"STUDY OF CORRELATION OF INSULIN RESISTANCE AND DYSLIPIDEMIA IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE

By

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DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION, KOLAR, KARNATAKA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

GENERAL MEDICINE

Under the guidance of

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MAY 2014

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Dr. ANIL KUMAR MANNAVA

ABSTRACT

THE STUDY OF CORRELATION OF INSULIN RESISTANCE AND DYSLIPIDEMIA IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE

Background:

Non Alcoholic Fatty Liver Disease (NAFLD) is emerging as an important cause of liver disease in India. Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). Non alcoholic steatohepatitis can even progress to hepatocellular carcinoma. Insulin resistance is believed to be the key factor that leads to increased lipolysis in peripheral adipose tissue and increased uptake of fatty acids by hepatocytes. Central or abdominal obesity that is more commonly associated with insulin resistance and has been observed in 80–90% of Indian patients with NAFLD.Dyslipidemia is a common feature, and is present in approximately 50% of Indian patients with NAFLD. Non-alcoholic fatty liver disease (NAFLD) is related to insulin resistance and the metabolic syndrome and may respond to treatments originally developed for other insulin-resistant states (e.g. diabetes mellitus type 2) such as weight loss, metformin and thiazolidinediones.

Objectives:

1. To estimate HOMA-IR and serum lipid profile among patients with Non Alcoholic Fatty Liver Disease.

2. To study the correlation of insulin resistance, dyslipidemia in patients with Non Alcoholic Fatty Liver Disease.

Materials and Methods:

This is an Observational clinical correlational study conducted in Sri Devaraj Urs Medical College, Tamaka, Kolar. 50 patients of Non Alcoholic Fatty Liver Disease were included in the study. Height, Weight, Waist circumference of the patients is noted, BMI is calculated. Fasting blood sugar, Lipid profile, Serum Insulin, LFT were measured. Insulin resistance is calculated by HOMA-IR index. Results were tabulated and Descriptive and Inferential statistical analysis was done.

Results:

Maximum number of subjects were seen in age group 41- 60 yrs. Mean age of Distribution is 55.08. Males contributed to 46% and females 54%. Out of the 50 patients, BMI < 25 is seen in 11 patients and > 25 is seen in 39 patients. No patient is with BMI <18.5.

BMI > 25 significantly correlates with NAFLD with p value of 0.035. The mean Waist Circumference for Males is 94.00±6.46 and for Females is 89.52±4.54.Waist Circumference alone does not significantly correlate with NAFLD. Out of the 50 patients, 35 patients have FBS< 110, 5 patients have FBS between 110-125 and 10 patients have FBS > 126. Out of 50 patients, 39 have Dyslipidemia, which contributes to 78%. Serum Insulin Mean Value for Males is 30.26±70.07 and for Females is 23.62±28.50. Insulin resistance is seen in 62% of patients. The p value for Insulin Resistance in NAFLD is 0.013 which is significant.

The mean value of Insulin Resistance for Males is 12.43±33.16 and for Females is 7.54±11.83.

Among the 31 patients with Insulin resistance, 22 patients are non diabetic(66%). Triglycerides correlate positively with Insulin Resistance in NAFLD patients with p value 0.005. Insulin resistance >2.6 is positively associated with incidence of Dyslipidemia with P=0.0201.

CONCLUSION:

The results of present study suggest that multiple factors contribute to NAFLD. BMI more than 25kg/m^2 is a risk factor for NAFLD and Insulin Resistance. The Incidence of Dyslipidemia and Insulin resistance is significantly high in NAFLD. Diabetes Mellitus alone does not significantly contribute to NAFLD. Increased Triglyceride level significantly correlates with Insulin Resistance compared to other Lipid parameters in NAFLD. Dyslipidemia positively correlates with Insulin resistance in NAFLD.

Key Words: Non Alcoholic Fatty Liver Disease, Insulin resistance, Dyslipidemia.

LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alananine Transaminase
AST	Aspartate transaminase
ATP	Adenosine Tri Phosphate
BMI	Body Mass Index
CAMP	Cyclic Adenosine Monophosphate
DM	Diabetes Mellitus
FBS	Fasting Blood Sugar
FFA	Free Fatty Acids
GLUT	Glucose Transporter
IR	Insulin Resistance
IRS	Insulin Receptor Substrate
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
LDL	Low Density Lipoprotein
LFT	Liver Function Tests
NAFLD	Non Alcoholic Fatty Liver Disease
PDK	Phosphoinositide Dependent Kinase
PKB	Protein Kinase B
PPBS	Post Prandial Blood Sugar
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
VLDL	Very Low Density Lipoprotein

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INTRODUCTION

Non Alcoholic Fatty Liver Disease (NAFLD) is emerging as an important cause of liver disease in India. Non Alcoholic Fatty Liver Disease (NAFLD) is an entity that was described a few years ago. Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). All of the stages of NAFLD have in common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes) and these occur in the absence of alcoholic usage. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD this being regarded as a major cause of cirrhosis of the liver of unknown cause. Non alcoholic steatohepatitis can even progress to hepatocellular carcinoma[1]. Hospital-based studies from west report that around 10-24% of general population, and 57-57% of obese individuals may have NAFLD. The corresponding rates for NASH are 3-4% and 15-20%, respectively.[2]

Non-alcoholic fatty liver disease (NAFLD) is related to insulin resistance and the metabolic syndrome and may respond to treatments originally developed for other insulin-resistant states (e.g. diabetes mellitus type 2) such as weight loss, metformin and thiazolidinediones.

Insulin resistance is a state in which a given concentration of insulin produces a less than expected effects. Insulin resistance is a physiological condition where the natural hormone insulin becomes less effective at lowering blood sugars. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects, Insulin resistance is believed to be the key factor that

leads to increased lipolysis in peripheral adipose tissue and increased uptake of fatty acids by hepatocytes. Hyperinsulinemia resulting from insulin resistance also adds to fatty acid content of hepatocytes by increasing glycolysis and by decreasing apolipoprotein B-100 production, and hence export of fatty acids as very lowdensity lipoproteins (VLDL). The end result is an increase in fatty acids and triglycerides in the hepatocytes leading to steatosis. Insulin resistance is almost universal in patients with NAFLD and is related to an imbalance between proinsulin (adiponectin) and anti-insulin cytokines (TNF-α), particularly those secreted from adipose tissue (adipokines).[3] Studies from some Indian centers have reported insulin resistance to be common in patients with NAFLD.[4,5,6]

Central or abdominal obesity that is more commonly associated with insulin resistance and has been observed in 80–90% of Indian patients with NAFLD.[5,6,7]

Dyslipidemia is a common feature, and is present in approximately 50% of Indian patients with NAFLD.[7] Both components of metabolic syndrome (high triglycerides and low HDL) were observed with almost equal frequency, being present in 53% and 66% patients with NAFLD, respectively.[7]

My present study is intended to study the role of insulin resistance, dyslipidemia in patients with NAFLD.

AIMS AND OBJECTIVES

1. To estimate HOMA-IR and serum lipid profile among patients with Non Alcoholic Fatty Liver Disease.

2. To study the correlation of insulin resistance, dyslipidemia in patients with Non Alcoholic Fatty Liver Disease.

REVIEW OF LITERATURE

NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

History: It was first described by Ludwig in 1980.[8] It was first recognized as a complication of jejunoileal bypass procedures for morbid obesity.[9] The epidemiological impact and the number of recent publications on this condition have been increased. The prevalence of NAFLD in the general population is estimated to be 20%. NAFLD occurs in 63% of patients with type 2 diabetes mellitus.[10] Many names have been synonymously used, Non alcoholic steatohepatitis (NASH),[8] Fatty Liver Hepatitis,[11] Non alcoholic steatonecrosis, non alcoholic fatty liver disease,[12] diabetic hepatitis.

Information is scarce on the natural history of this disease, which can progress to the following consecutive stages in some patients: Fatty liver, Steatohepatitis, Steatohepatitis with fibrosis and cirrhosis.

Epidemiology:

Prevalence: True prevalence of NAFLD is difficult to assess without large scale epidemiological studies. Steatosis is the most common cause of raised transaminases and affects nearly 10-24% of general population[13]. While only 2-3% in the general population have steatohepatitis. In patients undergoing liver biopsy, prevalence of NAFLD and steatohepatitis range from 15-39%[14] and 1.2-4% respectively. However on imaging the prevalence of steatohepatitis ranges from 9.7-2.3% and 1.2-4.8% respectively.

Risk Factors: Obesity, Type 2 DM and Hyperlipidimia are coexisting conditions frequently associated with NAFLD.[15] Prevalence of obesity in NAFLD ranges from 30-100%.[15] The prevalence of type 2 DM varies between 10-75% and prevalence of hyperlipidemia varied between 20-92% .NAFLD may affect persons of any age and has been described in most racial groups.

Diabetes is an independent risk factor for liver disease in NAFLD patients; hepatic fibrosis is more common in them. In presence of NASH there is increased progression to end-stage liver disease and an increase in heart and vascular deaths in type II DM.

Factors that may imply a higher risk of steatosis developing to NASH include[16]; Age >40yrs, BMI>40kg/m^2, AST/ALT >1 and the presence of at least two causes of NASH, such as diabetes and hyperlipemia.

Causes of Fatty Liver Disease:[15]

Nutritional

- Protein Calorie malnutrition
- Starvation
- Total parental nutrition
- Rapid weight loss
- Gastro intestinal surgery for obesity

Drugs

- Glucocorticoids
- Synthetic estrogens

- Aspirin
- Calcium channel blockers
- Amiodarone
- Tamoxifen
- Tetracycline
- Methotrexate
- Valproic acid
- Zidovudine
- Didanosine
- Fialuridine

- **Metabolic or Genetic**
- Lipodystrophy
- Dysbetalipoproteinemias
- Acute fatty liver of pregnancy

Others:

- Inflammatory bowel disease
- Small bowel diverticulosis
- HIV infection
- Bacillus cereus toxins.

Conditions associated with NASH

- Obesity
- Diabetes mellitus

- Hyperlipidemia
- Female sex
- Advanced Age
- Rapid weight loss
- Insulin resistance
- Changes in iron stores.

Pathogenesis of NAFLD[15]

The Pathogenesis of NAFLD has remained poorly understood. Much current thinking remains hypothetical. It is not yet understood why simple steatosis develops in some patients, where as steatohepatitis and progressive disease develops in others: differences in body fat distribution or antioxidant systems, possibly in the context of genetic predisposition, may be among the explanations.

1. A net retention of lipids within hepatocytes, mostly in the form of triglycerides, is a pre requisite for the development of NAFLD. The primary metabolic abnormalities leading to lipid accumulation could consist of alterations in the pathways of uptake, synthesis, degradation, or secretion in hepatic lipid metabolism resulting from insulin resistance.

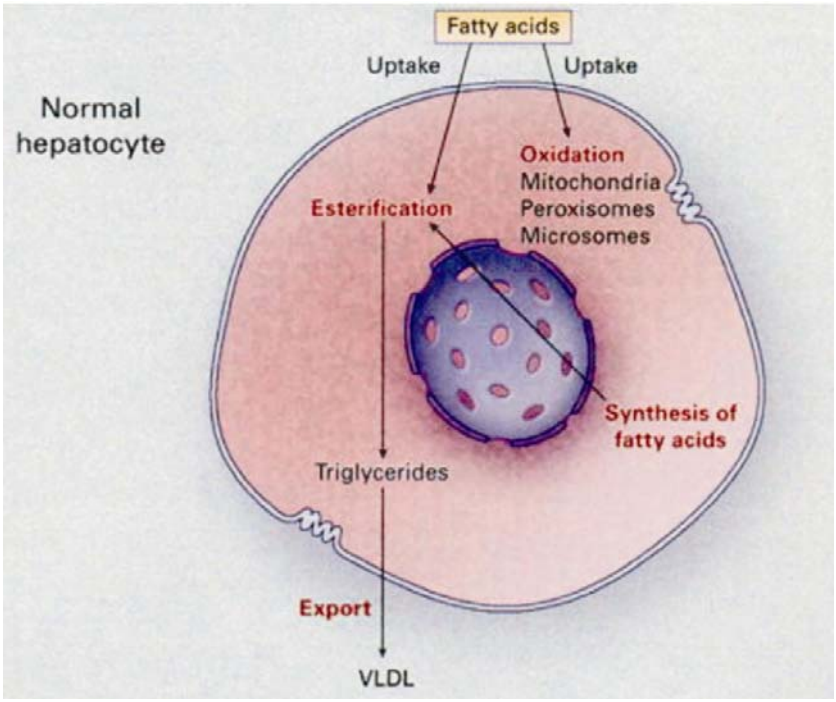


Figure 1: Role of fatty acid uptake and lipid metabolism in hepatocyte in the pathogenesis of NAFLD.

2. Insulin resistance is the most reproducible factor in the development of NAFLD.[17] The molecular pathogenesis of insulin resistance seems to be multifactorial, and several molecular targets involved in inhibition of insulin action have been identified. These include Rad[18] (ras associated with diabetes) which interferes with essential cell functions (growth, differentiation, vascular transport, and signal transduction); pc-1[19] (a membrane glycol protein that has a role in insulin resistance) which reduces insulin stimulated tyrosine kinase activity: Leptin[20] which induces dephosphorylation of insulin – receptor substrate -1. Fatty acids; which inhibit insulin- stimulated peripheral glucose uptake; and TNF-α[21] which down regulates insulin induced phosphorylation of IR substrate-1 and reduces the expression of insulin dependent glucose transport molecule GLUT4. Insulin

resistance leads to fat accumulation by two main mechanisms; lipolysis and hyper insulinemia.

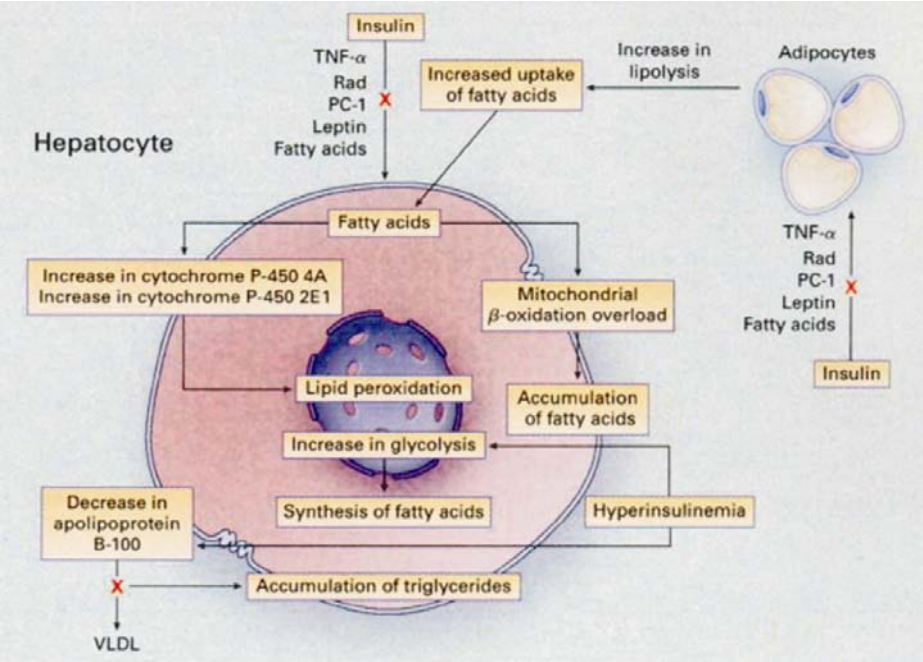


Figure 2: The molecular pathogenesis of insulin resistance.

