بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / مني مغربي أحمد

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic hepatic diseases in many developed countries. It is also a serious health problem all over the world ***(Sumida et al., 2014).***

NAFLD is an important cause of morbidity and mortality worldwide and is rapidly becoming the leading cause of end-stage liver disease and liver transplant ***(Carr et al., 2016).*** It is also the most common liver disorder in Western countries, affecting 17–46% of adults, with differences according to the diagnostic method, age, sex and ethnicity ***(Vernon et al., 2011).*** It parallels the prevalence of Metabolic syndrome and its components, which also increases the risk of more advanced disease, both in adults and in children ***(Younossi et al., 2012).***

Metabolic syndrome is not only highly prevalent in patients with NAFLD, but its components also increase the risk of developing NAFLD ***(Fujioka et al., 2015).*** Therefore, for instance, improving insulin resistance may reduce the incidence of NAFLD and NASH ***(Ratziu et al., 2010).***

NAFLD covers a wide spectrum of histological lesions ranging from simple steatosis to Non-alcoholic steatohepatitis (NASH) with/without fibrosis, cirrhosis, and hepatocellular carcinoma ***(Younossi et al., 2016).***

Approximately 50% of NASH patients have complications such as diabetes mellitus, cardiovascular disease and hyperlipidemia ***(Lazo et al., 2011).***

Survival is lower in patients with NASH based on the findings from long-term longitudinal studies ***(Ekstedt et al., 2006; Soderberg et al., 2010).***

It is therefore imperative to distinguish simple steatosis from NASH to provide risk stratification and intervention slowing down disease progression for patients with the latter condition ***(Wieckowska et al., 2007).***

Liver biopsy is currently the most reliable approach for identifying the presence of steatohepatitis (SH) and fibrosis in patients with NAFLD, but it is generally acknowledged that biopsy is limited by cost, sampling error, and procedure-related morbidity and mortality. Therefore, there has been significant interest in developing clinical prediction rules and non-invasive biomarkers targeting to replace liver biopsy or identify candidates for liver biopsy ***(Chalasani et al., 2018).***

Imaging studies such as ultrasonography, computed tomography (CT), and magnetic resonance imaging have been used to diagnose NAFLD ***(Rotman et al., 2010).***

These modalities have the advantage of being noninvasive and can be repetitively performed over a while ***(Rotman et al., 2010).***

Nevertheless, none of them have sufficient sensitivity and specificity for staging the disease and cannot distinguish between simple steatosis and NASH with or without fibrosis ***(Wieckowska et al., 2007).***

Several biomarkers that are associated with inflammation and apoptosis have been individually studied for their performances in distinguishing NASH from simple steatosis and through the use of genome- wide association studies, several genetic factors have recently been shown to be associated with NAFLD ***(Pearce et al., 2013).***

Thymosin Beta4 (Tβ4) is a G-actin sequestering peptide and is mainly related to the regulation of actin polymerization in living cells ***(Ballweber et al., 2002).*** It is involved in many critical biological processes, including apoptosis, cell migration, angiogenesis, fibrosis, and wound healing ***(Sosne et al., 2010).***

Aim of the Work

The aim of this study is to evaluate the role of Thymosin Beta 4 in the diagnosis of Nonalcoholic Fatty Liver and its relation to Metabolic syndrome in Egyptian patients.

Chapter 1

NONALCOHOLIC FATTY LIVER DISEASE AND NONALCOHOLIC STEATOHEPATITIS

Definition:

NAFLD is characterized by excessive hepatic fat accumulation, associated with insulin resistance (IR), and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a proton density fat fraction (providing a rough estimation of the volume fraction of fatty material in the liver) >5.6% assessed by proton magnetic resonance spectroscopy or quantitative fat/water selective magnetic resonance imaging (MRI) ***(EASL-EASD-EASO Clinical Practice Guidelines, 2016).***

NAFLD can be categorized histologically into nonalcoholic fatty liver (NAFL) or nonalcoholic steatohepatitis (NASH; Table 1). NAFL is defined as the presence of 5% hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning. NASH is defined as the presence of 5% hepatic steatosis and inflammation with hepatocyte injury (e.g., ballooning), with or without any fibrosis (AASLD, 2018).

