PREVALENCE OF NONALCOHOLIC FATTY LIVER DISEASE IN TYPE 2 DIABETES MELLITUS

By

DR. BASAVESHWAR MHETRE

Dissertation submitted to

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In partial fulfillment of the requirements for the degree of

MD

in

GENERAL MEDICINE

Under the guidance of

Dr. R.M.HONNUTAGI **M.D.**

Professor,

Department of Medicine,

SHRI B.M. PATIL MEDICAL COLLEGE, VIJAYAPUR

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Date:

Place: Vijayapur

DR.BASAVESHWAR MHETRE

B.L.D.E UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, HOSPITAL &

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Place:

Date:

Dr. R. M. HONNUTAGI M.D.
Professor,
Medicine Department,
Shri B. M. Patil Medical
College, Vijayapur.

B.L.D.E UNIVERSITY'S

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HOD of Medicine

Seal & Signature

Dr. R.M.HONNUTAGI
M.D.(Medicine)
BLDEU's Shri B.M. Patil
Medical College, Hospital and
Research Centre, Vijayapur.

Date:

Place: Vijayapur

Principal

Seal & Signature

DR.M.S.BIRADAR
M.D.(Medicine)
BLDEU's Shri B.M. Patil
Medical College, Hospital and
Research centre, Vijayapur

Date:

Place: Vijayapur

B.L.D.E UNIVERSITY'S

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Place: Vijayapur

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DR. BASAVESHWAR MHETRE

LIST OF ABBREVIATIONS USED

AMPK	Adenosine monophosphate activated protein kinase
ALT	Alanine transaminase (SGPT)
AST	Aspartate transaminase(SGOT)
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
BMI	Body mass index
CBC	Complete blood count
CKD	Chronic kidney disease
CSF	Colony stimulating factor
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
ER	Endoplasmic reticulum
ESR	Erythrocyte sedimentation rate
FABP	Fatty acid binding protein
FAD	Flavin adenosine dinucleotide
FADH	Flavin adenosine dinucleotide hydride
FBS	Fasting blood sugar
FFA	Free fatty acid
HbA1c	Glycosylated Haemoglobin
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein
HGP	Hepatic glucose production
HSL	Hormone sensitive lipase
IHTG	Intrahepatic triglyceride

IMCL	Intramyocellular lipid
IL	Interleukin
LDL	Low density lipoprotein
LFC	Liver fat content
LPL	Lipoprotein lipase
MCD	Methionine choline deficient
MRS	Magnetic resonance spectroscopy
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydride
NEFA	N-ethyl tetrahydro fluorenamine
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
PDI	Protein disulphide isomerase
PPAR	Peroxisome proliferator activated receptor
PPBS	Postprandial blood sugar
RBP	Retinol binding protein
ROS	Reactive oxygen species
SF	Subcutaneous fat
SOD	Superoxide dismutase
SREBP	Sterol regulatory element binding protein
T2DM	Type 2 diabetes mellitus
TAG	Triacyl glycerol
TG	Triglyceride
TGF	Transforming growth factor
TNF	Tumor necrosis factor

USG	Ultrasonography
VF	Visceral fat
VLDL	Very low density lipoprotein

ABSTRACT

BACKGROUND AND OBJECTIVES:

Microvascular and macrovascular complications of Type 2 DM are well studied, but association of T2DM with Non alcoholic fatty liver disease (NAFLD) has been recognized recently. The prevalence of NAFLD amongst T2DM is higher compared to non diabetics.

There is evidence that T2DM patients with NAFLD are at higher risk of developing cirrhosis compared to non diabetics.

Nonalcoholic fatty liver disease is commonly associated with obesity, type 2 diabetes mellitus, dyslipidemia and insulin resistance-components of metabolic syndrome. This strongly supports the notion that NAFLD is hepatic manifestation of metabolic syndrome.

Recent data suggest that the prevalence of NAFLD may also be linked to increased coronary artery disease risk, independent of risk conferred by the elements of metabolic syndrome. Indentifying people with NAFLD would also highlight a subgroup of diabetic patients who would be targeted to decrease their risk of future CAD events.

There are very few reports about the prevalence of NAFLD in T2DM patients. To highlight the problem and as this study has not been done in this part of state it has been taken for the study.

The aim of this study is determine prevalence of NAFLD in Type 2 Diabetes mellitus and to find liver function abnormalities in Type 2 Diabetes mellitus.

METHODS:

This study was carried out in B.L.D.E.U's Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapur, Karnataka; during the period from November 2013 to June 2015. A total of 122 patients who were known case of Type 2 diabetes mellitus and who satisfied inclusion criteria were included in the study.

RESULTS:

The mean age of patients include in study was 59.7±12 years, majority with age group of 56-65 years. Out of 122 patients included in study 58(47.5%) had NAFLD, the most common sonographic grade of NAFLD was mild fatty liver (62%), followed by moderate (36%), then severe fatty liver (2%). The mean SGOT, SGPT and ALP levels were 31±14.4 IU/L, 25±14.2 IU/L and 104±47.6 respectively. Elevated levels of ALP was found to be significantly higher in patients with NAFLD compared patients without NAFLD. 58.6% patients with NAFLD had BMI above normal compared to 36.2% of patients without NAFLD who had elevated BMI which is statistically significant p=0.0001, making obesity an important association. Triglyceride levels was found higher in patients with NAFLD compared to patients without NAFLD and was statistically significant with p value of 0.003. Higher prevalence of retinopathy (p=0.01), nephropathy (p=0.01), and coronary artery disease (p=0.03) were found in patients with patients with NAFLD.

CONCLUSION:

Prevalence of NAFLD was 47.5% in T2DM patients, was significantly associated with overweight, obesity, raised levels of TG, VLDL, ALP. Microvascular complications of T2DM, nephropathy and retinopathy were found to be significantly higher in patients with NAFLD. Macrovascular complication of T2DM Coronary artery disease was found to be significantly higher in patients with NAFLD.

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INTRODUCTION

Fatty liver is defined as fat, largely triglyceride, exceeding 5% of liver weight. It is caused by failure of normal hepatic fat metabolism, either due to a defect within the hepatocyte or to deliver excess fat, fatty acid or carbohydrate beyond the secretory capacity for lipid of liver cell.

Nonalcoholic fatty liver disease (NAFLD) which develops in absence of alcohol abuse includes a spectrum of hepatic changes from steatosis alone to nonalcoholic steatohepatitis, fibrosis and cirrhosis.

The clinical implications of NAFLD are derived mostly from its common occurrence in general population and its potential to progress to cirrhosis and liver cell failure[1]. Estimates suggest that about 20-30% of adults in developed countries have excess fat accumulation in liver[2], 44-70% among people with diabetes about 80% in the obese and morbidly obese[3].

Metabolic syndrome and associated co morbidities like type 2 diabetes mellitus (T2DM) and dyslipedemia are predisposing factors of NAFLD, and prevalence of NAFLD has increased parallel to these epidemics[4]. The association of T2DM with microvascular and macrovascular complications is well established, but association of T2DM with NAFLD as a major complication has been recently recognized.

The prevalence of NAFLD amongst T2DMpatients is described to be higher than non diabetic patients. Approximately 70% of T2DM patients have a fatty liver

and they also appear to have more severe forms of disease including non alcoholic steatohepatitis (NASH) and fibrosis[5].

Chronic liver disease is often identified by asymptomatic elevation of two serum transaminase; alanine transaminase (ALT) and aspartate transaminase (AST) during routine serum chemistry, but more often slight increase in levels are over looked. Nonetheless there is evidence to suggest that apparently mild elevation in levels of these enzymes may be marker for significant liver disease (i.e. bridging fibrosis and cirrhosis) [6]. Elevation of levels of any of two enzymes has been found to be in the range of 2.8%-13.3% in general population[7], and 7.8%-31.5% in T2DM patients[8-13].The studies have found that liver enzyme abnormalities plus T2DM constitutes a greater risk of CVD [14-16] and renal diseases[17]. This makes diagnosis of NAFLD in T2DM patients, not only essential for prevention of hepatic complications but also important for the prevention of CVD and renal impairment.

AIMS AND OBJECTIVES

1. To determine prevalence of NAFLD in Type 2 Diabetes mellitus.

2. To find liver function abnormalities in Type 2 Diabetes mellitus.

REVIEW OF LITERATURE

THE LIVER

The liver is a central and essential organ and performs several important functions. The most important function in view of the current work is the liver's buffer function between the gut and the systemic circulation, this allows the liver to maintain glucose, fat and amino acid homeostasis. Due to its anatomical location, the liver is a rather inaccessible organ for conventional (non-imaging) techniques.

Liver anatomy

The liver is positioned in the upper right part of the abdomen, right beneath the thoracic diaphragm. The organ is highly vascular and has a double blood supply: approximately 75% of the blood is supplied by the portal vein (Figure.1) and approximately 25% by the hepatic artery. Both vessels run in the free edge of the lesser omentum and enter the liver at the hilum. The portal vein mainly drains nutrients and hormones from the splanchnic area directly into to liver, having an important influence on liver metabolic processes. The hepatic artery mainly supplies the liver with oxygen rich blood.[18] The human liver is divided into eight functionally independent segments which are organized in four anatomic lobes: left lobe (segment 2 and 3), right lobe (segment 5, 6, 7, and 8), caudate lobe (segment 1) and quadrate lobe (segment 4a and 4b). Lobes are again subdivided in smaller lobules which form the actual functional units of the liver. Each lobule is approximately 1.0-2.5mm in size and consists of hepatic cells, has its own central vein, and a peripheral portal triad, including a portal vein, bile duct and hepatic artery. The liver is also innervated

by nerves which accompany the vessels and ducts into the lobules: parasympathetic innervation through the left vague nerve and also sympathetic innervation.

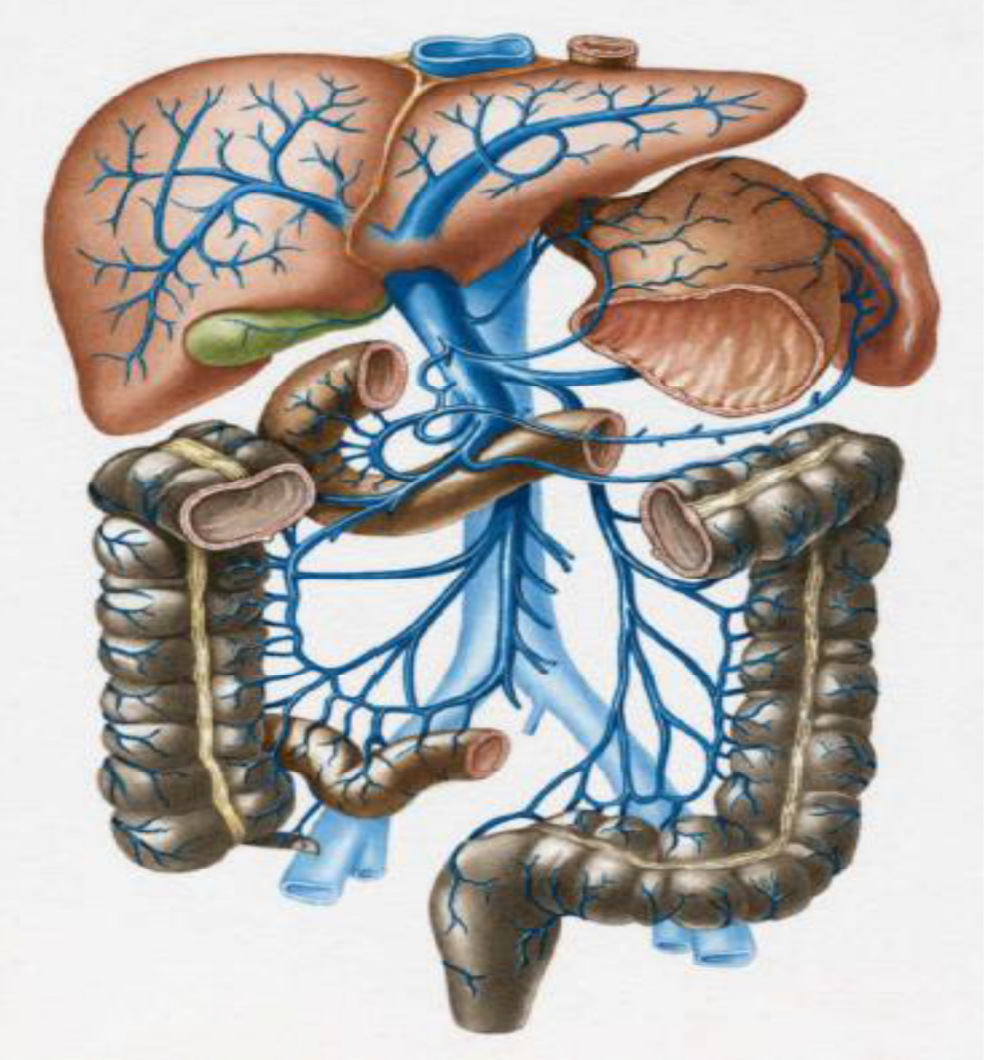


Figure1. Liver anatomy and venous blood supply. The main blood supply comes from the portal vein draining nutrients and hormones from the splanchnic area directly into to liver

Liver fat metabolism

Free fatty acids (FFAs) are delivered to the liver mainly by the portal route. FFAs are derived both from ingested meals as well as released from abdominal (visceral) fat depots. A third source of liver FFA is the triglycerides stored within the

liver. After a meal (postprandial) FFA are mainly stored in the liver as triglycerides, whereas during fasting they are mainly oxidized. During oxidation of FFA acetyl-CoA is formed, which can be used as a substrate for gluconeogenesis.