3. Deficiency of enzymes of peroxisomal B-oxidation has been recognized as an important cause of micro vesicular steatosis and steatohepatitis.[22] Deficiency of acyl-coenzyme A oxidase disrupts the oxidation of very long chain fatty acids and dicorboxylic acid, leading to extensive micro vesicular steatosis and steatohepatitis. PPAR-α has been implicated in promoting hepatic synthesis of uncoupling proten-2, which is expressed in the liver of patients with NAFLD[23]

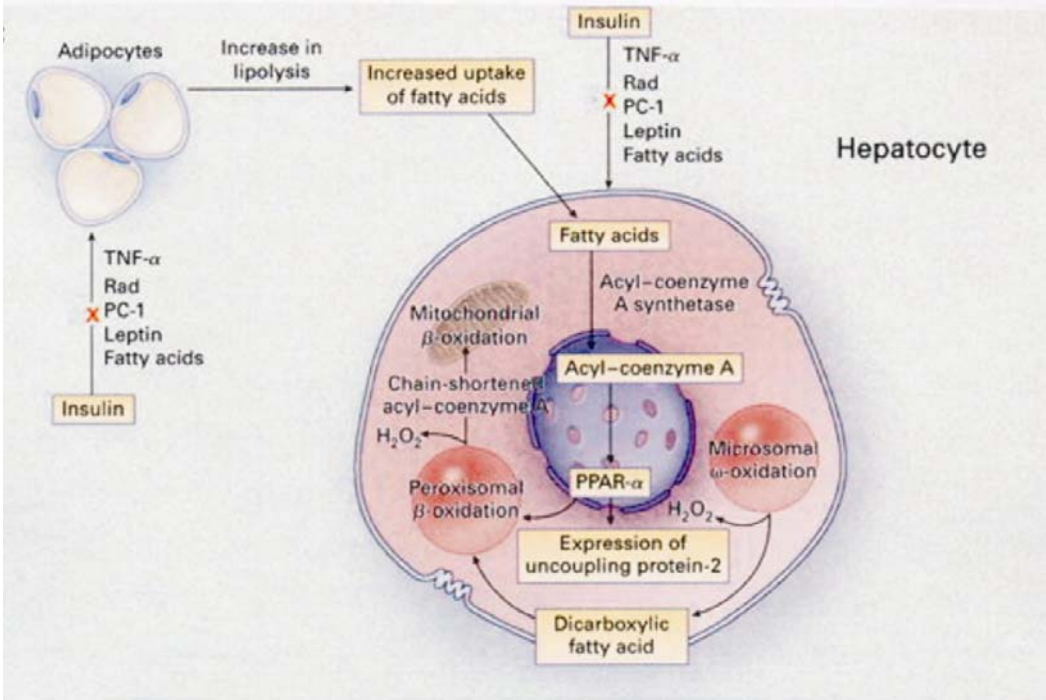


Figure 3: Relation between microsomal oxidation, peroxisomal oxidation and mitochondrial oxidation in pathogenesis of NAFLD.

4. Increased intrahepatic levels of fatty acids provide a source of oxidative stress, which may in large part, be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms: lipid per oxidation, cytokine induction and induction of Fas Ligand.

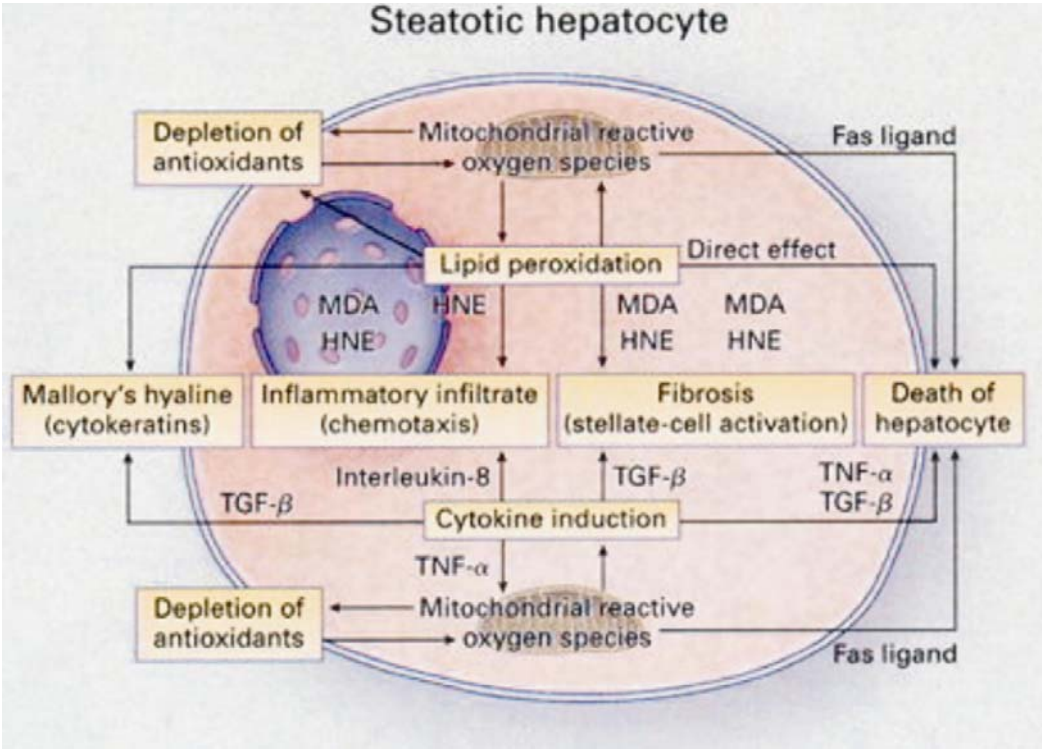


Figure 4: Role of mitochondrial reactive oxygen species in progression from steatosis to steatohepatitis.

MDA-Malondialdehyde TNFα -Tumour necrosis factor α.

HNE-4 hydroxynonenal TGFβ -Transforming growth factor β

Steatohepatitis – A tale of two hits [24]

Symptoms of liver disease rarely develop in patients with fatty liver who are obese, have diabetes, or have hyper lipidemia. The steatotic liver may be vulnerable to further injury when challenged by additional insults. This has led to the presumption that progression from simple steatosis to steatohepatitis and to advanced fibrosis results from two distinct events. First, insulin resistance leads to the accumulation of fat with in hepatocytes, and second mitochondrial reactive oxygen species cause lipid per oxidation, cytokine induction and the induction of Fas ligand; this leads to high

afflux of electrons to the mitochondrial respiratory chain, and an increased production of oxygen free radicals, which are responsible for the hepatic lesions of NASH.[25]

Clinical Manifestations of NAFLD:[15]

Most patients with NAFLD have no symptoms or signs at the time of diagnosis.

Many patients report fatigue or malaise and sensation of fullness or discomfort on the right upper abdomen.

Hepatomegaly is the only physical finding in most patients.

Finding of chronic liver disease and diminished numbers of platelets suggest that advanced disease with cirrhosis is present.

Laboratory Abnormalities[26]

Mild to moderately elevated serum levels of AST, ALT or both (2-5 fold increase in transaminases).

AST/ALT ratio usually less than 1 (65-90%). AST/ALT ratio increases as fibrosis advances.

Serum Alkaline phosphtase is above the normal range in many patients. Hypoalbuminemia.

Prolonged ALT levels PT.

Hyperbilirubinemia in cirrhotic stage of NAFLD.

Elevated serum ferritin.

Imaging Studies

1. Ultrasonography (USG):[27,28]

Fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of kidneys. USG has a sensitivity of 89% and specificity of 93% in detecting steatosis and sensitivity and specificity of 77% and 89% respectively in detecting increased fibrosis.

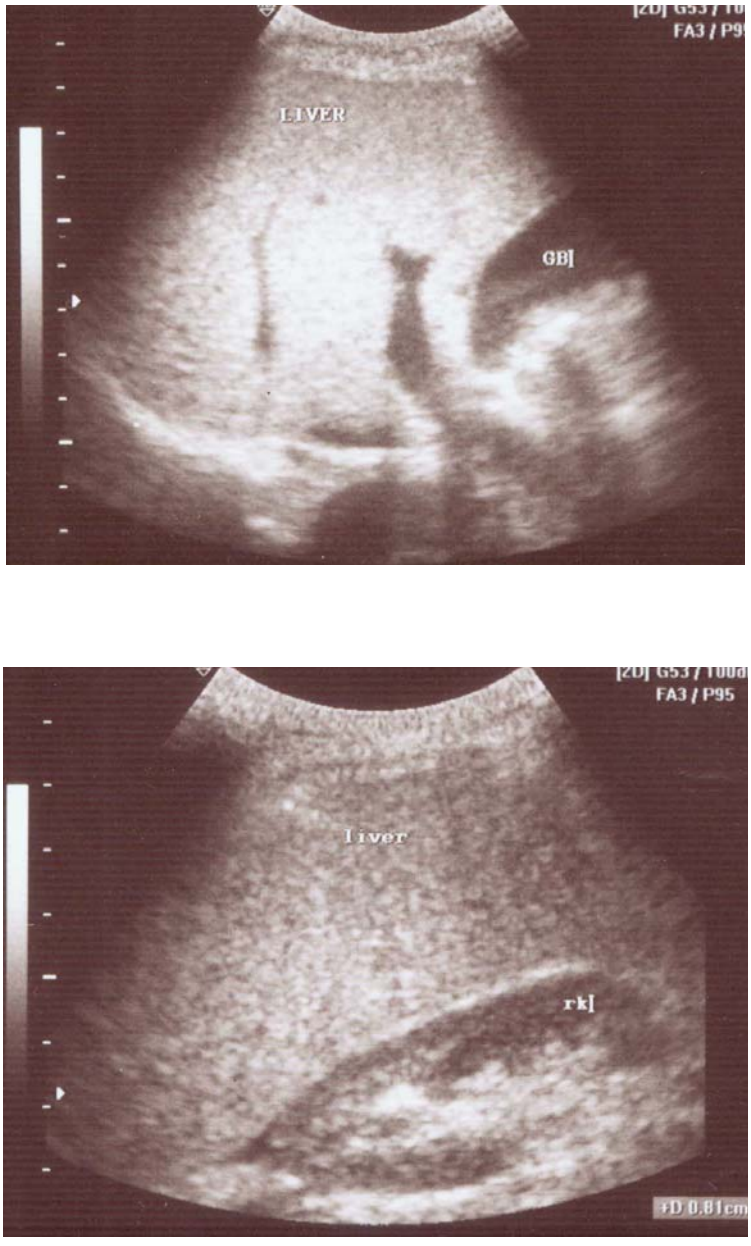


Figure 5: Ultrasonographic picture of fatty liver.

2. **CT Scan:**

Fatty infiltration of liver produces a low density hepatic parenchyma on CT scan. Steatosis is diffuse in most patients with non alcoholic fatty liver disease, but occasionally it is focal.

3. **MRI:**

MRI can distinguish space occupying lesions from focal fatty infiltration (characterized by isolated areas of fat infiltration) or focal fatty sparing (characterized by isolated areas of normal liver) [29]

4. **Magnetic Resonance spectroscopy** allows quantitative assessment of fatty infiltration of the liver.[30]

Histological Finding in NAFLD[15]

Non alcoholic fatty liver disease is histologically indistinguishable from the liver damage resulting from alcohol abuse.

Liver biopsy features include steatosis, mixed inflammatory cell infiltration, hepatocyte ballooning and necrosis, glycogen nuclei, Mallory's hyaline, and fibrosis.

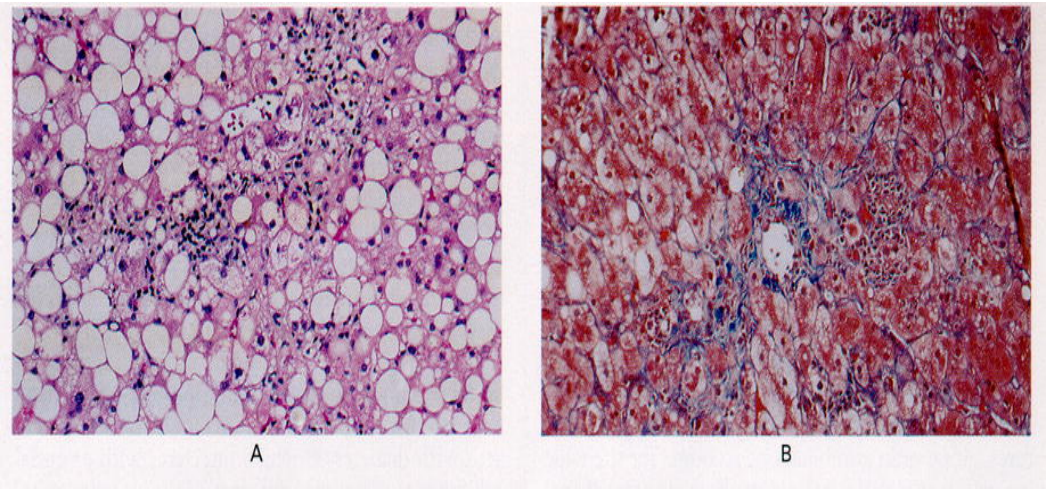


Figure 6: Characteristic findings of NAFLD on Liver –Biopsy Specimens.

Portal tracts are relatively spared from inflammation, although children with Non alcoholic fatty liver disease may show predominance of portal inflammation as opposed to lobular infiltrate.[31] Mallory's hyaline is notably sparse in children with NAFLD.[32]

The findings of fibrosis in Non alcoholic fatty liver disease suggests more advanced and serve liver injury.

The combination of Steatosis, infiltration by mononuclear cells or polymophonuclear cells and hepatocyte ballooning and spoty necrosis is known as non alcoholic steatohepatitis.

Grading and staging of the Histopatholigical lessons of Non alcoholic fatty liver disease[25,33]

Grading of Steatosis:

- Grade 1 : <33% hepatocytes affected
- Grade 2 : 33-66% hepatocytes affected
- Grade 3 : >66% of hepatocytes affected.

Grading of Steatohepatitis:

Grade 1, mild:

Steatosis: Predominantly macro vesicular, involves up to 66% of lobules.

Ballooning: Occasionally observed: zone 3 hepatocytes.

Lobular inflammation: Scattered and mild acute inflammation (polymorph nuclear cells) and occasional chronic inflammation (mono nuclear cells)

Portal Inflammation: None or mild.

Grade 2; Moderate

Steatosis: any degree, usually mixed macro vesicular and micro vesicular.

Ballooning: obvious and present in zone 3.

Lobular inflammation: Polymorphonuclear cells may be noted in association with

Ballooned hepatocytes: poricellurlar fibrosis: mild inflammation may be seen.

Portal inflammation: mild to moderate.

Grade 3; Severe:

Steatosis: typically involves >66% of lobules (panacinar): commonly mixed steatosis

Ballooning: Predominantly zone 3: marked

Lobular inflammation: Scattered acute and chronic inflammation: Polymaphonuclear cells may be concentrated in zone 3 areas of ballooning and persinusiodal fibrosis

Portal inflammation; mild to moderate.

Staging for fibrosis:

Stage 1: Zone 3 perivenular, persinusiodal, or pericellular fibrosis focal or extensive.

Stage 2: as above, with focal or extensive periportal fibrosis.

Stage 3: bridging fibrosis, focal or extensive

Stage 4: Cirrhosis.

