A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME

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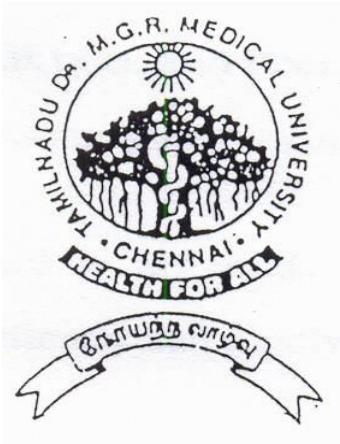

DEPARTMENT OF BIOCHEMISTRY

THANJAVUR MEDICAL COLLEGE,

THANJAVUR

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY,

CHENNAI

APRIL - 2016

CERTIFICATE

This is to certify that dissertation titled **"A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME"** is a bonafide work done by **Dr.S.ARUNA** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2013 to 2016.

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Remark of the Guide:

The work done by DR.S.ARUNA on **"A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME"** is under my supervision and I assure that this candidate will abide by the rules of the Ethical Committee.

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I, **Dr.S.ARUNA** hereby solemnly declare that the dissertation title **"A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department, **Dr.N.SASIVATHANAM,M.D(Bio).,DGO,** This dissertation is submitted to the Tamil Nadu Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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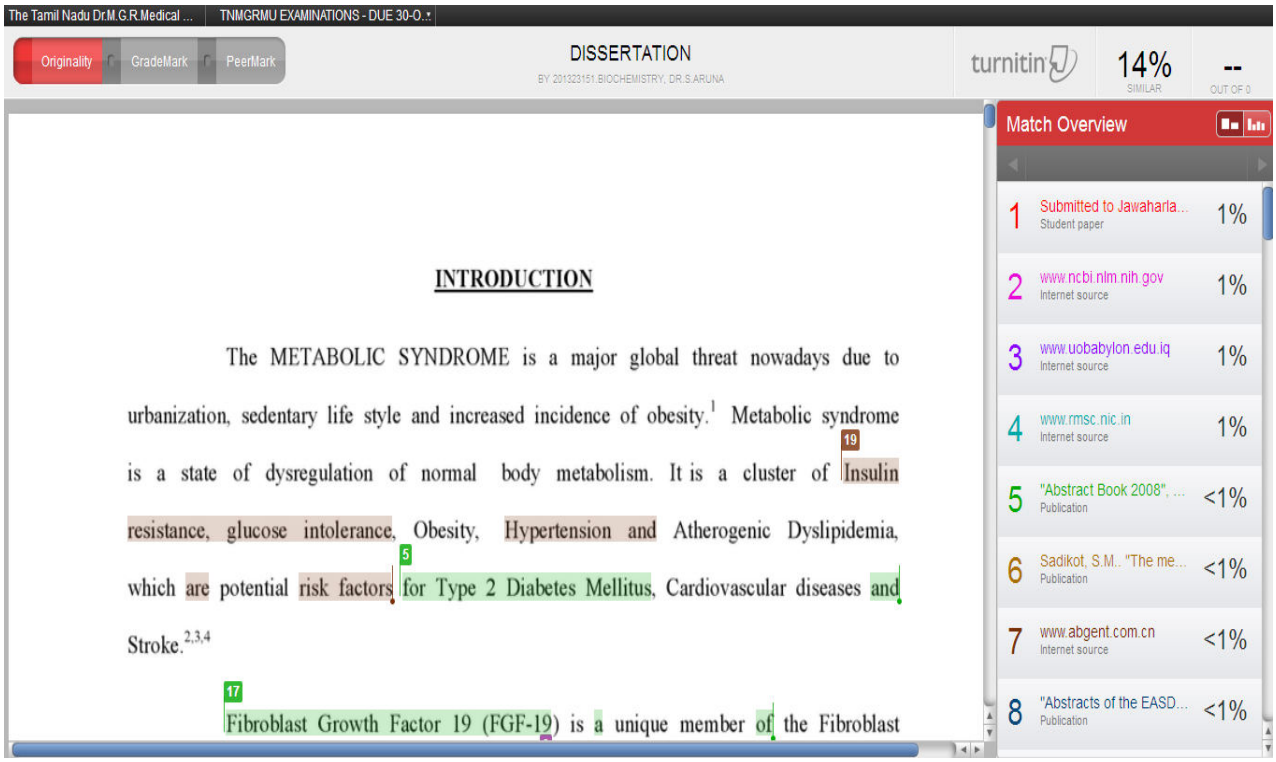

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CONTENTS

ABBREVIATIONS

MetS - Metabolic Syndrome

T2DM – Type 2 Diabetes Mellitus

FBS - Fasting Blood Sugar

PPBS – Post prandial Blood Sugar

TC – Total Cholesterol

TGL – Triglycerides

HDL-C - High density lipoprotein Cholesterol

LDL-C - Low density lipoprotein Cholesterol

VLDL - Very low density lipoprotein

TNF-α - Tumor necrosis factor α

INF α - Interferon α

IL- 6 - Interleukin 6

PAI - Plasminogen activator inhibitor

CETP - Cholesterol ester transfer protein

LPL - Lipoprotein lipase

PKA - Protein kinase A

HSL - Hormone sensitive lipase

CVD - Cardiovascular disease

VEGF - Vascular endothelial growth factors

IGF-1 – Insulin like Growth Factor-1

PPARγ - Peroxisome Proliferator-Activated Receptor γ

IRS-Insulin Receptor Substrate

PI3 K – 1-Phosphatidyl Inositol 3 Kinase

MAPK - Mitogen Activator Protein Kinase

GLUT - Glucose transporters

FFAs - Free Fatty acids

FGF – Fibroblast Growth Factor

FGFR – Fibroblast Growth Factor Receptor

FXR – Farnesoid X Receptor

CNS – Central Nervous system

WHO - World Health Organization

EGIR - European Group for the study of Insulin Resistance

NCEP ATP - The National Cholesterol Education Program Adult Treatment Panel

AACE - American Association of Clinical Endocrinologists

IDF - International Diabetes Federation

NHANES – National Health And Nutrition Examination Survey

WC – Waist Circumference

BMI – Body Mass Index

IFG – Impaired Fasting Glucose

PTPs - Protein Tyrosine Phosphatases

LAR - Leukocyte Antigen-Related Phosphatase

SHP2 - Src-Homology-Phosphatase 2

DNA – Deoxyribo Nucleic Acid

AGPAT2 - 1-acyl glycerol-3-phosphate-O-acyl transferase

Km - Michaelis constant

HBP - Hexosamine Biosynthetic Pathway

UDP-N-GlcNac - Uridine diphosphate-N-acetyl glucosamine

GFAT – Glutamine: Fructose-6-phosphate Amidotransferase

CoA – Coenzyme A

NAD – Nicotinamide Adenine Dinucleotide

CPT-1- Carnitine Palmitoyl Transferase-1

ROS - Reactive Oxygen Species

AGEs - Advanced Glycation End products

ERK - Extracellular signal Regulated protein Kinase

GSK – Glycogen Synthase Kinase

FHF - Fibroblast Homologous Factors

CYP7A1- Cytochrome P 7A1

ACC – Acetyl CoA Carboxylase

SCD – Stearoyl CoA Desaturase

SHP-1 - Small Heterodimer Partner-1

eIF - Eukaryotic Initiation Factor

mRNA – messenger RNA

GABA – Gamma Amino Butyric Acid

HRP – Horse Radish Peroxidase

TMB - 3,3’,5,5’ – Tetra Methyl Benzidine

ELISA – Enzyme Linked Immuno Sorbent Assay

GOD - Glucose oxidase

POD - Peroxidase

CHE - Cholesterol Esterase

CHO - Cholesterol Oxidase

4AAP - 4-AminoAntipyrine

GPO - Glycerol Phosphate Oxidase

LPL - Lipoprotein Lipase

GK - Glycerol kinase

ATP - Adenosine Tri Phosphate

DHAP - Di-Hydroxy Acetone Phosphate

DHBS -3,5 Dichloro-2 Hydroxy Benzene Sulfonate

CM - Chylomicrons

AIP - Atherogenic Index of Plasma

hsCRP – high sensitive C reactive Protein

HbA1C – Glycated Hemoglobin

CAD - Coronary Artery Disease

SBP- Systolic Blood Pressure

DBP – Diastolic Blood Pressure

HT- Height

WT - Weight

SD – Standard Deviation

A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME

ABSTRACT:

INTRODUCTION:

Metabolic syndrome is a state of dysregulation of normal body metabolism. It is a cluster of Insulin resistance, glucose intolerance, Obesity, Hypertension and Atherogenic Dyslipidemia, which are potential risk factors for Type 2 Diabetes Mellitus, Cardiovascular diseases and Stroke. FGF 19 (Fibroblast Growth Factor 19) is a newly identified metabolic regulator, influencing homeostasis of glucose and lipid metabolism.

AIMS AND OBJECTIVES:

1. To measure serum FGF19 in patients with Metabolic syndrome and to compare the serum level of FGF 19 with healthy individuals.
2. To analyze the correlation between serum FGF 19 and the components of Metabolic syndrome.

MATERIALS AND METHODS:

The study was conducted at Thanjavur Medical College, Thanjavur. The study included 50 patients with Metabolic syndrome (25 males, 25 females) and 50 age and sex matched healthy controls (25 males, 25 females). Anthropometric measurements and blood pressure were recorded and fasting blood samples were collected. Serum levels of FGF 19 were estimated using sandwich ELISA. Fasting and

post prandial blood sugars and a complete lipid profile were done for all the samples. AIP (Atherogenic Index of Plasma) was calculated for all the subjects.

RESULTS:

Student's t-test analysis shows a significant decrease in the mean serum FGF 19 in cases (135.02 ± 20.76 pg/ml), when compared to the mean serum FGF 19 in controls (266.34 ± 65.5 pg/ml), which is statistically significant. Pearson correlation between FGF 19 and other parameters shows a negative correlation between serum FGF 19 and BMI, Waist circumference, systolic and diastolic BP, AIP, fasting and post prandial blood sugar, serum TC, TGL, VLDL and LDL concentrations and a positive correlation with serum HDL concentration, which are also statistically significant.

CONCLUSION:

This study shows that serum levels of FGF 19 are low in patients with Metabolic syndrome. The negative relationship obtained between FGF 19 and several other known cardiovascular risk factors like TGL and log (TGL / HDL-C) suggests that FGF 19 can be used as a novel marker in assessing cardiovascular risk in patients with Metabolic syndrome. Hence, earlier intervention can be taken to reduce the cardiovascular complications.

KEY WORDS:

Fibroblast Growth Factor 19, Metabolic syndrome, Insulin resistance, glucose intolerance, Obesity, Atherogenic Dyslipidemia, Type 2 Diabetes Mellitus, glucose and lipid metabolism.

INTRODUCTION

The Metabolic syndrome is a major global threat nowadays due to urbanization, sedentary life style and increased incidence of obesity.[1] Metabolic syndrome is a state of dysregulation of normal body metabolism. It is a cluster of Insulin resistance, glucose intolerance, Obesity, Hypertension and Atherogenic Dyslipidemia, which are potential risk factors for Type 2 Diabetes Mellitus, Cardiovascular diseases and Stroke.[2,3,4]

Fibroblast Growth Factor 19 (FGF-19) is a unique member of the Fibroblast Growth Factor family of secreted proteins.[5] It is a hormone like protein that regulates Carbohydrate, Lipid and Bile acid metabolism. FGF -19 is synthesized from the small intestine and secreted in to the circulation when bile acids are taken up into the ileum after a meal and then acts on CNS to elicit its metabolic effects. [6,7,8] This hormone-like postprandial protein has recently been shown to stimulate glycogen synthesis and inhibit gluconeogenesis through Insulin independent pathways.[7,6] It stimulates Hepatic protein synthesis and Glycogen synthesis.[8]

FGF-19 has recently been introduced as a novel regulator of metabolism, reversing diabetes mellitus, hyper lipidemia, hepatic steatosis and adiposity[9].

In this study, the aim is to estimate Serum levels of FGF-19 in patients with Metabolic Syndrome and to evaluate the relationship between FGF-19 and other cardiovascular risk factors, there by predicting future cardiovascular complications.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

METABOLIC SYNDROME

HISTORIC TRENDS:

The concept of Metabolic syndrome (MetS) started in 1920, when a Swedish physician, Kylin associated Hypertension, Hyperglycemia and Gout[1,10]. Then, Vague in 1947, demonstrated the association between Visceral Obesity and the metabolic abnormalities in Cardiovascular diseases and Type 2 Diabetes Mellitus[1]. Later, in 1965, an abstract, which again described an entity comprising of Obesity, Hypertension and Hyperglycemia, has been presented by Avagadro and Crepaldi, at the European Association for the study of Diabetes Annual Meeting.[1]

Then came the very famous Banting lecture in 1988, by Reaven, a significant move in the field, which described a cluster of risk factors for Cardiovascular disease and Diabetes, and he coined the word "SYNDROME X" for this entity [1, 11, 12]. He introduced the concept of Insulin Resistance, which was a major contribution. But, Visceral Obesity was surprisingly being missed in his definition and was later included as a major abnormality. Kaplan, in 1989, renamed the entity as "THE DEADLY QUARTET", consisting of glucose intolerance, hypertension, upper body obesity

and Hypertriglyceridemia. Again it was renamed as "THE INSULIN RESISTANCE SYNDROME" in 1992.[1]

Many groups then came forward to develop the diagnostic criteria for metabolic syndrome. The World Health Organization (WHO) diabetes group made the first attempt to give a definition of the syndrome in 1998, which was modified by the European Group for the study of Insulin Resistance (EGIR), in 1999[1,13]. The next definition was proposed by The National Cholesterol Education Program Adult Treatment Panel (NCEP ATP), in 2001. Subsequently, in 2003, the American Association of Clinical Endocrinologists (AACE) contributed to the Syndrome's definition. Thus, a desire for a single unifying definition arised. To accomplish this need, a new definition was proposed by the International Diabetes Federation (IDF), in April 2005.[1]

EPIDEMIOLOGY:

Due to epidemic of obesity, as a direct consequence, the prevalence of Metabolic syndrome is rising at an alarming rate. Currently, almost 25% of the US population is affected by MetS, and the problem is worse in individuals older than 60 years.[3] In diabetic patients, the prevalence was almost around 86%.[14]

In Europe, the prevalence, in individuals of age 40-55 years was between 7% and 36% for men and between 5% and 22% for women. The frequency of the syndrome varies largely between populations, because of the

differing frequencies of the components and different methodologies of measurement used.[14]

Decreased physical activities and increased intake of energy dense foods and sugars are the most important contributing factor in the drastic increase in the prevalence of Obesity and hence the Metabolic Syndrome[15]. A huge hike in the prevalence of obesity (BMI > 30.0) is also documented by the NHANES surveys:

NHANES I: 14.1%

NHANES II: 14.5%

NHANES III: 22.5%.

Metabolic syndrome is a growing health problem, even in countries with lower gross national product, due to ingestion of cheap vegetable oils and increased urbanization rate[15].

CURRENT SCENARIO IN INDIA:

In Asian Indians, the prevalence of the metabolic syndrome differs according to the area, the extent of urbanization, patterns of life style and cultural or socio-economic factors. According to a recent data, in large cities in India, the prevalence accounts for about one-third of the urban population[16].

The prevalence of the individual risk factors in Indians is:

1. Abdominal obesity - 31.4%

2. Hypertriglyceridemia - 45.6%

3. Low HDL - 65.5%

4. Hypertension - 5.4%

5. Raised fasting plasma glucose - 26.7% [16]

The prevalence of metabolic syndrome in rural areas are comparatively low. According to a recent survey, the overall prevalence was around 5.0% in rural adults[16].

The prevalence of metabolic syndrome in paediatric and adolescent population is not uncommon. Because, obesity is prevailing in all age groups, it is not surprising that children are not immune to it. The prevalence of obesity in urban children in Delhi was found to be 16% in 2002, which increased to 29% in 2007. The overall prevalence of metabolic syndrome in pediatric population of developed countries varies from 3.1% to 12.7%. In India, in adolescent age group, it is about 4.2%. No gender difference in distribution was found[16]. The increasing prevalence of obesity in pediatric age group is the main initiating factor of MetS in children[17].

As pediatric metabolic syndrome is a precursor to adult metabolic syndrome, there is no surprise that the prevalence of metabolic syndrome will rise up in the upcoming years[16].

Almost one third of the population in urban south Asia has MetS. Prevalence of Metabolic syndrome, according to various criteria is:

- ATP III - 40.3%
- WHO - 30.6%
- IDF - 34.9%[18]

There is variation in the occurrence of MetS among different ethnic groups. Reassessment should be done in the criteria defining MetS especially in Asian Indians. Cut-offs of WC and BMI in the definition can be modified to yield higher rates of diagnosis of MetS[19]. .

Even though the risk of developing Metabolic syndrome has been attributed extensively to life style factors, like smoking, physical inactivity, and unhealthy eating habits, certain studies have suggested that the predisposition begins in utero[20].

In 1992, Hales and Barker proposed "the Thrifty Phenotype", which said that the susceptibility of an offspring to acquire adult chronic diseases occurs as a consequence of exposures or insults in the prenatal and postnatal periods[20].

In offsprings, epigenetic changes may occur in glucose-insulin metabolism causing Insulin resistance and defects in secretion of insulin, as a consequence of maternal obesity and Insulin resistance[20].

The growing evidence for the association between intrauterine environment and the Metabolic syndrome, suggests that the mechanisms causing epigenetic changes in the offspring in response to prenatal exposures have to be

investigated. With a better understanding of the mechanisms, prediction and prevention of complications of pregnancy can be improved, thereby improving the betterment of the mother and the offsprings[20].

CLINICAL IMPORTANCE OF METABOLIC SYNDROME:

1. It predisposes to multiple associated disorders.
2. It serves as a target for treatment and prevention of these disorders.
3. It is extremely common nowadays[21].

DEFINITION AND DIAGNOSIS:

MetS is considered as disturbance in body metabolism. It involves insulin resistance and inflammation. It predisposes to Hypertension, diabetes mellitus and atherosclerosis. These diseases in turn increase the risk of CVD[22].

The Metabolic syndrome is also designated as:

- The Insulin Resistance Syndrome,
- The Syndrome X,
- The Dysmetabolic Syndrome,
- HONDA [Hypertension, Obesity, Non-Insulin-Dependent Diabetes Mellitus, Dyslipidemia, And Atherosclerotic Cardiovascular Disease]
- The Deadly Quartet[23, 24].

The syndrome is a constellation of clinical findings and laboratory values, comprising of Insulin Resistance, Hyper Insulinemia, high blood glucose,

Obesity with Dyslipidemia [high Triglycerides and low High-Density Lipoprotein Cholesterol] And Hypertension[23, 21, 25, 26, 27].

Obesity predisposes to some extent of Insulin Resistance almost always. Failure of tissues to respond to Insulin may be due to two reasons:

i. Reduction in affinity or number of Insulin receptors

ii. Normal binding of Insulin, but abnormal post receptor responses.

It is a general rule, that with increase in amount of body fat, there is increase in the resistance to Insulin, in the tissues which are normally Insulin sensitive[28].

The syndrome usually occurs in individuals with frank obesity, but can occur in non-obese individuals, with increased abdominal fat[29].

The factors defining Metabolic syndrome are interconnected and include physiological, biochemical, metabolic and clinical factors[1]. The risk factors include atherogenic dyslipidemia, hypertension, glucose intolerance, proinflammatory and a prothrombotic state[1,11]. They predispose to the risk of Cardiovascular disease, Type 2 Diabetes Mellitus and all cause mortality[1].

Prothrombotic state is due to:

i. Increase in pro-coagulant factors – fibrinogen, factor VII.

ii. Increase in antifibrinolytic factors – Plasminogen Activator Inhibitor –1.

iii. Platelet aberrations.

iv. Endothelial dysfunction[11, 12].

Proinflammatory state is due to increase in circulating levels of cytokines and acute phase reactants like C-reactive protein[11].

The state of chronic, systemic and low-grade inflammation, associated with MetS, is involved in the pathogenesis of consequent Insulin resistance and atherosclerotic lesions[30].

The concurrent presence of MetS and insulin resistance is a high risk for developing CVD[25].

Insulin sensitivity varies widely in normal individuals. The metabolic traits associated with insulin resistance include:

1. Hyper insulinemia
2. Some degree of glucose intolerance
3. Abdominal obesity
4. Dyslipidemia [high triglycerides, low high-density lipoprotein cholesterol]
5. Elevated blood pressure
6. Elevated c-reactive protein
7. High Plasminogen Activator Inhibitor – 1 levels
8. A positive family history of type 2 diabetes mellitus[31, 29].

In patients with Metabolic syndrome, the risk of mortality from Cardiovascular problem is about two times compared to normal individuals. Also, there is a fivefold increased risk of developing frank Type 2 Diabetes Mellitus[32, 33].

An Italian study based on large population, proposed that the risk of mortality from all causes including cardiovascular accidents, increased with the number of metabolic abnormalities:

- Increased blood sugar
- Increased blood pressure
- High Triglycerides
- Low HDL cholesterol, in both gender[34].

The Framingham offspring study reported that individuals with at least three risk factors associated with Metabolic syndrome, had 2.4 fold increased risk for Cardiovascular disorders in men and 5.9 fold in women[34].

Another study, Lakka et al also emphasized the importance of CVD in patients with MetS. The study was done in middle-aged men and they were followed for 11.4 years. The result is that, even in the absence of Diabetes or prior CVD, there is significant increased risk of CVD in the presence of MetS[34].

Another population based study revealed that the risk of CVD increases with the number of components of MetS.

These studies highlight two facts:

1. The importance of creating awareness among clinicians regarding the strong association between MetS and CVD.
2. The urgent need to intervene and modify the fatal cascade of events in patients with MetS, which has got significant impact on the society[34].

Even normal-weight subjects can develop Metabolic syndrome. Also the syndrome is not seen in all obese individuals[32,17].

DIAGNOSTIC CRITERIA PROPOSED FOR THE CLINICAL DIAGNOSIS OF THE METABOLIC SYNDROME:

One of the main reasons of defining criteria for MetS is to identify individuals at risk of developing CVD[13].

