Prof. Dr. SUFALA SUNIL VISWAS RAO, MD,
Principal,
Karpaga Vinayaga Institute of Medical Sciences,
Madhuranthagam.

HEAD OF THE DEPARTMENT
Department of Biochemistry
Karpaga Vinayaga Institute of Medical Sciences,
Madhuranthagam.

DECLARATION

I, **Dr. Prabhasuresh** hereby declare that this dissertation "" is a presentation of my own work and that it has not been submitted anywhere for any award.

Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to literature and discussion.

This work was done under the guidance of **Professor Dr. ARUNA KUMARI .R, MD,** at Karpaga Vinayaga Institute of Medical Sciences, Madhuranthagam.

Candidate's Name : Dr. Prabha Suresh

Candidate's Signature :

Date :

In the capacity as guide for the candidate's dissertation work, I certified that the above statements are true to the best of my knowledge.

Prof. Dr. ARUNA KUMARI .R, MD,
Professor and Guide,
Department of Biochemistry
Karpaga Vinayaga Institute of Medical Sciences,
Madhuranthagam.

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Introduction

INTRODUCTION

According to the World Health Organization (WHO), obesity is the most common public health problem in both developed and developing countries [1]. According to the World Health Statistics Report 2012, one among six adults is obese and approximately 2.8 million individuals die every year due to overweight or obesity [2], now obesity is being recognized as a disease because of its increased risk of morbidity and mortality . In India the prevalence of obesity (generalized and abdominal) is higher with females which include 20 million obese women in 2014 compared to 9.8 million obese men [3].

Obesity is strongly associated with diabetes mellitus, gall bladder disease, certain cancers (endometrial, breast, prostate and colon), cardiovascular diseases (such as stroke and coronary heart disease) and non-fatal diseases including gout, respiratory conditions, gastro-esophageal reflux disease, osteoarthritis and infertility. A high level of Low Density Lipid (LDL) is also a risk factor for Coronary Heart Disease [4]. Due to an evolving societal prejudice against fatness obesity induces serious implications for psychosocial health. The risk for this disorder depends on the body mass index (BMI) of about 21kg/m^2 [5].

Several reports suggested that obesity is associated with the polymorphisms in genes controlling appetite and metabolism. Development of obesity is linked with the variation in such genes which are more than 41 in number [6]. In man lipoprotein complexes with one or more apolipoproteins are transported in blood which are derived from insoluble lipids. Low-density

lipoprotein (LDL) is comprises of 75% lipid (cholesterol and cholesteryl esters) and 25% protein (7). ApolipoproteinB (ApoB) the main protein component of Very Low Density Lipid (VLDL) and LDL acts as a key role in lipid metabolis in addition for removal of LDL from the circulation by LDL-receptor-mediated endocytosis it serves as the ligand (8). The primary responsibility for transporting cholesterol in LDL to tissues, the protein ApoB plays the major role. ApoB on the LDL particle acts as a ligand for LDL receptors in various cells throughout the body. Atherosclerosis is caused by high plasma ApoB levels [9]. Variation in the apoB gene is involved in the pathogenesis of obesity. The gene encoding for human ApoB [ID: 338] is of 43 kb in length, located exactly on the short arm of Chromosome 2 (2p23-p24) [10].

Several single nucleotide polymorphisms in the apoB gene particularly of Xbal, EcoRI and MspI have been closely associated with variation in lipid levels and obesity (11). This polymorphism is found to be associated with generalized obesity and increases the levels of various lipoprotein subfractions like triglycerides (TGL), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C).

Epidemiological studies have revealed an association between apoB gene polymorphisms and obesity and an increase in various lipoprotein subfractions (total cholesterol [TC], low density lipoprotein cholesterol [LDL-C], triglyceride [TG]) and atherosclerosis.

It has been reported that the for the development of cholelithiasis (gallstones) in patients, X+ allele of the Apo B gene is a risk factor. Some of the study explains that there is no association between the obesity and apoB XbaI polymorphism [12]. In this study we have attempted to correlate apoB XbaI gene polymorphisms in obese and non-obese patient with suspected coronary heart diseases.

This study has been done in Kancheepuram district covering the area of 120 villages near the 500 bedded tertiary care centre.

Aims & Objectives

AIMS AND OBJECTIVES

The study aims to determine the detection and comparison of LDL receptor gene –Apo B polymorphism in obese and non – obese patients with suspected coronary heart disease.

1. To determine the correlation between glucose levels in obese and non obese patients with suspected coronary heart disease.
2. To determine the correlation between lipid profile in obese and non obese with suspected coronary heart disease patients .
3. To determine the Thyroid profile in obese and non obese with suspected coronary heart disease patients.
4. To identify the LDL receptor Apo B polymorphism gene in obese and non obese with suspected coronary heart disease patients by Molecular Technique Polymerase Chain Reaction.

Review of Literature

REVIEW OF LITERATURE

Worldwide obesity has risen to higher proportions and has become a major worldwide health problem[13]. The highest rise in obesity levels among women has been found over the past three decades.

People who are overweight have an increased risk of cardiovascular disease, cancer, diabetes, osteoarthritis, and chronic kidney disease, with most deaths due to cardiovascular problems, including heart attack and stroke. The obese patients have a history of diabetes mellitus, genetic predisposition for diabetes and coronary heart disease in family etc.

The obese state has long been recognized to accentuate the common risk factors for atherosclerotic disease-hyperlipidemia, hypertension, and glucose intolerance. Evidence has also been presented that even after correction of obesity to be an "independent" risk factor for atherosclerotic disease by its association with risk factors.

The associated risk factors along with obesity are high blood pressure, cigarette smoking, cholesterol (TC), LDL-C, HDL-C, and diabetes. Patients with obesity, left ventricular hypertrophy, and family history of premature coronary heart disease have also considered in defining risk.

Family history of risk factors such as coronary heart disease, physical activity, and obesity are included because these factors and diseases, lifestyle and obesity are helpful to co-relate the coronary heart disease prediction.

Usually elevated blood pressure are associated with abnormal cholesterol values, increased body in mass index, diabetes etc. High levels of body mass index, the more prevalence of diabetes mellitus, Triglyceride, LDL – cholesterol, high density lipoprotein cholesterol and blood pressure are the main cause of CVD. These are the preventive and intervention risk factors[14].

Persons who exercise typically have a lower risk of CHD[15] information on physical activity was not available at the baseline examinations used to develop this CHD risk prediction algorithm, but cigarette smoking, low HDL-C levels, and diabetes are less common among those who are physically active[16- 19]. Regular and vigorous exercise is often associated with higher levels of HDL-C, an important determinant for reduced CHD risk[20- 22].

Similarly, "body mass index, an obesity index that expresses weight in kilograms divided by height in meters squared, has been considered a candidate variable for the CHD prediction algorithm. Greater obesity has been associated with higher TC, lower HDL-C, higher blood pressure, and diabetes, and the residual impact of obesity on CHD has typically been slight after incorporation of these other variables into the regression model[23]".

It is uncommon for persons to have four or five hour risk factors, and estimates of CHD risk tend to be more precise for individuals with fewer risk factors. The average CHD rates reported in those tables are roughly comparable to the myocardial infarction and coronary death rates among middle-aged men who participated in the west of Scotland trail of cholesterol lowering[24, 25].

Past 10 years the risk for coronoary heart disease have a range of age, myocardial infarction and chest pain.

A study that considered CHD prediction using TC, LDL-C, TC/HDL-C ratio, and LDL-C/HDL ratio[26]. The lipid profile and lipoprotein abnormalities have also been reported to be associated with measures of central obesity, such as waist-to-hip ratio in adults.

Concluded that “total cholesterol / HDL is a superior measure of risk for CHD compared with either total cholesterol or LDL cholesterol, and that current practice guidelines could be high risk factor was based on this ratio other then the LDL – Cholesterol level.”

The acceleration of atherosclerosis is due to factor of obesity risk that obese subjects have abnormalities in lipoprotein metabolism. For example, “obese subjects without hyperlipidemia have been reported to have increases in synthesis of very low density lipoprotein (VLDL)-triglycerides (TG), VLDL-apolipoprotein B (apo B) (VLDL-B)[27, 28], low density lipoprotein (LDL)-apo B (LDL-B)[29], and

total body cholesterol[30]". The abnormalities in VLDL, the precursor of LDL will enhance the rate of atherogenesis.

Researchers reported that body composition and thyroid hormones appear to be associated since thyroid hormones are involved in the basal metabolism and thermo genesis. The body metabolism is depends on lipid levels, glucose levels and metabolism, food intake and fat oxidation.

Hypothyroidism, the low levels of thyroid hormones causes increased weight and decrease the basal metabolism and thermo genesis. "There is an inverse correlation between free thyroxin (FT4) values and body mass index (BMI), even when FT4 values remain in the normal range.

Always abnormalities in thyroid function may be secondary to weight excess"[31]. In various studies revealed that adult obese patients, thyroid hormone and thyroid stimulating hormone (TSH) concentrations have been described as normal, elevated or reduced. The high conversion rate of T4 been described as normal, elevated or reduced.

The high conversion rate of T4 to T3 in obese patients has been also interpreted as a defense mechanism; capable of counteracting the accumulation of fat is increasing the energy expenditure, based metabolic rate[32].

Abnormalities in lipid levels and thyroid hormone in obesity patients increases the risk of cardiovascular disease regular monitoring of total cholesterol and thyroid hormone levels are mandatory in obese patients[33].

Obese related glomerulopathies are recognized complication of obesity. Renal biopsies and autopsy studies have reported that focal and segmental glomerulosclerosis (FSG) is the commonest histologic lession in obesity patients with symptoms of proteinuria[34].

Many experimental studies also showed the dyslipidaemia has major contribution renal injury, due to proliteration of mesangial cells and their extracellular discuss matrix induced by LDL cholesterol. The experimental study on zucker rat with obesity, researchers demonstrated hyperinsulinemia, hyperlipidaemia that leads to glomerulosclerosis and progressive renal failure[35].

The present study also included the factor of renal failure as a risk factor associated with lipid abnormality and ApoB gene polymorphism. Obese patients with hypertension are invariably associated with renal involvement become these abnormalities are interrelated pathogenically.

Obesity is characterized by inflammation with systemic involvement. Adipose tissue in obesity can be characterized by macrophage infiltration and associated inflammation in turn has been associated with anemia of chronic disease.

This study reported and hypothesized that, “obesity may be associated with the features of anemia of chronic disease, including low hemoglobin. Concentration low serum iron and transferring saturation (TS) and elevated serum ferritin”.

Overweight and obesity were associated with changes in serum iron, TS, and ferritin that would be expected to occur in the setting of chronic, systemic inflammation. However, overweight and obese persons were not more likely to be anemic compared with normal-weight persons.

"A recently discovered 25-amino acid peptide called hepcidin is thought to be a key mediator of anemia of inflammation[36-37]. Hepcidin is synthesized and secreted mainly by the liver and is detectable in blood and urine.

Hepcidin is induced by elevated iron stores or inflammation and functions as a signal inhibiting intestinal iron absorption and sequestering iron in macrophages. A recent study reported that hepcidin is synthesized not only by the liver, but also by adipose tissue, and that hepcidin messenger RNA expression was increased in adipose tissue of obese patients[38]".

Compared to normal-weight persons, those in higher BMI categories were progressively less likely to have low serum ferritin. In contrast, persons with moderate or severe obesity were more likely to have low serum iron or TS.

However, compared to normal-weight persons, all other higher BMI categories were not more likely to be anemic. As shown in previous studies, increasing BMI category was very strongly associated with elevated CRP, consistent with the presence of low-grade inflammation[39].

Obesity:

Obesity is one of the most common health problems in industrial societies (Grundy and Barnet 1990). Obesity is a health threat in part because it is associated with many other disease, including type II diabetes, gallstones and certain cancers (Grundy and Barnet 1990)[39] with excessive mortality (NIH 1985)[40].

Data from National Health Nutrition Examination Surveys (NHANES) show a high correlation between obesity and risk for coronary artery disease (NIH 1985). It has also been shown that most obese people experience hypertriglyceridemia and hypercholesterolemia (Grundy and Barnet 1990).

In addition, obesity is a very strong risk factor for hypertension. Obesity is epidemic in the United States (US) [Wilding 1998][41] and its prevalence is increasing (Grundy and Barnet 1990).

Currently, the criteria for inclusion into the overweight and obese categories are a relationship of height and weight (Bray 1992b)[42]. Body mass index (BMI) is body weight in kilograms divided by height in meters squared. In adults aged 19 – 34 years, BMI of 19 – 25kg/m^2 is considered normal, 25 – 30 kg/m^2 is overwight, >30 kg/m^2 represents obese.

In the US population of adults aged 25 years or more, 42% of men and 28% of women are overweight and 21% of men and 27% of women are obese

(Must et al.1999)[43]. In 1991, the prevalence of obesity was 12.0% (Mokdad et al. 1999)[44] and increased to 17.9% in 1998.

The incidence of obesity in every state, in both sexes and in all age groups, races and educational levels was increased. Nearly 10% of total health care costs are related to obesity (Wilding 1998)[41].

We often blame excessive eating and insufficient exercise for obesity; however there is still no concrete explanation for the fact that some people become obese, despite attempts not to, and others, apparently without much effort, do not (Ravussin and Danforth 1999)[45].

Genetic contributions to the development and maintenance of obesity cannot be eliminated. Studies in twins demonstrated an inherited tendency to gain weight in response to overfeeding (wilding 1998). Additionally, it was recently shown that there is unaccounted for physical activity in some people that prevents weight gain (Levine et al. 1999).

Symptoms and complication associated with obesity:

In obese person may have the symptoms of the medical condition mentioned below

They are include:

- Breathing disorders includes sleep apnea, chronic obstructive pulmonary disease)
- Types of cancers (e.g., prostate and bowel cancer in men, breast and uterine cancer in women)
- CHD
- Depression
- Hyper tension and type 2diabetes
- Liver disease
- High blood pressure
- Increased cholesterol content
- Stroke
- Psychological problems

1. The concept of BMI

Quatelet in 1869 was the first person to observe that among adults of normal build but different heights, weight was roughly proportional to height square. Quatelet Index (Weight in kg. /Height in meters^2) was later renamed '**body mass index (BMI)**' by Keys et al. (1972)[46].

BMI, Fat patterning and Mortality

Waist circumference and waist – to- hip ratio, as opposed to BMI may be more specific indicators of total body fat and may therefore provided an improved means of determining the risk of mortality associated with obesity, especially in

the elderly. Many reports have shown that abdominal or central obesity is equally important as total adiposity and may be a better indicator of CVD risk[46].

Waist circumference, used as a surrogate of abdominal obesity, is highly correlated with visceral adipose tissue (Lemieux S, et al., 1996)[47] and has been strongly linked with components of the metabolic syndrome, including glucose intolerance, hypertension, dyslipidemia and insulin resistance (Zhu S et al., 2002)[48].

Few studies have evaluated the usefulness of waist circumference or waist – to – hip ratio, compared to BMI, when predicting mortality and those that have been inconsistent (Baik I, et al., 2000; Kalmijn S. et al., 1999; Price GM et al.,2006; Visscher TL. Et al., 2001)[49- 51].

The data from the Rotterdam Study (Visscher et al. 2001) of 6, 296 men and women from The Netherlands was used to study the relation of BMI, waist circumference and waist- to – hip ratio as predictors of all – cause mortality.

Although this study had several methodological limitations, it showed that waist circumference and not BMI may prove useful for the prediction of all-cause mortality in the elderly.

Waist-to-hip ratio however was significantly positively related to all – cause and CVD mortality among nonsmoking men and women. Waist circumference was not associated with mortality from all – causes or CVD among

men or women. The investigators were able to control for a wide range of potential confounders and effect modifiers. “healthy”.

This study provides powerful evidence that BMI may not be indicative of increased adiposity and mortality in the elderly and waist- to- hip ratio may add clinical utility when recommending a healthy body size in older adults.