Effects of (increased) supply of FFA to the liver are not entirely clear. It seems that FFA in general increases hepatic gluconeogenesis and that the hepatic autoregulation prevents the hepatic glucose production (HGP) from actually increasing in the healthy individual with normal liver insulin sensitivity.[19] However, in patients with type 2 diabetes hepatic autoregulation seems to be defective, resulting in an increased HGP.

Triacylglycerol (TAG)

Fat in the form of triacylglycerol (TAG) is the most concentrated form of energy that is available for biological tissues.[20] The high energy content of TAG is strikingly illustrated by the fact that the first unaided crossing of the polar icecap was possible due to the very high butter-fat content of the expedition's 220 kg food reserves. The food reserves were transported on man- powered sledges, and the weight of the similar amount of energy in carbohydrate form would have made the expedition impossible.[21]

Triacylglycerol are made of a backbone of glycerol to which three fatty acids are attached (Figure 2), demonstrating the property of TAG to store free fatty acids in a compound with low biological toxicity, compared to fatty acids by themselves. Furthermore, high levels of TAG for a short or medium period of time are very well tolerated, and therefore TAG provides a safe way to store and transport fatty acids. For the fatty acids to become available and mobilized, TAGs have to undergo lipolysis. In humans the main storage pool for TAG in the entire body is the

subcutaneous tissue, which exports part of its storage as fatty acids. In addition, the liver has a vast capability of storing TAG which is exported in the form of very-low-density lipoprotein (VLDL) and ketone bodies.

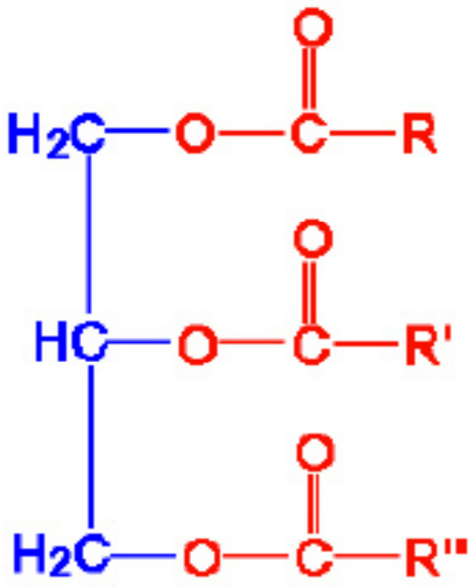


Figure 2.Basic structure of Triacylglycerol (TAG) consisting of a glycerol backbone (in blue) and three chains of fatty acids (in red, R-groups).

There is a fine balance between the adipose tissue and the liver, which together constitute a metabolic cycle: VLDL TAG fatty acids produced by the liver can be incorporated into the adipose tissue by the action of lipoprotein lipase (LPL). In turn, due to hormone-sensitive lipase (HSL), fatty acids are released by the adipose tissue, which can be used for liver VLDL synthesis.

HSL is expressed mainly in adipose tissue and not in the liver and promotes actively the release of TAG stored in cytosolic droplets in the adipocyte. Insulin and catecholamine and their mutual balance regulate the activity of HSL, depending on

the nutritional status of the body.[22] Insulin and catecholamine work antagonistically, with insulin inhibiting the activation of HSL and adrenalin promoting the activation of HSL, resulting in a release of fatty acids. The liver of a normal human adult has a TAG concentration of about 5 micromoles/g fresh weight, [23] but the normal range has been considered to extend as high as 45 micromoles/g fresh weight. The hepatic storage capacity of a normal adult (liver weight 1.5 kg) would, therefore, range from 7.5 to 67.5 mmol (6.7-60.7 g).

Due to its ability to store high amounts of TAG the liver works as a buffer, taking up excess of (cytotoxic) fatty acids and storing them as TAG or VLDL. In this way the liver protects the body from fatty acid lipotoxicity[24]. There are three potential sources for fatty acids which enter the hepatic TAG pool: FFAs in the plasma (originating mainly from adipose tissue), de-novo Lipogenesis and remnant lipoproteins. Animal experiments with perfused rat livers have shown that in the normal state the main contribution to the liver TAG synthesis is the plasma FFA level. Most likely plasma FFA levels are also the main contributor to liver TAG synthesis in humans. Measurement of de-novo Lipogenesis is problematic but recently great advances have been made using stable isotope labeling techniques.[25] Studies using the aforementioned stable isotope techniques have shown that the extent of dietary fatty acid recycling via serum FFA and VLDL-TAG is determined by the rate of delivery of dietary fat to the intestine.[26]

NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease(NAFLD) represents a spectrum of conditions characterized histologically by macrovascular hepatic steatosis and occurs in those who do not consume alcohol in amounts generally considered to be harmful to the liver.[27] Several (often incorrectly used) synonyms for the same disease are diabetes hepatitis, fatty-liver hepatitis, alcohol-like liver disease, Laennec's disease and nonalcoholic steatohepatitis (NASH).[28]

NAFLD disease is an increasingly recognized condition, fuelled by the increasing prevalence of obesity, and is very rapidly becoming a major health problem world-wide. [29- 30]

NAFLD in the early stage usually asymptomatic,[31] but it is by itself a risk factor for hepatocellular carcinoma (HCC) and is also part of the natural (progressive) history of NASH, which can lead to cryptogenic fibrosis.[32]

Due to this risk of progression to more severe liver disease through the is consequences of its fibro-inflammatory risk, NAFLD has been predicted to be the major cause of liver transplantation in 2020.[33] stressing the great need for early detection of the disease.

Metabolic disorders related to excess nutrient intake have been on the rise in recent decades. Increased incidences of metabolic syndrome, obesity and type diabetes have also lead to an increased awareness of the significance of co morbidities associated with these diseases, one of which is nonalcoholic fatty liver disease (NAFLD).

Hepatic steatosis, or fatty liver, is characterized by the excessive accumulation of triglycerides in the form of lipid droplets in the liver. This, in the absence of excessive alcohol consumption, is termed nonalcoholic fatty liver disease (NAFLD), the most common liver abnormality in the western countries. Besides obesity, NAFLD is associated with type 2 diabetes, Dyslipidemia, and hypertension

Other potential causes of hepatic steatosis are listed in Table.1

Nutritional	**Drugs***	**Metabolic or genetic**	**Others**
-Protein colorie malnutrition † -Starvation† -Total parentralnitrition† -Rapid weight loss† -GI surgery for obesity†	Glucocorticoids† Synthetic estrogen† Aspirin‡ Ca- channel blocker† Amiodarone€ Tamoxifen† Tetracycline‡ Methotrexate† Valproate‡ Cocaine‡ Antiviral agents Zidovudine† Didanosine‡	Lipodystrophy † Dysbetalipoprotenemia† Weber-christian disease† Acute fatty liver of pregnancy‡ Wolmans disease€	Inflammatory bowel disease† Small bowel diverticulosis with bacterial over growth† HIV infection† **Environmental toxin** Phosphorus‡ Petrochemicals‡† Toxic mushrooms† **Organic solvents** Bacillus cereus toxin‡

*This is partial list of agents that produce that produce fatty liver. Some drugs produce inflammation as well. The association of fatty liver with calcium channel blocker and valproic acid is weak, whereas with amiodarone is strong

† This factors predominantly cause macrovascularsteatosis.

‡ This factors predominantly cause microvascularsteatosis.

€ This factor cause hepatic phospholipidosis (mostly owing to accumulation of phospholipids in lysosomes).

NAFLD is a metabolic-related disorder characterized by fat infiltration of the liver in the absence of chronic alcohol consumption. Clinically, NAFLD encompasses

a broad spectrum of hepatic derangements ranging from fat accumulation (steatosis) to severe inflammation and fibrosis (NASH, nonalcoholic steatohepatitis) that can lead to cirrhosis (Figure 3).

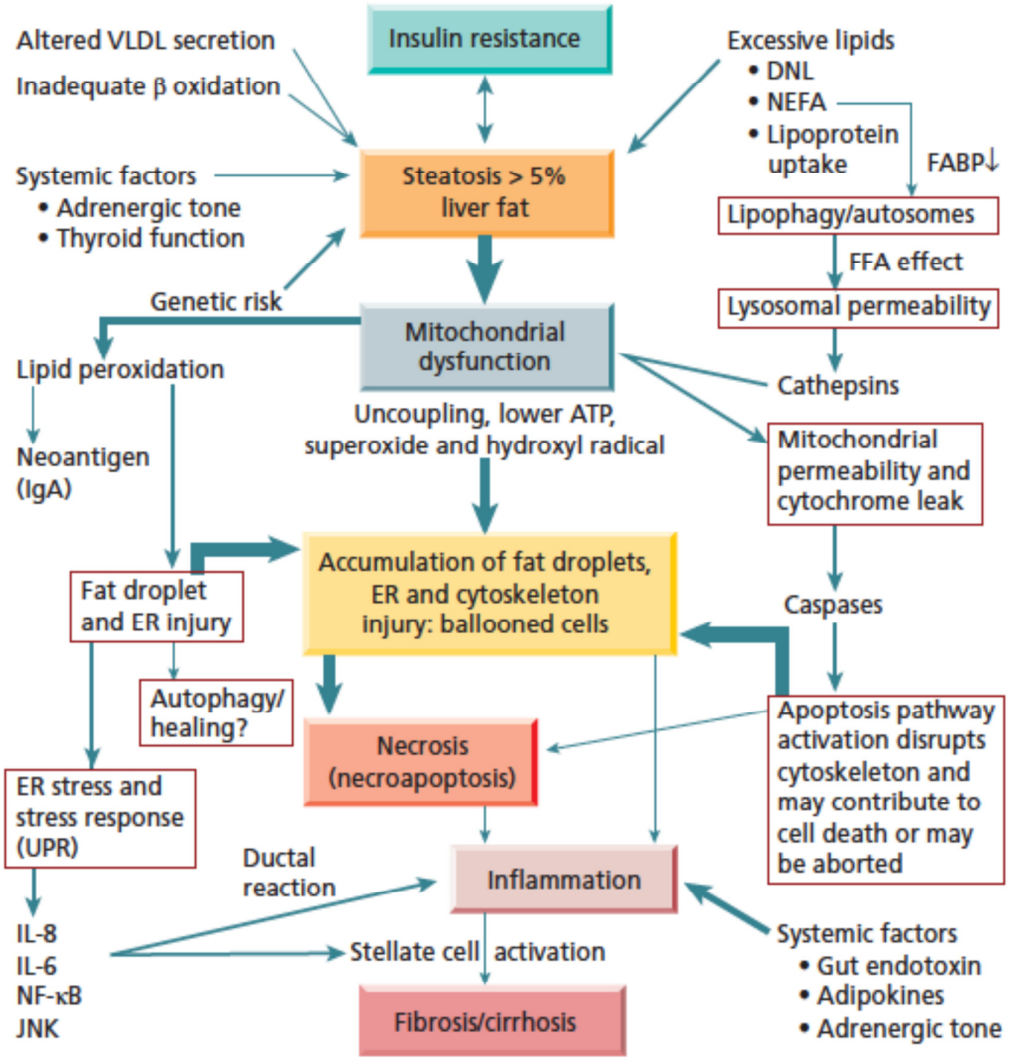


Figure 3. Progression of NAFLD. Fat accumulation (steatosis) occurs as a consequence of either increased import of triglyceride into hepatocytes or decreased export of triglyceride from hepatocytes. Steatosis predisposes the liver to susceptibility to a second "hit" trigger that can lead to inflammation, fibrosis, and hepatocellular injury characterized by NASH. If NASH further progresses, it can develop into cirrhosis of the live

Epidemiology of NAFLD

The prevalence of nonalcoholic fatty liver, NAFLD, is clearly increased in many obesity-related disorders. It has been shown that there is an increased relative risk for NALFD of 4.6 in obese persons with a body-mass index (BMI) of a least 30 kg/m2.[34]Truncal obesity is an important risk factor for NAFLD, also in patients with a normal BMI.[35]

Type 2 diabetes increases not only the risk of NAFLD but also the severity of the disease regardless of BMI.[36] A prevalence of NAFLD in T2DM of approximately 47% in the has been observed in some countries.[37]In patients with T2DM and severe obesity 100 percent had at least mild steatosis, 50 percent had steatohepatitis and 19 percent had cirrhosis.[38] Insulin resistance and hyperinsulinemia are associated with NAFLD also in subjects without T2DM.[39] Furthermore, patients with a history of T2DM are at increased risk to develop NASH. Hypertriglyceridemia, but not hypercholesterolemia, is also an important risk factor for NAFLD. An ultrasound study.[40] detected fatty infiltration of the liver in 50% of the patients with hypertriglyceridemia.[41]

The first important problem in determining the prevalence of NAFLD is that the observed prevalence greatly depends on the sensitivity of the instrument used to measure liver fat content (LFC). Liver biopsy and also Magnetic Resonance Spectroscopy (MRS) are currently considered to be the two most sensitive techniques for measuring LFC.[42]The second problem is that NAFLD is poorly detected by measurement of liver enzymes in blood samples, since these enzymes are normal in up to 78% of the patients with NAFLD.[43]

The fact that metabolic imbalance at the level of the liver can be detected at an early stage, was shown in a study by Machann et al. showing LFC to vary from 0.5% to as high as 39.3% in healthy subjects, with impaired glucose tolerance, overweight and a family history of type 2 diabetes.[44] This might explain the occasional observed high values of LFC in subjects described as "healthy" volunteers.