DIAGNOSTIC CRITERIA FOR METABOLIC SYNDROME:

MEASURES	WHO(1998)[1,26]
INSULIN RESISTANCE	IGT, IFG, T2DM, or Lowered insulin sensitivity plus any 2 of the following
GLUCOSE	IGT, IFG, OR T2DM
BODY WEIGHT	Men: waist-to-hip ratio > 0.90 Women: waist-to-hip ratio > 0.85 and/or BMI > 30 kg/m^2
BLOOD PRESSURE	≥ 140/90 mm Hg
LIPIDS	TGs ≥ 150 mg/dL and/or HDL-C < 35 mg/dL in men or < 39 mg/dL in women
OTHERS	Microalbuminuria: Urinary excretion rate of > 20 mg/min or Albumin : Creatinine ratio of > 30 mg/g.

DIAGNOSTIC CRITERIA FOR METABOLIC SYNDROME:

MEASURES	EGIR(1999)[1]
INSULIN RESISTANCE	Plasma insulin > 75th percentile plus any 2 of the following
GLUCOSE	IGT or IFG (but not diabetes)
BODY WEIGHT	Waist circumference ≥ 94 cm in men or ≥ 80 cm in women
BLOOD PRESSURE	≥ 140/90 mm Hg or on Hypertension treatment
LIPIDS	TGs ≥ 150 mg/dL and/or HDL-C < 39 mg/dL in men or women

DIAGNOSTIC CRITERIA FOR METABOLIC SYNDROME

MEASURES	ATPIII (2001)[1,31,23,35,36]
	Any 3 of the following 5 features
INSULIN RESISTANCE	None
GLUCOSE	>110 mg/dL (includes Diabetes)
BODY WEIGHT	Waist circumference ≥ 102 cm in men or ≥ 88 cm in women
BLOOD PRESSURE	≥ 130/85 mm Hg
LIPIDS	TGs ≥ 150 mg/dL HDL-C < 40 mg/dL in men or < 50 mg/dL in women

DIAGNOSTIC CRITERIA FOR METABOLIC SYNDROME:

MEASURES	AACE(2003)[1]
INSULIN RESISTANCE	IGT or IFG, plus any of the following based on the clinical judgement
GLUCOSE	IGT or IFG (but not Diabetes)
BODY WEIGHT	BMI ≥ 25 kg/m^2
BLOOD PRESSURE	$\geq$ 130/85 mm Hg
LIPIDS	TGs $\geq$ 150 mg/dL and/or HDL-C < 35 mg/dL in men or < 39 mg/dL in women
OTHERS	Other features of Insulin Resistance (includes family history of Diabetes Mellitus, Polycystic Ovary Syndrome, Sedentary life style, advancing Age, and Ethnic groups susceptible to Type 2 Diabetes Mellitus)

DIAGNOSTIC CRITERIA FOR METABOLIC SYNDROME:

MEASURES	IDF(2005)[1,37,35,38,39]
INSULIN RESISTANCE	None
GLUCOSE	≥ 100 mg/dL (includes diabetes)
BODY WEIGHT	Increased waist circumference (population specific) plus any 2 of the following
BLOOD PRESSURE	≥ 130 mm Hg systolic or ≥ 85 mm Hg diastolic or on hypertension treatment
LIPIDS	TGs ≥ 150 mg/dL or on TGs treatment HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on HDL-C treatment
OTHERS	Other features of insulin resistance (includes family history of Diabetes Mellitus, Polycystic Ovary Syndrome , Sedentary Life Style, Advancing Age, and ethnic groups susceptible to Type 2 Diabetes Mellitus)

But, the inclusion of Abdominal adiposity in the criteria may miss the mortality due to CVD in non-obese individuals[40].

Even in patients with frank Diabetes mellitus, a subgroup of patients with the greatest risk of CVD was missed, using the IDF definition of Metabolic syndrome. The definition proposed by IDF is more restrictive as it requires the presence of abdominal obesity for diagnosing MetS. As a consequence, patients despite having Dyslipidemia and Hypertension, without abdominal obesity will not be diagnosed as having MetS[41].

ANTHROPOMETRIC MEASUREMENTS:

BODY MASS INDEX (BMI):

BMI is defined as the weight in kilograms divided by the height in meters, squared. If height cannot be measured, the Demispan or the Knee height can be used to calculate height with the following formulae:

1. Height = 0.73 x (2 x demispan) + 0.43

[Demispan: It is measured from the Sternal notch to the middle finger]

2. Height (cm) = [knee height (cm) x 1.91] – [age in years x 0.17] + 75

For females (60 to 80 years)

3. Height (cm) = [knee height (cm) x 2.05] + 59.01

For males (60 to 80 years)

BMI is increased by muscle mass and does not discriminate between lean body mass and fat mass. Normal BMI should be around 18.5 - 24.9 kg/m^2 .[42]

WAIST CIRCUMFERENCE (WC):

WC indicates the degree of abdominal obesity and is measured at the level of umbilicus. Hip circumference is measured at the level of the greater trochanters. Waist - hip ratios help to define the fat distribution whether it is Android or Gynoid[42.]

BODY FAT DISTRIBUTION:

The distribution appears to be more important compared with the absolute amount of excess adipose tissue, in terms of complications of obesity. Central obesity (increased intra-abdominal fat) is more closely associated with type 2 diabetes mellitus, metabolic syndrome and cardiovascular diseases, when compared to Generalised obesity.

Other names for Central obesity:

- Abdominal obesity,
- Visceral obesity,
- Apple-Shaped obesity or
- Android type of obesity;

Other names for Generalised obesity:

- Pear-Shaped obesity or
- Gynoid type of obesity.[42]

PATHOGENESIS:

A cluster of metabolic derangements are often seen associated with insulin resistance which are collectively referred to as Dysmetabolic syndrome or Syndrome X. The central abnormality predisposing to the Metabolic syndrome is the Insulin Resistance[24].

"Insulin Resistance" usually denotes resistance towards the functional effects of insulin in glucose uptake, metabolism, or storage[43]. It can be defined as the functional inability of insulin to exert its normal biological functions at concentrations, which are effective in normal individuals[32, 33].

It is characterized by reduced insulin mediated glucose transport and metabolism in Adipose tissue and Skeletal muscle and also by impaired suppression of glucose synthesis in the liver[43,33]. The resulting hyperglycemia, leads to the compensatory rise in secretion of Insulin[32].

The defects are also due to impairment of insulin signalling in the target tissues and also from down regulation of GLUT4, the major Insulin responsive glucose transporter, in Adipose tissue. The entire cascade of Insulin signalling is affected, which includes:

1. Binding of insulin to its receptor,

2. Receptor phosphorylation,

3. Activation of Tyrosine kinase activity,

4. Phosphorylation of insulin responsive substrate proteins[43].

In adipose tissue, a proposed mechanism for the signalling defect is excessive expression and activity of many PTPs (Protein Tyrosine Phosphatases), which by dephosphorylation terminates the signalling cascade, in obese individuals. The expression and functional activities of the following three PTPs is increased in Skeletal muscle and Adipose tissue of the obese people, leading to Insulin Resistance:

1. PTP1B,
2. LAR (Leukocyte Antigen-Related Phosphatase),
3. SHP2 (Src-Homology-Phosphatase 2).[43]

In adipose tissue, Insulin Resistance results in elevated levels of free fatty acids in circulation, because of impairment of suppression of lipolysis by Insulin. This abnormal Insulin mediated suppression of producing circulating free fatty acids is considered as an early manifestation in people with genetic predisposition for insulin resistance[15].

Inflammation occurs in Adipose tissue with infiltration of Macrophages and increase in Cytokines like TNF-alpha and IL-6, which antogonizes actions of Insulin. With weight loss, this inflammation is partly reversible. Weight loss decreases expression of genes responsible for recruitment of macrophages[32].

EFFECTS OF BODY FAT DISTRIBUTION IN OBESITY:

The Intra-abdominal fat drains into the Portal vein, and so directly reaches the liver. It is metabolically active and releases enormous amounts of Free Fatty Acids (FFAs), Adipokines (Tumour Necrosis Factor - Alpha, Adiponectin, and Resistin) and Steroid hormones. All these molecules reach the liver in high concentrations from the adipose tissue. The FFAs predispose to Insulin Resistance because they compete with glucose for oxidation in peripheral tissues as a fuel supply and hence to Type 2 Diabetes Mellitus[42]. Adipokines are structurally similar to Cytokines and hence their name. They exert their actions on specific receptors to influence Insulin sensitivity in the tissues. So, Central obesity have a profound influence on insulin sensitivity in the liver and affects Carbohydrate and Hepatic Lipid metabolism[39].

In normal individuals, the origin of majority of fatty acids reaching the liver is from lipolysis of the subcutaneous adipose tissue. Only 5-10% comes from the visceral adipose depot, but in individuals with visceral obesity, it is increased to 30%. Normally, less than 5% of fatty acids come from the denovo synthesis by liver, and this is also significantly increased in fatty liver patients[32].

The high lipolytic rate of the intra abdominal visceral fat (Omental and Mesenteric), contributes to approximately 26% of the FFA release from the upper body. It is out of proportion to the actual mass. This in turn, increases the supply of substrates for gluconeogenesis, contributing to the failure of suppression

of gluconeogenesis by insulin, in turn leading to excessive Hepatic glucose production. Also, the FFAs in Liver affect the Insulin mediated disposal of glucose to the peripheral tissues, mainly the skeletal muscle. The increased FFAs in the liver also contribute to peripheral Hyperinsulinemia by reducing the extraction of Insulin into the Liver tissue[44].

Weight loss rapidly reduces Hepatic fat content and improves Insulin sensitivity[32]. The presence of enlarged adipocytes in the visceral adipose tissue is a predictor of upper body obesity and its complications. The enlargement of the adipocytes can also be a consequence of the resistance to insulin mediated suppression of lipolysis[44]. Patients with abdominal obesity have an insulin-resistant type of fibres in their skeletal muscle[32].

Although Obesity is clearly associated with CVD, it is mainly mediated by other cardiovascular risk factors like hypertension, diabetes and lipid profile imbalances[45].

EFFECTS OF FREE FATTY ACIDS:

1. Inhibits Insulin mediated glucose uptake.
2. Impairs Insulin mediated suppression of endogenous glucose production.
3. Impairs glucose mediated suppression of endogenous glucose production.
4. Stimulates short-term Insulin secretion.
5. Inhibits long-term Insulin secretion, possibly due to Islet toxicity.
6. Reduces Hepatic clearance of Insulin.
7. Increases the production of VLDL triglycerides. [44]

HEPATIC INSULIN RESISTANCE :

Normally, Insulin action of lowering plasma glucose level is by

1. Suppression of hepatic gluconeogenesis
2. Decreasing uptake of amino acids and free fatty acids from muscle and adipose tissue to liver and
3. Favouring glucose uptake by skeletal muscle and adipose tissue.

Compared to adipose tissue, the Insulin mediated uptake of glucose by muscle is ten times more. This is because of the greater muscle mass.[15] For Insulin to effectively suppress gluconeogenesis in the liver; normal Insulin responsiveness is required at the Adipocytes. So, suppression of endogenous glucose production is in part depends on the ability of Insulin to decrease the levels of FFAs. [15]

INSULIN RESISTANCE AND DYSLIPIDEMIA:

The risk factor for Coronary artery disease in the syndrome is the existing Dyslipidemia. The main cause for Dyslipidemia is excessive synthesis of apolipoprotein B containing VLDL (very low density lipoprotein) particles. [24]

The physiological function of Insulin is to suppress VLDL production, in particular Apolipoprotein B particles, in the liver, by the following mechanisms:

1. Decreases FFA availability by inhibiting lipolysis in adipose tissue.

2. Direct hepatic effect - by affecting the assembly and hence the synthesis of VLDL.[32, 33]

In Metabolic syndrome, the synthesis of apolipoprotein B by the liver is increased. It is also increased by the occurrence of large quantities FFAs in the portal circulation, which are released from the visceral adipose tissue as a consequence of increased lipolysis. Insulin and FFAs also mediate the transfer of lipids to apolipoprotein B, by increasing the levels of Microsomal Triglyceride Transfer Protein and moreover, they reduce the degradation of apolipoprotein B by Ubiquitination-dependant pathway. [24]

In skeletal muscle, Insulin resistance is directly related to the intra-myocellular Triglyceride concentration[29].

As a consequence of the VLDL triglyceride overproduction, there occurs exchange of VLDL triglyceride to HDL (High Density Lipoprotein) – cholesterol esters by the action of Cholesterol Ester Transfer Protein[24].

The triglyceride-rich HDL molecules are good substrates for degradation by Hepatic Lipase[32]. Hepatic lipase hydrolyze these HDL molecules, to produce small HDL molecules, which are easily degraded by the kidney leading to low levels of serum HDL[24].

The VLDL triglycerides are also exchanged for LDL – cholesterol esters by the same Cholesterol Ester Transfer Protein. Again these triglycerides are

hydrolyzed by the hepatic lipase to produce small, dense LDL particles, which are commonly seen in patients with Insulin Resistance[24]. The small dense LDL particles are highly atherogenic, predisposing the individuals to cardiovascular complications[32].

The major risk factors for developing atherosclerosis include the components of MetS like Dyslipidemia, hypertension, diabetes mellitus and smoking. The normal function of the vascular endothelium is altered leading to subintimal aggregation of fat, smooth muscle cells and fibroblasts to form atherosclerotic plaque[46].

So, the patients with metabolic syndrome are subjected to aggressive treatment with lipid lowering agents to prevent cardiovascular complications[24].

INSULIN RESISTANCE AND HYPERTENSION:

The concurrence of Hypertension and Insulin resistance doubles the risk of Cardiovascular complications. In normal individuals, Insulin induces the endothelial cells to increase the production of Nitric Oxide, a potent vasodilator[24]. Insulin resistance thus causes defect in vasodilatation and sodium absorption by the kidney is preserved, leading on to hypertension[17]. Increased plasma FFAs also contributes to the defect. Insulin activates the sympathetic nervous system and also causes expansion of plasma volume by promoting salt and water reabsorption by the kidney tubules; and so Hyper insulinemia contributes to Hypertension[24].

Conversely, Hypertension also contributes to Insulin resistance. The defect in vasodilatation reduces the surface area of the vessels perfusing the skeletal muscles, thereby reducing glucose uptake[24].

CENTRAL CONTROL OF GLUCOSE METABOLISM:

The hypothalamus and certain other regions of the brain are capable of sensing metabolic requirements and causing alterations in the metabolism as needed. For example, the fatty acids which are taken up by the Mediobasal Hypothalamus alter the feeding behaviour and reduce glucose production by the Liver[24]. The receptors for Insulin are seen widely in the brain tissue. Insulin regulates satiety by centrally suppressing appetite. The Hypothalamic insulin receptor is very important in regulating hepatic glucose metabolism, because the central inhibition of insulin also decreases the effect of exogenously administered Insulin in suppressing Hepatic glucose production[15].

GENETIC CAUSES OF INSULIN RESISTANCE:

The high prevalence of Insulin resistance in certain populations, suggests a strong genetic basis, in addition to the westernized life style with high calorie intake and being sedentary. Between monozygotic twins, there is nearly 100% concordance in diagnosis of type 2 diabetes mellitus, but only 20% between dizygotic twins[15].

EXTREME INSULIN RESISTANCE:

Extreme Insulin resistance is due to some rare mutations in genes associated with Insulin action. Associated conditions include Hyper Insulinemia, Dyslipidemia, Hypertension, and Impaired Glucose Tolerance. The skin lesion, Acanthosis Nigricans is characteristic of Insulin resistance. It occurs in the flexures and neck, as a papillomatous pigmented hyperkeratotic lesion. Women with Extreme Insulin Resistance are manifested with Hyper Androgenism, Hirsuitism and Menstrual disorders[15].

INSULIN RECEPTOR MUTATIONS:

More than 100 mutations of Insulin receptor gene are known. The complete absence of Insulin receptors have also been reported[15].

i. **LEPRECHAUNISM**: The most severe form of the syndromes with severe Insulin resistance. It is characterised by Intrauterine Growth Retardation, prominent eyes, upturned nostrils, thick lips, posteriorly rotated low set ears, and thick skin lacking subcutaneous fat tissue. Individuals succumb within one year of age[15].

ii. **RABSON-MENDENHALL SYNDROME:** It is a milder syndrome with incomplete absence of Insulin receptors. Patients have life expectancy up to 15 years of age and characterised by growth retardation and several dysmorphic features including gingival hyperplasia and dysplastic teeth.

Due to inappropriately elevated fasting insulin levels, the affected children exhibits fasting hypoglycemia, but they have post prandial hyperglycemia[15].

iii. **TYPE A INSULIN RESISTANCE:** It is the mildest form of Insulin receptor gene mutations. It constitutes a triad of Insulin Resistance, Hyperandrogenism (in females) and Acanthosis Nigricans. The patients have mostly a heterozygous mutation often in the receptor's tyrosine kinase domain. Most of the people do not develop diabetes. Patients with homozygous alleles are prone to develop frank diabetes in late childhood or adolescence[15].

iv. **INSULIN MEDIATED PSEUDO ACROMEGALY:** A syndrome of severe Insulin resistance associated with pathologic tissue growth similar to that of Acromegaly, but there is no elevation of Growth hormone and IGF-1. The defect lies in the post receptor signalling of Insulin. The severe Hyperinsulinemia activates the mitogenic signalling pathways, which are intact resulting in Acromegaloid tissue growth[15].

v. **MUTATIONS IN THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ (PPARγ):** This receptor has a vital role both in differentiation of adipocytes and Insulin action. Heterozygous loss of function type of mutations has been reported in the ligand-binding domain of the receptor. The defect is characterised by features of severe Insulin Resistance, partial lipodystrophy of limbs and buttocks, sparing the face and central abdominal adipose tissue. The inability to trap the free fatty

acids after a meal, by the subcutaneous adipose tissue results in lipodystrophy[15].

LIPODYSTROPHY:

It includes a group of disorders which are characterised by complete or partial absence of adipose tissue and Insulin resistance.

1. **FAMILIAL PARTIAL LIPODYSTROPHY:** The patients appear normal at birth, but after puberty they tend to lose the subcutaneous tissue from the gluteal region and extremities. So, the muscles look prominent in these areas. Fat deposition is increased in the face, neck, axilla, abdominal cavity and labia majora. Imaging studies show complete absence of adipose tissue in the affected regions. The adipose tissue is preserved in intra-thoracic, intra-abdominal, inter-muscular and bone marrow. Inter-muscular fat is excessively accumulated. Several mutations were identified by genetic studies in these patients, in the gene LMNA, which codes for Lamin A and Lamin C. These are structural proteins present in the nuclear membranes of myocytes and adipocytes and aid in DNA replication in these cells[15].
2. **CONGENITAL GENERALISED LIPODYSTROPHY:** This disorder is characterised by Insulin resistance and a complete absence of adipose tissue from birth. The patients show features of Insulin resistance along with Muscular Hypertrophy, Hypertrophic Cardiomyopathy and Hepatomegaly collectively referred to as THE ANABOLIC SYNDROME. Death occurs

usually in early childhood due to cardiac complications. The mutations associated with this syndrome are found in two different genes on different chromosomes:

i. AGPAT2 gene coding for the enzyme, 1-acyl glycerol-3-phosphate-O-acyl transferase

ii. BSCL2 gene encoding a protein called, SEIPIN, whose function is not known.

The enzyme, 1-Acyl Glycerol-3-Phosphate-O-Acyl Transferase catalyzes a key step in triacylglycerol synthesis. It helps in the formation of Phosphatidic acid by acylation of Lyso-phosphatidic acid. So, if this gene gets mutated, triacyl glycerol synthesis is affected. Hence, storage of triacylglycerol in adipose tissue is not possible leading to Congenital generalised lipodystrophy[15].

COMMON POLYMORPHISMS CAUSING INSULIN RESISTANCE:

1. P85 subunit of PI3K (1-Phosphatidyl Inositol 3-Kinase) codon 326 Methionine → Valine; This resulted in reduced insulin sensitivity.
2. IRS-1-G972R polymorphism. This results in reduced binding between IRS-1 and the p85 subunit of PI3K and thereby reduces IRS-1 mediated PI3K functional activity.
3. Gene 3q27 coding for Adiponectin, resulting in decreased levels of circulating Adiponectin.[15]

Adiponectin is synthesized in Liver. Its hormonal action in liver is to decrease inflammation and to increase sensitization to Insulin, thereby decreasing Hepatic lipid content. Serum levels of Adiponectin is considerably lowered in patients with Metabolic syndrome, compared to normal individuals.[32]

INSULIN SECRETION IN OBESITY AND INSULIN RESISTANCE:

To maintain glucose homeostasis, normal Insulin secretion is essential. So, in patients with obesity and Insulin resistance, as a compensatory mechanism, hyper-secretion of insulin occurs. The blood glucose level is determined by the ability of the pancreatic beta cells; the patient may be normo-glycemic or may develop impaired glucose tolerance or Diabetes. The hyper secretion of Insulin, even in the presence of normo-glycemia, occurs due to increase in sensitivity of beta cells to glucose[47]. This increase in sensitivity is due to two factors:

1. Increased beta cell mass of pancreas.
2. Increased expression of Hexokinase compared to Glucokinase expression in the beta cells[47].

The Michaelis constant(Km) of Hexokinase is much lower than that of Glucokinase for glucose; so, the activity of increased Hexokinase leads to increased Insulin secretion, over a wide range of blood glucose concentrations. This causes a shift to left in the glucose-Insulin dose response curve. This hyper-insulinemia is due to both increased Insulin secretion and also decreased Insulin

clearance. The rate of Insulin secretion is three to four times higher in obese people and it has a strong correlation with the Body mass index. 50% of the total Insulin produced daily comes from the basal secretion; and Insulin secretory pulses occur every 2 hours. This is similar to the normal temporal pattern of Insulin secretion found in non-obese individuals. The difference lies in the amplitude of the post prandial pulses, which is much higher in obese people[47].

