INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) includes a variety of histological conditions (ranging from liver steatosis and steatohepatitis, to fibrosis and hepatocellularcarcinoma) that are characterized by an increased fat content within the liver. The accumulation of fat within the liver is essential for diagnosis of NAFLD and might be associated with alterations in the hepatic and systemic inflammatory state ***(Braunersreuther et al., 2012)*.**

NAFLD is regarded as a hepatic manifestation of metabolic syndrome (MS), and patients with NAFLD, particularly those with NASH, often have one or more components of the MS: obesity, hypertension, dyslipidemia and raised fasting plasma glucose levels or overt type 2 diabetes (T2DM) ***(Sanja et al., 2014).***

Finding a means to noninvasive diagnosis of NAFLD and its entities has been the aim of many research efforts since recently, and seems to remain a very much needed goal among many clinicians and researchers in the field of hepatology ***(Barsic et al., 2012).***

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological activities in immune regulation, hematopoiesis, inflammation and oncogenesis ***(Kishimoto, 2010).***

The role of IL-6 in liver pathology is very complex, and its participation in the development of NAFLD remains unclear. IL-6 activates several cells, such as immune cells, hepatocytes, hematopoietic stem cells, and osteoclasts. Furthermore, IL-6 has a wide range of biological functions, including induction of inflammation and oncogenesis, regulation of immune response, and support of hematopoiesis ***(Kishimoto, 2010).***

AIM OF THE WORK

The aim of the present study is to assess the correlation between serum levels of the Interleukin-6 and the severity of liver affection in non-alcoholic fatty liver disease.

NON ALCOHOLIC FATTY LIVER DISEASE

Definition:

Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity, insulin resistance, and dyslipidemia. Two broad types are recognized- simple steatosis is typically stable while non alcoholic steatohepatitis is characterized by significant cell injury and the potential for progression to cirrhosis ***(Caldwell and Argo, 2011).***

Hepatic steatosis describes the accumulation of fat, mostly as triglyceride, cholesterol and phospholipids, in excess of 5-10% of liver weight ***(Caldwell and Argo, 2011).***

The term 'NASH' was introduced by Ludwig and collegues (1980) describing the lesion in various degrees of severity in patients without significant ethanol exposure. The subsequent observation that NASH could progress over years to cirrhosis was followed by others showing that NASH is a common cause of 'cryptogenic' cirrhosis, which accounts for 10-20% of all cirrhosis. These issues came into sharper focus with recognition that NAFLD exists as a spectrum, from simple steatosis without evidence of cell injury, which tends to be stable over time, to steatohepatitis, which may progress to cirrhosis. In later stages, NASH-related cirrhosis loses diagnostic fatty infiltration and may have the appearance of 'cryptogenic' cirrhosis ***(Caldwell and Argo, 2011).***

The term 'NAFLD' was introduced as a means of grouping all of the variants under one broad term. The names NAFLD and NASH have become established. NASH remains, by definition, a clinical-Pathological diagnosis requiring both exclusion of ethanol as a major contributor, and the presence of cell injury, as evidenced by cellular ballooning, focal necrosis, fibrosis and inflammation **(*Baumeister et al., 2008).***

Incidence of NAFLD

1-Epidemiology of NAFLD:

NAFLD has become a global epidemic, affecting 20–40% of the general adult population ***(Marchesini et al., 2011)***. The annual incidence of NAFLD in prospectively followed adult populations is estimated to be about 3-5% ***(Lazo and Clark, 2008).***

The prevalence is significantly influenced by ethnicity and familial factors. It can also occur in non-obese patients most of whom are physically deconditioned and thus can be said to be metabolically obese ***(Caldwell and Argo, 2011).***

Fatty liver has been documented in up to 10% to 15% of normal individuals and 70% to 80% of obese individuals. Obesity has been reported in 70 to 100 % of cases of NASH ***(Duvnjak et al., 2007)***. A study in Japan showed that approximately 20 to 25% of diabetic patients showed NAFLD ***(Okanou et al., 2011).***

Among patients with hyperlipidaemia, at least two-thirds with hypertriglyceridaemia and one-third with hypercholesterolaemia have fatty liver by ultrasound imaging ***(Assy et al., 2000).***