2. Obesity Models

There are many types of genetic mutations that result in pathophysiological signaling and metabolic alterations and which cause obesity in rodents (Guerre - Millo 1997). Two such models are the obese (ob/ ob) and diabetes (db/db) mice, both of which exhibit hyperphagia, profound early- onset obesity, hyperglycemia, hyperinsulinemia, infertility Colleman 1973), and defective thermoregulation (Coleman 1978)[52] indicative of a hypothalamic defect.

The phenotypes of the ob/ ob and db /db mice are identical and the strains can only be distinguished by genetic mapping or through parabiosis studies (Coleman 1973; Co1eman 1978)[52]. The results of parabiosis experiments suggest that there are satiety centers in the ob/ob and normal mice that respond to a circulation factor that is produced by db/db mice.

The fa/fa genetic defect in rats was first discovered in the laboratory of Zucker and Zucker in 1961 as an autosomal recessive mutation mapped to chromosome 5 (Truett et al. 1991)[53].

The Koletsky strain of rat develops obesity, hyperinsulinemia, hypertension, and proteinuria (Koletsky et al.1973)[54] due to a single recessive gene with a mutation in the extracellular domain of the leptin receptor (Takaya et al.1996)[55].

3. Theories of Food Intake

Several theories have been proposed to describe the mechanism of initiation of food consumption. Most of the theories revolve around the dual center hypothesis (Anand and Brobeck, 1951)[56].

The dual center hypothesis suggested that the lateral region of the hypothalamus, the "feeding center", played a role in the initiation of feeding and the ventromedial region, the "satiety center", inhibited feeding.

The amino static theory of food intake (Rogers and Leung, 1973) suggests that rats eat less when fed a diet devoid of certain amino acids, a diet with an amino acid imbalance, or a diet high in protein.

The glucostatic theory (Mayer, 1953)[57] suggest that there are glucose-sensitive receptors, glucoreceptors, in the lateral hypothalamus that initiate feeding when blood glucose is low.

3.1 Physical activity

In sedentary live and less physical activity persons obesity is common. Obesity is common in the middle aged people though it can occur in all age group who have less physical activity and consume more calories.

3.2 Endocrine factor

Cushing's disease, hypothyroidism and hypogonadism are the hormone factors for obesity.

3.3 Trauma

Damage of hypothalamus in head injuries will alter satiety and regular appetite functions that cause obesity.

3.4 Prosperity and civilisation

Obesity is common in prosperous countries like UK, USA, and in people of the higher economic strata of society, in developing countries.

4. Statistical analysis of overweight and obese people in India:

WHO recently stated that the growth in the number of severely overweight adults are expected to be double than that of under-weight during 1995-2005 (WHO 1998). Globalization is also playing an important role for modernization and sedentary life. So in near future obesity would emerge as a challenging problem for India.

Table 1.1 lists the states of India ranked in order of percentage of people who are overweight or obese.

States	Males (%)	Males Rank	Females (%)	Female Rank
India	**12.1**	**14**	**16**	**15**
Punjab	30.3	1	37.5	1
Kerala	24.3	2	34	3
Goa	20.8	3	27	3
Tamil Nadu	19.8	4	24.4	4
Andhra Pradesh	17.6	5	22.7	10
Sikkim	17.3	6	21	8
Mizoram	16.9	7	20.3	17
Himachal Pradesh	16	8	19.5	12
Maharashtra	15.9	9	18.1	13
Gujarat	15.4	10	17.7	7
Haryana	14.4	11	17.6	6
Karnataka	14	12	17.3	9
Manipur	13.4	13	17.1	11
Uttarakhand	11.4	15	14.8	14
Arunachal Pradesh	10.6	16	12.5	19
Uttar Pradesh	9.9	17	12	18
Jammu and Kashmir	8.7	18	11.1	5
Bihar	8.5	19	10.5	27
Nagaland	8.4	20	10.2	22
Rajasthan	8.4	20	9	20
Meghalaya	8.2	22	8.9	26
Odisha	6.9	23	8.6	25
Assam	6.7	24	7.8	21
Chhattisgarh	6.5	25	7.6	27
West Bengal	6.1	26	7.1	16
Madhya Pradesh	5.4	27	6.7	23
Jharkhand	5.3	28	5.9	28
Tripura	5.2	29	5.3	24
Delhi	45.5	36	49.8	64

5. Obesity and CVD:

Obesity and overweight increase the risk of several serious chronic diseases, such as type 2 diabetes, cardiovascular disease, hypertension and stroke, hypercholesterolemia, hypertriglyceridemia, arthritis, asthma and certain forms of cancer and weight gain is associated with a high risk of developing cardiovascular and metabolic diseases such as coronary heart disease, hypertension, diabetes and dyslipidemia.

Epidemiological studies have documented a close relationship between body mass index and cardiovascular events (Berchtold P et al., 1981)[58]

The association of increased body weight (Obesity) with elevated levels of triglycerides and diminished HDL cholesterol has been described in both adults and children.

Persons who exercise typically have a lower risk of CHD. Risk of CHD and diabetes are less common among those who are physically active. Regular and vigorous exercise is often associated with higher levels of HDL-C, an important determinant for reducing the risk of Coronary Heart Disease.

Similarly, "body mass index, an obesity index that expresses weight in kilograms divided by height in meters squared, has been considered a candidate variable for the CHD prediction algorithm. Obesity is appreciated with Triglycerides levels, elevated blood pressure and diabetes mellitus. The obesity on

coronary heart disease of these variables associated with regression model (Berns et al., 1989)[59].

Persons with less CHD risk factors is not possible to compare the persons with 2 or 3 risk factors. The score sheet used in the target persons for the prevention of CHD. It is used by a tabular form called Sheffield tabular column.

It is used to estimate risk of coronary heart disease. It is a threshold for risk interventions. The average CHD rates reported in those tables are roughly comparable to the prevalence of myocardial infarction and coronary death rates among middle-aged men who participated in a trial study done at the west of Scotland.

Kinosian B ET AL., 1955, they concluded that while predicting CHD using TC, LDL-C, TC/HDL-C ratio and LDL-C/HDL ratio, the total cholesterol / HDL is a superior measure of risk for CHD when compared with either total cholesterol or LDL cholesterol, and the guidelines for the current practice could be more efficient if risk stratification was based on this ratio (Albrink MJ et al., 1981)[60].

The lipid profile and lipoprotein abnormalities have also been reported to be associated with measures of central obesity, such as waist-to-hip ratio in adults.

The acceleration of atherosclerosis is due to the risk of obesity since those subjects have abnormalities in lipoprotein metabolism. For example, obese subjects without hyperlipidemia have been reported to have increased synthesis of very low density lipoprotein (VLDL)-triglycerides (TG), VLDL-apolipoprotein B

(apo B) (VLDL-B), low density lipoprotein (LDL)-apo B (LDL-B) and total body cholesterol.

Several studies showed that these abnormalities in VLDL, the precursor of LDL will enhance the rate of atherogenesis (Ostlund RE et al., 1900; Streja et al., 1980)[61, 62].

Alternatively there is significant weight loss has been shown to result in decreased triglyceride and increased HDL cholesterol concentrations.

Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. By the year 2020, it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability.

Atherosclerotic vascular disease (ASVD), which encompasses coronary heart disease, creberovascular disease, and peripheral arterial disease, is responsible for the majority of cases of CVD in both developing and developed countries.

In Atherosclerosis the lipids and fibrous materials are accumulated in coronary arteries. It will progress the atherosclerosis and enhance the cardio vascular diseases. The link between lipid metabolism and atherosclerosis dominated the thinking until the 1980s. Over the last fifteen years, however, a prominent role for inflammation in the pathogenesis of atherosclerosis has been established (Wood et al., 1980; Haarbo et al., 1990)[63, 64].

Haarbo et al. found positive associations in women especially after 50 years of age, between central fat and total cholesterol, triglycerides levels and LDL cholesterol and a negative association with HDL cholesterol in postmenopausal women. Walton et al.

The concept that inflammation governs atherosclerosis and its complication has provided a new unifying hypothesis of the link between risk factors and the cellular and molecular alterations that underlie this disease.

Endothelial injury is the first and crucial step in the pathogenesis of atherosclerosis. A plethora of genetically determined and epigenetic factors, such as oxidized low-density lipoprotein (LDL), free radicals (e.g., due to cigarette smoking), hypertension, diabetes mellitus, elevated plasma homocysteine, infectious microorganisms, autoimmune reactions, and combinations thereof, have been identified as etiological principles.

Endothelial injury triggers inflammation with increased adhesiveness and activation of leukocytes, platelets, which is accompanied by the production of cytokines, chemokines, vasoactive molecules and growth factors (Haarbo et al., 1989)[65].

Haarbo et al. found positive associations in women especially after 50 years of age, between central fat and total cholesterol, triglycerides levels and LDL cholesterol and a negative association with HDL cholesterol in postmenopausal women. Walton et al.

The hallmark of the early atherosclerotic lesion is the Cholesterol ester-laden (CE-laden) macrophage foam cell. The free cholesterol causes macrophage lesions. This will leads to phospholipids associated events.

Macrophage death by necrosis leads to lesional necrosis, release of cellular proteases, inflammatory cytokines, and prothrombotic molecules, which could contribute to plaque instability, plaque rupture, and acute thrombotic vascular occlusion.

Currently, atherosclerosis is considered as an inflammation-mediated disease driven by complex interactions between leukocytes, especially lymphocytes and monocytes (collectively termed as peripheral blood mononuclear cells or PBMC), platelets and cells of the vessel wall (Carlesson et al., 1986)[66].

Literature on regional adipose tissue distribution and metabolism has flourished over the past 25 years. They concluded that the, "proportion of abdominal adipose tissue is a key correlate; and perhaps driver of the health risk associated with overweight and obesity.

Visceral obesity has now been established as being part of a complex phenotype including adiposetissue storage dysfunction and ectopic triglyceride accumulation in several sites including the liver.

The demonstrated beneficial role of anti-inflammatory drugs in reducing the incidence of nonfatal events in many large clinical trials has demonstrated the major role of PBMC in the atherosclerotic process.

Hence, a potential insight into the mechanistic and signaling pathways that leads to inflammatory events in PBMC becomes necessary for tailoring efficient therapeutic strategies for CVD (Freedman et al., 1997; Priestley et al 1985)[67, 68].

Nazare et al. have reported ethnic differences in risk and prevalence of metabolic diseases including obesity.

Taken together, obesity has become an all too common condition in many of the world's industrialized societies today and is continuing to rise at an alarming rate.

Although at first sight it might seem that this is simply the result of changes to our diet and a dramatic drop in the level of physical activity in recent years, the reality is that scientific studies into the causes of obesity show that the condition results from a complex combination of factors (Hunaga et al 1986; Wang et al., 2011)[69, 70].

The important and potentially atherogenic circulating compounds in the circulating lipid systemis Apolipoprotein B (ApoB) including low-density lipoproteins (LDLs). ApoB is easily oxidized and responsible for inducing an inflammatory reaction and leads to the formation of plaques in the arterial wall.

Therefore, the ApoB considered as a marker in the status of pro- and anti-atherogenic lipoproteins and may serve as a good marker for assessing cardiovascular disease (CVD) risk in future.

One such factor which has come under increasing examination over past ten years or so is genetics and in particular, the genes involved in the regulation of LDL receptor within the body (Hamilton et al 2006; Barter et al., 2006)[71, 72].

6. Obesity and thyroid hormones

Researchers reported that body composition is associated with the levels thyroid hormones since thyroid hormones are involved in the basal metabolism and thermo genesis. The body metabolism in turn depends on lipid levels, glucose metabolism, food intake and fat oxidation.

The Apo B gene is located on the short arm of chromosome.]. Polymorphism has been found to be associated with generalized and regional obesity and an increase in various lipoprotein subfractions (total cholesterol [TC], lowdensity lipoprotein cholesterol [LDL-C], and triglycerides [TG]), but the results are conflicting.

Apo B gene variations in persons with varying lipid levels will cause obesity. (Faraj et al., 2006; Chatziste et al., 2013)[73, 74].

Hypothyroidism characterized with low levels of thyroid hormones causes an increase in the body weight and decrease in basal metabolism and thermogenesis. Several single nucleotide polymorphisms in the Apo B gene have been described (Biersac et al 1991)[75].

There is an inverse correlation between total thyroxine (tT4) values and body mass index (BMI), even when T4 values remain in the normal range.

Abnormalities in thyroid function may be secondary to body weight. Increased rate of convertion of T4 to T3 in obese patients which plays as a defense mechanism by the high levels of expenditure of energy and increased basal metabolic rate[75].

Abnormalities in lipid levels and thyroid hormones in obese patients increases the risk of cardiovascular disease and regular monitoring of total cholesterol and thyroid hormone levels are mandatory in such patients.

7. Obesity and Renal Function

Obesity related glomerulopathies are recognized as a complication of obesity. Renal biopsies and autopsy studies have reported that focal and segmental glomerulosclerosis (FSG) is the commonest histological lesion in obesity patients with symptoms of proteinuria .

Many experimental studies also showed the dyslipidaemia is a major contributor of renal injury, due to proliferation of mesangial cells and their extracellular matrix induced by LDL cholesterol.

In an experimental study done on zucker rats with obesity, researchers demonstrated that hyperinsulinemia and hyperlipidaemia leads to glomerulosclerosis and progressive renal failure. Chylomicrons, LDL

lipoproteins, VLDL are the components of ApoB. These are the ligand for LDL receptor to transport the serum cholesterol.

Thus dyslipidemia is as a result in dysfunction of ApoB gene.In ApoB gene region many typical sites of single nucleotide polymorphism are observed. Deletion and insertion also plays major role. The Insertion/Deletion polymorphism is within the first exon. The frequent allele (93bp) encodes a 27 amino acid peptide (INS; SP-27) and the deletion of 9bp results in 24 amino acid peptide (Del; SP-24) (Jemaa et al., 2004)[76].

The present study also included the factor of renal failure as a risk factor associated with lipid abnormality and Apo B gene polymorphism. Obese patients with hypertension are invariably associated with renal involvement because these abnormalities are interrelated pathogenically.

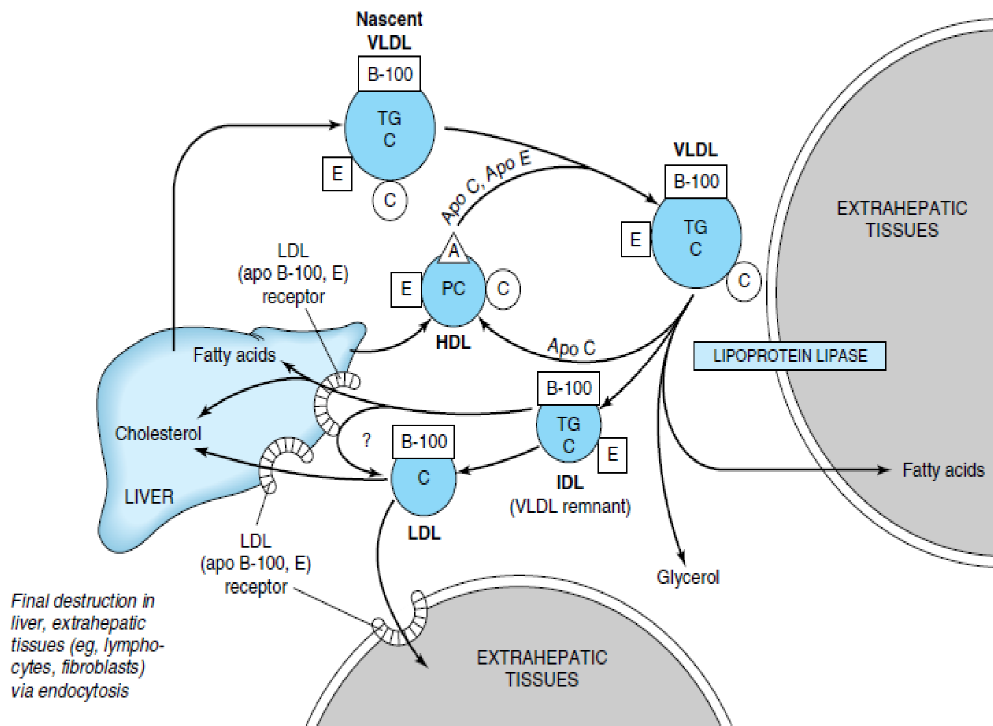
8. Obesity with LDL receptor

LDL receptor is a Mosaic protein containing 839 amino acids which mediates endocytosis of cholesterol rich LDL. Apo – protein B100 is embedded in the outer layer of phospholipid. Apo B100 is recognized by LDL receptors.