In Impaired glucose tolerance, this temporal pattern of Insulin secretion is altered. The impairment lies in the capability of beta cells in sensing and responding to the changes in the plasma glucose levels. This altered temporal pattern of Insulin secretion is considered to be an early manifestation of dysfunction of beta cells, leading on to Type 2 Diabetes Mellitus[47].

In Type 2 Diabetes Mellitus, again in response to insulin resistance, hyperinsulinemia occurs. But the degree of hyperinsulinemia is low compared to the plasma glucose levels. Abnormality of beta cells lies in the first phase of insulin secretion[47].

MEDICAL CONDITIONS CAUSING INSULIN RESISTANCE:

1. Auto-immune disorders, including Systemic Lupus Erythematosus, due to the presence of antibodies against insulin receptor.
2. Renal failure and Uremia, accumulation of Uremic toxins, raised levels of Growth Hormone and Glucagon and Metabolic Acidosis.
3. Hepatic Cirrhosis, due to elevation in circulating FFAs.

4. Familial Hemochromatosis, where Insulin secretion and Insulin action are related to the extent of hepatic iron accumulation.
5. Thalassemia major, due to transfusion-induced iron overload.
6. Many types of cancer - Gastrointestinal and Pancreatic tumours, due to inhibition of Insulin action by cytokines, TNF-alpha and IL-6.[15]

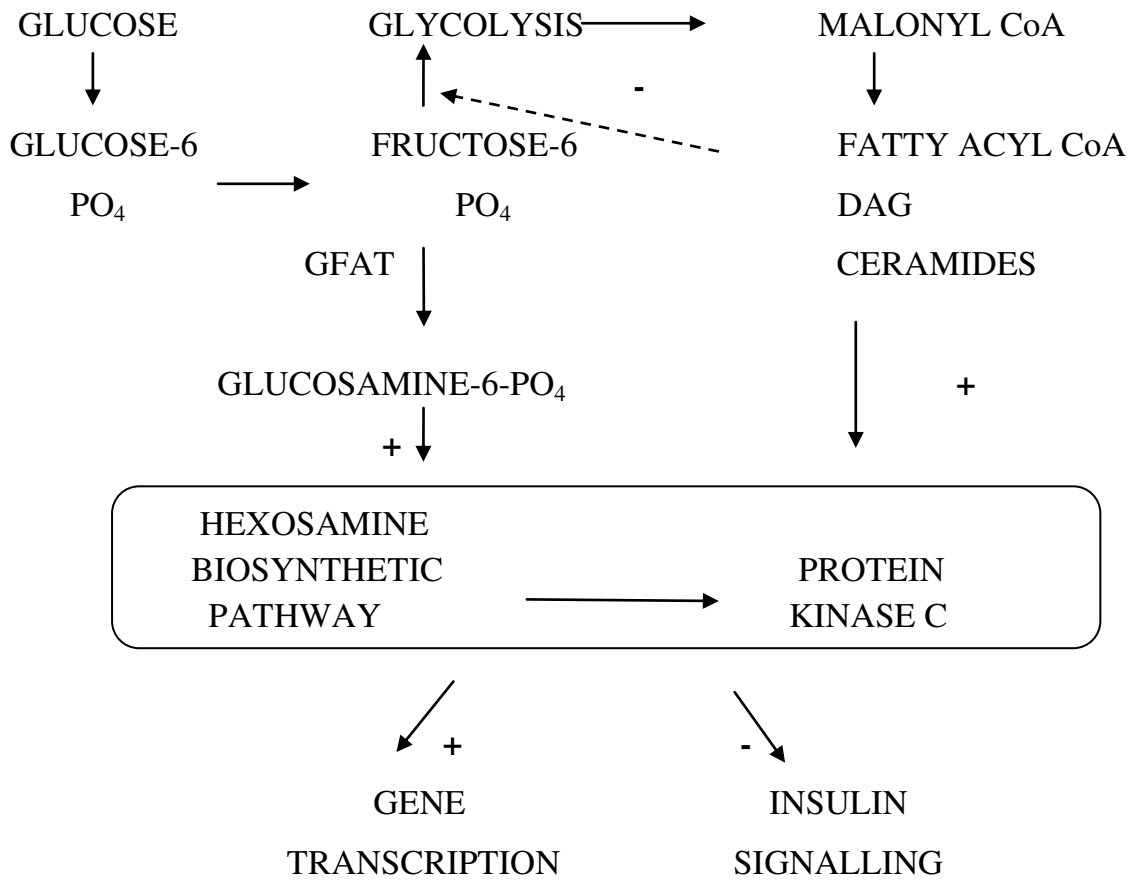
NUTRIENT EXCESS AND ITS EFFECTS ON SYSTEMIC INSULIN ACTION:

Our body cells have the capability to sense the availability of nutrients, and accordingly regulate insulin signalling or glucose transport or both[15].

1. **GLUCOSE TOXICITY:** Chronic hyperglycemia has inhibitory action on insulin secretion and action. The proposed mechanism of insulin resistance mediated by hyperglycemia includes:
    a. Down regulation of glucose transport system
    b. Defect in Insulin mediated synthesis of glycogen[15].

HEXOSAMINE BIOSYNTHETIC PATHWAY: (HBP pathway)

The entry of glucose into this pathway is essential for hyperglycemia induced Insulin resistance.



Normally, only 1 to 3% of glucose, which gets converted to fructose-6-phosphate, enters this pathway to synthesize Uridine diphosphate-N-acetyl glucosamine (UDP-N-GlcNac). UDP-N-GlcNac is the main substrate for glycosylation of proteins and its intracellular concentration is nutritionally regulated. The activities of many enzymes and transcription factors are regulated by the glycosylation of their serine or threonine residues. In hyperglycemia, when more glucose enters the pathway, glucose will not be available for glycogen synthesis and glycolysis[15].

The first and rate-limiting step of this pathway is catalyzed by the enzyme Glutamine: Fructose-6-phosphate Amidotransferase (GFAT). Glutamine is required for this rate limiting step of the pathway and hence glutamine analogues are being used to hinder the pathway and hence insulin resistance mediated by hyperglycemia[15].

2. **LIPID TOXICITY:** Persistent increase in circulating free fatty acids is a hallmark of Insulin resistance[15].

IMPACT OF FREE FATTY ACIDS ON INSULIN:

a. **SKELETAL MUSCLE:** The main effect is inadequate suppression of oxidation of fatty acids by Insulin. An increase in the availability of free fatty acids leads to increased in the mitochondrial Acetyl CoA/CoA ratio and $NADH/NAD^+$ ratio. This in turn inactivates the enzyme, Pyruvate dehydrogenase and Phosphofructokinase by feedback mechanism. So, the intracellular concentrations of glucose-6-phosphate gets elevated which again by feedback mechanism causes inhibition of Hexokinase activity, which further leads to increased intracellular glucose concentration and reduced glucose uptake[15].

FFA down regulates Insulin signalling in skeletal muscles[15]. Also, the raised FFA levels increases the phosphorylation of serine/threonine residues on the insulin receptor substrates, IRS-1 and IRS-2, thereby reducing IRS mediated glucose transport[15].

FFAs inhibit Fructose-6-Phosphate from entering into glycolytic pathway, shunting it into the HBP pathway forming increased amount of glucosamine-6-phosphate[15].

b. LIVER:

The extent of hepatic Insulin resistance correlates well with the fat content of Liver[32]. As already mentioned, the FFAs increases endogenous production of glucose by inhibiting Insulin mediated suppression of Hepatic gluconeogenesis. The FFAs increases the activity of Hepatic Glucose-6-phosphatase more compared to the increase in the activity of Glucokinase, thus increasing the glucose output from liver. Fatty acid oxidation is inhibited by suppressing the enzyme, CPT-1, which in turn is caused by the elevation of malonyl CoA. This favours fatty acid esterification resulting in increased synthesis of Triglycerides and VLDL, contributing to the dyslipidemia of Insulin resistance[15].

OXIDATIVE STRESS:

Oxidative stress decreases Insulin responsiveness and impairs Insulin signalling. Signalling pathways involving nuclear factor-KB, p38 MAPK and NH2-Terminal Jun Kinases and some protein kinases, which usually undergo stress induced activation are enhanced by FFAs and glucose, leading to Impaired Insulin Secretion and also Insulin Resistance. [15]

Increased availability of nutrients leads to consequent increase in reactive oxygen species (ROS). The ROS activates isoforms of Protein kinase C, leading to synthesis of AGEs (Advanced Glycation End products). [15]

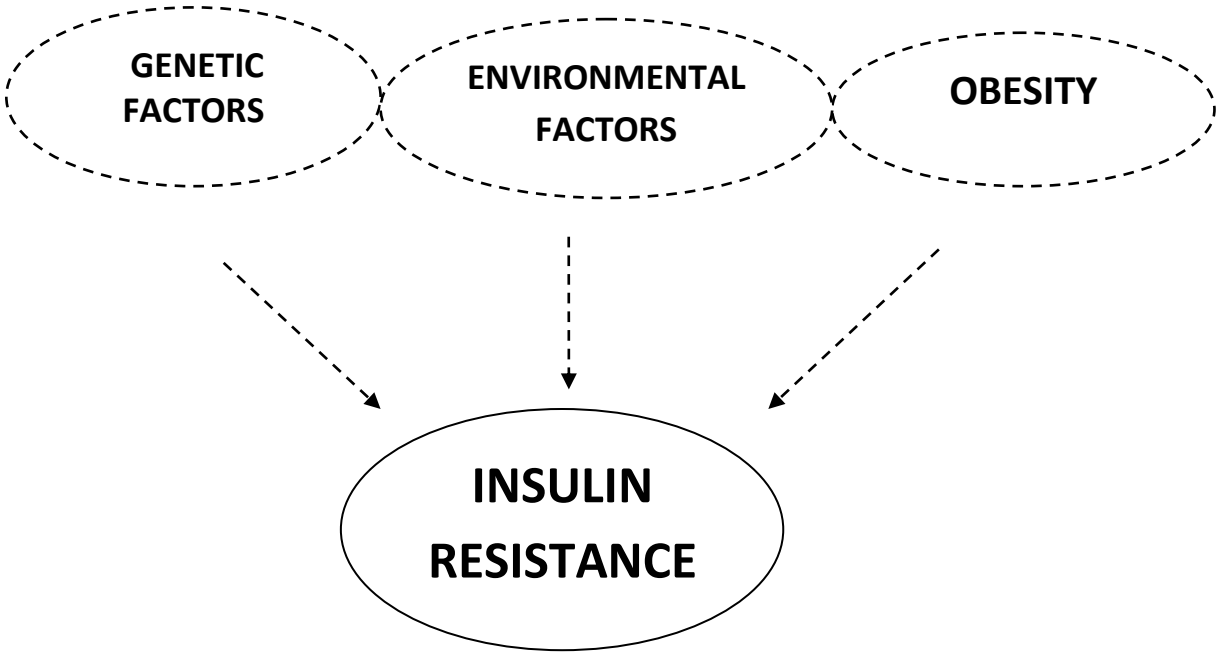
AGING:

With advancing age, increased incidence of Insulin Resistance is seen[15]. In most populations in the world, the age dependency for the prevalence of the syndrome is found to be true[17].

The causes are:

1. Increased fat depot, in particular visceral fat.
2. Increased cytokines levels in circulation.
3. Increased accumulation of Triglycerides in the cells.
4. Decline in Mitochondrial functions mainly Oxidative Phosphorylation.
5. Resistance to effects of Leptin on fat distribution leading to increased lipid accumulation in tissues.[15]

So, the development of central factor of Metabolic Syndrome, the Insulin Resistance depends on a complex interplay consisting of Genes, Obesity and Environment, which in turn includes nutritional factors, hormones and finally advancing age.[15]

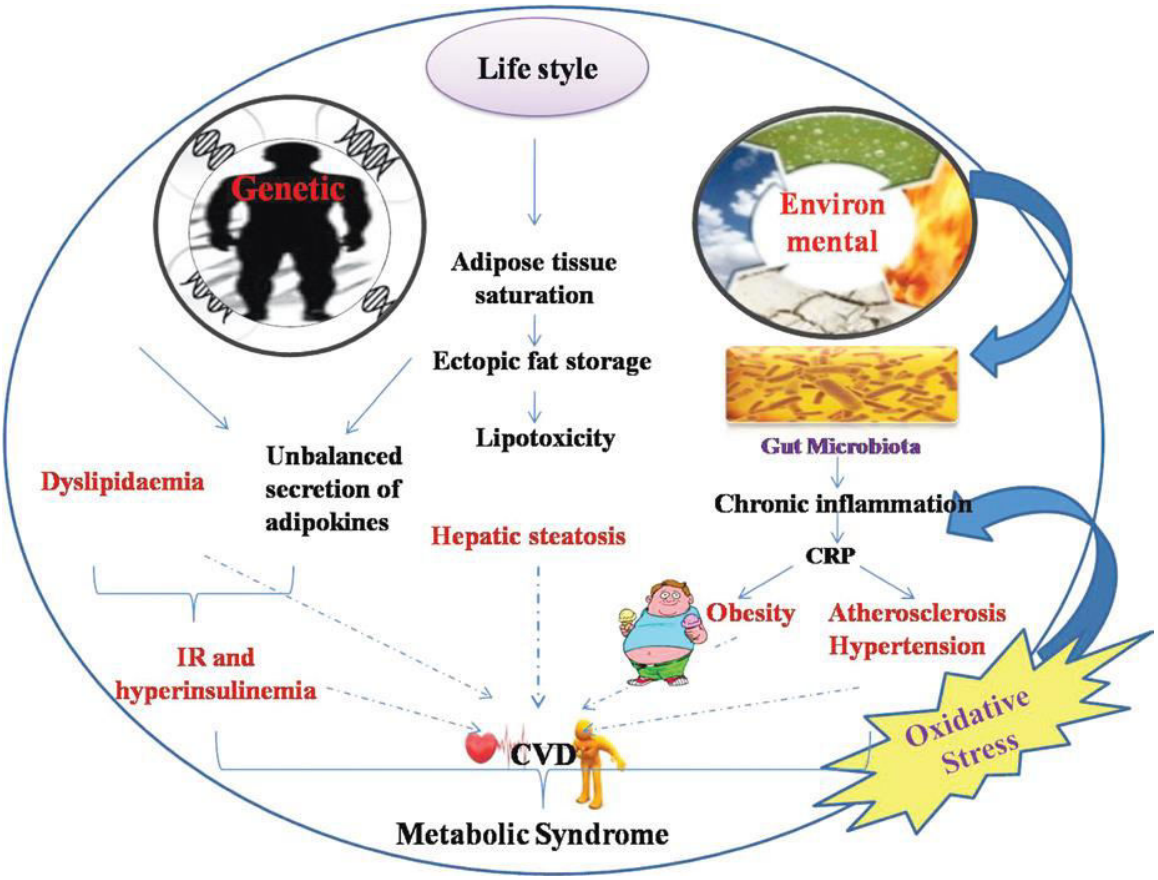


METABOLIC SYNDROME AND CVD:

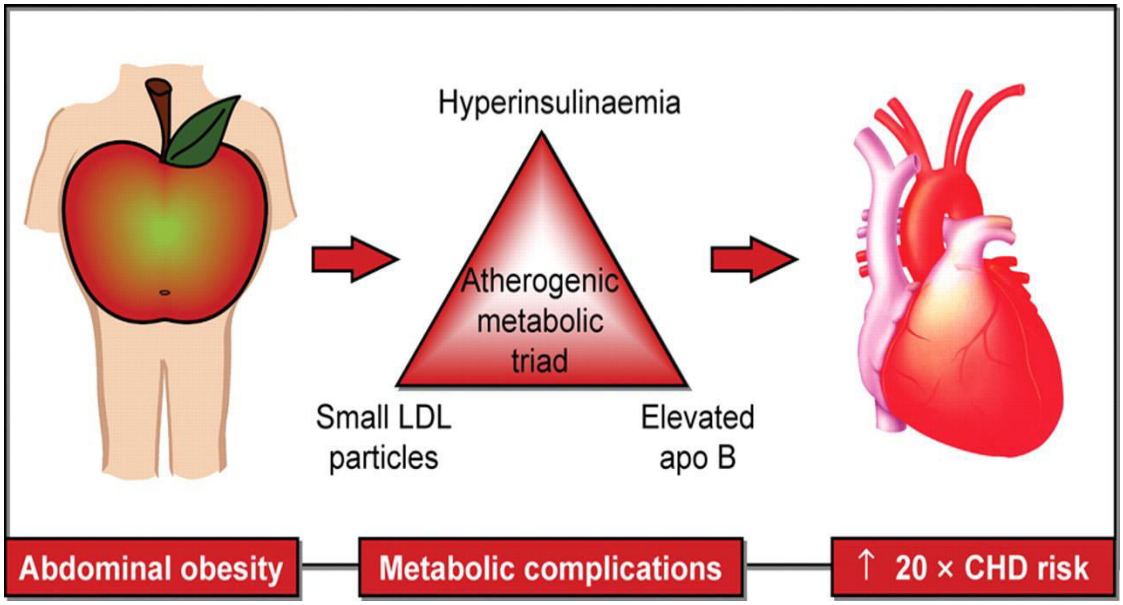
Individuals with MetS are at increased risk of CVD and total mortality, especially in men aged 45 years and women aged 55 years[26,48]. And those with frank diabetes or prior CVD are at a even higher risk[26]. Simultaneous occurrence of Hypertension and impaired glucose metabolism has the highest risk and accounted for the most of the deaths[36]. Patients with manifestations of atherosclerosis and MetS are at increased risk of CVD and total mortality, irrespective of the presence or absence of Type 2 Diabetes mellitus[38].

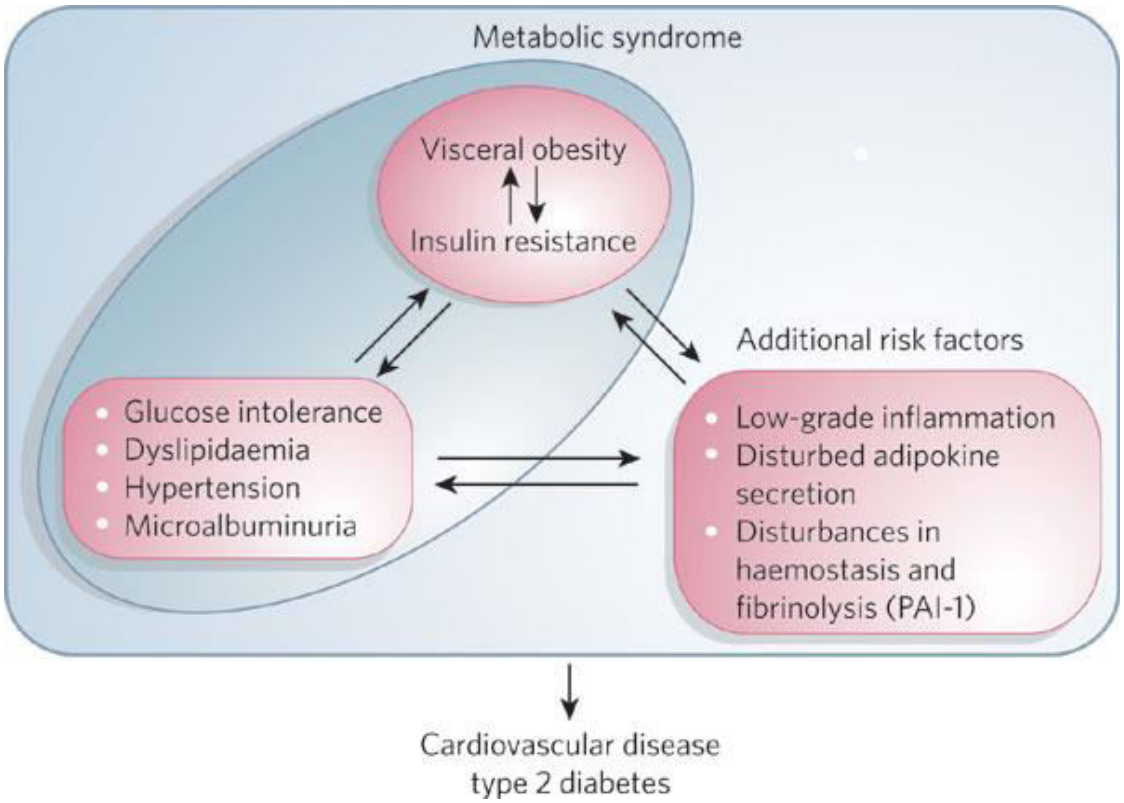
In Coronary Heart Disease patients, Metabolic syndrome prevails in almost 50%. With proper Cardiac rehabilitation and life style modifications, the

PATHOGENESIS OF METABOLIC SYNDROME



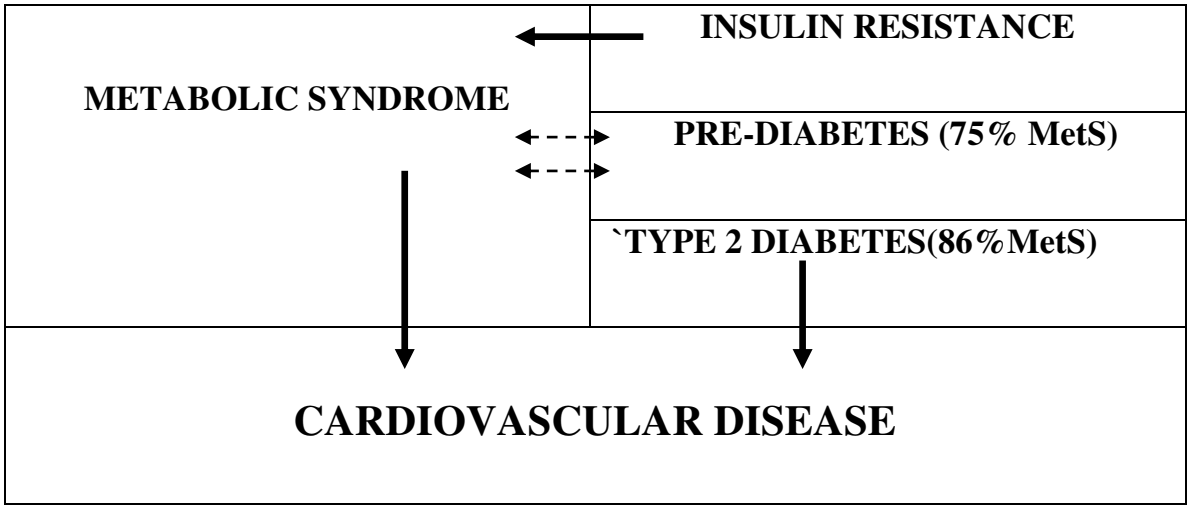
METABOLIC SYNDROME AND RISK OF CVD





prevalence of the syndrome can be markedly lowered[37]. Both overweight and non-overweight individuals with metabolic risk factors should be targeted to bring down the burden due to CVD in general population[35].