The diagnosis of NAFLD requires the exclusion of both secondary causes and daily alcohol consumption 30 g for men and 20 g for women ***(Ratziu et al., 2010).***

NAFLD may be categorized as primary or secondary depending on the underlying pathogenesis. Primary NAFLD is associated with insulin resistance and metabolic syndrome. Other conditions associated with NAFLD are total parenteral nutrition, acute starvation, abdominal surgery (e.g., extensive small bowel resection, biliopancreatic diversion, and jejunal bypass), use of several drugs (e.g., amiodarone, tamoxifen, glucocorticoids, synthetic estrogens, diltiazem, aspirin, methotrexate, highly active antiretroviral therapy). It is also associated with hepatitis C, HIV, and metabolic disorders i.e., hypobetalipoproteinemia, lipodystrophy, hypopituitarism, hypothalamic obesity, Weber-Christian syndrome, acute fatty liver of pregnancy, Reyes syndrome, and Mauriac syndrome ***(Adams and Lindor, 2007; Duvnjak et al., 2007).***

Table (1): Nonalcoholic fatty liver disease and related definitions ***(AASLD, 2018)***

Nonalcoholic fatty liver disease **(NAFLD)**	Encompasses the entire spectrum of fatty liver disease in individuals without significant alcohol consumption, ranging from fatty liver to steatohepatitis and cirrhosis.
Nonalcoholic fatty liver **(NAFL)**	Presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes or no evidence of fibrosis. The risk of progression to cirrhosis and liver failure is minimal.
Nonalcoholic steatohepatitis **(NASH)**	Presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis. This can progress to cirrhosis, liver failure, and rarely liver cancer. NASH cirrhosis: Presence of cirrhosis with current or previous histological evidence of steatosis or steatohepatitis.
Cryptogenic cirrhosis	Presence of cirrhosis with no obvious etiology. Patients with cryptogenic cirrhosis are heavily enriched with metabolic risk factors such as obesity and metabolic syndrome.
NAFLD activity score **(NAS)**	An unweighted composite of steatosis, inflammation, and ballooning scores. It is a useful tool to measure changes in liver histology in patients with NAFLD in clinical trials.

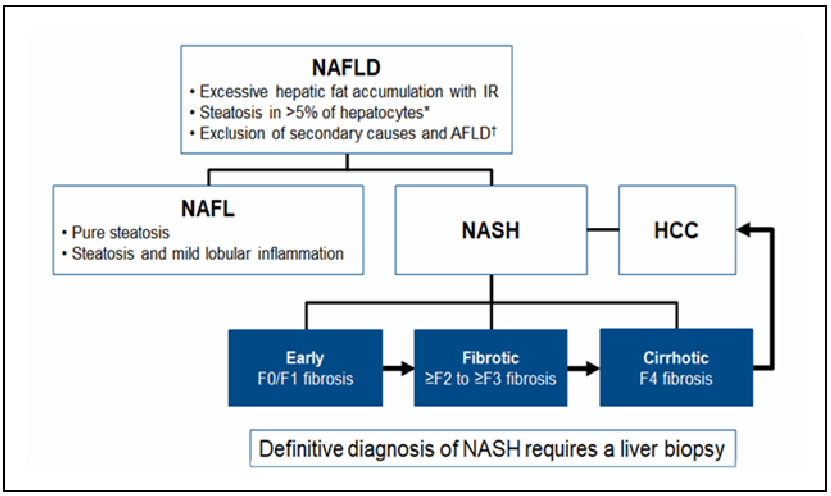

Figure (1): Definition of NAFLD, NAFL and NASH ***(EASL-EASD-EASO Clinical Practice Guidelines, 2016).***

Epidemiology

In 2016, the World Health Organization (WHO) estimated that more than 1.9 billion adults (39% of the adult population) were overweight, and 650 million (13% of the adult population) were obese ***(Afshin et al., 2017).***

In the United States, data from the National Health and Nutrition Examination Survey (NHANES) for 2015 to 2016 estimated that 39.8% of adults were obese. In parallel with the obesity epidemic, there has been a rise in obesity-related complications including NAFLD ***(Hales et al., 2017).***

