Abstract

NAFLD is recognized as a leading cause of liver diseases wide world. It ranges from simple steatosis without fibrosis to non-alcoholic steatohepatitis (NASH) with varying stages of fibrosis and NASH-related cirrhosis.

Liver biopsy is the gold standard means to evaluate fibrosis in NAFLD. However, it cannot be used as a routine screening tool to detect or monitor fibrosis progression in NAFLD due to its invasive nature and its rare potential risks of death and sampling variability.

aLiver biopsy samples were taken from each patient after informed consent and then staged according to the biopsy findings. Calculation of the non-invasive scoring systems were done for each patient and compared to the biopsy stage.

After comparing the noninvasive fibrosis scores with the results of liver biopsy, 36 patients out of 40 (according to NFS) and all patients (according to FIB-4 and BARD score) were correlated with the results of liver biopsy.

Keywords: Noninvasive Scoring Systems, Liver Biopsy, NAFLD Patients

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a condition where there's excessive accumulation of fat in form of triglycerides (TGs) in liver ***(LaBrecque et al., 2014).***

NAFLD is primarily caused by obesity, type 2 Diabetes Mellitus (DM), dyslipidemia and insulin resistance. Secondary causes, like nutritional diseases, metabolic or genetic disorders and medications were also incriminated; however they are less common but important ***(Kneeman et al., 2012).***

Although chronic liver diseases prevalence has remained the same or decreased, NAFLD prevalence has doubled during the last 20 years. NAFLD was confirmed to play an important role in the Middle East, Far East, Africa, the Caribbean and Latin America ***(LaBreque et al., 2014).***

The prevalence of NAFLD in the adult general population ranges from 10% to 24% worldwide and from 57.7% to 74% in obese people. NAFLD accounts for 90% of cases with elevated liver function tests, after exclusion of common hepatic disorders (e.g., viral hepatitis, alcoholism, inherited liver disease, or medications) ***(Obika and Noguchi, 2011).***

Patients with NAFLD are more likely to die prematurely than their matched controls from cardiovascular and end stage liver diseases and hepatocellular carcinoma ***(Tapper et al., 2014).*** So it's essential to identify patients with advanced fibrosis in the course of NAFLD. Till now the Gold Standard tool for diagnosis and assessment of liver fibrosis is biopsy ***(Cichoz-Lach et al., 2012).*** Biopsy may not be suitable for many of those patients; so other non-invasive tests depending on simple clinical and laboratory tests have been proposed to detect fibrosis in NAFLD patients as:

- FIB-4 index (age × AST) / (platelet × √ALT) ***(McPherson et al., 2010).***
- NAFLD fibrosis score [-1.675 + 0.037 × age + 0.094 × BMI + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet – 0.66 × albumin] ***(McPherson et al., 2010).***

BARD score (weighted sum of BMI > 28 = 1 point, AST/ALT ratio > 0.8 = 2 points, diabetes = 1 point) ***(McPherson et al., 2010)***.

AIM OF THE WORK

The aim of this study is to evaluate the accuracy of non-invasive scoring systems versus liver biopsy in staging of fibrosis in patients with NAFLD.

NON-ALCOHOLIC FATTY LIVER DISEASE OVERVIEW

Epidemiology of NAFLD

In the United States the prevalence of NAFLD is estimated to be 34%, in Europe it is estimated to be 25%. In Asia it seems to be equivalent to the West with reported rates about 30%, there are few data on NAFLD prevalence in the Middle East and Africa. Studies reported 20% for Sudan and 16.6% for Saudi Arabia ***(Demir et al., 2015).***

It was expected, by the year 2015, that the number of overweight subjects exceeds 2.3 billion. More than 20% of the Western population, 60% diabetic individuals, and 90% morbidly obese patients will present steatosis. Furthermore, up to 15% of the Western population, 25%-30% of subjects with either obesity or type-2 diabetes mellitus, and over 35% of the morbid obese individuals will develop NASH ***(Ferolla et al., 2015).*** Over a follow-up of 10–15 years, about 20% of patients with fatty liver will progress to NASH and about 10% of patients with NASH will progress to cirrhosis. For patients with NASH and cirrhosis, the 10-year liver-related mortality is about 15% and all-cause mortality about 20%. The mortality for NASH with cirrhosis approximates that of chronic hepatitis C with cirrhosis ***(Yatsuji et al., 2009).*** 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).***