Diagnosis [15]

The diagnosis of non alcoholic fatty liver disease is usually suspected in persons with asymptomatic elevation of aminotransferase levels, radiological findings of fatty liver, or unexplained persistent hepatomegaly.

Imaging studies are helpful in determining the presence and amount of fatty infiltration of the liver.

Severity of NAFLD can only be confirmed with liver biopsy.

Diagnosis of NAFLD requires the exclusion of alcohol abuse as a cause of liver disease. A daily intake as low as 20g in females and 30g in males may be sufficient to cause alcohol induced liver disease in some patients (350ml of beer 120ml of wine, and 45ml of hard liquor each contain 10g of alcohol)[34-36]

Natural history:

The natural history of non alcoholic fatty liver disease is not well defined, but it seems to be determined by the severity of histological damage.

Patients found to have pure steatosis on liver biopsy seem to have the best prognosis within the spectrum of Non alcoholic fatty liver disease.[37] Whereas features of steatohepatitis or more advanced fibrosis is associated with worse prognosis.[32,38,39]

Management

In fact that there is no universal effective treatment for NASH leads some to avoid invasive diagnostic tests such as liver biopsy.[13]

1. Change in habits – Diet and Physical exercise:

Diet and physical exercise significantly reduce the risk of developing type-2 Diabetes. Given the important relationship between insulin resistance and NAFLD a change in habits is advisable.

The degree of fatty infiltration usually decreases with weight loss in most patients, although the degree of necro inflammation and fibrosis may worsen.[33, 34]

The rate of weight loss is important and may have a critical role in determining whether liver histological findings will improve or worsen. In patients with a high degree of fatty infiltration, rapid weight loss may promote necro inflammation, portal fibrosis and bile stasis.[40, 41]

A weight loss of about 500g per week in children and 1600 gm per week in adults[34] has been advocated. Nevertheless the most effective rate and degree of weight loss still have to be established.

2. Treatment of associated conditions:

In patients with Diabetes mellitus and hyperlipidemia good metabolic control is always recommended, but it is not always effective in reversing NAFLD.

Improvement in Liver function test results is almost universal in obese adults and children.

3. Drug Therapy:

No medications have been proved to directly reduce or reverse liver damage independently of weight loss, but such medications would be desirable.[19]

a) **Gemtibrozil**[42]: 600 mg daily for 4 weeks showed a significant improvement of transaminases.

b) **Vitamin E**[43]: (α-tocopherol) 400-1200 IU daily improves hepatic enzymes in patients with NAFLD.

c) **Metformin.**[44]Reduces hyper insulinemia and improves hepatic insulin resistance, Liver enzymes and steatosis also significantly improved, perhaps due to a reduced hepatic expression of TNF-α.

d) Ursodiol,[45] Betaine,[46] and Trogiltazone:[47] led to improvement in liver test results as well as histological findings.

PLASMA LIPIDS AND LIPOPROTEINS

Bloor's classification of lipids[48]

I. **Simple Lipids**

Esters of fatty acids with various alcohols:

a. **Neutral fat:** Triglycerides.

b. **Waxes:** True waxes, cholesterol esters, Vitamin A and Vitamin D.

II. **Compound lipids**

Esters of fatty acids containing group other than and in addition to alcohol and fatty acids.

a. Phospholipids

b. Glycolipids

c. Sulfolipids

d. Amino lipids

e. Lipoproteins

III. **Derived Lipids**

Derivatives obtained by hydrolysis of group I and II lipids.

a. Fatty acids

b. Monoglycerols

c. Alcohols

Lipids are carried in the plasma in the form of lipoprotein complexes. These complexes impart solubility to the otherwise insoluble lipids.

Structure of a lipoprotein particle

The lipoproteins are globular particles of high molecular weight. Each particle contains a hydrophobic core of triglyceride and cholesterol ester surrounded by a coat

containing polar phospholipids, free cholesterol and apo proteins. The apo protein decides the role of a lipoprotein like binding to specific enzyme or onto cell membrane, thus directing the lipoprotein to the site of metabolism.

Apo proteins AI and AII are the major apoproteins of HDL. They play a major role in removing excess cholesterol from the surface of the cells. There are two major proteins in apoprotein B family. $B_{48,}$ major structural protein of chylomicron and is responsible for the secretion of the same.[41] B_{100}, major structural protein of VLDL and LDL. It is essential for secretion of VLDL from liver and as a ligand for removal of LDL by LDL receptors.

Apoprotein-C, which is found in all lipoproteins, regulates the activity of lipoprotein lipase and inhibits removal of chylomicrons and VLDL by liver. Apo C_2 activates the enzyme.Its absence prevents normal lipolysis and causes hypertriglyceridemia. Apo C_3 retards catabolism of VLDL and chylomicrons.[49]

Apo E also present in VLDL and chylomicrons. They are required for normal catabolism of remnants.

Table 1. Major lipoprotein classes[49]

Lipoprotein	Density g/ml	Size nm	Apo lipoproteins		Other constituents
			Major	Other	
Chylomicrons	0.930	75-1200	Apo B-48	A-I, IV C-I, II, III	Retinyl esters
Chylomicron remnant	0.930-1.006	30.80	Apo B-48	E, A – I IV, C-I, II,III	Retinyl esters
VLDL	0.930-1.006	30.80	Apo B-100	E, A-I II V C-I II III	Vit E
IDL	1.006-1.019	25.35	Apo B	E, C-I II III	Vit E
LDL	1.019-1.063	18.25	Apo B	-	Vit E
HDL	1.063-1.210	5.12	Apo A-I	A-II, IV E C-III	LCAT, CETP Paroxanose
Lp(a)	1.050-1.120	25	Apo B-100	Apo (a)	-

LCAT – Lecithin cholesterol acyl transferase

CETP – Cholesteryl ester transfer proteins

Normal lipoproteins metabolism[49]

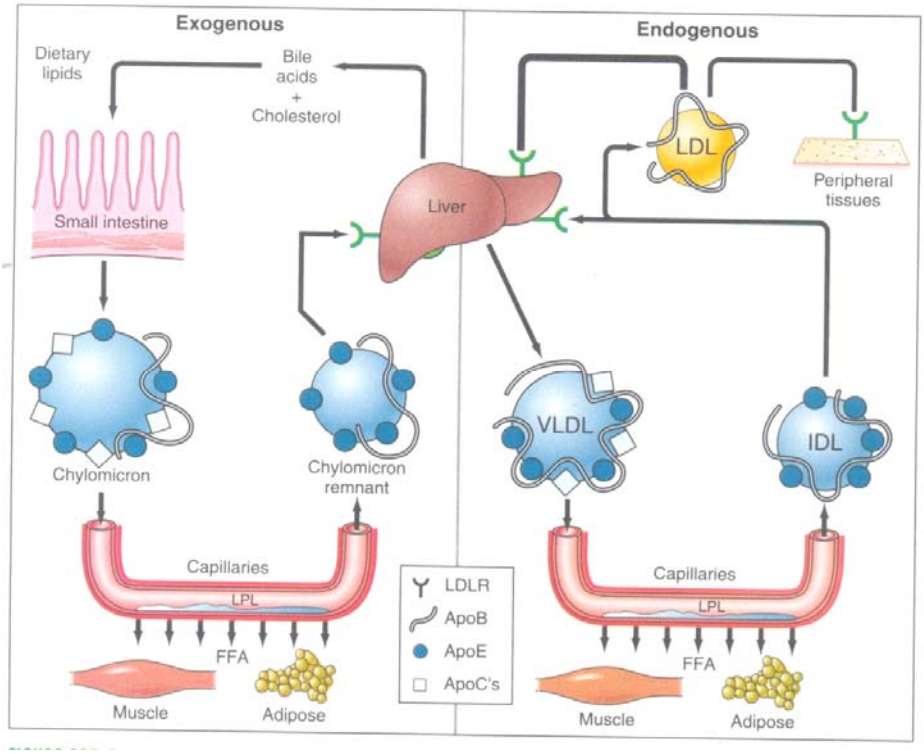


Figure 7: Exogenous and endogenous lipoprotein pathways. The exogenous pathway transports dietary lipids to the periphery and the liver. The endogenous pathway transports hepatic lipids to the periphery.

LPL – Lipoprotein lipase; FFA – Free Fatty Acids;

LDL-R – Low-density lipoprotein Receptor

Chylomicrons are formed from dietary fats and cholesterol, absorbed in the intestine. They are secreted into the lymph, through thoracic duct enter systemic circulation, and come in contact with lipoprotein lipase located on the surface of endothelial cells particularly in adipose tissue and muscle. Lipoprotein lipase needs insulin for maintenance of adequate tissue levels. This results in hydrolysis of

triglycerides into fatty acids and glycerol. After lipolysis is complete, chylomicron remnant is released back into circulation and is cleared by liver.

VLDL is synthesized endogenously by liver. This transports triglycerides to tissues to be used as fuel or to adipose tissue for storage. After lipolysis by lipoprotein lipase, a VLDL remnant (IDL) is produced; this is converted to LDL or removed by the liver.

The majority of cholesterol in plasma is found in LDL. It delivers cholesterol to tissue via a specific high affinity LDL receptor that controls uptake of cholesterol by cells. When the cholesterol needs of the cells are met by uptake of plasma cholesterol there is inhibition of rate limiting enzymes of cholesterol biosynthesis and vice versa.

HDL is important for removal of cholesterol from peripheral tissues to the liver and for metabolism of chylomicrons and VLDL.

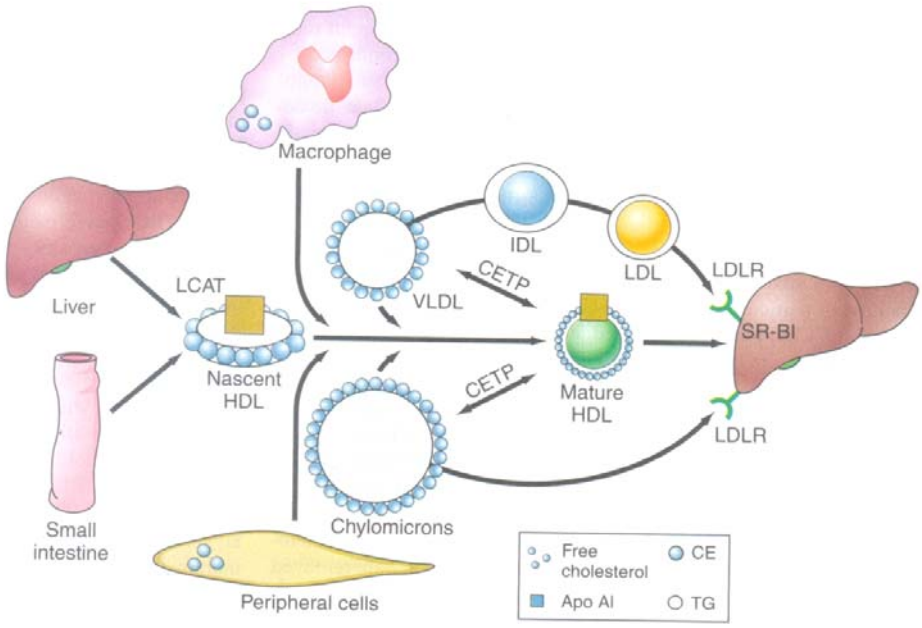


Fig 8 : HDL metabolism and reverse cholesterol transport. This pathway transports excess cholesterol from the periphery back to the liver for excretion in the bile. The liver and the intestine produce nascent HDL. Free cholesterol is acquired from macrophages and other peripheral cells and esterified by LCAT, forming mature HDL. HDL cholesterol can be selectively taken up by the liver via SR-BL. Alternatively; HDL cholesteryl ester can be transferred by CETP from HDL to VLDL and Chylomicrons, which can then be taken up by the liver.

LCAT – Lecithin Cholesterol Acyl Transferase;

CETP – Cholesteryl Ester Transfer Protein

Dyslipidemia and Type 2 Diabetes.[50.51]

The most common anomaly in type 2 diabetes is hyper triglyceridemia caused by increase in VLDL. The effect on triglycerides moderate. The mechanism of over production of VLDL- TG because of increased flow of glucose and free -fatty acids to the liver and the impaired clearance is by impaired lipoprotein lipase activity. The

mechanism of increased LDL-C is both increased production and decreased clearance. Non-enzymatic glycosylation of LDL apo B occurs with poor diabetic control and interferes with LDL catabolism.

Metabolic Syndrome: (MES) [52]

Metabolic Syndrome has been given a variety of names:

- Syndrome X
- Insulin resistance syndrome
- Deadly Quartet
- Multiple metabolic syndromes

Major characteristics of MES include: Insulin resistance (IR), Abdominal obesity, elevated blood pressure and dyslipidemia (low HDL and high triglycerides)

Insulin Resistance (IR) has been implicated in the polycystic ovarian syndrome and non alcoholic steatohepatitis (NASH).

ATP III diagnostic criteria for MES (three of the following)

1. Abdominal obesity: male > 102cm, Female >88 cm
2. Triglycerides > 150 mg / dl
3. HDL less than 40 mg / dl in men, less than 50 mg / dl in women.
4. Blood pressure of 130/85 mmHg or Higher.
5. Elevated fasting glucose > 100mg / dl

INSULIN

Our cells communicate using a molecular postal system: the blood is the postal service and hormones are the letters. Insulin is one of the most important hormones, carrying messages that describe the amount of sugar that is available from moment to moment in the blood.

Insulin is a peptide hormone, produced by beta cells of the pancreas, and is central to regulating carbohydrate and fat metabolism in the body. Insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. In the liver and skeletal muscles, glucose is stored as glycogen, and in fat cells (adipocytes) it is stored as triglycerides.[53]

The insulin molecule contains 51 amino acids; it is made up of two peptide chains linked by disulphide bonds. Although it is active as a monomer, during its biosynthesis and storage it assembles to dimers and in the presence of zinc, to hexamers.

X-ray analysis has revealed the 3-dimensional structure of the insulin molecule in its hexameric, dimeric and monomeric states. Two main conformations of insulin which differ in the extent of helix in the B chain (B9–B20 and B1–B20, respectively) have been identified.

Other variations are seen in insulin when dimeric or monomeric. Reagenmts such as chloride and phenol govern the conformations present in the insulin hexamers and this can influence the behavior and properties of insulin preparations employing them.[54]

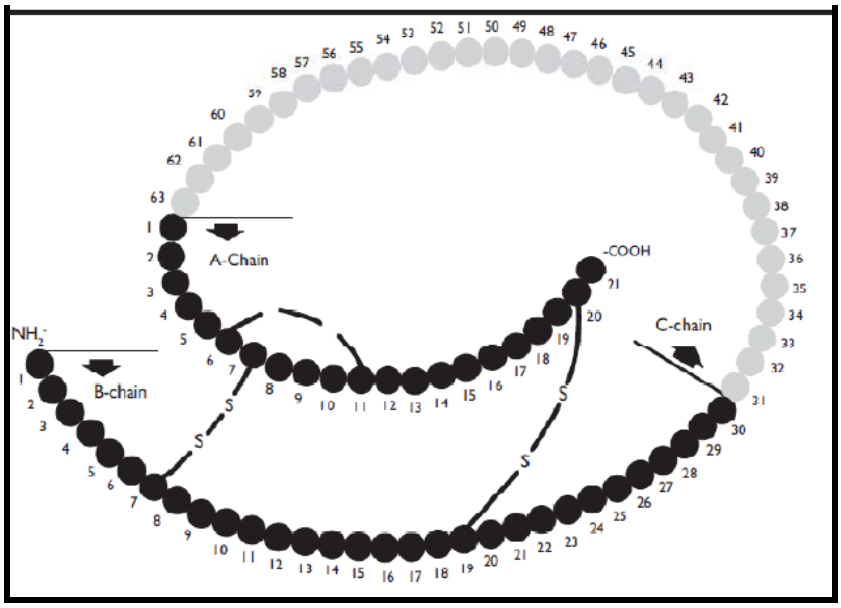


Figure 9: Structure of Insulin

Synthesis of Insulin:

Insulin is a hormone produced by the β-cells of the Islets of Langerhans in the pancreas. At birth about $3x10^5$ islets are present, it becomes $1x10^6$ during the first years of life. The islets contain various cell types which each produce different hormones. The β-cell produces insulin. Other important hormones are somatostatin, produced in the δ-cell, and glucagon, produced in the α-cell. The latter counteracts the effect of insulin in many ways. The β-cell is situated central in the islet of Langerhans whereas the other cells are located peripherally.