The large impact of NAFLD on patient survival was recently emphasized in a study showing that NAFLD accompanied by elevated liver enzymes is associated with a clinically significant risk of developing end-stage liver disease, resulting in a lower survival in patients with NASH. Furthermore, this study showed that most patients with NAFLD will develop diabetes or impaired glucose tolerance in the long term and that progression of liver fibrosis is associated with more severe insulin resistance and weight gain.[45] These findings stress the need for methods to detect (and treat) NAFLD at an early stage.

Etiology of NAFLD

Since the first description of the disease.[46] the pathogenesis of NAFLD has remained to be poorly understood. It is evident that an accumulation of triglycerides within the hepatocytes takes place, but the mechanism by which this happens is very unclear.

A popular hypothesis for the development of NAFLD is the "Second hit hypothesis" which was first described and has continued to be used to described possible phases of NAFLD and NASH.[47]

This hypothesis explains lip toxicity as the reverse sequence in which the relative contribution of liver fat may be amplified in patients with overt steatosis.2 In NAFLD the liver is not only receiving more lipid from outside, but also de-novo

Lipogenesis contributes to the accumulation of hepatic and lipoprotein fat in NAFLD.[48] These observations together with the increasing awareness of the molecular interplay between lipid and carbohydrate metabolism, have led to a "lipocentric" view of the pathogenesis of insulin resistance and T2DM.[49]

Certain studies using 1H MRS in obese individuals have shown NAFLD to develop easier in obesity[50] and a correlation between LFC and BMI has been observed.[51] However, the relation between liver fat content and BMI is not completely straightforward depending on the study population.[52]

Currently the visceral fat mass in healthy subjects is regarded to be directly related to LFC, possibly by providing FFAs to the liver through the portal blood flow.[53] In contrast, the amount of (abdominal) subcutaneous adipose tissue seems not to be related to LFC.[54]

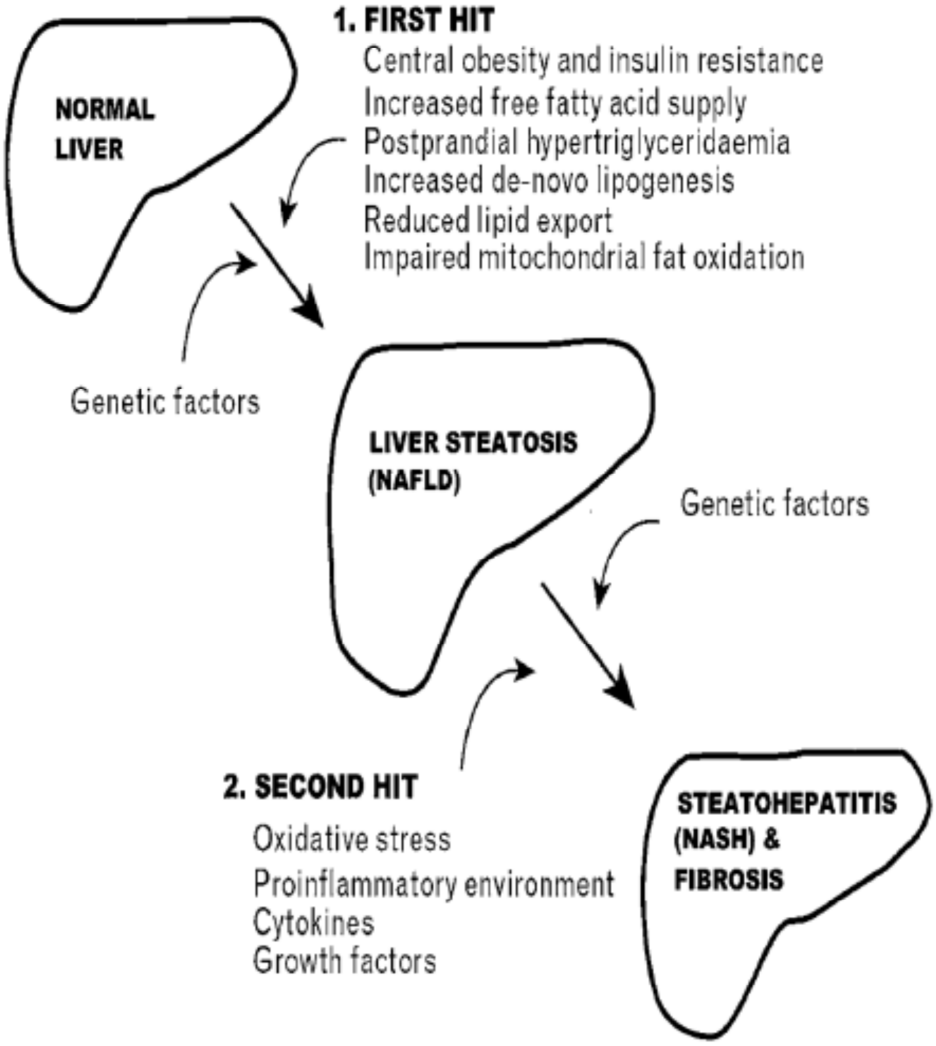


Figure 4. Recent version of the "Second hit hypothesis" for the development of fatty liver. Modified from "the trials and tribulations of the treatment of nonalcoholic fatty-liver disease".[55]

CONSEQUENCES OF HEPATIC STEATOSIS

Lipid accumulation in insulin sensitive tissues has been linked to insulin resistance in both human and animal studies. Thus one potential consequence of hepatic steatosis is the development of insulin resistance. One study showed high fat diet in rats, demonstrated that hepatic fat accumulation was followed by hepatic insulin resistance. In this study, hepatic fat accumulation decreased insulin activation of glycogen synthase and increased gluconeogenesis.

These changes were associated with activation of proteins, protein kinase C-e and c-Jun terminal kinase, which interfere with tyrosine phosphorylation of IRS-1 and IRS-2 and therefore insulin signaling. Several studies have demonstrated that hepatic steatosis is more closely linked to hepatic insulin resistance than factors such as whole body obesity, visceral obesity or circulating fatty acids in humans.

Fat accumulation in the liver leads to a variety of consequences, namely systemic insulin resistance and impaired insulin extraction by the liver. Hwang et al. sought to elucidate what lipid depot (i.e. intramyocellular lipid (IMCL), intrahepatic triglyceride (IHTG), visceral fat (VF) and/or deep abdominal subcutaneous fat (SF)) was correlated with insulin resistance.

Using 1H-magnetic resonance spectroscopy and magnetic resonance imaging, they were able to show significant inverse correlation between IHTG and whole body. This correlation suggests hepatic triglyceride accumulation can have systemic consequences involving impaired insulin sensitivity in non-hepatic tissues. Recent studies have emphasized the role of liver fat on hepatic insulin clearance.

In non-diabetic subjects, 50-70% of the insulin secreted by the pancreas is removed by the liver during first-pass transit. In advanced liver disease, hepatic insulin clearance is reduced, which is considered to be a major cause of hyperinsulinemia in liver cirrhosis. Increased liver fat is associated with impaired insulin clearance in nondiabetic human subjects.[56] Thus; it is possible that the link between liver fat and non-hepatic insulin resistance involved a sequence of events that include reduced hepatic insulin clearance, increased systemic insulin concentrations, and down regulation of insulin receptors and insulin signaling.

TRIGGERS INDUCING NASH DEVELOPMENT

NASH is characterized by steatosis and the induction of inflammation, fibrosis, and hepatocellular injury. It is interesting that not all of individuals with hepatic steatosis develop NASH. It has been estimated that 15-25% of cases of early stage NAFLD progress to NASH, and 15-20% of NASH cases further develop into cirrhosis. As a result of this data, it has been suggested that steatosis increases susceptibility of the liver to various other triggers, and those individuals characterized by the appropriate combination of triggers will be at highest risk for development of NASH.

This concept has experimental support. In a study, mice were fed a high fat diet (60% daily caloric intake) for 8 months to induce steatosis, obesity, insulin resistance and dyslipidemia.[57] there was no evidence of significant liver damage or inflammation produced by the diet. Hepatocytes were then isolated; steatosis hepatocytes had increased production of intracellular ROS and were more susceptible to TNF-a induced apoptosis than nonsteatotic hepatocytes. Studies such as these imply that a combination of steatosis and triggers such as increased cytokines may promote the development of NASH.

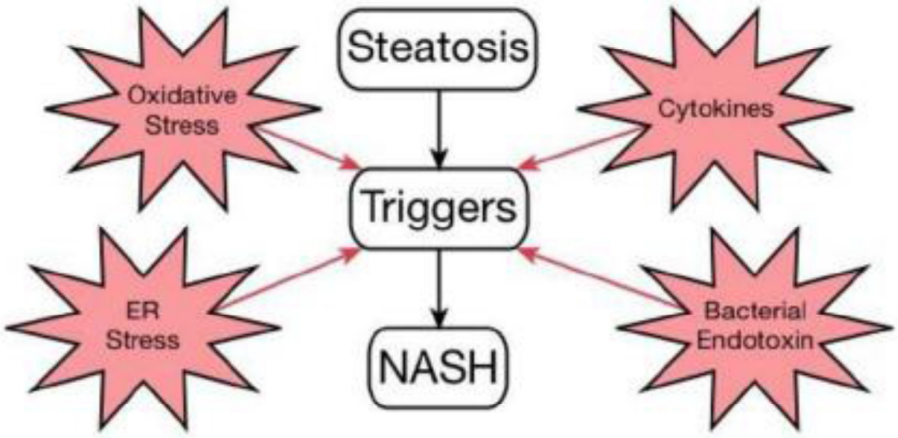


Figure 5. Types of triggers in the progression of NAFLD .Steatosis predisposes the liver to injury from 2nd hit triggers, which can include oxidative stress, cytokines, bacterial endotoxin, or ER stress.

Several so called second "hit" triggers, in addition to cytokines, have been proposed including oxidative stress, bacterial endotoxin, and/or ER stress. While simple steatosis occurs primarily in hepatocytes, the development of inflammation and fibrosis (characteristic features of NASH) involve Kupffer cells and stellate cells, respectively. Thus, the development of NASH is much more complex than the development of steatosis and involves multiple triggers and cell types within the liver.

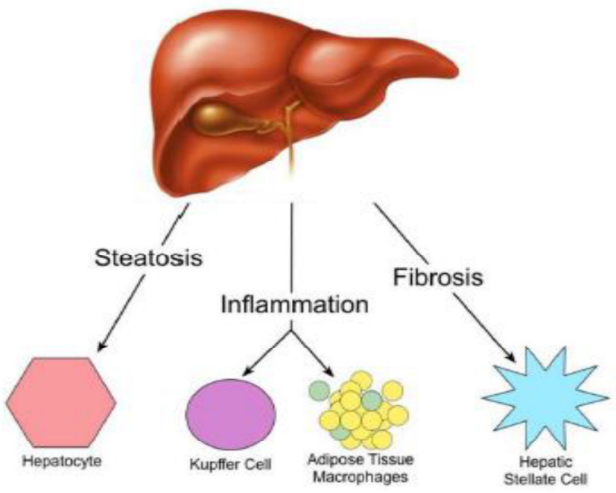


Figure 6 .Liver cell types involved in the progression of NAFLD. Lipid accumulation occurs primarily in hepatocytes, while inflammation occurs as a result of cytokine release from Kupffer cells and adipose tissue. Inflammation leads to hepatic stellate cell activation and the induction of fibrosis.

OXIDATIVE STRESS

Oxidative stress occurs when reactive oxygen species (ROS) are produced in excess of antioxidant defenses. ROS is a collective term that includes both oxygen radicals such as singlet oxygen and the superoxide anion, and certain non-radicals that are oxidizing agents and/or are easily converted into radicals, such as hydrogen peroxide and peroxynitrite.