Although earlier studies found higher prevalence of NASH in women, other studies have shown that NASH occurs with equal frequency in both sexes ***(Duvnjak et al., 2007).***

Average age for NASH patients is 40-50 years and for NASH-related cirrhosis is 50-60 years. However, the emerging obesity epidemic has resulted in increasing numbers of children with this disease sometimes with advanced fibrosis. About 20% of patients report a family history of unexplained liver disease ***(Schwimmer et al., 2003).***

2-Ethnic variations in NAFLD:

The relationships between obesity, diabetes, hyperlipidaemia and NAFLD are influenced by ethnicity. People of African-American descent have significantly less hepatic steatosis in spite of a relatively high prevalence of obesity and diabetes ***(Caldwell et al., 2002).*** In contrast, people of Hispanic-American descent have a higher prevalence, while those of primarily Northern European and Asian-American descent have an intermediate prevalence of steatosis ***(Weston et al., 2005).***

Although there is a constant relationship between liver triglyceride content and intraperitoneal fat (visceral adiposity) within ethnic groups, there is a substantial degree of dissociation

between insulin resistance and both steatosis and visceral adiposity in some ethnic groups ***(Guerrero et al., 2009).*** A possible genetic basis for ethnic variation has been identified ***(Romeo et al., 2008).***

3-Familial associations:

Familial clustering of NASH and NAFLD could represent inherited genetic predisposition or common environmental factors such as dietary habits or activity levels ***(Abdelmalek et al., 2006).***

Consistent with a strong familial component, there is a high prevalence of NAFLD among the adult relatives of affected children ***(Schwimmer et al., 2009).***

Causes

In the great majority of cases, NAFLD arises in association with one or more features of the metabolic syndrome ***(Olufadi and Byrne, 2008).***

Other causes of steatosis which should be considered include nutritional causes, e.g. rapid weight loss and total parenteral nutrition, and drug-induced steatosis. Commonly implicated agents include glucocorticoids, amiodarone, synthetic oestrogens and highly active antiretroviral drugs (HAART) (Table 1) **(*Preiss and Sattar, 2008)*.**

Steatosis can also be seen in a variety of inherited metabolic diseases including Wilson's disease, and childhood

disorders such as glycogen storage diseases and the lipid storage diseases ***(Caldwell and Argo, 2011).***

The distribution of fat (predominantly in reticulo-endothelial cells) and typical presentation in infancy (although not exclusively so) distinguish the lipid storage disorders from NAFLD/NASH ***(Caldwell and Argo, 2011).***

Table (1): Causes of secondary NAFLD ***(Caldwell and Argo, 2011).***

Specific conditions associated with fatty liver
• Lipodystrophies Wilson's disease
• Primary mitochondrial diseases
Bariatric surgery
• Jejunoileal bypass (no longer used)
Nutrition related disorders
• Total parenteral nutrition Coeliac sprue
Medications
• Amiodarone Methotrexate
• Nucleoside analogues (HAART, chemotherapy agents)
• Tamoxifen
Toxins
• Carbon tetrachloride (CCI4)—fire extinguishers, refrigerants, dry cleaning pre-1940
• Ethyl bromide (EtBr)—organic chemistry solvent
• Perchloroethylene (C2CI4)—dry cleaning, degreasing in automotive uses, paint stripping
• Various petrochemicals

Classification of NAFLD

The major indicators of injury have been incorporated into a score commonly called the NAFLD Activity Score (NAS) and staging system of fibrosis, which is being used in most clinical trials ***(Kleiner et al., 2005).***

The following features have also been used to divide NAFLD into four types:

(1) Simple steatosis.

(2) Steatosis with inflammation alone.

(3) Steatosis with inflammation and ballooning.

(4) Steatosis with inflammation and fibrosis.