The human insulin gene is located on the short arm of chromosome 11. *Via* DNA/RNA resynthesis, a precursor molecule known as pre-pro-insulin (98 amino acids, molecular weight [MW] 11.500) is produced in the endoplasmatic reticulum of the pancreatic β-cells. It is cleaved to proinsulin (86 amino acids, MW approximately 9000) directly after the molecule has left the ribosome.

The proinsulin is transported to the Golgi apparatus, where packaging into clathrin-coated secretory granules takes place. Maturation of the secretory granule is associated with the loss of the clathrin coating. In addition, the proinsulin is converted into insulin and C-peptide (MW 3000) by proteolytic cleavage at two sites. Normal granules shed insulin and C-peptide in equimolar amounts, along with some proinsulin and so-called split products (only partially cleaved proinsulin). Insulin (MW 5808) itself consists of an A-chain of 21 amino acids and a B-chain of 30 amino acids, which are connected by two disulfide bonds.

The secreted insulin first passes the liver where a proportion of insulin is cleared *via* a receptor-mediated process after exerting its action.[55-57] The proportion of insulin cleared during first-pass through the liver has been estimated to be about 50% in dogs[56] and approximately 40 to 80% in humans.[58-61] The plasma half-life time ($t1/2$) of insulin is only 5-10 minutes. C-peptide, the 31 amino acid residue, has no known biological function. Since C-peptide is produced in equimolar amounts with insulin it can be used as a marker for insulin secretory capacity, because it is not cleared by the liver but by the kidney and has a longer $t_{1/2}$. than insulin.[62,63]

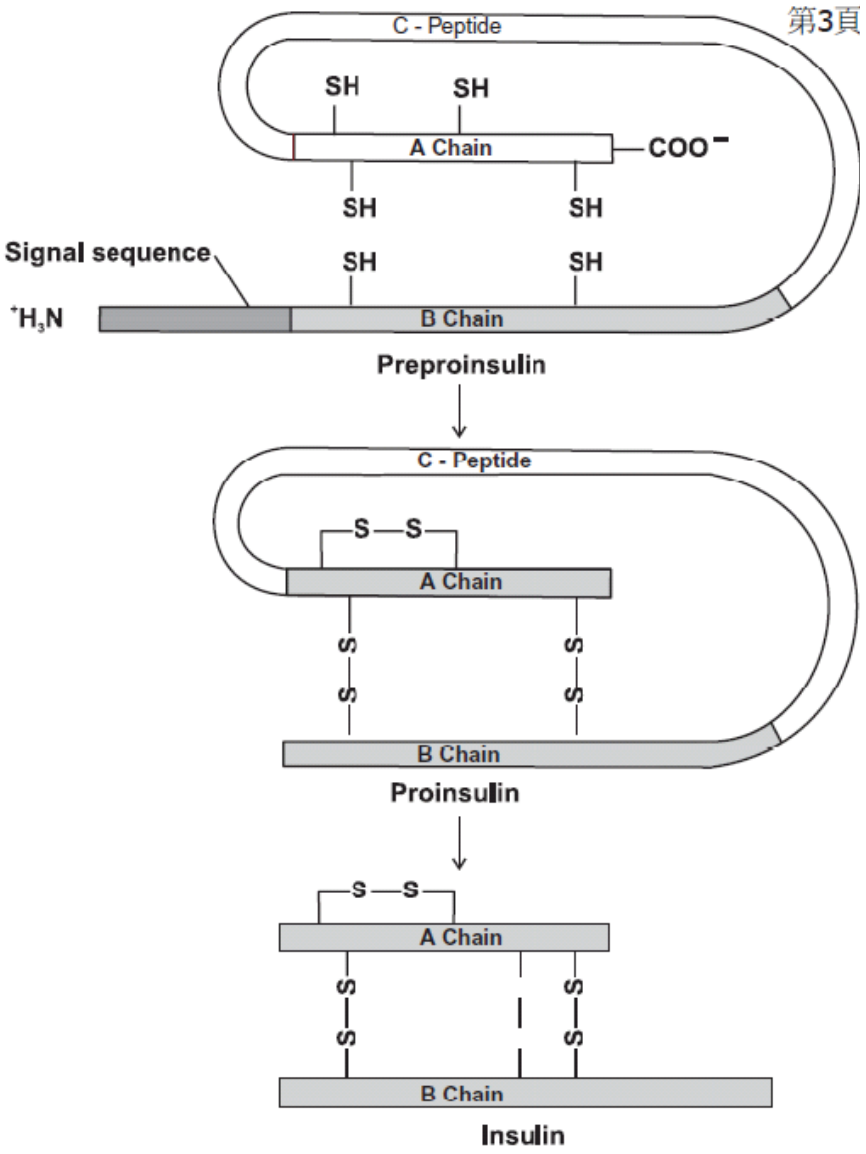


Figure 10: Synthesis of Insulin

Secretion of Insulin:

The main trigger for insulin release is an increase in the plasma glucose concentration in the portal circulation. Plasma glucose is sensed and taken up by the β-cell *via* facilitated diffusion by the specific glucose transporter (GLUT)-2. Subsequently, glucose is metabolised by the cell, which sets free energy in the form of adenosine tri-phosphate (ATP). The increase in intracellular ATP induces a closure of the ATP-dependent potassium channel at the cell membrane of the β-cell. This causes a depolarisation of the cell membrane, which leads to an opening of the voltage-dependent calcium channels and an inflow of calcium ions into the cell. The increase in intracellular calcium concentration eventually leads to the release of insulin from the granulae *via* exocytosis.[62,63]

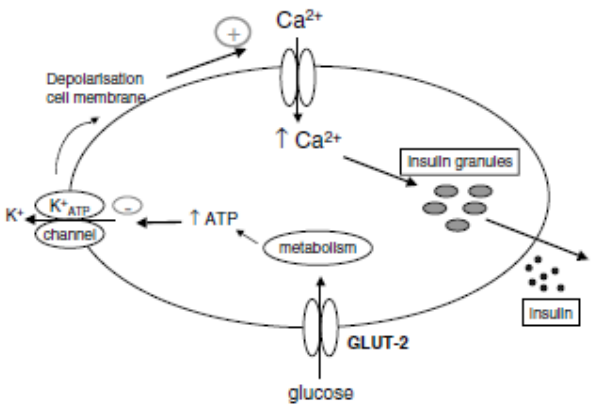


Figure 11: Secretion of Insulin

Several phases of insulin secretion can be identified: (i) basal insulin secretion is the way insulin is released in the post-absorptive state; (ii) the cephalic phase of insulin secretion is evoked by the sight, smell, and taste of food (before any nutrient is absorbed by the gut), and is mediated by pancreatic innervation; (iii) first-phase insulin secretion is defined as the initial burst of insulin, which is released in the first

5–10 min after the β-cells are exposed to a rapid increase in glucose (or other secretagogues); (iv) after the acute response, there is a second-phase insulin secretion, which rises more gradually and is directly related to the degree and duration of the stimulus; (v) finally, a third phase of insulin secretion has been described, albeit only *in vitro*. During all these stages, like many other hormones, insulin is secreted in a pulsatile fashion, resulting in oscillatory concentrations in peripheral blood. Oscillations include rapid pulses (recurring every 8-15 min) superimposed on slower, ultradian oscillations (recurring every 80-120 min) that are closely related to fluctuations in the glucose concentration.[56-59] This pulsatile pattern of insulin delivery to the liver is regulated mainly by modulation of insulin pulse mass in response to stimuli. The mass of insulin pulses through the liver is the predominant determinant of hepatic insulin clearance.[61]

Action of Insulin

Insulin is an anabolic hormone, which means that insulin facilitates the storage of energy sources, such as fat and glycogen, and stimulates protein synthesis. Because, physiologically, insulin is secreted following energy intake, insulin not only directs these energy sources towards storage, but simultaneously prevents endogenous release of energy sources (free fatty acids through lipolysis, proteolysis, *de novo* glucose production by the liver and ketogenesis), because these substrates are redundant in times of plenty.[62-67]

Table 2: Metabolic actions of Insulin

	Stimulation of	Inhibition of
Liver	glycogen synthesis	gluconeogenesis
	protein synthesis	glycogenolysis
	lipogenesis	ketogenesis
Muscle	glucose transport	
	glycogen synthesis	
	protein synthesis	proteolysis
Adipose tissue	glucose transport	
	lipogenesis	lipolysis

Glucose Homeostasis:

Blood glucose levels are usually tightly regulated between 4-8 mmol/L. Low blood glucose levels are dangerous because brain function depends on glucose, and lack of glucose in the brain can cause seizures, loss of consciousness and death. On the other hand, elevated blood glucose levels can lead to either ketoacidosis or hyperglycemic hyperosmolar dehydration in the acute situation, which can both eventually result in a coma. Furthermore, prolonged elevation of blood glucose levels can result in micro- (retinopathy, nephropathy, neuropathy) and macrovascular long-term complications.

The tight regulation of plasma glucose levels is achieved by the finely tuned hormonal regulation of glucose uptake by the tissues (rate of disappearance, Rd) on the one hand and glucose production on the other hand (rate of appearance, Ra).[68] Glucose uptake by peripheral tissues is either insulin-independent (in the brain) or insulin dependent (in muscle and adipose tissue). The brain cannot store glucose and is critically dependent on glucose for its function. Therefore, in the non-fed (= postabsorptive) state a certain level of endogenous glucose production is necessary.

Glucose appearing in the post-absorptive state is mainly derived from the liver,[69] although the kidney is also capable of glucose production.

The amount of glucose produced by the kidney has been reported to be less than 5% after an overnight fast to 20% after a 60-h fast.[69] However, higher estimates of the contribution of the kidney to total post-absorptive gluconeogenesis have been reported. These differences depend on the techniques used to quantify renal glucose production. A significant role for the kidney in carbohydrate metabolism in type 2 diabetes has recently been proposed.[70,71]

In healthy individuals the amount of endogenous glucose production (EGP, both liver and kidney) in the post-absorptive state averages 1.8-2.3 mg.kg-1.min-1[69-74] which is about 10.0-12.8 μmol.kg-1.min-1. Endogenous glucose production comprises 2 pathways: glycogenolysis, which is the breakdown of glucose stored as glycogen, and gluconeogenesis, which is the synthesis of new glucose molecules from precursor molecules like amino acids (mainly alanine), glycerol and lactate.

Endogenous glucose production is mainly regulated by fluctuations in the insulin/glucagon ratio in the portal vein.[75,76] Following a meal, insulin secretion is stimulated and the increase in portal vein insulin concentration inhibits endogenous glucose production *via* inhibition of glycogenolysis and gluconeogenesis. When the meal has been absorbed, plasma glucose levels decrease, even to a level a little below normal post-absorptive levels. This relative hypoglycaemia leads to increased secretion of glucagon. The subsequent elevation in portal vein glucagon concentration stimulates glycogenolysis and hepatic glucose production[77].

Endogenous glucose production is also influenced by other hormones (cortisol, growth hormone), free fatty acids (FFA), gluconeogenic precursors, paracrine substances (cytokines, prostaglandins) and the autonomic nervous system. All these factors keep endogenous glucose production relatively constant, a process called hepatic autoregulation.[78-80] Insulin-stimulated glucose uptake primarily takes place in skeletal muscle and amounts about 0.5 mg.kg-1.min-1 (the remainder of the average basal glucose uptake of 2.0-2.2 mg.kg-1.min-1 being utilised by the brain [1.0-1.2 mg.kg-1.min-1] and red blood cells).[81,82]

Glucose taken up in the muscle can either be oxidised to pyruvate (aerobic glycolysis) or lactate (anaerobic glycolysis) or stored as glycogen (non-oxidative glucose metabolism). Insulin-stimulated glucose oxidation seems to be bound to a maximum, making non-oxidative glucose disposal quantitatively the most important.[83]

The differences in the insulin dose-response curve between the various tissues are necessary for normal glucose and lipid metabolism. During an overnight fast, serum insulin levels are sufficiently low as to not to inhibit lipolysis (which provides free fatty acids and hence ketone bodies for the brain and glycerol for gluconeogenesis) and endogenous glucose production (providing glucose for the brain), but, on the other hand, are not high enough for maximum stimulation of (skeletal muscle) glucose uptake.

After a meal, serum insulin levels rise, which stimulates glucose uptake and inhibits lipolysis and glucose production. The latter is achieved directly, by inhibition of gluconeogenesis and glycogenolysis, as well as indirectly, *via* inhibition of lipolysis, which diminishes the supply of glycerol and free fatty acids to the liver.[62,63]

Insulin Resistance:

Insulin resistance at target organs leads to decreased glucose uptake, increased glucose production and increased whole-body lipolysis. Therefore, in patients with type 2 diabetes mellitus, basal glucose production is significantly elevated, leading to fasting hyperglycaemia. In addition, following a meal, insulin resistance leads to inadequate stimulation of (skeletal muscle) glucose uptake and insufficient suppression of endogenous glucose production and lipolysis. The result is postprandial hyperglycaemia.

The incapability to suppress whole-body lipolysis substantially contributes to the increased endogenous glucose production and diminished glucose uptake. Firstly, NEFAs increase endogenous glucose production by stimulating key enzymes involved in gluconeogenesis and by providing the energy needed for glucose production. Secondly, the glycerol formed by triglyceride hydrolysis serves as a gluconeogenic substrate. Thirdly, free fatty acids impair insulin stimulated glucose uptake. Besides substrate competition (Randle effect),[84] impairment of insulin signalling appears to be responsible for this effect[85]

Molecular mechanisms of insulin resistance:

Skeletal muscle

Over 80% of insulin-stimulated glucose disposal takes place in skeletal muscle.[82] The main defect in patients with type 2 diabetes mellitus seems to reside in non-oxidative glucose disposal (NOGD), i.e., glycogen synthesis,[86] the major pathway for overall glucose metabolism. With increasing obesity and insulin resistance, insulin-stimulated NOGD becomes more impaired.[87,88]

In patients with overt diabetes mellitus, the rate of glycogen formation was 60% that of normal subjects.[86] Possible mechanisms involved in decreased glycogen synthesis could either be decreased hexokinase activity, diminished glycogen synthase activity or impaired GLUT-4 translocation. Shulman *et al*. using 31P-and 13-C-nuclear magnetic resonance (NMR) spectroscopy showed that the defects were not at the level of hexokinase[89] or glycogen synthase[90] activity, but that impaired glucose transport appears to be the prime defect in insulin-stimulated glycogen synthesis in type 2 diabetic patients.

The defects in glucose transport can either be due to defects in the glucose transporter itself or in translocation of GLUT-4 to the cell membrane. The translocation of GLUT-4 from intracellular compartments to the plasma membrane is the prime defect.