Unfortunately, obesity affects all ages, and childhood NAFLD has become a serious problem affecting over 70% of obese children and adolescents. The progression to NASH and/or cirrhosis may have a much higher impact on younger individuals, ultimately necessitating liver transplantation at very young ages. Indeed, NASH cirrhosis has been reported in children as young as 8 years of age ***(Darwish and Metselaar, 2015).***

Racial and ethnic differences have been reported in the prevalence of NAFLD, where it is most common in East Asian Indians, followed by Hispanics, Asians, Caucasians, and less frequently in African Americans ***(Macaluso et al., 2015).***

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

Etiology of NAFLD

NAFLD is subdivided into primary and secondary types. The primary type results from insulin resistance, and thus frequently occurs as part of the metabolic changes that accompany obesity, type 2 diabetes and dyslipidemia ***(Angulo et al., 2007).*** The secondary type can be associated with the use

of certain medications and a variety of miscellaneous disorders that include infectious, nutritional, and inborn errors of metabolism (Table 1) ***(Abdelmalek and Diehl, 2007).***

Table (1): Secondary causes of NAFLD.

1. Genetic / Inborn errors of metabolism ▪ Abetalipoproteinemia. ▪ Galactosemia. ▪ Hypoproteinemia. ▪ Familial combined hyperlipidemia. ▪ Wilson's disease. ▪ Glycogen storage disease. ▪ Weber Christian disease. ▪ Lipodystrophy.	**2. Drugs and Toxins** ▪ Amiodarone. ▪ Methotrexate. ▪ Tamoxifen. ▪ Glucocorticoids. ▪ Estrogen. ▪ Organic solvents. ▪ Phosphorus. ▪ Highly Active Antiretroviral Therapy.
3. Surgical ▪ Jejunoileal bypass. ▪ Gastric bypass. ▪ Biliopancreatic diversion. ▪ Extensive small bowel resection.	**4. Nutritional** ▪ Total parentral nutrition. ▪ Starvation and cachexia. ▪ Protein calorie malnutrition. ▪ Inflammatory bowel disease. ▪ Jejunal diverticulosis with bacterial overgrowth.
5. Others ▪ Celiac disease and hepatitis virus.	

(Kneeman et al., 2012)

Classification of NAFLD

NAFLD is classified into two categories*:* non-NASH fatty liver (NNFL), in which only steatosis or steatosis with inflammation are observed, and non-alcoholic steatohepatitis (NASH), in which, in addition to steaosis, lobular inflammation, ballooning and liver cell injury are observed (Table 2) ***(Tzeng et al., 2015).***

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

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

(Tzeng et al., 2015)

Pathogenesis of NAFLD and NASH

Different theories have been formulated, leading initially to the 'two hits hypothesis. The "first hit" is thought to be lipid hepatic accumulation secondary to sedentary lifestyle, high fat intake, obesity and insulin resistance. Further hepatic insults act as "second hit". Recently, this view is evident to be too simple to summarize the nature of human NAFLD. Consequently, a multiple-hit hypothesis is now used to explain the pathogenesis and the progression of NAFLD ***(Leamy et al., 2013)*.**

Multiple hit hypothesis-an overview

Dietary, environmental and genetic factors can contribute to the development of insulin resistance, which in turn results in development of NAFLD/ NASH, increase de novo lipogenesis (DNL), impaired inhibition of adipose tissue lipolysis causing increased fatty acid flux to the liver and finally, adipose tissue dysfunction releasing adipokines and inflammatory cytokines. Fat accumulates in liver in form of TGs with increased lipotoxicity and mitochondrial dysfunction with oxidative stress producing reactive oxidative stress (ROS) and endoplasmic reticulum (ER) stress ***(Cusi, 2009).***