INTER-RELATION AND OVERLAP OF METABOLIC SYNDROME WITH INSULIN RESISTANCE, PRE-DIABETES AND TYPE 2 DIABETES[11]:



Population based preventive measures should be undertaken by increasing the awareness of the association of risk factors, in particular for South Asians[18]. Epidemiologists from India and international agencies like WHO (World Health Organization) proposed the rapidly rising impact of CVD as a potential alarm for the past 15 years. The estimate is about 2.6 million among Indians, by 2020, CVD being the foremost cause of morbidity and mortality[49].

FGF-19 (FIBROBLAST GROWTH FACTOR 19)

A large number of signals getting released from enterocytes are known to regulate feeding, blood glucose and induce satiety. Some of them include Cholecystokinin, Ghrelin, peptide YY, Oxyntomodulin and Glucagon-like peptide. Recently, FGF 19 is being added to the list of Gastrointestinal hormones regulating metabolism.[50]

FGF 19 is a unique member of the Fibroblast Growth Factor family.[51]

The members of the family are known to be involved in:

- Embryonic development
- Cell growth and survival
- Morphogenesis
- Tissue repair - Wound healing
- Tumour growth and Angiogenesis[51,52].

The FGF signaling pathway is a ubiquitous micro environmental regulator of adult homeostasis and cell to cell communication[4].

FGFRS (FIBROBLAST GROWTH FACTOR RECEPTORS):

The FGF family members exert their actions by binding to receptors on the cell surface, referred to as FGFRs[51].

After binding, dimerisation of receptors occurs followed by activation of Tyrosine kinases. They are encoded by four different genes, Fgfr1 to Fgfr4[53].

Initially FGF proteins were characterized by their ability to promote proliferation of fibroblasts. FGFRs 1, 2 and 3 mediated this mitogenic activity. FGFR4 was identified then to possess the ability to bind FGFs, but lacks mitogenic proliferation[54].

FGFR signalling plays a major role in regulating glucose homeostasis and energy balance[55,56].

Their interactions with FGFRs is promoted by their high affinity to Heparan sulphate, a glycosaminoglycan found on the cell surface.[51]

The structure of FGFRs1-4 constitutes three domains each:

1) An extracellular ligand binding domain,

2) A single transmembrane domain,

3) An intracellular Tyrosine kinase domain.[5]

FGF FAMILY:

The FGF family constitutes about 22 members, including FGF 1 to FGF 23. FGF 15 is not found in Humans. They are further divided into three subfamilies:

1. Canonical

2. Intra cellular

3. Hormone-like.[57]

The canonical FGFs have binding sites for Heparin for stable interactions with FGFRs. Hormone like FGFs have reduced affinity to heparin, and hence they are endocrine in function. This property of hormone-like FGFs is acquired during evolution[57]. Instead they require klotho proteins, which acts as a co-receptor/co-factors to facilitate their binding with their cognate receptors in the target tissues[57,58].

FGF 19 subfamily consists of FGF 19, FGF 21 and FGF 23[58,59] . They are involved in regulation of bile acid, glucose, lipid, phosphate, energy and vitamin D homeostasis, by acting in an endocrine fashion. They require interaction of α or β klotho proteins[58].

The extremely low affinity of FGF 19 subfamily to Heparan sulfate, permits their circulation to reach distant tissues, as a hormone, to bind with FGFR and Klotho proteins.[60]

β klotho protein is expressed in muscle, adipose tissue, liver and pancreas[60]. β klotho protein mediates the tissue responsiveness to FGF 19 and their levels are found to be low in adipose tissue in individuals with Obesity.[61]

To interact with these cofactors, amino acid sequences present in the C-terminal end is important. This C-terminal βklotho binding domain is absent in Canonical FGFs, which do not require interaction with the co-receptors. The C-terminal tail is proposed to be acquired during evolution, as a compensatory mechanism for its weakened affinity towards Heparan sulfate.[62]

Human FGFs comprises of about 150 to 300 amino acids. A core residue with 120 amino acids is conserved among the family members. The members have 30 to 60% identity among themselves[57]. Based on the similarities in sequences and functions, human FGFs can be classified in to seven subfamilies.[5]

FGF FAMILY MEMBERS:

- FGF1 subfamily
  - FGF1 (Acidic FGF)
  - FGF2 (Basic FGF)
- FGF4 subfamily
  - FGF4
  - FGF5
  - FGF6
- FGF7 subfamily
  - FGF3
  - FGF7
  - FGF10
  - FGF22
- FGF8 subfamily
  - FGF8
  - FGF17
  - FGF18

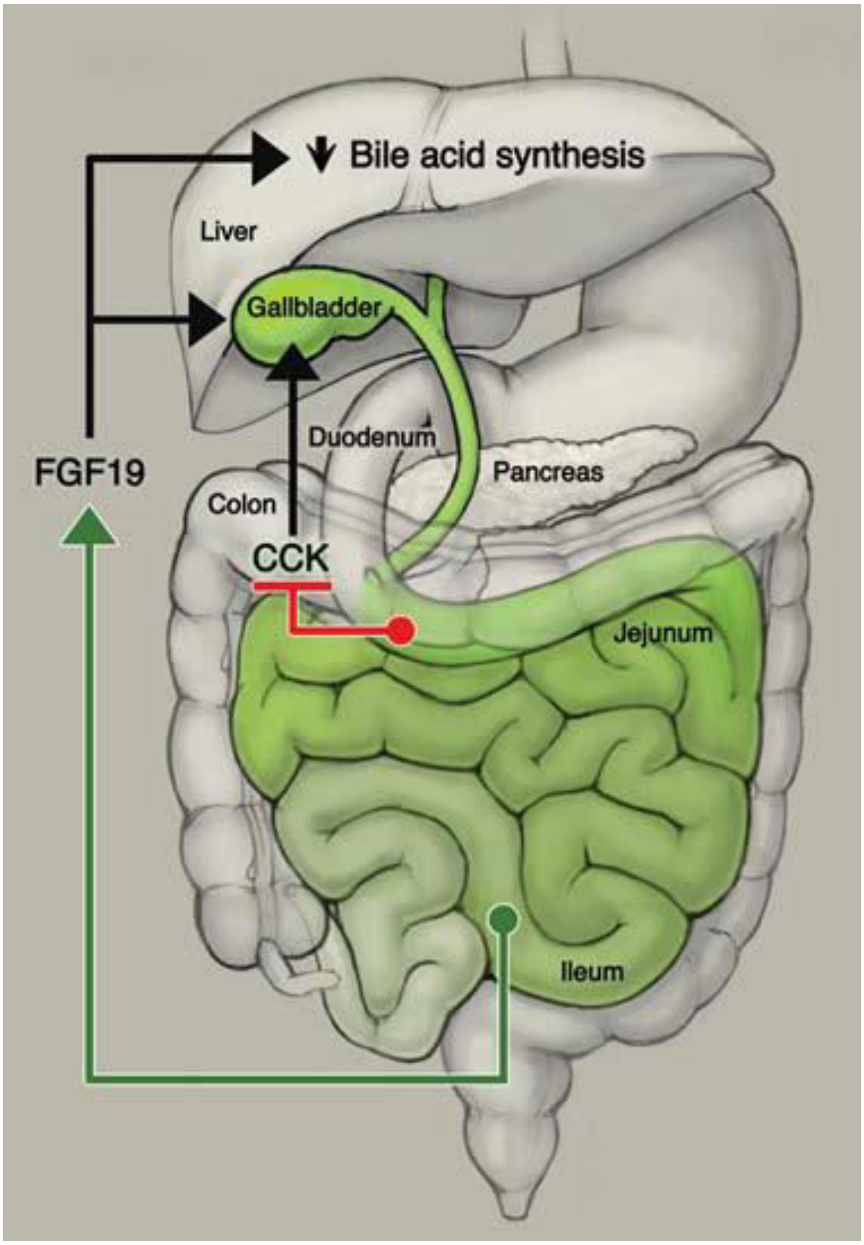
- FGF9 subfamily
  - FGF9
  - FGF16
  - FGF20
- FGF11 subfamily
  - FGF11(FHF 3)
  - FGF12 (FHF 1)
  - FGF13 (FHF 2)
  - FGF14 (FHF 4)
- FGF19 subfamily
  - FGF19
  - FGF21
  - FGF23.[63]

FGF 19:

Human FGF 19 is a 24 kD protein.[64] Following a meal, FGF 19 is secreted from the ileum, binds to FGFR4 and βklotho protein, and mediates postprandial responses[65]. FGF 19 from ileum reaches the hepatocytes through portal circulation, to exert its effects, acting as a entero-hepatic signal[66,7,67].

The complex triggers the activation of cellular kinases, namely ERK(Extracellular signal Regulated protein Kinase) and GSK, inhibiting Hepatic

SITE OF FGF 19 SYNTHESIS



synthesis of bile acids and glucose respectively. Synthesis of Glycogen and protein is stimulated.[65, 8]

Bile acid not only induces FGF 19 secretion from the intestine; they also activate the FGF 19/FGFR4 signalling in hepatocytes. This helps in inhibition of bile acid synthesis and also prevents excess accumulation of toxic bile contents in the Liver.[66]

FGF 19 is unique in the way that:

1. It has lower affinity towards Heparan sulphate; instead they use transmembrane receptors called as β-klotho.[51]

2. Unlike other FGFs, it acts specifically on FGFR4 alone, which has no mitogenic response[51,54,63].

3. It performs endocrine functions in addition to its autocrine and paracrine functions. [51]

- Endocrine functions - Bile acid, Glucose and Lipid Metabolism.
- Paracrine functions - Embryogenesis, Cell Growth and Differentiation, and Angiogenesis.
- Autocrine functions - Promotes proliferation and invasion of cancerous tissues.[51]

Due to its reduced affinity towards Heparan sulfate, it is loosely held on the cellular membrane and gets detached from the extra cellular matrix, escaping into circulation, facilitating the endocrine actions[51,68].

FGF 19 transcripts are seen in many tissues like Brain, Skin, Gall Bladder, Kidney, Umbilical cord, Cartilage and Intestine, but it is primarily expressed in Ileum[6,51,69,70]. Even though it is secreted in the ileum, the specific FGFR4 receptors occurs in Liver in large numbers[51,52]. This contributes to the metabolic effects of FGF 19.

FGF 19 is a postprandial enterokine, derived from Ileum that governs metabolic processes[71].FGF 19 is a peptide hormone consisting of 216 amino acids[9]. It was originally identified in chick embryos as a promoting factor in inner ear development[2].

FGF 19 consists of two disulfide bonds. The disulfide bonds are important for stability, protein folding and activity and are more common in mammalian proteins[72]. The intra-molecular disulfide bond contributes to their plasma stability and facilitates them to function as hormones on distant tissues[58].

FGF 19 is stable for up to ten minutes in blood and brain. Entry of FGF 19 in to brain depends on its serum concentration. In the basal state, the role of FGF 19 in regulating metabolism dominates its action on the central nervous system. Excretion of FGF 19 is by the Kidneys. In end stage renal disease, FGF 19 concentration is found to be elevated in the blood.[73]

Bile acids acts as the ligand for FXR, thus the receptors are referred to as the “bile acid sensor”. FXR in turn increases the expression of FGF 19 from

the enterocytes[9]. So, in response to food intake, concentration of serum FGF 19 is increased[8].

Bile salts negatively regulate their own production.[74,67] They help in digestion and absorption of dietary lipids in the proximal parts of small intestine. After fulfilling their function, bile salts reach the terminal ileum and activate FXR to secrete FGF 19[74]. The secretion of FGF 19 represents a transition between the fed and the fasting states, acting as a late postprandial signal[6].

FXR is capable of controlling various metabolic pathways. They regulate synthesis of bile acids, conjugation and transport, upon activation by bile acids and also different aspects of Carbohydrate and lipid metabolism[3].

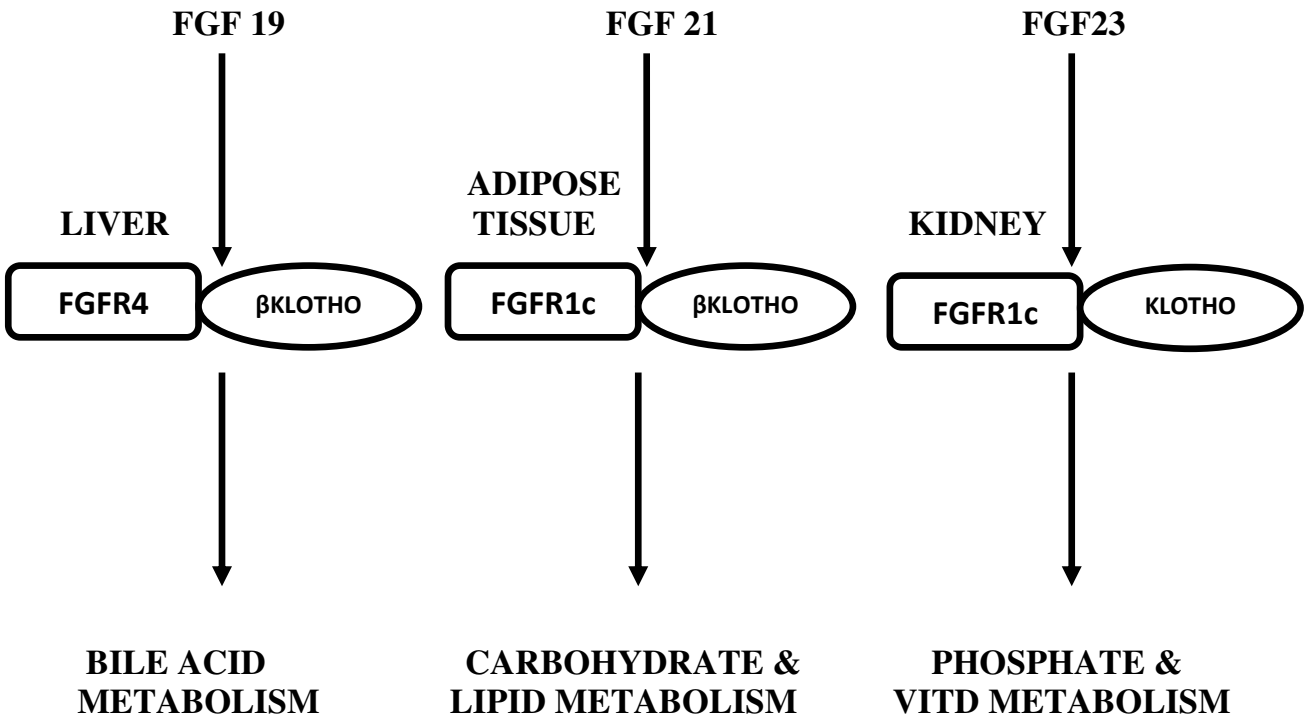
The functional FGF receptor complex comprises of:

- Trans membrane tyrosine kinase
- Heparan sulfate
- Activating FGF.[52, 5]

FIBROBLAST HOMOLOGOUS FACTORS(FHFs):

These factors have sequence and structural similarity with FGFs, including high affinity to Heparin, but cannot bind and activate FGFRs. The intra-cellular domains of the voltage-gated sodium channels are the main target for FHFs. In humans, mutations in FHFs resulted in Cerebellar Ataxia.[70]

MODE OF ACTIONS OF FGF 19 FAMILY MEMBERS[63]:

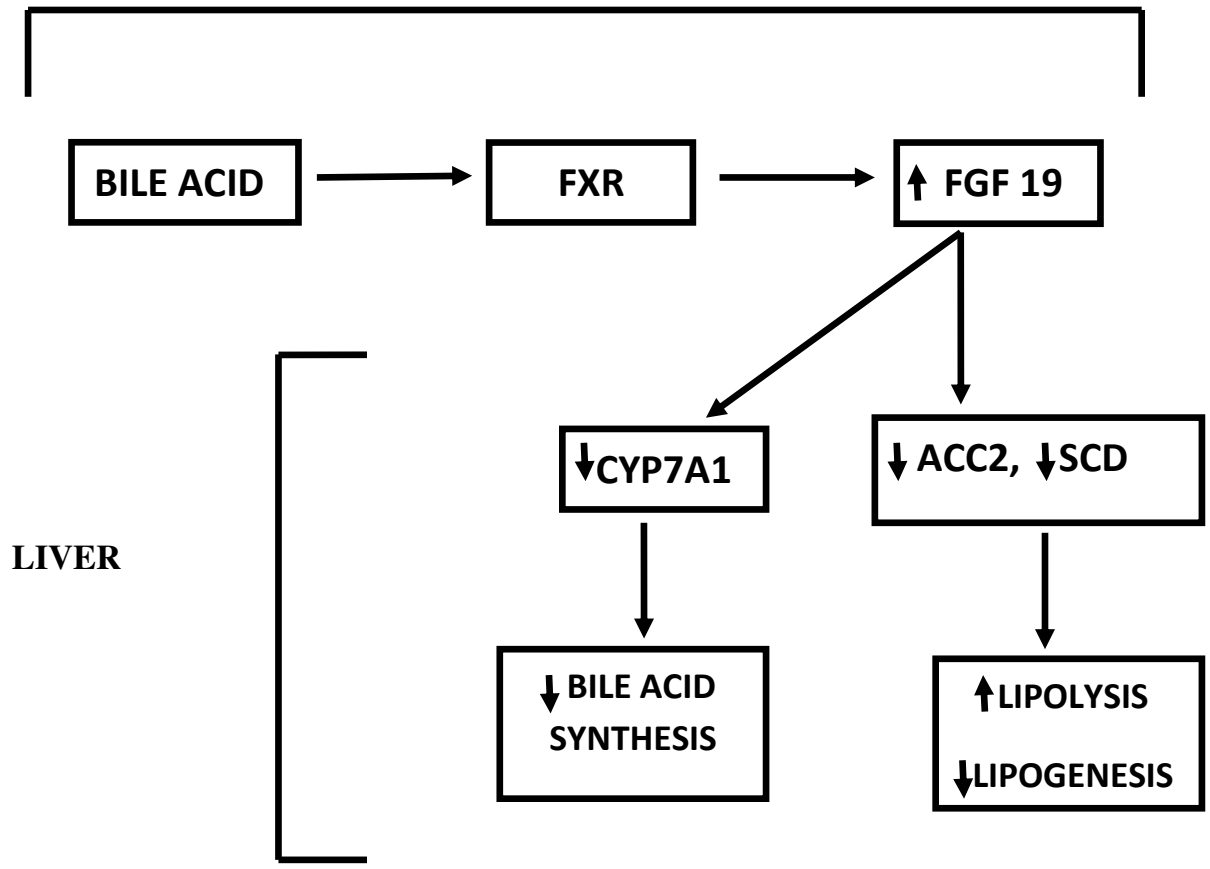


ROLE OF FGF 19 IN BODY METABOLISM:

1. BILE ACID METABOLISM:

Bile acid synthesis forms a major pathway for catabolism of cholesterol in humans. Cholesterol is hydroxylated at position 7 by the enzyme cholesterol 7 α hydroxylase (Cyp7a1) in Endoplasmic reticulum. This is the rate limiting step in bile acid synthesis. This step is under feedback inhibition[51].

