BENGHAZI UNIVERSITY

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"BIOCHEMICAL STUDIES RELATED TO NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND GALLSTONES DISEASE IN A LOCAL LIBYAN SUBJECTS"

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY TO THE FACULTY OF MEDICINE

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿1﴾ خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ

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صدق الله العظيم

سورة العلق

من الآيه رقم ﴿1﴾ إلى الآيه ﴿5﴾

الإهداء

بسم الله الرحمن الرحيم

- (وقل اعملوا فسيرى الله عملكم ورسوله والمؤمنون)

صدق الله العظيم

- "إلهى لايطيب اليل إلا بشكرك.. ولايطيب النهار إلا بطاعتك .. ولا تطيب اللحظات إلا بذكرك.. ولا تطيب الأخره إلا بعفوك ..ولاتطيب الجنه إلا برؤيتك الله جل جلالك."
- إلى من بلغ الرساله وأدى الأمانه ..ونصح الامه ..إلى نبى الرحمه ونور العالمين ..

سيدنا محمد صلى الله عليه وسلم

- مهما كتبت من كلام وجفت الأحبار بحثا عن تعبير يشرح التقدير لكل من أخذ بيدى ووضعنى على هذا الدرب ما وجدت.
- الأمس كنت بصفه واليوم صفه جديده أثنى على العالى القدير الذى سهل وسخر الحلم المستحيل فغديت به لمستقبلى القريب .
- "اللهم آت نفسي تقواها , وزكّها, أنت خير من زكّاها , أنت وليّها ومولاها ,اللهم إني أعوذ بك من علم لا ينفع, وقلب لا يخشع , ونفس لا تشبع , ودعوة لا يستجاب لها ."

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ý ABBREVIATIONSQ

ABCA1	----------	ATP-binding cassette transporter.
ACC	----------	Acetyl Co A Carboxylase.
Acetyl CoA	----------	Acetyl Coenzyme A.
ADP	----------	Adenosine Diphosphate.
AGE	----------	Aminoguanidine.
ALT	----------	Alanine Aminotransferase.
apo B	----------	Apolipoproteins B.
apobec1	----------	Apob Editing Complex-1.
AST	----------	Aspratate Aminotransferase .
ATGL	----------	Adipose Triglycerols Lipase.
ATP	----------	Adnenosin triphosphate.
B.P	----------	Blood Pressure .
BMI	----------	Body Mass Index.
CDC	----------	Center For Disease Control.
CETP	----------	Cholesteryl Ester Transfer Protein.
CHD	----------	Coronary Heart Disease.
CHO	----------	Carbohydrate.
ChREBP	----------	Carbohydrate Response Element–Binding Protein.
CLAS	----------	Cholesterol–Lowering Atherosclerosis Study.
CS	----------	Cholesterol Stones.
CVD	----------	Cardiovascular Disorders.
DM	----------	Diabetes Mellitus.
DNL	----------	De Nova Lipogenesis .

EDTA ---------- Ethylene Diamine Tetra Acetic Acid.

ER ---------- Endoplasmic Reticulum.

FA ---------- Fatty Acid.

FABP ---------- Fatty Acid Binding Proteins.

FAT ---------- Fatty Acid Translocase.

FAT/CD36 ---------- Fatty Acid Translocase/ Clusterdomain 36.

FATP ---------- Fatty Acid Transport Proteins.

FFA ---------- Free Fatty Acids.

FSIGT ---------- Frequently Sampled Glucose Tolerance Test.

FXR ---------- Farnesoid X Receptor.

GD ---------- Gallstone Disease.

GLUT4 ---------- Glucose Transporter-4.

GOD ---------- Glucose Oxidase.

GOT ---------- Glutamate Oxaloacetate Transminase.

GPO ---------- Glycerophosphate Oxidase.

GPT ---------- Glutamate Pyruvate Transaminase.

HbA1c ---------- Glycated Hemoglobin.

HBV ---------- Hepatitis B Virus.

HCL ---------- Hydrochloric Acid.

HCV ---------- Hepatitis C Virus.

HDL ---------- High Dinsty Lipoprotein.

HIR ---------- Hepatic Insulin Resistance.

HNF-1a---------- Hepatocyte Nuclear Factor 1a.

HNF-4a---------- Hepatocyte Nuclear Factor-4a.

HOMA ----------	Homeostasis Model Assessment.
HSL ----------	Hormone Sensitive Lipase .
IDL ----------	Intermediate Density Lipoprotein.
IGT ----------	Impaired Glucose Tolerance.
IKK beta ----------	Inhibitor Of Kappa Kinase Beta.
IMT ----------	Intimal Medical Thickness.
IR ----------	Insulin Resistance.
LDH ----------	Lactate Dehydrogenase.
LDL ----------	Low Density Lipoprotein.
LPL ----------	Lipoprotein Lipase.
LRP ----------	LDL Receptor Related Protein.
LXR ----------	Liver Xenobiotic Receptor.
MODY-1----------	Monogenic Autosomal Dominant Non-Insulin-Dependent Diabetes Mellitus Type 1.
MRI ----------	Magnetic Resonance Imaging.
mRNA ----------	Messenger Ribonucleic Acid.
MS ----------	Metabolic Syndrome.
MTTP ----------	Microsomal Triglyceride Transfer Protein.
NAD ----------	Nicotinamide Adenine Dinucleotide.
NAFLD ----------	Non Alcoholic Fatty Liver Disease.
NASH ----------	Non Alcoholic Steatohepatitis.
NCCT ----------	Non Contrast Computed Tomography.
NCEP ----------	National Cholesterol Education Programme.
NEFA ----------	Non-Esterified Fatty Acid.

NIDDM	----------	Non-Insulin Dependent Diabetes Mellitus
Nm	----------	Nanometer.
NR1H4	----------	Nuclear Receptor subfamily 1, group H, member 4.
NS	----------	Non- Significant.
O-GGT	----------	Oral Glucose Tolerance Test.
PAI-I	----------	Plasminogen Activator Inhibitor-I.
PAP	----------	Peroxidase.
PEPCK	----------	Phosphoenol pyruvate Carboxykinase.
PPAR-a	----------	peroxisome proliferator-activated receptor -a.
PS	----------	Pigment Stones.
PSC	----------	Prospective Complications.
PUFAs	----------	Poly Unsaturated Fatty Acids.
PVS	----------	Polyvinyl Sulphate.
RXR	----------	Retinoid Xenobiotic Receptor.
SD	----------	Standard Deviation.
SHBG	----------	Sex Hormone Binding Globulin.
SHP	----------	Short Heterodimer Partner.
SOCS	----------	Hepatic Suppressors Of Cytokine Signaling.
SPSS	----------	Statistical Package For Social Sciences.
SREBP-1c	----------	Sterol Regulatory Element Binding Protein-1c.
SUA	----------	Serum Uric Acid.
TAGs	----------	Triacylglycerols.
TGF	----------	Tumor Growth Factor.
TGH	----------	Triglyceride Hydrolase.

TNF-α ---------- Tumor Necrotic Factor-Alpha.

TPA ---------- Tissue Plasminogen Activator.

U.S. ---------- Ultrasonography.

VLDL ---------- Very Low Density Lipoprotein.

Vs ---------- Versus.

γ-GT ---------- Gamma-Glutamyl Transpeptidase.

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1.0. INTRODUCTION

"Fatty Liver and Gall Stone Disease in Libyan Subjects- A Biochemical Study**"**

Fatty liver is one of the common problems associated with truncal obesity, metabolic syndrome and dyslipidemia. In countries which do not consume alcohol, non alcoholic fatty liver disease (NAFLD) is considered to be one of the common problems encountered in hepatology unit. NAFLD is considered to be the hepatic expression of metabolic syndrome. Metabolic syndrome is considered to be a cluster of factors associated with it. Obesity, dyslipidemia, hypertension, decreased High Density Lipoprotein. (HDL cholesterol), increased serum blood into skeletal muscle and adipose tissue; serum non-esterified fatty acid triglyceride, including microalbumnuria are the components of metabolic syndrome (MS). The most probable cause of NAFLD is reported to be insulin resistance. Insulin resistance leads to greater breakdown and over flow of fatty acids into hepatocytes which results in hepatitis. Insulin resistance impairs uptake of glucose from Non-Esterified Fatty Acid (NEFA) levels may also be elevated due to the failure of insulin to suppress Hormone Sensitive Lipase (HSL) mediated lipolysis (Donnelly et al., 2005, Lewis et al., 2002)

Influx of lipids into adipose tissue may be limited by other mechanisms (Lewis et al., 2002) which induce insulin resistance (IR) in adipose tissue or defects in the transporters of lipids into adipose tissue. Defects in the control mechanisms involved in the release of lipids from adipocytes and defects in normal growth such as in fatty liver. As in the case of adipocytes, hepatic fat over load and proliferation of adipose tissue, could lead pooling of plasma lipids causing ectopic fat deposition hepatic insulin resistance (HIR) (Samuel et al., 2004, Thomas et al., 2005).

Over flow of fat to liver could be due to increased dietary intake. Even after a short term fat feeding liver fat increases three fold without increase in visceral or skeletal muscle fat (Samuel et al., 2004, Thomas et al., 2005). Indeed the adipose tissue fat is an indicator of liver fat. The intrahepatic lipids increase by 22% for any 1% increase in total adipose

tissue, by 21% for any 1% increase in subcutaneous adipose tissue and by 104% for 1% increase in intra-abdominal adipose tissue (Diraison et al., 2003). Patients with low HDL-cholesterol and abnormal cellular lipid efflux due to ATP-binding cassette transporter (ABCA1) gene defects (Tangier disease) also have elevated plasma triglycerides (Lonardo et al., 2006) and fatty liver.

In NAFLD nearly a quarter of the accumulated fat comes from de novo lipid (DNL) synthesis (Donnelly et al., 2005) and hepatic lipid synthesis is markedly increased in NAFLD (Stefkova et al., 2004). It may be noted that NAFLD is classically associated with gall stone disease and hypertriglyceridemia (Lonardo et al., 2006, Loria et al., 2005).

The increased incidence of gall stones at least partly is due to decreased secretion of bile salts, which are potent emulsifiers, and the consequent instability of bile pigments resulting in precipitation and stone formation. It would be logical to hypothesize that defective Farnesoid X Receptor (FXR) expression or signaling and consequent deficiency in bile inhibition of fatty acid synthesis might play a role in certain cases of hepatic steatosis associated with biliary stones.

Cholelithiasis or gallbladder stones are one of the major surgical problems in the Libyan population and account for many hospital admissions and surgical interventions. Most patients with gallstones present with severe abdominal colic requiring investigations and treatment. Many of them need surgical intervention by the time they are symptomatic. This problem is probably related to obesity, cardiovascular disorders (CVD), metabolic syndrome and dietary habits, especially excessive consumption of meat, which is known to contain large amounts of cholesterol. Obese individuals with a Body Mass Index (BMI) > 30 kg/m2 have 95% cholesterol-dominant gallstones and are at a high-risk for cholesterol stones (Schafmayer et al., 2006).

Pigment Stones (PS) was the most common type of gallstones, cholesterol seemed to be the major component in all types of stones. High cholesteroI content in Cholesterol Stones (CS) especially suggests supersaturation of cholesterol in bile consequent to dyslipidemia

(excessive cholesterol and altered lipid metabolism) is an etiological factor. Higher triglycerides content in mixed stones also suggests that dyslipidemia plays a key role in its pathogenesis. Therefore NAFLD could be associated with gall stone disease.

1.1. Objectives of the Present Study:

a) To identify the presence of Nonalcoholic fatty liver disease (NAFLD) in a local hospital based population.
b) To diagnose Gall Stone Disease patients among these cases.
c) To understand the possible link between fatty liver disease and gall stones.

1.2. Outcome of the Study:

The study will bring out the presence of fatty liver patients along with the said biochemical parameters. The identification and analyses of gall stones for various constituents (Like cholesterol stones, Pigment stones and mixed stones) will be done. Identification of the presence of and the percentage of patients with gall stone disease in the said fatty liver cases will bring out the link between gall stone disease and fatty liver. This will help understand the pathogenesis of gall stone disease as well as devise methods to understand the aetiopathogenesis specific to Benghazi region.

2.1. NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD):

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a major cause of liver related morbidity and mortality, because of its potential to progress to cirrhosis and liver failure (Adams et al., 2005). The pathologic picture of non-alcoholic fatty liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis, resembles that of alcohol induced liver disease, but it occur in patients who do not abuse alcohol (Adams et al., 2010).

2.2. What is Fatty liver? And what is NASH?

Nonalcoholic fatty liver disease (NAFLD) has become the most common form of liver disease, affecting 20% to 30% of the US population. Its clinical manifestations are usually absent or subtle, and it usually comes to medical attention incidentally when aminotransferase levels are found to be elevated or a radiographic study reveals that the liver is fatty. Primary NAFLD is now considered the hepatic manifestation of the metabolic syndrome (Kim and Younossi, 2008) & (Younossi, 2008, Kruger et al., 2011). The pathogenesis is thought to be a multiple-hit process involving insulin resistance, oxidative stress, apoptosis, and adipokines. The primary event of NAFLD is the accumulation of triacylglycerols (TAGs) in hepatocytes (Lonardo et al., 2006). NAFLD is the most common cause of abnormal results in liver-function tests. This clinical situation includes simple (benign) fatty liver, NASH, cirrhosis, and hepatocellular carcinoma. NAFLD is defined as liver steatosis in patients who do not consume enough alcohol to cause hepatic injury. Although some drugs or genetic abnormalities can cause NAFLD, the majority of cases are associated with obesity, insulin resistance, and type II diabetes (Kruger et al., 2011).

2.3. The Plasma Non-Esterified Fatty Acid (NEFA) pool:

Plasma Non-Esterified Fatty Acid (NEFA) pool accounts for approximately 60% of TAGs content in the liver of NAFLD patients, which reflects the importance of the NEFA pool in the pathogenesis of NAFLD (Donnelly et al., 2005) .The hepatic uptake of fatty

acids is not regulated and, as a result, plasma fatty acid concentration is directly related to the influx of fatty acids to the liver. In the fasted state, adipose tissue contributes approximately 80% of fatty acid content to the plasma NEFA pool, and in the fed state, its contribution remains at approximately 60%. Thus, the over production of fatty acids in adipose tissue that flow to the liver via the NEFA pool is the most likely explanation for excess TAGs accumulation in NAFLD. In insulin-resistant states, insulin does not fully suppress the activity of Hormone Sensitive Lipase (HSL), which catalyzes the hydrolytic release of fatty acids from the TAGs moiety and results in enhanced lipolysis and flux of fatty acids to the plasma NEFA pool. In addition, reduced glucose uptake due to insulin resistance reduces glycerol-3-phosphate levels, thereby reducing the reutilization of fatty acids for TAGs synthesis. Thus, insulin resistance in adipose tissue is important as a pathogenic factor of NAFLD. Recently, dysregulated adipocytokines such as adiponectin and tumor necrotic factor-α (TNF-α) have been examined as causative candidates of insulin resistance, and it was recently reported that oxidative stress in accumulated fat causes dysregulated adipocytokine production. In light of these findings, it seems possible that the reduction of oxidative stress as well as the use of insulin-sensitizing agents such as thiazolidinediones and metformin may prove to be successful treatments for NAFLD (Gill and Wu, 2006, Cohen-Naftaly and Friedman, 2011, Marchesini et al., 2001) **.**

2.4. De nova Lipogenesis (DNL):

In healthy human subjects, the contribution of DNL in the liver to TAGs content in the fasted state is very small (less than 5% for (VLDL) Very low density lipid -TAGs). It has been reported that DNL in the liver is elevated in insulin-resistant states and in NAFLD. It was found that DNL accounted for 26% of liver TAGs content in hyperinsulinemic subjects with NAFLD. In healthy subjects, DNL is elevated following meals (23% for VLDL-TAGs), which can be accounted for by elevation in the circulating levels of lipogenic precursors. However, in NAFLD, DNL remaining elevated in the fasted state, and further postprandial elevation is not observed. Indeed, constant elevation of DNL was observed in

subjects fed a diet high in simple carbohydrates for 25 days. These observations reflect the sustained elevation of enzymes involved in hepatic DNL (Diraison et al., 2003).

Insulin activates the membrane-bound transcription factor Sterol regulatory element binding protein 1c (SREBP-1c), which transcriptionally activates most genes required for lipogenesis. In mice, even in the insulin-resistant state, insulin stimulates hepatic SREBP-1c transcription and increases lipogenesis (Tamura and Shimomura, 2005) Lipogenesis is also regulated by glucose: glucose activates the carbohydrate response element–binding protein (ChREBP), which induces gene expression of liver-type pyruvate kinase, a key regulatory enzyme in glycolysis; this enzyme in turn provides the precursors for lipogenesis. In addition (ChREBP) stimulates gene expression of most enzymes involved in lipogenesis (Yamashita et al., 2001). Hyperinsulinemia and hyperglycemia may induce these transcriptional factors in humans, although this remains to be directly demonstrated.

2.5. Other factors causing fatty liver:

Mice with peripheral insulin resistance due to adipose- and muscle-specific glucose transporter-4 (GLUT4) double knockout show increased DNL accompanied by hepatic elevation of SREBP-1c and acetyl-CoA carboxylase. However, in these mice, increased DNL does not result in increased TAGs level in the liver, nor does it increase steady-state serum levels of TAGs or NEFAs; this could be the result of a compensatory enhancement of peripheral fatty acid usage. Unlike in this animal model, such compensation is assumed to be insufficient in human subjects with type 2 diabetes. The reduced use of fatty acids in peripheral tissue in combination with elevated DNL may contribute to TAGs accumulation in the livers of subjects with NAFLD. TAGs accumulation in the liver can be effectively reduced by muscle exercise which, improves insulin resistance and enhances peripheral fatty acid disposal (Tamura and Shimomura, 2005).

2.6. Common causes of fatty liver:

The accumulation of TAGs in the liver results from an imbalance among the uptake, synthesis, export, and oxidation of fatty acids. In type 2 diabetes, secretion of VLDL is increased due to insulin resistance. It has been reported, however, that in NAFLD, VLDL secretion is not increased compared with VLDL secretion in controls. Methodological differences could be the reason for the reported differences in the findings reported... Secretion rates of hepatic VLDL should be quantified in hyperinsulinemic subjects with and without hepatic steatosis. β- Oxidation of fatty acids in the liver was found to be increased in patients with NASH. However, the increase is not sufficient for overcoming elevated rates of hepatic TAGs synthesis. The increase in NEFA oxidation may account for the apparent oxidative stress that causes hepatic injury in NAFLD patients (Tamura and Shimomura, 2005) .

2.7. NAFLD and the Metabolic Syndrome (MS):

Individuals with NAFLD have significantly higher levels of glucose, insulin, triglycerides, serum ALT (Alanine Amino Transferase), and higher Body Mass Index (BMI) and waist circumference (central adiposity) than controls (Szabo et al., 2003). The strongest independent predictors of NAFLD are waist circumference, BMI, and triglycerides content. NAFLD predicts the MS independently of insulin and BMI. In addition, NAFLD is a stronger predictor for the combination of hyperglycemia, low levels of high-density lipoprotein (HDL), hypertriglyceridemia, and hypertension than is central obesity (Gill and Wu, 2006).

Although the deleterious effects of central adiposity on glucose and lipid metabolism are well recognized, independent favorable effects of peripheral adiposity on features of the MS have been suggested. Few studies on obese women have been carried out to test the hypothesis that trunk fat mass and leg fat mass have independent and opposite effects on liver injury (Perlemuter et al., 2008). In univariate analyses, serum ALT was positively correlated with BMI and trunk fat mass, Aspartate AminoTransferase (AST) was

negatively correlated with height and leg fat mass, and gamma-glutamyl transpeptidase (γ-GT) was positively correlated with total and trunk fat mass. Leg fat mass may confer independent protective effects against obesity-associated liver dysfunction in contrast to the deleterious effects of trunk fat mass. Regional body composition must be taken into account in the assessment of the relationship between body weight and shape, as well as in NAFLD (Zhang et al., 2012).

NAFLD is associated with an increased risk for mortality in patients with type 2 diabetes mellitus. In this context, it was shown that certain newly diagnosed type 2 diabetes mellitus subsequently developed NAFLD and about 29% of these subjects died (Adams et al., 2010)

In a multivariable analysis adjusted for differences in age, sex, and date of diabetes diagnosis, death was significantly associated with a diagnosis of NAFLD.

NAFLD remained an independent risk factor for mortality when controlling for smoking, hypertension, obesity, or hyperlipidemia. Only ischemic heart disease was independently associated with higher mortality among patients with NAFLD ((Bhatia et al., 2012).

2.8. NAFLD IN RELATION TO THE MS AND DM2:

In the analysis of the third National Health and Nutrition Examination Survey (NHANES III), up to 31% of the elevated ALT activity could be explained by high alcohol consumption, hepatitis B or C infection and/or high transferrin saturation, whereas in the remaining 69%, the elevated ALT activity was significantly associated with higher BMI, waist circumference, triglycerides, fasting insulin and lower (HDL) cholesterol (Deurenberg et al., 1998). The putative role of the liver in the pathogenesis of DM2 has gained much interest and several cross-sectional studies have demonstrated that NAFLD is related to features of the metabolic syndrome and DM2 (Contos et al., 2001, Adams et al., 2005). Several studies have addressed the prospective relation of ALT and the MS and DM2 (Unwin et al., 1997, Marchesini et al., 2001) In patients with DM2, elevated serum ALT enzyme activity is more frequently observed than in the general population (Pagano et

al., 2002, Ioannou et al., 2005) In addition, some (Nakanishi et al., 2004, Vozarova et al., 2002) but not all studies (Sattar et al., 2004, Wannamethee et al., 2005) have demonstrated independent and significant associations of ALT with future DM2. The observed association between ALT and incident DM2 in the mentioned studies may be explained by the fact that they were performed in high-risk populations that may not be representative for the general population. Overall, these studies show that patients with NAFLD are at increased risk for developing DM2 and the MS, suggesting that the increased CVD risk may be mediated via components of the MS and DM2. Since the pathophysiology linking NAFLD with either the MS and/or diabetes has not been clarified, it is difficult to make the distinction between confounding and mediating variables in the epidemiological analyses.

The presence of fat in the liver can also be suggested by the use of several imaging modalities; however, no current noninvasive method can distinguish NASH from NAFLD. Because liver biopsy remains the gold standard for grading and staging (i.e., recognition of inflammation and fibrosis), a critical issue in management is whether individual patients should undergo biopsy to detect progressive disease. This study reported a clear relationship between serum hyaluronic acid and fibrosis; inclusion of this marker into a composite score predicted NASH and improved selection of patients with NAFLD for liver biopsy (Marchesini et al., 2003).

2.9. Other Noninvasive Diagnostic Approaches:

Magnetic Resonance Imaging (MRI) is an accurate tool for quantifying liver fat. MRI measurements of liver fat content correlate with image analysis of histologic sections and with a pathologist's estimate of liver fat (both $r = 0.92$, $P < .001$) (Choudhury and Sanyal, 2005) "Dynamic" breath tests can detect specific alterations in metabolic pathways. The ^{13}C-methacetin breath test, a microsomal liver function test, enables quantitative evaluation of cytochrome P450-dependent liver function, whereas the ^{13}C-octanoate breath test evaluates hepatic mitochondrial beta-oxidation. Both mechanisms increase oxidative stress, which is implicated in the pathogenesis of NASH, as discussed below. A unique breath test system incorporating ^{13}C-methacetin breath test and ^{13}C-octanoate breath test assesses the

extent of hepatic injury and reliably distinguishes NASH from steatosis (Leevy, 1962) ^{13}C-octanoate breath test findings correlated with insulin resistance and Hyperinsulinemia, and 13C-methacetin breath test predicted the extent of hepatic fibrosis.

Decreased production of adiponectin, an anti-inflammatory cytokine secreted by adipocytes, may be the link between the MS, DM2, and NAFLD. It was shown that serum levels of adiponectin were significantly lower, and serum levels of type IV collagen, iron, and ferritin were all significantly higher in NASH patients; 90% of patients with NASH were diagnosed by combined evaluation of serum adiponectin and collagen levels. Subclinical glucose intolerance is common in NAFLD ((Kanemasa and Sumida, 2006).

A simple model incorporating C-peptide levels, glucose level at 60 minutes during oral glucose tolerance test (O-GTT), and serum ALT also distinguishes NASH from steatosis (Nomura et al., 1987).

Nonalcoholic fatty liver disease (NAFLD), which includes nonalcoholic steatosis and nonalcoholic steato- hepatitis (NASH), describes the clinicopathologic spectrum of alcohol-like liver disease in nonalcoholic patients.

Although it may be observed as an iatrogenic complication (due to drugs or anti-obesity surgery) or secondary to various other conditions (toxins, lipodystrophic syndromes, hypobetalipoproteinemia), NAFLD most commonly occurs as a primary (idiopathic) disease.

2.10. The clinical importance of primary NAFLD appears to rest on three main observations:

- Ø It commonly occurs in the general population worldwide and among patients presenting with unexplained mild to moderate raised aminotransferase levels.
- Ø It is not a sign/symptom of disease but it is a pathological condition that has the potential to progress to advanced hepatic and extrahepatic disease, and to interact with other etiologies of liver disease.

- It may recur following orthotopic liver transplantation and poses a heavy burden of complications in the setting of major extrahepatic and liver-related surgery.

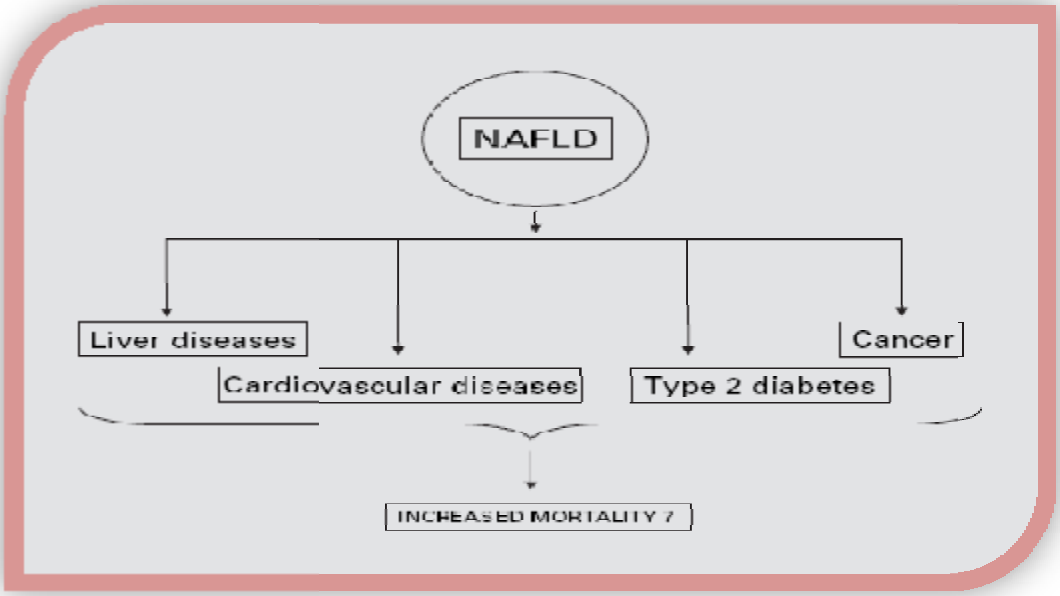


Figure (2.1):

The link between NAFLD and hepatic and Extrahepatic Disease States

(Marchesini and Forlani, 2002).

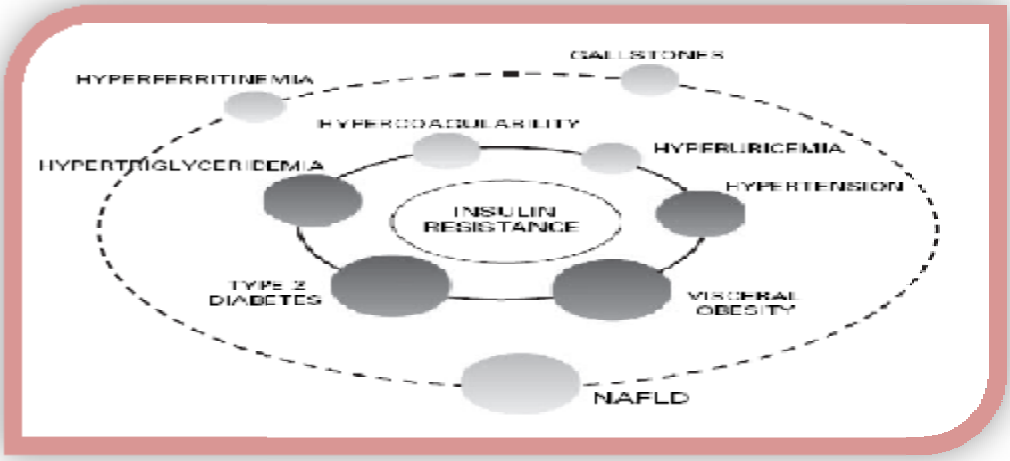


Figure (2. 2):

The Expanding Galaxy of the Insulin Resistance Syndrome.

Beyond the deadly quartet (dark satellites) NAFLD as one of the additional components (light satellites) of the IR syndrome (or MS) (Lonardo, 1999)**.**

2.11. Relationship between Metabolic Syndrome (MS) and Non-Alcoholic Fatty Liver Disease (NAFLD):

As NAFLD is considered as a hepatic expression of metabolic syndrome the following definitions describe the features of MS and its relevance.

2.11.1. National Cholesterol Education Programme (NCEP) definition of metabolic syndrome is (Cotrim et al., 1999)**:**

- Waist circumference >102 cm (40 inch) in men, > 88 cm (35inch) in women
- Serum triglycerides >150 mg/dL (1.7mmol/L)
- HDL cholesterol < 40 mg/dl (1.0mmol/L) in men and < 50 mg/dL (1.3mmol/L) in women
- Blood pressure >130 / 85 mm Hg
- Serum fasting glucose >100 mg/dL (5.6mmol/L)

2.11.2. World Health Organization Definition:

- Glucose intolerance, impaired fasting glucose, diabetes, or insulin resistance (assessed by clamp studies) plus at least two of the following criteria:
- Waist/hip ratio of > 0.90 in men, 0.85 in women
- Serum triglycerides > 150 mg/dL (1.7 mmol/L)
- HDL cholesterol < 35 mg/dL (0.9 mmol/L) in men < 39 mg/dL (1.0 mmol/L) in women
- Blood pressure >140 / 90 mm Hg
- Urinary albumin excretion > 20 ug/min or Albumin:creatinine ratio > 30 mg/g

2.11.3. International Diabetic Federation:

Central obesity defined as waist circumference of > 94 cm for Europid men > 80 cm for europid women with ethnicity specific values for other groups, plus any of two of the following:

- Raised serum triglyceride level of > 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality.
- Reduced HDL cholesterol < 40 mg/dL (0.9 mmol/L) for men, < 50 mg/dL (1.1 mmol/L) for women or specific treatment for this abnormality.
- Raised blood pressure, systolic BP >130 or diastolic BP > 85 mm Hg or treatment of previously diagnosed hypertension.
- Fasting blood glucose > 5.6 mmol/L or previously diagnosed type 2 diabetes, if > 5.6 mmol/L.

The increased prevalence of the underlying causes of the MS syndrome (obesity and sedentary life style) portends enormous increase in cardiovascular disease and type 2 diabetes world wide. The diagnosis provides a focus on the cluster of cardiovascular risk factors that require attention and emphasizes the multi factorial nature of the risk for the disease. Metabolic syndrome therefore needs careful attention to understand its varied expression in different organs. The basic pathological basis for all these altered metabolic machinery is related to insulin sensitivity. Insulin resistance therefore takes the central stage for the expression of the cluster of risk factors shown. Abnormal insulin signaling and secretion, impaired glucose disposal, lipotoxicity and proinflammatory cytokines exacerbate insulin resistance and result in the perturbation of the metabolic syndrome.

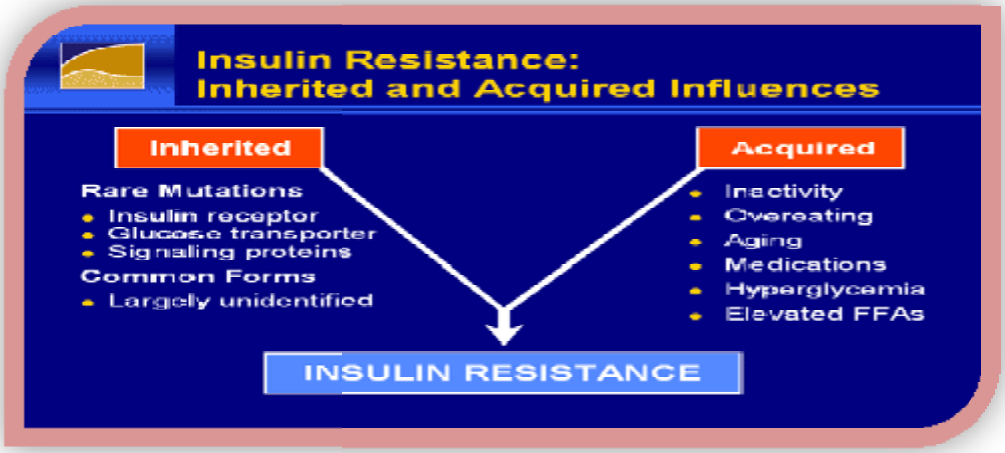


Figure (2.3): Insulin Resistance Inherited and Acquired Influences.

2.12. Among the spectrum of diseases associated with Insulin Resistance is Non alcoholic fatty liver disease (NAFLD).

NAFLD is considered as the hepatic expression of IR. Therefore it will be prudent and pertinent to review the available literature related to metabolic syndrome and its association with NAFLD and Gall stone disease (GD).

2.12.1. Metabolic Syndrome:

Previously, physicians had often treated coexisting diabetes, hypertension, or dyslipidemia as separate diseases, without considering the impact of treatment for one on the other. The importance of the concept for everyday clinical practice was only highlighted in 1988. In view of the rather limited understanding of the nature of the association of these features at that time, Reavan used the term syndrome X (Reaven, 1988). He suggested that insulin resistance played a central etiologic role in providing a link between these components. The common synonyms for Insulin resistance syndrome

are CHO syndrome, carbohydrate intolerance, hypertension, obesity) Reaven's syndrome, Chronic cardiovascular factor syndrome.

Although Reaven formulated his concept in terms of the harmful effects of insulin resistance (low insulin mediated glucose disposal) many studies have used increased insulin concentrations (Reaven, 1988) as a surrogate for directly measured insulin resistance because the measurement, by the hyperinsulinemic euglycemic clamp insulin suppression test or frequently sampled glucose tolerance test (FSIGT) is expensive and has limited patient acceptance. In non-diabetic subjects, increased insulin concentration generally reflects insulin resistance (Zelber-Sagi et al., 2006)

There is now a considerable body of evidence to support the existence of a metabolic syndrome, perhaps the most compelling being that provided by the San Antonio Heart study (Laurin et al., 1996) It was found that a combination of three or more risk factors for coronary artery disease in the same cardiac patient was more prevalent than either one factor alone or two factors in combination. This study suggested that hyperinsulinemia might provide the common etiologic link (Szabo et al., 2003)

It is important to emphasize, that although a common etiologic thread may account for the association between the components of the syndrome, large ethnic differences exist, both in the pattern of risk factors and in the manifestations of the syndrome. For example based on the prevalence of obesity and hypertension, it was surprising that there were fewer cardiovascular deaths in North American Samoans, compared with Americans of European origin. Similarly, less risk of cardiovascular death has been reported in Nauruans and Pimas when compared with Caucasians, based on obesity and frequency of non-insulin dependent diabetes mellitus [NIDDM] in these populations (Cortez-Pinto et al., 1999).

In diabetic African-Americans, Serum high density lipoprotein cholesterol levels tend to be much higher and triglyceride levels much lower when compared with Caucasians and obesity appears to be better reflected by body-mass index in black women. This combination of protective factors may account for the reduced risk of coronary heart disease in individuals of African origin, whereas the increased prevalence of

cardiovascular disease is explained by the higher frequency and severity of hypertension in this population (Opie and Seedat, 2005).

In individuals of South Asian origin, metabolic syndrome components are of a more classical nature as reflected by the increased incidence of ischemic heart disease. In this population, hyperinsulinemia, high plasma triglycerides, low HDL Cholesterol, and diabetes [rather than smoking, hypertension, or altered hemostatic factors] appear to account, at least in part, for the increased cardiovascular mortality. The pronounced tendency toward central adiposity in South Asians, despite similar body mass indices to Europeans, may help explain this pattern of risk factors and increased mortality (Marchesini et al., 2001).

It is likely that both genetic and environmental factors are involved in the development of the metabolic syndrome. Insulin resistance, to a degree is similar to that seen in type-2 diabetes, is found in up to 25% of the general population. Most of the studies and theories relating to the etiology of the syndrome have seen a focus on the role of insulin resistance in the development of type-2 diabetes.

2.12.2. Hyperinsulinemia:

Hyperinsulinemia and insulin resistance have been shown to increase the risk of cardiovascular disease or atherosclerosis in a diabetic patient, as well as being a potential risk factor in the development of hypertension, not only in diabetic patients but also in the general population. The mechanism by which hyperinsulinemia or insulin resistance increases the risk of atherosclerosis is still unclear. Many theories have been suggested, including insulin-induced salt retention, alteration of catechol production, and direct enhancement of the proliferation of vascular smooth muscle cells (Sattar et al., 2004).

The existence of multiple cardiovascular risk factors in pre-diabetic subjects and the possible relationship of these factors to hyperinsulinemia and insulin resistance provide some of the strongest data supporting the importance of insulin resistance to cardiovascular risk. In two recent Finnish studies, the duration of diabetes and elevation of glycosylated

hemoglobin levels were statistically significant, although fairly weak, predictors of coronary heart disease in type-2 diabetes subjects. The weak association between duration of diabetes and severity of glycemia and cardiovascular disease suggests that a common antecedent might underlie both atherosclerotic heart disease and type-2 diabetes (Hanley et al., 2004).

Hyperinsulinemia and insulin resistance strongly predict the development of diabetes in populations that are at high risk and those that are at low risk for the development of type-2 diabetes. A number of studies have documented a relationship between the risk of coronary heart disease events and hyperglycemia, increased levels of glycated hemoglobin [HbA_1C] and hyperinsulinemia. Whether these abnormalities play a direct role in the pathogenesis of atherosclerosis or increase the risk of CHD is being investigated. The EURODIAB prospective complications study (PSC) showed that waist–hip-ratio, and fasting triglyceride were almost as strong risk factors as HbA_1C. In addition these two risk factors are key components of the insulin resistance syndrome (Wannamethee et al., 2005) .

2.12.3. Atherosclerosis and insulin resistance:

Atherosclerosis is a multi-factorial process, which can lead to clinical sequelae and requires extensive accumulation of smooth muscle cells within the intima of the affected artery. The three fundamental biological processes involved are:

a) The accumulation of intimal smooth muscle cell with macrophages and T-lymphocytes.
b) Formation of connective tissue matrix including collagen elastic fibres and proteoglycans.

Accumulation of lipids, in the form of cholesterylesters and free cholesterol within the cells (Kontush and Chapman, 2006).

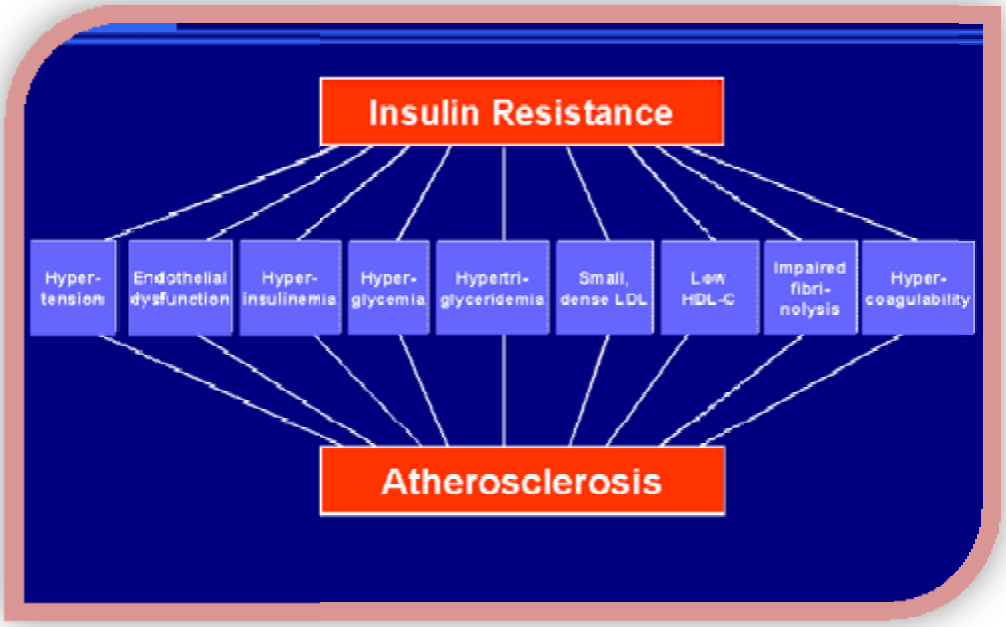


Figure (2.4):

Interrelationship between Insulin Resistance and Atherosclerosis.

The earliest lesion of atherosclerosis is called fatty streak found in young children whereas the fibrous plaque appears in early adulthood and progresses with age.

The Framingham Study, as well as other longitudinal population studies, has made available extensive information on the risk of CAD by level of total blood cholesterol, apart and in combination with other major modifiable risk factors for CAD like cigarette smoking, diabetes and high blood pressure. Each of these risks was multiplicative in the sense that each potentiated the risk from the other factors. Finding among populations, and for persons within populations consistently exhibit a strong, continuous, and direct association between total cholesterol levels and manifestations of CAD (Su et al., 2006).

Atherosclerosis is considered as a chronic inflammatory process that can be converted into acute cardiovascular event, by plaque rupture or erosion. The fatty streak plays a significant role in the events that lead to plaque progression and rupture. Formation of

fatty streak is induced by the transport of lipoproteins across the endothelium and retention in the vessel wall.

It was shown that for any given plasma lipoprotein concentration, the degree of lipoprotein retention in the artery wall is more important than the rate of transport of the same lipoprotein into the artery wall (Toledo et al., 2006).

Once LDL is transported across the artery wall and binds to the extracellular matrix, lipid oxidation is initiated, since micro-environment conditions excluding plasma soluble antioxidants are established. With oxidation of LDL, endothelial cells are stimulated to release potent chemoattractants, such as monocytes chemoattractant protein-I, monocyte colony stimulating factor and growth related oncogene chemokine. These chemoattractants promote the recruitment of monocytes into the subendothelial spaces, which lead to further oxidation of LDL. Heavily modified LDL is cytotoxic to endothelial surface and smooth muscle cells so that it is no longer recognized by LDL receptor. Heavily modified LDL is taken up by the macrophage scavenger receptor, leading to massive accumulation of cholesterol in the macrophages and their transport into cells form the hallmark of atherosclerotic process. Besides promoting the transformation of macrophages into foam cells, oxidized LDL is a potent inducer of inflammation molecules and stimulates the immune system.

There are multiple mechanisms behind the transformation into the form cells. The best known mechanisms are the uptake of lipoproteins of abnormal composition by the macrophages and the uptake of modified LDL by macrophages. It was shown that the degree of susceptibility to oxidation of LDL isolated from 35 male survivors of myocardial infarction was positively co-related with the severity of coronary atherosclerosis (Regnstrom et al., 1992)

The presence of antibodies to oxidized LDL has been described in the sera of several patients and controls. They are found to occur naturally in humans and to be detectable in higher proportion of patients with advanced atherosclerosis, mostly in those with inflammatory reactions to the atherosclerotic plague (Choudhury and Sanyal, 2005) the

endothelium controls leukocyte adhesion, platelet reactivity, capillary permeability, the regulation of vascular smooth muscle cells and blood clotting. The regulation of smooth muscle cells is mediated by the release of potent vaso relaxing agents like [Nitric oxide, prostacyclin] and vasoconstrictor agent's thromboxane and endothelin.

2.12.4. Thrombosis:

The fibrinolytic system controls the potency of the vascular tree and is probably a critical regulator of thrombosis. One hypothesis is that small amounts of fibrin are constantly deposited on the endothelium and that these fibrin deposits are continuously dissolved resulting in a dynamic balance between coagulation and fibrinolysis. The generation and activity of plasmin, the enzyme responsible for degradation in deposits and thrombi are regulated mainly by the production of two critical proteins by the vascular endothelium, tissue plasminogen activator [TPA] and the main inhibitor of TPA, PAI-I. Tissue plasminogen activator converts inactive plasminogen into plasmin at the site of fibrin formation. Impaired fibirinolytic activity is characterized by low TPA activity and high PAI-I antigen and activity. Studies in humans have shown that TPA antigen concentration associated with high PAI-I and low basal stimulated activity may be high in subjects in pre-clinical atherosclerosis and may be a marker for the development of coronary and cerebrovascular events (Lopes-Virella et al., 2008).

2.12.5. Hypertension:

Similarities are found in the vascular injury resulting from hypertension, atherosclerosis and diabetes. There is a complex relation among insulin sensitivity, hypertension and endothelial function. There is some evidence that insulin resistance precedes the onset of established hypertension in high risk patients. Because insulin is a vasodilator it would need to activate a variety of other potential physiological mechanism to play a casual role in the pathogenesis of hypertension.

IMT (intimal medial thickness) thickening indicates both an intimal atherosclerotic process and medial hypertrophy by the influence of pressure. Insulin insensitivity is a strong risk factor for carotid IMT thickening and plaque formation compared with other risk factors, to co-relate between IMT and coronary or cerebrovascular disease was evaluated for clinical significance and relation to insulin resistance in essential hypertension (Kanemasa and Sumida, 2006).

Insulin sensitivity to glucose utilization was evaluated by newly modified SSPG method, which demonstrated that insulin resistance was a strongest predictor of IMT in subjects with non-diabetic mild hypertension. Elevated B.P. is associated with IMT/Atherosclerosis by hemodynamic factors, endothelial injury and / or dysfunction and cell membrane abnormalities (Wanless and Lentz, 1990).

Systolic B.P. is probably associated with IMT thickening in part because of arterial medial hypertrophy (Ridker et al., 1993) On the other hand; increased insulin may have a stimulatory effect on the proliferation of arterial smooth muscle cells.

In a recent study the potential mechanism linking hypertension with insulin resistance, a direct measure characterization of nitric oxide production from umbilical vein endothelial cells in response to insulin was undertaken. The effect of insulin to promote renal tubular reabsorption of sodium, sympathetic nervous system activity and proliferation of vascular smooth muscle cells tend to increase plasma volume, cardiac output and peripheral resistance. However, these effects are opposed by direct vasodilatory action of insulin in some vascular beds (Neuschwander-Tetri and Caldwell, 2003).

Thus the net hemodynamic effect of insulin, if any, is a tendency to lower blood pressure. Interestingly drugs that improve insulin sensitivity also lower blood pressure in hypertensive humans and rats this suggests that it maybe abnormalities underlying insulin resistance rather than insulin per se that are casually related to hypertension. A number of reports have confirmed that elevated insulin levels are associated “cross sectionally“with increased triglyceride levels decreased HDL and hypertension. There are relatively few

data to help determine whether insulin concentrations predict the development of metabolic disorders. In the San Antonio Heart Study, increased fasting insulin levels significantly predict the development of type-2 diabetes, low HDL levels, high TAGs levels and hypertension over an 8year follow up (Clark et al., 2002).

Insulin resistance has been strongly associated with hypertension in lean subjects (Marchesini and Forlani, 2002).

The relationship between insulin resistance and hypertension remain the most controversial part of the insulin resistance syndrome. For example, a significant association between insulin resistance and hypertension in lean type-2 diabetes subjects, but not in obese subjects was observed. (Laakso et al, 1990). An association between insulin resistance and hypertension in black subjects compared to white ones in one study(Saad et al 1991) and in another study in white subjects were reported.(Falkner 2003). Because the blacks in Falkner's study were much leaner than the blacks in Saad's study, it is possible that the failure to find a significant relationship in one report would have been related to obesity rather than ethnicity (Saad et al., 1991)

2.12.6. Obesity:

Obesity is a chronic and increasingly common disease characterized by excess body fat. It develops gradually and often persists throughout life. It is estimated to affect 1/3rd of all adult Americans and more importantly it causes 3,000,000 deaths yearly in the United States. In addition, it can be attributed to obesity-related conditions (Angulo, 2002) as a preventable cause of death; obesity is second only to smoking. Traditionally, obesity was believed to be associated with affluent lifestyles in the West. However, obesity is a fast growing problem in developing countries. Several studies in India have shown that changes in dietary patterns, physical activity levels, lifestyle associated with affluence, and migration to urban areas all result in increased obesity incidence (Kalhan et al., 2001).

2.12.7. Body Mass Index [BMI]:

In 1998, the U.S National Heart, Lung and Blood institute established guidelines to define overweight and obesity. The parameter set for obesity assessment is BMI [also called Quetelet Index] and is calculated using the formula:

BMI = Weight (Kg) / Height (M) ^{2}.

Overweight is defined as a BMI of 25-29.9 kg/m^2 and obesity as a BMI of more than 30 kg/ m^2. While BMI is simple and generally an accurate measure, it does not account for weight distribution and lean body mass. In addition very muscular or very short individuals could be classified as obese when they are not (Reid, 2001).

2.12.8. Waist circumference:

Waist-to-hip ratio provides information about the distribution of body fat. To find the ratio, the circumference of the waist at the navel is measured while the patient stands relaxed. The next step is to measure around the hips at the point where the buttocks protrude the most. The waist measure is then divided by the hip measure resulting in waist-hip-ratio.

2.12.9. Fat distribution:

Based on the waist to hip ratio obesity can be described by fat distribution. A collection of fat on the hips and buttocks [below the waist or gluteo-femoral] may be characterized as gynoid obesity [pear-shaped]. A collection of fat mostly in the abdomen [above the waist] may be characterized as Android obesity or abdominal obesity or central obesity [apple-shaped]. Android obesity is associated with an increased risk of metabolic complications such as coronary heart diseases, hypertension, dyslipidemia, diabetes mellitus and cancers, while gynoid obesity makes the person more prone to mechanical disorders such as varicose veins and disorders of the joints (Kalhan et al., 2001) Even at

the same levels of overweight, as individual with a greater amount of visceral fat is more likely to have or develop, many of the serious health conditions associated with obesity.

Since men typically carry excess weight in the upper body and women in the lower body, men rather than women should be targeted for weight reduction (Misra et al., 2006).

Obesity should be considered a multifactorial condition. Genetic, cultural, socio-economic, behavioral and situational factors all play a role in eating and weight control. Obesity is mostly primary, that is, no obvious cause exist other than an imbalance in energy intake and expenditure. Genetic alterations, endocrine diseases [including Cushing's syndrome, hypothyroidism and hypogonadism], drugs or neurological disorders rarely cause obesity. Many of these conditions often involve obesity as a sign, but only a small fraction of cases of obesity are actually caused by these factors and their associated pathophysiology (Castelli, 1984) Triglycerides are the main storage form of fat and comprise about 95% of the fat of the body. Unlike for carbohydrates the body has an extensive, amount of storage space for fats. Excess energy intake or insufficient energy utilization results in fat storage and, if uncorrected eventually leads to obesity (Downs et al., 1998) the risk of developing diabetes increases as the degree of overweight increases. The prevalence of diabetes is approximately three times higher in overweight than in non-overweight persons. It is estimated that 90% of those with NIDDM in the U.S. are obese (Pedersen et al., 2004) Studies have shown that visceral adiposity increases the risk of hyperinsulinemia and glucose intolerance at a given BMI.

2.12.10. Coronary heart disease and Obesity:

The risk of coronary heart diseases is doubled if the BMI is > 25 and nearly quadrupled if the index is > 29.Obesity has an association with coronary heart diseases presumably through its impact on risk factors, including hypertension, dyslipidemia, impaired glucose tolerance and type-2 diabetes mellitus.

2.12.11. Hyperlipidemia:

In more than half of all diabetic patients, especially those with type-2 diabetes and insulin-resistance patients decrease in high density lipoproteins cholesterol and hypertriglycemia have been reported (Austin et al., 1988)

Hyperinsulinemia and central obesity, which are commonly accompanied by insulin resistance and type-2 diabetes, can lead to an overproduction of very low-density lipoproteins (VLDLs). VLDL particles contain a number of apoproteins and triglycerides. Increased free fatty acid and glucose levels can increase VLDL output from the liver, and elevated triglycerides levels can inhibit apolipoproteins (apo) B degradation, resulting in increased secretion of VLDL. Lipoprotein lipase (LPL) activity is decreased in diabetic patients since insulin is a major regulator of LPL activity. Because LPL is necessary for the breakdown of chylomicrons and triglycerides and its activation results in decreased clearance of VLDL, decrease in LPL activity are one of the causes for the increase in VLDLs. A decrease in LDL levels results in more triglycerides rich particles, fewer HDL particles, and smaller, dense LDL particles in type-2 diabetic patients. Increased VLDL levels can accelerate the atherosclerotic process in several ways: VLDLs could be toxic for the metabolism and growth of endothelial cells (Austin et al., 1988).

Prolonged exposure to hyperglycemia is now recognized as the primary casual factor in the majority of diabetic complications. The presence of amino groups on certain phospholipids such as phosphotidylethnolamine and phosphoditylserine provides appropriate sites with which glucose can react with lipid amines to form aminoguanidine (AGE). Oxidation-reduction reactions occurring normally during glycation can oxidize fatty acid residues, independently of transition metals or exogenous free radical generating systems. Significantly, LDL oxidation follows formation of AGE-LDL, and both occur in direct proportion to glucose concentration and are inhibited in the presence of the AGE inhibitor aminoguanidine. It is apparent therefore, that free amine-glucose interactions with lipids are spontaneous and natural in vivo steps, leading to fatty acid glycation and oxidation products. The apo B component of LDL is a relatively large protein with many

potential lysine and arginine AGE modification sites, although the predominant site of such modification has been found distally to the N-terminus of the LDL receptor binding domain. AGE-apoB levels are up to four fold higher in diabetic patients. The pathological implications of this AGE modifications have been demonstrated in a study in which AGE-LDL was injected into transgenic mice over expressing the human LDL receptor: the modification delayed LDL clearance compared with native LDL (Hilden et al., 1977).

This suggested that advanced glycation of Apo B can lead to hyperlipoproteinaemia and thus actively contribute to atherosclerosis by reducing LDL clearance and by facilitating AGE-LDL deposition in the vessel wall via AGE-receptor interaction (Ground, 1982) Increased insulin resistance and hyperinsulinemia are the hallmark of impaired glucose tolerance. Impaired early phase of insulin secretion, disturbance in the oscillatory cycles of insulin release and impaired suppression of hepatic glucose output have all been reported in IGT. IGT is considered a prediabetic state, because within a 5 year period 30% to 40% of subjects with impaired glucose tolerance will develop permanent type-2 diabetes (Clark et al., 2002).

Both insulin resistance and insulin defiance reciprocally promote and aggravate each other establishing chronic hyperglycemia. Conceptually insulin resistance could be the primary abnormality, overcome by β cell hypersecretion to maintain normo glycemia. Its compensatory mechanism progressively fails with transition from normoglycemia to impaired glucose tolerance and impaired glucose tolerance to clinical diabetes, with both fasting and post prandial hyperglycemia.

Glycemic control is usually assessed by HbA1c, an inability to achieve target levels in spite of adequate control of fasting and pre-meal levels could be due to elevated post-prandial glucose levels. Several studies show a good correlation between post-prandial glucose and HbA1c levels. Therefore after establishing IGT in hyperinsulinemic patients is essential to estimate HbA1c as most patients detected as diabetic are not as new as presumed and HbA1c estimation would reflect glucose levels 3 months lack HbA1c is an early glycation product that is formed when the cells exposed to glucose which rapidly

attach to amino group of protein through non enzymatic process of nucleophilic addition to form Sciff base adducts. Within hours these adducts undergo Amadori rearrangement to form stable glycation products. Excess formation of HbA1c enhance free radical mediated damage.It is very likely that the combination of insulin resistance, hyperinsulinemia, and increased free fatty acid flux in patients with type-2 diabetes is the underlying abnormality driving increased hepatic assembly and secretion of VLDL, IDL, and /or LDL particles (with or without absolute hypertriglyceridemia). In the presence of increased secretion of apoB-containing lipoproteins and concomitant hypertriglyceridemia, CEPT-mediated transfer of HDL cholesteryl ester to those lipoproteins would result in lower levels of HDL cholesterol (increased HDL triglyceride) in type-2 diabetes (Ruhl and Everhart, 2003).

The comparative lack of the effect of hypergylcemia on coronary heart disease in diabetics may be due partly to the many years and perhaps decades, of hyperinsulinemia that characterize the prediabetic phase, during which increased risk factors for coronary heart disease are present. It has been suggested that as many as 50% of newly diagnosed patients with type-2 diabetes might already have evidence of coronary heart disease.

It is conceivable that the insulin resistance that often precedes the development of clinical signs and symptoms of type-2 DM is responsible for the increased risk of coronary heart disease. Middle aged people of South Asian and African Caribbean descent in the UK have a prevalence of type-2 diabetes which is three-four times higher, respectively than in general population. Although it has been known for years that DM is a major coronary risk factor, the precise mechanism for the increased risk of coronary heart disease has not been well defined. South Asian people have a predisposition to central distribution of body fat and this fat is highly resistant (Larason H., 2000).The susceptibility of South Asians to heart disease on one hand and the protection from heart disease observed in African Caribbean’s on the other hand, appears largely related to the differing interrelationships between insulin resistance, central obesity and lipoprotein patterns in these groups.

Recent data from the Center for Disease Control (CDC) showed an increase in prevalence of DM across all ethnic group and all ages, the most striking increase being (70%) observed in people aged between 30 and 39 years. These data suggest that there is not only an increase in prevalence of DM, but that the disease is occurring at younger ages and that such trends, if uninterrupted, can potentially lead to a new epidemic of cardiovascular disease during this millennium. Early identification and implementation of appropriate therapeutic strategies would be necessary to contain that the emerging new epidemic of cardiovascular disease related to diabetes.

2.13. Liver Disorders Progressing From NAFLD to NASH.

In 1980, Ludwig concluded that No therapy for NAFLD has been proven effective, but preliminary studies of lipid-lowering agents, insulin sensitizing agents, antioxidants, and other cytoprotective agents are intriguing. During that time they published the first systematic description of what was then an “unnamed and poorly understood” condition. Using liver biopsy, their findings resembled those of alcoholic hepatitis, but because the patients did not have a history of heavy drinking, the condition was named NASH**,** and it is now believed to be part of a spectrum of disorders that comprise the following:

- Simple steatosis (fat accumulation within liver cells).
- Steatosis with nonspecific inflammation.
- Steatohepatitis (fat accumulation and liver cell injury).
- Cirrhosis (fibrosis, scarring, and nodule formation).
- Hepatocellular carcinoma.

NAFLD encompasses a broad clinicopathologic spectrum ranging from simple steatosis to NASH, which may advance to cirrhosis and end-stage liver disease. Steatosis alone does not appear to be progressive. NAFLD is a spectrum of disorders that range from simple hepatic steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma. The most common-and often the only-laboratory abnormalities of patients with NAFLD are mild to moderate elevations of AST, ALT, or both. The diagnosis of NAFLD requires that the patient have no history of significant alcohol intake, no other liver disease, and findings on

liver biopsy that are compatible with the disorder. Biopsy should be reserved for patients at risk of more serious disease, e.g., those with persistently elevated liver enzyme levels and other risk factors.

2. 13.1. PATHOGENESIS:

2. 13.1. 1 Genes And Environmental Factors:

NAFLD and steatohepatitis probably result from a complex interplay between genes and environment. all factors a genetic predisposition is suggested by the observed clustering of NASH and cryptogenic cirrhosis within families (Struben et al., 2000, Willner et al., 2001) Suggestive are polymorphisms of genes that encode proteins such as TNF-α promoter, microsomal triglyceride transfer protein (MTTP) (involved in the export of triglycerides from the liver), and HFE gene involved in hemochromatosis (Bernard et al., 2000, George et al., 1998).

2.13.1.2. Multiple 'hits' to Steatohepatitis:

The "two-hit" hypothesis is the leading theory of the pathogenesis of NASH.

2.13.2.1. First hit: Insulin Resistance IR:

IR is believed to lead to the accumulation of triglyceride es in hepatocytes as a result of more fatty acids being synthesized, more free fatty acids being delivered to the liver, less fatty acids being degraded, and less triglycerides being released from the liver. This link is supported by findings that many patients with NAFLD have hyper insulinemia, IR, and the MS (Marchesini et al., 2001, Chitturi et al., 2002) even if they do not have diabetes mellitus and are not obese (Marchesini et al., 2001, Chitturi et al., 2002) NAFLD has also been reported in patients with severe IR, such as those with congenital and acquired lipid dystrophies (Arioglu et al., 2000). Excessive fat in the hepatocytes may set the stage for the necro inflammation and fibrosis as seen in NASH

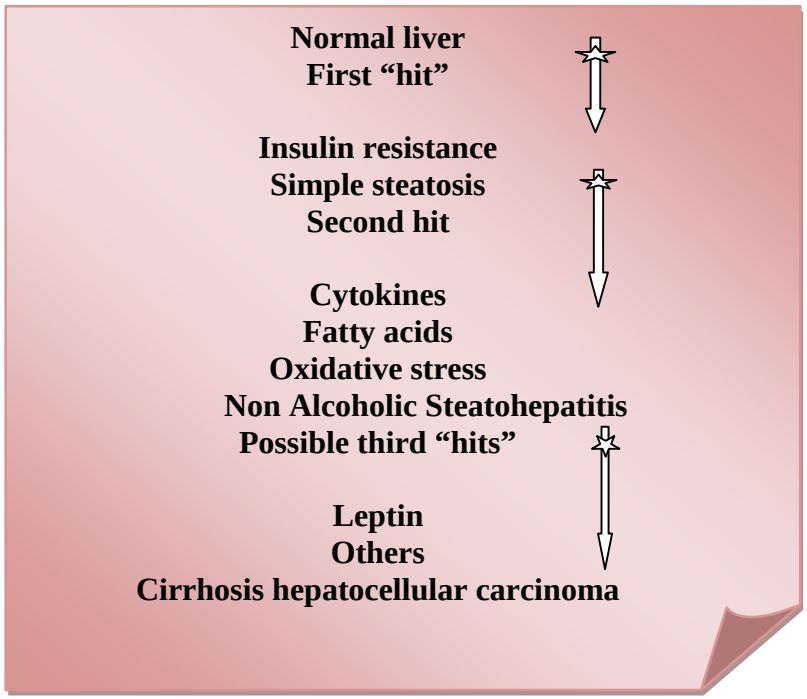


Figure (2.5)

Pathogenesis of NAFLD The "multiple hit" hypothesis

2.13.1.2.2. Second Hits: Oxidative Stress, Cytokines.

A variety of second hits could account for the progression from simple steatosis to steatohepatitis. Increased FFA levels, in addition to mediating IR and causing oxidative stress, can be directly hepatotoxic, leading to cellular injury. Oxidative stress occurs when more oxidant substances are produced than the antioxidant processes of the liver can handle. Oxidative stress can cause lipid peroxidation, leading to activation of hepatic stellate cells and hepatocytes death, contributing to hepatocellular injury and fibrosis. Sources of oxidative stress in steatohepatitis include reactive oxidative species that leak from the mitochondria during oxidation of fatty acids; cytochrome P450 enzymes (CYP2E1 and CYP4A); and hepatic iron (George et al., 1998, Kaplan, 1998) Cytokine production is increased in NASH and is believed to play a role in its pathogenesis. In the

liver, TNF-α can contribute to oxidative stress (Marchesini et al., 2003) and may contribute to IR through activation of the inhibitor of kappa kinase beta (IKK beta).

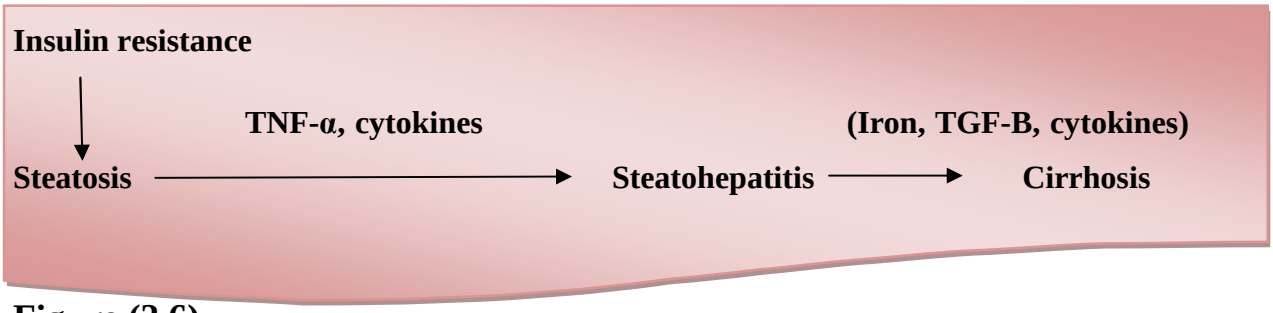


Figure (2.6)

Possible pathogenesis of steatosis to cirrhosis in NAFLD.

2.13.2.3. Third hit: Leptin

Leptin, a protein primarily derived from adipocytes, regulates appetite and energy expenditure. It promotes IR, contributes both to oxidative stress and to enhanced secretion of inflammatory cytokines (Chitturi et al., 2002) and may play a role in causing fibrosis. Produced by activated hepatic stellate cells, leptin can contribute to fibrosis either directly or indirectly through transforming growth factor-beta-1 (Honda et al., 2002). However, while patients with NASH have elevated leptin levels, leptin levels do not correlate with the severity of fibrosis Although separation of steps involved in the multiple-hit hypothesis of NASH provides a convenient scheme, there are probably significant overlaps among these steps. Future research can elucidate the importance of each step in the pathogenesis of this disease.

2.13.3. Clinical Features of NAFLD:

NAFLD has no specific signs or symptoms, although some patients complain of fatigue, malaise, and right upper quadrant abdominal pain. Hepatomegaly, found in about half of patients, is sometimes the only physical finding (Powell et al., 1990) Jaundice,ascites,gynecomastia,and spider angiomas suggest advanced disease. Mildly elevated AST, ALT are the most common finding, and often the only, laboratory

abnormalities of patients with NAFLD. Commonly mild to moderate (twofold to threefold) elevations of AST, ALT, or both are seen (Powell et al., 1990). However, their levels can be as high as 10 to 15 times normal, but this is rare. In 65% to 90% of patients, the ratio of AST to ALT is less than 1.

However, as fibrosis advances, this ratio can reverse and lose its diagnostic value in assessing steatohepatitis (Angulo et al., 1999). Other liver enzymes (alkaline phosphatise or gamma-glutamyltransferase) may be elevated two to three times above the normal range. Hypoalbuminemia, prolonged prothrombin time, and hyper bilirubinemia are less common and, when present, suggest advanced disease. Serum ferritin levels and transferrin saturation may be elevated, but the hepatic iron index and hepatic iron quantitative levels are usually normal.

2.13.4. Imaging can detect NAFLD, but not tell the severity:

Ultrasonography (US) is sensitive in detecting liver steatosis (Saadeh et al., 2002) Non contrast computed tomography (NCCT scan) and magnetic resonance imaging (MRI) can also detect fatty infiltration. However, none of these three imaging methods is especially good for diagnosing steatohepatitis or detecting fibrosis. While all three can detect significant grades of steatosis, none can distinguish between steatohepatitis and the other types of NAFLD (Ong et al., 2001).

2.13.5 How to Evaluate Suspected NAFLD?

Clinical Evaluation (Exclude significant alcohol consumption, drugs and other conditions that cause Non Alcoholic Fatty Liver disease)

2. 13.5.1. Assess risk factors:

a. Obesity (at least moderate "central" obesity).
b. Diabetes Mellitus (NIDDM or impaired glucose tolerance).
c. Hyper triglyceridemia may have NAFLD which can be substantiated by ultrasonography or be liver biopsy.
d. Insulin resistance.
e. Hypertension.
f. Gender.

2. 13.5.2. Measure serum levels of:

- Aspartate Amino transferase (AST).
- Alanine Amino transferase (ALT) (ALT greater than twice normal).
- Gamma -Glutamyl trransferase.

2. 13.5. 3. Exclude other causes of liver disease by assessing;

- Hepatitis B or C serology.
- Auto immune markers.
- Ceruloplasmin.
- Alpha -1 antitrypsin level and phenotype.
- Iron studies.

2. 13.5.4. Imaging studies:

1. Ultrasonography,
2. Computed tomography, or
3. magnetic resonance imaging for hepatic steatosis(≤33%fat may be normal)

2.13.5.5. Liver biopsy.

2.13.6. Determine need based on risk (persistently abnormal liver enzymes, obesity, diabetes mellitus, or older age)

The AST/ALT ratio is however greater than 1 in alcoholic liver disease and less than 1 in NASH which is being followed in certain studies (Das, 2003). The role of liver biopsy in routine clinical practice is controversial. Although most experts believe that a biopsy is important for determining both diagnosis and prognosis, few would recommend biopsy for all patients suspected to have NAFLD. Arguments against liver biopsy include its cost and risk, the lack of effective therapy for NAFLD, and NAFLD has generally good prognosis (Dixon et al., 2001) found that hypertension, elevated ALT, and insulin resistance predict steatohepatitis.

2.13.7. NAFLD; Predictors of NASH and Advanced Fibrosis (Dixon et al., 2001)

HAIR score(presence of 2 or 3) factors Predicts NASH **H**ypertension **A**LT > 40 U/L **I**nsulin **R**esistance index > 5	**BAAT** score (presence of 0 or 1 factors excludes septal fibrosis or cirrhosis) **B**MI ≥ 28 kg/m2 **A**ge ≥ 50 yrs **A**LT ≥ 2 times normal **T**riglycerides ≥1.7 mmol/L

The prevalence of NAFLD averages 20% and that of NASH, 2% to 3%, making these conditions the most common liver diseases in the United States. NAFLD is associated with insulin resistance, which may be evident clinically with obesity, type 2 diabetes mellitus, and Hypertriglyceridemia. The pathogenesis of NAFLD consists of hepatic fat accumulation and oxidative stress with formation of free radicals. The clinical diagnosis is based on the presence of the IR syndrome and exclusion of alcohol abuse as well as viral, autoimmune, genetic, and drug induced liver diseases. Liver biopsy is essential for diagnosis but may not be necessary for clinical management.

Truncal obesity, dyslipidemia (Serum triglyceride level > 200mg/dL; HDL - Cholesterol < 40mg/dL; small dense LDL particle >150 mg/dl) associated with metabolic syndrome are also implicated in the pathogenesis of non alcoholic steato -hepatitis (NASH)

2.13.8. Probable Causes of NAFLD A Liver Manifestation of a Generalized Fat Storage Disorder :

The probable ways of fat accumulation in liver could be:

a. Abnormal fat input (Increased availability of lipids and lipid precursors)
b. Decreased fat out flow/secretion;
c. Increased fat synthesis; and
d. Decreased fat oxidation.

2.13.8.1. Abnormal Input:

It has been observed that nearly 80% of people who undergo liver transplantation following cirrhosis from NASH get back the disease in few years (Contos et al., 2001) this point is added to the fact that at least in the majority of the patients the cause lays outside the liver. Adipose tissue functions as an energy reserve; where energy can be stored in the form of lipids (more concentrated form of energy) safely unlike other tissues where it could cause 'lipid toxicity (Unger and Orci, 2002). This way it acts as an energy sink in times of plenty. Soon after feeding the blood would be over loaded with energy rich compounds and the adipose tissue has an important role in clearing these compounds, especially lipids which are potentially harmful. However in chronic over nutrition adipocytes may become overloaded and may no longer be able to take up circulating lipids and glucose. Insulin is a stimulator of lipoprotein lipase the enzyme which mediates extraction of fatty acids from circulating lipoproteins, which are the carriers of lipids as triglycerides (TAGs). Through the tissue-specific action of lipoprotein lipase, the TAGs-derived free fatty acids (FFA) are taken-up by peripheral tissues. Similarly the type-4 glucose transporters on adipocyte surface are also insulin dependent (Lewis et al., 2002) Probably the over-loaded adipocytes may adopt insulin resistance (IR) as a strategy to save themselves from further overloading, damage and cell death (apoptosis?). As soon as the adipose tissue gets saturated, the energy rich substrates starts 'over flowing' to other tissues like liver and muscle. This could be only one but the most common mechanism of fatty liver. Nearly 60% of TAGs deposited in liver in NAFLD comes from circulating nonesterified fatty acids.

The TAGs contained within adipose tissue are continuously being hydrolyzed into fatty acids and glycerol by the enzyme hormone-sensitive lipase (HSL). Insulin resistance impairs uptake of glucose from blood into skeletal muscle and adipose tissue; serum non-esterified fatty acid (NEFA) levels may also be elevated due to the failure of insulin to suppress HSL mediated lipolysis (Lewis et al., 2002).

Influx of lipids into adipose tissue may be limited by other mechanisms (1), which induces IR in adipose tissue or defects in the transporters of lipids into adipose tissue Defects in the control mechanisms involved in the release of lipids from adipocytes, and defects in normal growth and proliferation of adipose tissue, could lead pooling of plasma lipids causing ectopic fat deposition such as in fatty liver. As in the case of adipocytes, hepatic-fat over load may lead to hepatic insulin resistance (HIR) (Samuel et al., 2004). Liver is another immediate buffer that has been shown to have a high capacity for accumulating fat and redirecting or oxidizing it later depending on the energy homeostatic signals. Within the liver, these fatty acids are either oxidized or re-esterified into TGs and secreted into the blood bound to VLDL (Lewis et al., 2002, Samuel et al., 2004) The fatty acids re-esterified by the liver into TG are almost exclusively from adipose tissue lipolysis (McDevitt et al., 2001).

Over flow of fat to liver could be due to increased dietary intake. Even after a short term fat feeding liver fat increases three fold without increase in visceral or skeletal muscle fat (Samuel et al., 2004) indeed the adipose tissue fat is an indicator of liver fat. The intrahepatic lipids increase by 22% for any 1% increase in total adipose tissue, by 21% for any 1% increase in subcutaneous adipose tissue and by 104% for 1% increase in intra-abdominal adipose tissue (Thomas et al., 2005) Thus liver bears the brunt as soon as adipose tissue buffering reaches its limit.

As the buffering capacity of liver exceeds its limit lipid starts getting accumulated in the cardiovascular system and other organs. The macrophages associated with blood vessels, the evolutionary kin of adipocytes and hepatocytes, will try to “buffer” the excess lipids by phagocytosis and later oxidize them partly to form toxic free radicals and immunogenic compounds. They become ‘foamy cells’ as their capacity to accumulate fat reaches the limit (Libby et al., 2002) this eventually leads to “fibrosis” of vessel wall (atherosclerosis is homologous to cirrhosis of liver) and an increase in CVS related mortality. Indeed some studies published recently found the seeds of CVS mortality in NAFLD (Brea et al., 2005).Defects in fatty acid transporters in adipocytes could result in hypertriglyceridemia

and subsequent increased inflow of FA to liver For example CD36-/-mice lacking the fatty acid transporter that is normally present in muscle and adipose tissue showed increased hepatic TAGs content and a decreased sensitivity of hepatic glucose production to insulin (Goudriaan et al., 2003)

2.13.8.2.Increased intake:

Lipid intake is determined by lipoproteins which are carriers of lipids to liver predominantly from adipose tissue and digestive tract through the circulatory system. The lipids are further transported into hepatocytes by various 'lipid transporters' which are membrane bound channels and cellular lipid binding proteins like fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), fatty acid binding proteins (FABP), caveolin-1 etc. Tissue accumulation of FA requires intracellular trapping involving association between many membranes' intracellular FA binding proteins.

FATP is highly expressed in hepatocytes and adipocytes that reveal high-level FA uptake for both metabolism and storage. FATP1 is found in adipose tissue and in the heart. FATP2 and FATP5 are expressed in the liver, while FATP4 is expressed in the intestine (Pohl et al., 2004). Overexpression of FATP5 in cultured cells has been shown to increase FFA uptake while knock out of FATP-5, results in decreased accumulation of fat in liver and decreased production of conjugated bile acids (Stahl et al., 2001, Doege et al., 2006). A recent study found an upregulation of FABP4 and FABP5 in NAFLD independent of obesity (Westerbacka et al., 2007).Thus FATP5 is an important membrane protein involved in fatty acid accumulation by the liver. In mice, which are FAT/CD36-deficient, the flux of fatty acids toward the liver is increased, precipitating steatosis, but without any evidence of an increase in hepatic VLDL production (den Boer M, 2004). Fatty acids taken up by liver are oxidized and excess is esterified and accumulated or secreted. Esterification is most efficient with mono-unsaturated lipids as monounsaturated fatty acyl-CoAs are the preferred substrates for the synthesis of triacylglycerol (TAGs) in the endoplasmic reticulum (ER) (Shi and Burn, 2004). This is possibly one reason why loss of members of desaturation enzyme family, stearoyl-CoA desaturase, which catalyses the rate-limiting

step in the synthesis of monounsaturated fatty acids, particularly oleate (18:1) and palmitoleate (16:1), protects mice against fatty liver in (Ntambi et al., 2002).

2. 13.8.3. The decreased Secretion/Excretion:

The increased hepatic uptake and biosynthesis of FAs are compensated through increased removal of lipids from the liver. In this process, VLDL plays a central role. The principal apoprotein for this particle is apoB100, but apoE and apoC-I, C- I I , and C- I I I are incorporated as well (Tulenko and Sumner, 2002). Lipid homeostasis in mammalian cells is regulated by a family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs).

In the liver, three SREBPs regulate the production of lipids for export into the plasma as lipoproteins and into the bile as micelles. nSREBP-1a transgenic mice develop a massive fatty liver engorged with both cholesterol and triglycerides (Shimano et al., 1996).The bile acid receptor farnesoid X receptor (FXR; NR1H4) is a central regulator of bile acid and lipid metabolism. FXR protects the liver from the deleterious effect of bile acid overloading by inhibiting their biosynthesis and stimulating their excretion. FXR regulates the expression of several apolipoproteins involved in the transport and metabolism of lipids (Claudel et al., 2005, Watanabe et al., 2004). Bile acid reduces the secretion of VLDL by repressing microsomal triglyceride transfer protein (MTTP) which mediates lipidation of apoB100 to form VLDL (Castro et al., 2007) This effect is possibly mediated through FXR. Pharmacologic agents that induce hepatic steatosis like amiodarone, tetracycline, pirprofen, tianepine inhibit MTTP activity. However it is still unclear whether FXR mediated decrease in triglyceride rich VLDL secretion and consequent decrease in lipid out flow from liver is important in the pathogenesis of fatty liver.

Abetalipoproteinemia, a genetic disease which is associated with fatty liver is caused by mutations in the MTTP gene resulting in blockage of VLDL assembly and secretion (Castro et al., 2007) MTTP-493 G/T polymorphism may influence NASH by modulating postprandial lipemia and lipoprotein metabolism; homozygous GG carriers have a more

atherogenic postprandial lipid profile than the other genotypes, independently of adipokines and insulin resistance (Gambino et al., 2007). Hepatic fatty acid level is sensed by genes like PPAR α which promote lipid secretion with the help of FABP, MTTP and apoB100 which are upregulated (Landrier et al., 2004, Linden et al., 2002) Indeed this could be one mechanism by which fibrates known PPAR-α agonists used in the treatment of NAFLD exerts their beneficial effect. Lipid synthesis should go hand in hand with lipid secretion and hepatic VLDL formation for maintenance of lipid metabolism.Therefore,selective modulators of nuclear receptors involved in lipid homeostasis could thus revolutionize the treatment of NAFLD, Gall Stone disease, obesity and type 2 diabetes mellitus.

HDL particles participate in reverse cholesterol transport, the mechanism by which cholesterol from extrahepatic tissues returns to the liver for excretion as biliary cholesterol (Tulenko and Sumner, 2002).

Low HDL-cholesterol is associated with metabolic syndrome and NAFLD (Brea et al., 2005). ABCA1 is a lipid binding protein which increases reverse cholesterol transport to pre-beta HDL. Patients with low HDL-cholesterol and abnormal cellular lipid efflux due to ABCA1 gene defects (Tangier disease) also have elevated plasma triglycerides (Tulenko and Sumner, 2002, Stefkova et al., 2004) and fatty liver.

2.13.8.4. The increased synthesis

In NAFLD nearly a quarter of the accumulated fat comes from de novo lipid (DNL) synthesis. Lipid synthesis is markedly increased in NAFLD (Diraison et al., 2003) Acetyl coenzyme A (acetyl CoA) is a crucial metabolic intermediate in carbohydrate and protein catabolism towards lipid synthesis. Acetyl CoA carboxylase (ACC) and Fatty acid synthetase are two major enzymes that drive DNL in the liver (Sanyal, 2005). Inhibitors of ACC decreases lipid accumulation by hepatocytes and might prove useful in the development of novel therapeutic agents to combat fatty liver (Sanyal, 2005) .

Synthetic pathways for triacylglycerol (TAGs), cholesterol and its esters, and phospholipids are separate, but transcriptionally co-regulated (Horton et al., 2002) in insulin sensitive tissues and particularly in the liver, the transcription factor SREBP-1c transduces the insulin signaling regulating lipid synthesis. Overexpression of nSREBP-1c in the liver of transgenic mice bypasses insulin requirement and activates the same genes stimulated by insulin and produces a triglyceride-enriched fatty liver with no increase in cholesterol. The mRNAs for fatty acid synthetic enzymes and rates of fatty acid synthesis are elevated fourfold in liver (Horton et al., 2002). Alcohol induces fatty liver partly by impairing PPAR- α and PPAR-γ activity and activity SREBP-1 (You et al., 2002). Overexpression of nSREBP-2 in liver increases the mRNAs encoding all cholesterol biosynthetic enzymes (Horton et al., 2002, Horton et al., 1998).

Under physiological conditions, SREBP-1c is transiently induced in the liver by insulin through activation of IRS-2; this causes a switch from glycogen synthesis to lipid synthesis.

To complete a feedback loop, SREBP-1c then suppresses IRS-2 transcription. Under certain pathogenic conditions, expression of SREBP-1c in the liver remains elevated, and this increases lipid synthesis with resultant accumulation of fat (Ide et al., 2004).

Hepatitis B virus (HBV) infection is associated with fatty liver in a significant proportion of patients. (HBV) encoded protein (HBx) causes lipid accumulation in hepatic cells which is mediated through SREBP1 and PPAR-g (Kim et al., 2007)

Obesity and insulin resistance is a pro-inflammatory state characterized by increased levels of pro-inflammatory cytokines (Libby, 2002, Wellen and Hotamisligil, 2005) Cytokines like IL-6 and TNF-a further promotes insulin resistance by increasing hepatic suppressors of cytokine signaling (SOCS) expression (Sanyal, 2005, Wellen and Hotamisligil, 2005, Ueki et al., 2004).

Over expression of SOCS-1 and SOCS-3 in liver causes insulin resistance and an increase in the key regulator of fatty acid synthesis in liver, SREBP-1c. In obesity, increased SOCS proteins enhance SREBP-1c expression by antagonizing STAT3-mediated

inhibition of SREBP-1c promoter activity (Ueki et al., 2004) Interestingly n-3 PUFAs downregulate SREBP 1-c, which increases transcription of genes responsible for fatty acid synthesis such as fatty acid synthase and stearoyl Co-A desaturase (Levy et al., 2004)

The liver has a central role in glucose homeostasis. On feeding, glucose influx triggers gene expression changes in hepatocytes to suppress endogenous glucose production and convert excess glucose into glycogen or fatty acids to be stored in adipose tissue. This process is controlled by insulin, although debate exists as to whether insulin acts directly or indirectly on the liver. Transcriptional activation of glycolytic and lipogenic genes requires the presence of both insulin and glucose, neither of which is active alone. Recently, carbohydrate responsive element binding protein (ChREBP) emerged as a pivotal transcription factor implicated in the regulation of lipogenic genes by glucose (Dentin et al., 2006).

Liver xenobiotic receptor (LXR) is another glucose sensor which is activated by glucose to switched on several genes involved in fatty acid synthesis (Mitro et al., 2007) An LXR-binding site in the SREBP-1c promoter activates SREBP-1c transcription in the presence of LXR agonists. When lipogenesis is increased by pharmacological activation of the liver X receptor, hepatic VLDL production is increased 2.5-fold, and the liver produces large TGs-rich VLDL particles (Grefhorst et al., 2002).

Interestingly, glucose induces the expression of LXR target genes involved in cholesterol homeostasis like ABCA1 which is defective in Tangier disease (Mitro et al., 2007) a common feature of many metabolic pathways is their control by retinoid xenobiotic receptor (RXR) heterodimers. It is interesting to note that LXR heterodimerizes with RXR. Promiscuous RXR cans also heterodimerizes with PPAR members. PPAR-a plays a pivotal role in fatty acid catabolism in liver by upregulating the expression of numerous genes involved in mitochondrial fatty acid oxidation. Thus RXR is a common partner of two nuclear receptors acting in opposite directions with regard to fatty acid metabolism. Therefore both LXR and PPAR-α compete for the limited pool of RXR and this dynamic equilibrium determines the direction of lipid metabolism (Yoshikawa et al., 2003) FXR

also plays a key regulatory role in by inhibiting the activity of LXR and eventually other transcription factors that stimulate SREBP-1c expression (Watanabe et al., 2004;(Zhang et al., 2006).

An increase in serum triglyceride concentrations and fatty liver have been observed in patients with malabsorption of bile acid as found in chronic inflammatory bowel disease ileal resection and cholestyramine treatment (Canbay et al., 2006). This could be due to loss of bile salt mediated inhibitory effect on fatty acid synthesis mediated through FXR and further research is required in this area.

It may be noted that NAFLD is classically associated with gall stone disease and hypertriglyceridemia (Lonardo et al., 2006, Loria et al., 2005). The increased incidence of gall stones at least partly is due to decreased secretion of bile salts, which are potent emulsifiers, and the consequent instability of bile pigments resulting in precipitation and stone formation. It would be logical to hypothesize that defective FXR expression or signaling and consequent deficiency in bile inhibition of fatty acid synthesis might play a role in certain cases of hepatic steatosis associated with biliary stones.

Hepatocyte nuclear factor-4a (HNF-4a) is a transcription factor which is mutated in monogenic autosomal dominant non-insulin-dependent diabetes mellitus type 1 (MODY-1), controls the expression of several genes, including hepatocyte nuclear factor 1a (HNF-1a), a transcription factor which regulates the expression of several hepatic genes and the human CYP7A1 gene in bile acid synthesis and phosphoenolpyruvate carboxykinase (PEPCK) gene in gluconeogenesis (Ntambi et al., 2002). Long-chain fatty acids, including palmitic acid, have been identified as endogenous HNF-4a ligands and this allows the transcriptional control of gluconeogenesis during active lipid synthesis. It is interesting to note that glucose and fructose induces lipogenesis and reduces hepatic HNF-4a levels, which in turn attenuates the expression of sex hormone binding globulin (SHBG), a biomarker of the metabolic syndrome (Selva et al., 2007). Thus HNF-4a is another major protein at the cross roads of sex hormones, diabetes, fatty liver, dyslipidemia and gall stone

disease. It may be noted that many of the de novo lipid synthesis pathways described above are shared by both alcoholic and nonalcoholic fatty liver disease.

2.14. Whole-body fatty acid and triglyceride homeostasis:

TAGs molecules are the most energy-dense molecules in mammalian physiology and consist of three fatty acids esterified to a glycerol backbone. Normally, a surplus of (dietary) energy is incorporated into TAGs and stored in adipose tissue. Upon fasting, the adipose TAGs store is used to deliver energy in the form of free fatty acids (FFA) to maintain whole-body energy balance. Transport of dietary and de novo synthesized lipids from the liver to the peripheral tissues (*i.e.*, adipocytes), and from the adipocytes back to the liver is important in this balance.

The liver plays a crucial role because it can synthesize, store, secrete and oxidize fatty acids. Because TAGs are very hydrophobic, they need to be transported in blood as the lipoproteins, together with cholesterol, phospholipids and proteins. The core of a lipoprotein contains TAGs and esterified cholesterol whereas the surface consists of phospholipids and free cholesterol. Embedded in the lipoprotein surface are the so-called apolipoproteins, proteins needed for stabilization of the particle and solubility of the core lipids (Ginsberg et al., 2005). Moreover, apolipoproteins act as ligands for receptors and are required for the actions of specific enzymes (Ginsberg et al., 2005).

2.14.1. Triglyceride-rich lipoproteins:

In the enterocytes, dietary TAGs are incorporated into lipoproteins called chylomicrons. Apolipoprotein B (apoB) is the main protein of TAGs-containing lipoproteins (chylomicrons and VLDL). In the surface of chylomicrons, a truncated form of apoB is present, consisting of only 48% of the N-terminal part and is therefore called apo B 48. Editing of the apoB100 mRNA into apo B48 mRNA is regulated by the apoB editing complex-1 (apobec1) (Anant et al., 2001). In the circulation, the TAGs content of these particles is lipolysed primarily by lipoprotein lipase (LPL) secreted by muscle and adipose

tissue. ApoC-III inhibits the actions of LPL while apoC-II enhances its lipolytic actions. The released fatty acids can be taken up and are reesterified into TAGs (*e.g.*, in adipocytes) or used as an energy source (*e.g.*, in muscle). As a result of lipolysis, chylomicrons are depleted of TAGs, become smaller and are referred to as chylomicron remnants. Both chylomicrons and chylomicron remnants are cleared by the liver upon binding to the apo E receptors or the LDL receptor related protein (LRP) or by hepatic lipase (HL) (Cooper, 1997) TAGs transport from the liver to peripheral tissues induces their incorporation into VLDL particles. VLDL-TAGs are lipolyzed by LPL in a similar way as chylomicrons and the fatty acids are taken up by the peripheral tissues. Upon lipolysis, the TAGs content of the VLDL particle becomes depleted and, as a result, the particle sizes decreases and the relative cholesterol concentration increases. The cholesterol-dense VLDL remnant particles are called intermediate density lipoprotein (IDL) or LDL particles, depending on their size and density. With increasing VLDL particles size, the ratio of TAGs over phospholipid will also increase (Fraser, 1970) and the resulting LDL particle will contain relatively more cholesterol: small, dense LDL particles. It is known that small, dense LDL particles are associated with increased cardiovascular risk (Austin and Krauss, 1995, Carmena et al., 2004).

2.14.2. Reverse fatty acid flux:

β- Oxidation of fatty acids is considered the primary source of energy and reducing equivalents (ATP, NADH) needed for *de novo* synthesis of glucose (gluconeogenesis) during fasting. Moreover, ß-oxidation generates ketone bodies, an additional fuel source for the brain when glucose levels are low. In ß-oxidation process, fatty acyl-CoA is broken down into shorter chains by a series of dehydrogenases. For this process, FFAs are released from the adipose stores after lipolysis of TAGs by triglyceride hydrolase (TGH) (also called adipose TAGs lipase (ATGL) (Zechner et al., 2005), and hormone sensitive lipase (HSL) (Gilham and Lehner, 2004), and carried by serum albumin to the liver. Insulin inhibits lipolysis in peripheral tissues. Thus, upon fasting, when insulin levels are low, the insulin-mediated inhibition of lipolysis is absent and FFAs are released into the circulation,

taken up by the liver and partly used in the ß-oxidation process and partly reesterified to form TAGs. This latter process gives rise to the fasting induced hepatic steatosis (Heijboer et al., 2005).

2.14.3. Direct effects of insulin:

Elegant *in vitro* studies showed that insulin is able to inhibit VLDL production via acceleration of the apoB degradation (Fisher et al., 2001). This process is mediated by PI3K (Brown and Gibbons, 2001) But is probably independent of PKB (Au et al., 2004). Via this route, insulin affects the number of VLDL particles secreted. On the other hand, insulin inhibits transcription of the gene encoding for MTTP (Hagan et al., 1994, Wetterau et al., 1997) probably via activation of the transcription factor sterol regulator element binding protein-1c (SREBP-1c) (Sato et al., 1999).

Activation of PLD is an important process in the lipidation of VLDL, is prevented when PIP2 levels are low under conditions of high insulin (Brown and Gibbons, 2001, Phung et al., 1997) Taken together, these data show that insulin impairs the production of large VLDL1 particles via its effects on MTTP and PLD. Thus, the overall effect of insulin is the secretion of less and smaller VLDL particles. We recently confirmed this effect of insulin *in vivo* in mice in which we measured the VLDL-TGS production rate under hyperinsulinemic, euglycemic conditions (Grefhorst et al., 2005).

VLDL-TAGs production rate decreases during hyperinsulinemia, partly due to the secretion of smaller particles. Because a VLDL particle contains a single apoB molecule, the amount of apo B secreted directly reflects the number of VLDL particles produced. As discussed, the amount of apoB secreted by the liver depends, in part, on its lipidation (Gibbons et al., 2004) Therefore, one can imagine that when the amount of TGS available for VLDL is increased to a larger extent than the amount of apoB, the size of VLDL particles will increase. Moreover, insulin regulates whole-body energy balance, including TAGs storage and fatty acid flux via its inhibitory effects on peripheral lipolysis. Thus, insulin might also affect VLDL secretion via its effects on hepatic lipid availability.

2.14.4. VLDL production and hepatic lipid availability:

2.14.4.1. Sources of fatty acids:

TAGs are needed for lipidation of both the apo B and the pre-VLDL particle (Gibbons et al., 2004) the fatty acids that constitute the hepatic TAGs pool originate from three sources:

1. The diet.
2. The peripheral (adipose) stores.
3. De novo lipogenesis.

Fatty acids taken up by the hepatocyte are not directly used for VLDL production but are first esterified and stored as a cytoplasmic lipid droplet (Gibbons et al., 2004, Gibbons et al., 2000). Utilization of TAGS from this pool for VLDL assembly requires hydrolysis followed by re-esterification.

3.0. MATERIALS AND METHODS:

3.1. Patients:

In the present study the histories for100 cases available including gender and age, referred to the 7th October Hospital, Benghazi between (Jan *2008*- Dec *2009*) for various gall-bladder surgical conditions were studied. In 100 subjects taken for the present study the presence of fatty liver and its relation to GD were studied.

Among the 100 cases, (58 females; 42-males) 69 (33 males; 36-females) were diagnosed as NAFLD cases. Among 69 NAFLD patients the presence of GD was evaluated. 50 age matched male subjects (50 ± 5 years) and 50 female subjects (48 ±5 years) who came for routine health check up were included as control subjects. To include cases in the study the following criteria are used:

(1) Absence of alcohol consumption.

(2) Evidence of fatty liver (FL) using ultrasound scanning.

(3) Absence of alternative etiologies of chronic liver disease, notably viral, autoimmune, thyroid, drug induced, hemodynamic or genetic-metabolic (alpha1-antitrypsin, hemochromatosis, Wilson disease).

In addition to abdominal ultrasonography all patients answered a questionnaire, and underwent physical examination and blood sampling for biochemical analysis including the serum transaminase level.

Criteria for inclusion in the study were those cases that have undergone liver ultrasonographic assessment in the same day or, in any case, not later than 12 months apart. Focal liver lesions, ascites or other ultrasonographic findings like portal hypertension (such as ascites, splenomegaly, presence of patent umbilical vein, and varies in the hepatic-gastric ligament or spontaneous spleen-renal shunts) were criteria for exclusion.

100 patients taken for the present study were included based on:

(1) Absent-to-low alcohol consumption (≤ 30 g alcohol daily for men and ≤ 20 g for women);
(2) Evidence of fatty liver based on ultrasound scanning as detailed above; and
(3) Absence of alternative etiologies of chronic liver disease,

Gallstone disease:

Gall stone disease was defined by the presence of one or more echogenic, distal acoustic shadowing, and possibly movable structures in the gallbladder or empty gallbladder fossa in a subject with a history of cholecystectomy (Loria et al., 2005).

Most of biochemical parameters were analyzed by the kits manufactured by Bicon (Germany).

These kits were calibrated by quality controls for every parameters analyzed, intra assay variation and inter assay variation are given.

3.2. ESTIMATION OF SERUM INSULIN:

PRINCIPLE:

Elecsys system was used which employs two monoclonal anti bodies, both together are specific for human insulin (Sapin, 2003).

SANDWICH PRINCIPLE:

1st incubation: 20µl sample and biotinylated monoclonal insulin specific antibody and a monoclonal insulin specific antibody labeled with the Ruthenium complex reacted to form sandwich complex.

2nd incubation: After the addition of streptavadin coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavadin.

The reaction mixture was aspirated into measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with the Pro cell. Application of voltage to the electrode then induced chemi luminescent emission, which were measured by a photomultiplier. Results were determined via a calibration curve which is instrumenting specific generated by a point calibration and master curve provided via the reagent barcode.

REAGENTS:

Elecsys insulin reagent.

Streptavadin coated micro particles 6.5 ml:

Streptavidin-coatedmicroparticles 0.72 mg/ml/binding capacity: 470ng biotin/mg micro particles Preservative.Anti insulin-Ab-biotin

Biotinylated monoclonal anti-insulin antibodies (mouse) 1mg/1; MES buffer 50 mmol/l pH 6.0; preservative anti insulin Ab Ru (bpy) 23. 10 ml.

Monoclonal anti-insulin antibodies (mouse) labeled with ruthenium complex 1.75 mg/l; MES buffer 50mmol/l, pH 6.0; preservative.

Insulin reagent set has a bar coded label containing the specific information for calibration of the particular reagent lot.

3. 3. BLOOD GLUCOSE ESTIMATION

By Enzymatic Colorimetric (GOD-PAP) (Glucose is oxidized- peroxidase) (Trinder, 1969).

PRINCIPLE:

Glucose is oxidised by glucose [GOD] to give Gluconic acid and hydrogen peroxide. Hydrogen peroxide is broken down to oxygen and water by peroxidase [POD]. Phenol acts

as an oxygen acceptor and the product is reacted with four aminophenazone to produce a colored product, which is measured spectrophotometrically.

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

$$H_2O_2 + \text{4amino phenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{4-[P.benzoquinone-mono-imino]-phenazone (red chinoimin)} + 4H_2O$$

REAGENTS: Tris/Phosphate buffer (150mmol/1pH 7.5), Phenol (7.5 mol/l), GOD (1200U/l), POD (660U/l), 4aminophenazone (0.8m mol/l), Peroxidase (> 0.9U/ml), Glucose Oxidase (>15U/ml).

Samples: Serum

Procedure: Wavelength: Hg 546 nm, Temperature: 37ºC, Cuvette (1 cm).

Zero adjustment: For each series one reagent blank only was used.

	Blank	Standard/ Sample
Working reagent	l000 µl	l000 µl
Distilled water	l0 µl	----
Standard/ Sample	----	10 µl

The content was mixed and absorbance measured after incubating at 37ºC for 15 minutes measured were taken within 60 minutes against reagent blank.

Calculation: $\Delta A\text{sample} / \Delta A$ standard x standard conc. (100 mg/dl)

Calculation of HOMA index

Formula: Fasting serum insulin (µU/mL) ×fasting serum glucose (mmol/l)/22.5 (Matthews et al., 1985).

3.4. ESTIMATION OF LIPID PROFILE:

3.4.1. SERUM CHOLESTEROL ESTIMATION:

PRINCIPLE: Enzymatic colorimetric test (Allain et al., 1974).

Cholesterol esters are hydrolysed by cholestrol esterase to free cholesterol, which is then oxidized by cholesterol oxidase to hydrogen peroxide and delta-4-Choleston

Cholesterol ester + $H_2O \xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + $O_2 \xrightarrow{\text{cholesterol oxidase}}$ Cholesten-4-en-3-One + H_2O_2

$2H_2O_2$+Aminoantipyrine+Phenol $\xrightarrow{\text{peroxidase}}$ quinoeimine + $4H_2O$

REAGENTS:

Reagent 1: Pipes buffer, (pH6.9 (90 mmol/l), Phenol (26 mmol/l))

Reagent 2: Cholestrol Oxidase (200 U/l), Cholestrol Esterase (300U/l), Peroxidase (1250 U/l), Amino-4-Phenazone (0.4mmol/l), Cholesterol (200 mg/dl)

Perpetration: Working solution reagent (1) added to reagent (2)

Specimen: Serum collected using standard sampling tubes. Heparinized or EDTA plasma.

Testing procedure: Wavelength: Hg 546 nm , Temperature 37 ºC, Cuvette: 1 cm, Zero adjustment: one reagent blank.

	Blank	Standard/ Sample
Working reagent	l000 µl	l000 µl
Distilled water	l0 µl	----

Standard/ Sample	----	10 µl

Contents were mixed, Incubated 5 min.at 37 º C. and absorbance read for calibrator and sample against reagent blank with in 60 min.

Calculation: ΔA sample ⁄ ΔA standard x standard conc. (200 mg /dl).

3.4.2 ESTIMATION OF HDL-CHOLESTEROL

Test principle:

The Chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL (high density lipoproteins)-fraction, their cholesterol content is determined enzymatically (Nauck et al., 1997).

Reagent Concentration:

Phosphotungstic acid (0.55 mmol/l), Magnesium chloride (25 mmol/l)

Specimen: Serum collected using standard sampling tubes Serum,

Testing procedure:

PRECIPITATION: Pipette into centrifuge tubes.

Sample	500 µl
HDL reagent	1000 µl

Contents were mixed well and let to stand for 10 min.at 25°C then centrifuged for 10 minutes at 4000g. After centrifugation the clear supernatant was separated from the precipitate within 1 hour and the cholesterol concentration determined.

Cholesterol determination:

	Blank	Standard/ Sample
Working cholesterol reagent	l000 µl	l000 µl
Standard/ Sample	----	100 µl

Contents were mixed& Incubated for 5 min at 37 º C.

Absorbance of calibrator and sample against reagent blank. Sample was read within 60 min.

Calculation: ΔA sample ╱ ΔA standard x standard conc. (50 mg/dl)

3.4.3.ESTIMATION OF SERUM TRIGLYCERIDES:

PRINCIPLE: [Automated Method] (Young et al., 1975)

The method involves enzymatic colorimetcic test hydrolysis of triglycerides with subsequent enzymatic determination of liberated glycerol by colorimeter.

Triglycerides+$3H_2O \xrightarrow{LPL}$ Glycerol + Fatty acid.

Glycerol + ATP $\xrightarrow{Glycerokinase}$ glycerol 3-Phosphate + ADP .

Glycerol-3-Phosphate+$0_2 \xrightarrow{GPO}$ Dihydroxyacetone phosphate+H_2O_2 .

H_2O_2+4aminophenazo+4 cholorophenol $\xrightarrow{peroxidase}$ 4-[P.benzoquinone-monoimino]-phenazone + 2 H_2O + Hcl

Reagent Concentration:

R1: Pipes Puffer pH 7.2 (50 mmol/l), p-Chlorophenol (2 mmol/I).

R2: Lipoproteinlipase (150000 U/I), Glycerolkinase (800 U/I), Glycerol-3-P-oxidase (4000 U/I), Peroxidase (440 U/l), 4-aminoantipyrln (0.7mmol/l), ATP (0.3mmol/l).

Manual procedure:

Wavelength: 546 nm, Temperature: 37ºC, Cuvette: 1 cm Zero adjustment: reagent blank, one reagent blank per series only

	Blank	Standard/ Sample
Working reagent	l000 µl	l000 µl
Distilled water	l0 µl	----
Standard/ Sample	----	10 µl

Contents were mixed& Incubated for 5 min at 37 º C.

Absorbance of calibrator and sample against reagent blank. Sample was read within 60 min.

Calculation: ΔA sample/ΔA standard x standard conc. (200 mg/dl **.**

3.4.4. ESTIMATION OF LDL CHOLESTEROL

Polyvinyl Sulphate [PVS] Precipitation Method

PRINCIPLE:

LDL are precipitated by adding Poyvinyl Sulphate [PVS] to the sample; their concentration is calculated from the difference between the serum cholesterol and the cholesterol in the supernatant after centrifugation.(Assmann et al., 1983) .

REAGENTS: Polyvinyl Sulphate (supplied by Boeringenher Mannheim)

PROCEDURE:

0.2 mL of serum was taken in a test tube, to that 0.1 ml of PVS was added. Contents mixed, & allowed to stand for 15 minutes at room temperature and then centrifuged for

2 minutes at 10,000 g. After centrifugation, the supernatant was separated and used for cholesterol estimation by CHOD PAP method.

CALCULATION:

LDL Cholesterol=Total cholesterol-cholesterol in supernatant (mg %).

LDL cholesterol estimation using Fried Wald formula:

When serum triglycerides level is lesser than 400mg/100ml, the following formula is used for the estimation of the same.

LDL-C [mg%] = Total cholesterol-(Triglycerides/5+ HDL-C].

3.5. Uric Acid Estimation (uricase method)

PRINCIPLE

Uric acid is converted by uricase into allantoin (Barham and Trinder, 1972) and hydrogen peroxides. According to following equatoin:

$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_20_2$$

$$2H_20_2 + 4H^+ + \text{Phenol} + \text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinonimine dye} + 4H2o.$$

Reagent concentration:

R1: Phosphate buffer pH8.0 (50mml/l), Chlorophenol (7.5 mmol/l)

R 2: Uricase (300U/l), Peroxidase (> 1 KU/L), 4-Aminoantipyrine (3mmol/l).

Manual procedure:

Wavelength: 546 nm, Temperature: 25ºC, Cuvette: 1 cm Zero adjustment: reagent blank, one reagent blank per series only

	Blank	Standard/ Sample
Working reagent	l000 µl	l000 µl
Distilled water	40 µl	----
Standard/ Sample	----	40 µl

Contents were mixed& Incubated for 5 min at 37 º C.

Absorbance of calibrator and sample against reagent blank. Sample was read with in 60 min.

Calculation: ΔA sample ⁄ ΔA standard x standard conc (6 mg/dl).

3.6. ESTIMATION OF SERUM TRANSAMINASES

3.6.1. Glutamate pyruvate transaminase (GPT)

PRINCIPLE:

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarat, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm , by means of lactate dehydrogenase (LDH) coupled reaction (Bergmeyer et al., 1978) King, 1965;

2-oxoglutarat+L-alanin $\xrightarrow{ALT}$ L-gluatmat+Pyruvat

Pyruvat +NADH+H+ $\xrightarrow{LDH}$ L-Lactat+NAD^+

REAGENT CONCENTRATION:

R1: Tris buffer pH 7.8 (100mmol/l), L-Alanine (500mmol/l).

R2: NADH (0.18 mmol/l), LDH (1200U/l), Oxoglutrate (15 mmol/l)

3.6.2. Glutamate Oxaloacetate Transminase (GOT)

2-oxoglutarat+Aspartate $\xrightarrow{AST}$ L-gluatmat+Pyruvat

Oxalacetat+NADH+H+ $\xrightarrow{LDH}$ L-Lactat+NAD^+

REAGENT CONCENTRATION:

R1: Tris buffer pH 7.8 (80mmol/l), L-aspratate (200mmol/l).

R2: NADH (0.18 mmol/l), LDH (800U/l), MDH (600 U/l), Oxoglutrate (12 mmol/l)

PROCEDURE:

Wavelength: 334 nm, Temperature: 25, C, Cuvette: 1 cm light path Zero adjustment: reagent blank, one reagent blank

500 µl of working reagent + Sample 50 µl were mixed. Incubated for 1 min. at 37 º C and absorbance taken every minute 3 times.

Calculation: ΔA/min * 1750 = (ALT)

ASPARTATE TRASAMINASSE (AST), ALANINE AMINO TRANSFERASE (ALT)

3.7. Analyses of Gall stones:

Gallstones from 41 patients of cholelithiasis were collected after cholecystectomy from the department of Surgery, Seventh of October Hospital, Benghazi, Libya. The 41cases gallstones were collected from 6 males and 35 females. The stones were classified into 3 types depending upon their color and character, yellow and whitish stones identified as cholesterol stones, black and blackish brown as pigment stones and brownish yellow or green identified as mixed stones. The other information about the patients such as age, sex and number of stones were obtained from hospital records. Various physical characters of

stones such as number, shape, size, texture and cross-section were noted. The stones were powdered in a pestle and mortar and dissolved in different solvents depending upon the type of chemical constituent to be analyzed. To determine total cholesterol and total bilirubin, 30mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. The stone solution thus obtained was used for determination of total cholesterol and total bilirubin. To determine calcium, oxalate, inorganic phosphate, magnesium, chloride, triglycerides, sodium and potassium, 30 mg of stone powder was dissolved in 3 ml 1N HCl in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water. The tubes were kept in boiling water bath for 1 hr. To analyze phospholipids, stone powder (20 mg) was dissolved in 15 ml $CHCl_3$+CH3OH in 2:1 ratio, containing 1N HCl. To measure bile acids, the stones were dissolved in chloroform-methanol (2:1) mixture and ethyl alcohol-solvent ether in (3:1 mixture) respectively.

Total cholesterol was estimated by ferric chloride-sulfuric acid method,

Total bilirubin was estimated by the colorimetric method method of Accurex (Gambino and Meiter. 1965), triglycerides was estimated by enzymic colorimetric method.(Buccolo *et al*, 1973), oxalate as described by Satyapal and Pundir based on enzymic colorimetric method (Satyapal and Pundir. 1993), calcium by OCPC kit method (Young *et al*, 1975), phospholipid & inorganic phosphate was estimated by colorimetric method of Fiske and Subba Row (Fiske and Subba 1925), magnesium was estimated by colorimetric method of Neill and Neely (Neill and Neely 1965), chloride was estimated by colorimetric method (Schoenfeld.1964), sodium & potassium by Flame photometer and bile acids was estimated by colorimetric method of Carey (Carey.1958),were estimated. The dissolved stone solutions were stored at 2-8°C, when not in use.

4.0. Statistical analysis:

In this study, mean ± SD were calculated for all groups and subgroups and the following statistical analysis were conducted according to fowler et al., 1998:

4.1-student s t-test:

This test was done to compare two groups and to obtain the differance between these groups, by using Microsoft Office Excel, 2007

4.2- Correalation Coefficient (Persons correalation):

This test was estimated by using the **S**tatistical **P**ackage for **S**ocial **S**ciences **SPSS** version, 17 program for windows softwar to obtain the strength and the significance of the association between all studied parameter.

5.0. Results:

In the present study 100 cases, referred to the 7th October Hospital, Benghazi between (Jan 2008-and Dec 2009) were taken and information on gender, age, presence/ absence of fatty liver was evaluated.

Among the hundred patients 58 females and 42males were diagnosed to be NAFLD 69 patients had high insulin levels (Hyperinsulinemia) as Insulin Resistance (IR) (Male 33 and Females 36).

general dyslipidemia was present in these cases. Gall Stones were present in 33 cases and 19 had undergone cholecystectomy. Of the 33 NAFLD GD cases 23 were females and 10 were males.

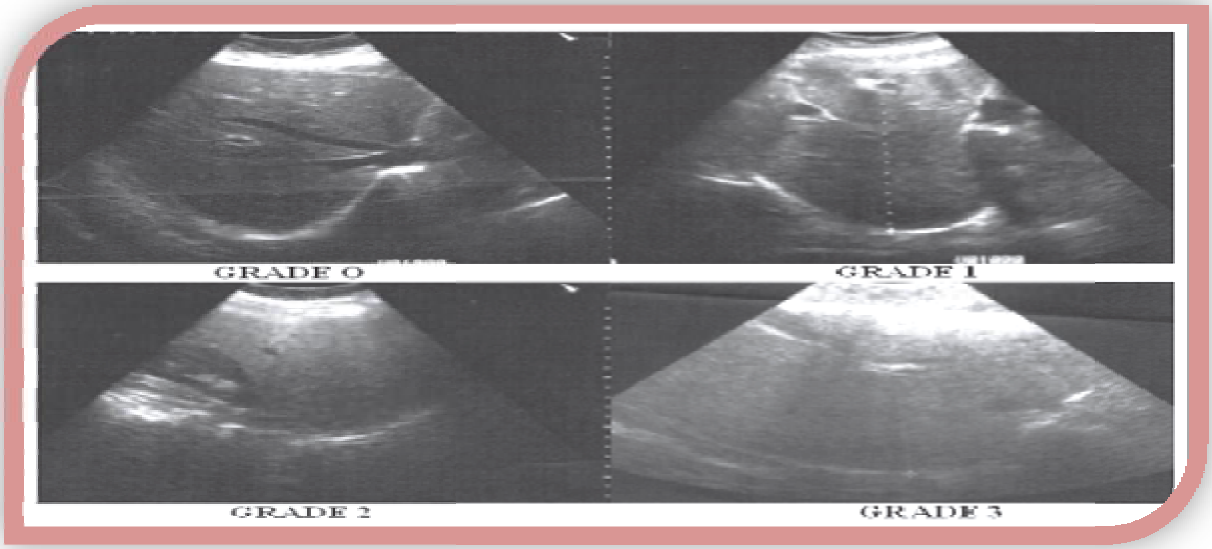


Figure (5.1): Ultrasonographic fatty liver stages (in 4 different patients).

0- Grade 0-no fatty liver.
1- Grade 1- mild fatty liver.
2- Grade 2- moderate fatty liver.
3- Grade severe fatty liver.

Gallstone disease (GD): GD was defined by the presence of one or more echogenic, distal acoustic shadowing, and possibly movable structures in the gallbladder or empty gallbladder fossa in a subject with a history of Cholecystectomy (Loria et al., 2005, Jaraari et al., 2010).

Informed written consent was obtained from all participating individuals before taking blood samples. Institute's Ethics Review Committee approved the project.

Data were expressed as mean ± SE for variables normally distributed and as median (25th-75th percentile) for those not normally distributed. Statistical significance was calculated using SPSS version, 17.

5.0 Results:

One hundred cases were diagnosed as NAFLD based on ultrasonographic findings and serum transaminase levels. Of the 100 NAFLD cases 42 cases were males and 58 cases were females. Among the 100 NAFLD cases, 33 cases were with GD (23 cases were females and 10 cases were males cases female to male ratio was 2.3:1.

Table (5.1): Provides the clinical and laboratory characteristics of male patients with NAFLD and controls.

Parameter	Controls n=50	NAFLD Males n=42	P value
BMI (wt in Kg/height in m^2)	27±4.2	29.5±3.5	p<0.05
Systolic blood pressure (mmHg)	113.6±13.4	127.9±16.2	P<0.01
Diastolic blood pressure (mmHg)	69.6±11.4	78.8±11.8	0.002

From the results it is observed mean values ± SD of the BMI, systolic pressure, diastolic blood pressure, were significantly increased compared to controls.

Significant at P<0.05, Non- Significant at P> 0.05.

Figure (5.2): Show Mean Values ± SD of the BMI, systolic pressure, diastolic blood pressure.

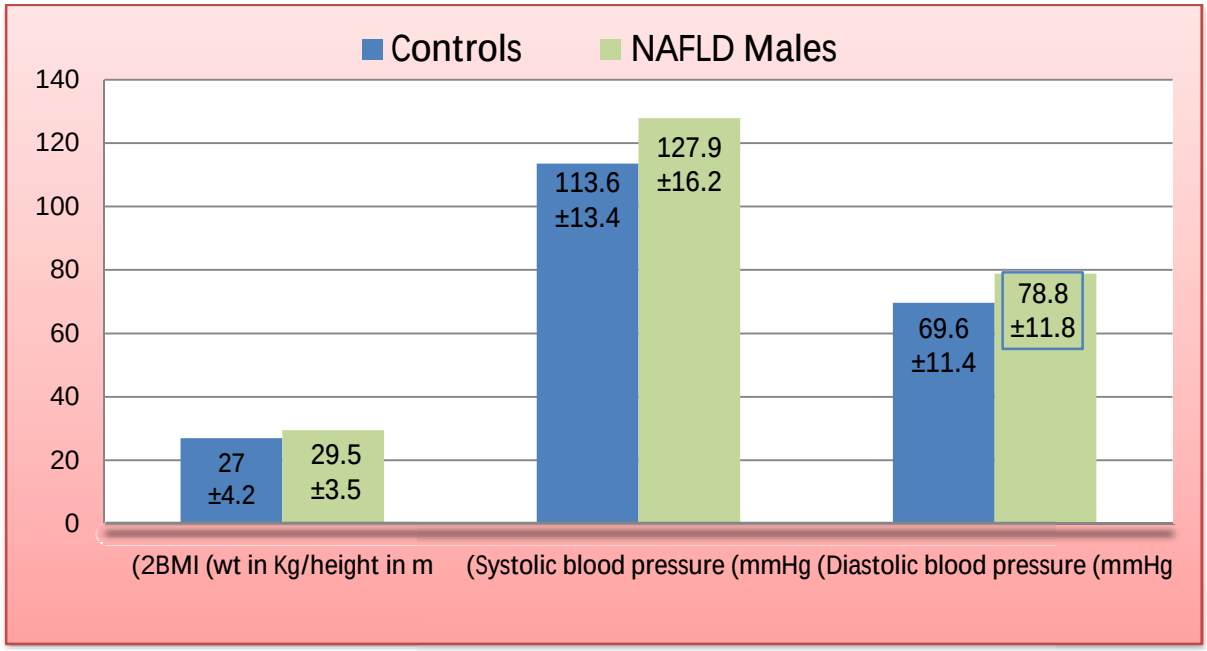


Table (5.2): Show mean value ± SD of serum total cholesterol (mg/dL), Triglycerides (mg/dL) and HDL cholesterol (mg/dL).

Parameter	Controls n=50	NAFLD Males n=42	P value
S. Cholesterol(mg/dL)	160±5.8	188.74±10.62	P<0.01
S.Triglycerides(mg/dL)	108.4±7.88	185.0±7.66	P<0.001
S.HDL-cholesterol (mg/dL)	44.77±2.12	34.74±2.21	p<0.001

Serum total cholesterol and triglycerides were significantly increased compared to controls and the level of HDL cholesterol was reduced. The increase in serum cholesterol was significant compared to the control subjects. It can not be considered as hypercholesterolemia as it was below 200 mg/dL

Figure (5.3): Show Mean Value ± SD of Serum Total Cholesterol (mg/dL), Triglycerides (mg/dL) and HDL Cholesterol (mg/dL).

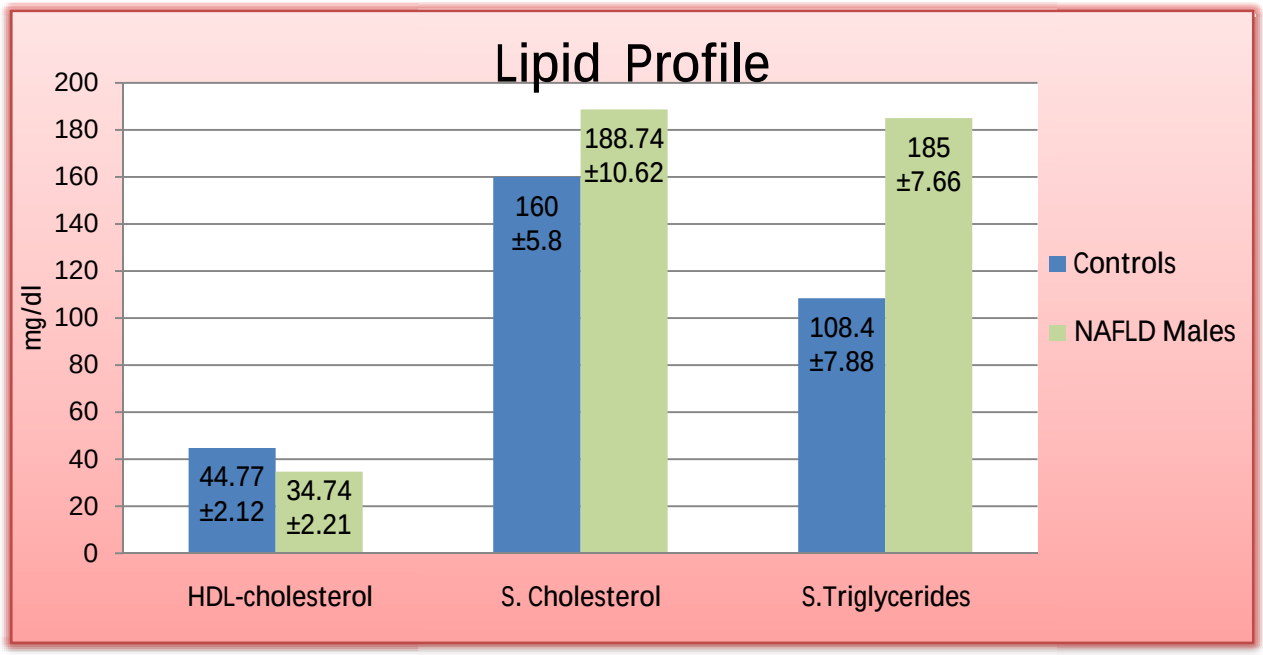


Table (5.3):

Show mean value ± SD of the levels of serum AST and ALT.

Parameter	Controls n=50	NAFLD Males n=42	P value
AST(U/L)	19.45±4..8	35.0±2.12	P<0.01
ALT(U/L)	18.62±4.2	68.0±8.2	P<0.001
S. Uric acid (mg/dL)	5.45±2.5	7.95± 2.6	P<0.01

Both enzymes were higher in NAFLD male patients compared to control subjects .The increase of serum ALT was greater than AST indicating <1 ratio between AST and ALT. Serum uric acid (SUA) levels were significantly increased in male NAFLD patients compared to controls.

Figure (5.4):

Serum Levels of Transaminases and uric acid in NAFLD-male subjects

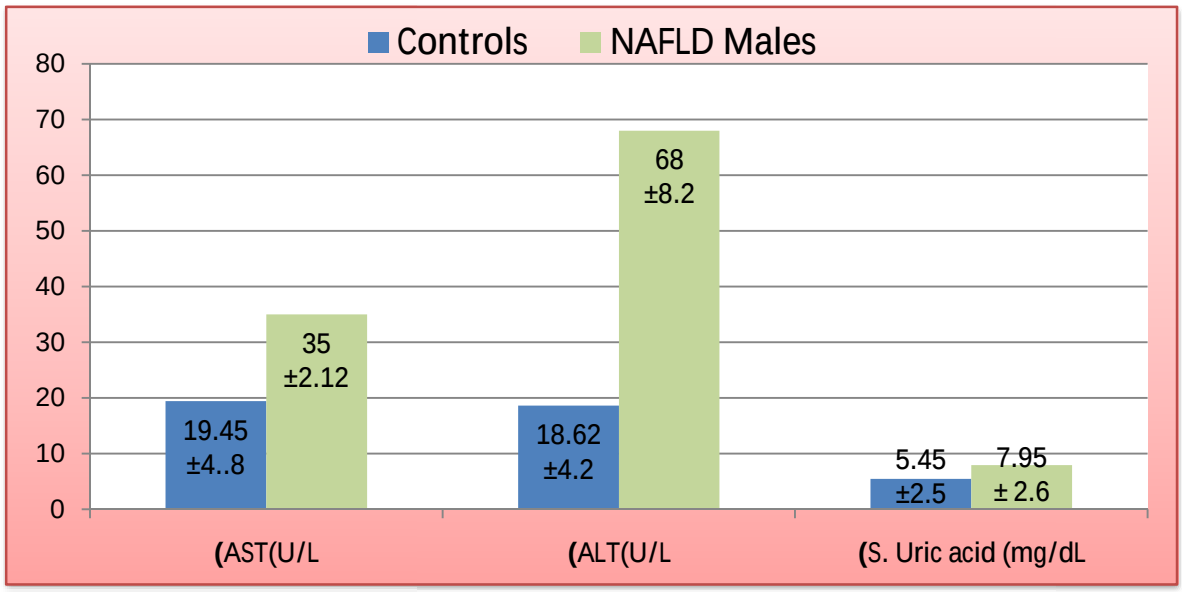
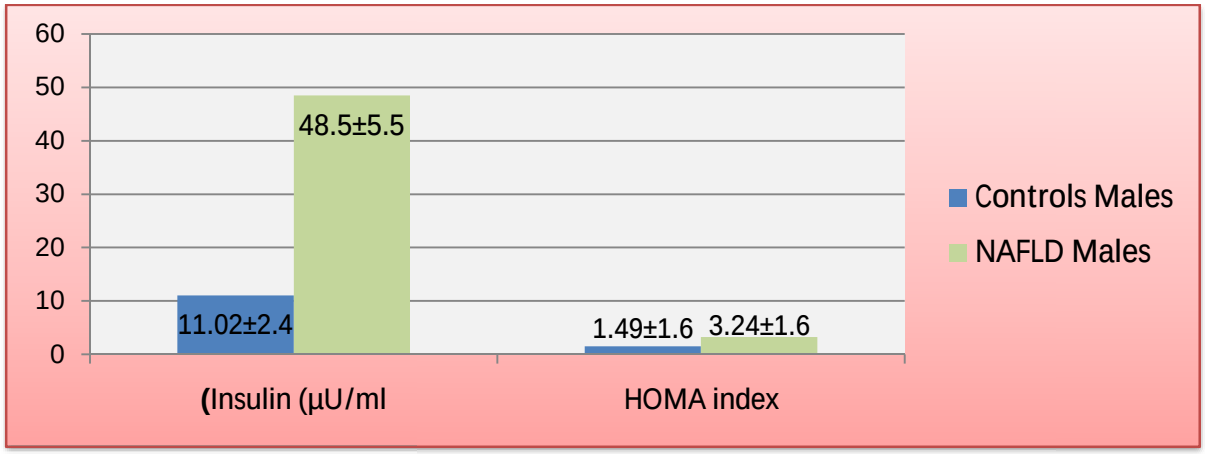


Table (5.4): Show means value ± SD of the levels of age, serum insulin & levels of HOMA index in male NAFLD patients compared to control subjects

Parameter	Controls Males n=50	NAFLD Males n=42	P value
Age (Years)	50±50	48±4.50	
Insulin (µU/ml)	11.02±2.4	48.5±5.5	P<0.001
HOMA index	1.49±0.71	3.24±1.6	P<0.001

The levels of serum insulin and HOMA index were higher in NAFLD male patients compared to control subjects. The mean age of male NAFLD patients was 48±4.5. The insulin levels, HOMA index indicated the presence of insulin resistance (IR) in the NAFLD patients.

Figure (5.5): Show means value ± SD of the levels, serum insulin & levels of HOMA index in male NAFLD patients' compared to control subjects



The levels of serum insulin and HOMA index were higher in NAFLD male patients compared to control subjects.

Table (5.5): Clinical and laboratory characteristics of NAFLD male patients without and with gall stone disease (GD)

Parameter	NAFLD Males n=42	NAFLDGD-Males n=10	P value
Age (Years)	**48±4.5**	**48±5.0**	**NS**
BMI (wt in Kg/height in m^2)	**29.5+3.5**	**29.0±2.5**	**NS**
Systolic blood pressure (mmHg)	**127.9±16.2**	**128.5±13.5**	**NS**
Diastolic blood pressure (mmHg)	**78.8±11.8**	**74.5±5.4**	**NS**
Serum Uric acid (mg/dL)	**7.95± 2.4**	**8.10± 2.45**	**NS**

NS : non significant

Table (5.6): Show means value ± SD of the levels, of serum TAGs, HDL cholesterol levels and total cholesterol levels in male NAFLD patients and male NAFLD GD**.**

Parameter	NAFLD Males n=42	NAFLDGD-Males n=10	P value
AST (U/L)	35.0±2.12	28.5±4.5	P<0.05
ALT (U/L)	68.0±8.2	47.5±4.5	P<0.05
HDL-cholesterol (mg/dL)	34.75±2.21	30.5±2.5	P<0.01
S. Cholesterol (mg/dL)	188.74±10.62	188.0±5.5	NS
S.Triglycerides (mg/dL)	185.0±7.66	190.5±7.5	NS

Details of parameters studied indicating that NAFLD male and male NAFLD GD patients had increased serum triglyceride and total cholesterol levels but the increase in serum cholesterol in the patients were lower than 200 mg/dl suggesting that these patients were dyslipidemic with hypertriglyceridemia and lowered HDL cholesterol levels

Figure (5.6): Show means value ± SD of the levels, of serum Triglycerides, HDL cholesterol levels and total cholesterol levels in male NAFLD patients and male NAFLD GD**.**

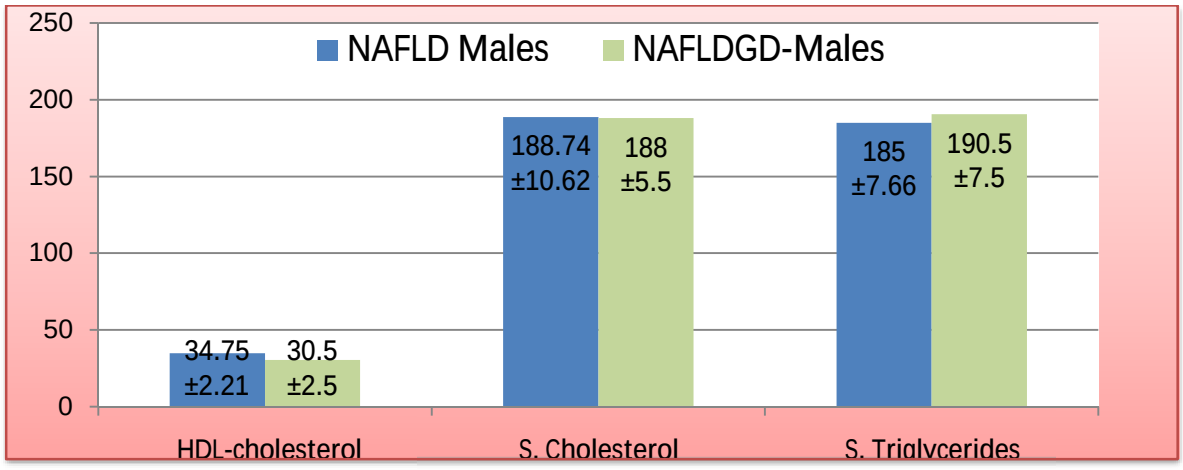


Table (5.7): Show means value ± SD of the levels,of serum insulin & HOMA index in male NAFLD patients and male NAFLD GD**.**

Parameter	NAFLD Males n=42	NAFLDGD-Males n=10	P value
Insulin (µU/ml)	48.5±5.5	38.0±2.5	P<0.05
HOMA index	3.24±1.6	3.5±0.77	NS

Details of parameters studied indicating that male NAFLD and male NAFLD GD patients had hyperinsulinemia, IR (HOMA index)

Figure (5.7): Show means value ± SD of the levels of serum insulin & HOMA index in male NAFLD patients and male NAFLD GD**.**

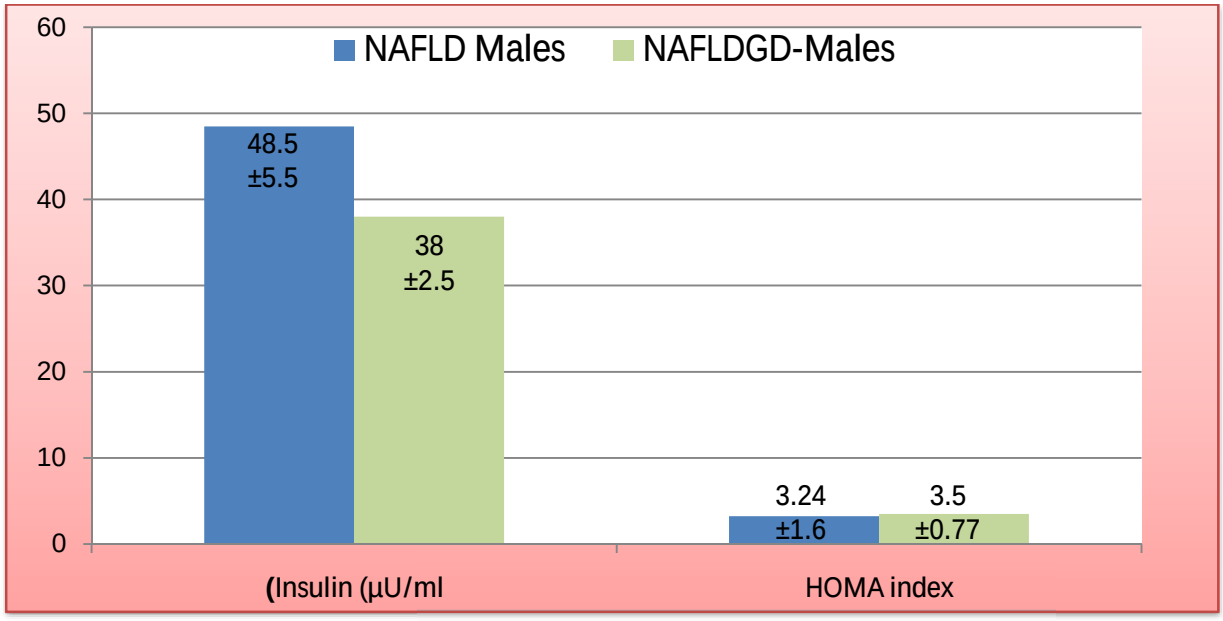


Table (5.8): Show means value ± SD of the levels, of age, BMI, Systolic blood pressure, Diastolic blood pressure, AST, ALT, Insulin, HOMA and Serum Uric acid in female NAFLD compared to control subjects

Parameter	Controls Females n=50	NAFLD Females n=58	P value
Age (Years)	48±5	52±3.5	
BMI (wt in Kg/height in m2)	27±4.8	31.5+4.5	p<0.05
Systolic blood pressure (mmHg)	110.56±13.4	128.59±16.2	P<0.01
Diastolic blood pressure (mmHg)	75.6±11.4	78.8±11.8	0.002
AST(U/L)	19.85±3.8	29.45±2	P<0.01
ALT(U/L)	18.62±4.2	55.45±6.4	P<0.001
Insulin (µU/ml)	11.02±2.4	44.5±6.5	P<0.001
HOMA index	1.51±0.91	3.14±1.8	P<0.001
S.Uric acid (mg/dL)	5.65± 2.65	6.10± 2.45	NS

Clinical and laboratory finding for the female NAFLD patients.The mean age of the patients were 52±3.5 years which were comparatively higher than in male NAFLD subjects. BMI, systolic pressure, serum transaminases, insulin and HOMA index were significantly higher among female NAFLD and NAFLD GD patients.

Figure (5.8a): Show means value ± SD of the levels, of age, BMI, Systolic blood pressure, and Diastolic blood pressure in female NAFLD compared to control subjects

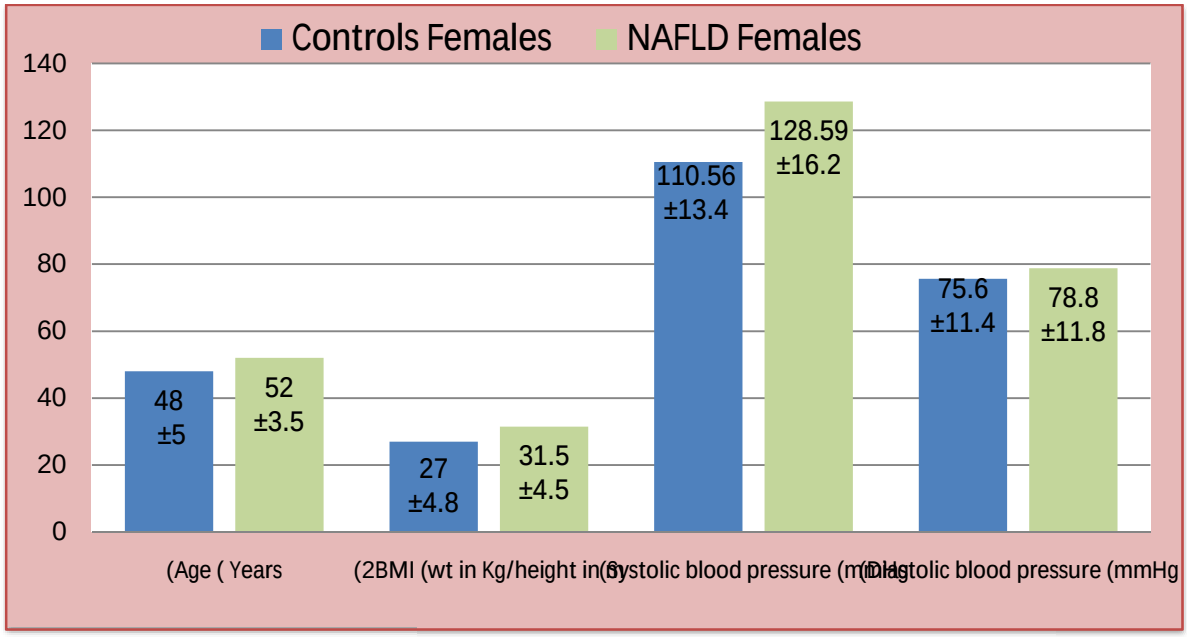


Figure (5.8b): Show means value ± SD of the levels, of, AST, ALT, Insulin, HOMA and S.Uric acid in female NAFLD compared to control subjects.

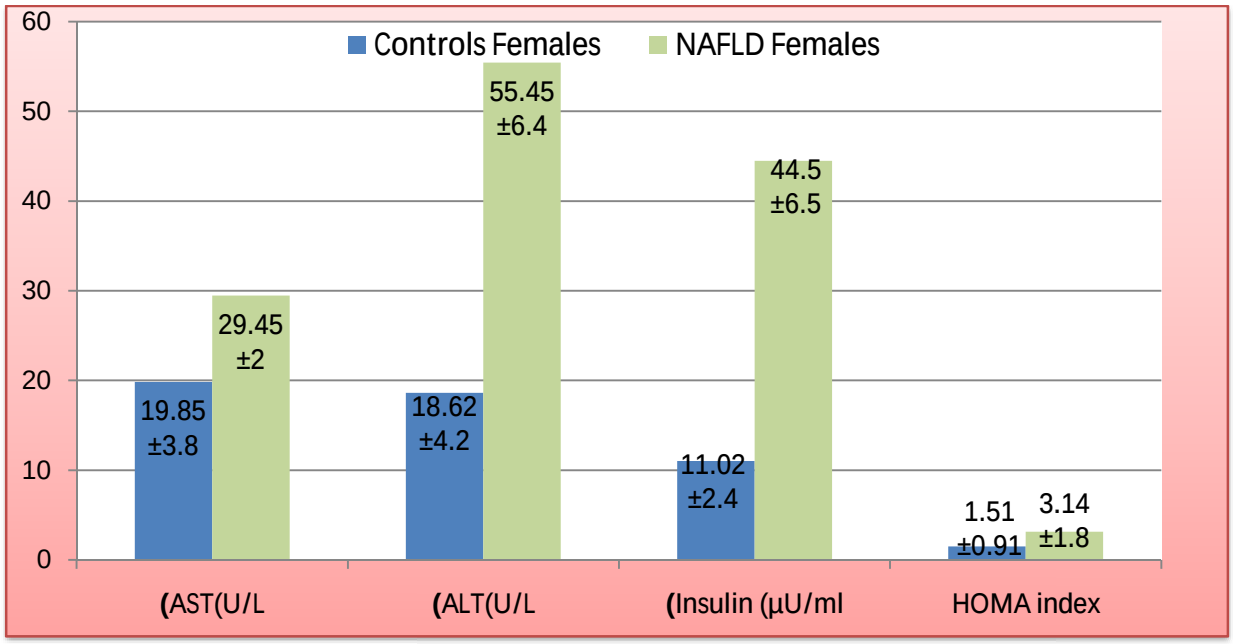


Table (5.9): Show means value ± SD of s.TGs levels, HDL cholesterol levels and total cholesterol levels in Females NAFLD patients compared to control subjects**.**

Parameter	Controls Females n=50	NAFLD Females n=58	P value
HDL-cholesterol (mg/dL)	47.8±2.12	30.65±2.21	p<0.001
S.Cholesterol(mg/dL)	158±6.28	185.18±7.18	P<0.01
S.Triglycerides(mg/dL)	105.45±7.6	184.850±7.5	P<0.001

The lipid profile in NAFLD& NAFLD GD was similar to NAFLD male, NAFLDGD male patiens with an increase in HDL cholesterol level in NAFLDGD patients.

Figure (5.10):

Show means value ± SD of s.TGs levels, HDL cholesterol levels and total cholesterol levels in Females NAFLD patients compared to control subjects

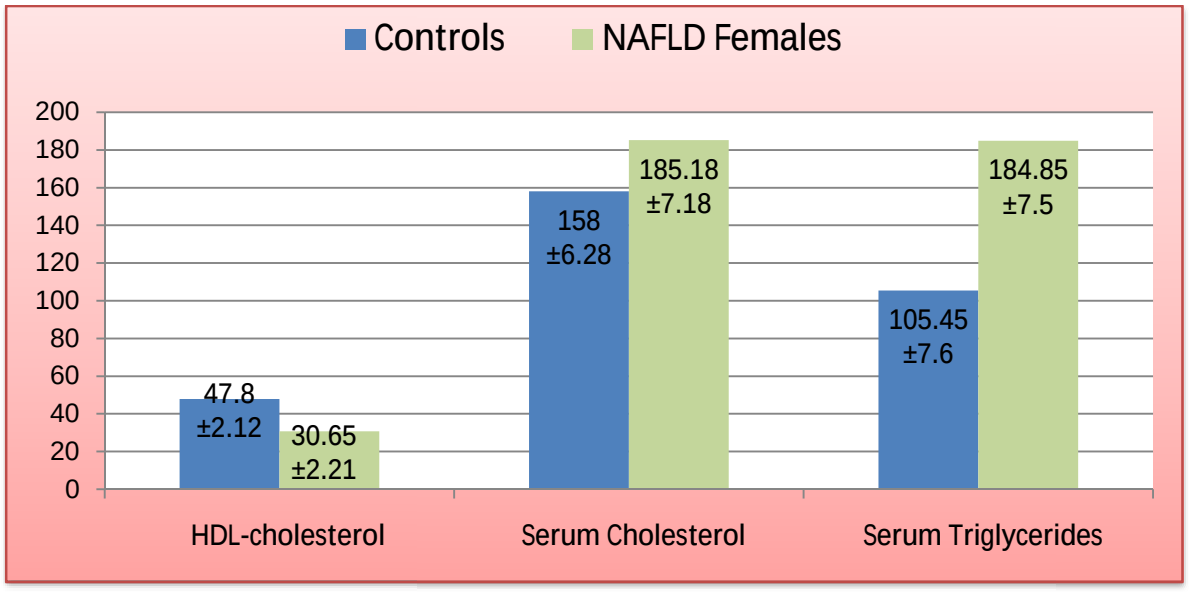


Table (5.10):

Clinical and laboratory characteristics of female NAFLD patients with out and with Gall Stone Disease (GD)

Parameter	NAFLD Females n=58	NAFLDGD-Females n=23	P value
Age (Years)	52±3.5	50.0±5	NS
BMI (wt in Kg/height in m^2)	31.5+4.5	30.0±4.5	NS
Systolic blood pressure (mmHg)	128.59±16.2	130.5±12.4	NS
Diastolic blood pressure (mmHg)	78.8±11.8	74.5±10.5	NS
HDL-cholesterol (mg/dL)	30.65±2.21	29.8±2.12	NS
Serum Cholesterol(mg/dL)	185.18±7.18	188±6.24	NS
Serum Triglycerides(mg/dL)	184.850±7.5	185.45±7.4	NS
AST(U/L)	29.45±2.0	30.85±3.4	NS
ALT(U/L)	55.45±6.4	48.60±4.2	P<0.05
Insulin (µU/ml)	44.5±6.5	38.02±2.4	P<0.05
HOMA index	3.14±1.8	3.51±0.89	NS
Serum Uric acid (mg/dL)	5.85±2.30	6.15± 2.40	NS

Though in general there was significant reduction in HDL cholesterol levels both in male and female NAFLD cases compared to the controls, HDL cholesterol level was higher in these patients when compared to patients with NAFLD GD.

6.0 Discussion

NAFLD is now considered as one of the insulin resistant states being expressed in the liver. In other words NAFLD is a hepatic expression of the metabolic syndrome. Cholelithiasis or gallbladder stones are one of the major surgical problems in the Libyan population and account for many hospital admissions and surgical interventions. Most patients with gallstones present with severe abdominal colic requiring investigations and treatment. Many of them need surgical intervention by the time they are symptomatic. This problem is probably related to obesity, cardiovascular disorders (CVD), metabolic syndrome and dietary habits, especially excessive consumption of meat, which is known to contain large amounts of cholesterol. Obese individuals with a BMI > 30 kg/m^2 have 95% cholesterol-dominant gallstones and are at a high-risk for cholesterol stones formation (Clemens et al., 2006). Pigment Stones (PS) was the most common type of gallstones, and cholesterol seemed to be the major component in all types of stones. High cholesteroI content in CS especially suggests supersaturation of cholesterol in bile consequent to dyslipidemia (excessive cholesterol and altered lipid metabolism) is an etiological factor. Higher triglycerides content in mixed stones also suggests that dyslipidemia plays a key role in its pathogenesis (Lonardo et al., 2006). It may be noted that NAFLD is classically associated with gall stone disease and hypertriglyceridemia (Lewis et al., 2002, Loria et al., 2005)

Fatty liver is one of the common problems associated with truncal obesity, metabolic syndrome and dyslipidemia. In countries which do not consume alcohol, non alcoholic fatty liver disease (NAFLD) is considered to be one of the common problems encountered in hepatology units. NAFLD is considered to be the hepatic expression of the metabolic syndrome. Metabolic syndrome is considered to be a cluster of factors used to define it. Obesity, dyslipidemia, hypertension, decreased HDL cholesterol, increased serum triglyceride, including microalbumnuria are the components of the metabolic syndrome (MS). The most probable cause of NAFLD is reported to be insulin resistance (Chouhar and Sanyal, 2005; Pragna et al. 2002) in the present study NAFLD patients had

hyperinsulinemia and the values of HOMA index were increased indicating the presence of insulin resistance. These patients had elevated levels of serum transaminases. The levels of ALT were higher than AST levels with an AST: ALT ratio of<1 . Insulin resistance leads to greater breakdown and over flow of fatty acids into hepatocytes, which results in hepatitis. Insulin resistance impairs uptake of glucose from blood into skeletal muscle and adipose tissue; serum non- esterified fatty acid (NEFA) levels may also be elevated due to the failure of insulin to suppress hormone sensitive lipase(HSL) mediated lipolysis (Jaraari et al., 2010, Donnelly et al., 2005).

Influx of lipids into adipose tissue may be limited by other mechanisms (Donnelly et al., 2005). This induces IR in adipose tissue or defects in the transporters of lipids into adipose tissue. Defects in the control mechanisms involved in the release of lipids from adipocytes and defects in normal growth and proliferation of adipose tissue, could lead to pooling of plasma lipids causing ectopic fat deposition such as in fatty liver. As in the case of adipocytes hepatic fat overload may lead to hepatic insulin resistance (HIR) (Jaraari et al., 2010, Samuel et al., 2004) over flow of fat into the liver could be due to increased dietary intake. Even after a short term of fat feeding, liver fat increases three fold without increase in visceral or skeletal muscle fat (Jaraari et al., 2010, Samuel et al., 2004)

Patients with low HDL-cholesterol and abnormal cellular lipid efflux due to ABCA1 gene defects (Tangier disease) also have elevated plasma triglycerides (Mendez *et al*, 2005) and fatty liver for NAFLD patients nearly a quarter of the accumulated fat comes from de novo lipid (DNL) synthesis (Samuel et al., 2004). And hepatic lipid synthesis is markedly increased in NAFLD (Thomas et al., 2005). The increased incidence of gall stones at least partly is due to decreased secretion of bile salts, which are potent emulsifiers, and the consequent instability of bile pigments resulting in precipitation and stone formation. It would be logical to hypothesize that defective FXR expression or low HDL-cholesterol and abnormal cellular lipid efflux due to ABCA1 gene defects (Tangier disease) have elevated plasma triglycerides (Mendez *et al*, 2005) and fatty liver.

It would be logical to hypothesize that defective FXR expression or Likewise, increased VLDL and decreased HDL, primarily HDL2, were common in obese persons with gallstones; therefore, elevation of VLDL-C and LDL-C is closely related to excessive dietary cholesterol (Stefkova et al., 2004). The decreased HDL may bear a close relationship to the elevated activity of plasma CETP (cholesteryl ester transfer protein) which promotes the lipoprotein cholesterol of HDL to be transferred to other lipoproteins such as VLDL and LDL in patients with cholesterol gallstones (Diraison et al., 2003).

In the present study an increase in plasma Total Cholesterol, TAGS, VLDL-C, LDL-C and a decrease HDL-C were observed. It was reported that an increase in the dietary cholesterol resulted in an increase in biliary cholesterol secretion in gallstone patient compared to controls suggesting that dietary cholesterol might be important in the pathogenesis of cholesterol gallstones.(Kern, 1994) These findings support the hypothesis that hepatic metabolism of cholesterol in gallstone patients differs from those without stones.

It was suggested that cholesterol precursor secreted by the gallbladder was transported directly from plasma through the plasma membrane of hepatocytes to the biliary canaliculi without entering the interior of the cell (Robins and Brunengraber, 1982).

The concentration of apolipoproteins in bile is about 10% of that in plasma. Although apolipoproteins are potential antinucleating agents in bile, their functional role in vivo as a factor in the solubilization of biliary cholesterol is relatively unexplored. In vitro apoA1 in low concentrations can delay the shift from micelles to vesicles, thereby enhancing the cholesterol-solubilizing capacity of bile acids. Another finding (Donnelly et al., 2005) is that apoA1 stabilizes phospholipid lamellae and thus prolongs nucleation time in model bile systems (Kern, 1994, Robins and Brunengraber, 1982). current study showed that in GD patients the serum levels of HDL cholesterol was high compared to control subjects an unusual observation which could be a result of poor hepatic uptake of HDL cholesterol in GD patients (Jaraari et al., 2010).The increase in HDL cholesterol in NAFLDGD patients

in the present study compared to NAFLD patients may reflect similar mechanism though the levels were comparatively lower than the normal controls.

7.0. Conclusion

The present study indicates that NAFLD could be the early sign of cholelithiasis and gallstone disease, particularly for a local Libyan population susceptible to stone disease. The presence of hyperlipidemia (dyslipidemia, decreased HDL cholesterol levels and hypertriglyceridemia) in these cases could also indicate a risk for the future precipitation of CVD.

Therefore, the high prevalence of NAFLD in patients with GD may justify doing a routine liver biopsy during cholecystectomy to establish the diagnosis of nonalcoholic steatohepatitis (NASH) in such patients. Creating awareness of the association of NAFLD in GD patients may help with the early diagnosis and prevention of the progression of NAFLD towards GD in susceptible populations.

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8.1. SUMMARY

Aim: To identify the presence of nonalcoholic fatty liver disease and evaluate its association with gallstone disease in local Libyan patients.

In the present study 100 cases, referred to the 7th October Hospital, Benghazi between (Jan *2008*-and Dec *2009*) was taken. Information on gender, age, presence/ absence of Fatty liver was noted.

Among the 100 patients (58 females; 42 males) that were diagnosed NAFLD 69 patients had high insulin levels (hyperinsulinemia as insulin resistance) (IR) (Male33 and Females 36).

Among the 100 NAFLD patients the prevalence of gallstone disease was determined. In general dyslipidemia was present in these all cases. Among the 100 NAFLD cases 33 cases were with gallstone disease (GD) (23 cases were females and 10 cases were males) Of the NAFLD gallstone disease cases female to male ratio was 2.3:1.

50 age matched male subjects (50±5 years) and 50 female subjects (48±5 years) who came for routine health check up were included as control subjects.

Lipid profile (total cholesterol, High Dinsty Lipoprotein (HDL)-cholesterol, Low Dinsty Lipoprotein (LDL)-cholesterol, triglycerides), fasting glucose, fasting insulin, and uric acid, serum transaminases were determined by standard procedures. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated for IR. Ultrasonography and serum transaminase levels were used to diagnose NAFLD cases and gallstone disease (GD) by specific ultrasonographic pattern.

Results and Discussion: Hyperlipidemia, insulin resistance, in additional to a fall in High Dinsty Lipoprotein (HDL) cholesterol were observed in NAFLD and NAFLD gallstone disease patients compared to control subjects. The level of serum uric acid was increased more in male patients compared to female patients. The level of High Dinsty Lipoprotein (HDL) cholesterol though reduced in NAFLD gallstone disease patients

compared to control subjects, was found to be more elevate in NAFLD gallstone disease compared to NAFLD patients in both male and female cases.

The association of NAFLD in gallstone disease patients suggests that NAFLD could be a risk factor for gallstone disease in Libyan population known to be prone for gallstone disease. The presence of NAFLD in gallstone disease patients validates carrying out a liver biopsy after cholecystectomy which will help diagnose the presence of non alcoholic steatohepatitis (NASH) cases. The presence of obesity along with hyperlipidemia may suggest the need to evaluate the possible progression of gallstone disease patients to coronary vascular disease (CVD).

Key words: Nonalcoholic fatty liver disease (NAFLD), gallstone disease (GD); lipid profile, insulin resistance (IR).

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جامـــــعة بنـــــغازى

كلية الطب – بنغازى

قسم الكيمياء الحيوية

"الدراسه البيوكيميائيه لمرضى الكبد الدهنى الغير كحولى وعلاقته بمرض حصى المرارة عند المرضى الليبيين"

هذه الرساله للحصول على درجة الماجستير فى علوم الكيمياء الحيوية

كلية الطب – جامعة بنغازى

بنغازى - ليبيا

مقدمة من

هيـــام عبدالله بالقـاسم العـوامى .

تحت إشراف

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جامعة بنغازى- كلية الطب – قسم الكيمياء الحيوية .

- 2012 -

الملخـــــص العـــــــربى

"الدراسه البيوكيميائيه لمرضى الكبد الدهنى الغير كحولى وعلاقته بمرض حصى المرارة عند المرضى الليبيين"

- يعتبر مرض الكبد الدهنى الغير كحولى أحد حالات مقاومه الانسولين فى الكبد ، أى أنه هو أحد مكونات المتلازمه الأيضيه .
- ومرض حصى المراره هو أحد المشاكل الجراحيه الرئيسيه لدى المرضى الليبيين والتى تستدعى غالبا التدخل الجراحى .
- وهذه المشكله فى الغالب تكون لها علاقه بالسمنه المفرطه وأحتشاء الشريان التاجى أضف على ذلك طبيعه الأكل وبالأخص اللحوم الحمراء والدهون الحيوانيه والتى تحتوى على كميات كبيره من الكوليسترول والأحماض الدهنيه المشبعه .
- تهدف هذه الدراسه للتعرف عن كيفيه وجود مرض الكبد الدهنى الغير كحولى وكذلك تحديد مدى علاقته بحصى المرارة عند المرضى الليبين .
- تضمنت هذه الدراسه 100 حاله مرضيه حيث تم تحديدها من ناحيه العمر والجنس وايضا وجود أو عدم وجود مرض الكبد الدهنى الغير كحولى وذلك بمستشفى السابع من أكتوبر بمدينه بنغازى فى الفتره من يناير 2008 م وحتى ديسمبر 2009 م .
- كما تضمنت الدراسه أيضا 100 حاله غير مرضيه (حاله دليل) من ضمن المترددين على المستشفى لعمل بعض الفحوصات الروتينيه منهم 50 حاله من الذكور (أعمارهم 50± 5 سنوات) و 50 حاله من اللإناث (أعمارهم 48 ± 5) .
- تبين من خلال هذه الدراسه أنه 100 مريض قد تم تشخيصهم كمصابيين بمرض الكبد الدهنى الغير كحولى منهم 58 أناث و42 ذكور.
- ومن ضمن ال100 مريض وجد منهم عدد69 مريض الديهم ارتفاع فى معدلات الانسولين فى الدم (مقاومة الانسولين) حيث كان عدد الذكور 33 وعدد الاناث 36 .
- بالنسبه لمدى انتشار مرض حصى المراره بهذه الحالات المرضيه أى المائه مريض قد تم تحديده .
- وبشكل عام كانت نتائج تحليل الدهون لكافه الحالات غير طبيعيه .

- **وكان من خلال ال 100 مريض والذين يعانون من مرض الكبد الدهنى الغير كحولى حيث وجد منهم 33 مريض يعانون من مرض حصى المراره .**
- **ومن بين هؤلاء 33 مريض وجد عدد الذكور 10 وعدد الاناث 23 وبنسبه الإناث إلى الذكور 2.3:1 .**
- **تم إجراء العديد من التحاليل منها الدهون مثل الكوليسترول الكلى والكوليسترول قليل الكثافه وكذلك عالى الكثافه بالإضافه إلى الدهون الثلاثيه , كما تم تحليل نسبه السكر فى الدم والأنسولين , وحمض اليوريك وبعض الانزيمات الخاصه بأختبار وظائف الكبد.**
- **ومن خلال النتائج لوحظ ارتفاع فى نسبه الدهون بالدم وأيضا أرتفاع فى مقاومه الإنسولين بالإضافه إلى إنخفاض فى مستوى الكوليسترول العالى الكثافه أو مايسمى بالكوليسترول الحميد فى مرضى الكبد الدهنى الغير كحولى وهذه الملاحظه وجدت أيضا فى مرضى الكبد الدهنى المصاحب بحصى المراره .**
- **وقد تم مقارنه النتائج بالمجموعات الغير مرضيه (حاله الدليل) والمتضمنه فى هذه الدراسه.**
- **والنتائج يلاحظ عليها زياده مستوى حمض اليوريك لدى المرضى الذكور مقارنه بالمرضى الإناث.**
- **وتمت الملاحظه أيضا من خلال النتائج وبالرغم من انخفاض مستوى الكوليسترول العالى الكثافه بمرضى الكبد الدهنى الغير كحولى المصاحب بحصى المراره ومقارنته بالمجموعات الاخرى الغير مرضيه أو السليمه ولكن وجد معدله مرتفع بمرضى الكبد الدهنى الغير كحولى والمصاحب بحصى المراره مقارنه بمرضى الكبد الدهنى الغير كحولى والغير مصاحب بحصى المراره وهذه الملاحظه لوجظت فى حالات الذكور والاناث معا .**
- **لوحظ بهذه الدراسه ان مرضى الكبد الدهنى الغير كحولى معرضون للإصابه بحصى المراره ويتضح هذا من خلال دراسه حالات مرضى الكبد الدهنى الغيركحولى وعلاقته بالمرضى الذين يعانون من مرض حصى المرارهولهذا يمكن القول بأن مرضى الكبد الدهنى الغير كحولى قد يكون عامل خطر يؤدى للإصابه بحصى المراره فى المرضى الليبين .**
- **وللتأكد من الإصابه بالكبد الدهنى الغير كحولى فى المرضى الذين يعانون من حصى المراره فمن الممكن أخذ عينه من نسيج الكبد وذلك بعد استئصال المراره وهذا يؤدى**

إلى المساعده فى تشخيص وجود حاله التهاب الكبد الدهنى الغير كحولى للحالات المرضيه .

- **أيضا أرتفاع نسبه الدهون فى الدم و بالاضافه إلى السمنه وأزدياد الوزن يعتبر مؤشر للحاجه إلى تقييم أحتماليه أصابه مرضى حصى المراره بأمراض القلب والاوعيه الدمويه .**

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