Several defects in the insulin-signalling pathway have already been found and will be discussed below.

IRS-1 is the first molecule downstream in the insulin-signalling cascade and plays a key role in skeletal muscle insulin signalling. In humans, IRS-1 polymorphisms are significantly more common in type 2 diabetic patients than in controls[91,92] but their role in the development of insulin resistance and type 2 diabetes is unclear. Furthermore, in obese insulin- resistant subjects[93,94] and moderately overweight type 2 diabetic patients[93,95] insulin-stimulated IRS-1 phosphorylation in skeletal muscle is decreased as compared to control subjects, whereas protein expression is not altered.[93,96,97] This defect can already be found in normoglycaemic relatives of type 2 diabetic patients. [98]

PI3-kinase is central in the insulin-signalling cascade; however, its activation is necessary but not sufficient for the metabolic actions of insulin. Insulin-stimulated PI3K activity is impaired in obese subjects,[93] as well as in moderately overweight type 2 diabetic patients.[99] Little is known about the physiological regulation of PDK-1, but thus far insulin action on PDK-1 appears to be normal in insulin-resistant skeletal muscle.[95] With respect to PKB/Akt, unravelling its role in insulin resistance has been complicated by the existence of three isoforms.

It appears that Akt 2 is essential in glucose homeostasis, Akt 2 knockout mice having insulin resistance and a diabetes mellitus-like syndrome. In humans, recent studies have detected a missense mutation in the kinase domain of PKB-β (Akt2) in a family of severely insulin-resistant patients that was preserved over three generations.[100]

Liver:

Insulin signalling in the liver differs from that in skeletal muscle (and adipose tissue). In muscle, IRS-1 (*via* PI3K) controls both activation of aPKC and PKB/Akt, whereas in the liver aPKC is controlled (again *via* PI3K) by IRS-2 and PKB/Akt by IRS-1. In muscle and adipocytes, aPKC and PKB/Akt stimulate the transportation of GLUT-4 to the cell membrane. In the liver, aPKC regulates the expression of SREBP-1c, a transcription factor that activates numerous genes, including FAS and acetyl-coenzyme A carboxylase, that control lipid synthesis in the liver. PKB/Akt in the liver is involved in the control of glucose production.

When insulin activates PKB/Akt (*via* IRS-1), this results in the phosphorylation of Foxo-family transcription factors. Defective IRS-1 signalling to PKB/Akt leads to lack of inhibition of enzymes involved in gluconeogenesis and increased glucose production.[101] IRS-2-mediated signalling to aPKC in the liver of diabetic rodents is largely intact or elevated. This might explain the increased very-low-density lipoprotein (VLDL)-triglyceride synthesis in type 2 diabetes.

Hepatocyte nuclear factor (HNF) may also play a role in insulin-mediated glucose metabolism in the liver. On the molecular level HNF-4 seems to interact with Foxo-1.[102] However, although genetic defects of some of the HNF transcription factors play a role in some forms of maturity-onset diabetes of the young (MODY), thus far no evidence exists that HNF-transcription factors are involved in type 2 diabetes mellitus.

GSK-3, an enzyme regulating glycogen synthesis, is a substrate of PKB/Akt. Normally, GSK-3 is constitutively active, phosphorylating glycogen synthase (GS), which becomes inactive and thus glycogen synthesis is inhibited. Insulin promotes glycogen synthesis *via* PKB-mediated inhibition of GSK-3. Defective glycogen synthesis is not only evident in skeletal muscle of patients with insulin resistance but also in the liver.

Polymorphisms in the glycogen synthase gene have been described in insulin-resistant patients. [103]

In conclusion, in the liver impaired insulin signalling from IRS-1 to PKB/Akt leads to increased glucose production *via* inhibition of gluconeogenic enzymes. In addition, glycogen synthesis is inhibited and, at least in rodents, impaired IRS-2 signalling to aPKC leads to increased VLDL synthesis.

Adipose Tissue:

About 10% of whole-body glucose uptake occurs in adipose tissue. This might suggest that adipose tissue is of minor importance in insulin-stimulated glucose disposal and in insulin resistance. Muscle GLUT-4 depletion is associated with a markedly enhanced glucose uptake in adipose tissue.[104] Hence, there seems to be cross-talk between adipose tissue and skeletal muscle, and adipose tissue seems to be of major importance in the development of insulin resistance.

Insulin-stimulated glucose uptake in adipose tissue takes place *via* the same mechanism as in skeletal muscle: insulin signalling leading to GLUT-4 translocation. However, discrepancies have been found as to the defects in the insulin-signalling

cascade in type 2 diabetic patients, between adipose tissue and skeletal muscle cells. In adipose tissue defects are related to decreased protein expression, whereas this is normal in skeletal muscle. Hence, IRS-1 phosphorylation in adipose tissue of patients with type 2 diabetes is decreased because of a decreased IRS-1 protein expression (by 70%) and PI3K activity is decreased to the same extent by decreased protein expression.[105] In addition, in adipose tissue IRS-2 is capable to compensate for changes in IRS-1,[105] a phenomenon that does not seem to occur in skeletal muscle149. PKB/Akt activation is also impaired in adipose tissue of type 2 diabetic subjects, primarily *via* a reduction in insulin-stimulated serine phosphorylation.[106] GLUT-4 protein and mRNA expression are also substantially reduced in adipose tissue of type 2 diabetic patients,[107] in contrast to the normal expression in skeletal muscle.[108]

The main interest in the role of adipose tissue in whole-body insulin resistance has been on so called adipocytokines (or even better, adipokines, since not all proteins secreted by adipocytes are cytokines), proteins secreted by the adipocyte that might induce insulin resistance.

Insulin resistance is a state in which a given concentration of insulin produces a less than expected effects. Insulin resistance is a physiological condition where the natural hormone insulin becomes less effective at lowering blood sugars. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects, Insulin resistance is believed to be the key factor that leads to increased lipolysis in peripheral adipose tissue and increased uptake of fatty acids by hepatocytes. Hyperinsulinemia resulting from insulin resistance also adds to fatty acid content of hepatocytes by increasing glycolysis and by decreasing apolipoprotein B-100 production, and hence export of fatty acids as very lowdensity

lipoproteins (VLDL). The end result is an increase in fatty acids and triglycerides in the hepatocytes leading to steatosis. Insulin resistance is almost universal in patients with NAFLD and is related to an imbalance between proinsulin (adiponectin) and anti-insulin cytokines (TNF-α),particularly those secreted from adipose tissue (adipokines).[109]

Insulin resistance is calculated by **Homeostasis model assessment index(HOMA).** HOMA-IR= fasting Insulin(μU/l)× fasting plasma glucose(mmol/l)/22.5.(or) fasting insulin(μU/l) × fasting plasma glucose(mg/dl)/405. Insulin resistance is diagnosed if this value is more than 2.6.[110]

The HOMA model is used to yield an estimate of insulin sensitivity and β-cell function from fasting plasma insulin and glucose concentrations. HOMA has been compared with a number of well-validated methods used to measure IR and β-cell function[110].

There is good correlation between estimates of IR derived from HOMA and from the euglycemic clamp and between HOMA and the minimal model.

Estimates of β-cell function using HOMA have been shown to correlate well with estimates using continuous infusion glucose model assessment (CIGMA), hyperglycemic clamps, and the acute insulin response from the intravenous glucose tolerance test (IVGTT).

Clamps are complex stress tests with insulin and glucose concentrations and flux well outside the normal range.

Central or abdominal obesity that is more commonly associated with insulin resistance and has been observed in 80–90% of Indian patients with NAFLD.[111-113]

Dyslipidemia is a common feature, and is present in approximately 50% of Indian patients with NAFLD.[111] Both components of metabolic syndrome (high triglycerides and low HDL) were observed with almost equal frequency, being present in 53% and 66% patients with NAFLD, respectively.[111]

MATERIALS AND METHODS

Source of data:

The study group is selected from inpatients of RL Jalappa hospital and research centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar. This study includes a minimum of 50 subjects. The study population will comprise of men and women with age more than 18years.

Inclusion criteria.

1. Patients with ultrasound diagnosis of Fatty liver with no history of consumption of alcohol with age more than 18 years in RL Jalappa hospital and research center, Tamaka, Kolar are included in the study.
2. Patients with NAFLD are included in this study irrespective of history of diabetes mellitus.

Exclusion criteria

Patients with history of alcohol intake are excluded from this study.

Method of collection of data:

1. Informed consent is obtained from the patients under study. Patients who are found to be having fatty liver on ultrasound examination (from the patients who are subjected to ultrasound examination for various other conditions) are considered. After thorough history and clinical examination and applying inclusion and exclusion criteria the eligible patients are considered for the study. Persons height, weight, abdominal circumference will be noted.BMI is calculated.

2. After overnight fasting of minimum 8 hours, blood sample is collected for the estimation of plasma glucose, plasma insulin, fasting lipid profile and liver function tests by the standard procedures.
3. Insulin resistance is calculated using the Homeostasis Model Assessment Index(HOMA)

   HOMA-IR= [Fasting Insulin(μU/l)× Fasting plasma glucose(mmol/l)] /22.5

   (or)

   HOMA-IR= [Fasting insulin (μU/l) × Fasting plasma glucose (mg/dl)] /405.

   Insulin resistance is diagnosed if this value is more than 2.6.[114]
4. NAFLD (non alcoholic fatty liver disease) was diagnosed if person shows "fatty liver" on ultrasonography by diffuse increase in echogenicity. Liver ultrasound is more sensitive in assessing the severity of nonalcoholic fatty liver disease with homeostasis model assessment-insulin resistance[27,28].

Statistical analysis

Study design: An observational clinical correlation study.

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent

Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

1. **Chi-Square Test**: The chi-square test for independence is used to determine the relationship between two variables of a sample. In this context independence means that the two factors are not related. In the chi-square test for independence the degree of freedom is equal to the number of columns in the table minus one multiplied by the number of rows in the table minus one

   $\chi^2 = \frac{\sum (Oi - Ei)^2}{Ei}$, Where Oi is Observed frequency and Ei is Expected frequency with (n-1) df

The Assumptions of Chi-square test

The chi square test, when used with the standard approximation that a chi-square distribution is applicable, has the following assumptions:

- **Random sample** – A random sampling of the data from a fixed distribution or population.
- **Sample size (whole table)** – A sample with a sufficiently large size is assumed. If a chi square test is conducted on a sample with a smaller size, then the chi square test will yield an inaccurate inference. The researcher, by using chi square test on small samples, might end up committing a Type II error.

- **Expected Cell Count –** Adequate expected cell counts. Some require 5 or more, and others require 10 or more. A common rule is 5 or more in all cells of a 2-by-2 table, and 5 or more in 80% of cells in larger tables, but no cells with zero expected count. When this assumption is not met, Fisher Exact test or Yates' correction is applied.

2. **Fisher Exact Test:** The Fisher Exact Test looks at a contingency table which displays how different treatments have produced different outcomes. Its null hypothesis is that treatments do not affect outcomes-- that the two are independent. Reject the null hypothesis (i.e., conclude treatment affects outcome) if *p* is "small".

The usual approach to contingency tables is to apply the χ^2 statistic to each cell of the table. One should probably use the χ^2 approach, unless you have a special reason. The most common reason to avoid χ^2 is because you have small expectation values

	Class1	**Class2**	**Total**
Sample1	a	b	a+b
Sample2	c	d	c+d
Total	**a+c**	**b+d**	**N**

2x2 Fisher Exact Test statistic= $\sum p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!}\frac{1}{\sum a!b!c!d!}$

1: Fisher Exact test (rxc tables)

Let there exist two such variables $X$and Y, with $m$and $n$observed states, respectively. Now form an $m \times n$matrix in which the entries $a_{i\,j}$represent the number of observations in which $x = i$and $y = j$. Calculate the row and column sums $R_i$and C_j, respectively, and the total sum

$$N = \sum_i R_i = \sum_j C_j$$

of the matrix. Then calculate the conditional probability of getting the actual matrix given the particular row and column sums, given by

$$P_{\mathrm{cond}R} = \frac{(R_1!\,R_2!\cdots R_m!)(C_1!\,C_2!\cdots C_n!)}{N!\prod_{i,j} a_{i\,j}!},$$

which is a multivariate generalization of the hypergeometric probability function.

3. Student t test (Two tailed, independent)

Assumptions: Subjects are randomly assigned to one of two groups. The distribution of the means being compared are normal with equal variances.

Test: The hypotheses for the comparison of two independent groups are:

H_o: $u_1 = u_2$ (means of the two groups are equal)

H_a: u_1 u_2 (means of the two group are not equal)

The test statistic for is t, with $n_1 + n_2 - 2$ degrees of freedom, where n_1 and n_2 are the sample sizes for groups 1 and 2. A low p-value for this test (less than 0.05 for example) means that there is evidence to reject the null hypothesis in favor of the alternative hypothesis. Or, there is evidence that the difference in the two means are statistically significant. The test statistic is as follows

t-Test: Two-Sample Assuming Equal Variances

$$S_P = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

In all work with two-sample t-test the degrees of freedom or df is:

$$df = n_1 + n_2 - 2$$

The formula for the two sample t-test is:

$$T = \frac{\overline{X} - \overline{Y}}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Pre-test: Test for variance assumption: A test of the equality of variance is used to test the assumption of equal variances. The test statistic is F with n_1-1 and n_2-1 degrees of freedom.

t-Test: Two-Sample Assuming Unequal Variances

$$T = \frac{\overline{X} - \overline{Y}}{\sqrt{\frac{S_X^2}{n_1} + \frac{S_Y^2}{n_2}}}$$

Note in this case the Degree of Freedom is measured by

$$df' = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{\left(\frac{S_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{S_2^2}{n_2}\right)^2}{n_2 - 1}}$$

and round up to integer.

RESULTS OF THE T-TEST: if the p-value associated with the t-test is small (< 0.05), there is evidence to reject the null hypothesis in favor of the alternative. in other words, there is evidence that the means are significantly different at the significance

level reported by the p-value. if the p-value associated with the t-test is not small (> 0.05), there is not enough evidence to reject the null hypothesis, and you conclude that there is evidence that the means are not different.