The apo E protein which is found in VLDL remnants and chylomicrons remnants are also detected by LDL receptors.

LDL receptor gene encodes the LDL receptor protein on chromosome 19. It is expressed in adrenal cortex and epithelial cells of bronchus.

LDL receptor gene also contains 27small nuclear proteins associated with increased risk of coronary heart disease. Investigations have shown that the correlation among Ins/Del polymorphism and lipid and lipoprotein levels may contribute to the pathogenesis of obesity, cardiovascular and non-alcoholic fatty liver disease (Vimaleswaran et al 2015)[77].



8.a. Structure

The Low Density Lipoprotein receptor which contains resides on chromosome 19 at the band 19p13.2 and is split into 18 exons that gene produce 6 isoforms through alternative splicing mechanism.

Coding sequence Exon 1 contains a signal sequence which localizes the receptor to the endoplasmic reticulam for the transport to the cell surface.

Exons 2-6 codes the ligand binding region, 7-14 codes the epidermal growth factor (EGF) domain, 15 codes the oligosaccharide rich region, 16 (and some of 17) code the membrane spanning region and 18 code the cytosolic domain.

LDL receptor family made up of number of domains including 3EGF domains, 7LDL- R class A domains and 6LDLR class B domains. LDL receptor N-terminal domains involve ligand binding and composed of seven repeat sequences.

This repeat sequence called as class A repeat or LDL-A sequence, which contains forty amino acids, including repeat disulfide bonds of six cysteine residues. Each repeat has highly conserved acidic residues used for coordinate a single calcium ion. The endosome has acidic interior.

Coordinated disulphide bonds of calcium are important for the structural domain intergration. Ligand binding domain is EGF precursor homology domain, which contains three repeat growth factors like A, B and C.

A and B are closely linked with C region. In C- terminal domains contains fifty amino acids which involve receptor mediated endocytosis.

Researchers demonstrated that the Del allele is associated with decrease secretion and increase degradation of Apo B. (Peacock et al., 1995)[78].

Seven member of LDL receptors in mammals include

i. LDL Receptor

ii. VLDL receptor

iii. Apo ER2

iv. LDL R – Related Protein -4

v. LDLR- Related protein 1

vi. LDLR – related Protein 1b

vii. Megalin.

8.b. LDL Receptor Gene Mutation

LDL receptor gene mutation cause familial hyperchosterolaemia. LDL receptor mutation broadly classified into 5 types

They are

1. **Class - 1** mutation – LDL receptor synthesis affected in the endoplasmic reticulum (ER).
2. **Class - 2** mutations - inhibit the transport to the Golgi body needed for post translation modification to the receptor
3. **Class -3** mutations - inhibit the binding of LDL to the receptor.
4. **Class - 4** mutations inhibit the internalization of the receptor-ligand complex.
5. **Class -5** mutations causes receptors that cannot recycle properly.

8.c. Functions of LDL Receptor Gene

LDL receptor mediates the endocytosis of cholesterol and it is maintain plasma LDL level. This occurs in all nucleated cells but liver mainly involves

70% of LDL removed from blood circulation. LDL receptors contains clustered in clathrin coated pits which form coated endocytic vesicles that carry LDL into the cell.

After internalization, the receptors dissociate from their ligands and receptor folds to obtain a closed conformation and recycles to the cell surface.

This recycling of LDL receptors provides an efficient mechanism for delivery of cholesterol to cell. LDL receptors proteins are synthesized by ribosomes on the endoplasmic reticulum and post translational modified by Golgi apparatus before travelling in vesicles to the cell surface.

Study of Benhizia et al. The allele carriers of rat hapetoma cells when compared with Del/ Del genotype shows the increased levels of serum VLDL and TG(Watts et al., 2000)[79].

Materials & Methods

MATERIALS AND METHODS

This observational study conducted from February 2016 to June 2017 was started after getting approval from the Institutional Ethical Committee at Karpaga Vinayaga Institute of Medical Science Maduranthagam.

Number of study groups	:	Two
Sample size	:	100
Study design	:	Observational study

INFORMED CONSENT

An informed consent was obtained from all the subjects participating in the present study.

The participants of the study were 100 male and female between 18-55 years, 50 obese patients and 50 non obese patients with hypertension, diabetes mellitus, hyper cholestrolism who agree to participate in the study and attending general medicine outpatient department and Inpatient Ward in Karpaga Vinayaga institute of medical science, Kancheepuram District, Tamil Nadu.

The participants belong to different religious and socioeconomic backgrounds.

Blood samples of 10ml from each patients fasting lipid profile and LDL level estimation LDL receptor gene APO B detection by molecular technique- pcr

Sponsorship : No

Conflict of interest : Nil

Inclusion Criteria:

- Obese and non obese patients at the age group of 18 – 55 years, both male and female.
- Hypertension, Diabetes mellitus, Hyper cholesterolism.

Exclusion Criteria:

- Age less than 18 years and more than 55 years.
- Emergency care metabolic diseases patients.

General Information

The general information of all the participants such as age, education, marital status, occupation and income were obtained. Detailed history of the participants such as past medical history, surgical history, menstrual history, obstetric history, personal history and family history were collected through personal interview method.

Anthropometry

Anthropometric measurements viz. height (cm) and weight (kg), were recorded. The height was measured using anthropometric rod to nearest 0.1 cm. The subjects were weighed on portable platform weighing balance to nearest 0.5 kg with ordinary clothes.

BMI was calculated by using the formula expressed as the ratio of weight in kgs to height in square meters.

BMI=weight(kg)/Height(m^2)

BMI classification for adult Asians (WHO)

	Under weight	Ideal BMI	Over weight	Obese grade I	Obese grade II
BMI	<18.5	18.5 – 22.9	23- 24.9	>25-29.9	>30.0

BLOOD PRESSURE

After a rest of 15 min, blood pressure (BP) of each of the participants was measured by auscultatory method with a standardized calibrated mercury sphygmomanometer, in sitting position, in the right arm. The appearance of sound (phase 1) is used to define systolic blood pressure (SBP).

The disappearance of sound (phase 2) is used to define diastolic blood pressure (DBP). Two readings were taken at five min interval and average of the two values was as blood pressure at that moment.

BIOCHEMICAL PARAMETERS

Serum fasting and postprandial plasma glucose, lipid profile, thyroid function tests and LDL receptor ApoB gene expression were the biochemical parameters estimated in the study population.

COLLECTION OF BLOOD SAMPLE

The blood samples of the respondents were collected after an overnight fast, 1ml in sodium fluoride coated sugar tubes and 3ml in plain tubes between 8 am to 9 am. 1 ml of blood for postprandial blood sugar was collected 2 hrs after breakfast.

The blood drawn was allowed to coagulate and the serum was separated by centrifuging and stored at -20°C until assayed.

Apparatus and glassware

All glassware used for the experiments were thoroughly washed initially detergent solution and then washed with double distilled deionized water three times and dried before used.

All precautionary measures were taken to prevent contamination during all stages of procedure.

All colorimetric readings were taken using colorimeter.

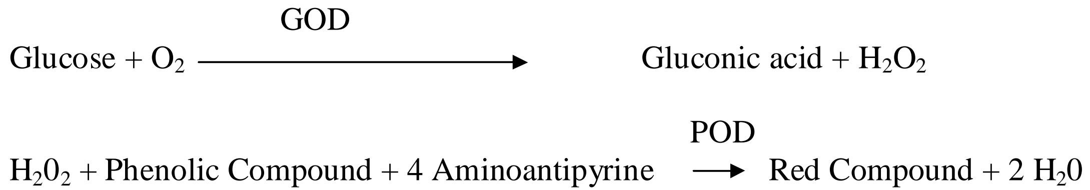
MEASUREMENT OF BLOOD GLUCOSE

METHOD: GOD/POD METHOD [83]

PRINCIPLE

Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. This hydrogen peroxide then reacts with phenolic compound/ amino antipyrine in the presence of peroxides to form a highly red coloured quinonaeamine.

The intensity of the red colored compound is proportional to the glucose concentration and is measured at 505 nm. The final colour is stable for 2 hours.



$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_20_2 + \text{Phenolic Compound} + 4\ \text{Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red Compound} + 2\ H_20$$

REAGENTS REQUIRED

Phosphate Buffer	-	95 mmol/L
4 Aminoantipyrine	-	0.2 mmol/L
P- Hydroxyl Benzoic Acid	-	5.9 mmol/L
Glucose Oxidase	-	>5000U/L
Peroxidase	-	>5000U/L
Glucose Standard	-	1 g/L

REAGENT RECONSTITUTION

Gently dissolve one tablet in 20 ml of distilled or deiodinised water in clean beaker with continuous stirring. Transfer the solution into a dark bottle. Write the reconstitution date on the label provided. This is stable for 6 weeks when stored at 2°-8°C.

PROCEDURE

	Blank	Standard	Test
Working Solution	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Incubate for 15 min at 37°C. Mix and read.

MEASUREMENT OF SERUM TOTAL CHOLESTEROL

METHOD: CHOLESTEROL OXIDASE AND PEROXIDASE METHOD [84]

PRINCIPLE

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipytine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of cholesterol present in the sample.

REAGENTS

REAGENT 1 (ENZYMES / CHROMOGEN)

Cholesterol esterase - ≥ 29 U/L

Cholesterol oxidase - ≥ 240 U/L

Peroxidase - ≥ 1000 U/L

4-Aminoantipyrine - 0.5 mmol/L

REAGENT 1A (BUFFER)

Pipes buffer, pH 6.95	-	50 mmol/L
Phenol	-	24 mmol/L
Sodium cholate	-	0.5 mmol/L

STANDARD (CHOLESTEROL 200 mg/dl)

Cholesterol - 2 g/L

REAGENT RECONSTITUTION

Allow the reagents to attain room temperature. Dissolve the contents in one bottle of reagent 1 with the contents in one bottle of reagent 1A. Mix by gentle swirling and write the date of reconstitution in the label provided. Wait for 5 min before using. This is stable for 3 months when stored at 2°C - 8°C

PROCEDURE

	Blank	Standard	Test
Working Solution	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Incubate for 5 min at at 37°C. Mix and read in a semi auto analyser programmed as an end point reaction with an increasing slope.

NORMAL VALUE: Serum: 130 – 250 mg/dl

LINEARITY:

The procedure is linear up to 1000 mg/dl. If the value exceeds this limit, dilute the serum with normal saline and repeat the assay. Multiply results by dilution factor.

MEASUREMENT OF SERUM HDL

METHOD: PHOSPHOTUNGSTATE METHOD [84]

PRINCIPLE

Chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) fractions in serum are separated from HDL (high density lipoproteins) by precipitating with phosphotungstic acid and magnesium chloride. The HDL fraction of cholesterol which remains in the supernatant, After centrifugation is assayed with the enzymatic cholesterol method using cholesterol esterase, cholesterol oxidise, peroxidise and the chromogen.

REAGENTS

REAGENT 1 (ENZYMES / CHROMOGEN)

Cholesterol Esterase	-	≥ 30 U/L
Cholesterol Oxidase	-	≥ 250 U/L
Peroxidase	-	≥ 1000 U/L
4-Aminoantipyrine	-	0.5 mmol/L

REAGENT 1A (BUFFER)

Pipes buffer, pH 6.95	-	50 mmol/L
Phenol	-	24 mmol/L
Sodium cholate	-	0.5 mmol/L

REAGENT 2 (PRECIPITATING REAGENT)

Phosphotungstic acid - 2.4 mmol/L

Magnesium chloride - 39 mmol/L

STANDARD (HDL CHOLESTEROL 50 mg/dl)

Cholesterol - 0.5 g/L

REAGENT RECONSTITUTION

Allow the reagents to attain room temperature. Dissolve the contents in one bottle of reagent 1 with the contents in one bottle of reagent 1A. Mix by gentle swirling till completely dissolved.

Write the date of reconstitution in the label provided. Wait for 5 min before using. The reconstituted reagent is stable for 3 months when stored at 2°C - 8°C.

PROCEDURE

Reagent	**Test**
Sample	0.20 ml
Precipitating Reagent 2	0.20 ml

Dispense into a centrifuge tube, 0.20 ml of serum and 0.20 ml of the precipitating reagent. Mix well and centrifuge at 3500-4000 rpm for 10 min. Separate the supernatant immediately and determine the cholesterol content. The HDL cholesterol standard should not be subjected to the precipitation step.

CHOLESTEROL PROCEDURE

	Blank	Standard	Test
Working Solution	1 ml	1 ml	1 ml
Standard	-	20 µl	-
Sample	-	-	20 µl

Incubate for 5 min at 2°C - 8°C. Mix and read in a semi auto analyser programmed as an endpoint reaction with an increasing slope.

MEASUREMENT OF SERUM TRIGLYCERIDE

METHOD: ENZYMATIC COLORIMETRIC METHOD [85]

PRINCIPLE

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with adenosine triphosphate (ATP) in the presence of glycerol kinase forms glycerol 3 phosphate which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidise to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglyceride present in the given sample.

The intensity of the purple colour is directly proportional to the triglycerides concentration and is measured at 546 nm.

NORMAL VALUES:

Serum/plasma :	Suspicious	: 150 mg/dl and above
	Elevated	: 200 mg/dl and above

REAGENTS

REAGENT 1 (ENZYME / CHROMOGEN)

Lipoprotein lipase	-	≥ 1100 U/L
Glycerol kinase	-	≥450 U/L
Glycerol3phosphate Oxidase	-	≥ 5000 U/L
Peroxidase	-	≥ 350 U/L
4-Aminoantipyrine	-	0.7 mmol/L
ATP	-	0.3 mmol/L

Reagent 1A (Buffer)

Pipe's buffer, pH 7.0	-	50 mmol/L
ADPS	-	0.9 mmol/L
Magnesium salt	-	17.8 mmol/L

REAGENT RECONSTITUTION

Allow the reagents to attain room temperature. Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A. Mix by gentle swirling and write the reconstitution date on the label provided. This is stable for 6 weeks when stored at 2° C - 8° C.

PROCEDURE

	Blank	Standard	Test
Working Solution	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Incubate for 5 min at 37°C. A purple colour develops. Mix and read at 546 nm in a semi auto analyser programmed as endpoint reaction with an increasing slope.

CALCULATION OF LDL

Serum total cholesterol was estimated by cholesterol oxidase and peroxidase method, serum HDL-Cholesterol by phosphotungstate method, serum triglycerides by enzymatic colorimetric method. From the values of total cholesterol, HDL-cholesterol and triglycerides,

LDL-cholesterol can be calculated using the Friedewald's formula.

Friedewald Formula = T.Cholesterol - (HDL + TGL / 5)

where TGL/5 is the concentration of VLDL cholesterol.

ESTIMATION OF SERUM CREATININE

METHOD: JAFF'S METHOD

KIT NAME: BioSystems

PRINCIPLE

Creatinine in alkaline medium reacts with picrate to form an orange – red complex. Under specific conditions of the assay, the rate of development of the colour is proportional to the concentration of creatinine in the sample when measured at 500nm (490 – 510nm).