NAFLD is the most common liver disease worldwide ***(Chalasani et al., 2018; Younossi et al., 2019***). It is now the second leading indication for liver transplantation (LT) in the USA narrowly behind only alcohol-related liver disease ***(Kim et al., 2018).***

Currently, it is estimated that the global prevalence of NAFLD is approximately 25% and the highest prevalence of NAFLD was reported from the Middle East, whereas the lowest prevalence rate was reported from Africa. In the US, NAFLD and its subtype, NASH, affect 30% and 5% of the population, respectively ***(Younossi et al., 2016).***

There is a noticeable variable prevalence of NAFLD among populations, and phenotypic expression of severity. These differences are attributable to numerous factors including metabolic co-morbidities, microbiome, environmental, genetic/epigenetic factors ***(Younossi et al., 2019).***

Pathogenesis

The underlying mechanism for the development and progression of NAFLD is complex and multifactorial. Different theories have been formulated leading initially to the 'two hits hypothesis. According to this, hepatic accumulation of lipids secondary to sedentary lifestyle, high fat diet, obesity, and insulin resistance, acts as the first hit, sensitizing the liver to further insults acting as a "second hit". The second hit activates inflammatory cascades and fibrogenesis ***(Peverill et al., 2014; Buzzetti et al., 2016).***

However, it became rapidly evident that this view is too simplistic to recapitulate the complexity of the human NAFLD where multiple parallel factors, acting synergistically in genetically predisposed individuals, are implicated in the development and progression of the disease ***(Buzzetti et al., 2016).***

Consequently, a multiple-hit hypothesis has now substituted the outdated two-hit hypothesis for the progression of NAFLD **(Fig. 2)** ***(Buzzetti et al., 2016).***

Dietary habits, environmental and genetic factors can lead to the development of insulin resistance, obesity with adipocyte proliferation, and changes in the intestinal microbiome ***(Buzzetti et al., 2016).***

Insulin resistance is one of the key factors in the development of steatosis/NASH and results in increased hepatic de novo lipogenesis and impaired inhibition of adipose tissue lipolysis, with the consequently increased flux of fatty acids to the liver ***(Bugianesi et al., 2010).*** Insulin resistance also promotes adipose tissue dysfunction with consequent altered production and secretion of adipokines and inflammatory cytokines ***(Guilherme et al., 2008).***

Fat accumulates in the liver in the form of triglycerides, and this happens contemporarily with increased lipotoxicity from high levels of free fatty acids, free cholesterol and other lipid metabolites, as a consequence, mitochondrial dysfunction with oxidative stress and production of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress associated mechanisms, are activated ***(Cusi, 2009).***

Also, altered gut flora leads to further production of fatty acids in the bowel, increased small bowel permeability, and thus increased fatty acid absorption and raised circulating levels

of molecules which contribute to the activation of inflammatory pathways and release of pro-inflammatory cytokines such as IL-6 and TNF-α ***(Kirpich et al., 2015).***

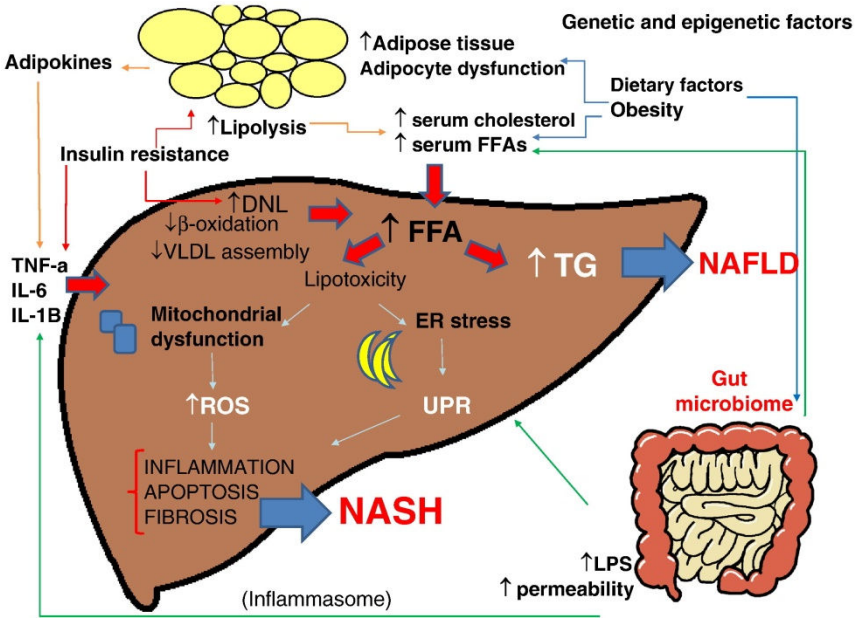

Figure (2): Multiple hit hypothesis for the development of NAFLD ***(Buzzetti et al., 2016).***