4. SIGNIFICANT FIGURES

+ Suggestive significance (p value: $0.05<P<0.10$)

* Moderately significant (p value:$0.01<P \leq 0.05$)

** Strongly significant (p value : $P \leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

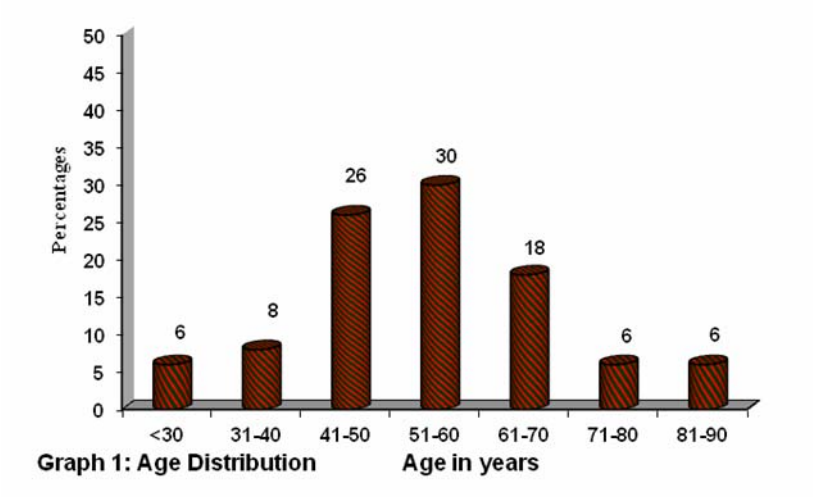
OBSERVATIONS AND RESULTS

The present study is done in R L Jalappa Hospital, attached to Sri Devaraj urs Medical College, Kolar. 50 patients with Non Alcoholic Fatty Liver Disease were identified and patients Height, Weight, Abdominal circumference is noted.BMI is calculated, Fasting blood glucose, Fasting plasma insulin, Fasting Lipid profile and Liver function tests are analyzed, Insulin Resistance is calculated using the HOMA-IR method. The results are as follows:

Age distribution:

Table 3: Age Distribution

Age in years	No. of patients	%
<30	3	6.0
31-40	4	8.0
41-50	13	26.0
51-60	15	30.0
61-70	9	18.0
71-80	3	6.0
81-90	3	6.0
Total	**50**	**100.0**



Graph 1: Age Distribution

Maximum number of subjects were seen in age group 41- 60 yrs. Mean age of Distribution is 55.08, standard deviation is 15.13 (55.08±15.13)

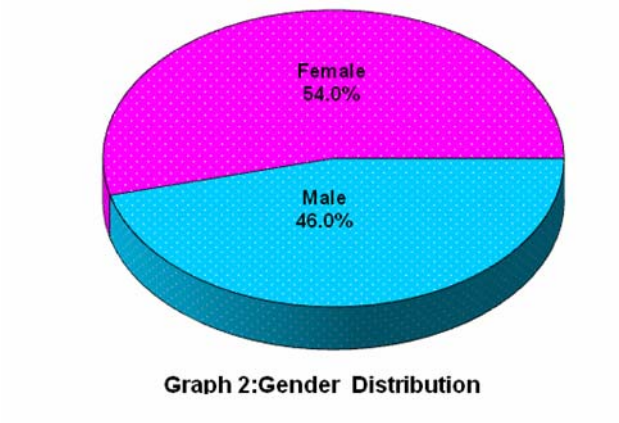
Males: 57.74±17.69, Females: 52.81±12.45.

Gender distribution:

In this study Males contributed to 46% and females 54%

Table 4: Gender Distribution

Gender	No. of patients	%
Male	23	46.0
Female	27	54.0
Total	**50**	**100.0**



Graph 2:Gender Distribution

Height Distribution:

Males: 165.96±5.54cms.

Females: 159.48±4.15cms.

Weight Distribution:

Males: 75.78±13.08

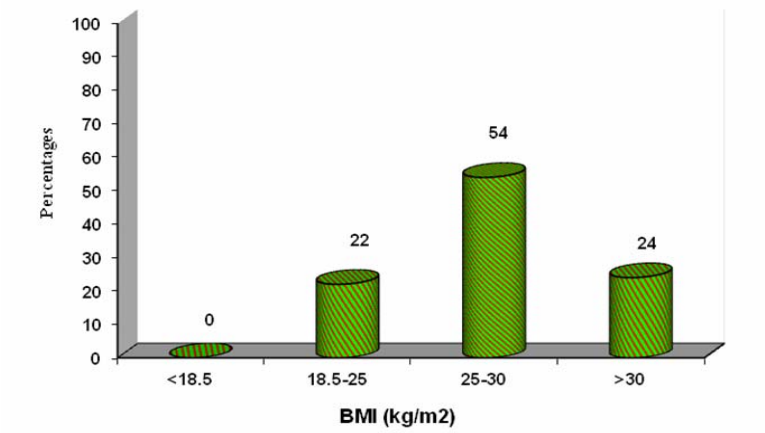
Females: 70.70±9.64

Body Mass Index:

Table 5: BMI Distribution

BMI (kg/m^2)	No. of patients	%
<18.5	0	0.0
18.5-25	11	22.0
25-30	27	54.0
>30	12	24.0
Total	**50**	**100.0**

Graph 3: BMI Distribution



Out of the 50 patients, BMI < 25 is seen in 11 patients and > 25 is seen in 39 patients.

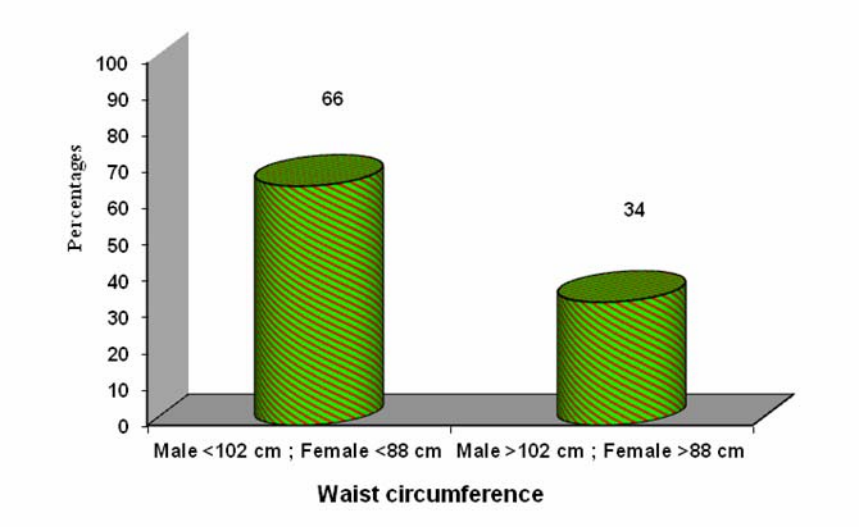
No patient is with BMI <18.5.

BMI > 25 significantly correlates with NAFLD with p value of 0.035

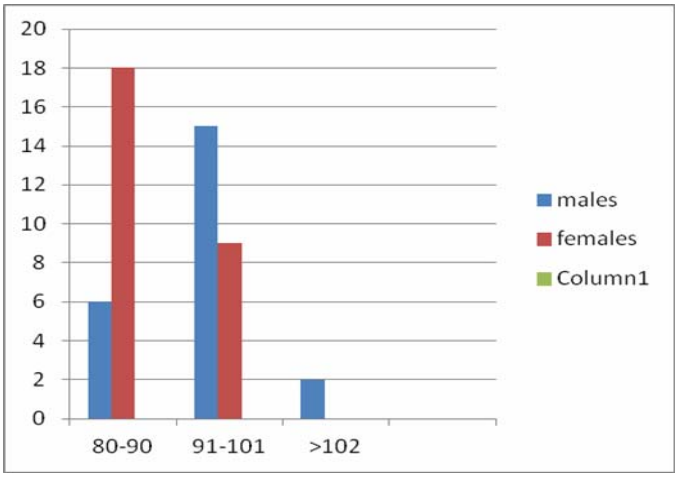
Waist Circumference:

Table 6: Waist Circumference

Waist circumference	No. of patients	%
Male <102 cm ; Female <88 cm	33	66.0
Male >102 cm ; Female >88 cm	17	34.0
Total	**50**	**100.0**



Graph 4: Waist Circumference



Graph 5: BMI- males and Females

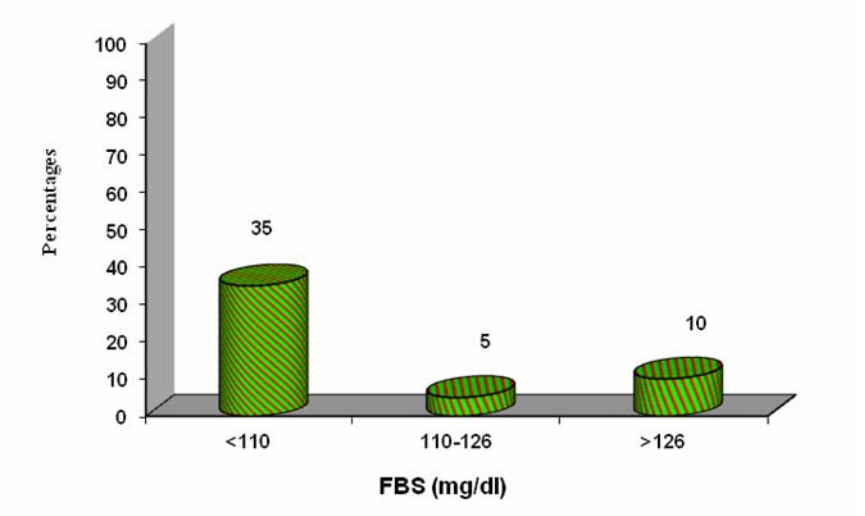
The mean Waist Circumference for Males is 94.00±6.46 and for Females is 89.52±4.54.Waist Circumference alone does not significantly correlate with NAFLD.

Blood Sugar : Out of the 50 patients, 35 patients have FBS< 110, 5 patients have FBS between 110-125 and 10 patients have FBS > 126.

Table 7: Sugar Parameters

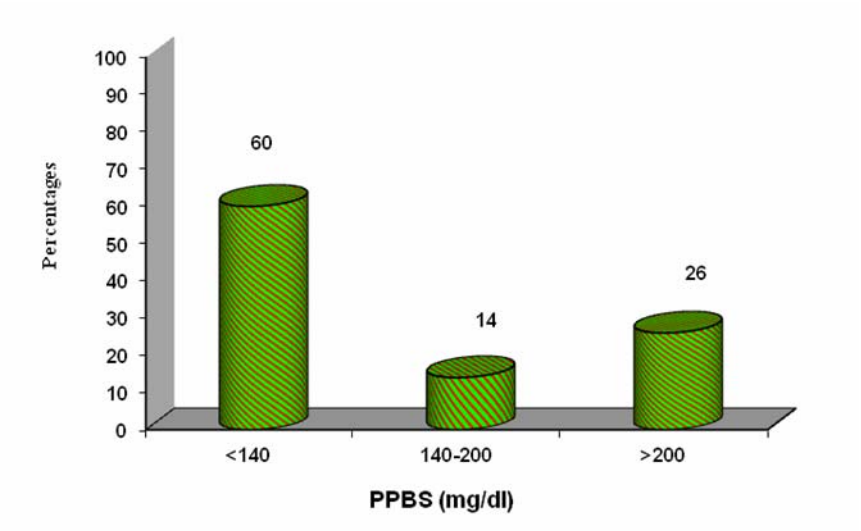
	No. of patients (n=50)	%
FBS (mg/dl)		
• **<110**	35	70.0
• **110-126**	5	10.0
• **>126**	10	20.0
PPBS (mg/dl)		
• **<140**	30	60.0
• **140-200**	7	14.0
• **>200**	13	26.0

Graph 6: FBS



The Mean FBS value of the Males is 135.74±84.33 and for the Females is 115.00±97.99

Graph 7: PPBS


100
90
80
70
60
50
40
30
20
10
0
Percentages
60
14
26
<140
140-200
>200
PPBS (mg/dl)

Lipid Profile:

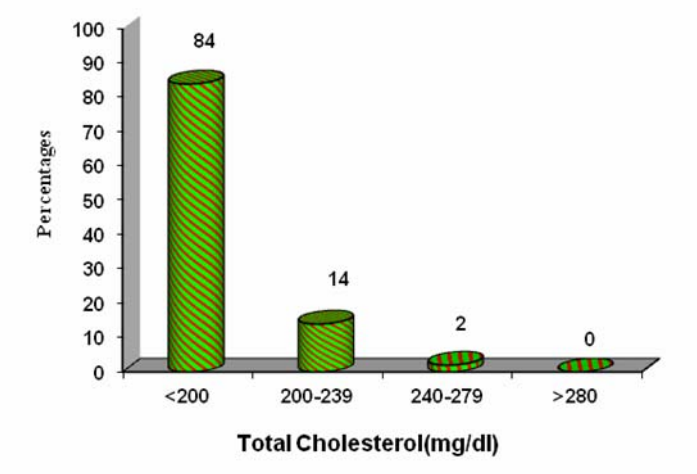
Table 8: Lipid Parameters

Lipid parameter	No. of patients (n=50)	%
Total Cholesterol(mg/dl)		
• **<200**	42	84.0
• **200-239**	7	14.0
• **240-279**	1	2.0
• **>280**	0	0.0
TGL(mg/dl)		
• **<150**	17	34.0
• **150-199**	9	18.0
• **200-499**	23	46.0
• **500+**	1	2.0
HDL(mg/dl)		
• **<35**	19	38.0
• **36-39**	11	22.0
• **40-59**	20	40.0
• **>60**	0	0.0
LDL(mg/dl)		
• **<70**	8	16.0
• **70-100**	20	40.0
• **100-129**	12	24.0
• **130-159**	8	16.0
• **160-189**	0	0.0
• **190 & above**	2	4.0

Out of 50 patients, 39 have Dyslipidemia, which contributes to 78%.

Out of 50 patients, 42 patients have normal total cholesterol value and rest 8 patients have elevated total cholesterol value. The Mean Total Cholesterol in Males is 167.13±37.65(mg/dl) and in females is 168.81±36.2(mg/dl).

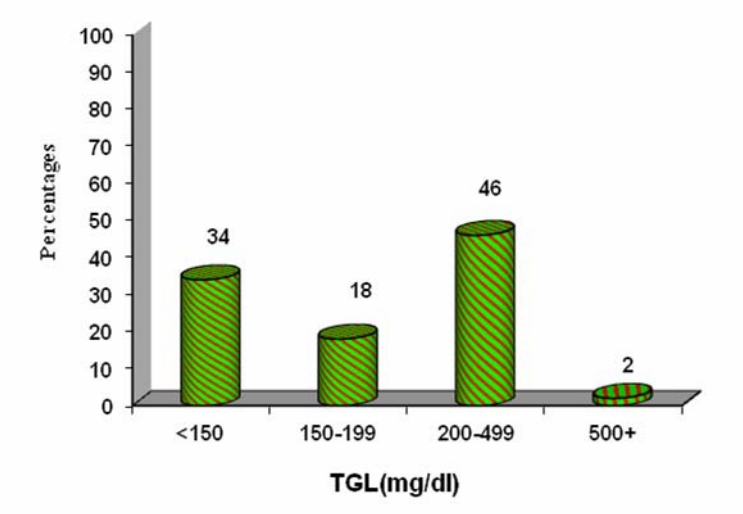
Graph 8: Total Cholesterol



17 patients have Triglycerides less than 150(mg/dl) and 33 patients have more than 150(mg/dl). Among the 33 patients 1 has value more than 500(mg/dl).