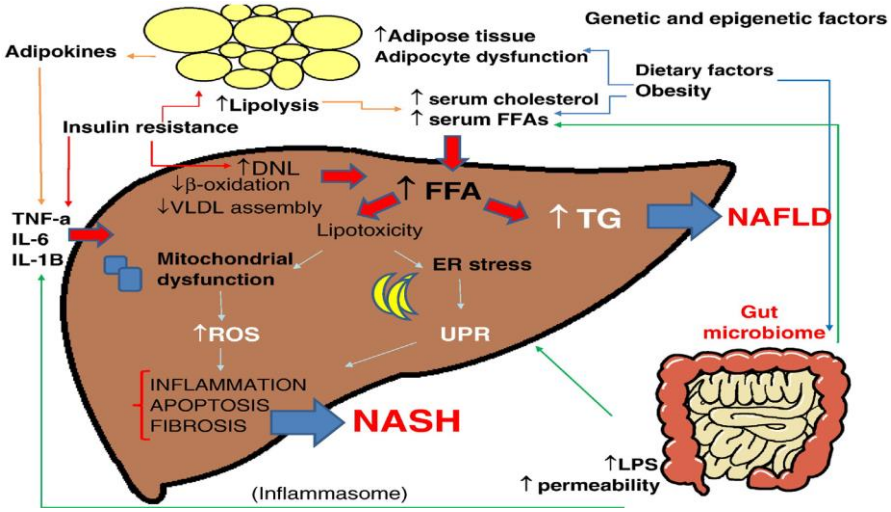

Figure (1): Multiple hit hypothesis for the development of NAFLD. LPS: lipopolysaccharides, TNF-a: tumor necrosis factor alpha, IL-6: interleukin-6, IL-1B: interleukin 1B, ROS: reactive oxidative stress, DNL: de novo lipogenesis, FFA: free fatty acids, ER stress: endoplasmic reticulum stress, TG: triglycerides, NASH: non-alcoholic steatohepatitis, UPR: un-folded protein response, VLDL: very low density lipoproteins ***(Buzzetti, et al., 2016).***

DIAGNOSIS AND TREATMENT OF NAFLD

An important key feature to make the diagnosis of NAFLD is the exclusion of ethanol exposure as a significant factor. This remains controversial, with little agreement between studies as to whether this means total abstinence or consumption below a threshold level ***(Dunn et al, 2008)*.** Some studies limit the use of alcohol per day <20 g (2.5 units) in women and <30 g (3.75 units) in men ***(Sattar et al., 2014)***.

Laboratory Evaluation

- **AST and ALT**

Approximately 80% of patients with NAFLD have liver function tests in normal ranges; only a small proportion exhibits mild elevation of aminotransferases (not exceeding four folds the upper limit of normal) ***(Adams and Angulo, 2007).*** The (AST / ALT) is predictive for the severity of the liver disease. It's < 1 when there's no or minimal fibrosis and > 1 suggesting cirrhosis or advanced fibrosis ***(Oh et al., 2008).***

- **Gamma-glutamyltransferas (GGT)**

It is frequently elevated in patients with NAFLD, and it has been reported to be associated with increased mortality ***(Ghouri et al., 2010).***

- **Tumor necrosis factor alpha (TNF-α)**

Increased level is correlated with the severity of inflammation and fibrosis ***(Obika and Noguchi, 2011).***

- **Adiponectin**

A significant decrease is observed in patients with early-stage NASH ***(Obika and Noguchi, 2011).***

- **Type IV collagen 7S domain and HA**

Both are used as potential fibrosis biomarkers ***(Obika and Noguchi, 2011).***

- **Cytokeratin-18**

It's markedly increased in patients with NASH and indicator of apoptosis ***(Obika and Noguchi, 2011).***

- **IL-6**

Plasma levels of IL-6 vary in proportional with the hepatic concentration and indicate inflammatory activity and the degree of fibrosis ***(Wieckowska et al., 2008).***

Scoring Systems

Although the liver biopsy is considered the gold standard test for diagnosis of liver fibrosis in NAFLD patients, it can't be used routinely due to its invasiveness carrying a very rare risk of death and sample variability. So, some non-invasive fibrosis scoring systems were formulated include the FIB-4 index, NAFLD fibrosis score (NFS) and BARD score ***(Sun et al., 2016).***

NAFLD Fibrosis Score (NFS)

It's generated using a panel including six variables of age, hyperglycaemia, BMI, platelet count, albumin, and *AST/ALT* ratio (AAR).

NAFLD fibrosis score [-1.675 + 0.037 × age + 0.094 × BMI + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet – 0.66 × albumin] ***(Cichoz-Lach et al., 2012).***

Two cutoff values were proposed, by ***Angulo et al. 2009***, to classify the probability of advanced fibrosis as:
NAFLD score is < -1.455 (proved to have no or mild fibrosis) or > 0.676 (proved to have advanced fibrosis) ***(Treeprasertsuk et al., 2013).***

BARD Score

It's simple scoring system, formulated by Harrison et al at 2006, which can be used as a predictive tool in assessing fibrosis in patients with NAFLD. It consists of three variables; the BMI, AAR, and the presence of diabetes. BARD score = weighted sum of (BMI > 28 = 1 point, AST / ALT ratio > 0.8 = 2 points, diabetes=1 point) ***(Raszeja-Wyszomirska et al., 2010).***

BARD Score is less than 2 (proved to have no or mild fibrosis) or 2 – 4 points (proved to have advanced fibrosis) ***(Raszeja-Wyszomirska et al., 2010)***.