Unresolved oxidative stress can interfere with normal cell metabolism and can cause damage leading to cell death. In the liver, oxidative stress can be generated through mechanisms involving mitochondrial dysfunction (leading to progressive impairment of b-oxidation, respiratory chain, and ATP synthesis), increased fatty acid oxidation in either peroxisomes (β-oxidation) by acyl-CoA oxidase or in the endoplasmic reticulum by CYP-2E1 and CYP4A isoforms or ER protein folding.

MITOCHONDRIA

FFA oxidation in mitochondria involves electron generation through redox reactions of the cofactors NAD+ and FAD to/from NADH and FADH2. These electrons are transferred to the electron transport chain where they generate an electrochemical potential required for oxidative phosphorylation and the generation of ATP. Normally electrons travel through the electron transport chain to cytochrome C oxidase, where they combine with oxygen and protons to form water. Sometimes, a fraction of these electrons "leak" from the electron transport chain and react directly with oxygen to form the ROS superoxide.

Manganese superoxide dismutase (Mg-SOD) converts superoxide into peroxide, which is further converted to water by mitochondrial glutathione peroxidase (which requires reduced glutathione to function). A dysfunctional mitochondrial environment where either the endogenous antioxidant enzymes Mg-SOD or glutathione peroxidase are down regulated or lacking in substrate to function properly promotes ROS formation.[52-54]

Mitochondrial structure can also play a role in its dysfunction; in NASH patients, at least 40% of mitochondria are structurally abnormal. These abnormalities (enlarged mitochondria, loss of mitochondrial cristae and Para crystalline inclusions) impair the electron transport chain enzyme activity and lead to uncoupling of oxidation from phosphorylation and production of ROS. Enhanced mitochondrial ROS formation has been shown in obese mice and hepatocytes and rat models of obesity and NASH. NASH patients have been reported to have enhanced hepatic levels of CYP2E1; studies in streptozotocin- induced diabetic rats showed increased levels and activity of mitochondrial CYP2E1 in the livers and other tissues, providing another potential source of ROS.

PEROXISOMES/MICROSOMES

Fatty acid β-oxidation or ω-oxidation can also occur in peroxisomes and microsomal, respectively; however, hydrogen peroxide is generated without the coupling of oxidative phosphorylation. The endogenous antioxidant enzyme of peroxisomes is catalase, which converts hydrogen peroxide to water. If the amount or activity of catalase is insufficient to reduce the hydrogen peroxide produced through β-oxidation, the hydrogen peroxide can react with cell components, particularly lipids, and causing cell damage and death. As paroxysmal FFA β-oxidation is viewed as a protective response to fatty acid overload in the liver mediated by PPAR-α, recent studies have shown PPAR-α expression in NAFLD patients is reduced.

Gene expression studies in NAFLD patients show enhanced expression of DNL, fatty acid uptake, fatty acid oxidation (however, reduced PPAR-α) and antioxidant genes, indicating antioxidant gene expression was adjusted to deal with the excess ROS generated from fatty acid oxidation. A study done in sodium valproate induced steatotic rats showed that, in addition to structural and functional alterations in hepatic mitochondria, increased lipid peroxidation was evident in hepatic peroxisomes and microsomes when compared to nonsteatotic control rats. These studies suggest there are impairments in fatty acid oxidation that generates ROS in NAFLD livers, making them more susceptible to lipotoxic conditions.

ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is an organelle responsible for many cell processes, including oxidative processes like protein folding that can generate ROS. Protein folding is an essential function of the ER mediated by protein disulphideisomerases (PDI), FAD dependent oxidases ERO1p, ERV2p and Fmo1p, with the final electron transfer being from ERO1p to 02 with peroxide and superoxide

as minor electron acceptors from ERO1p. Uncoupling of ERO1p, e.g. during ER stress (disruption of ER function leading to complex signaling cascades that attempt to ameliorate the stress), can lead to generation of ROS.

The cytochrome P450 family of proteins are a diverse group of enzymes, most of which are involved in catalyzing the oxidation of organic substances such as lipids, steroids, and xenobiotic. Two cytochrome P450 enzymes, CYP2E1 and CYP4A, are found in the ER and are responsible for a variety of detoxification reactions. CYP2E1 catalyzes the ω-1 hydroxylation of long chain fatty acids, while CYP4A catalyzes the ω and ω-1 hydroxylation of medium chain fatty acids (C6-C12). CYP2E1 catalyzes the NADH-dependent reduction of oxygen leading to lipid peroxidation. CYP2E1 expression has been shown to be increased in both rat dietary models of steatohepatitis as well as in the livers of patients with NASH. In mice fed a methionine choline deficient diet (MCD diet), steatohepatitis was induced as well as CYP2E1 expression and catalysis of lipid peroxides by hepatic microsomes.

This study showed that CYP2E1 can act as an initiator of oxidative stress in steatotic livers, however when CYP2E1 knockout mice were fed a MCD diet, steatohepatitis and lipid peroxidation were still induced, suggesting there are other catalysts to lipid peroxidation involved in the progression of steatohepatitis. One such alternative catalyst is CYP4A, which was discovered in vitro to play a role in lipid peroxidation in the absence of CYP2E1. Thus targeting a specific enzyme involved in lipid peroxidation may be futile due to the redundant nature of microsomal enzyme expression in lipid store management under conditions of NAFLD.

Oxidative stress can induce other forms of stress that further propagate the progression of NAFLD. Human Hematoma cells and hepatocytes to show that oxidative stress coupled with limited proteasome inhibition induced ER dysfunction

and inclusion formation. Inclusion formations (or Mallory bodies) in hepatocytes are significant markers of many liver diseases, including NASH. Prevention or alleviation of oxidative stress may prevent or reduce Mallory body formation and ER dysfunction in NAFLD.

CYTOKINES/ADIPOKINES

Cytokines are pleiotropic regulatory peptides that can be made by virtually all nucleated cells in the body. Types of cytokines include interferons, interleukins (IL), tumor necrosis factors (TNF), colony stimulating factors (CSF), transforming growth factors (TGF), erythropoietin, and thymopoietin. When the immune system is activated, cytokines activate and signal immune cells such as T-cells and macrophages to travel to the site of infection. Activated immune cells also release cytokines, propagating the immune response signal.

Cytokines can be either pro-inflammatory (i.e. tumor necrosis factor- (TNF-), interleukin-1 (IL-1), interleukin-6 (IL-6)) or anti-inflammatory (i.e. IL-1 receptor antagonist, interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13)).

Pro-inflammatory cytokines can up regulate the synthesis of secondary mediators and pro-inflammatory cytokines by macrophages and mesenchymal cells, stimulate production of acute phase proteins, attract inflammatory cells, or act as endogenouspyrogens (elevate thermoregulatory set point of the hypothalamus). Anti-inflammatory cytokines counteract inflammation through direct inhibition of pro-inflammatory cytokines or through other means. In the context of liver injury, TNF-α production occurs early and triggers production of other cytokines that recruit inflammatory cells, kill hepatocytes, and initiate fibro genesis.

The liver plays a secretory role in the development of NASH in the context of cytokine release from Kupffer cells (resident hepatic macrophages). TNF-α production occurs early and triggers production of other cytokines that recruit inflammatory cells, kill hepatocytes, and initiate fibro genesis. From a positional standpoint, both the liver and visceral adipose tissue share proximal associations between metabolic cells (hepatocytes and adipocytes, respectively) and secretory cells such as immune cells (NK and NKT cells), Kupffer cells, hepatic stellate cells, endothelial cells or macrophages, with each tissue having immediate access to an extensive network of blood vessels for continuous or dynamic immune and metabolic responses.

A subcategory of cytokines specific (but not exclusive) to adipose tissue is termed adipokines. White adipose tissue serves three known functions, energy storage, hydrolysis of triglyceride to free fatty acids, and release of adipokines, since adipose tissue contains adipokines-producing cells such as macrophages, fibroblasts and infiltrating monocytes.

In the context of NAFLD, obesity contributes to a higher prevalence of cirrhosis, suggesting that adipose tissue is a significant factor in the progression of the disease. Visceral adipose tissue seems to play an important role in the development of NASH by secreting FFA, hormones and adipokines.

Visceral adipose tissue, like the liver, shares proximal associations between metabolic cells (adipocytes) and secretory cells such as immune cells (NK and NKT cells), endothelial cells or macrophages; interspersed networking of blood vessels allows for expedited immune and metabolic responses. Different adipokines are expressed in subcutaneous versus visceral adipose tissue.

Subcutaneous adipose tissue predominantly expresses leptin, adiponectin, retinol binding protein-4 (RBP-4), and acylation stimulating protein, which is all involved in metabolic control.

Visceral adipose tissue predominantly expresses inflammatory proteins, such as tumor necrosis factor- (TNF-), interleukin-6, interleukin-8, adipsin, and resistin; proteins involved in tissue repair such as plasminogen activator inhibitor-1 and angiotensinogen, and proteins involved in regulation of metabolism, such as visfatin.Ectopic fat, such as visceral adipose or epicardial or 0control and inflammation in obesity-related disorders. In NAFLD, adiponectin is inversely correlated with hepatic fat content. RBP-4 appears to be positively correlated with peripheral insulin resistance and hepatic fat content; however, since RBP-4 is produced by both the liver and adipose tissue, it is unclear whether changes in RBP-4 occur as a result of changes in liver and/or adipose tissue production.

It has been hypothesized that, even though insulin resistance may lead to hepatic steatosis, the presence of hepatic fat may in turn worsen hepatic insulin resistance and could initiate a vicious cycle.[58] This hypothesis is supported by a study showing that the development of obesity leads to elevated liver enzymes, which in turn lead to glucose intolerance.[59] Although hepatic insulin resistance has been postulated as a main cause for increased LFC, at present no evidence has been found for a direct relationship between LFC and reduced insulin stimulated uptake of glucose by the liver.

Interestingly, heart and liver share common mechanisms of lipotoxicity, although the resulting damage is different. In the heart, triglycerides are not toxic in a direct sense, but ceramide accumulation, formed via de-novo synthesis from FFA, plays a central role in apoptosis of cardiomyocytes.[60]

In summary, NAFLD is a very common disease with yet unknown exact pathology. To enhance our knowledge of NAFLD and its pathogenesis, there is a great need for mechanistic studies in patients at high risk for developing NAFLD, such as patients with T2DM and/or obesity.

Diagnosis of NAFLD

Traditionally measurements of transaminase levels and good clinical history are regarded essential in diagnosing the underlying cause and treatment selection of fatty liver. However, in practice, transaminase elevation are nonspecific, lack diagnostic utility and are therefore of limited value[62-63], this poses a need for sensitive and specific methods for diagnosing fatty liver.

Ultrasound has high sensitivity (91.7%) and specificity in detecting fatty liver[64]. So serial ultrasound scan is an accurate tool for non invasive monitoring of efficacy of interventions in NAFLD patients.

Both computed tomographic scan and in particular magnetic nuclear resonance imaging seem to be more sensitive techniques for quantification of liver steatosis. Howerever none of these imaging technique have sufficient sensitivity and specificity for staging the disease and they cannot distinguish between simple steatosis and fibrosis[65].

The current gold standard for liver fat content estimation is liver biopsy, although an accurate fat quantification using this invasive procedure is complicated for several practical reasons. One of the main problems of liver biopsy is sampling variability's, a confounding factor which is often overlooked but should be considered especially when interpreting respected biopsies within same patient.

High variability in degree of fibrosis observed in repeated biopsies , performed in order to assess the progression of NAFLD , has been attributed to the fact that histological NASH lesions can be unevenly distributed throughout the liver parenchyma.

Treatment of NAFLD

Strategies in NAFLD Management

The first-line treatment of NAFLD is currently based on dietand lifestyle modifications. Most of the published studies inNAFLD population have shown that gradual weight loss (5–10%), calorie-restricted diet, and regular physical exerciselead to a decrease in the incidence of metabolic syndrome, improvement in liver enzyme profile, and resolution ofhepaticsteatosis[65-69]. Moreover, dietary treatment is limited by the lack of compliance and the frequent regain of weight at follow-up.

A pharmacological treatment in patients with NAFLD is not universally accepted yet. Given that insulin resistance plays a key role in the pathogenesis of NAFLD, many studies have evaluated the use of insulin sensitizers as a possible treatment for this disease. Biguanides (metformin) and thiazolidinediones (TZDs), including pioglitazone and rosiglitazone, are the two classes of insulin sensitizers studied in humans[70].

Several trials have shown a beneficial effect of TZDs in patients affected by NAFLD. Three studies, two open-label and one placebo-controlled trial, have evaluated the efficacy of rosiglitazone inNAFLD patients[71-73]. All of these studies have reported an improvement in transaminase levels and hepatic inflammation. However, nowadays rosiglitazone has been removed from the market because of its significant side effects.