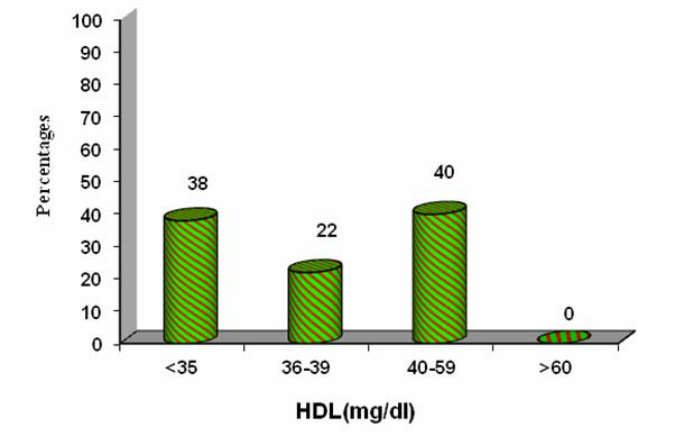
The Mean Triglycerides for Males is 191.43±102.47(mg/dl) and for Females is 200.11±84.61(mg/dl)

Graph 9: Triglycerides



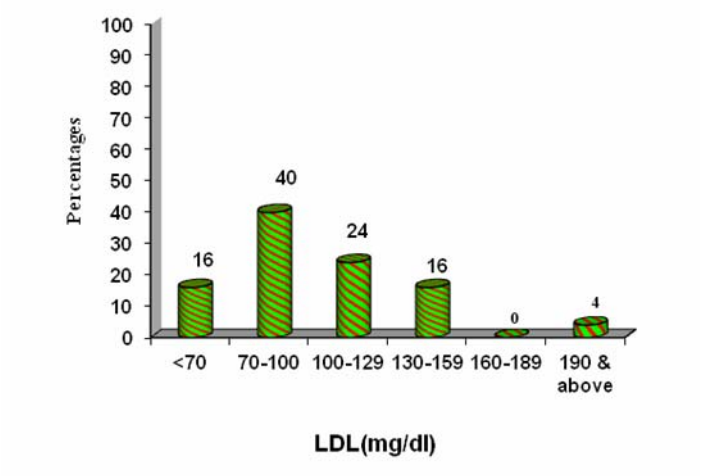
19 patients have HDL less than 35(mg/dl). And 31 patients have more than 35(mg/dl). No patient has more than 60(mg/dl). The Mean value for Males is 37.65±8.72(mg/dl) and for Females is 35.85±8.49(mg/dl)

Graph 10: HDL



40 patients have LDL value in the normal range and 8 patients have lower value and 2 patients have elevated LDL values. Mean value for Males is 90.64±34.70(mg/dl) and for Females is 101.19±35.54(mg/dl)

Graph 11: LDL


100
90
80
70
60
50
40
30
20
10
0
Percentages
16
40
24
16
0
4
<70
70-100
100-129
130-159
160-189
190 & above
LDL(mg/dl)

Liver Function Tests:

Table 9: Liver Function Tests

Liver Function tests	No. of patients (n=50)	%
Total Bilirubin (mg/dl)		
• **0**	0	0.0
• **0-1.3**	45	90.0
• **>1.3**	5	10.0
Direct Bilirubin (mg/dl)		
• **<0.3**	1	2.0
• **0.3-1.9**	48	96.0
• **>1.9**	1	2.0
SGOT (IU/L)		
• **0**	0	0.0
• **0-42**	37	74.0
• **>42**	13	26.0
SGPT (IU/L)		
• **0**	0	0.0
• **0-48**	40	80.0
• **>48**	10	20.0
ALP(U/L)		
• **<20**	1	2.0
• **20-140**	32	64.0
• **>140**	17	34.0
Total protein (g/dl)		
• **<6**	11	22.0
• **6-8.3**	38	76.0
• **>8.3**	1	2.0
Albumin(g/dl)		
• **<3.5**	27	54.0
• **3.5-5.5**	23	46.0
• **>5.5**	0	0.0
Globulin(g/dl)		
• **<2.3**	0	0.0
• **2.3-3.5**	41	82.0
• **>3.5**	9	18.0
A/G ratio		
• **<1.0**	11	11.0
• **>1.0**	39	39.0
GGT(U/L)		
• **0**	0	0.0
• **0-45**	40	80.0
• **>45**	10	20.0

Table 10: LFT mean values

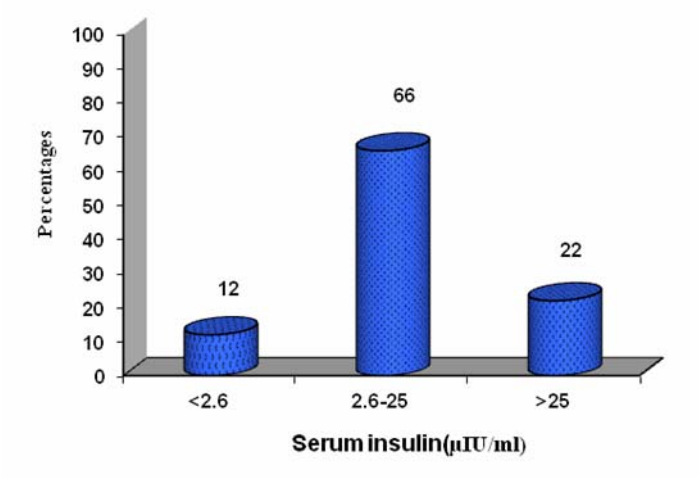
Total Bilirubin(mg/dl)	0.93±0.66	0.74±0.69
Direct Bilirubin(mg/dl)	0.32±0.35	0.31±0.52
SGOT(U/L)	40.26±19.20	43.22±42.76
SGPT(U/L)	41.57±17.58	38.56±17.19
ALP(U/L)	135.39±66.32	128.22±34.22
Total Protein(g/dl)	6.70±0.78	6.39±0.58
Albumin(g/dl)	3.43±0.45	3.35±0.42
Globulin(g/dl)	3.23±0.49	3.00±0.41
A/G	1.05±0.15	1.11±0.23
GGT(U/L)	40.09±21.39	37.07±32.68

Serum Insulin:

Table 11: Serum Insulin Distribution

Serum insulin(µIU/ml)	No. of patients	%
<2.6	6	12.0
2.6-25	33	66.0
>25	11	22.0
Total	**50**	**100.0**

Graph 12: Serum Insulin

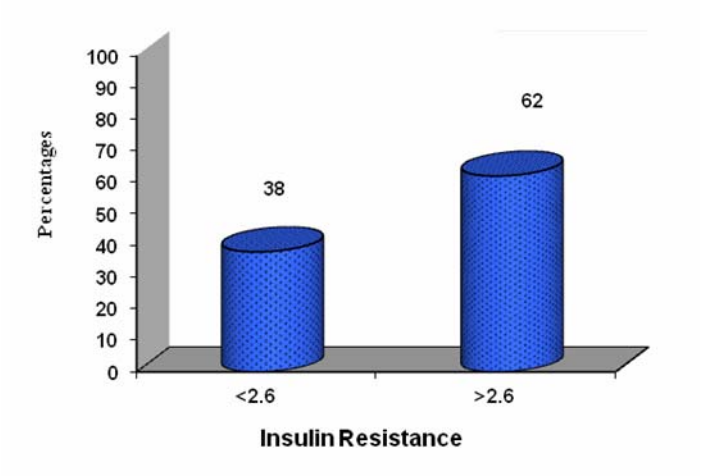


Serum Insulin Mean Value for Males is 30.26±70.07 and for Females is 23.62±28.50.

Insulin Resistance:

Table 12: Insulin Resistance Distribution

Insulin Resistance	No. of patients	%
<2.6	19	38.0
>2.6	31	62.0
Total	50	100.0



Graph 13: Insulin resistance

The p value for Insulin Resistance in NAFLD is 0.013 which is significant.

The mean value of Insulin Resistance for Males is 12.43±33.16 and for Females is 7.54±11.83.

Among the 31 patients with Insulin resistance, 22 patients are non diabetic(66%).

Comparison of Lipid parameters according to Insulin resistance:

Table 13: Comparison of Lipids to IR

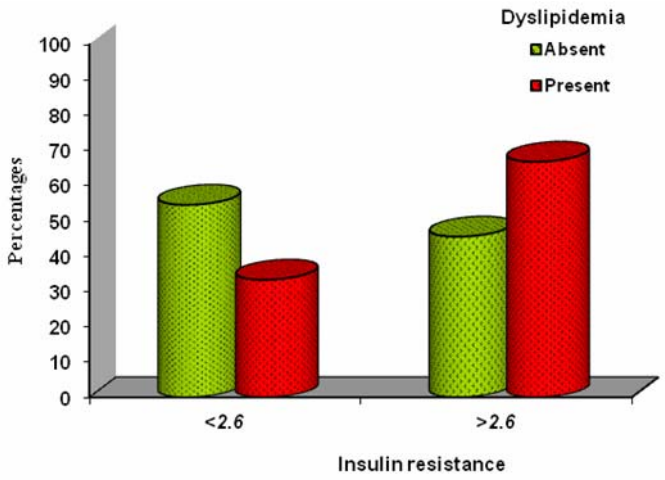
Lipid parameters(mg/dl)	**Insulin Resistance**		**P value**
	<2.6	**>2.6**	
Total Cholesterol	168.53±29.53	167.74±40.65	0.942
Triglycerides	150.68±51.02	223.97±101.32	0.005**
HDL	39.42±5.43	35.00±9.70	0.076+
LDL	95.63±28.66	96.97±39.25	0.899

Triglycerides correlate positively with Insulin Resistance in NAFLD patients with p value 0.005.

Correlation of Insulin resistance with Incidence of Dyslipidemia

Table 14: Correlation of IR with Dyslipidemia

Insulin resistance	Dyslipidemia		Total
	Absent	Present	
<2.6	6(54.5%)	13(33.3%)	19(38.0%)
>2.6	5(45.5%)	26(66.7%)	31(62.0%)
Total	**11(100.0%)**	**39(100.0%)**	**50(100.0$)**
Inference	**Insulin resistance >2.6 is positively associated with incidence of Dyslipidemia with P=0.0201**		



Graph 14: Correlation of IR and Dyslipidemia

Table 15: Comparison of LFT parameters according to Insulin resistance

LFT parameters	Insulin Resistance		p value
	<2.6	>2.6	
Serum Total Bilirubin(mg/dl)	0.88±0.69	0.8±0.68	0.674
Direct Bilirubin(mg/dl)	0.33±0.33	0.31±0.51	0.898
SGOT(U/l)	37.16±16.05	44.74±41	0.448
SGPT(U/l)	38.68±14.29	40.71±19.03	0.691
ALP(U/l)	132.84±54.05	130.71±50.07	0.888
Total Protein(g/dl)	6.29±0.61	6.68±0.71	0.057+
Albumin(g/dl)	3.18±0.45	3.51±0.37	0.07
Globulin(g/dl)	3.06±0.34	3.13±0.52	0.612
A/G	1.03±0.19	1.12±0.19	0.123

DISCUSSION

The study was conducted in Sri Devaraj Urs Medical College, 50 patients of NAFLD were included in the study. All cases met inclusion and exclusion criteria. The observations made in this study were discussed here and the results have been compared with other studies.

AGE:

In the present study the prevalence of NAFLD is more in the age group 41 to 60 years with 56%. In the study done by Targher G et.al,[115] the prevalence of NAFLD is more in the Population aged more than 60 years compared to the young with 74.6%. In the study done by Sookoian S et.al,[116] the mean age of prevalence of NAFLD is 50.3.

SEX DISTRIBUTION:

In the present study 46% of patients are males and 54% are females.

BMI:

In the present study 78% of the patients have BMI more than 25kg/m^2 .The mean BMI is 32.9 kg/m^2. In the study done by Sookoian S et.al,[116] the mean BMI is 31.9 kg/m^2 with a significant value of 0.03. in the study done by Targher G et.al., [115] the mean BMI of the patients with NAFLD is 28.3 kg/m^2. In the study done by Adamo D E et al.,[117] the mean BMI of the patients with high liver fat is 35.5 kg/m^2 and that of patients with low liver fat is 35.7 kg/m^2. The BMI in this study is comparable with that of other studies.

WAIST CIRCUMFERENCE:

The mean value of Waist circumference in this study is 91.58±5.89cms. The mean value of males is 94.00±6.46cms and that of females is 89.52±4.54cms. In the study done by Williamson M R et.al, the mean value is 106.7 ± 12.8cms and in that study as the grade of steatosis increases, the mean value of waist circumference increased.

FBS:

The mean FBS in the present study is 124.54±91 (mg/dl). 70% have FBS less than 110. 10% have values between 110-125(mg/dl) and 20% have values more than 126(mg/dl).In the study done by Adamo D E et.al. [117], the mean value is 96.7± 1.90(mg/dl). In the study done by Sookoian S et al[116] the mean value of FBS in NAFLD patients is 115.56 ± 40.5(mg/dl) and that of control group in that study is 96.3± 28.8(mg/dl). The values of FBS are comparable with other studies.

Among the 31 patients with Insulin resistance, 22 patients are non diabetic (66%).

LIPID PROFILE:

39 out of the 50 patients(78%) have Dyslipidemia.

The mean Total Cholesterol in this study is 168.04±36.50(mg/dl) . Elevated Total Cholesterol is found in 16%. In the study done by Sookoian S et al[116] the mean value of Total Cholesterol in NAFLD group is 209.97 ± 45.6(mg/dl) and in control group is 207.65 ± 42.92(mg/dl) with no significant change between two groups. In the study done by Williamson M R et.al, [118] the mean value in NAFLD group is 161 ± 31.70(mg/dl). The Total Cholesterol is comparable with other studies.

The mean Triglyceride value in the present study is 196.12±92.37(mg/dl). Elevated Triglyceride levels are found in 66%. In the study done by Sookoian S et al[116] the mean value of Triglycerides in NAFLD group is 180.69 ± 103.63(mg/dl) and in control group is 124 ± 53.14(mg/dl). In the study done by Williamson M R et.al,[118] the mean value in NAFLD group is 164.7 ± 88.57(mg/dl).

The mean HDL in this study is 36.68±8.55(mg/dl). Decreased HDL is found in 38%. In the study done by Sookoian S et al[116] the mean value of Total Cholesterol in NAFLD group is 52.59± 20.88(mg/dl) and in control group is 64.57 ± 10.82(mg/dl). In the study done by Williamson M R et.al,[118] the mean value in NAFLD group is 46.01 ± 12.37(mg/dl).

The mean LDL in this study is 96.45± 35.20(mg/dl). Elevated LDL is found in 4%. In the study done by Sookoian S et al[116] the mean value of Total Cholesterol in NAFLD group is 133 ± 38.6 (mg/dl) and in control group is 123 ± 35.57(mg/dl) . In the study done by Williamson M R et.al,[118] the mean value in NAFLD group is 82.75 ± 25.90(mg/dl).

Table 16: Mean values of Lipid profile in comparison with other studies:

	Total Cholesterol (mg/dl)	**Triglycerides (mg/dl)**	**HDL Cholesterol (mg/dl)**	**LDL Cholesterol (mg/dl)**
Present study	**168.04±36.50**	**196.12±92.37**	**36.68±8.55**	**96.45± 35.20**
Sookoian S et al[116]	209.97 ± 45.6	180.69 ± 103.63	52.59± 20.88	133 ± 38.6
Williamson M R et.al[118]	161 ± 31.70	164.7 ± 88.57.	46.01 ± 12.37	82.75 ± 25.90

SERUM FASTING INSULIN:

The mean Fasting Serum Insulin in the present study is 26.67± 51.44 (µIU/ML). Serum Insulin is less than normal in 12% of patients, Elevated Insulin values are seen in 22% of patients.

Table 17: Mean values of Fasting Serum Insulin in comparision with other studies:

Study	**Fasting Sreum Insulin (µIU/ML)**
Present study	**26.67±51.44**
Sookoian S et al[116]	49.3± 9.0
Perez M et al[119]	11.0± 5.1
Adamo DE et al[117]	33.6 ± 2.54

INSULIN RESISTANCE:

It is calculated according to the HOMA-IR. Insulin Resistance is seen in 62% patients in the present study. The pvalue for Insulin Resistance in NAFLD is 0.013.The mean value of insulin resistance is 9.79±23.96. In males the mean is

12.43±33.16 and in females it is 7.54±11.83. IR is comparable with other studies. Present study supports the findings of the studies done by Sookoian S et al[116] and Adamo DE et al[117]

Table 18: Mean HOMA-IR, Comparison with other studies

Study	Mean HOMA- IR	p value
Present study	**9.79±23.96**	**0.013**
Sookoian S et al[116]	3.5 ± 2.2	0.03
Adamo DE et al[117]	10.9 ± 1.14	0.002

LFT:

In the present study the p value for the various parameters of LFT is not significant. In the study done by Sookoian S et al,[116] LFT is not altered significantly. In the study done by Targher G et al,[115] SGOT and SGPT values are significantly elevated in NAFLD group with significant p value. In the study done by Adamo DE et al,[117] SGPT is significantly elevated in the high liver fat content group.