FIB-4 Index

It's a score that was developed to stage liver disease in patients with HIV – HCV co-infection. Also, it has been validated in patients with HCV infection alone. Its components are the age with three biochemical values; the platelet count, ALT, and AST, to detect fibrosis. FIB-4 has showed a better accuracy in diagnosing advanced fibrosis in NAFLD patients when compared to other simple noninvasive tests in several studies. FIB - 4 index = (age × AST) / (platelet × √ALT) ***(Shah et al., 2009).***

Fib4 score is < 1.30 (proved to have no or mild fibrosis) or > 2.67 (proved to have advanced fibrosis) ***(Martinez et al., 2011).***

AST / ALT Ratio (AAR)

The AST/ALT ratio is a simple noninvasive test that could be widely available and could be used in primary care, although data in NAFLD patients still scarce ***(Sorbi et al., 2009)*.**

Aspartate Aminotransferase to Platelet Ratio Index (APRI)

APRI has been designed to detect significant fibrosis ≥F2 and cirrhosis in patients with chronic liver disease including NAFLD patients ***(Yilmaz et al., 2011)***.

Enhanced liver fibrosis (ELF)

It consists of serum markers as hyaluronic acid, aminoterminal propeptide of type III collagen, tissue – inhibitor of matrix –metlaoproteinase– 1. ELF panel in NAFLD / NASH population, had good performance in distinguishing severe fibrosis (stages 3-4), but low performance for moderate fibrosis and absence of fibrosis ***(Guha et al., 2008).***

Fibro-Test (FT)

It consists of serum markers as alpha 2 - macroglobulin, apolipoprotein A1, haptoglobin, GGT, and bilirubin. It has shown a high diagnostic accuracy for the staging of fibrosis in NAFLD patient ***(Festi et al., 2013).***

Imaging

Ultrasound

It is the most common and less invasive imaging technique used for NAFLD diagnosis even in asymptomatic patients with elevated liver enzymes. Ultrasound has sensitivity of 80%, a specificity of 99% and a negative predictive value of 96% for diagnosing NAFLD ***(Khov et al., 2014).*** The sensitivity of ultrasonography decreases in morbid obesity, because ultrasonography examination is difficult to perform in such circumstances and being operator depended limits its use ***(Wieckowska et al., 2008)***.

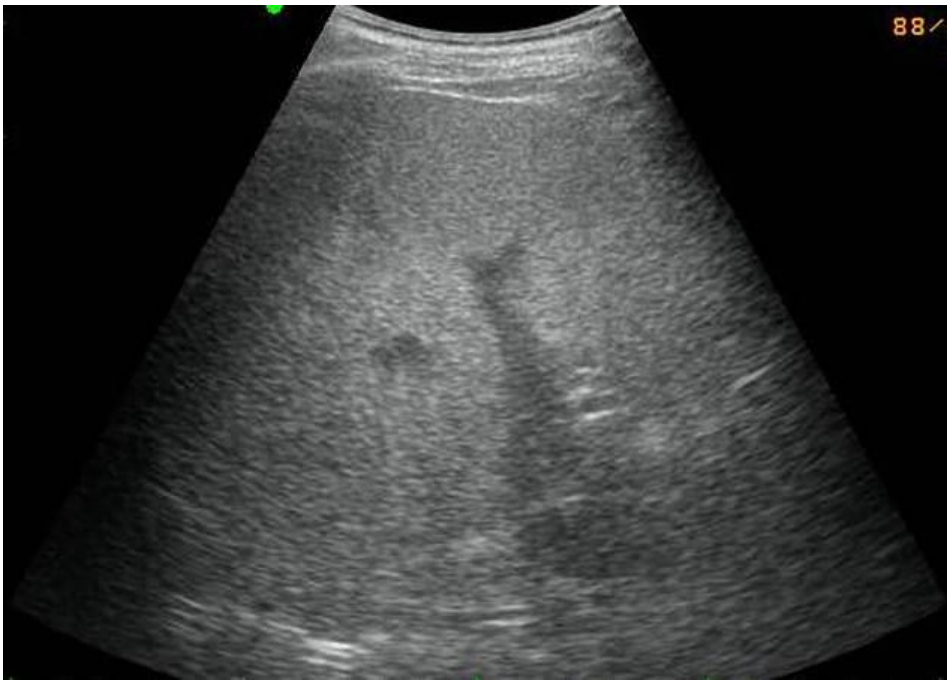

Figure (2): Diffuse fat accumulation in the liver at US ***(Khov et al., 2014).***