Role of Metformin in NAFLD

The effectiveness of metformin as an antidiabetic drug is explained by its ability to lower blood glucose by decreasing gluconeogenesis in the liver, stimulating glucose uptake in the muscle, and increasing fatty acid oxidation in adipose tissue. The final effect is an improvement of peripheral insulin sensitivity.

At molecular level, some of the beneficial effects of this drug have been related to the phosphorylation and nuclear export of LKB1. This latter kinase activates adenosine monophosphate-activated protein kinase (AMPK).

AMPK inhibits the sterol regulatory element-binding protein-1c (SREBP-1c), which is a transcription factor for genes involved in fatty acid synthesis[74]. SREBP-1c is induced by an excess of glucose and insulin and is inappropriately increased in NAFLD patients.

A study conducted between December 2010-March 2011 by Sanjay Kalra et al, across 101 cities of India ,out of 924 patients ,in age group of 25-84yrs ,a cohort of 522(56.5%) Type 2 Diabetes mellitus patients were identified as having NAFLD. Prevalence of disease was found to be higher in females (60%) than males (54.3%) type 2 diabetes mellitus patients; with prevalence of NAFLD varying from 44.1% in Western India to 72.4% in Northern India.Prevalence of NAFLD increased with increasing age, with 239(45.8%) identified patients in age group of 25-50yrs and 283(54.2%) among those age 51yrs with high prevalence recorded in 61-70yrs age group at 61.8%. The mean AST and ALT levels were 54.8±36.1IU/L and 55.6±39.8IU/L, respectively in NAFLD population and highest in age group 25-40yrs an lowest in 71-84 yrs age group [75].

A study conducted Vijay Vishwanathan et al in Department of Diabetology, M. V. Hospital for diabetes and Diabetes Research centre, Chennai, between December 2006 and July 2007 ,on Non alcoholic fatty liver disease with diabetic microvascular and macrovascular complications in South Indian Diabetic subjects , total 2161 patients were screened for presence of NAFLD,156(7.2%)were found to be positive for NAFLD. Patients with NAFLD had higher BMI, Higher triglyceride and low HDL levels compared to patients without NAFLD. Levels of ALT and AST were significantly higher in patients with NAFLD. In patients with NAFLD there was increased prevalence of retinopathy, neuropathy and nephropathy. The prevalence of Coronary Artery disease was high in patients with NAFLD. Prevalence of Peripheral vascular disease was similar in both groups [76].

A study conducted by Somalwar AM et al in department of medicine govt medical college Nagpur between December 2011 and November 2013, on study of association of nonalcoholic fatty liver disease with micro and macro vascular complications of type2 diabetes mellitus included 120 patients with type 2 diabetes, out of these 68(56.66%) had fatty liver on ultrasonography. An increase in the waist circumference, BMI, systolic and diastolic blood pressure and levels of HBA1c, AST, ALT, total cholesterol, triglyceride and decrease in HDL were observed in fatty liver group. NAFLD group had higher prevalence of retinopathy (67.67% vs 17.30%), neuropathy(52.9%vs19.23%), nephropathy (82.2%vs53.4%). Prevelence of CAD(70.58%vs21.11%) and POVD(10.25%vs0%) was higher in patients with NAFLD[77].

A cross sectional study conducted by Shivanand Pai et al at a tertiary care hospital in Mangalore on Non alcoholic fatty liver disease in patients with Type 2 Diabetes mellitus. 44 patients with type 2 diabetes mellitus attending clinic of tertiary care hospital participated,30 diabetic patients had fatty liver and 14 diabetic patients

without fatty liver acted as controls . Diabetic patients with fatty liver had high BMI, High waist hip ratio, elevated SGPT/SGOT ratio (>1) and deranged lipid profile when compared to diabetic patients without fatty liver. SGPT/SGOT ratio >1 could act as biochemical marker for prediction of development of fatty liver in diabetic patients[78].

A study conducted between January 2008 & January 2012 at department of internal medicine Affiliated Hospital of Medical college in Qingdao university, China,61% of inpatients with T2DM had NAFLD, which decreased significantly with increase in age and prolonged course of diabetes. The prevalence of NAFLD in patients presenting with Diabetic Nephropathy (DN), Diabetic Neuropathy(DPN) and Diabetic retinopathy(DR) was 49.4%, 57.2% and 54.9%, respectively. These rates were significantly lower than those of patients without DN, DPN and DR (65.9%, 65.6% and 66.1%, respectively)[79].

MATERIALS AND METHODS

This study was conducted in the Department of Medicine, BLDE University, Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapur on patients with Type 2 diabetes mellitus during the period of December 2013-July 2015.

SAMPLE SIZE:

With prevalence of Nonalcoholic fatty liver disease is 9% in type 2 diabetes mellitus at confidence interval 95% at ±5 margin of error the sample size is 125.

Total number of patients to be studied:125±5

It is calculated using formula: $n = \frac{Z\alpha^2 \times p \times q}{d^2}$

q=100-p

d=clinically expected variation

Z = standardized normal deviate

STATISTICAL ANALYSIS:

Data will be analysed using chi square test or Fishers exact test. Data will be represented diagrammatically and by Mean ± standard deviation.

METHOD OF COLLECTION OF DATA:

Patient enrolled in the study will undergo complete medical examination at the time of enrollment.

INCLUSION CRITERIA:

Type 2 Diabetes Mellitus patients .

EXCLUSION CRITERIA:

1. All Type 2 diabetic patients with alcohol consumption of >30 gm/day for 3years
2. Type 2 diabetic patients
    a) Suffering from acute and chronic hepatitis.
    b) On hepatotoxic drugs and other hepatic diseases
    c) Other hepatic diseases.

Data collection

Demographic data like gender and age were collected along with relevant history and recorded on predesigned and pretested proforma. A thorough clinical examination was conducted and the findings were also recorded.

TESTS PERFORMED:

After selecting the case for study careful history and physical examination done and relevant investigations will be done. NAFLD is diagnosed on USG abdomen and graded as

Mild(Grade I): Minimal diffuse increase in hepatic echopattern with normal visualisation of portal vein radicals and diaphragm

Moderate (Grade II): Moderate diffuse increase in hepatic echogenicity and slightly impaired visualisation of intrahepatic vessels and diaphragm.

Severe (Grade III): Marked increase in echogenicity with poor or non-visualisation of hepatic vessels and diaphragm.

INVESTIGATIONS:

1. Complete blood count
2. Liver function tests.
3. Fasting blood sugar.
4. Post prandial blood sugar.
5. USG abdomen: Hyperechogenccity of liver will be considered as fatty liver.

   Mild: Minimal diffuse increase in hepatic echogenicity.

   Moderate: Moderate diffuse increase in hepatic echogenicity and slightly impaired visualation of intrahepatic vessels and diaphragm.

   Severe: Marked increase in echogenicity, poor penetration of posterior liver and poor or nonvisualation of hepatic vessels and diaphragm.
6. HbA1c:
7. Lipid profile.
8. Blood urea and Serum creatinine.
9. ECG.
10. Urine complete.
11. Ophthalmoscopic examination.

OBSERVATIONS AND RESULTS

Careful statistical analysis was performed on the obtained raw data, and following explanatory tables and charts were constructed for better insight into the topic.

In this study 122 patients with Type 2 Diabetes Mellitus were enrolled, 77(63%) were males and 45(37%) were females.

FIGURE 1 SEX DISTRIBUTIUON

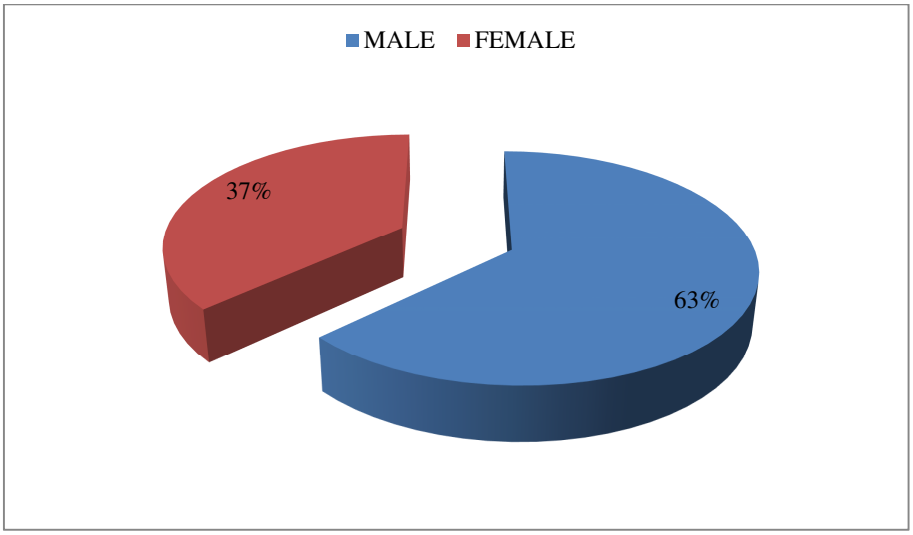
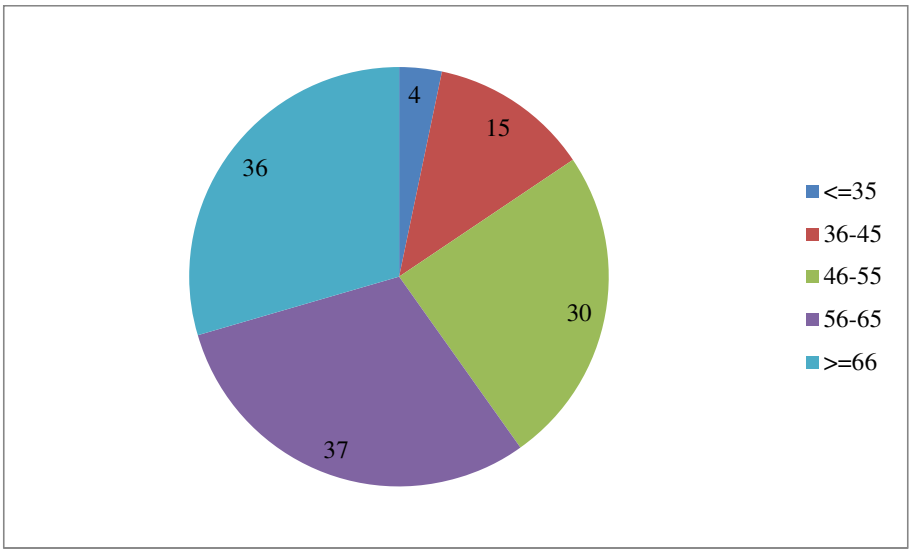


Table 1: Percentage distribution of patients according to age

Age	N	Percent
<=35	4	3.3
36-45	15	12.3
46-55	30	24.6
56-65	37	30.3
>=66	36	29.5
Total	122	100

Figure 2. Percentage distribution of patients according to age



Out of 122 patients included in study 37 patients were aged between 56-65 years, 36 were aged >=66 years, 30 patients between 46-55 years, 15 between 36-45 years and 4 were <= 35 years.

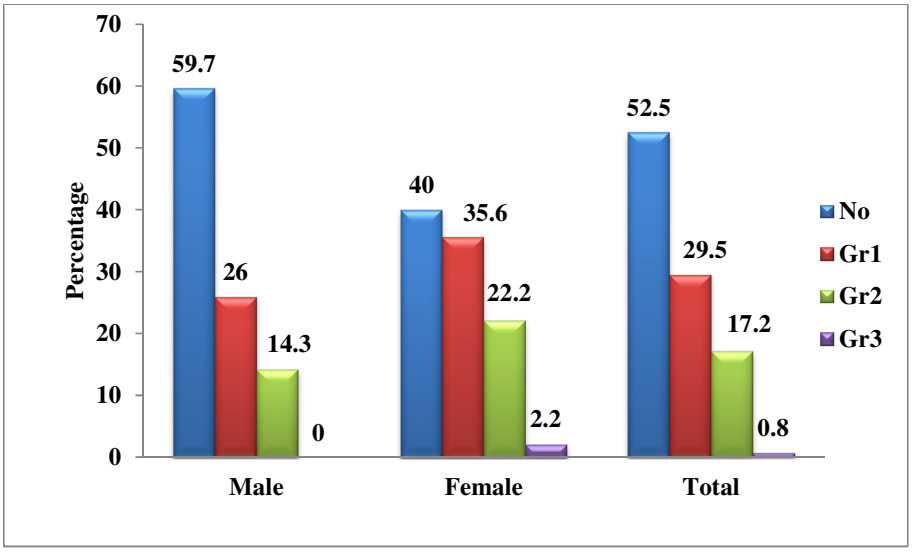
Table 2: Mean, Standard Deviation and Comparison of means of parameters by Gender