THE PHYSIOLOGY OF FGF 19:



In the intestine, the bile acids activate the nuclear receptor in the enterocytes, called the Farnesoid X receptor (FXR)[51,75]. Then, FXR increases the secretion of FGF 19 by the ileum, FGF 19 binds to FGFR4 in liver and inhibits transcription of Cyp7a1 gene, thereby repressing bile acid synthesis,[51,6]. This

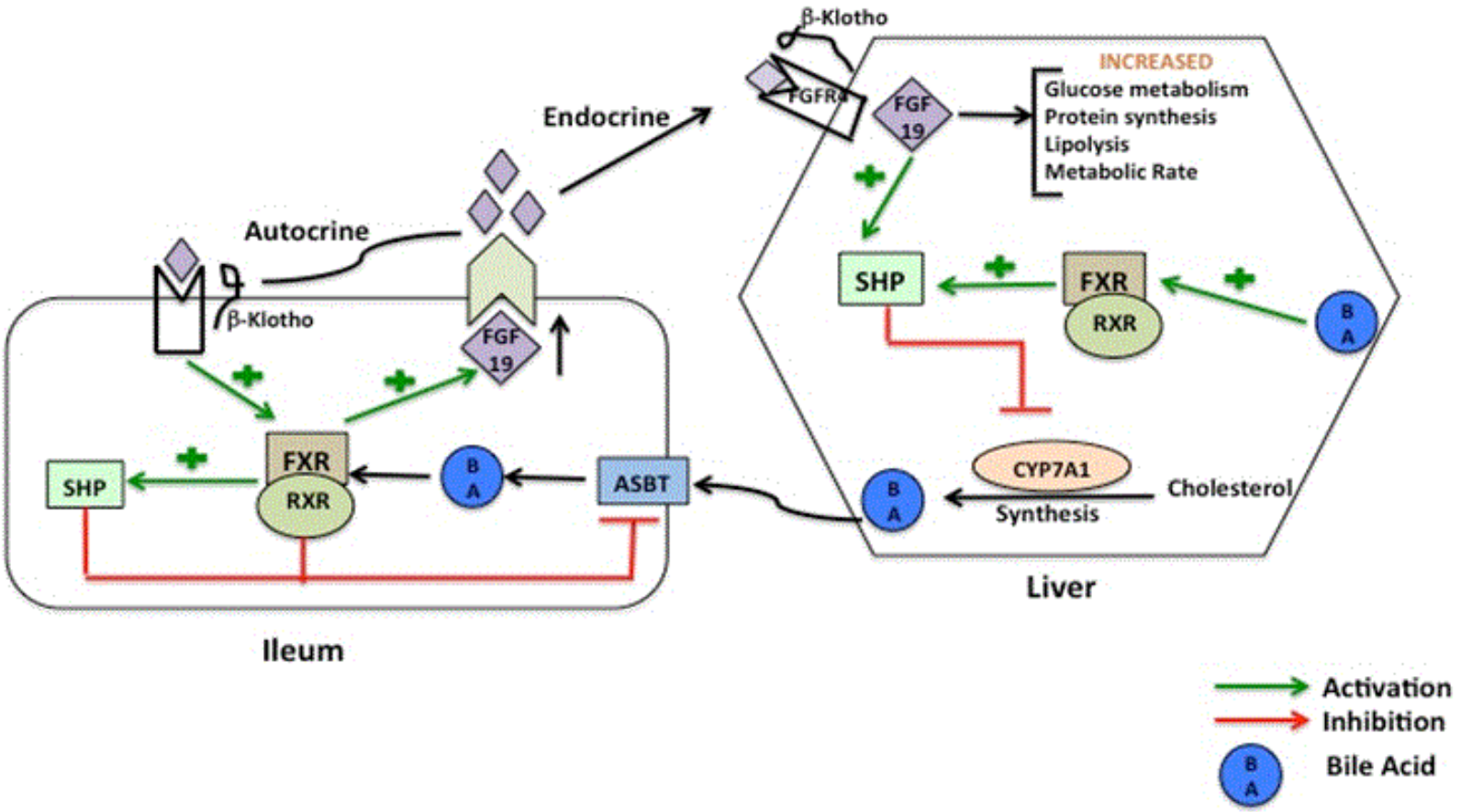
feedback inhibition is also mediated by increased expression of SHP-1(Small Heterodimer Partner-1), a member of the nuclear receptor family. This in turn inhibits the expression of Cyp7a1[51,76]. Increased flux of bile acids is followed by a peak in serum FGF 19 levels in about 90 to 180 minutes after a meal. FGF 19 deficiency is an important factor causing bile acid malabsorption. It can be treated with FXR agonists, which in turn stimulates FGF 19 secretion, which covers for FGF 19 deficiency and bile acid diarrhoea can be prevented. But the property of tumorigenesis of FGF 19 limits the use of such treatments leading to increased FGF 19 levels in serum than the physiological level[51]. But, now M70, a non-tumorigenic variant has been engineered, retaining all other biological functions is available[51,64]. So the hopes of using FGF 19 in treatment of metabolic diseases have increased tremendously.

2. GLUCOSE AND PROTEIN METABOLISM:

FGF 19 is known to lower blood glucose levels. It promotes glycogen synthesis and protein synthesis in muscle and liver, similar to Insulin[51,71,77].

Glycogen synthase is the rate limiting enzyme of the pathway of Glycogen synthesis. It is active in its dephosphorylated state and becomes inactive when phosphorylated. Glycogen synthase kinase 3α (GSK 3α) and GSK 3β are the enzymes responsible for phosphorylation of Glycogen synthase. These enzymes, themselves become inactive on phosphorylation. FGF 19 promotes activation of Glycogen synthase by phosphorylating these enzymes. Thus Glycogen synthesis is favoured[51].

MECHANISM OF ACTION OF FGF 19



The Eukaryotic Initiation Factor 4F (eIF4F) complex mediates protein synthesis. It is responsible for binding of mRNA to the ribosomes and hence mediates initiation of translation. The complex consists of two major proteins:

- eIF4B
- eIF4E

FGF 19 phosphorylates these proteins and activates them aiding in initiating translation. FGF 19 also increases the phosphorylation of rpS6 (ribosomal subunit protein S6), which in turn lead to increase in global protein synthesis[51].

Hence the actions of FGF 19 is similar to Insulin in phosphorylating the eukaryotic initiation factors and the ribosomal protein S6, but differs in the further pathway downstream. Insulin acts through the mTOR pathway, by increasing the phosphorylation of protein kinases AKT and p70 S6 kinase. In contrast, FGF 19 acts via phosphorylation of p90 S6 kinase by activating ERK1 and ERK2. So, FGF 19 acts in a parallel fashion but independent of the pathway of Insulin in governing protein synthesis in Liver[51].

3. LIPID METABOLISM:

FGF 19 down-regulates the enzyme, Acetyl CoA Carboxylase 2(ACC2), and inhibits conversion of Acetyl CoA to Malonyl CoA. Malonyl CoA is a repressor of the enzyme, Carnitine Palmitoyl Transferase 1(CPT1), which initiates Fatty acid oxidation. So, by reducing the activity of ACC2, FGF 19

induces Fatty acid oxidation. FGF 19 also down-regulates the enzyme Stearoyl CoA desaturase, which in involved in Lipogenesis[70].

FGF 19 by increasing the oxidation of fatty acids, reduces Triglyceride concentration[9]. Unlike Insulin which favours lipogenesis, FGF 19 inhibits fatty acid synthesis[51,71] and decreases the expression of enzymes of lipogenesis in liver cells[2].

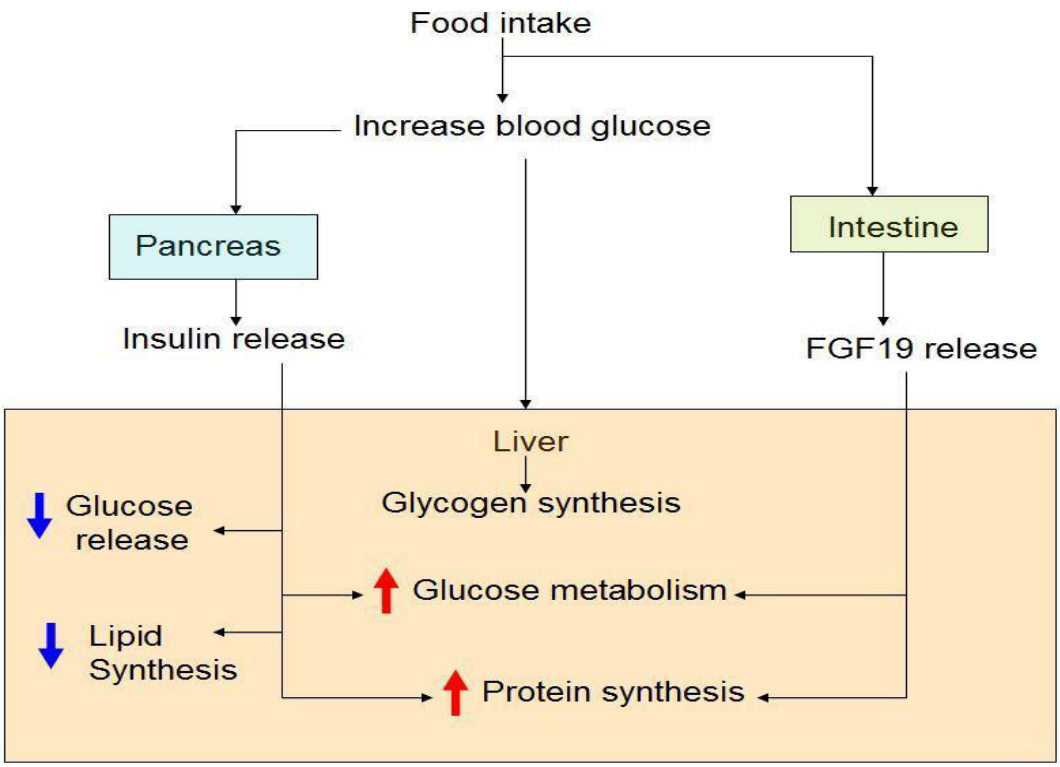
4. **MITOGENIC ROLE:**

Induction of tumour and cancer: FGF 19 has potent mitogenic activity. The FGFR-FGF 19 signalling is found to play a major role in development and progression of HCC (Hepato Cellular Carcinoma). FGF 19 is over expressed in cancerous liver. Treatment with recombinant FGF 19 resulted in increased proliferation and invasion of the cancerous tissue and also inhibits Apoptosis in cancer cells[51].

5. **ANGIOGENESIS:**

- FGF 19 acts s a major growth factor is expressed during development of retina. In lens cells, it is necessary for differentiation of lens fibres and its survival.
- It is involved in patterning of retina in the nasal-temporal region and in guiding the axons of retinal ganglion cells.
- It regulates cell division.
- It also plays a role in the development of forebrain and is significantly expressed in fetal brain tissue.

ACTIONS OF FGF 19



- It helps in development of embryonic spinal cord.
- It is critical for development of ventral regions of diencephalon and telencephalon.
- It is involved in the specification of oligo dendrocytes and GABAergic interneurons in the ventral region of diencephalon and telencephalon.
- Inhibition of functions of FGF 19 affects brain development during mid-segmentation stages, resulting in reduction in the size of cerebellum, forebrain and midbrain.
- FGF 19 helps in development of inner ear, by aiding in patterning of the neuro ectoderm.[51]

So, FGF 19 acts as a multifunctional regulator, influencing many physiological events.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

Aim of the study:

To measure serum Fibroblast Growth Factor 19 in patients with Metabolic syndrome and to compare the serum level of FGF 19 with healthy individuals.

Objective of the study:

To analyze the correlation between serum FGF 19 and the components of Metabolic syndrome.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College, Thanjavur, after being approved by the Ethical committee. Participants of the study group were selected from the Outpatient Department of Medicine.

The study included 50 patients with Metabolic syndrome (25 males, 25 females) and 50 age and sex matched healthy controls (25 males, 25 females), in the age group of 20-70 years. Informed consent was obtained from all the participants.

INCLUSION CRITERIA:

Patients with components of Metabolic syndrome were included in the study.

EXCLUSION CRITERIA:

- History of Myocardial infarction
- Coronary bypass surgery
- Chronic hepatic disease
- Chronic renal disease
- Cancer

- Alcohol abuse
- Pregnant females.

The participants of the study were routinely measured for height and weight. BMI was calculated with the formula weight/height^2 (kg/m^2). All the participants were informed about the study and informed consents were obtained from them. Waist circumference was measured for each subject.

SAMPLE COLLECTION:

Venous blood samples were drawn from each subject, under aseptic precautions, after an overnight fast of 12 hours. The samples were allowed to clot for 30 minutes and were centrifuged at 3000g for 10 minutes. The sera for estimating FGF 19 were stored in the deep freezer, until the estimation was done. The following parameters were estimated immediately after the serum separation.

1. Blood Glucose – fasting and postprandial.
2. Fasting lipid profile:
    - Total cholesterol
    - HDL cholesterol
    - Triacylglycerol.

LDL and VLDL cholesterol levels were calculated from the estimated parameters using Friedewald formula. AIP (Atherogenic Index of Plasma) was calculated as log (TG/HDL-C). Fasting serum FGF 19 was measured in all the

samples within one month of collecting the samples by Sandwich Enzyme – Linked Immuno Sorbent Assay.

ESTIMATION OF SERUM FGF 19:

Reagents provided in the kit purchased:

i. Item A – Human FGF 19 micro plate – consists of 96 wells coated with anti-human FGF 19(12 strips x 8 wells).
ii. Item B – 25ml of 20x concentrated wash buffer solution.
iii. Item C – Standards – Recombinant human FGF 19(2 vials)
iv. Item L – Assay diluent C - Diluent buffer – for standard/sample (serum/plasma) - (30ml)
v. Item E - Assay diluent B – Diluent buffer of 5x concentration - for standard/sample (cell culture medium/urine) - (15ml)
vi. Item F – Detection antibody – Biotinylated anti-human FGF 19 (2 vials)
vii. Item G – HRP conjugated Streptavidin concentrate – 500x (200 µl)
viii. Item H – TMB (3,3',5,5' – Tetra Methyl Benzidine) substrate reagent in buffer solution – (12ml)
ix. Item I - Stop solution – 0.2 M sulphuric acid (8ml).

Procedure:

The assay kit is an in vitro Enzyme – Linked Immuno Sorbent Assay for estimating FGF 19. An antibody specific for FGF 19 is coated on a 96-well plate.

Standards and samples are pipetted into the appropriate wells. FGF 19 present in the sample is bound with the antibodies coated on the wells. After washing the wells, a Biotinylated anti-human FGF 19 antibody is added. Again the wells are washed to remove unbound Biotinylated antibody. HRP-conjugated Streptavidin is added to the wells. Washing is repeated. A TMB substrate solution is added and the colour develops in proportion to the concentration of FGF 19 bound in each well. After adding Stop solution, the colour changes from blue to yellow. The intensity of the colour is measured with an ELISA reader at 450nm.

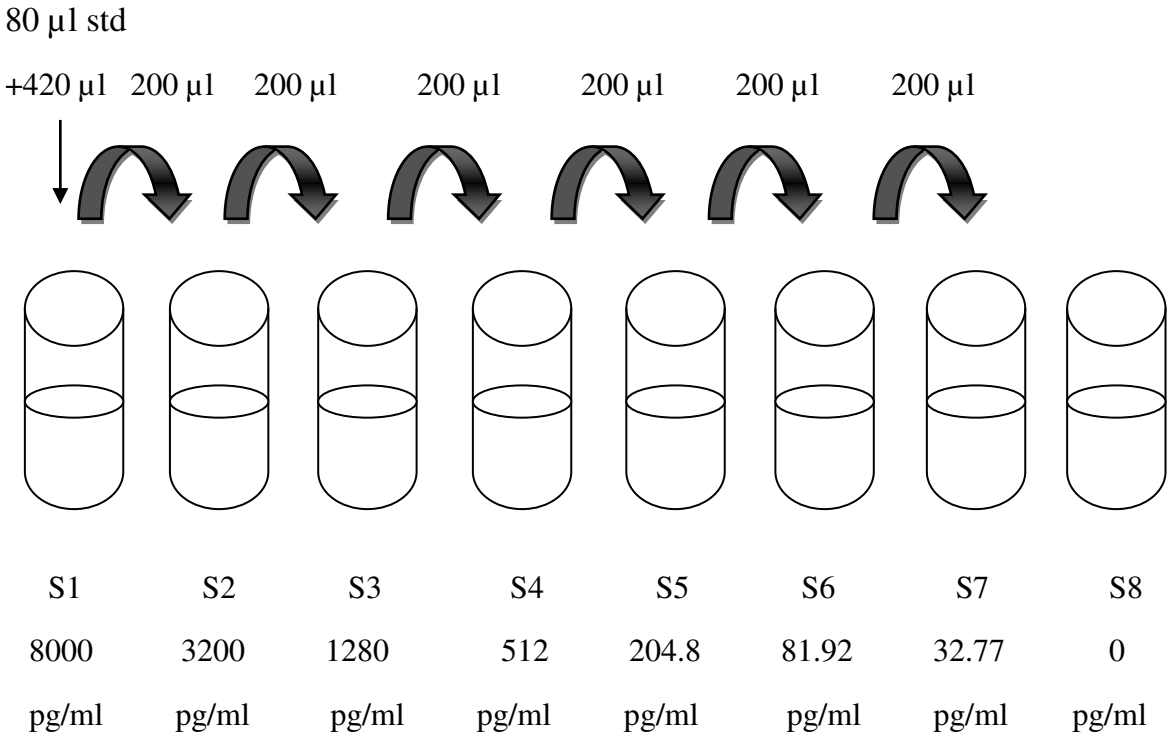
Preparation of Reagents:

i. All the reagents and samples were brought to room temperature before usage.

ii. STANDARD PREPARATION:

- 400 µl of Assay diluent C was added to the vials containing the standard (Item C), after spinning the vial briefly. The concentration of this preparation was 50 ng/ml.
- By a gentle mix, the powder was dissolved thoroughly.
- 80 µl of the prepared standard in vial (50 ng/ml) was added to the 420 µl of Assay diluent C in to a tube to prepare 8000 pg/ml standard solution.

- 300 µl of Assay diluent C was pipetted into each tube.
- A dilution series was produced from the 8000 pg/ml standard solution as follows.
- Each tube was mixed thoroughly mixed before transferring its contents to the next tube.
- The Assay diluent C serves as the zero standard (0 pg/ml)



iii. **WASH BUFFER:** 20 ml of wash buffer concentrate (20x) from Item B was diluted with distilled or deionised water to yield 400ml of 1x wash buffer.

iv. **DETECTION ANTIBODY:** After briefly spinning the vial (Item F), 100 µl of 1x Assay diluent B was added in to it. Then, the vial was mixed gently by pipetting up and down. This detection antibody concentrate was diluted with 8 ml of 1x Assay diluent B to make the dilution 80 fold.

v. **HRP STREPTAVIDIN**: HRP Streptavidin concentrate vial was pipetted up and down to mix thoroughly before use, after briefly spinning the vial. 20 µl of the concentrate was diluted with 10 ml 1x Assay diluent B to prepare a 500 fold diluted solution.

STEPS:

i. All reagents and standards were prepared as instructed and allowed to attain room temperature before use.

ii. 100 µl of the standard and sample was added to each well.

iii. The wells incubated for 2.5 hours at room temperature.

iv. After the incubation period, 100 µl of the prepared Streptavidin solution was added to each well.

v. Allowed incubation at room temperature for 45 minutes.

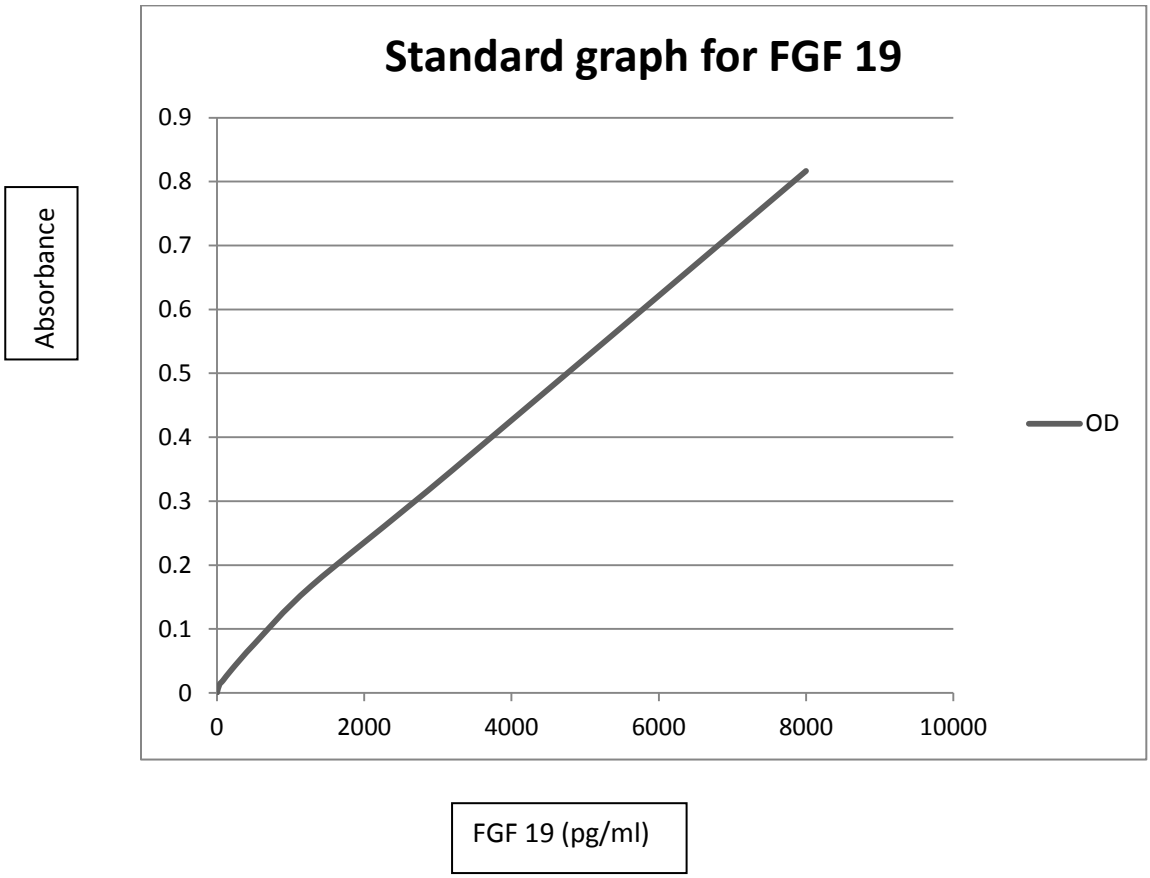
vi. 100 µl of TMB substrate reagent was added to each well. And again incubated for 30 minutes at room temperature.

vii. 50 µl of Stop solution was added to each well.

viii. Readings were taken at 450 nm immediately.

Calculation of results:

A Standard curve was plotted with the standard concentration on the x-axis and the absorbance on the y-axis. The best fit straight line was drawn through the points on the graph. Using the absorbance value for each sample, the corresponding concentration of FGF 19 was calculated from the standard curve.



ESTIMATION OF GLUCOSE:

Method:

Glucose Oxidase – Peroxidase method (End point)

Principle:

Glucose present in the sample is oxidized to Gluconic acid and hydrogen peroxide by Glucose oxidase (GOD). Peroxidase enzyme (POD) acts on hydrogen peroxide to yield water and nascent oxygen. This nascent oxygen oxidizes phenol, which in turn combines with 4-aminoantipyrine to form a coloured quinoneimine complex. The intensity of the colour is directly proportional to the concentration of Glucose in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 2H_2O$$

Glucose standard: 100 mg/dl

Enzyme Reagent Composition:

Glucose oxidase : ≥ 20000 U/L

Peroxidase : ≥ 2000 U/L

Phenol : 10 mmol/L

Phosphate buffer : 200 mmol/L.

Specimen: Fresh unhemolysed serum.

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Reaction time : 5 minutes

Reaction temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Blank Absorbance Limit : 0.2

Units : mg/dl

Assay procedure: (End point method)

Pipette into test tubes labelled as	Blank	Standard	Test
Enzyme reagent	1ml	1ml	1ml
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

The tubes were mixed well after each addition and incubated at 37ºC for 5 minutes. The absorbance of standard and test was read at 505 nm.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{concentration of Standard}$$

Linearity: Up to 500 mg/dl.