COMPARISION OF INSULIN RESISTANCE AND LIPID PARAMETERS:

The Triglycerides positively correlated with Insulin resistance group with p value of 0.005. In the study done by Adamo DE et al,[117] triglyceride values are significantly high in the high liver fat group with p value of 0.05 and other lipid parameters are not significant.

In this study insulin resistance >2.6 is positively associated with incidence of Dyslipidemia with P=0.020. The present study supports the findings of study done by Marchesini G et al[120] where it was concluded that in NAFLD Insulin resistance is positively associated with Dyslipidemia.

CONCLUSION

The results of present study suggest that multiple factors contribute to NAFLD.

- BMI more than 25kg/m^2 is a risk factor for NAFLD and Insulin Resistance.
- The Incidence of Dyslipidemia is significantly high in NAFLD.
- Diabetes Mellitus alone does not significantly contribute to NAFLD.
- The Incidence of Insulin Resistance is also significantly high in NAFLD.
- Increased Triglyceride level significantly correlates with Insulin Resistance compared to other Lipid parameters in NAFLD.
- Dyslipidemia positively correlates with Insulin resistance in NAFLD.

SUMMARY

The study was conducted in Sri Devaraj Urs Medical College, 50 patients of NAFLD were included in the study. The objective of the study was to estimate HOMA-IR and serum lipid profile among patients with Non Alcoholic Fatty Liver Disease and also also correlation between Insulin resistance and Dyslipidemia in NAFLD.

A total of 50 patients were included in the study:

- Majority of the patients were in the age group 41-60 yrs contributing to 56%.
- 46% were Males and 54% were Females.
- 78% has BMI more than 25kg/m^2 with the mean BMI 32.9 kg/m^2
- 70% of the patients are non diabetics. Diabetes Mellitus alone does not significantly contribute to NAFLD.
- Out of the patients with Insulin resistance more than 2.6, 71% are non diabetics.
- 78% of the patients have Dyslipidemia. The Incidence of Dyslipidemia is significant in NAFLD.
- Insulin resistance is seen in 62% of the patients. The p value is 0.013 which is significant.
- Increased levels of Triglycerides Positively correlated with Insulin resistance in NAFLD with p value of o.oo5.
- Dyslipidemia positively correlates with Insulin resistance in NAFLD.
- LFT does not correlate with Insulin resistance or Dyslipidemia in NAFLD.

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ANNEXURES

PROFORMA

CASE HISTORY OF THE PATIENTS

Case No : Date:

Name: OP No/ IP No:

Age:

Gender: Occupation:

CHIEF COMPLAINTS:

PAST HISTORY:

Hypertension : yes/no if yes , duration:

Diabetes : yes/no if yes , duration:

Other:

FAMILY HISTORY:

Diabetes : yes/no if yes , duration:

Hypertension : yes/no if yes , duration:

Other:

PERSONAL HISTORY:

Economic status :

Diet: vegetarian / mixed

Smoking : yes/no if yes, duration:

Alcohol : yes/no if yes , duration:

GENERAL PHYSICAL EXAMINATION:

Ht: Wt: BMI:

WAIST CIRCUMFERENCE:

Pulse rate : Blood pressure :

Oedema : Icterus :

Pallor : Clubbing :

Cyanosis : Lymphadenopathy :

SYSTEMIC EXAMINATION :

CVS :

RS :

PER ABDOMEN :

CNS :

DIAGNOSIS :

INVESTIGATIONS :

Fasting Blood Sugar (FBS): mg/dl

Post Prandial Blood Sugar (PPBS): mg/dl

LIPID PROFILE:

Total Cholesterol: mg/dl

Triglycerides: mg/dl

HDL: mg/dl

LDL: mg/dl

ULTRA SOUD ABDOMEN:

LIVER FUNCTION TEST:

Serum Total Bilirubin: mg/dl

Serum direct Bilirubin: mg/dl

SGOT/AST: U/l

SGPT/ALT: U/l

Alkaline Phosphatase: U/l

Total Protein: g/dl

Albumin: g/dl

Globulin: g/dl

A/G ratio:

Gamma GT: U/l

S.INSULIN: mcU/ml

INSULIN RESISTANCE:

OTHER TESTS:

ANNEXURE II

Patient details:

Name: **Age:** **Gender:** **Hospital No.**

Title of the Study: THE STUDY OF CORRELATION OF INSULIN RESISTANCE AND DYSLIPIDEMIA IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE

INFORMED CONSENT

I, _____, exercising my free power of choice, hereby give my consent to be included as a subject in the **"STUDY OF CORRELATION OF INSULIN RESISTANCE AND DYSLIPIDEMIA IN PATIENTS WITH NON ALCHOLIC FATTY LIVER DISEASE"** under the principal investigatorship of **Dr. ANIL KUMAR MANNAVA.** I understand that I remain free to withdraw from this study at any time.

I have read or had read to me and understand the purpose of this study and the confidential nature of the information that will be collected and disclosed during the study.

I have had the opportunity to ask my questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

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Participant's Name & signature Date

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Signature of the witness Date

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Signature of the principal investigator Date

MASTER CHART

Sl No	Hosp. No	Age	Sex	Height	Weight	BMI	Waist circumference	FBS	PPBS	Totl Cholesterol	Triglycerides	HDL	LDL	S. Total Bilirubin	Direct Bilirubin	SGOT	SGPT	ALP	Total Protein	Albumin	Globulin	A/G	GGT	USG Abdomen	S. Insulin	Insulin Resistance
1	717957	45	F	160	75	29.29	96	97	181	154	288	32	64	0.6	0.4	20	28	110	6.2	3.8	2.4	1.5	16	fatty liver	26.22	6.27
2	718573	45	F	156	64	26.3	92	119	206	153	263	41	59	0.7	0.2	14	21	89	6.1	3.2	2.9	1.1	18	fattyliver, hepatomegaly	33.51	9.69
3	729277	70	M	162	74	28.2	96	230	320	163	501	16		1.2	0.8	75	80	310	5.4	2..8	2.6	1.07	28	fatty liver, hepatomegaly	13.65	7.75
4	725324	52	F	160	70	27.34	83	89	229	201	170	42	125	0.8	0.6	38	30	145	64	3.4	3	1.13	14	fatty liver	2.46	0.54
5	713824	32	M	170	72	24.9	98	70	110	190	279	42	92	0.8	0.6	38	30	14	6.4	3.4	3	1.13	34	Fatty liver, cholecystitis	6.38	1.1
6	719599	30	F	160	70	27.3	82	73	218	169	214	32	94	0.8	0.4	48	32	100	6.4	3.2	3.2	1	20	Fatty Liver, Ovarian cyst	17.97	3.24
7	719264	45	F	158	64	25.66	85	98	160	168	298	32	76	0.6	0.3	35	32	110	5.8	3.5	2.3	1.5	18	Fatty liver	64.9	15.7
8	732543	43	F	158	68	27.2	88	94	139	178	234	34	97	0.4	0.3	130	40	35	6.8	3.9	2.9	1.3	26	Fatty liver	17.09	3.94
9	745892	65	M	168	70	24.8	97	122	188	225	255	37	137	1	0.2	42	38	208	7.1	3.6	2.5	1.1	42	Fatty liver, Prostatomegaly	15.11	4.54
10	858129	48	F	158	68	27.24	92	114	212	186	214	36	102	0.8	0.2	36	35	110	6.8	3.6	3.2	1.1	45	Fatty liver, fibroid Uterus	12.4	3.49
11	845870	72	M	172	84	28.3	98	355	304	103	73	50	40	0.41	0.1	15	40	163	8	4	4	1	20	fatty liver, hepatomegaly	3.71	3.25
12	843575	70	F	160	58	22.7	86	92	88	155	164	34	84	0.4	0.13	31	31	87	5.1	2.3	2.8	0.8	45	fatty liver	8.03	1.82
13	860820	60	F	154	78	31.4	90	598		99	280	16	27	4	2.8	222	102	129	6.6	3.1	3.5	0.9	191	fatty liver, hepatosplenomegaly	38.05	56.1
14	853844	88	M	155	59	25	88	100	119	155	132	35	72	0.4	0.08	32	40	110	5.8	2.6	3.2	0.8	44	Fatty liver	3.49	0.86
15	742793	55	M	170	75	26	92	350	384	168	155	42	78	2.6	1	84	88	248	6.4	3.5	2.9	1.2	66	fatty liver, hepatomegaly	2.13	1.84
16	832727	60	M	168	74	26.2	96	202	268	160	132	44	68	1.5	0.2	21	38	119	5.6	3	2.6	1.2	61	Fatty liver	11.91	5.93
17	853363	64	M	160	69	26.95	101	210	280	164	312	31	71	0.3	0.02	19	28	83	8.6	4.5	4.1	1.1	44	Fatty liver	128.9	66.8
18	854258	80	F	161	62	24	90	111	155	154	138	48	72	0.8	0.1	60	55	128	6	3.1	2.9	1	52	Fatty liver	16.4	4.49
19	856208	55	F	158	85	35	96	112	160	57	475	14	210	0.4	0.1	68	74	210	6.2	3.4	2.8	1.2	52	fatty liver	34.16	9.44
20	855137	60	F	155	56	23.33	94	87	82	195	164	42	120	0.58	0.1	23	44	125	6.8	3.6	3.2	1.1	31	fatty liver, hepatosplenomegaly	128.2	27.5
21	866937	62	F	154	65	27.42	87	87	118	154	138	52	74	0.2	0.08	32	40	126	5.8	2.6	3.2	0.8	48	fatty liver, hepatomegaly	11.27	2.42

MASTER CHART

22	847812	55	F	160	90	35.15	100	128	155	227	201	41	146	0.4	0.2	14	39	116	7	3.5	3.5	1	23	Fatty liver	46.92	14.8
23	836535	50	F	168	75	26.59	82	90	108	174	168	48	72	0.4	0.1	44	32	148	7.2	3.7	2.5	1.4	22	Fatty liver	2.52	0.56
24	811614	68	M	168	70	25.3	93	90	118	150	113	42	85	0.2	0.08	42	36	114	6.4	3.6	2.8	1.2	50	Fatty liver	18.95	4.03
25	820029	50	F	156	55	24.4	88	80	120	191	80	44	131	0.4	0.1	26	32	126	6.2	3.2	3	1.1	28	Fatty liver	0.2	0.03
26	806073	50	F	158	80	32.1	94	99	134	159	72	32	112	0.8	0.2	42	38	164	5.8	2.9	2.9	1	34	fatty liver, hepatosplenomegaly	3.21	0.78
27	869582	50	F	158	58	23.2	86	90	128	151	130	37	91	0.8	0.2	19	43	184	5.8	2.7	3.1	0.8	38	Fatty liver	11.12	2.47
28	812157	60	F	154	82	36.4	90	75	100	135	92	29	88	0.4	0.1	28	34	136	6.4	3.8	2.6	1.4	42	fattyliver, hepatomegaly	18.7	3.46
29	859569	24	F	163	77	29.5	88	142	218	169	214	32	94	0.4	0.08	32	28	124	7.6	3.8	3.8	1	24	Fatty liver	9.52	3.33
30	812570	52	M	160	78	30.4	92	78	117	210	95	56	135	0.2	0.06	34	28	142	6	3.2	2.8	1.1	30	Fatty liver	14.47	2.8
31	862533	20	M	165	60	22.05	82	90	110	154	100	30	104	2.8	1.2	36	52	101	6.9	3.1	3.8	0.8	45	Fatty liver, shrunken kidneys	4.8	1.06
32	812519	85	M	168	75	26.59	90	80	108	194	111	42	129	0.6	0.03	42	38	124	6.1	3.2	2.9	1.1	24	Fatty liver	6.81	1.34
33	714649	55	M	155	68	29.8	92	86	128	205	165	41	135	0.8	0.2	26	30	168	6.6	3.4	3.2	1.1	22	Fatty liver	15.63	3.31
34	883548	59	m	170	82	28.37	98	186	317	164	256	28	85	0.74	0.38	31	16	83	7.5	3.2	4.3	0.7	24	Fatty liver	330	151.55
35	885796	82	M	164	65	262	96	86	160	186	210	39	105	1.2	0.1	64	56	240	5.8	2.7	3.1	0.8	52	Fatty liver	1.39	0.29
36	919280	60	F	165	80	29.41	90	90	118	235	239	38	149	0.71	0.25	17	24	114	7.3	3.5	3.8	0.9	26	Fatty liver, cystitis	1.75	0.38
37	917915	75	F	164	73	27.2	94	100	110	181	151	31	120	1.24	0.47	62	56	148	6.4	3.1	3.3	0.8	40	Fatty liver	14.67	3.62
38	928412	38	m	169	75	26.31	95	84	125	135	144	33	74	0.4	0.1	22	30	118	7.2	4	3.2	1.2	27	Fatty liver, cystitis	9.98	2.07
39	927758	60	M	169	86	30.17	104	185	280	184	224	39	100	1.4	0.95	77	57	60	6.8	3.2	3.6	0.9	118	Fatty liver, MRD	17.1	7.81
40	720625	65	F	166	75	27.2	89	94	118	213	277	39	118	0.6	0.2	28	18	162	7.4	3.8	3.6	1.05	22	Fatty liver	15.7	3.64
41	937238	67	M	170	80	27.68	86	88	128	169	54	42	116	1	0.1	16	24	142	7	3.6	3.4	1.05	33	Fatty liver	16.58	3.6
42	927189	41	M	177	101	32.26	101	86	110	93	269	21	18	0.64	0.22	32	35	138	6.8	3.7	3.1	1.2	26	Fatty liver	16.27	3.45
43	711165	60	F	164	72	26.8	86	71	89	199	154	38	130	0.56	0.09	24	36	177	5.9	3.4	2.5	1.3	31	Fatty liver	5.78	1.01
44	918000	29	M	170	115	39.79	106	87	118	190	200	42	108	0.61	0.16	51	65	79	7.5	3.8	3.7	1.01	50	Fatty liver	17.7	3.8
45	871391	68	M	158	54	21.68	80	80	110	116	127	39	51	1.1	0.4	49	32	100	6.1	3.2	2.9	1.1	22	Fatty liver	8.8	1.73
46	836535	50	F	166	75	27.5	90	96	128	186	210	36	106	0.5	0.1	16	24	124	6.4	4	2.4	1.6	36	Fatty liver	10.93	2.61
47	705480	56	M	164	82	30.5	93	90	118	121	170	42	45	0.75	0.4	40	31	94	7.2	3.8	3.4	1.1	28	Fatty liver	5.86	1.3
48	705479	42	m	165	75	27.57	88	87	110	245	326	33	146	0.84	0.09	38	44	156	6.9	3.8	3.1	1.2	32	Fatty liver	26.44	5.67
49	868054	45	F	152	54	23.37	85	72	104	164	120	40	99	1	0.6	26	32	104	6.2	3.2	3	1.06	27	Fatty liver	5.79	1.02
50	918246	37	F	160	80	31.25	94	107	117	151	255	28	72	0.6	0.08	32	41	131	5.9	3.1	2.8	1.1	32	Fatty liver	80.2	21.1