Parameters	N=122 (Total)		N=77 (Male)		N=45 (Female)		t value	p value
	Mean	SD	Mean	SD	Mean	SD		
AGE	59.7	12.0	62.2	11.7	55.5	11.4	3.12	0.002*
T2DM (YRS)	6.8	4.5	7.3	4.8	6.0	3.9	1.64	0.104
HTN(YRS)	2.9	4.6	3.5	5.1	1.8	3.3	1.96	0.053
IHD(YRS)	0.6	2.9	0.8	3.6	0.3	1.0	0.90	0.368
BMI	25.6	4.3	25.4	3.9	25.9	4.9	-0.68	0.500
T BIL	0.7	0.4	0.7	0.2	0.7	0.6	0.07	0.943
SGOT	30.2	15.4	31.6	16.1	27.8	14.1	1.33	0.185
SGPT	24.5	14.0	26.4	15.0	21.3	11.5	1.94	0.054
ALP	97.9	42.7	97.4	48.0	98.7	31.9	-0.16	0.877
FBS	184.2	75.1	180.8	72.6	190.0	79.6	-0.65	0.518
PPBS	240.1	74.9	234.8	74.0	249.0	76.5	-1.01	0.314
HbA1C%	8.9	2.1	9.0	2.3	8.7	1.8	0.76	0.452
T CHOL	175.5	45.6	168.5	46.2	187.4	42.4	-2.26	0.026*
TG	146.7	73.9	142.4	68.4	154.0	82.8	-0.84	0.404
HDL	36.5	18.1	35.2	19.3	38.8	15.9	-1.05	0.298
LDL	110.2	38.2	103.7	40.8	121.4	30.6	-2.52	0.013*
VLDL	30.0	15.2	29.2	13.6	31.5	17.7	-0.81	0.422
SR CREAT	1.6	2.0	1.8	2.3	1.3	1.2	1.36	0.176
B UREA	40.1	32.6	42.9	36.9	35.1	23.1	1.29	0.201
HB	11.7	2.3	12.2	2.3	10.9	2.0	3.12	0.002*
TC	12242.0	5514.1	11895.0	5621.4	12835.0	5335.0	-0.91	0.365
ESR	59.7	101.5	49.4	36.7	77.2	159.8	-1.47	0.145

Table 3: Percentage distribution of Fatty Liver disease

USG LIVER	Male		Female		Total	
	N	Percent	N	Percent	N	Percent
Normal liver	46	59.7	18	40	64	52.5
Fatty liver Grade1	20	26	16	35.6	36	29.5
Fatty liver Grade2	11	14.3	10	22.2	21	17.2
Fatty liver Grade3	0	0	1	2.2	1	0.8
Total	77	100	45	100	122	100

Figure 3: Percentage distribution of Fatty Liver disease



For female patients with NAFLD, 59.2% (n=16),37% (n=10) and 1(0.8%) had grade 1, grade2 and grade 3 NAFLD respectively. For males with NAFLD 64.5% (n=20), 35.5(n=11), 0 had grade 1, grade2 and grade 3 NAFLD respectively.

Table 4. Comparison of means of parameters by presence and absence of NAFLD

Parameters	WITH NAFLD (N=58)		WITHOUT NAFLD (N=64)		p value
	Mean	SD	Mean	SD	
AGE	58.9	11.8	60.5	12.2	0.481
T2DM (YRS)	6.0	3.2	7.6	5.3	0.044*
HTN(YRS)	2.8	4.1	2.9	5.1	0.924
IHD(YRS)	0.3	1.0	0.9	3.9	0.233
BMI	27.3	4.8	24.0	3.1	0.000*
T BILIRUBIN	0.8	0.6	0.7	0.2	0.182
SGOT	31.0	14.4	29.5	16.4	0.582
SGPT	25.0	14.2	24.0	14.0	0.709
ALP	104.0	47.6	92.4	37.2	0.134
FBS	194.8	78.1	174.5	71.5	0.136
PPBS	247.8	72.3	233.1	77.1	0.281
HbA1C%	9.2	2.0	8.5	2.1	0.067
T CHOL	180.7	47.8	170.7	43.3	0.226
TG	167.2	92.3	128.1	45.1	0.003*
HDL	39.3	24.3	34.1	9.1	0.115
LDL	111.9	39.0	108.7	37.7	0.652
VLDL	34.0	19.0	26.4	9.6	0.005*
SR CREAT	1.2	0.9	2.0	2.5	0.014
B UREA	34.8	28.4	44.8	35.6	0.091
HB	11.9	2.2	11.6	2.4	0.385
TC	12766.0	5264.4	11766.0	5730.6	0.319
ESR	51.7	32.8	66.8	136.8	0.414

Note: $p < 0.05$ is taken as significant level

In this study duration of diabetes, BMI, triglyceride levels, VLDL levels are found significant.

Table 5: Bivariate analysis of deranged liver parameters of the study population

		NAFLD	NON NAFLD	TOTAL	OR	P VALUE
ELEVATED ALP	YES	21	10	31	3.06	0.009*
ELEVATED SGOT	YES	14	9	23	1.9	0.15
ELEVATED SGPT	YES	8	5	13	1.8	0.2

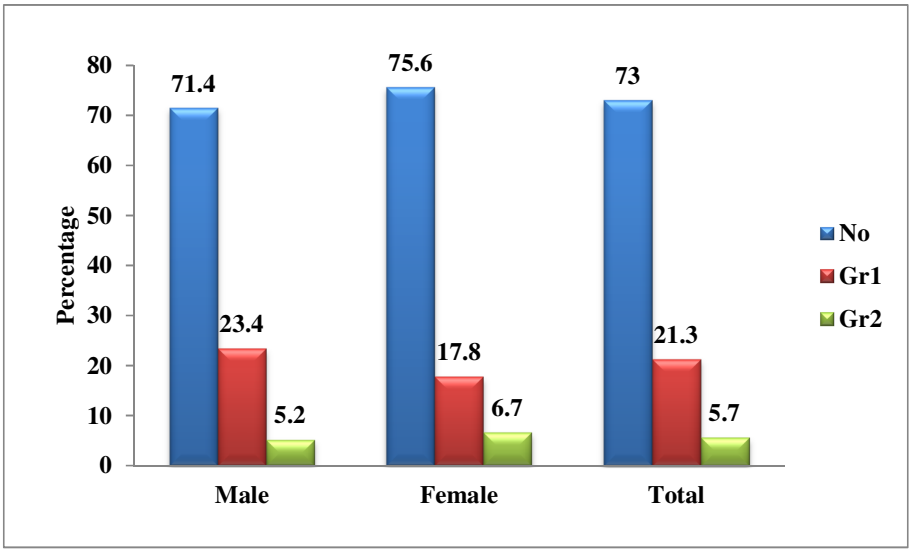
Note :P value by chi square test,* P<.005 is taken as significant

In this study elevated levels of Alkaline phosphatase (ALP) was found significant

Table 6: Percentage distribution of NPDR

NPDR	Male		Female		Total	
	N	Percent	N	Percent	N	Percent
No	55	71.4	34	75.6	89	73
Grade1	18	23.4	8	17.8	26	21.3
Grade2	4	5.2	3	6.7	7	5.7
Total	77	100	45	100	122	100

Figure 4: Percentage distribution of NPDR

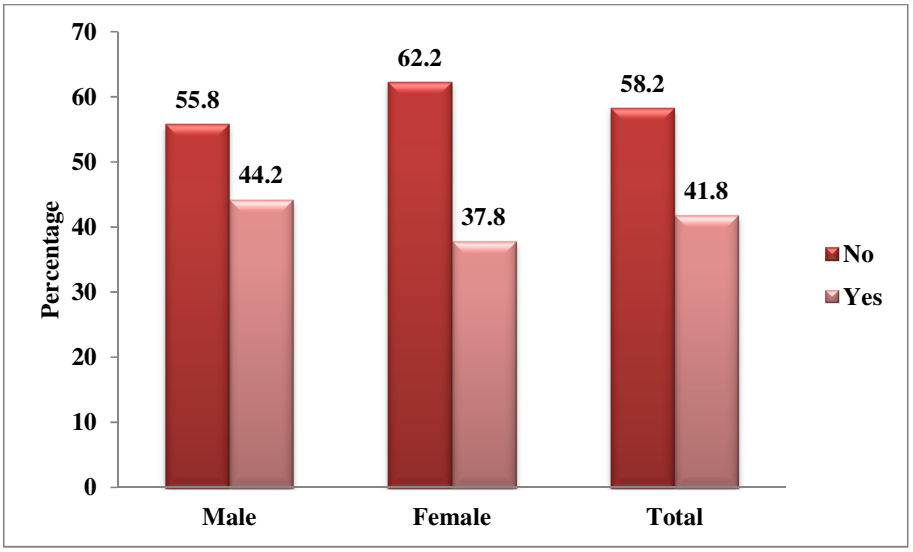


In the present study out of 122 patients 22(29%) males and 11(24%) female had Non proliferative diabetic retinopathy, none had proliferative diabetic retinopathy.

Table 7: Percentage distribution of Presence of Ischemic Heart Disease

IHD	Male		Female		Total	
	N	Percent	N	Percent	N	Percent
No	43	55.8	28	62.2	71	58.2
Yes	34	44.2	17	37.8	51	41.8
Total	77	100	45	100	122	100

Figure 5: Percentage distribution of Ischemic Heart Disease

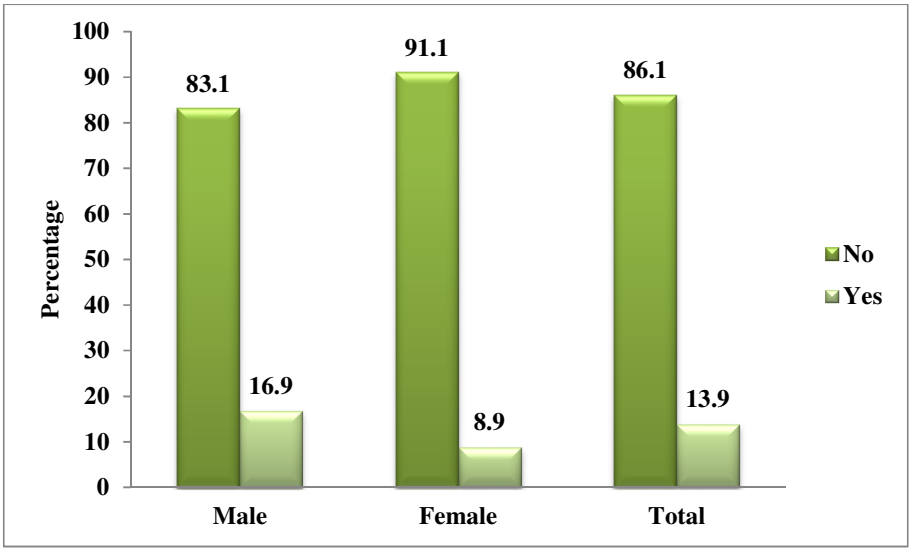


In the present study out of 122 patients 34(44%) males and 17(38%) female had Ischemic heart disease.

Table 8: Percentage distribution of Presence of Chronic Kidney Disease

CKD	Male		Female		Total	
	N	Percent	N	Percent	N	Percent
No	64	83.1	41	91.1	105	86.1
Yes	13	16.9	4	8.9	17	13.9
Total	77	100	45	100	122	100

Figure 6: Percentage distribution of Chronic Kidney Disease

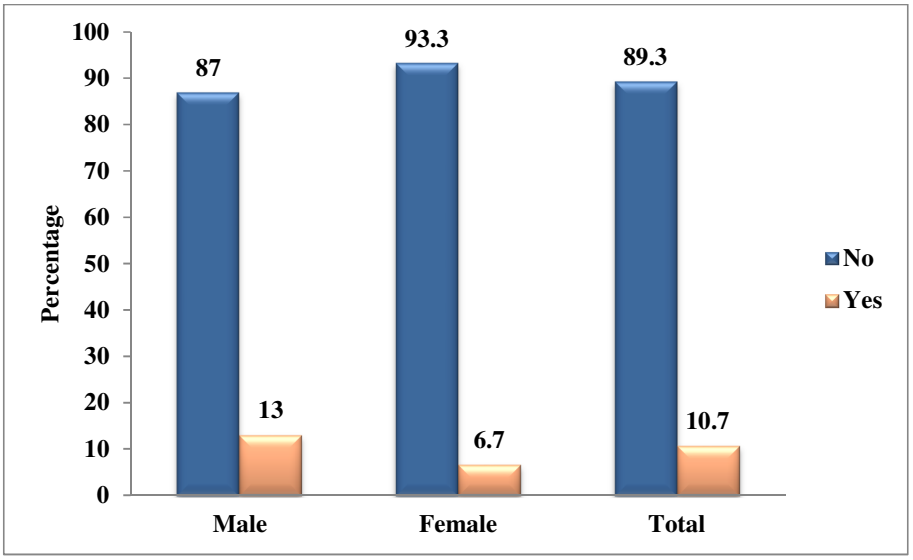


In the present study out of 122 patients 13(17%) males and 4(9%) female had chronic kidney disease.

Table 9: Percentage distribution of Presence of Cerebrovascular Accident

CVA	Male		Female		Total	
	N	Percent	N	Percent	N	Percent
No	67	87	42	93.3	109	89.3
Yes	10	13	3	6.7	13	10.7
Total	77	100	45	100	122	100

Figure 7: Percentage distribution of Cerebrovascular Accident



In the present study out of 122 patients 10(13%) males and 3(7%) female had cerebrovascular accident.

Table 10.Comparison of presence or absence of complications of T2DM in patients with and without NAFLD.

		NAFLD	NON NAFLD	TOTAL	OR	P VALUE
CAD	YES	30	21	31	2.19	0.03
CKD	YES	13	4	23	4.33	0.01
RETINOPATHY	YES	22	5	11	2.94	0.01
CVA	YES	8	5	13	1.8	0.28

Note: P value by chi square.* p <0.05 is taken as significant level

In this study presence of chronic kidney disease, non proliferative diabetic retinopathy and coronary artery disease were significant in patients with NAFLD.

Table 11. Comparison of presence of HTN, Obesity, Over weight in patients with and without N AFLD

	NAFLD	**NON NAFLD**	**TOTAL**	**P value**
HTN	29	26	53	0.22
OBESE	14	7	21	0.00*
Over Weight	20	14	34	0.00*

Note: P value by chi square test,* P<.005 is taken as significant

In this study presence of obesity and overweight were significant.

DISCUSSION

Nonalcoholic fatty liver disease is defined as having hepatic steatosis either by imaging or by histology in absence of secondary hepatic steatosis like alcohol consumption, use of steatogenic drugs or hereditary disorder. This study is first cross sectional study to report on prevalence of NAFLD in Type 2 diabetes mellitus in this part of country.

The study population was mostly urban and from diverse occupational backgrounds. The age range of study subject was 32- 80 years, with mean age of 59.7±12 years. The majority of all subjects studied were in age group of 56-65 years.

The prevalence of ultrasonographic NAFLD among type 2 diabetic subjects in this study was 47.5%, the majority being in the age group 45-49 years, followed by the age group 40-44 years. Our findings were comparable with those of a study carried out by Matteoni et al that found the highest prevalence of NAFLD in a similar age group [80]. The most common sonographic grade of NAFLD was mild fatty liver (62%), followed by moderate (36%) and then severe fatty liver (2%). A similar Italian study by Giovanni et al, also employing U/S as a screening tool, found a much higher prevalence of 69.5%. 38% (n=64) of the female study subjects had NAFLD, while 30% (n=48) of male study subjects had NAFLD. There was no statistically significant association of gender with NAFLD. This finding was comparable to that of Ludwig et al [81], who found no statistically significant association between NAFLD and gender, with the disease occurring in similar proportion among males and females.

The mean SGOT, SGPT and ALP levels were 31±14.4 IU/L and 25±14.2 IU/L and 104 ±47.6 respectively in NAFLD population. Elevated levels of SGOT, SGPT and ALP were seen in 14, 8 and 21 patients with NAFLD respectively. A level of ALP was found to be significantly higher in patients with NAFLD compared to patients without NAFLD. There was no significant difference in levels of SGOT and SGPT, this is similar to study by Jali MV et al, in which they found 30% had abnormal SGOT and 22% had abnormal SGPT[82]. It is evident that the level of liver enzymes will provide little diagnostic or prognostic value when assessing NAFLD patients.

In our study 58.6% patients with NAFLD had a BMI that was above normal (27.3± 4.8), compared to 36.2% of patients without NAFLD (24±3.1) that had an elevated BMI. This was statistically significant with a p value of 0.0001, making obesity an important association. Though in our study only BMI was taken as a marker for obesity, raised BMI showed strong correlation with presence of fatty liver. This finding is similar to shobhaluxmi et al[83] where BMI was 30.17±3.92 in patients with NAFLD and 23.7±2.55 in patients without NAFLD which was statistically significant with p value of 0.03.

Our study showed triglyceride level of 167.2±92.3 in patients with NAFLD compared to 128.1 ±45 in patients without NAFLD and VLDL level of 34±19 in patients with NAFLD and 26.4±9.6 in patients without NAFLD both were significant with P value of 0.003 and 0.005 for triglyceride and VLDL respectively. Total cholesterol levels were 180±47.8 and 170.7±40.3 in patients with and without NAFLD respectively and were not significant, these are similar to Jin HB et al which showed increased TG($p<0.01$) , total cholesterol ($P=0.88$)[77].

In our study NAFLD patients had higher prevalence of retinopathy (P= 0.01), nephropathy (P=0.01) and Coronary artery disease (P=0.03), which were significant and prevalence and prevalence of cerebrovascular accident(P=0.28) was not significant. It is similar to study by Somalwar AM et al who found significantly higher prevalence of retinopathy (P=<0.001), nephropathy (P=<0.05) and coronary artery disease (P= <0.001)[84].

CONCLUSION

1. In our study we found the prevalence of NAFLD in Type 2 diabetes mellitus patients being 47.5%.
2. NAFLD was significantly associated with over weight and obesity.
3. NAFLD was significantly associated with biochemical abnormalities of hyper-triglyceridemia and raised VLDL levels.
4. Raised levels of Alkaline phosphatase was found significant in patients with NAFLD.
5. There were no significant changes in SGOT and SGPT levels compared to non NAFLD patients.
6. Microvascular complications of Type 2 Diabetes mellitus, nephropathy and retinopathy were found to be significantly higher in patients with NAFLD.
7. Macrovascular complication of Type 2 Diabetes mellitus coronary artery disease was found significantly higher in patients with NAFLD.

Early detection of NAFLD and its early treatment is necessary as these patients are at risk of developing cirrhosis, end stage liver failure and hepatocellular carcinoma. Furthermore, diabetic patients with NAFLD are at increased risk of developing cardiovascular disease, retinopathy and nephropathy.

LIMITATION

HBV and HCV markers were not done owing to financial constraints, and viral hepatitides may occasionally mimic NAFLD.

It was not possible to completely rule out previous use of medications that can cause secondary fatty liver disease owing to limited patient recall and poor medical record keeping by most study subjects.

Finally, some study patients may not have been truthful pertaining to alcohol consumption.

BIBLIOGRAPHY

1. Angulo P Nonalcoholic Fatty Liver Disease. N Engl J Med. 2002; 346(16):1221-1231.
2. Neuschwander –Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis : summary of an AASLD Single Topic Conference. Hepatology. 2003;37(5) :1202 -1219.
3. Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med.2000; 132(2): 112-117.
4. Collantes R, Ong JP, Younossi ZM, Nonalcoholic fatty liver disease and the epidemic of obesity, Cleveland. Clin J Med. 2004;71(8):657–64.
5. Cusi K., Nonalcoholic fatty liver disease in type 2 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes. 2009;16(2):141-9.
6. Ferreira VS, Pernambuco RB, Lopes EP, Morais CN, Rodrigues MC, Arruda MJ, et al., Frequency and risk factors associated with non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. Arq Bras Endocrinol Metabol.2010;54(4):362-8.
7. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98(5):960–967.
8. Yildirim B, Ozugurlu F, Sahin S, Ozyurt H, Atis O, Akbas A, etal., Association between elevated aminotransferase levels and the metabolic syndrome in Northern Turkey. Ann Hepatol.2010;9:161-5
9. Elizabeth H Harris. Elevated liver function tests in Type 2 Diabetes. Clinical Diabetes 2005;23(3):115-9.

10. West J, Brousil J, Gazis A, Jackson L, Mansell P, Bennett A. et al.,Elevated serum alanine transaminase in patients with type 1 or type 2 diabetes mellitus. QJM. 2006;99(12):871-876.
11. Meybodi MA, Afkhami-Ardekani M, Rashidi M., Prevalence of abnormal serum alanine aminotransferase levels in type 2 diabetic patients in Iran. Pak J BiolSci.2008;11(18):2274-2277.
12. Judi L, Toukan A, Khader Y, Ajlouni K, Khatib MA., Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus. Ann Saudi Med 2010;30(1):25-32.
13. Esteghamati A, Jamali A, Khalilzadeh O, Noshad S, Khalili M, Zandieh A. et al., Metabolic syndrome is linked to a mild elevation in liver aminotransferases in diabetic patients with undetectable non-alcoholic fatty liver disease by ultrasound. Diabetol Metab Syndr.2010;2:65.
14. Ioannou GN, Weiss NS, Boyko EJ, Mozaffarian D, Lee SP. Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. Hepatology.2006;43(5):1145–51.
15. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30(5):1212-8.
16. Grundy SM, Cleeman J I ,Merz CN, etal . Co-ordinating Committee of the National Cholesterol Education Program; National Heart , Lung , and Blood Institute ; American College of Cardiology Foundation ; American Heart Association . Implications of recent clinical trials for the National Cholesterol

Education Program Adult Treatment Panel III guidelines Circulation. 2004; 110(2);227-39.

17. Treeprasertsuk S, Jimenez FL, Lindor KD Nonalcoholic fatty liver disease and the coronary artery disease; Dig Dis Sci.2011:56(1):35-45.
18. Lautämaki R, Borra R, Iozzo P, Komu M, Lehtimaki T, Salmi M, et al. Liver steatosis coexists with myocardial insulin resistance and coronary dysfunction in patients with type 2 diabetes. Am J Physiol Endocrinol Metab.2006;291(2):282-90.
19. Targher G, Bertolini L, Rodella S, Zoppin G, Lippi G, Day C, Muggeo M. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients.Diabetologia.2008;51(3):444-50.
20. Berg J M, Tymoczko J, Stryer L, Biochemistry. 5th ed. New York;W H Freeman :2002.
21. Guyton AC and Hall JE Text book of Medical Physiology. W.B. Saunders, Philadelphia:1996.
22. Seppala -Lindroos A, Vehkavaara S , Hakkinen AM, Goto T, Westerbacka J , Sovijarvi A, Halavaara J and Y ki - Jarvinen H. Fat Accumulation in the Liver Is Associated With Defects in Insulin Suppression of Glucose Production and Serum Free Fatty Acids Independent of Obesity in Normal Men . J Clin Endocrinol Metab.2002:87(7):3023 -3028.
23. Gibbons GF, Islam K and Pease RJ Mobilization of Triacylglycerol Stores. Biochem Bio phys Acta1.2000:483(1):37-57.
24. Stroud M .Shadows on the Wasteland, Penguin.1993:69

25. Holm C, Langin D, Manganiello V, Belfrage P and German DE. Regulation of Hormone -Sensitive Lipase Activity in Adipose Tissue Methods, Enzymo;1997: 286:45 -67 .
26. Cairns SR and Peters TJ Isolation of Micro - and Macro –Droplet Fractions From Needle Biopsy Specimens of Human Liver and Determination of the Sub cellular Distribution of the Accumulating Liver Lipids in Alcoholic Fatty Liver . Clin Sci (Lond) ;1984:67(3): 337 -345 .
27. Prashant M, Ganesh HK, Vimal MV, John M, Bandagar T, Joshi SR, etal Prevalence of Nonalcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus. J Ass Physi Ind.2009:57:205-210.
28. Parks EJ and Hellerste in MK Thematic Review Series : Patient Oriented Research . Recent Advances in Liver Triacyl glycerol and Fatty Acid Metabolism Using Stable Isotope Labeling Techniques. J Lipid Res.2006;47:1651-1660.
29. Barrows BR, Timlin MT and Parks EJ Spillover of Dietary Fatty Acids and Use of Serum Non esterified Fatty Acids for the Synthesis of VLDL-Triacyl glycerol Under Two Different Feeding Regimens.Diabetes.2005;54(9):2668-2673.
30. Dharel N, Fuchs M Nonalcoholic Fatty Liver Disease- A major public health challenge for the 21st century. JSM Gastroenterol Hepatol;2014: 2(2):1018-1020
31. Hui E, Xu A, Yang H B, Lam K S Obesity as common soil of Non Alcoholic Fatty Liver disease and diabetes: Role of adipokines. J of Diab investigation;2013:4(5):413-422.

32. Angulo P GI Epidemiology :Nonalcoholic Fatty Liver Disease. Aliment Pharmacol Ther;2007:25:883-889.
33. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM and Hobbs HH Prevalence of Hepatic Steatosis in an Urban Population in the United States: Impact of Ethnicity. Hepatology.2004: 40(6):1387-1395 .
34. Adams LA and Lindor KD Nonalcoholic Fatty Liver Disease. Ann Epidemiol.2007;17(11):863-869.
35. Bugianesi E Nonalcoholic Fatty Liver Disease (NAFLD) and Cardiac Lipotoxicity: Another Piece of the Puzzle. Hepatology.2008;47(1):2-4.
36. Charlton M Nonalcoholic Fatty Liver Disease: a Review of Current Understanding and Future Impact. Clin Gastroenterol Hepatol. 2004;2(12):104 -105.
37. Elmakki E, Aqeely H, Bani I, Omer H, Solan Y, Taher A et al Nonalcoholic Fatty Liver Disease in Saudi Patients with T2DM in Jazan Region: prevalence and associated factors .BJMMR.2015;5(7);872-879.
38. Ruderman N, Chisholm D, Pi-Sunyer X and Schneider S. The Metabolically Obese, Normal-Weight Individual Revisited. Diabetes.1988 47:699-713.
39. Silverman JF, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, Norris HT, Caro JF. Liver Pathology in Morbidly Obese Patients With and Without Diabetes. Am Gastroenterol.1990;85(10):1349-1355.
40. Amarapurka D, Kamani P, Patel N, Gupte P, Kumar P, Agal S et al. Prevelence of non-alcoholic fatty liver disease: population based study. Annals of Hepatology.2007:6(3);161-163.

41. Silverman JF, Pories WJ and Caro JF Liver Pathology in Diabetes Mellitus and Morbid Obesity. Clinical, Pathological, and Biochemical Considerations. Pathol Annu. 1989;24(pt 1):275-302.

42. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of Nonalcoholic Fatty Liver Disease With Insulin Resistance. Am J Med.1999;107(5):450-455

43. Assy N, Kaita K, Mymin D, Levy C, Rosser B and Minuk G. Fatty Infiltration of Liver in Hyper lipidemic Patients. Dig Dis Sci. 2000; 45(10) :1929 1934.

44. Adams LA and Lindor KD Nonalcoholic Fatty Liver Disease. Ann Epidemiol 2007;17:863-869.

45. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH .Prevalence of Hepatic Steatosis in an Urban Population in the United States: Impact of Ethnicity. Hepatology.2004; 40:1387-1395.

46. Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, Fritsche A, Schick F. Hepatic Lipid Accumulation in Healthy Subjects: a Comparative Study Using Spectral Fat-Selective MRI and Volume-Localized 1H-MR Spectroscopy. Magn Reson Med.2006;55:913-917.

47. Sorrentino P et al. A Clinical-Morphological study on Cholestatic Presentation of Non-Alcoholic fatty liver disease. Digestive Diseases and Sciences 2005;50(6):1130-1135

48. Ludwig J, Hashimoto E, Porayko MK, Moyer TP and Baldus WP. Hemosiderosis in Cirrhosis: a Study of 447 Native Livers. Gastroenterology.1997;112:882-888.

49. Day CP and James OF. Steatohepatitis: a Tale of Two "Hits"? Gastroenterology.1998;114:842- 845.
50. Sastre J, Serviddio G, Pereda J, Minana JB, Arduini A, Vendemiale G, Poli G, Pallardo FV and Vina J. Mitochondrial Function in Liver Disease. Front Biosci. 2007;12:1200-1209.
51. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD and Parks EJ. Sources of Fatty Acids Stored in Liver and Secreted Via Lipoproteins in Patients With Nonalcoholic Fatty Liver Disease. J Clin Invest. 2005; 115:1343-1351.
52. Savage DB, Petersen KF, Shulman GI. Disordered Lipid Metabolism and the Pathogenesis of Insulin Resistance. Physiol Rev.2007;87:507-520.
53. Schonfeld G, Patterson BW, Yablonskiy DA, Tanoli TS, Averna M, Elias N, Yue P and Ac kerman J. Fatty Liver in Familial Hypo beta lipoproteinemia : Triglyceride Assembly into VLDL Particles Is Affected by the Extent of Hepatic Steatosis. J Lipid Res.2003;44 : 470-478.
54. Jansen HJ, Vervoort GM, Graaf VD, Stienstra R, Tack CJ, Liver fat content is linked to inflammatory changes in subcutaneous adipose tissue in type 2 diabetes patients. Clin Endocrinol.2013:79(5)661-666.
55. Dowman JK, Pathogenesis of non-alcoholic fatty liver disease. QJ Med 2010; 103:71-83
56. Gan SK, Adams LA, Watts GF. the Trials and Tribulations of the Treatment of Nonalcoholic Fatty-Liver Disease. Curr Opin Lipidol.2008 ;19:592-599.
57. Thamer C, Machann J , Haap M, Stefan N, Heller E, Schnodt B, Stumvoll M, Claussen C, Fritsche A, Schick F and Haring H. Intra hepatic Lipids Are

Predicted by Visceral Adipose Tissue Mass in Healthy Subjects Diabetes Care.2004;27 : 2726 -2729 .

58. Hoyumpa AM, Greene HL, Dunn GD, Schenker S. Fatty Liver: Biochemical and Clinical Considerations. Am J Dig Dis.1975;20:1142- 1170.
59. Kotronen A, Lindroos SA, Bergholm R, Yki-Jarvinen H . Tissue Specificity of Insulin Resistance in Humans: Fat in the Liver Rather Than Muscle Is Associated With Features of the Metabolic Syndrome. Diabetologia.2008;51(1);130-138
60. Zimmet P, Shaw J, Alberti KG. Preventing Type 2 Diabetes and the Dysmetabolic Syndrome in the Real World: a Realistic View. Diabet Med.2003;20:693-702.
61. Gorter CJ and Broer LJF;. Negative Result of an Attempt to Observe Nuclear Magnetic Resonance in Solids. Physica.1942;9(6):591-596.
62. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ and Shulman GI. Mechanism of Hepatic Insulin Resistance in Non- Alcoholic Fatty Liver Disease. J BiolChem.2004:279:32345-32353.
63. Bugianesi E, McCullough AJ and Marchesini G. Insulin Resistance: a Metabolic Pathway to Chronic Liver Disease. Hepatology.2005;42:987-1000.
64. Hamaguchi, M., T. Kojima, N. Takeda. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Annals of Internal Medicine. 2005;143(10): 722–728.
65. A. Kotronen, J. Westerbacka, R. Bergholm, K. H. Pietilainen, and H. Yki-Jarvinen, “Liver fat in the metabolic syndrome,” The Journal of Clinical Endocrinology & Metabolism: 2007;92:3490–3497.

66. Huang M A, GreensonJ K, Chao C, "Oneyearintense nutritional counseling results in histological improvement in patients with nonalcoholic steatohepatitis: a pilot study," American Journal of Gastroenterology, 2005:100(5)1072–1081.

67. Palmer, M and Schaffner, F. Effect of weight reduction on hepatic ... Gastroenterology. 1990;99:1408–1413.

68. Wang RT, Koretz RL, and YeeHF, "Is weight reduction an effective therapy for nonalcoholic fatty liver? Asystematic review," Am J Med 2003; 115(7): 554–559.

69. Kadayifci A, Merriman RB, Bass NM. Medical treatment of non-alcoholic steatohepatitis. Clin Liver Dis.2007;11:119-140.

70. Vuppalanchi R and Chalasani N, "Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management," Hepatol,2009:49(1); 306–317.

71. G. A. Marlatt, M. E. Larimer, P. D. Mail et al., "Journeys of the circle: a culturally congruent life skills intervention for adolescent Indian drinking," Alcoholism:2003:27(8):1327–1329.

72. M. Tiikkainen, A. M. Hakkinen, E. Korsheninnikova, T.Nyman, S. M¨ akimattila, and H. Yki-Jarvinen, "Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes," Diabetes:.2004;53(8):2169–2176.

73. M. Kohjima, N, Higuchi, Kato M. "SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease," Inl J of Mor Med 2008: 21(4):507–511.

74. Kalra S, Vithalani M, Gulati G, Kulkarni CM , Kadam Y , Pallivathukkal J et al,: Study of prevalence of nonalcoholic fatty liver disease in Type 2 Diabetes mellitus in India (SPRINT). Journal Ass Phys of India: 2013:61:448-453.

75. Vishwanathan V, Kadari M, Meimpudi S, Kumpatala S. Association of NAFLD with diabetic complication in India .International Journal of Diabetes in Developing C:2010;30(4):208-212 .

77 Somalwar AM, Raut AD. Study of association of nonalcoholic fatty liver disease (NAFLD) with micro and macrovascular complications of type 2 diabetes mellitus(T2DM). Int J Res Sci.2014;2(2):493-497.

78 Pai S, Chakrapani M, Madi DR, Achappa B, Unnikrishnan. Nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. Int J Boil Med Res. 2012;3(3):2189-2192.

79 Lv WS, Sun RX, Gao YY, Wen JP, Pan RF, Li L et al . A study on Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. World Journal Gastroentrol 2013:28:19(20):3134-42.

80 Matteoni CA, Younossi ZM, Gramlich T et al. Nonalcoholic fatty liver disease: A spectrum of clinical pathological severity. Gastroenterology 1999; 116:1413- 1419.

81 Ludwig J, Viggiano RT, McGill DB &Ott BJ. Nonalcoholic steatohepatitis. Mayo clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980; 55: 434-438.

82 Jali MV, Kambar S, Jali SM, Hiremath MB. Prevelence of nonalcoholic fatty liver disease among type 2 diabetes mellitus patients- A cross sectional hospital based study. Al Ameen J Med Sci 2015; 8(1):50-54

83 ShobhaLuxmi, Rukhsana Abdul Sattar and Jamal Ara. Association of Non Alcoholic Fatty Liver with type 2 Diabetes Mellitus. JLUMHS 09:2008 188-193.

84 Jin, H.B., Gu, Z.Y., Yu, C.H., Li, Y.M. Association of nonalcoholic fatty liver disease with type 2 diabetes: clinical features and independent risk factors in diabetic fatty liver patients. Hepatobiliary Pancreat Dis Int. 2005;4:389–392.

ANNEXURES

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. SD/13
Date. 16-11-13
BLDE U's Shri B.M. Patil Medical College,

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Prevalence of nonalcoholic fatty liver disease in type 2 diabetes Mellitus patients"

—x—x—x—x—

Name of P.G. student Dr Basaveshwar Mhetre.
Department of Medicine

Name of Guide/Co-investigator Dr R.M. Honnutagi,
Prof of Medicine

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

INFORMED CONSENT FORM

TITLE OF RESEARCH: "STUDY OF PREVALENCE OF NON ALCOHOLIC FATTY LIVER DISEASE IN TYPE 2 DIABETES MELLITUS PATIENTS"

GUIDE : DR R.M. HONNUTAGI

P.G.STUDENT : DR BASAVESHWAR. MHETRE

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to ascess the prevalence of nonalcoholic fatty liver disease in type 2 diabetes mellitus.

PROCEDURE:

I understand that I will undergo detailed history an clinical examination and investigations.

RISKS AN DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to assess the prevalence of nonalcoholic fatty liver disease in Type 2 Diabetes mellitus in this part of state.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed.

REQUEST FOR MORE INFORMATION :

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION :

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT :

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation.

_____ _____

(Signature of Guardian) (Signature of patient)

STUDY SUBJECT CONSENT FORM:

I confirm that Dr. Basaveshwar Mhetre has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

SIGNATURE OF PARTICIPANT DATE

SIGNATURE OF WITNESS DATE

PROFORMA

Name of the patient :

Age in years :

Sex :

Aresss:

Religion:

Occupation:

IP no/OP no:

Presenting Complaints :

Past history: Type 2 Diabetes mellitus, Hypertension, Ischemic heart disease

Personal history:

1. Tobacco chewing
2. Smoking
3. Alcoholism
4. Diet- Veg/Mixed
5. No habits

Family history:

GENERAL PHYSICAL EXAMINATION :

Built :

Nourishment :

Ht(Cm) :

Wt(Kg) :

BMI:

Pallor

Icterus

Clubbing

Cyanosis

Edema

6. Vital parameters a. Pulse :

b. BP :

c. Respiratory rate :

d. Temperature

e. Waist circumference

SYSTEMIC EXAMINATION :

ABDOMEN EXAMINATION

CARDIOVASCULAR SYSTEM

RESPIRATORY SYSTEM

CENTRAL NERVOUS SYSTEM

BIOCHEMISTRY

1)LIVER FUNCTION TESTS	
Total bilirubin	
Conjugated	
Unconjugated	
SGOT SGPT	
Total protein Albumin Albumin: Globulin	
2)Fasting Blood sugar	
3)Postprandial Blood sugar	
4)HbA1c	
5)LIPID PROFILE	
Total Cholesterol	
Triglycerides	
HDL-Cholesterol	
LDL-Cholesterol	
VLDL-Cholesterol	
6) Serum creatinine	
7) Blood urea	

PATHOLOGY 1)Urine coplete	
Urine albumin	
Urine sugar Urine bile salts Urine bile pigments	
Urine microscopy RBC's Pus cells Cast's Epithelial cells	
2)Complete blood count:	
Hb	gm/dl
Total count	Cells/cumm
Differential count Neutrophils	%
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%
ESR	At end of 1st hour.

USG ABDOMEN:

FUNDOSCOPY:

ECG:

CONCLUSION:

KEY TO MASTER CHART

1) USG Liver

1. = Grade I Fatty liver

2. = Grade II Fatty liver

3. = Grade III Fatty liver

2) ECG

1. Ischemic heart diseases

3) NPDR - Non proliferative diabetic retinopathy

1. = Grade I

2. = Grade II

3. = Grade III

4) CKD

0 - No

1 – Yes

5) CVA

0 - No

1 – Yes