Normal values: Glucose (fasting): 70 – 110 mg/dl

Glucose (post prandial): 90 – 140 mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL:

Method:

Cholesterol oxidase-Peroxidase Enzymatic, endpoint method.

Principle:

The free cholesterol, liberated from the cholesterol esters by cholesterol esterase (CHE), is oxidized by cholesterol oxidase (CHO) to cholestenone with the simultaneous production of hydrogen peroxide. The

hydrogen peroxide reacts with 4-aminoantipyrine (4AAP) and a phenolic compound in the presence of peroxidase (POD) to yield a red coloured complex.

Cholesterol ester + Water $\xrightarrow{\text{CHE}}$ Cholesterol + Fatty acid

Cholesterol + Oxygen $\xrightarrow{\text{CHO}}$ Cholest-4-en-3-one + H_2O_2

$2\,H_2O_2$ + 4AAP+ Phenol $\xrightarrow{\text{POD}}$ Quinoneimine dye + $4H_2O$

Absorbance of quinoneimine formed is directly proportional to cholesterol concentration.

Reagent Composition:

Goods buffer (pH – 6.4) : 100 mmol/L

Cholesterol oxidase : >100 U/L

Cholesterol esterase : >200 U/L

Peroxidase : >3000 U/L

4 – Amino antipyrine : 0.3 mmol/L

Phenol : 5 mmol/L

Cholesterol standard: 200mg/dl

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Incubation time : 10 minutes

Incubation temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Absorbance Limit : 0.4

Blank with : Reagent

Units : mg/dl

Assay procedure: (End Point Method)

Pipette into test tubes labelled as	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

Mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against reagent blank at 505 nm.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Reference range: 150-200 mg/dl

Linearity: Up to 1000 mg/dl

Sensitivity: 1mg/dl

Interference: Hemoglobin upto 200mg/dl, Ascorbate upto 12mg/dl, Bilirubin upto 10mg/dl and Triglycerides upto 700 mg/dl do not interfere with the test.

ESTIMATION OF SERUM TRIGLYCERIDES:

Method: GPO-PAP method, Endpoint.

Methodology: Colorimetric, Enzymatic method with Glycerol Phosphate Oxidase (GPO).

Principle: Lipoprotein lipase (LPL) catalyzed hydrolysis of Triacylglycerol, yields Glycerol which is phosphorylated by Glycerol kinase (GK) using ATP to Glycerol-3-phosphate, which upon oxidation by the enzyme Glycerol Phosphate

Oxidase (GPO), yields Di-Hydroxy Acetone Phosphate and Hydrogen peroxide. The Hydrogen peroxide reacts with Phenolic compound and 4-Amino Anti Pyrine in the presence of Peroxidase (POD) to form a coloured complex. The intensity of Quinoneimine dye formed is proportional to the Triglyceride concentration in the sample.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol 3 phosphate + ADP}$$

$$\text{Glycerol 3 phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP+ 3,5-DHBS} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 2H_2O$$

[DHAP -Di-Hydroxy Acetone Phosphate

ATP - Adenosine Tri Phosphate

4-AAP - 4 Amino Anti Pyrine

DHBS -3,5 Dichloro-2 Hydroxy Benzene Sulfonate]

Triglycerides standard concentration- 200mg/dl

Reagent composition:

Buffer (pH – 7.0) : 40 mmol/L

4-AAP : 0.4 mmol/L

ATP : 2.0 mmol/L

Mg^{2+} : 2.5 mmol/L

DHBS : 0.2 mmol/L

Glycerol kinase : 1500 U/L

Glycerol 3 – phosphate oxidase : 4000 U/L

Peroxidase : 2200 U/L

Lipoprotein lipase : 4000 U/L

Reagent Preparation:

Reagent 1 (R1) - Enzymes / chromogen

Reagent 2 (R2) - Buffer

The working reagent was prepared by mixing 4 parts of R1 with 1 part of R2.

Sample: Unhemolysed serum collected after 12 hrs of fasting.

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Incubation time : 10 minutes

Incubation temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Absorbance Limit : 0.5

Blank with : Reagent

Units : mg/dl

Assay Procedure:

Pipette into test tubes labelled as	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

Mixed and incubated for 10min, at room temperature. Absorbance was read at 505nm for standard and sample against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Reference values: 50 -150 mg/dl

Linearity: Upto 1000mg/dl

Sensitivity: 2mg/dl

Interferences: Hemoglobin upto 300mg/dl, Ascorbate upto 3mg/dl and Bilirubin upto 20mg/dl do not interfere with the test.

ESTIMATION OF SERUM HDL - CHOLESTEROL:

METHOD: Phosphotungstic acid method, Endpoint.

PRINCIPLE: Chylomicrons (CM), LDL and VLDL are precipitated from serum or plasma with Phosphotungstate in the presence of divalent cations such as Magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent.

Phosphotungstic acid, Mg^{2+}

Serum/plasma ⟶ HDL + [LDL+VLDL+CM]

(supernatant) (precipitate)

REAGENT COMPOSITION:

Precipitating reagent:

Phosphotungstic acid	0.4 mmol/L
Magnesium chloride	20 mmol/L

HDL cholesterol standard – 15mg/dl

Sample: Unhemolysed serum

PRECIPITATION:

Precipitation of LDL, VLDL and Chylomicrons done as follows:

Pipette into tubes	Volume
Sample	200µl
Precipitating reagent	500µl

Mixed well and the reaction mixture was allowed to stand for 10 min at room temperature, centrifuged at 4000 rpm for 10min and collected the

clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

Assay procedure:

Pipette into tubes marked	Blank	Standard	Test
Cholesterol working reagent	1000µl	1000µl	1000µl
Distilled water	100µl	-	-
HDL Cholesterol standard	-	100µl	-
Sample Supernatant	-	-	100µl

Mixed well and incubated for 10 minutes at room temperature.

The absorbance of the standard and the test samples were read at 500 nm against reagent blank.

Calculation:

$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X Conc. of std. X Dilution factor}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 52.5$$

= mg/dl HDL Cholesterol.

Reference values: 35 – 60 mg/dl

Linearity: 150 mg/dl; **Detection limit**: 3.0 mg/dl

ESTIMATION OF SERUM LDL CHOLESTEROL:

BY FRIEDEWALD FORMULA:

LDL = TOTAL CHOLESTEROL – (HDL + VLDL)

VLDL = TGL/5, if TGL is less than 400mg/dl.

Reference values:

Serum / plasma LDL: 100 – 129 mg/dl

VLDL: < 40 mg/dl.

CALCULATION OF ATHEROGENIC INDEX OF PLASMA (AIP)

AIP = log (TGL / HDL)

RESULTS AND STATISTICS

MASTER CHART – I

CONTROL GROUP

S.No.	AGE	SEX	SBP (mmHg)	DBP (mmHg)	WC (cm)	WT (Kg)	HT (m)	BMI	LIPID PROFILE					FBS (mg/dl)	PPBS (mg/dl)	AIP	FGF 19 (pg/ml)
									TC	TGL	HDL	LDL	VLDL				
1	43	F	126	82	83	55	1.52	23.81	154	122	45	84.6	24.4	83	126	0.43315	313
2	49	F	130	80	80	61	1.60	23.83	160	134	48	85.2	26.8	93	133	0.44586	217
3	31	F	126	80	92	53	1.52	22.94	146	128	52	68.4	25.6	72	138	0.39121	327
4	29	F	114	70	84	55	1.58	22.03	158	114	53	82.2	22.8	83	125	0.33263	181
5	26	F	130	82	88	62	1.56	25.48	142	106	41	79.8	21.2	80	130	0.41252	176
6	24	F	120	80	90	52	1.54	21.93	166	104	45	100.2	20.8	77	138	0.36382	283
7	32	F	110	70	85	60	1.58	24.03	172	118	47	101.4	23.6	95	121	0.39978	367
8	53	F	130	80	79	53	1.60	20.70	146	98	48	78.4	19.6	86	129	0.30998	296
9	49	F	114	80	87	47	1.55	19.56	150	102	52	77.6	20.4	91	115	0.2926	224
10	46	F	126	76	85	51	1.53	21.79	160	96	45	95.8	19.2	79	120	0.32906	322
11	45	F	120	80	86	64	1.60	25.0	176	124	42	109.2	24.8	74	118	0.47017	226
12	38	F	112	80	82	48	1.53	20.50	162	118	50	88.4	23.6	85	114	0.37291	259
13	43	F	124	84	85	54	1.59	21.36	158	104	44	93.2	20.8	90	120	0.37358	232
14	28	F	116	70	90	58	1.62	22.10	172	112	48	101.6	22.4	89	131	0.36798	294
15	42	F	128	82	95	58	1.59	22.94	168	96	41	107.8	19.2	96	119	0.36949	185
16	52	F	112	70	93	52	1.54	21.93	154	82	45	92.6	16.4	93	123	0.2606	180
17	42	F	100	80	91	51	1.50	22.67	150	94	48	83.2	18.8	82	130	0.29189	320
18	39	F	126	76	89	45	1.42	22.32	146	78	40	90.4	15.6	77	132	0.29003	346
19	48	F	124	80	84	54	1.60	21.09	168	118	52	92.4	23.6	90	128	0.35588	201
20	39	F	116	72	84	54	1.52	23.37	158	110	44	92	22	74	122	0.39794	192
21	52	F	104	76	87	53	1.52	22.94	142	128	40	76.4	25.6	83	119	0.50515	207
22	31	F	114	78	88	54	1.59	21.36	164	132	48	89.6	26.4	89	132	0.43933	323
23	38	F	124	80	91	53	1.51	23.24	172	120	51	97	24	95	137	0.37161	324
24	29	F	128	78	93	60	1.58	24.03	166	114	42	101.2	22.8	91	122	0.43366	294
25	49	F	120	70	85	54	1.51	23.68	168	122	47	96.6	24.4	95	111	0.41426	167

26	37	M	128	80	83	56	1.60	21.88	154	102	48	85.6	20.4	84	135	0.32736	168
27	42	M	136	82	88	55	1.57	22.31	146	96	52	74.8	19.2	90	128	0.26627	334
28	48	M	130	80	83	62	1.60	24.22	132	76	41	75.8	15.2	73	131	0.26803	360
29	30	M	128	74	94	54	1.62	20.58	146	82	45	84.6	16.4	85	138	0.2606	244
30	41	M	126	80	92	52	1.56	21.37	178	108	48	108.4	21.6	83	129	0.35218	231
31	60	M	130	82	82	60	1.62	22.86	166	114	44	99.2	22.8	94	125	0.41345	347
32	32	M	120	74	98	64	1.60	25	141	84	47	77.2	16.8	90	130	0.25218	355
33	52	M	126	76	83	62	1.58	24.84	154	118	54	76.4	23.6	86	112	0.33949	315
34	33	M	130	80	91	56	1.59	22.15	134	80	42	76	16	76	113	0.27984	373
35	40	M	124	82	88	54	1.56	22.19	146	92	45	82.6	18.4	92	136	0.31058	306
36	46	M	128	76	90	46	1.54	19.39	178	96	48	110.8	19.2	78	125	0.30103	297
37	38	M	110	70	87	50	1.64	18.59	159	120	46	89	24	88	132	0.41642	273
38	49	M	120	78	79	56	1.53	23.92	165	111	41	101.8	22.2	72	145	0.43254	229
39	33	M	124	80	82	60	1.62	22.86	149	99	50	79.2	19.8	92	139	0.29667	175
40	50	M	130	84	87	52	1.56	21.37	144	109	44	78.2	21.8	75	122	0.39397	171
41	45	M	126	76	80	56	1.63	21.08	178	92	45	114.6	18.4	88	139	0.31058	267
42	41	M	122	80	92	60	1.57	24.34	168	124	41	102.2	24.8	94	136	0.48064	173
43	55	M	120	70	94	62	1.65	22.77	152	118	52	76.4	23.6	74	127	0.35588	285
44	22	M	120	84	82	58	1.63	21.83	167	95	49	99	19	92	133	0.28753	287
45	42	M	110	70	85	58	1.60	22.66	146	102	44	81.6	20.4	78	115	0.36515	296
46	26	M	124	78	79	62	1.70	21.45	170	110	45	103	22	87	121	0.38818	231
47	53	M	120	78	94	60	1.71	20.52	154	114	41	90.2	22.8	94	127	0.44412	364
48	39	M	114	70	83	54	1.63	20.32	163	98	44	99.4	19.6	95	118	0.34777	179
49	52	M	130	82	80	60	1.68	21.26	180	126	54	100.8	25.2	83	137	0.36798	362
50	26	M	126	80	79	64	1.75	20.89	146	104	48	77.2	20.8	90	120	0.33579	239

MASTER CHART – II

STUDY GROUP

S.No.	AGE	SEX	SBP (mmHg)	DBP (mmHg)	WC (cm)	WT (Kg)	HT (m)	BMI	LIPID PROFILE					FBS (mg/dl)	PPBS (mg/dl)	AIP	FGF 19 (pg/ml)
									TC	TGL	HDL	LDL	VLDL				
1	46	F	140	96	104	90	1.51	39.47	260	272	32	173.6	54.4	125	186	0.92942	110
2	52	F	130	88	95	81	1.55	33.71	232	240	36	148	48	118	172	0.82391	125
3	43	F	132	90	96	84	1.53	35.88	238	252	31	156.6	50.4	118	167	0.91004	130
4	54	F	138	94	102	92	1.60	35.94	256	268	34	168.4	53.6	122	182	0.89666	116
5	48	F	140	92	100	90	1.53	38.45	252	250	36	166	50	123	182	0.84164	114
6	41	F	130	86	92	77	1.50	34.22	212	186	40	134.8	37.2	112	152	0.66745	142
7	43	F	132	90	98	91	1.60	35.55	236	248	34	152.4	49.6	124	176	0.86297	122
8	46	F	136	92	98	84	1.53	35.88	243	252	36	156.6	50.4	120	180	0.8451	120
9	64	F	142	94	102	87	1.56	35.75	264	292	30	175.6	58.4	124	188	0.98826	104
10	42	F	132	86	87	82	1.62	31.25	206	164	41	132.2	32.8	114	164	0.60206	157
11	53	F	134	90	96	99	1.60	38.67	236	248	37	149.4	49.6	118	176	0.82625	124
12	49	F	132	88	90	77	1.55	32.05	212	172	40	137.6	34.4	118	167	0.63347	153
13	47	F	130	84	86	73	1.56	29.99	210	166	40	136.8	33.2	111	143	0.61805	171
14	51	F	132	88	94	86	1.58	34.45	232	244	38	145.2	48.8	116	174	0.80761	126
15	45	F	134	90	94	91	1.60	35.55	240	234	38	155.2	46.8	114	163	0.78943	138
16	52	F	130	84	90	81	1.55	33.71	213	204	40	132.2	40.8	106	152	0.70757	145
17	49	F	138	90	102	90	1.55	37.46	242	248	37	155.4	49.6	121	181	0.82625	118
18	62	F	130	88	92	81	1.55	33.71	230	240	39	143	48	115	170	0.78915	130
19	58	F	134	90	96	91	1.60	35.55	256	244	36	171.2	48.8	118	172	0.83109	128
20	71	F	132	88	100	81	1.55	33.71	247	236	37	162.8	47.2	118	180	0.80471	119
21	54	F	132	86	90	77	1.55	32.05	228	234	41	140.2	46.8	116	162	0.75643	132
22	63	F	135	92	98	91	1.58	36.45	214	261	36	125.8	52.2	119	175	0.86034	123
23	49	F	134	92	96	84	1.53	35.88	240	270	30	156	54	120	172	0.95424	129
24	53	F	130	86	88	86	1.65	31.59	210	168	42	134.4	33.6	116	165	0.60206	160
25	46	F	134	86	94	84	1.61	32.41	224	196	41	143.8	39.2	109	150	0.67947	144

26	45	M	142	94	102	93	1.52	40.25	265	284	31	177.2	56.8	124	187	0.96196	103
27	38	M	132	90	95	83	1.54	34.99	232	256	32	148.8	51.2	118	165	0.90309	134
28	43	M	144	94	106	94	1.52	40.69	273	285	29	187	57	123	191	0.99245	102
29	42	M	130	86	93	84	1.56	34.52	216	234	35	134.2	46.8	114	153	0.82515	148
30	42	M	132	85	94	82	1.62	31.25	219	195	42	138	39	111	148	0.66679	153
31	52	M	136	90	100	88	1.54	37.11	240	234	39	154.2	46.8	118	176	0.77815	127
32	44	M	138	94	98	85	1.52	36.79	226	265	34	139	53	123	182	0.89177	113
33	39	M	146	94	107	94	1.51	41.23	276	281	29	190.8	56.2	125	194	0.98631	100
34	48	M	130	82	86	72	1.53	30.76	205	163	43	129.4	32.6	110	146	0.57872	167
35	46	M	132	80	88	75	1.6	29.29	209	159	44	133.2	31.8	112	145	0.55794	172
36	52	M	130	82	88	87	1.62	33.15	226	232	39	140.6	46.4	116	164	0.77442	145
37	39	M	134	88	96	81	1.52	35.06	235	224	35	155.2	44.8	123	167	0.80618	141
38	38	M	136	94	97	88	1.57	35.70	264	259	34	178.2	51.8	126	182	0.88182	118
39	52	M	128	88	96	81	1.54	34.15	208	228	37	125.4	45.6	109	143	0.78973	158
40	49	M	134	86	98	82	1.61	31.63	226	199	39	147.2	39.8	120	158	0.70779	133
41	37	M	132	86	92	81	1.56	33.28	231	243	35	147.4	48.6	124	172	0.84154	124
42	42	M	136	94	106	87	1.52	37.66	246	239	36	162.2	47.8	125	186	0.8221	117
43	48	M	135	86	98	88	1.58	35.25	239	231	36	156.8	46.2	122	165	0.80731	144
44	37	M	130	82	88	74	1.57	30.02	217	178	40	141.4	35.6	115	158	0.64836	163
45	41	M	128	86	92	73	1.56	29.99	223	184	38	148.2	36.8	119	171	0.68503	161
46	60	M	130	80	91	70	1.55	29.14	224	178	39	149.4	35.6	111	156	0.65936	171
47	46	M	132	85	95	87	1.63	32.74	228	234	38	143.2	46.8	121	162	0.78943	144
48	52	M	128	86	87	70	1.57	28.39	220	198	42	138.4	39.6	113	161	0.67342	175
49	63	M	142	90	108	99	1.65	36.36	276	308	32	182.4	61.6	126	187	0.9834	102
50	40	M	132	87	88	77	1.61	29.71	235	228	38	151.4	45.6	119	173	0.77815	156

STATISTICAL ANALYSIS

- Student's t-test was employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- 'p' value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

STATISTICS

TABLE 1

DESCRIPTIVE STATISTICS OF CONTROL AND CASES

Variables	Control (n=50)				Cases (n=50)			
	Min	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**
SBP	100.0	136.0	121.920	7.6127	128.0	146.0	133.840	4.3064
DBP	70.0	84.0	77.640	4.4020	80.0	96.0	88.380	3.9790
WC	79.0	98.0	86.620	4.9193	86.0	108.0	95.380	5.7743
WT	45.0	64.0	55.880	4.7623	70.0	99.0	84.100	7.0313
HT	1.42	1.75	1.5828	0.05838	1.50	1.65	1.5652	0.0384
BMI	18.59	25.47	22.305	1.5341	28.39	41.22	34.369	3.1321
TC	132.0	180.0	157.880	12.1834	205.0	276.0	233.840	19.1326
TGL	76.0	134.0	106.880	14.7864	159.0	308.0	230.160	37.8015
HDL	40.0	54.0	46.320	3.9354	29.0	44.0	36.760	3.7881
VLDL	15.2	26.8	21.376	2.9573	31.8	61.6	46.032	7.5603
LDL	68.4	114.6	90.184	11.5157	125.4	190.8	151.048	16.1828
FBS	72.0	96.0	85.500	7.3158	106.0	126.0	118.040	5.1109
PPBS	111.0	145.0	126.920	8.2853	143.0	194.0	168.860	13.4271
AIP	.2521	0.5051	0.3603	0.06328	0.55794	0.99244	0.79287	0.11597
FGF 19	167.0	373.0	266.340	65.5070	100.0	175.0	135.020	20.7556

TABLE 2: STATISTICAL ANALYSIS OF SERUM FGF 19

BETWEEN CASES AND CONTROLS

T-TEST			
FGF 19 (pg/ml)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	266.340	65.5070	p value = .000 <0.05 – Significant
Cases (n=50)	135.020	20.7556	

BAR CHART 1

COMPARISON OF SERUM FGF 19 LEVEL

BETWEEN CONTROLS AND CASES

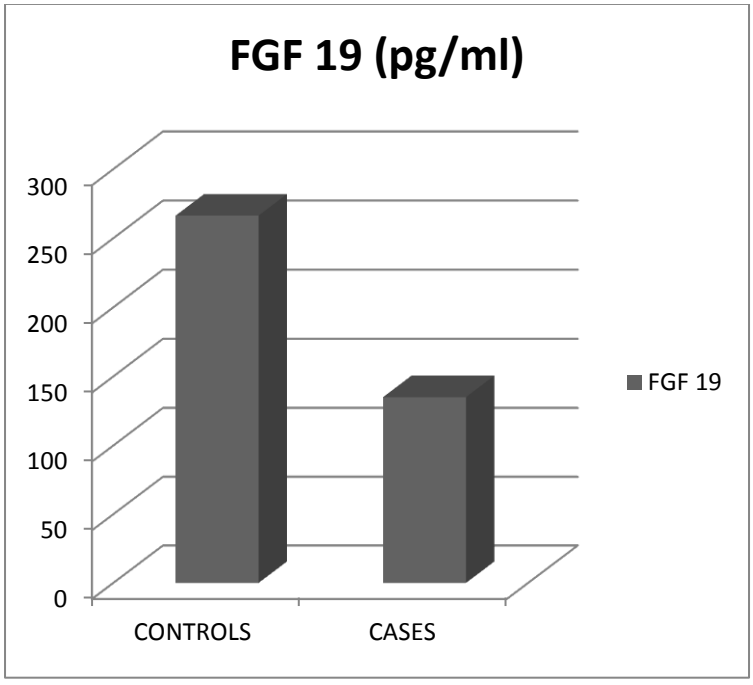


TABLE 3: STATISTICAL ANALYSIS OF AIP

BETWEEN CASES AND CONTROLS

T-TEST			
AIP	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	0.3603	0.06328	p value = .000 <0.05 – Significant
Cases (n=50)	0.79287	0.11597	