REAGENT

Reagent 1 : Picric acid 34.9 mmol/L

Sodium hydroxide 45 mmol/L

Reagent 2 : Sodium hydroxide 0.26 mol/L

Creatinine Standard – 2 mg/L

Preparation of working reagent – All reagents are brought to room temperature before use. Equal volumes of reagent 1 and 2 are mixed in a clean test tube. The working solution is stable for 3 days at 15 – 25° C.

PROCEDURE

The working reagent, standard and samples were brought to room temperature before the test. Working reagent 1ml and creatinine standard 100 µL are dispensed into a test tube and read immediately for factor calculation in a RA-50 Semi-automatic analyzer. After calibration 100 µL of serum of a subject was added to 1 ml of working reagent and read immediately to get the serum creatinine value for that subject.

MEASUREMENT OF SERUM BUN – BY UREASE METHOD [84]

PRINCIPLE

Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced combines with α-Ketoglutarate and NADH in the presence of glutamate dehydrogenase to yield glutamate and NAD. The amount of urea nitrogen may be calculated by determining the absorbance decrease per minute relative to urea nitrogen standard at 340 nm.

REAGENTS

Reagent 1 (Enzymes) : ADH 0.7 mmol/L

GLDH ≥ 1000 U/L

Urease ≥ 30000 U/L

NADH 0.3 mmol/L

A- Ketoglutarate 9 mmol/L

Reagent 2 (Buffer) : Standard (BUN 20 mg/dL):

Urea 0.428 g/L

PROCEDURE

	Standard	Test
Reagent	1000 µl	1000 µl
Standard	10 µl	-
Sample	-	10 µl

Mix and read immediately.

CALCULATION

BUN value X 2.14 = Urea

THYROID HORMONE ASSAY

The blood samples of all the participants were collected after an overnight fast and all following parameters were estimated on the day of collection.

1. **T3 assay**
2. **T4 assay**
3. **TSH assay**

ASSAY OF TOTAL T3

Total T3 assayed in serum according to method of Timothy J. Wilke 1986 [86].

Principle

In the type of enzymatic immune assay, the competitive reaction between enzyme antigen and native antigen is present. They conjugate for a selected number of binding sites which are insoluble. Immobilized T3 antibody, native T3 antigene, are main reagents for this assay.

TBG and T3 enzyme which bind to serum proteins are measured. This method achieves this goal. The fraction of the enzyme bound antigen or native antigen from the sample that does not bind to the coated well is washed away.

The substrate is added to the antigen concentration. The enzyme activity between antigen and antibody is inversely proportional to antigen concentration which is measurable.

Assay Protocol

Before starting the assay, allow all the serum reference and controls to reach room temperature [21-25°c].

- ❖ Format the micro plate wells for each calibrator and patient specimen to be assayed. Replace the unused micro well strips back in the aluminum foil, seal and store at 2-8°c.
- ❖ Id 50µl of the calibration and the patient specimen to assigned well.
- ❖ Add 100µl of T3enzyme conjugate to each well. Care should be taken to dispense the entire reagent to the bottom of the coated well
- ❖ Shake the micro plate gentle for 20-30 seconds to mix and cover.
- ❖ Incubate for 60 minutes at room temperature [21-25°c]
- ❖ Aspirate the contents of the wells and fill them completely [approximately 300ul] with the diluted washing solution. Repeat the washing procedure two times. After the last wash, blot the micro plate on absorbent tissue to remove excess liquid from wells.
- ❖ Add 100µl of working substrate solution to all the wells add the reagents in the same order which minimize reaction time difference.
- ❖ Incubate for 15 mins at room temperature.
- ❖ Add 50µl of stop solution to each well and mix well for 15-20 seconds.
- ❖ In a microplater redder at 450 nm, the absorbace is read.

ASSAY OF TOTAL T4

T4 assayed in serum according to the method of young. D.S.,et al [1975] and modified by Stjernholm, Mr., et al [1977][86].

Principle

In a competitive EIA, there exists a competitive reaction between native antigen and enzyme – antigen conjugate for a limited number of insolubilized binding sites on the antibody coated on the micro well. After the antigen, antibody reaction has taken place, the fraction of the antigen in the conjugate or native antigen from the sample, which does not bind to the coated well, is washed away.

The enzymatic activity in the antibody bound fraction, which is inversely proportional to the native antigen concentration, is measured by addition of the substrate. By utilizing calibrators of known antigen values, a dose response curve can be generated from which the antigen concentration in a sample can be found out.

Assay Protocol

Before starting the assay, allow all the reagents serum references and controls to reach room temperature (21-25°c).

Format the micro plate wells for each calibrator and patient specimen to be assayed. Replace the unused micro well strips back in to the aluminium foil, seal and store at 2-8°c.

- Add 50µl of the calibrator and the patient specimen to assign well.
- Add 100µl of T3 enzyme conjugate to each well. Care should be taken to dispense the entire reagent to the bottom of the coated well.
- Shake the micro plate gentle for room temperature(21-25°c)
- Aspirate the contents of the wells and fill them completely (approximately 300µl) with the diluted washing solution. Repeat the washing procedure two times. After the last wash, blot the micro plate on absorbent tissue to remove excess liquid from wells.
- dd 50µl of stop solution to each well at 450nm (using a reference wave length of 620-630nm to minimize well imperfections) in a micro plate reader.

ASSAY OF TSH

Serum TSH assayed in according to the method of Spenser. CA, et al (1995) and modified by fisher D.A. (1996)[87].

Principle

In a quantitative EIA, high affinity antibodies react with antigen to from an insoluble sandwich complex on the surface of a coated micro plate.

The antigen from the specimen gets linked at the surface of the well through interaction of reaction IgG coated on the well and affinity purified x-antigen IgG conjugated with HPR. X antigen fraction immune globulin G is conjugated with enzyme.

If it is not bind to coated well washed thoroughly. The enzyme activity, which is proportional to antigen concentration in the sample, is measured by addition of substrate. From the known antigen value the response curve is produced.

Thus the antigen concentration in a sample can be easily measured from the curve.

Assay Protocol

Before starting the assay, allow all the reagents serum references and controls to reach room temperature (21-25°c).

- Format the micro plate wells for each calibrator and patient specimen to be assayed. Replace the unused m00icro well strips back in to the aluminium foil, seal and store at 2-8°c.
- Add 50μl of the calibrator and the patient specimen to assign well.
- Add 100μl of T3 enzyme conjugate to each well. Care should be taken to dispense the entire reagent to the bottom of the coated well.
- Shake the micro plate gentle for room temperature(21-25°c)
- Aspirate the contents of the wells and fill them completely (approximately 300μl) with the diluted washing solution. Repeat the washing procedure two times. After the last wash, blot the micro plate on absorbent tissue to remove excess liquid from wells.
- Add 100μl of working substrate solution to all the wells.
- Incubate at room temperature (21-25°c) for fifteen (15) minutes.
- Add 50μl of stop solution to each well for 15-20 seconds.

- Read the absorbance in each well at 450nm (using a reference wave length of 620-630nm to minimize well imperfections) in a micro plate reader.

EXTRACTION OF GENOMIC DNA FROM WHOLE BLOOD [88]

The protocol is simple and fairly rapid. The organic solvents are not used. But it is used to precipitate contaminated protein in salt extraction. The DNA extracted from it is readily used in PCR techniques. One can obtain approximately 100-200 ug of DNA from 4-8 ml of fresh or frozen whole blood.

Reagents

Buffer A (Red blood cell lysis buffer) composition

- 0.32 M sucrose
- 10 mMTrisHCl
- 5 mM $MgCl_2$
- 0.75% Triton-X-100

Adjust pH to 7.6

Buffer B (Proteinase K buffer) composition

- 20 mMTris-HCl
- 4 mM Na_2EDTA
- 100 mMNaCl

Adjust pH to 7.4

All solutions should be sterile. Autoclaved is done to Buffer A. Then add Triton X - 100

Procedure

1. Tke 4ml of blood with this and add 4ml of buffer A and sterile, distilled, cold deionized water of 8ml.
2. Vortexed gently or invert tube 6-8 times and leave to incubate on ice for 2-3 minutes.
3. Spined at 3500 rpm for 15 minutes at $4^{o}C$. The supernatant is added to 2.5% bleach solution. To the vertexed pellet add 6ml of water and 2ml of buffer A.
4. Spined at 3500 rpm for 15 minutes at $4^{o}C$. The pellet should be white to cream in color. If the pellet still appears red, continue the washing process.
5. To the pellet add 5ml of buffer B and 500 µl of 10% sodium dodecyl sulfate. Re-suspend pellet by vortexing vigorously for 30-60 seconds.
6. Then added 50 µl of Proteinase K solution (20mg/ml). Prepare fresh proteinase K solution and refrigerate the solution.
7. In a water bath of $55^{o}C$ incubate the solution for 2 hours. Remove samples and leave to cool to room temperature (or leave for 2-3 minutes on ice).
8. Added 4 ml of 5.3 M NaCl solution. Vortex gently for 15 seconds.
9. Spined at 4500 rpm for 15-20 minutes at $4^{o}C$. Pour off supernatant into a fresh tube. Take care not to dislodge pellet.
10. Added an equal volume of cold isopropanol (stored at $-20^{o}C$). Invert 5-6 times gently to precipitate DNA.

11. Remove DNA with a wide bore tip and transfer to a micro centrifuge tube. Wash with 1 ml of 70% ethanol. Leave DNA to dry for 15-20 minutes at 37°C.
12. Re-suspend in 300-400 µl of TrisHCl, pH 8.5 (not TE!). Leave to re-dissolve overnight at room temperature. DNA can be safely refrigerated for up to a year. Long-term storage may involve ethanol at -70°C.

VERIFYING DNA EXTRACTION BY GEL ELECTROPHORESIS

10 X TBE buffer:

Tris base - 54 gm

Boric acid - 27.5 gm

0.5 M EDTA (pH 8)- 20 ml

Make upto 500ml using dH2O water

6X Tracking Dye (10 ml):

Bromophenol blue (0.25%)

Xylene cyanol (0.25%)

Glycerol (30%) – 6 ml

Make upto 10ml using autoclaved dH2O water.

Protocol

1. Prepared 1% of Agarose gel by adding Agarose in 1X TBE buffer.
2. Melted it in microwave oven until the Agarose uniformly dissolved in the buffer.

3. Added 2µl of Ethidium bromide to the Agarose solution and pour it on a clean gel platform and place the comb and set it aside to solidify.
4. Placed this set up in an electrophoresis tank containing 1X TBE buffer, pull out the comb.
5. Mixed the PCR product with tracking dye and load the mixture in the Agarose well.
6. Electrophoresis was done at 100 – 150V until the tracking dye reaches 75% of gel.
7. Analyzed the DNA bands using GEL DOC.

PCR SETUP PREPARATION

After DNA extraction verification by Gel electrophoresis, the extracted DNA is amplified.

Components	Volume
PCR Master Mix	25µl
Primer FP1/FP2 (100µMl)	2µl
Primer RP1/RP2 (100µMl)	2µl
Sample	1µl of Extracted DNA Sample
H2O	Up to 5µl

Set of Primers	Upstream primer 5'-GGAGACTATTCAGAAGCTAA-3' as and downstream primer 5'-GAAGAGCCTGAAGACTGACT-3' as.	Validated with 10 samples DNAs from the thesis subjected sample.

PCR SETUP CONDITIONS

CYCLE Steps	1 Step		2 step		Cycles
	Temperature	**Time**	**Temperature**	**Time**	
Initial denaturation	98°C	2 min	98°C	2min	1
Denaturation Annealing Extension	98°C - 72°C	15 s 30 s 30 s	98°C 56°C 72°C	15 s 30 s 30 s	35
Final extension	72°C	3 min	72°C	3min	1

DNA PREPARATION FOR SEQUENCING (Pre-Sequencing)

Required reagents

1. **ddNTPS -"A"** ddATP, **"G"**ddGTP,**"T"** ddTTP, **"C"**ddCTP,
2. All four dNTP's
3. DNA polymerase
4. Template
5. Primer set
6. MQ water

As the DNA is synthesized, nucleotides are added on to the growing chain by the DNA polymerase. In a normal nucleotide some occasion dideoxynucleotide will be incorporated. This leads in chain termination. The fragment of CT, CTA, and CTAT and so on, are thus generated. All these fragments are then undergone gel electrophoresis based on their length (Mol.Wt) in the machine capillaries.

The laser detector detects the corresponding fluorescent nucleotide and translates into the colored peaks called as chromatogram.

PCR PRODUCT PURIFICATION FROM GEL

(using EZ10 spin column DNA Gel Extraction Kit- Biobasicinc)

1) Added 700 µl of binding buffer II to the gel piece in eppendorf.
2) Kept in water bath at 55ºC for 20 min.
3) Added 100 µl of Isopropanol to that and mix well by inverting tube.
4) Transferred the solution into spin column and let it stand for 2 min
5) Centrifuged at 12000 rpm for 1 min. Discard the filtrate.
6) Added 500 µl of wash solution to the column and centrifuge at 12000 rpm for 1 min. Discard the filtrate. (Repeat step 6)
7) Empty spin of the column was done.
8) Added 25 µl of elution buffer in the center of the column placed on a fresh tube.
9) Incubated at RT for 2 min.
10) Spined at 12000 rpm for 1 min.
11) Removed the filter column and store DNA content at 4^0 C.

CYCLIC PCR

In cyclic PCR the chain termination reaction takes place to generate the fragments to be visualized in the sequencing machine.

Primers for all amplification of LDL receptor exon

PCR Mix	Amplified Region	Forward Primer	Reverse Primer	Frag Size (bp)
A	Promoter	CAGCTCTTCACGGG AGACC	ACCTGCTGTGTCCTAG CTGG	287
	Exon 1	CACATTGAAATGTA ATGAGG	CTATTCTGGCTGGAGC AAGCC	215
	EXON 5	AGAAAATCAACACA CTGTGTCCTG	GGAAAACAAGCEAGAT GGCCAGCG	180
B	EXON 9	CCTGACCTCGCTCC CCGGACCCCCA	GGCTGCAGGCAGGGC GACGCTCAC	223
	EXON 2	TTGAGAGACCCTTT CTCCTTTTCG	GCATAATCATGCCCAA AGGGG	183
	EXON 16	CCTCCTTTAGACCT GGGCC	CAAAGCGGGGAGGCT GTGAC	173

PCR amplified product was digested with AvaII generate exon containing 157bp and 280bp sub fragments for single stand polymorphism analysis.

Statistical Analysis

STATISTICAL ANALYSIS

The individual student't' test was done to evaluate the significance of difference of means of two groups, using SPSS (statistical package for social science) statistical package, version 17. The data's were also subjected to independent samples test (unpaired) where ever necessary to evaluate the significance of difference of mean of control and study groups using SPSS software. The values are presented as mean +SD and p valve <0.05 was considered significant. 9ZarJH., 1984)[89]

Results

RESULTS

The present study was conducted in the Department of Biochemistry, Department of Medicine at Karpaga Vinayaga Institute of Medical Science, Maduranthagam. The individuals enrolled in the study were grouped into two based on their BMI and blood pressure values.

- Obese Group (50)
- Non Obese Group (50)

The obese and the non – obese subjects were all between 18 and 55 years of age. The age distribution in four ranges namely, 18-30, 31-40, 41-50 and 51-55 in the obese group and non- obese group is given in **Figure 1 and Table 1**.

FIGURE .1. CASE DISTRIBUTION IN RELATION TO AGE

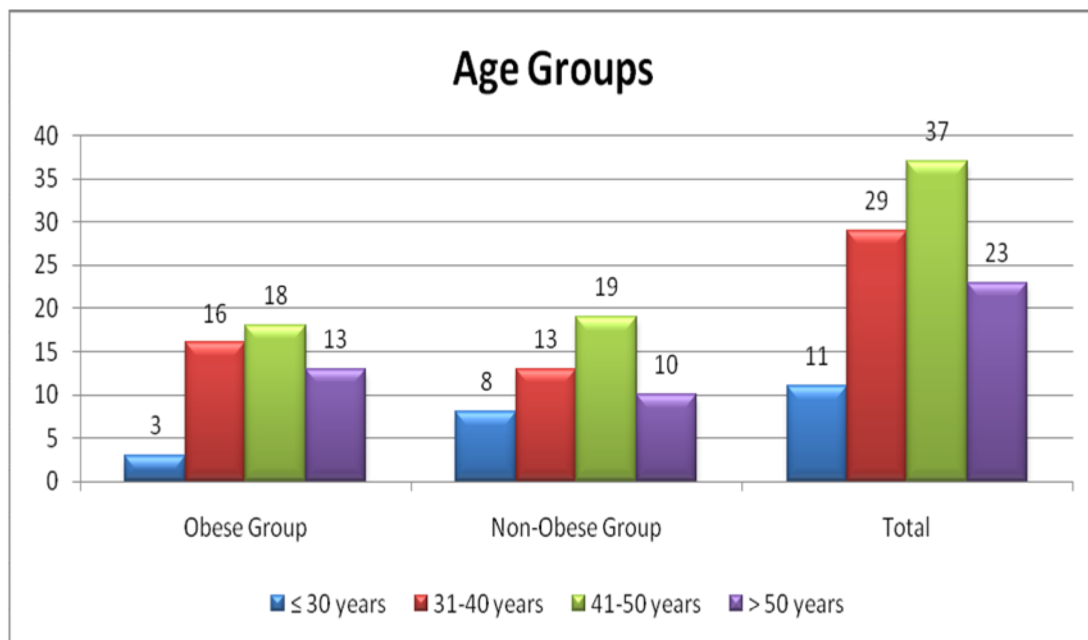


Table.1. CASE DISTRIBUTION IN RELATION TO AGE

Age Groups	Obese Group	Non-Obese Group	Tot al	Obese Group (%)	Non-Obese Group (%)	Total (%)
18- 30 years	3	8	11	6.00	16.00	11.00
31-40 years	16	13	29	32.00	26.00	29.00
41-50 years	18	19	37	36.00	38.00	37.00
51-55 years	13	10	23	26.00	20.00	23.00
Total	50	50	100	100.00	100.00	100.00

Age Distribution	Obese Group	Non-Obese Group	Total
Mean	44.08	42.64	43.36
SD	8.45	9.28	8.86
P value Unpaired t Test		0.4189	

The gender distribution in the two groups is given in **Figure 2 and Table 2.** In obese subjects age and gender there was no significant compared with non-obese subjects (P values of age and gender is 0.4189 and 0.0718 respectively).

FIGURE. 2. CASE DISTRIBUTION IN RELATION TO GENDER

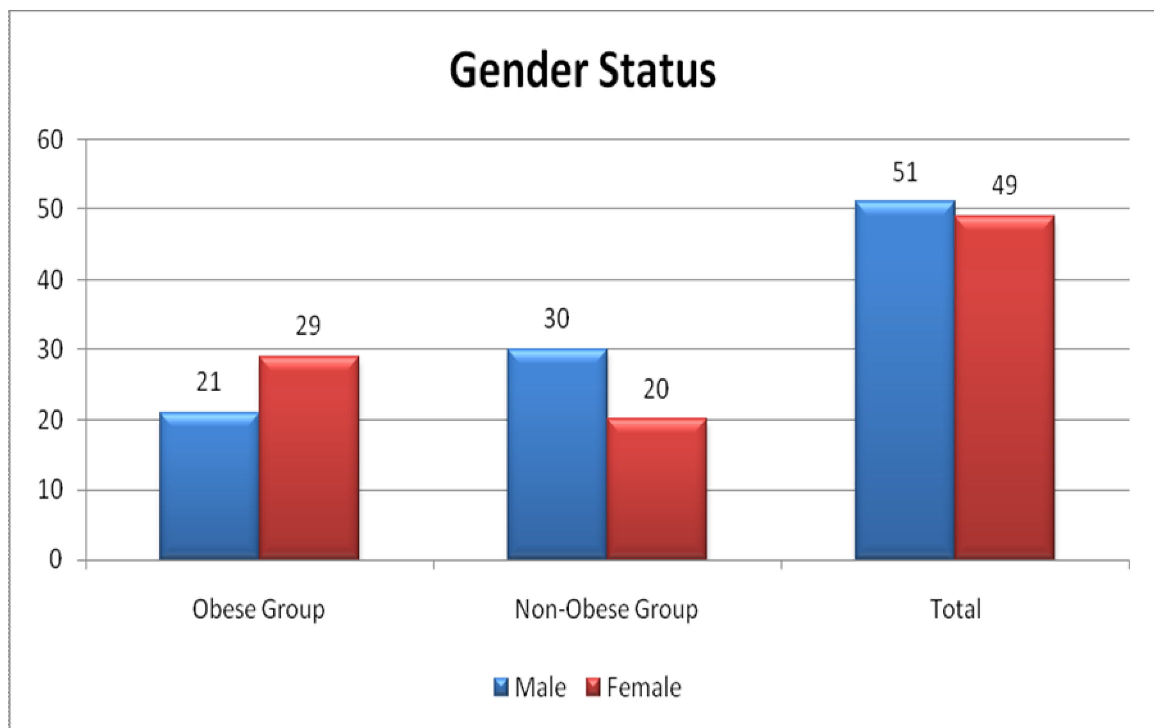


Table. 2. CASE DISTRIBUTION IN RELATION TO GENDER

Gender Status	Obese Group	Non-Obese Group	Total	Obese Group (%)	Non-Obese Group (%)	Total (%)
Male	21	30	51	42.00	60.00	51.00
Female	29	20	49	58.00	40.00	49.00
Total	50	50	100	100.00	100.00	100.00
P value Chi Squared Test		0.0718				

The parameters analyzed in all the subjects were

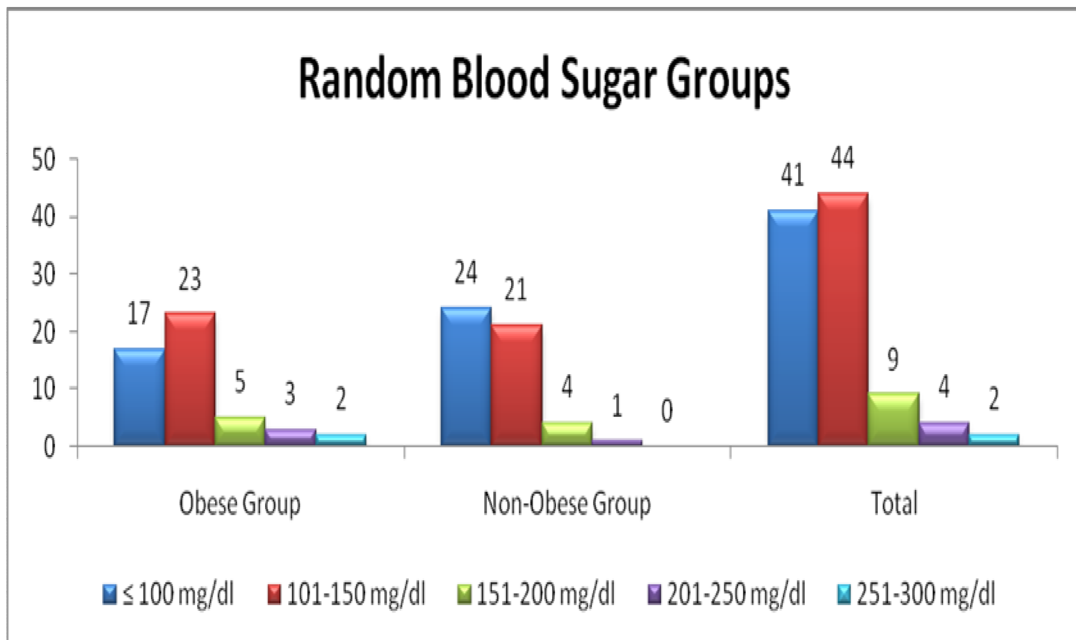
1. Blood Glucose
2. Serum Lipid Profile
3. Serum Urea
4. Serum Creatinine
5. Serum Thyroid Profile

The results expressed in their respective units are given in the master sheets at the Annexure I

RANDOM BLOOD SUGAR AND OBESE

Figure 3 and Table.3 shows the relation between the blood glucose levels in obese and non obese patients. The blood levels of random glucose were observed in both the groups of patients. The levels of random glucose were found to be significantly ($p < 0.02$) increased in non obese patients. The concentrations of random blood sugar were expressed as mean±SD in Table 4

Figure. 3. Random Blood sugar in obese and non obese patients



Random Blood Sugar Distribution	Obese Group	Non-Obese Group	Total
Mean	124.47	106.34	115.41
SD	12.26	11.73	40.14
P value Unpaired t Test		0.0232	

Table.3. Random Blood sugar in obese and non obese patients

Random Blood Sugar Groups	Obese Group	Non-Obese Group	Total	Obese Group (%)	Non-Obese Group (%)	Total (%)
≤ 100 mg/dl	17	24	41	34.00	48.00	41.00
101-150 mg/dl	23	21	44	46.00	42.00	44.00
151-200 mg/dl	5	4	9	10.00	8.00	9.00
201-250 mg/dl	3	1	4	6.00	2.00	4.00
251-300 mg/dl	2	0	2	4.00	0.00	2.00
Total	50	50	100	100.00	100.00	100.00

BLOOD PRESSURE WITH OBESE AND NON OBESE PATIENTS

Figure. 4 and Table.4. Shows that the relation between the blood pressure levels in obese and non obese patients. The levels of systolic and diastolic blood pressure were observed in both the groups. The mean SBP and DBP for the study participants with obese were 142.60±13.69 and 82.64±11.88 mmHg and with non obese were 118.20±18.92 and 79.32 ±10.7 in mmHg respectively as shown in Table 4. The observed results clearly depicts that the blood pressure was found to be significantly ($p < 0.05$) decreased in non obese patients when compared to obese patients

Figure.4. Blood Pressure in obese and no obese patients

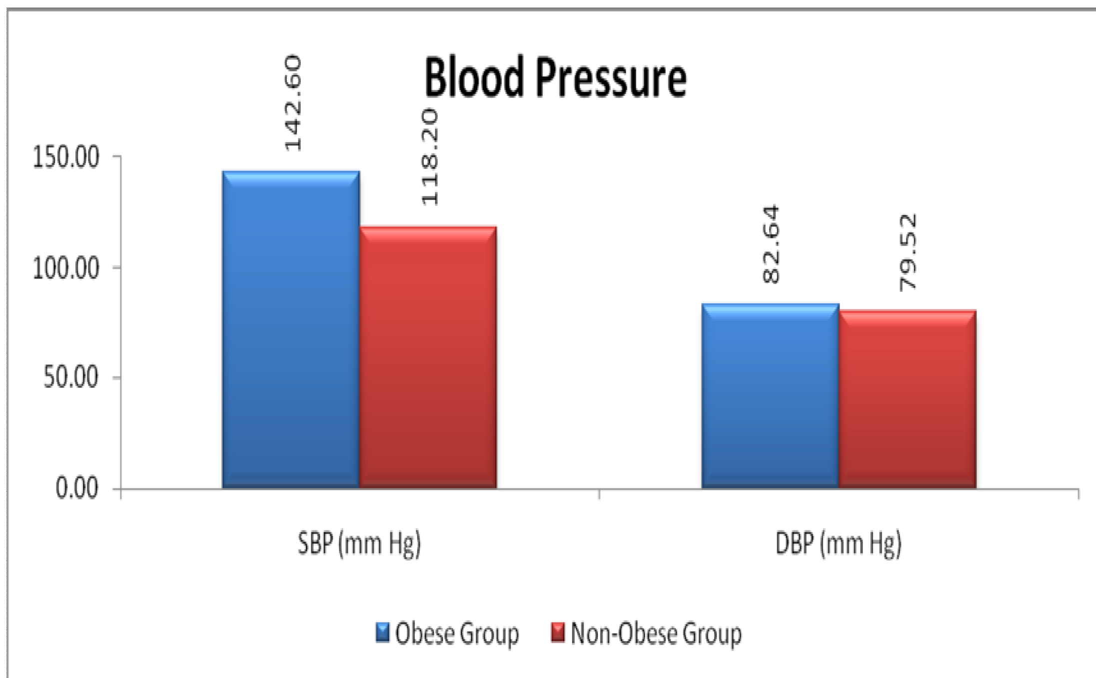


Table.4. Blood Pressure in obese and non obese patients

Blood Pressure		SBP (mm Hg)	DBP (mm Hg)
Obese Group	Mean	142.60	82.64
	SD	139.69	11.88
Non-Obese Group	Mean	118.20	79.52
	SD	18.92	10.71
P value Unpaired t Test		0.2239	0.1709

LIPID PROFILE IN OBESE AND NON OBESE

Figure 5 and Table.5. illustrates the levels of Total Cholesterol (TC), Triglycerides (TGL) were significantly increased and decreased in High Density Lipoprotein (HDL) in obese patients when compared with non obese patients. The levels of LDL and VLDL were found to be significantly decreased ($P<0.001$) in non -obese patients when compared with obese patients. The concentrations of TC, TGL, HDL, LDL, VLDL in mg/dl for the study sample were expressed in Table 5.

Figure.5. Lipid profile in obese and non obese levels

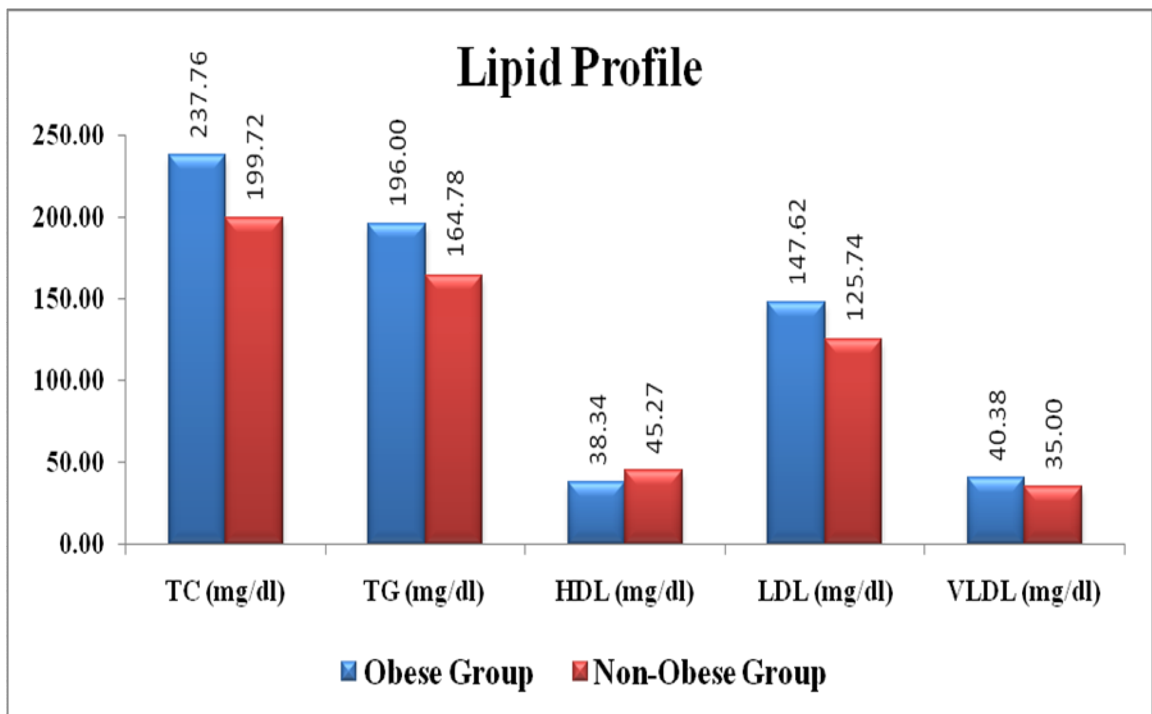


Table.5. Lipid profile in obese and non obese levels

Lipid Profile		Total Cholesterol (mg/dl)	Trigly cerides (mg/dl)	High Density Lipo protein (mg/dl)	Low Density Lipo protein (mg/dl)	Very Low Density Lipo protein (mg/dl)
Obese Group	Mean	237.76	196.00	38.34	147.62	40.38
	SD	56.43	72.77	21.69	69.36	14.34
Non-Obese Group	Mean	199.72	164.78	45.27	125.74	35.00
	SD	47.17	85.63	21.27	56.24	23.68
P value Unpaired t Test		0.0004	0.0423	0.1098	0.0864	0.1726

RENAL PROFILE IN OBESE AND NON OBESE LEVELS

Figure 6 and Table.6 shows that the levels of Urea and creatinine levels were no significant increased in obese patients when compared with non obese patients.

Figure.6. Renal Profile in obese and non obese patients

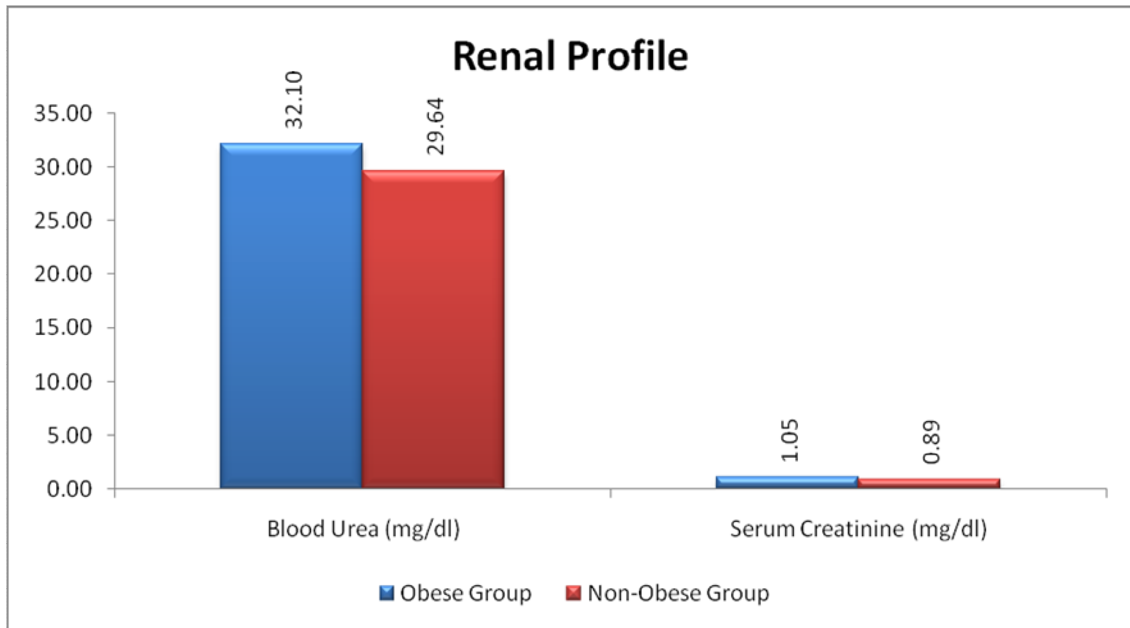


Table.6. Renal Profile in obese and non obese patients

Renal Profile		Blood Urea (mg/dl)	Serum Creatinine (mg/dl)
Obese Group	Mean	32.10	1.05
	SD	12.12	0.94
Non-Obese Group	Mean	29.64	0.89
	SD	8.63	0.53
P value Unpaired t Test		0.2452	0.2977

THYROID PROFILE IN OBESE AND NON OBESE PATIENTS

Figure 7 and Table.7 shows that the levels of TSH levels were significantly increased in obese patients when compared with non obese patients but there were no significant changes in T3 and T4 levels in obese patients when compared with non- obese patients.

Figure.7. Thyroid profile in obese and no obese patients

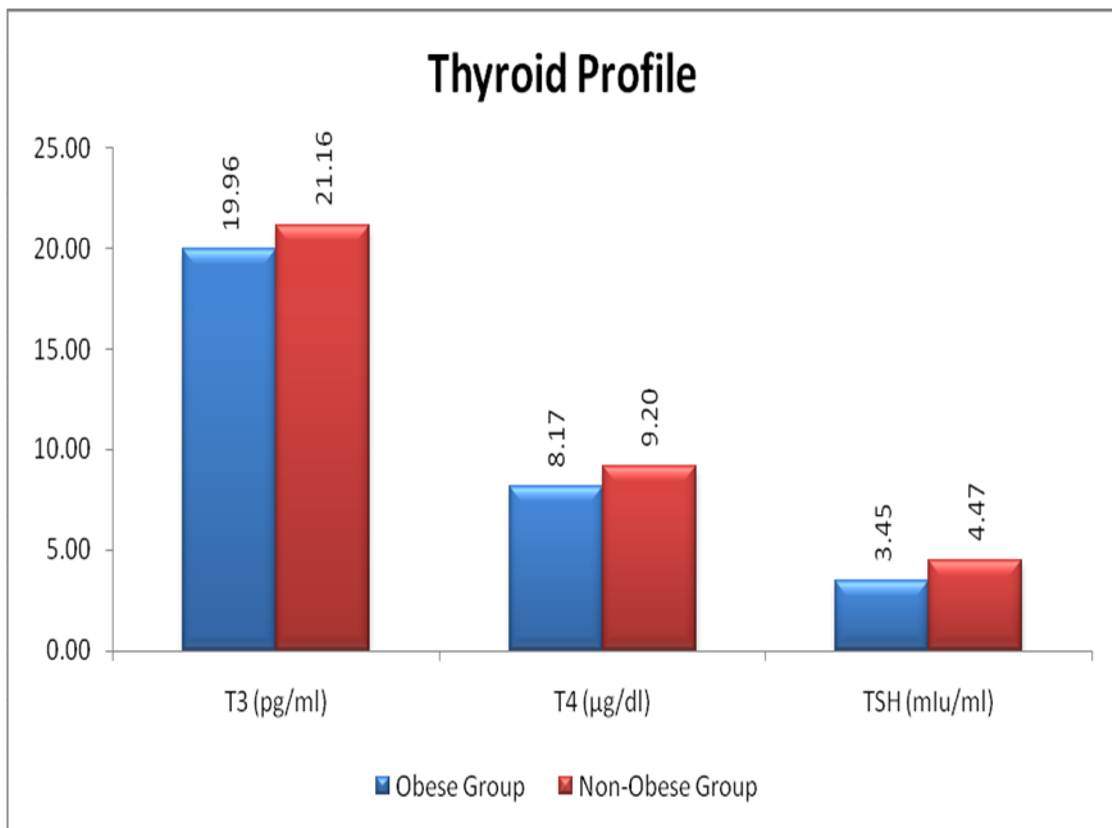


Table.7. Thyroid profile in obese and no obese patients

Thyroid Profile		T3 (pg/ml)	T4 (microgram /dl)	TSH (mIu/ml)
Obese Group	Mean	19.96	8.17	3.45
	SD	9.21	3.12	1.37
Non-Obese Group	Mean	21.16	9.20	4.47
	SD	9.83	3.90	2.94
P value Unpaired t Test		0.5300	0.1505	0.0286

LDL ApoB GENE POLYMORPHISM

Figure 8 and Table.8 shows that the LDL ApoB gene polymorphism like –ve polymorphism and +ve polymorphism levels. –ve LDL ApoB gene polymorphism X negative alleles were significantly increased in non obese patients when compared with obese patients but +ve LDL ApoB gene polymorphism X Positive alleles were significantly increased in obese patients when compared with non obese patients.

Figure.8. LDL Apo B Gene Polymorphism

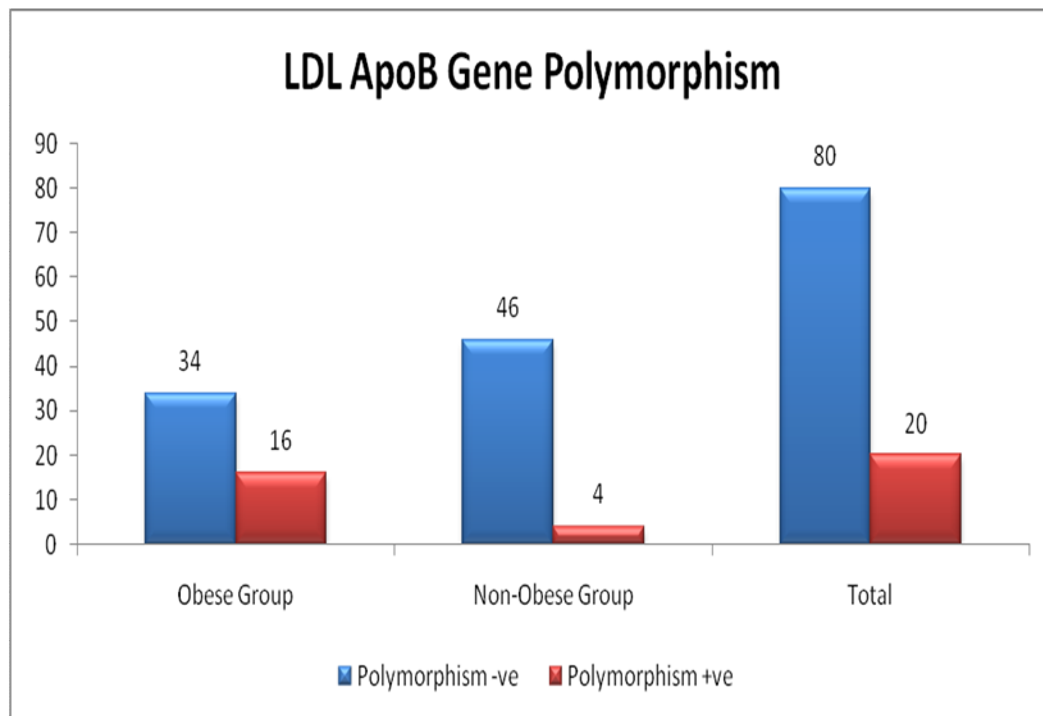


Table.8. LDL Apo B Gene Polymorphism

ApoB Gene Polymorphism	Obese Group	Non-Obese Group	Total	Obese Group (%)	Non-Obese Group (%)	Total (%)
Polymorphism -ve	34	46	80	68.00	92.00	80.00
Polymorphism +ve	16	4	20	32.00	8.00	20.00
Total	50	50	100	100.00	100.00	100.00
P value Chi Squared Test		0.0027				

LDL APOB GENE POLYMORPHISM ALLELES IN OBESE AND NON OBESE PATIENTS

Table.9. Indicates that the LDL ApoB gene polymorphism alleles like –ve and +ve polymorphism levels were significantly increased in Obese patients when compared with non obese patients.

LDL ApoB Gene Polymorphism	Obese Group	Non-Obese Group
Polymorphism –ve (X^-)	179	168
Polymorphism +ve (X^+)	45	56
P value Chi Squared Test	0.001	

Discussion

DISCUSSION

Obesity is unhealthy excess of body fat, which increases the risk of coronary heart disease. The prevalence of obesity is increasing in all age groups, including older persons.

Age and Sex distribution of obesity with coronary Heart Disease:

In the present study the mean obesity in the four age groups 18-30, 31-40, 41-50 and 50 - 55 years were mean of age groups of obese and non obese patients was 44.08 + 8.45 and 42.64 + 9.28 years respectively. Flegal KM et al and Mokdad et al showed 20 to 60years of age increase prevalence of obesity and decrease after 60 years age. Though the mean was higher in higher age groups it was not statistically significant (p value > 0.05).

In above 80years old male or female persons, the prevalence of rate of obesity is one-half when compared with 50-60years age old due to survival advantage of being lean, which makes lean and to develop very old.

BMI and Obesity with coronary Heart Disease:

Body mass index does not involve directly measure the body mass, but also to asses health risk factors with being obese. A normal BMI range is 18.5 to 24.9m.

The prevalence of obesity among the coronary Heart disease patients in the present study was similar to that of the general population in India. In our study, 71.7% coronary Heart disease patients of were obese which was almost

similar to study conducted in West Bengal in India (Park K. Text book of preventive and socialMedicine 19th edition page 335)[90].

The international diabetes foundation has accepted BMI > 25kg/m2 and >23kg/m2 as cut off value for obesity for Asian men & women respectively and according to this the prevalence of Obesity among males was 32%and among females was 52% which was alarming. In the present study in obese patients contain high BMI compared with non obese patients.

BLOOD SUGAR AND OBESE

In our study, the random blood glucose levels were observed in both obese and non-obese patients. The random blood sugar mean levels of obese patients are 124.47 + 12.26mg/dl and non obese mean values are 106.34 + 11.73mg/dl respectively. The levels of random blood glucose were found to be significantly increased in obese patients compared with non obese patients.

Wu L et al., 2013[91] observed the levels glucose 6- phosphate dehydrogenase in obesity induced by high fat diet and also increased in obese rat.

One mechanism for the signaling defects in obesity may be the high activity of protein tyrosine phosphates. It is dephosphorylated and by passing through tyrosyl phosphorylation it terminates the signaling. In some data, the expression and activity in muscle and adipose tissue of the obese humans and rodents indicate that, three PTP's including, leukocyte antigen related phosphate, src-homology-phosphate 2 and PTP1B levels are increased.

BLOOD PRESSURE WITH OBESE AND NON OBESE PATIENTS

There was a significant increase in the obese patients with increase in SBP and DBP compared with non obese patients.

A several Studies have shown that the relationship between BMI and systolic and diastolic blood pressure is nearly linear. In a heart study of Framingham provide results in excess weight gain in primary hypertension is 78% in men and 65% in women. Several studies indicates excessive weight gain raise to BP, but not all obese person are hypertensive. Thus some obese people will have less than 140/90mm Hg BP this level indicates hypertension[92].

LIPID PROFILE IN OBESE AND NON OBESE

Obesity mainly refers to excess of body fat. In the present study of lipid profile compared and observed between obese and non obese patients. Lipid profile value of total cholesterol, Triglycerides, LDL,VLDL and HDL levels were significantly difference in obese patients compared with non obese patients (G. Yuan J Wand., 2006)[93, 94].

Gunnel Ds et al.,1998[95] observed and reported in obese patients have number of metabolic abnormalities in lipid, glycemia, insulin and blood pressure. In this present study indicated hyperlipidaemia and hypercholesterolaemia that occur in obesity , which associated with increased incidence of coronary heart disease and strokes .

Haarbo et al[96]., found positive associations between central fat and total cholesterol, triglycerides levels and LDL cholesterol in women especially after 50 years of age, and a negative association with HDL cholesterol in postmenopausal women.

Walton et al done a study among healthy men between 21 to 77 years of age and found a relationship of increasing android fat distribution and elevated serum triglycerides and decreased HDL C_2 concentrations. But they did not report an association between fat distribution with either LDL or HDL cholesterol.

Reinehr et al. observed cardiac risk factors including higher triglyceride (TG) and low density lipoprotei (LDL) and lower high density lipoprotein (HDL) in obese children compared to children with normal weight. Roger et al., evaluated families who referred to family-oriented clinics to control cardiac risk factors.

Biochemical mechanisms responsible for the association between obesity have not been completely elucidated. The triglyceride levels are increases the storage is associated with a linear, increase in the production of cholesterol, which in turn is associated with increased cholesterol secretion in bile and an increased risk of gallstone formation and the development of gall bladder diseases.

Similarly, increased levels of circulating triacylglycerol in obesity are associated with decreased concentrations of high-density lipoprotein, which may account for the increased risks for cardiovascular disease and heart attack in obese patients (Benhizia., 1997)[97].

RENAL PROFILE IN OBESE AND NON OBESE LEVELS

Overweight and obesity is a one of the risk factors for the development of renal function loss. Several studies observed an association between obesity and progressive renal damage in subjects with renal disease , in renal transplant recipients and even in the general population (Lamia R et al., 2013) [98, 99].

Obesity subjects have an increased risk of developing hypertension, dyslipidaemia, insulin resistance/diabetes mellitus and cardiovascular complications, all of which promote chronic kidney disease (CKD)(Saha et al., 1993)[100].

However, even in the absence of these risks, obesity itself is associated with the development of CKD and accelerates its progression . Obesity and/or a central body fat distribution are associated with an unfavourable renal haemodynamic profile, which may play a role in the susceptibility and progression of chronic renal damage (John et al., 2008)[101].

In human, renal function measured by using indirectly and mainly in urea and creatinine in serum. In the present study clearly explains in obese patient's serum urea and Creatinine levels were significantly increased when compared with non obese patients.

THYROID PROFILE IN OBESE AND NON OBESE PATIENTS

Thyroid hormones are responsible for basal metabolism and thermo genesis. The body metabolism is mainly depends on protein levels, lipid levels, glucose levels and metabolism, food intake and fat oxidation(Becker et al., 1983)[102].

Hypothyroidism, the low levels of thyroid hormones causes increased weight and decrease the basal metabolism and thermo genesis. "There is an inverse correlation between free thyroxin (FT4) values and body mass index (BMI), even when FT4 values remain in the normal range (Racadot., 1991)[103, 104].

Always abnormalities in thyroid function may be secondary to weight excess". thyroid hormone and thyroid stimulating hormone (TSH) concentrations have been described as normal, elevated or reduced.

Matzen LE et al., 1989[105,106] observed the high conversion rate of T4 to T3 in obese patients has been also interpreted as a defence mechanism, capable of counteracting the accumulation of fat be increasing the energy expenditure, based metabolic rate.

Abnormalities in lipid levels and thyroid hormone in obesity patients increases the risk of cardiovascular disease regular monitoring of total cholesterol and thyroid hormone levels are mandatory in obese patients. In the present study explained thyroid hormone levels were significantly decreased in non obese patients[107-110].

LDL ApoB GENE POLYMORPHISM

In the present study observed the genotypic and allelic frequencies of LDL receptor Apo B gene polymorphisms on obese and non obese patients, it shows that there was significance difference in the genotype and allele frequencies of LDL Apo B polymorphism between obese and non obese patients.

S.H. Lye et al., 2013[111, 112] have reported ethnic differences in risk and prevalence of metabolic diseases including obesity. This may be related to patterns of ethnic-specific body fat distribution. Dyslipidemias, a group of biochemical disorders, is frequently encountered in obese individuals. The important characters of dyslipidemia is increased level of triglycerides , high levels of free fatty acids, .(atherogenic) low density lipoprotein cholesterol (LDL-C) particles and raised apolipoprotein B (Apo B) values.

However, there is a considerable heterogeneity of plasma lipid profile among obese people. The precise cause of this heterogenecity is not entirely clear but has been partly attributed to the degree of visceral adiposity (Ashavaid et al., 2000)[112-114].

S.E.A. Leigh et al., 2008[115, 116] also reported that significant difference in allelic frequencies of LDL receptor Apo B gene polymorphism. LDL receptor Apo B polymorphism does not leads to changes in the amino acid sequence and cannot be implicated at structure level. It is possible that some polymorphism might be present.

Saha et al showed a strong disequilibrium between insertion/deletion polymorphism and the LDL receptor Apo B gene.

E. Langenhoven[117- 119] all in 2007 studied the apolipoprotein B gene polymorphism in patients with essential hypertensive and related obesity compared with healthy controls in northern Chinese population.

In the present study clearly observed that there was no evidence associated LDL receptor Apo B gene polymorphism with obesity and lipid profile.

Summary

SUMMARY

- Metabolic syndrome is also known as metabolic syndrome X, cardio metabolic syndrome, syndrome X, insulin resistance syndrome, Reaven's syndrome.
- The prevalence of LDL receptor Apo B gene polymorphism was studied in two groups based on obesity and non obesity patients with coronary heart disease patients. Out of 100 patients including 50 is obesity patients and 50 were non obese patients.
- BMI were significantly increased in obese patients when compared with non obese coronary heart disease patients.
- Urea and creatinine levels were significantly increased in obese patients when compared with non obese patients.
- The blood levels of random glucose were estimated in two groups of coronary heart disease patients. The random glucose levels were significantly elevated in obese patients when compared with non obese coronary heart disease patients.
- Obesity is probably the most important modifiable acquired risk factor in the pathogenesis of types 2 diabetes.

- Blood pressure levels were significantly increased in obese patients.
- If obesity to increase in adiposity characterized by decreased HDL-C and increased triglycerides levels in body.
- LDL Receptor Apo B gene polymorphism gene alleles and genotype were significantly difference in obese and non obese coronary heart disease patients.
- LDL Receptor ApoB gene polymorphism is a one of the marker to differentiate Obese and non obese coronary heart disease patients.

Conclusion

CONCLUSION

- Obesity increase the risk of several serious chronic diseases, such as type 2 diabetes, cardiovascular disease, hypertension and stroke, hypercholesterolemia, hypertriglyceridemia, arthritis, asthma and certain forms of cancer.
- In our study 20% of the study population have LDL receptor Apo B gene polymorphism with coronary heart disease.
- The present study has some clinical significance that, LDL receptor Apo B gene polymorphism and lipid profile levels are very important marker for obese with coronary heart disease patients.
- The study has scientific importance since it is directed at the health concern of the young generation.

Limitations of the study

LIMITATIONS OF THE STUDY

- The limitation of this study is its cross-sectional nature. The cross sectional design of the study also means that the data was collected at one point of time, so causal relationship cannot be ascertained and there is a high potential for confounding.
- Despite these weaknesses, the cross sectional design was observed to be the most appropriate design due its ability to establish associations, low participant burden and usefulness within limited timeframes and budget of the study.

Scope for further studies

FUTURE SCOPE

- The study has significance since it is done in obese and non obese coronary heart disease patients it is directed towards the health of the young generation.
- The study can be extended with the other markers.

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Annexures

INSTITUTIONAL ETHICAL COMMITTEE

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTRE

MADURANTHAGAM - 603 308

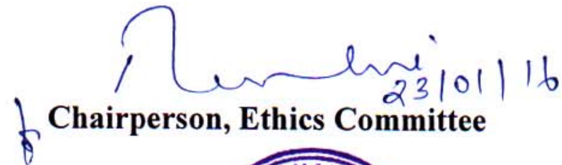
EC Ref. No: 08/2016

CERTIFICATE FOR APPROVAL

The Institutional Ethical Committee of Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam reviewed and discussed the application for approval **"DETECTION AND COMPARISON OF LDL RECEPTOR GENE APO B POLYMORPHISM IN OBESE AND NON OBESE PATIENTS WITH SUSPECTED CORONARY HEART DISEASE"** submitted by **Dr. PRABHA SURESH**, 1st Year Biochemistry Post Graduate, Guided by, "**DR. R. ARUNA KUMARI**, Professor, Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam.

The proposal is **APPROVED**

The Institutional Ethics Committee expects to be informed about the progress of the study, and Adverse Drug Reaction occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.



Date: 23/01/2016

Chairperson, Ethics Committee

ABBREVIATIONS

ADP	-	Adenosine Di Phosphate
ATP	-	Adenosine Tri Phosphate
AMPK	-	Adenosine Monophosphate Activated Protein Kinase
APPROX.	-	Approximately
BMI	-	Body Mass Index
CCK	-	Cholecystokinin
CYP27B1	-	Cytochrome P 450,family 27,subfamily B,Polypeptide 1
ELISA	-	Enzyme Linked ImmunoSorbent Assay
GOD	-	Glucose Oxidase
HDL	-	High Density Lipoprotein
HPA	-	Hypo Pituitary Axis
HRP	-	Horse Radish Peroxidase
IU	-	International Units
ICMR	-	Indian Council Of Medical Research
JAK	-	Janus Kinase
Kg/m^2	-	Kilogram/metre square
LDL	-	Low Density Lipoprotein
ml	-	Millilitre
mg/dl	-	Milligrams/decilitre
Mmol/L	-	Mill moles/litre

MSH - Melanocyte Stimulating Hormone

MAPK - Mitogen Activated Protein Kinase

nm - nanometre

ng/ml - nanogram/millilitre

nmol/L - nanomoles/millilitre

NHS - National Health Service

NPY - NeuroPeptide Y

NIMR - National Institute for Medical Research

P/D1cells - Cells lining the fundus of human stomach

POD - Peroxidase

PTH - Parathyroid hormone

PTHrP - Parathyroid hormone related protein

PYY3-36 - Peptide YY3-36 consisting of 36 amino acids

STAT - Signal Transducer and Activation of Transcription

SD - Standard Deviation

TC - Total Cholesterol

TGL - Triglycerides

THC - Tetrahydrocannibol

UV - UltraViolet

UV B - UltraViolet B

WHO - World Health Organisation

INFORMATION SHEET

- Your blood sample has been accepted.
- We are conducting a study on apparently healthy individuals attending Karpaga Vinayaga Institute of Medical Sciences,Kanchipuram Distric-603308 and for that your blood sample may be valuable to us.
- The purpose of this study is to identify **Detection and comparison of LDL receptor gene Apo B polymorphism in obese patients and non obese patients with suspected coronary heart disease** with the help of certain special tests.
- We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of participant　　　　　　Signature of investigator

PATIENT CONSENT FORM

Title of the study **: Detection and comparison of LDL receptor gene Apo B polymorphism in obese patients and non obese patients with suspected coronary heart disease.**

Name :   Date :

Age :   OP No :

Sex :   Individual ID :

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study.

Signature of participant   Signature of investigator

PROFORMA FOR PATIENT DETAILS

NAME:

AGE:

GENDER:

OP.NO.:

INDIVIDUAL ID. NO:

ADDRESS:

HISTORY:

GENERAL EXAMINATION:

HEIGHT:

WEIGHT:

BLOOD PRESSURE: **PULSE:**

SYSTEMIC EXAMINATION:

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM:

ABDOMEN:

NERVOUS SYSTEM:

INVESTIGATION:

DIAGNOSIS:

MASTER CHART

S.no	Age	Sex	BMI	RBS	FBS	PP	T.CHO	TGL	HDL	LDL	VLDL	Urea	creatinine	BP	T3	T4	TSH	HB
1	49	M	26	270			233	260	26	189	57	77	2	110/80	11	5	5	13
2	49	M	30	110			289	320	52	198	62	31	0.9	90/72	16	6	4	12.4
3	51	M	19.8	105			153	254	89	142	30.6	25	0.8	100/70	39	9	13	12
4	55	M	20	100			165	67	35	54	7.2	24	0.9	100/70	14	8	4.4	11.9
5	53	F	25.2		73	130	265	300	76	231	60	26	0.6	120/80	23	6	3	13
6	29	M	21.7	99			157	78	34	187.85	15	27	0.5	110/70	23	8	3.4	13
7	32	F	19.4		97	138	220	211	87	189	42.2	31	0.9	120/82	12	7	6	14.2
8	25	M	21	83			220	234	29	160	46.8	21	0.8	160/90	37	17	7.56	12.9
9	40	M	29	140			240	190	41.1	134	38	33	0.7	110/80	17	9	4.2	13
10	55	M	16.5	105			150	91	41	47.8	18.2	34	0.8	90/72	19	11	2.9	14.1
11	50	M	15.4	80			156	101	57.4	78	20.2	26	0.9	110/80	12	8	3.1	12
12	38	F	21	163			290	300	23	167	60	32	0.6	150/100	15	8	3.4	12
13	22	F	14.6	154			230	200	23	189	40	34	0.5	110/70	14	6	4.2	12
14	42	M	20	102			178	143	47.7	189	28	25	0.9	110/70	18	8	3	10.5
15	45	M	19	110			220	90	12	176	18	24	0.7	110/80	12	9	4.3	14.2
16	55	F	27.3	110			231	123	12	378	24.6	26	0.8	150/100	35	7	3.8	12.6
17	40	M	23		110	138	150	189	52	178	37	56	1.9	110/80	59	2.9	4.2	12
18	50	F	17.4	110			245	259	79	167	51.8	24	0.6	150/100	13	9	4.1	14.2
19	41	M	29	101			230	200	21	136	40	21	0.7	110/70	56	2.1	4.3	13.2
20	39	F	20	90			176	350	50	57	70	27	0.8	90/70	14	8	5	6.4
21	40	M	26		98	123	234	189	28	46.8	37	35	0.9	110/70	20	8	4	14
22	52	M	21.4	110			150	92	53.9	168	11.4	56	0.5	110/80	21	7	4.2	12
23	53	F	25.4		98	139	276	251	21	245	50.2	27	6	110/80	13	8	9	12
24	49	F	21	90			209	300	29	123	60	29	0.7	110/70	11	6	4.6	15

25	35	F	16	85			175	99	39.5	115.7	19.8	28	0.9	110/80	21	10	3.1	12
26	32	M	27	109			289	254	26	180	50.8	28	0.7	120/80	23	7	2.4	12.9
27	55	M	18	74			167	280	53	45	56	23	0.8	90/70	19	8	3.6	13.2
28	50	M	21		102	128	278	220	67	113.6	44	12	0.9	100/70	22	6	4.4	13.7
29	28	M	16.2	110			159	234	21	72.2	46.8	32	0.7	130/90	39	18	12.6	13
30	49	M	26	110			189	141	88	107	28.2	56	3.1	160/100	12	9	2.3	12
31	51	M	21	167			158	137	23	82.4	27.4	26	0.8	120/80	18	7	2.6	14.2
32	42	M	21	72			234	190	29	66.5	38	21	0.5	110/80	16	8	4.2	13.9
33	45	F	25	180			160	278	30	89	55.6	27	0.7	100/70	29	6	3.4	12
34	55	F	28.8	110			321	120	23	77.9	24	23	0.9	90/70	16	7	3.1	12.6
35	39	F	27	109			341	89	25	92.7	17.4	34	0.9	110/70	17	8	2.4	12.3
36	33	F	31		88	121	241	300	67	189	60	23	0.8	120/80	17	6	4.2	8.9
37	50	M	22	77			200	99	54	180	49	22	0.7	120/70	14	7	3.2	13.5
38	39	F	31.2	86			289	210	30	177	42	45	1.7	110/70	12	9	2.1	14.5
39	48	M	26.4	77			300	220	45	231	44	25	0.7	120/80	17	8	3.2	12
40	51	M	21	96			187	125	82	78	147	24	0.8	100/70	34	6	2.5	13.2
41	38	F	19	102			234	321	24	178	64.2	26	0.9	110/80	38	7	15	13
42	39	M	28	88			320	178	54	120	34	28	0.8	100/70	17	11	4.2	13.4
43	29	M	23	98			290	200	23	19.2	40	26	0.7	110/80	14	21	3.5	12
44	50	F	20	98			290	342	43	123	68.4	25	0.8	120/80	29	21	5.3	13.2
45	39	F	32	100			150	239	26	189	47.8	28	0.9	120/80	14	7	3.2	12.6
46	43	F	31.3	177			300	290	27	167	58	24	0.3	110/80	16	9	2.4	15
47	49	F	17.3	98			267	200	26	100	40	23	0.4	130/80	21	7	2.1	6.9
48	50	F	20	201			178	98	55.2	56	19.6	34	0.5	180/90	34	10	4.2	14.2
49	45	F	28	97			290	276	45	193	55.2	36	0.6	110/90	12	9	3.1	12
50	45	M	21	176			150	123	19	200	24	31	0.7	150/90	12	9	3.21	12.5

51	50	F	20.3	110			167	98	26	189	19.6	28	0.5	110/70	17	21	3.4	12
52	50	F	20.4	111			165	98	45	85.8	19.6	27	0.6	100/70	16	11	4.1	12
53	50	F	22	96			165	89	40	189	17.8	55	0.7	100/70	23	7	5	12
54	50	F	22	106			156	78	36	178	15.6	45	2.1	130/90	14	11	3.2	13.7
55	55	F	29	90			173	110	21	67.9	22	34	0.5	110/70	13	7	3.5	12.9
56	38	M	22.6	98			156	52	52	28	10.4	29	3.4	100/60	14	8	4.3	11.6
57	32	M	27.4	85			200	145	23	134	29	18	0.5	120/90	21	21	1.7	13.3
58	34	F	19	110			183	132	54	134	26.4	24	0.5	110/60	16	9	4.5	12.4
59	40	F	28		111	105	231	121	15	70	24.2	25	0.6	120/80	21	8	3.23	12
60	30	M	23	90			230	90	45	78	18	28	0.7	140/80	16	8	5	12
61	41	M	26.2	88			167	123	42	43	24	28	0.6	150/90	14	9	4.2	7.9
62	26	F	28	76			290	220	26	190	44	45	2.8	160/100	13	9	3.3	10
63	55	F	29	100			160	102	23	49.8	20.4	32	1.9	110/70	15	8	3.2	12.9
64	48	M	29	180			156	240	19	234	48	25	0.5	120/90	18	9	2.3	13
65	53	F	26.7	110			290	267	30	102.4	53.4	29	0.7	120/80	11	8	4.3	12
66	50	M	24	102			178	89	34	190	17.8	28	0.8	130/90	21	9	3.5	12.8
67	30	M	26.9	89			230	200	76	167	40	21	0.8	170/100	15	7	5	7
68	42	F	27	81			287	249	12	68.6	49.8	30	0.9	120/90	12	12	5	11.9
69	53	M	21	98			265	267	45	231	53.6	27	0.7	120/90	23	6	3.2	12.2
70	43	F	30		155	247	183	300	24	190	60	29	0.6	150/100	15	8	3.2	12
71	49	M	26.9	200			256	134	45	190	31.8	30	0.5	150/100	23	7	2.62	11
72	35	F	21		70	96	150	100	45	150	20	23	0.6	120/80	14	6	3.2	9.1
73	47	M	28.3	112			278	200	23	56	40	30	0.7	150/100	12	9	1.89	5
74	38	F	29		117	134	211	134	39	178	26	25	0.8	120/90	21	6	2.65	5.9
75	52	M	23	109			264	189	23	83.2	19.2	28	0.9	120/90	23	9	3	14.2
76	50	F	27	100			208	240	87	190	48	36	0.8	160/100	21	7	3.1	6.9

77	34	F	21	98			287	152	89	200	30.4	32	1.9	120/70	43	8	13.6	13.4
78	30	F	25.2	110			230	280	44	198	56	39	0.6	120/90	14	6	3.1	6.4
79	50	F	25	300			278	231	89	162	46.2	25	0.7	110/70	39	8	2.1	11.9
80	28	M	20.9	82			150	92	36	84.6	18.4	32	0.9	120/90	14	11	2.4	5.8
81	53	M	21		83	107	167	89	89	89	23	36	1.6	150/100	21	12	2.1	15.3
82	48	M	29.4		116	153	300	123	56	178	43.2	26	0.7	120/90	23	8	2.9	6.9
83	32	M	28	89			210	143	43	81	54	29	1.8	150/90	38	9	4.2	15.8
84	35	F	19		110	123	179	290	50	108.2	58	26	0.6	110/72	12	7	3.4	7
85	48	M	26.3	170			245	280	67	108.2	56	26	0.6	160/90	34	8	3.4	12
86	40	M	23.1	74			183	75	100	117	15	28	0.8	110/90	21	7	4.1	12
87	28	M	21.3	100			156	73	43	92.4	14.6	35	0.7	110/60	23	9	1.1	6.6
88	32	F	29	81			300	234	21	179	46	28	0.9	100/60	37	9	3.1	7.9
89	36	M	27.3	109			321	167	23	180	33	49	2.1	150/90	12	6	2.3	5.5
90	45	M	22.6	110			260	89	44	129.2	17.8	31	0.8	150/100	21	7	3.1	6.7
91	42	M	21.6	109			278	180	21	38.5	36	27	0.7	120/90	21	8	1.1	16.4
92	32	M	28.1	99			150	132	29	30.4	38.6	30	0.6	100/70	21	0.6	3.1	5.7
93	55	F	29	109			156	89	54	14.2	17.8	40	0.9	100/80	12	19	2.1	13
94	55	F	29.8		150	310	150	78	21	156	15.6	26	0.5	150/100	24	8	2.1	13.6
95	36	M	27.2	106			210	180	29	190	36	27	0.5	120/80	16	6	8.4	12
96	55	F	32.9	110			150	71	43	167	14.2	80	2.4	120/70	17	9	3.1	13.8
97	38	F	22	108			211	290	46	190	58	45	2.1	120/90	21	9	3.1	12
98	55	F	33.5	100			150	56	29	123	11.2	20	0.9	110/60	18	10	4.34	14
99	53	F	27	204			268	289	12	82	57.8	40	0.7	110/70	19	11	3.23	12
100	52	F	25.2		101	134	262	234	89	231	46.8	30	0.7	170/100	36	9	2.36	11.7

MASTER CHART

S.no	Age	Sex	BMI	RBS	FBS	PP	T.CHO	TGL	HDL	LDL	VLDL	Urea	creatinine	BP	T3	T4	TSH	HB
1	49	M	26	270			233	260	26	189	57	77	2	110/80	11	5	5	13
2	49	M	30	110			289	320	52	198	62	31	0.9	90/72	16	6	4	12.4
3	51	M	19.8	105			153	254	89	142	30.6	25	0.8	100/70	39	9	13	12
4	55	M	20	100			165	67	35	54	7.2	24	0.9	100/70	14	8	4.4	11.9
5	53	F	25.2		73	130	265	300	76	231	60	26	0.6	120/80	23	6	3	13
6	29	M	21.7	99			157	78	34	187.85	15	27	0.5	110/70	23	8	3.4	13
7	32	F	19.4		97	138	220	211	87	189	42.2	31	0.9	120/82	12	7	6	14.2
8	25	M	21	83			220	234	29	160	46.8	21	0.8	160/90	37	17	7.56	12.9
9	40	M	29	140			240	190	41.1	134	38	33	0.7	110/80	17	9	4.2	13
10	55	M	16.5	105			150	91	41	47.8	18.2	34	0.8	90/72	19	11	2.9	14.1
11	50	M	15.4	80			156	101	57.4	78	20.2	26	0.9	110/80	12	8	3.1	12
12	38	F	21	163			290	300	23	167	60	32	0.6	150/100	15	8	3.4	12
13	22	F	14.6	154			230	200	23	189	40	34	0.5	110/70	14	6	4.2	12
14	42	M	20	102			178	143	47.7	189	28	25	0.9	110/70	18	8	3	10.5
15	45	M	19	110			220	90	12	176	18	24	0.7	110/80	12	9	4.3	14.2
16	55	F	27.3	110			231	123	12	378	24.6	26	0.8	150/100	35	7	3.8	12.6
17	40	M	23		110	138	150	189	52	178	37	56	1.9	110/80	59	2.9	4.2	12
18	50	F	17.4	110			245	259	79	167	51.8	24	0.6	150/100	13	9	4.1	14.2
19	41	M	29	101			230	200	21	136	40	21	0.7	110/70	56	2.1	4.3	13.2
20	39	F	20	90			176	350	50	57	70	27	0.8	90/70	14	8	5	6.4
21	40	M	26		98	123	234	189	28	46.8	37	35	0.9	110/70	20	8	4	14
22	52	M	21.4	110			150	92	53.9	168	11.4	56	0.5	110/80	21	7	4.2	12
23	53	F	25.4		98	139	276	251	21	245	50.2	27	6	110/80	13	8	9	12
24	49	F	21	90			209	300	29	123	60	29	0.7	110/70	11	6	4.6	15

25	35	F	16	85			175	99	39.5	115.7	19.8	28	0.9	110/80	21	10	3.1	12
26	32	M	27	109			289	254	26	180	50.8	28	0.7	120/80	23	7	2.4	12.9
27	55	M	18	74			167	280	53	45	56	23	0.8	90/70	19	8	3.6	13.2
28	50	M	21		102	128	278	220	67	113.6	44	12	0.9	100/70	22	6	4.4	13.7
29	28	M	16.2	110			159	234	21	72.2	46.8	32	0.7	130/90	39	18	12.6	13
30	49	M	26	110			189	141	88	107	28.2	56	3.1	160/100	12	9	2.3	12
31	51	M	21	167			158	137	23	82.4	27.4	26	0.8	120/80	18	7	2.6	14.2
32	42	M	21	72			234	190	29	66.5	38	21	0.5	110/80	16	8	4.2	13.9
33	45	F	25	180			160	278	30	89	55.6	27	0.7	100/70	29	6	3.4	12
34	55	F	28.8	110			321	120	23	77.9	24	23	0.9	90/70	16	7	3.1	12.6
35	39	F	27	109			341	89	25	92.7	17.4	34	0.9	110/70	17	8	2.4	12.3
36	33	F	31		88	121	241	300	67	189	60	23	0.8	120/80	17	6	4.2	8.9
37	50	M	22	77			200	99	54	180	49	22	0.7	120/70	14	7	3.2	13.5
38	39	F	31.2	86			289	210	30	177	42	45	1.7	110/70	12	9	2.1	14.5
39	48	M	26.4	77			300	220	45	231	44	25	0.7	120/80	17	8	3.2	12
40	51	M	21	96			187	125	82	78	147	24	0.8	100/70	34	6	2.5	13.2
41	38	F	19	102			234	321	24	178	64.2	26	0.9	110/80	38	7	15	13
42	39	M	28	88			320	178	54	120	34	28	0.8	100/70	17	11	4.2	13.4
43	29	M	23	98			290	200	23	19.2	40	26	0.7	110/80	14	21	3.5	12
44	50	F	20	98			290	342	43	123	68.4	25	0.8	120/80	29	21	5.3	13.2
45	39	F	32	100			150	239	26	189	47.8	28	0.9	120/80	14	7	3.2	12.6
46	43	F	31.3	177			300	290	27	167	58	24	0.3	110/80	16	9	2.4	15
47	49	F	17.3	98			267	200	26	100	40	23	0.4	130/80	21	7	2.1	6.9
48	50	F	20	201			178	98	55.2	56	19.6	34	0.5	180/90	34	10	4.2	14.2
49	45	F	28	97			290	276	45	193	55.2	36	0.6	110/90	12	9	3.1	12
50	45	M	21	176			150	123	19	200	24	31	0.7	150/90	12	9	3.21	12.5

51	50	F	20.3	110			167	98	26	189	19.6	28	0.5	110/70	17	21	3.4	12
52	50	F	20.4	111			165	98	45	85.8	19.6	27	0.6	100/70	16	11	4.1	12
53	50	F	22	96			165	89	40	189	17.8	55	0.7	100/70	23	7	5	12
54	50	F	22	106			156	78	36	178	15.6	45	2.1	130/90	14	11	3.2	13.7
55	55	F	29	90			173	110	21	67.9	22	34	0.5	110/70	13	7	3.5	12.9
56	38	M	22.6	98			156	52	52	28	10.4	29	3.4	100/60	14	8	4.3	11.6
57	32	M	27.4	85			200	145	23	134	29	18	0.5	120/90	21	21	1.7	13.3
58	34	F	19	110			183	132	54	134	26.4	24	0.5	110/60	16	9	4.5	12.4
59	40	F	28		111	105	231	121	15	70	24.2	25	0.6	120/80	21	8	3.23	12
60	30	M	23	90			230	90	45	78	18	28	0.7	140/80	16	8	5	12
61	41	M	26.2	88			167	123	42	43	24	28	0.6	150/90	14	9	4.2	7.9
62	26	F	28	76			290	220	26	190	44	45	2.8	160/100	13	9	3.3	10
63	55	F	29	100			160	102	23	49.8	20.4	32	1.9	110/70	15	8	3.2	12.9
64	48	M	29	180			156	240	19	234	48	25	0.5	120/90	18	9	2.3	13
65	53	F	26.7	110			290	267	30	102.4	53.4	29	0.7	120/80	11	8	4.3	12
66	50	M	24	102			178	89	34	190	17.8	28	0.8	130/90	21	9	3.5	12.8
67	30	M	26.9	89			230	200	76	167	40	21	0.8	170/100	15	7	5	7
68	42	F	27	81			287	249	12	68.6	49.8	30	0.9	120/90	12	12	5	11.9
69	53	M	21	98			265	267	45	231	53.6	27	0.7	120/90	23	6	3.2	12.2
70	43	F	30		155	247	183	300	24	190	60	29	0.6	150/100	15	8	3.2	12
71	49	M	26.9	200			256	134	45	190	31.8	30	0.5	150/100	23	7	2.62	11
72	35	F	21		70	96	150	100	45	150	20	23	0.6	120/80	14	6	3.2	9.1
73	47	M	28.3	112			278	200	23	56	40	30	0.7	150/100	12	9	1.89	5
74	38	F	29		117	134	211	134	39	178	26	25	0.8	120/90	21	6	2.65	5.9
75	52	M	23	109			264	189	23	83.2	19.2	28	0.9	120/90	23	9	3	14.2
76	50	F	27	100			208	240	87	190	48	36	0.8	160/100	21	7	3.1	6.9

77	34	F	21	98			287	152	89	200	30.4	32	1.9	120/70	43	8	13.6	13.4
78	30	F	25.2	110			230	280	44	198	56	39	0.6	120/90	14	6	3.1	6.4
79	50	F	25	300			278	231	89	162	46.2	25	0.7	110/70	39	8	2.1	11.9
80	28	M	20.9	82			150	92	36	84.6	18.4	32	0.9	120/90	14	11	2.4	5.8
81	53	M	21		83	107	167	89	89	89	23	36	1.6	150/100	21	12	2.1	15.3
82	48	M	29.4		116	153	300	123	56	178	43.2	26	0.7	120/90	23	8	2.9	6.9
83	32	M	28	89			210	143	43	81	54	29	1.8	150/90	38	9	4.2	15.8
84	35	F	19		110	123	179	290	50	108.2	58	26	0.6	110/72	12	7	3.4	7
85	48	M	26.3	170			245	280	67	108.2	56	26	0.6	160/90	34	8	3.4	12
86	40	M	23.1	74			183	75	100	117	15	28	0.8	110/90	21	7	4.1	12
87	28	M	21.3	100			156	73	43	92.4	14.6	35	0.7	110/60	23	9	1.1	6.6
88	32	F	29	81			300	234	21	179	46	28	0.9	100/60	37	9	3.1	7.9
89	36	M	27.3	109			321	167	23	180	33	49	2.1	150/90	12	6	2.3	5.5
90	45	M	22.6	110			260	89	44	129.2	17.8	31	0.8	150/100	21	7	3.1	6.7
91	42	M	21.6	109			278	180	21	38.5	36	27	0.7	120/90	21	8	1.1	16.4
92	32	M	28.1	99			150	132	29	30.4	38.6	30	0.6	100/70	21	0.6	3.1	5.7
93	55	F	29	109			156	89	54	14.2	17.8	40	0.9	100/80	12	19	2.1	13
94	55	F	29.8		150	310	150	78	21	156	15.6	26	0.5	150/100	24	8	2.1	13.6
95	36	M	27.2	106			210	180	29	190	36	27	0.5	120/80	16	6	8.4	12
96	55	F	32.9	110			150	71	43	167	14.2	80	2.4	120/70	17	9	3.1	13.8
97	38	F	22	108			211	290	46	190	58	45	2.1	120/90	21	9	3.1	12
98	55	F	33.5	100			150	56	29	123	11.2	20	0.9	110/60	18	10	4.34	14
99	53	F	27	204			268	289	12	82	57.8	40	0.7	110/70	19	11	3.23	12
100	52	F	25.2		101	134	262	234	89	231	46.8	30	0.7	170/100	36	9	2.36	11.7