In general, the latter two types constitute NASH (because ballooning and fibrosis usually parallel each other and are typically associated with inflammatory infiltrates). The first two types can be grouped into non-NASH fatty liver (NNFL) (table 2) ***(Caldwell and Argo, 2011).***

Table (2): Working classification of non-alcoholic fatty liver disease

NAFLD:
Type 1 NAFLD: Steatosis with no inflammation or fibrosis
Type 2 NAFLD: Steatosis with non-specific lobular inflammation but absent of fibrosis or hepatocyte ballooning
NASH:
Type 3 NAFLD: Steatosis with inflammation and fibrosis of variable levels
Type 4 NAFLD: Steatosis, inflammation, hepatocyte ballooning, and fibrosis or Mallory-Denk bodies

(Caldwell and Argo, 2011)

Risk factors for NAFLD:

1-Obesity

Obesity is a state of an excess body fat that causes increased risk of metabolic derangement (***Park and Jeon, 2010).*** In severely obese patients (usually defined as body mass index**"BMI"** >35kg/m^2), the prevalence of steatosis is over 90% from series of patients undergoing bariatric surgery **(*Machado et al., 2006).***

Hepatic steatosis develops when the rate of hepatic fatty acid input (uptake and synthesis) exceeds the rate of fatty acid output (oxidation and secretion) ***(Fabbrini et al., 2010).*** Insulin resistance in liver, adipose tissue, and skeletal muscle are directly related to intrahepatic triacylglycerol content. However, it still remains a matter of debate whether insulin resistance is a cause or consequence of NAFLD ***(Maher et al., 2008)*.**

Adipose tissue inflammation and unfavourable adipokine secretion are enhanced in individuals with NAFLD compared to individuals with normal intrahepatic triacylglycerol content ***(Kolak et al., 2007)***, indicating that inflammatory factors released by the adipose tissue could be involved in the pathogenesis of NAFLD and hepatic insulin resistance ***(Tsochatzis et al., 2009).*** Liver macrophages were reported to be increased in number and to promote hepatic steatosis and insulin resistance in obesity. Chemokines such as monocyte chemoattractant protein-1 (MCP-1) contribute to obesity-induced recruitment of macrophages to the liver ***(Lanthier et al., 2009)*.**

Obesity modulates inflammation and activation of immune pathways which can affect hepatic lipid metabolism leading to hepatic injury, NASH and fibrosis ***(Valenti et al., 2009).***

2-Metabolic syndrome (MS)

In the great majority of cases, NAFLD arises in association with one or more features of the MS, namely insulin resistance, glucose intolerance or diabetes, central obesity, dyslipidaemia and hypertension **(*Olufadi and Byrne, 2008).*** Criteria for clinical diagnosis of MS are shown in table (3). Approximately one third of the population worldwide has the metabolic syndrome. The prevalence increases with age ***(Grundy, 2008)***. NAFLD is considered to be the hepatic component of metabolic syndrome ***(Tarantino et al., 2009)***.

Table (3): National Cholesterol Education Program: Adult Treatment Program III (NCEP ATP-III) Guidelines—metabolic syndrome components

Risk factor	Defining level
Abdominal obesity	
Men	>102 cm (> 40 inches)
Women	>88 cm (> 35 inches)
Triglycerides	≥150 mg/dl
HDL cholesterol	
Men	<40 mg/dl
Women	<50 mg/ dl
Blood pressure	> 135/85 mg/dl
Fasting blood glucose	≥100 mg/dl

Reaching the defining level for any three of the parameters satisfies the clinically applicable definition of metabolic syndrome **(Grundy et al., 2004).**

A simple laboratory test was designed to evaluate the insulin profile. It is known as homeostasis model assessment (HOMA), and is defined as the fasting insulin level (mU/L) multiplied by the fasting glucose level (mg/dl) and divided by 405 **(Tarantino et al., 2009).** Insulin resistance can be demonstrated in the liver, skeletal muscle and adipose tissue. Indeed, resistance is likely to result from systemic lipotoxicity (Table 4) ***(Taylor, 2004).***

Table (4): Key sites of insulin resistance

Tissue	Dysfunction
Adipose	Failure to suppress hormone sensitive lipase, activates release of free fatty acids from triglyceride stores
Liver	Failure to suppress glucose production/release from glycogenolysis and gluconeogenesis
Muscle	Failure in glucose uptake due to: ➢ decreased translocation of GLUT-4 transporter ➢ increased myocyte lipid stores ➢ impaired mitochondrial function

(Caldwell and Argo, 2011)

Most patients also have impaired exercise tolerance (measured by oxygen consumption during graded exercise). These findings are consistent with 'metabolic obesity' even in those with relatively low BMI ***(Wildman et al., 2008).***

3- Genetic and environmental factors

Studies of familial associations together with studies of ethnic variations, suggest a strong genetic component in the development of both NAFLD in general, and NASH in particular, although it remains to be seen whether the pattern of severity also follows predictable patterns ***(Caldwell and Argo, 2011).*** Candidate genes include genes determining the magnitude and pattern of obesity, genes determining insulin sensitivity, genes involved in hepatic lipid storage, genes involved in fatty acid oxidation, cytokines genes, genes influencing oxidative stress and genes encoding proteins involved in response to oxidative stress ***(Wedemeyer and Micheal, 2003).***

Pathogenesis of NAFLD and NASH:

Although the mechanism of hepatocellular injury in NASH can be encapsulated in the 'two hit' hypothesis (accumulation of fat followed by oxidative injury), it is increasingly evident that the mechanism involves a complex cascade of events leading to hepatocellular injury, most evident as cellular ballooning and cell death. Studies of the ballooned cell from various perspectives lead to an emerging concept of 'multiorganelle' failure, with impairment of critical organelles and inadequate compensatory pathways. These pathways are both driven by and contribute to systemic abnormalities, especially related to insulin resistance and disturbed energy homeostasis ***(Caldwell and Argo, 2011).***

1- **Mechanism of steatosis:** Lipid accumulation in the liver results from an imbalance between overall calorie intake and systemic caloric utilization characteristic of the metabolic syndrome. Hepatic fat results from several possible mechanisms including synthesis of new fatty acids, uptake of circulating free fatty acids, or uptake of diet-derived chylomicron. Liver fat can be disposed of by either oxidation or lipoprotein secretion. NAFLD appears to be driven especially by Non-esterified fatty acids (NEFA) uptake, de novo lipogenesis and altered lipid export ***(Caldwell and Argo, 2011)***. There are also two major pathways of fatty acid disposal: mitochondrial β-oxidation to ATP and ketone bodies, and secretion into blood as triglyceride in very low-density lipoprotein (VLDL). Disturbances in these processes can be inherited or acquired, resulting in accumulation of triglycerides in the liver ***(Neuschwander-Tetri, 2006).***

2- **Regulation of lipid synthesis:** Within the hepatocyte, lipid stores are primarily regulated by two main transcription factors: sterol regulatory element binding protein (SREBP), governed by insulin and dietary fatty acids, and carbohydrate response element binding protein (CREBP), governed by ambient glucose levels ***(Tamura and Shimomura, 2005*)**. SREBP and CREBP stimulate nuclear transcription of the enzymes responsible for fatty acid synthesis and subsequently their esterification into

triglyceride, stored as triacylglycerides within cytosolic fat droplets, or exported as VLDL ***(Caldwell and Argo, 2011).***

3- **Steatosis in humans:** The high burden of NEFA appears to derive predominantly from visceral fat ***(Villanueva et al., 2009).*** Compared to normal, de novo lipogenesis is increased from 5 to 25% and may also be a source of additional oxidative stress ***(Musso et al., 2008).*** Opposing the accumulation of liver fat, VLDL secretion is increased in NAFLD but plateaus at a hepatic triglyceride content of 10%, indicating limited compensation for high circulating NEFA ***(Fabbrini et al., 2008)***.

4- **Mitochondrial dysfunction:** Mitochondrial morpho-logical changes are readily evident in human NASH and include swelling and intramitochondrial crystals ***(Caldwell et al., 2009).*** Electron transport chain activity is reduced to 40-70% of normal in human NASH **(*Perez-Carrera et al., 2003)***. A clearer consensus exists regarding changes in mitochondrial permeability, leading to release of mitochondrial cytochrome c and apoptosis signalling ***(Ricciet et al., 2003).*** Increased mitochondrial cholesterol has been proposed as a mechanism contributing to mitochondrial dysfunction and associated changes in permeability ***(Caballero et al., 2009)***.

5- **Lipid composition in NAFLD:** Fat composition studies in NAFLD showed that most of the stored lipid is composed of triglyceride, with a lesser component of free fatty acids, although both were significantly increased compared to