BAR CHART 2

COMPARISON OF SERUM AIP

BETWEEN CONTROLS AND CASES

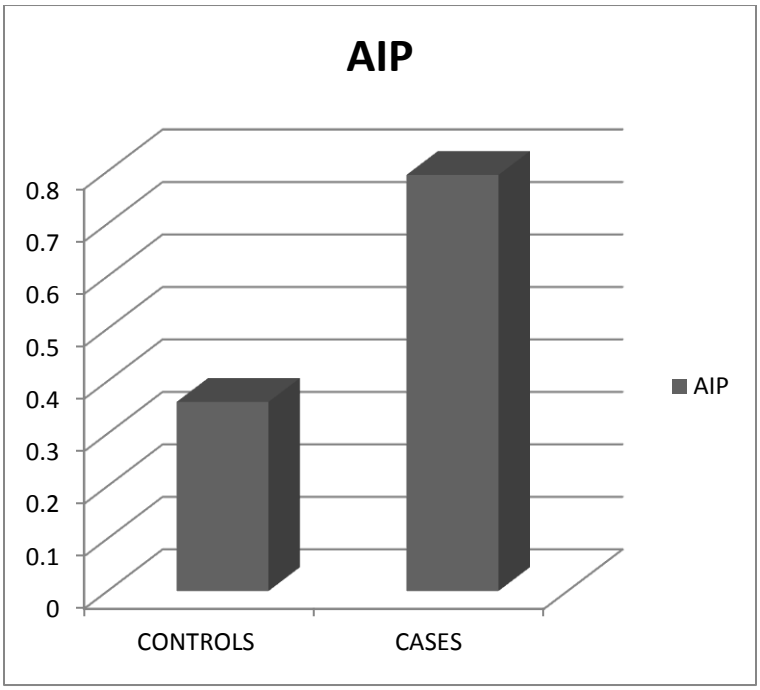


TABLE 4: STATISTICAL ANALYSIS OF FBS

BETWEEN CASES AND CONTROLS

T-TEST			
FBS (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	85.500	7.3158	p value = .000 <0.05 – Significant
Cases (n=50)	118.040	5.1109	

BAR CHART 3

COMPARISON OF SERUM FBS

BETWEEN CONTROLS AND CASES

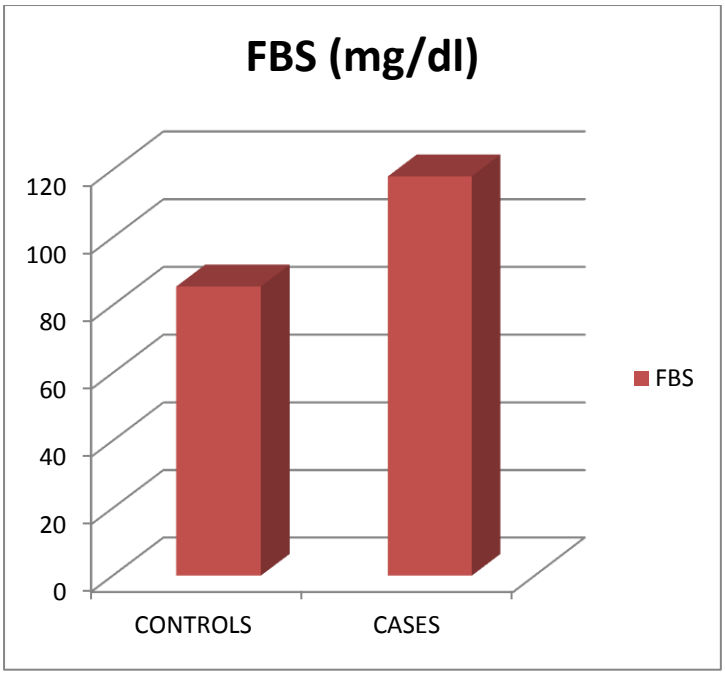


TABLE 5: STATISTICAL ANALYSIS OF PPBS

BETWEEN CASES AND CONTROLS

T-TEST			
PPBS (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	126.920	8.2853	p value = .000 <0.05 – Significant
Cases (n=50)	168.860	13.4271	

BAR CHART 4

COMPARISON OF SERUM PPBS

BETWEEN CONTROLS AND CASES

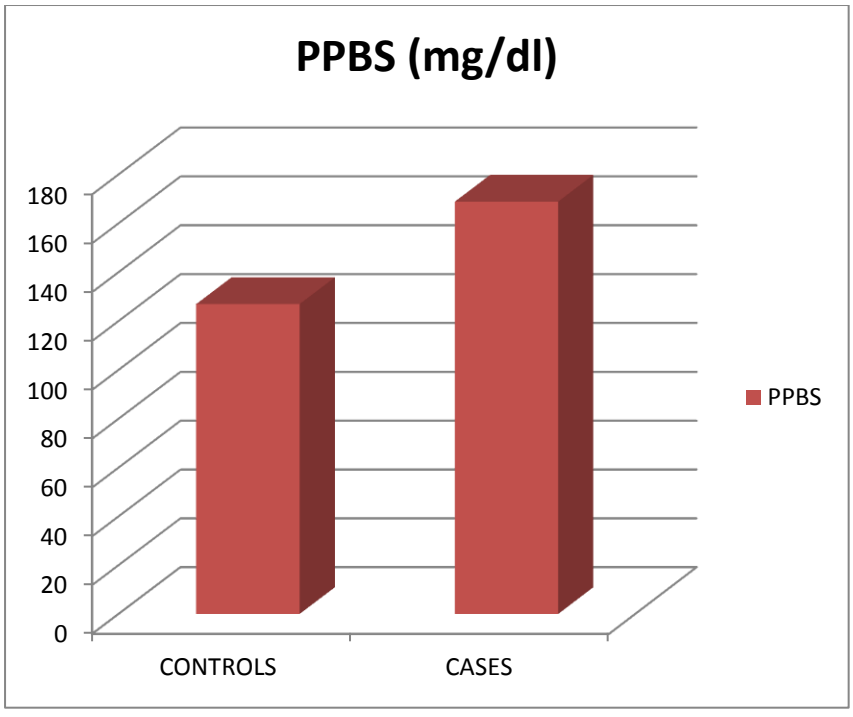


TABLE 6: STATISTICAL ANALYSIS OF TC

BETWEEN CASES AND CONTROLS

T-TEST			
TC (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	157.880	12.1834	p value = .000 <0.05 – Significant
Cases (n=50)	233.840	19.1326	

BAR CHART 5

COMPARISON OF SERUM TC

BETWEEN CONTROLS AND CASES

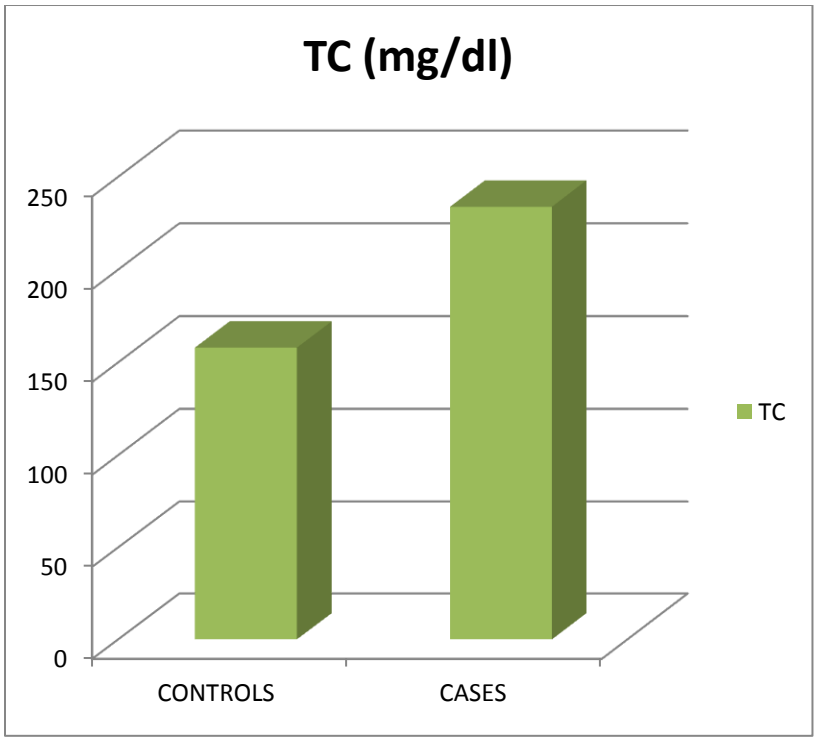


TABLE 7: STATISTICAL ANALYSIS OF TGL

BETWEEN CASES AND CONTROLS

T-TEST			
TGL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	106.880	14.7864	p value = .000 <0.05 – Significant
Cases (n=50)	230.160	37.8015	

BAR CHART 6

COMPARISON OF SERUM TGL

BETWEEN CONTROLS AND CASES

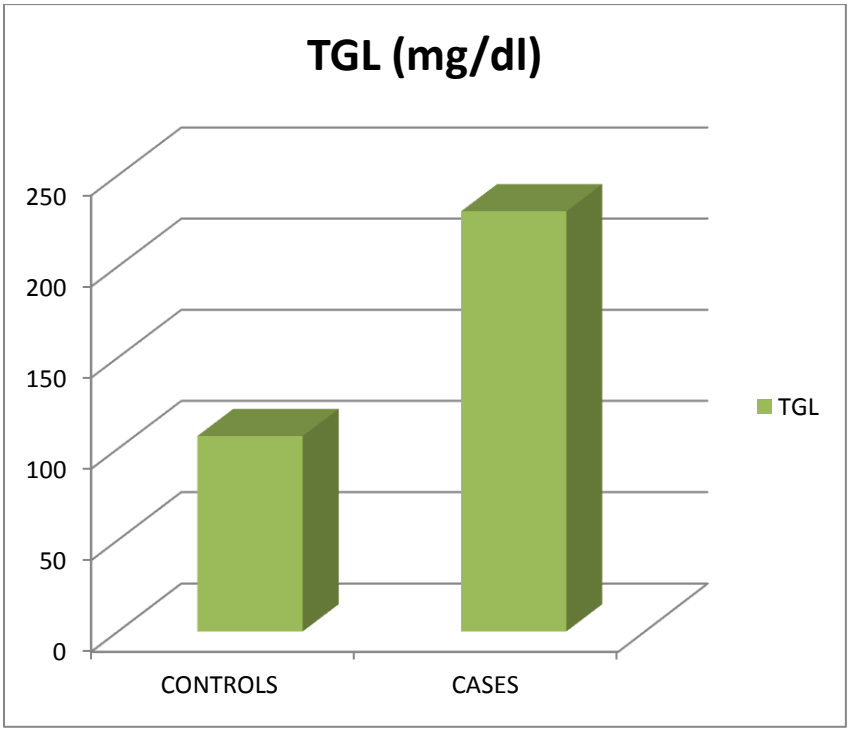


TABLE 8: STATISTICAL ANALYSIS OF HDL

BETWEEN CASES AND CONTROLS

T-TEST			
HDL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	46.320	3.9354	p value = .000 <0.05 – Significant
Cases (n=50)	36.760	3.7881	

BAR CHART 7

COMPARISON OF SERUM HDL

BETWEEN CONTROLS AND CASES

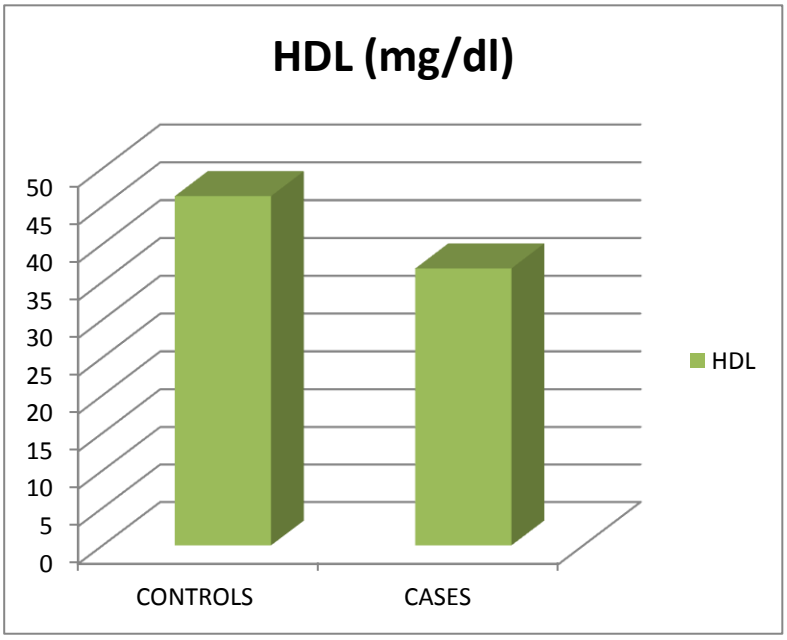


TABLE 9: STATISTICAL ANALYSIS OF VLDL

BETWEEN CASES AND CONTROLS

T-TEST			
VLDL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	21.376	2.9573	p value = .000 <0.05 – Significant
Cases (n=50)	46.032	7.5603	

BAR CHART 8

COMPARISON OF SERUM VLDL

BETWEEN CONTROLS AND CASES

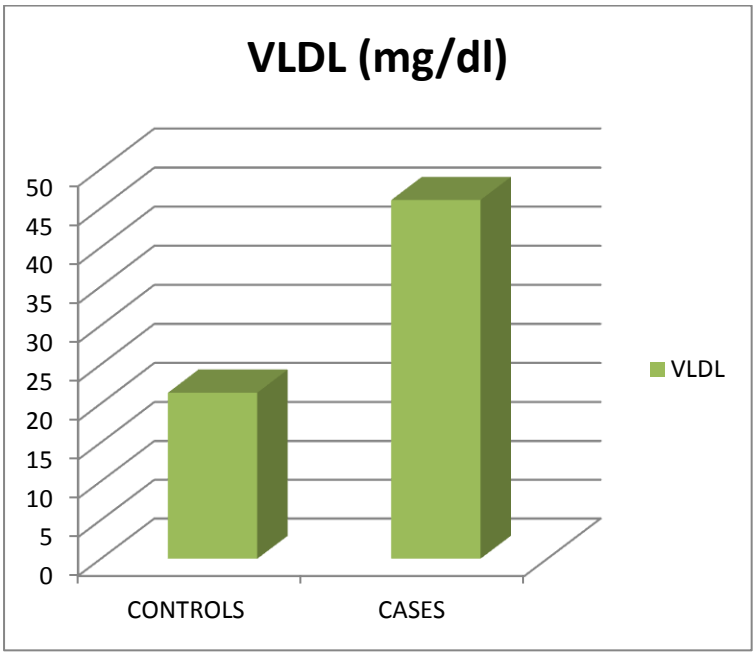


TABLE 10: STATISTICAL ANALYSIS OF LDL

BETWEEN CASES AND CONTROLS

T-TEST			
LDL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	90.184	11.5157	p value = .000 <0.05 – Significant
Cases (n=50)	151.048	16.1828	

BAR CHART 9

COMPARISON OF SERUM LDL

BETWEEN CONTROLS AND CASES

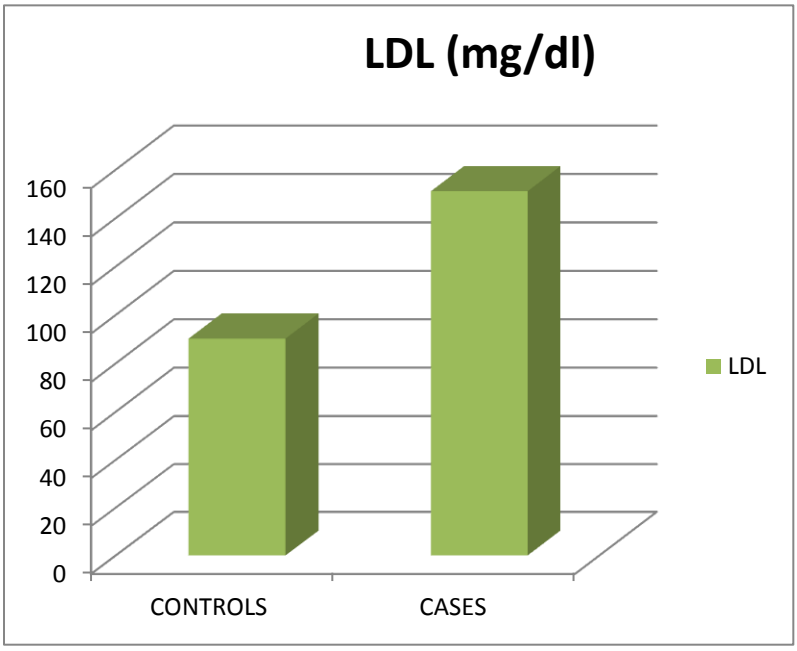


TABLE 11: STATISTICAL ANALYSIS OF HEIGHT

BETWEEN CASES AND CONTROLS

T-TEST			
HEIGHT (mt)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	1.5828	0.05838	p value = .042 <0.05 – Significant
Cases (n=50)	1.5652	0.0384	

BAR CHART 10

COMPARISON OF HEIGHT

BETWEEN CONTROLS AND CASES

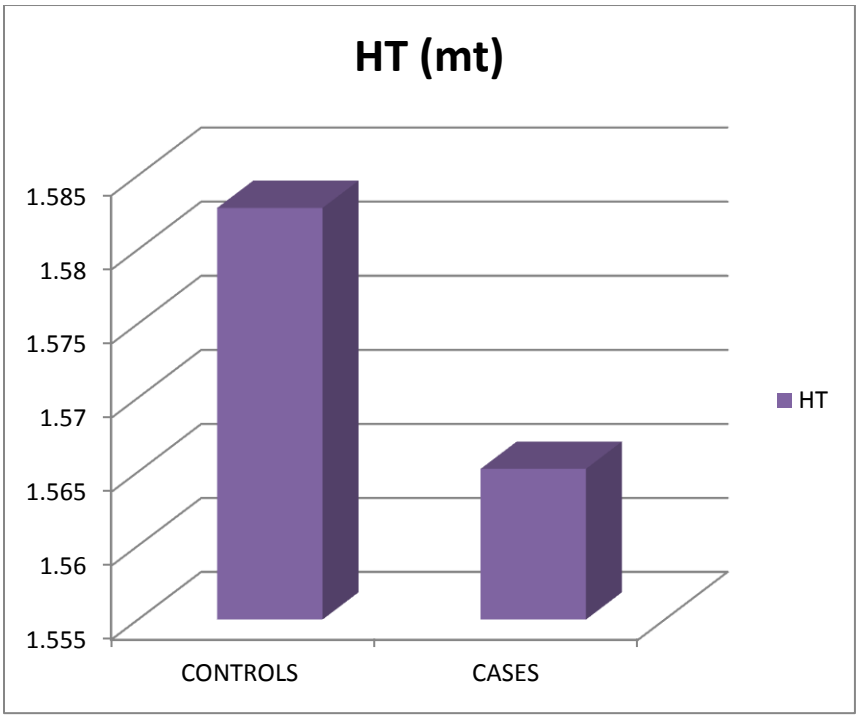


TABLE 12: STATISTICAL ANALYSIS OF WEIGHT

BETWEEN CASES AND CONTROLS

T-TEST			
WEIGHT (kg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	55.880	4.7623	p value = .000 <0.05 – Significant
Cases (n=50)	84.100	7.0313	

BAR CHART 11

COMPARISON OF WEIGHT

BETWEEN CONTROLS AND CASES

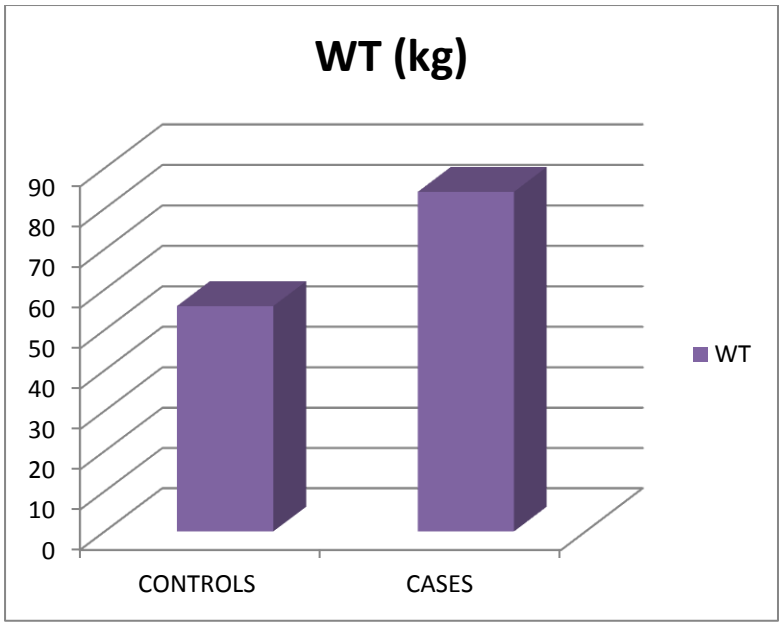


TABLE 13: STATISTICAL ANALYSIS OF BMI

BETWEEN CASES AND CONTROLS

T-TEST			
BMI (kg/m^2)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	22.305	1.5341	p value = .000 <0.05 – Significant
Cases (n=50)	34.369	3.1321	