Several studies from the last years have revealed alterations in the gut microbiome in people with NAFLD compared with healthy controls. NAFLD subjects accompanied by advanced fibrosis exhibit a gut microbiome signature with increased concentrations of Proteobacteria and Escherichia coli ***(Loomba et al., 2017).***

Gut bacteria derived metabolites such as phenylacetic acid and endotoxin are associated with the degree of steatosis in female NAFLD patients ***(Zhu et al., 2013).***

During the past decade, evidence of cross-talk between the gut microbiome, the liver, the immune system, and metabolism has emerged, suggesting that the gut–liver axis is an important factor in the development of NAFLD, including its progressive subtype nonalcoholic steatohepatitis (NASH). Indeed, NAFLD has been associated with an increased proportion of Gram-negative bacterial species in the gut microbiome ***(Zhu et al., 2013).***

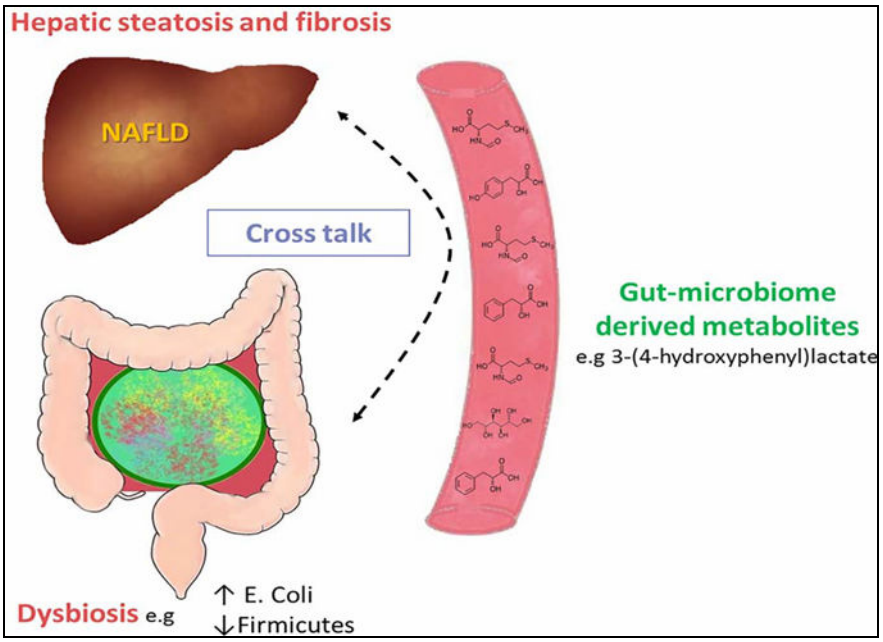

Figure (3): Gut-microbiome derived metabolites and development of NAFLD ***(Caussy et al., 2017).***

Microbial populations of patients with NASH have been suggested to have an increased ability to produce ethanol ***(Zhu et al., 2013).***

Some members of the gut microbiota can convert choline to trimethylamine, both of which can induce liver injury leading to hepatic steatosis and steatohepatitis ***(Chen et al., 2016).***

Furthermore, gut dysbiosis is associated with changes in the level of serum metabolites, including branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs). Moreover, BCAAs are increased in individuals with insulin resistance, which is considered as the major risk factor of hepatic steatosis ***(Pedersen et al., 2016).***

Therefore, evidence is increasing that the intestinal microbiota and its metabolites might play a crucial role in the pathogenesis of liver diseases including NAFLD ***(Hoyles et al., 2018).***

Several genetic modifiers of NAFLD have been identified, but a minority has been robustly validated. The best-characterized genetic association is with PNPLA3, initially identified from genome-wide association studies and confirmed in multiple cohorts and ethnicities as a modifier of NAFLD severity across the entire histological spectrum ***(Valenti et al., 2010; Liu et al., 2014).*** Recently, the TM6SF2 gene has been reported as another disease modifier and may have clinical utility assisting risk stratification for liver-related vs. cardiovascular morbidity ***(Liu et al., 2014; Dongiovanni et al., 2015).***

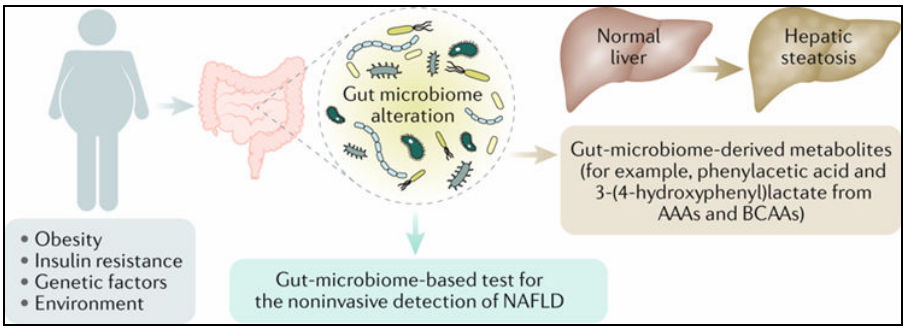

Figure (4): An altered gut microbiome and its derived metabolites can facilitate the development of hepatic steatosis in patients at risk of nonalcoholic fatty liver disease (NAFLD). A new gut microbiome signature derived from metagenomic and phenomic data might have the potential for the diagnosis of NAFLD. AAAs, aromatic amino acids; BCAAs, branched-chain amino acids (***Caussy and Loomba, 2018).***

Etiopathogenesis & risk factors

NAFLD is tightly associated with insulin resistance (IR) not only in the liver, but also in muscle and adipose tissues, and also with the metabolic syndrome or Syndrome X ***(Alberti et al., 2005; Gaggini et al., 2013).***

Metabolic syndrome is defined according to the modified National Cholesterol Education Program Adult Treatment Plan III (NCEP ATP III) guidelines, as the presence of 3 of the following 5 parameters: (1) fasting blood glucose > 100 mg/dL (or on antidiabetic drug treatment), (2) high-density lipoprotein (HDL) < 40 mg/dL in men or < 50 mg/dL in women, (3) triglycerides > 150 mg/dL (or taking a lipid lowering agent), (4) waist circumference >102 cm in men, >88 cm in women (5) blood pressure > 130/85 mm Hg (or taking antihypertensive medication) ***(Stone et al., 2005; American Heart Association, 2015).***

The metabolic syndrome is a clustering of risk factors that greatly increases an individual's probability of developing atherosclerotic cardiovascular disease (ASCVD), type 2 diabetes mellitus, and chronic kidney disease. The predominant underlying risk factors appear to be abdominal obesity, atherogenic dyslipidemia, hypertension, elevated plasma glucose, a prothrombotic state, and a proinflammatory state ***(Grundy, 2005; Reisin et al., 2005; Reynolds and He, 2005).***

As all components of metabolic syndrome correlate with liver fat content, independently of BMI, the presence of metabolic syndrome in any given patient should lead to an evaluation of the risk of NAFLD, and vice versa the presence of NAFLD should lead to an assessment of all components of metabolic syndrome ***(Koliaki et al., 2015).***

Hepatic triacylglycerol accumulation is accompanied by abnormal hepatic energy metabolism ***(Koliaki et al., 2015)*** and impaired insulin -mediated suppression of hepatic glucose and very low-density lipoprotein production leading to hyperglycemia, hypertriglyceridemia, and hyperinsulinemia ***(Yki-Jarvinen, 2014)***.

In nondiabetic persons, the product of fasting glucose (in mmol/L) and insulin (in mU/ml), divided by 22.5 (HOMA-IR), can serve as a surrogate for IR ***(Matthews et al., 1985),*** and it is an acceptable alternative to more expensive and time-