BAR CHART 12

COMPARISON OF BMI

BETWEEN CONTROLS AND CASES

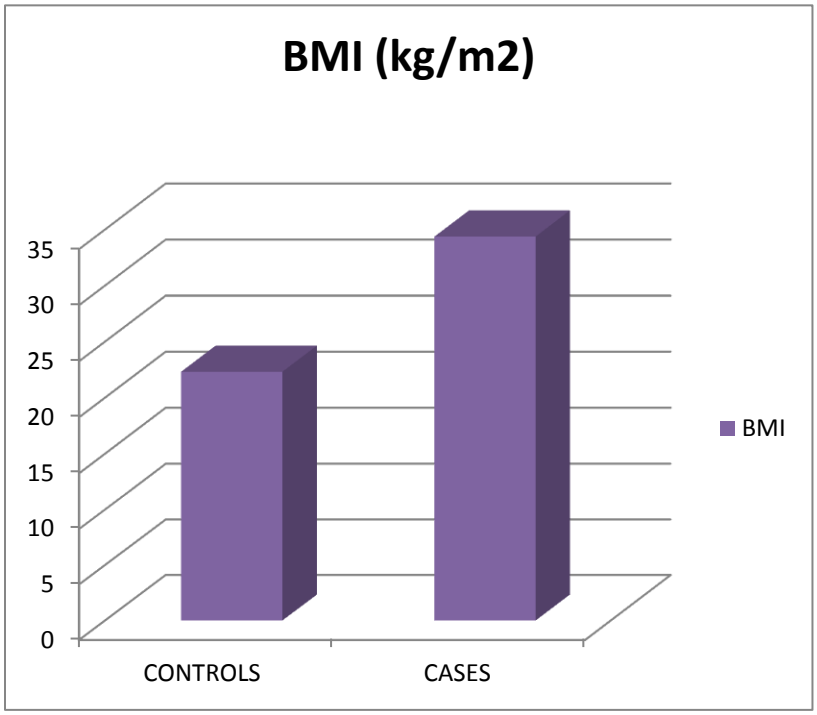


TABLE 14: STATISTICAL ANALYSIS OF WAIST CIRCUMFERENCE BETWEEN CASES AND CONTROLS

T-TEST			
WC (cm)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	86.620	4.9193	p value = .000 <0.05 – Significant
Cases (n=50)	95.380	5.7743	

BAR CHART 13

COMPARISON OF WAIST CIRCUMFERENCE BETWEEN CONTROLS AND CASES

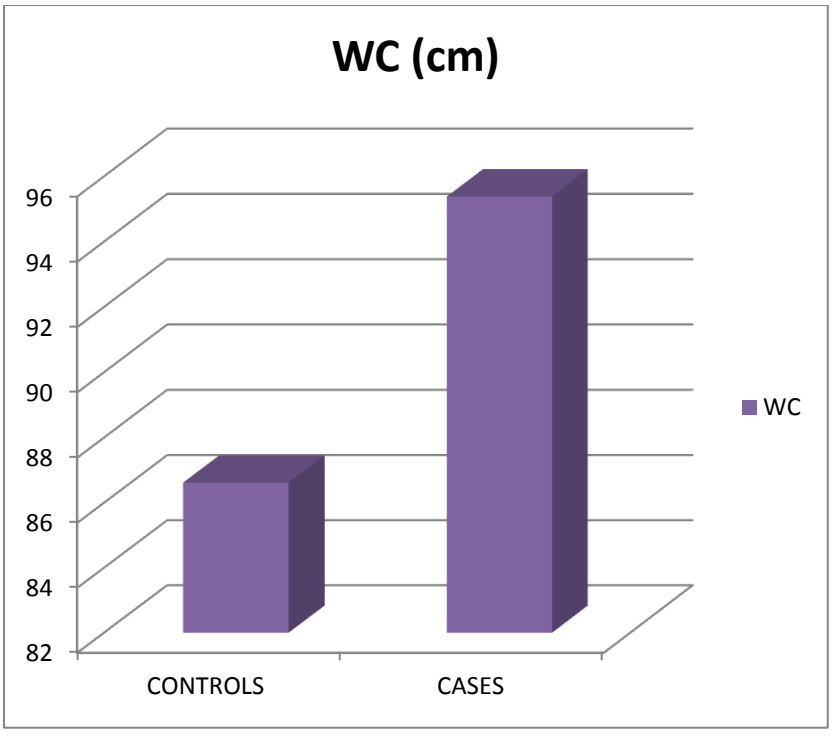


TABLE 15: STATISTICAL ANALYSIS OF SYSTOLIC BP

BETWEEN CASES AND CONTROLS

T-TEST			
SYSTOLIC BP (mmHg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	121.920	7.6127	p value = .000 <0.05 – Significant
Cases (n=50)	133.840	4.3064	

BAR CHART 14

COMPARISON OF SYSTOLIC BP

BETWEEN CONTROLS AND CASES

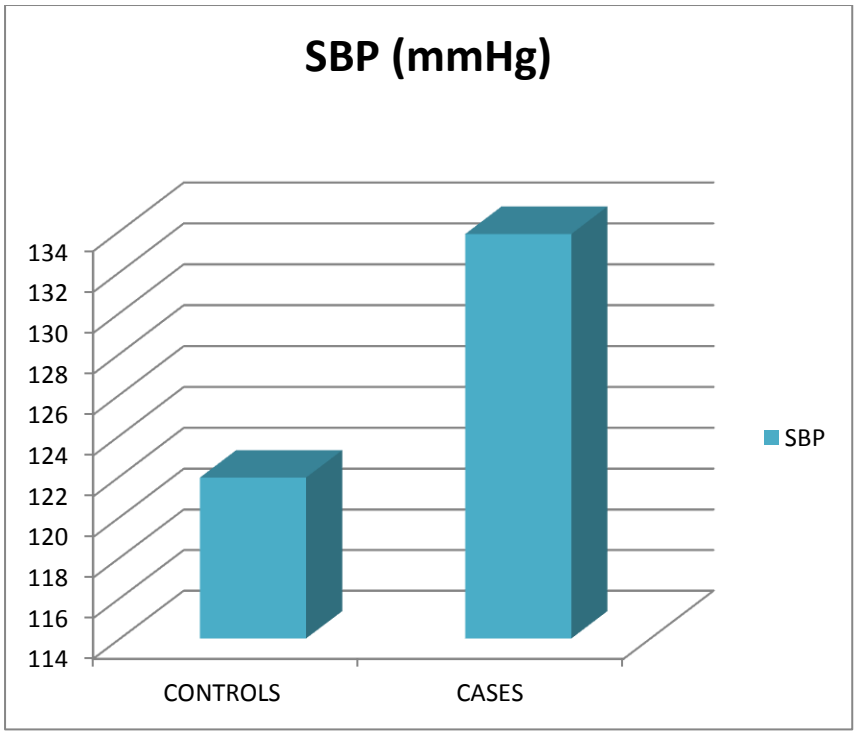


TABLE 16: STATISTICAL ANALYSIS OF DIASTOLIC BP

BETWEEN CASES AND CONTROLS

T-TEST			
DIASTOLIC BP (mmHg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	77.640	4.4020	p value = .000 <0.05 – Significant
Cases (n=50)	88.380	3.9790	

BAR CHART 15

COMPARISON OF DIASTOLIC BP

BETWEEN CONTROLS AND CASES

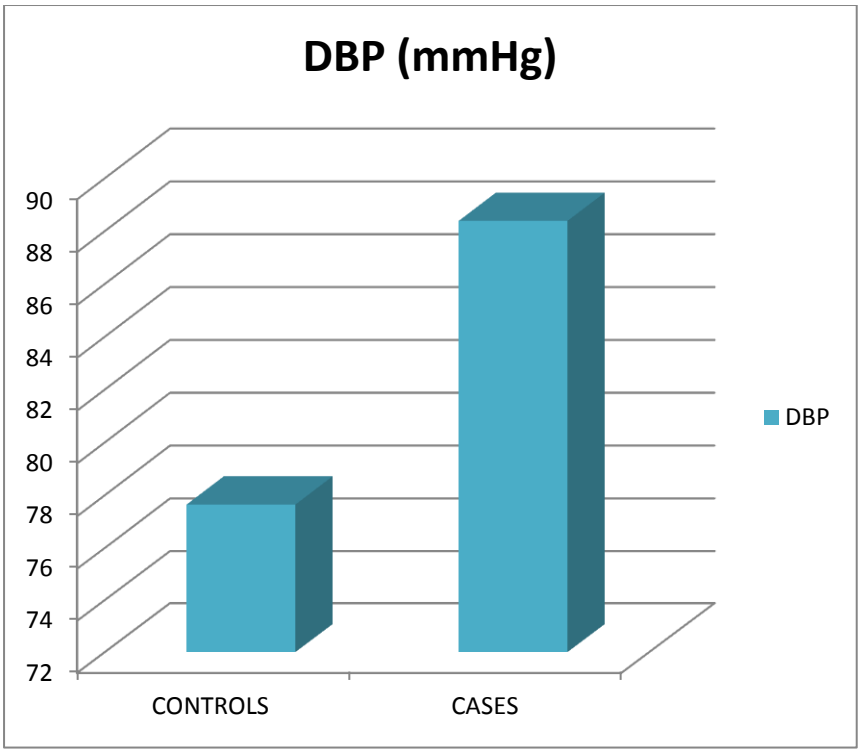


TABLE 17:

PEARSONS CORRELATION BETWEEN FGF 19

AND OTHER PARAMETERS

CASES – FGF 19	CORRELATION VALUE	STATISTICAL INFERENCE
BMI	-0.875	P < 0.01 Significant
WAIST CIRCUMFERENCE	-0.864	P < 0.01 Significant
SBP	-0.808	P < 0.01 Significant
DBP	-0.841	P < 0.01 Significant
FBS	-0.754	P < 0.01 Significant
PPBS	-0.853	P < 0.01 Significant
TC	-0.827	P < 0.01 Significant
TGL	-0.892	P < 0.01 Significant
HDL	+0.773	P < 0.01 Significant
VLDL	-0.892	P < 0.01 Significant
LDL	-0.742	P < 0.01 Significant
AIP	-0.873	P < 0.01 Significant

RESULTS

A total of 100 participants were included in the study. Out of these, 50 were grouped under controls and 50 were under cases.

The serum value of FGF 19, fasting and post prandial blood sugar, Total Cholesterol, HDL and TGL were estimated for all the samples in both the groups. BMI, VLDL, LDL and AIP were calculated. The values obtained for the cases and controls are represented in the master chart I and II respectively.

TABLE: 1

Shows the descriptive base line statistics of the controls and the cases. It includes the mean values of Anthropometric data, Blood pressure, serum FGF 19, AIP, Fasting and Post prandial blood sugars and Lipid profile.

TABLE: 2

Shows student's t-test analysis of serum FGF 19 level between cases and controls.

There is decrease in the mean serum FGF 19 in cases (135.02 ± 20.76 pg/ml), when compared to the mean serum FGF 19 in controls (266.34 ± 65.5 pg/ml), which is statistically significant. (p value < 0.05).

TABLE: 3

Shows student's t-test analysis of serum AIP between cases and controls.

There is increase in the mean AIP in cases (0.79± 0.12), when compared to the mean AIP in controls (0.36± 0.06), which is statistically significant. (p value < 0.05).

TABLE: 4

Shows student's t-test analysis of serum FBS between cases and controls.

There is increase in the mean FBS in cases (118.04 ± 5.11), when compared to the mean FBS in controls (85.5 ± 7.32), which is statistically significant. (p value < 0.05).

TABLE: 5

Shows student's t-test analysis of serum PPBS between cases and controls.

There is increase in the mean PPBS in cases (168.86 ± 13.43), when compared to the mean PPBS in controls (126.9 ± 8.29), which is statistically significant. (p value < 0.05).

TABLE: 6

Shows student's t-test analysis of serum TC between cases and controls.

There is increase in the mean TC in cases (233.84 ± 19.13), when compared to the mean TC in controls (157.88 ± 12.18), which is statistically significant. (p value < 0.05).

TABLE: 7

Shows student's t-test analysis of serum TGL between cases and controls.

There is increase in the mean TGL in cases (230.16 ± 37.8), when compared to the mean TGL in controls (106.88 ±14.79), which is statistically significant. (p value < 0.05).

TABLE: 8

Shows student's t-test analysis of serum HDL between cases and controls.

There is decrease in the mean HDL in cases (36.76 ± 3.79), when compared to the mean HDL in controls (46.32 ± 3.94), which is statistically significant. (p value < 0.05).

TABLE: 9

Shows student's t-test analysis of serum VLDL between cases and controls.

There is increase in the mean VLDL in cases (46.03 ±7.56), when compared to the mean VLDL in controls (21.38 ± 2.96), which is statistically significant. (p value < 0.05).

TABLE: 10

Shows student's t-test analysis of serum LDL between cases and controls.

There is increase in the mean LDL in cases (151.05 ± 16.18), when compared to the mean LDL in controls (90.18 ± 11.52), which is statistically significant. (p value < 0.05).

TABLE: 11

Shows student's t-test analysis of Height between cases and controls.

There is decrease in the mean Height in cases (1.57 ± 0.04), when compared to the mean Height in controls (1.58 ±0.06), which is statistically significant. (p value < 0.05).

TABLE: 12

Shows student's t-test analysis of Weight between cases and controls.

There is increase in the mean Weight in cases (84.1 ± 7.03), when compared to the mean Weight in controls (55.88 ± 4.76), which is statistically significant. (p value < 0.05).

TABLE: 13

Shows student's t-test analysis of BMI between cases and controls.

There is increase in the mean BMI in cases (34.37 ± 3.13), when compared to the mean BMI in controls (22.31 ± 1.53), which is statistically significant. (p value < 0.05).

TABLE: 14

Shows student's t-test analysis of Waist circumference between cases and controls.

There is increase in the mean Waist circumference in cases (95.38 ± 5.77), when compared to the mean Waist circumference in controls (86.62 ± 4.92), which is statistically significant. (p value < 0.05).

TABLE: 15

Shows student's t-test analysis of Systolic BP between cases and controls.

There is increase in the mean Systolic BP in cases (133.84 ± 4.31), when compared to the mean Systolic BP in controls (121.92 ± 7.61), which is statistically significant. (p value < 0.05).

TABLE: 16

Shows student's t-test analysis of Diastolic BP between cases and controls.

There is increase in the mean Diastolic BP in cases (88.38 ± 3.98), when compared to the mean Diastolic BP in controls (77.64 ± 4.4), which is statistically significant. (p value < 0.05).

TABLE: 17

Pearson correlation between FGF 19 and other parameters.

This table shows negative correlation between serum FGF 19 and BMI, Waist circumference, systolic and diastolic BP, AIP, fasting and post prandial blood sugar, serum TC, TGL, VLDL and LDL concentrations and a positive correlation with serum HDL concentration, which are statistically significant. (p < 0.05)

DISCUSSION

DISCUSSION

FGF 19 is a newly identified metabolic regulator, influencing homeostasis of glucose and lipid metabolism. It has been concluded that the expression of FGF 19 in liver is induced by FXR (Farnesoid X receptor), a transcription factor. The natural ligand for the FXR receptor was identified as bile acids. So, FXR acts as a "bile acid sensor" inducing the expression of FGF 19. FGF 19 inhibits the enzyme CYP7A1 in liver, thereby inhibiting the rate limiting step of Bile acid synthesis from Cholesterol. The repression of bile acid synthesis is the net result of FXR activation. FXR not only regulates bile acid metabolism, but also metabolism of cholesterol, triglyceride, lipoprotein and glucose. The dysregulations of glucose, cholesterol and triglyceride metabolism lead to Metabolic syndrome.

Animal studies revealed that Recombinant FGF 19 increased the metabolic rate, decreased body weight and reversed diabetes. This led to further research which suggested that FGF 19 increases oxidation of lipids and increases the activity of Carnitine Acyl transferase 1, favouring fatty acid oxidation. Therefore it was concluded that FGF 19 might improve dyslipidemia, reduce adiposity and body weight and also improve insulin sensitivity[78].

In this study, in patients with Metabolic syndrome, serum levels of FGF 19 (135.02 ± 20.76 pg/ml) are significantly lower than that of the healthy controls (266.34 ± 65.5 pg/ml).

Additionally, Serum TGL and HDL-C also differed significantly between cases and controls. Comparison of mean value of AIP of the controls (0.36± 0.06) and the cases (0.79± 0.12) showed a significant rise in the cases.

Pearson correlation showed negative correlation between FGF 19 and other cardiovascular risk factors like TGL and AIP and a positive correlation with cardio protective factors like HDL.

In patients with Metabolic syndrome, obesity is a main factor contributing to Insulin resistance, which plays a potent role in the pathogenesis of Cardiovascular diseases. Obesity also promotes atherogenic dyslipidemia. Dyslipidemia favours development of CVD. Hyper triglyceridemia is an independent risk factor for CVD. FGF 19 increases fatty acid oxidation, which decreases the concentration of triglycerides. Hence, a low FGF 19 level is invariably associated with increased atherogenity of plasma, leading on to Cardiovascular diseases.

Hence these observations suggest that FGF 19 is a novel marker of Metabolic syndrome and is used to assess cardiovascular risk in patients with Metabolic syndrome.

CONCLUSION

CONCLUSION

This study shows that serum levels of FGF 19 are low in patients with Metabolic syndrome. The negative relationship obtained between FGF 19 and several other known cardiovascular risk factors like TGL and log (TGL / HDL-C) suggests that FGF 19 can be used as a novel marker in assessing cardiovascular risk in patients with Metabolic syndrome.

Hence, earlier intervention can be taken to reduce the cardiovascular complications.

LIMITATIONS OF THE STUDY

LIMITATIONS OF THE STUDY

The study has the following limitations:

1. This is a pilot study including a small number of patients and controls.
2. Other valuable relevant markers like fasting Insulin, hsCRP, HbA1C were not included in the study.

FUTURE SCOPE OF THE STUDY

FUTURE SCOPE OF THE STUDY

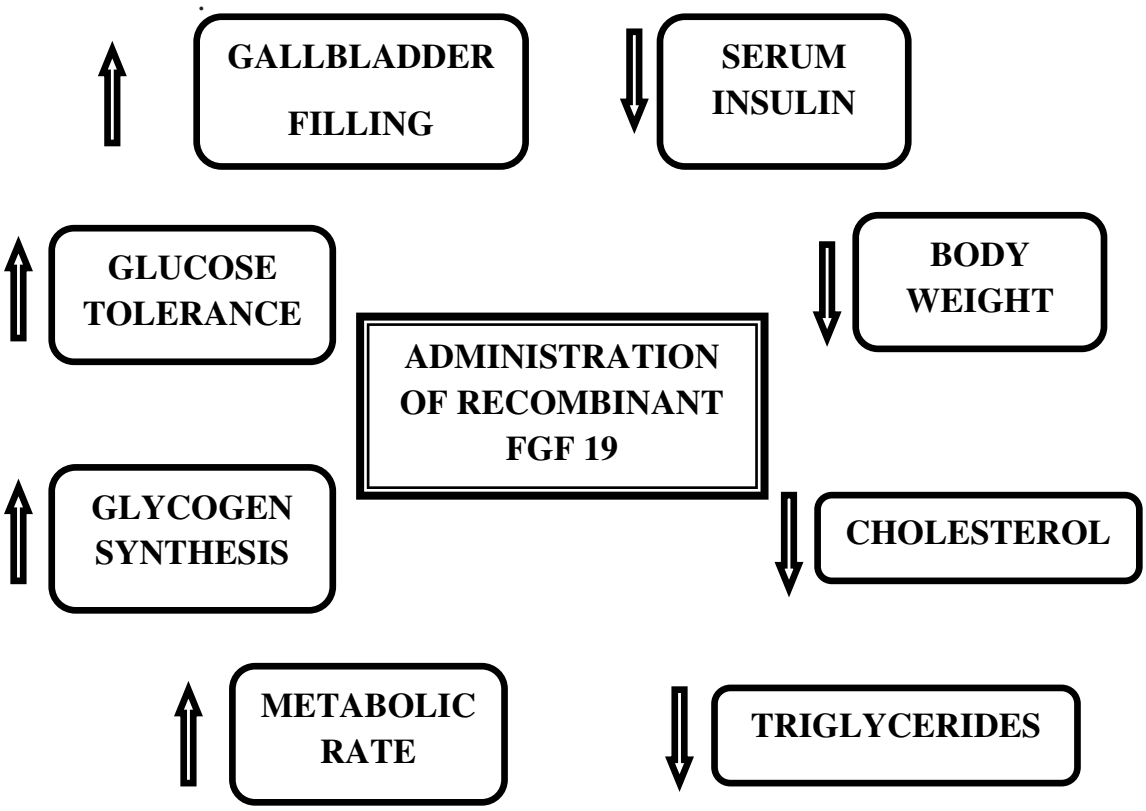
TREATMENT WITH FGF 19 IN METABOLIC SYNDROME:

In patients with obesity, metabolic syndrome and non alcoholic fatty liver, FGF 19 serum levels has been decreased [56,61]. And these three diseases predisposes to Coronary Artery Disease (CAD). Hence studies concluded that FGF 19 has its own contribution to CAD[56].

FGF 19 has beneficial effects on metabolism of Carbohydrates and Lipid. FGF 19 can be supplemented as a treatment modality in metabolic disorders[69]. FGF 19 can be used as a potential target for treating dyslipidemia[3].

Therapies with recombinant FGF 19 is under research to treat diabetes and bile acid disorders[51]. FGF 19 is considered as a new weapon to combat increasing incidence of obesity, metabolic syndrome and type 2 Diabetes. It helps to explore potential targets for treating the metabolic disorders[78].

METABOLIC EFFECTS OF FGF 19 TREATMENT[69]



ANNEXURE

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PROFORMA

A STUDY OF SERUM LEVEL OF FIBROBLAST GROWTH FACTOR-19 IN METABOLIC SYNDROME

PROFORMA

NAME OF THE PATIENT :

AGE :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

HT: WT: BMI:

BP: PR: WAIST CIRCUMFERENCE:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1. BLOOD SUGAR: FBS :

   PPBS :

2. LIPID PROFILE: TOTAL CHOLESTEROL:

   TRIGLYCERIDES :

   HDL-C :

   LDL-C :

   VLDL :

3. SERUM FGF 19:

CONSENT FORM

CONSENT FORM

Dr.S.ARUNA, post graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing '**A study of serum level of Fibroblast Growth Factor-19 in Metabolic Syndrome'.** The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature:

Name:

Place: