



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY

INTRODUCTION

Changes in human behavior and lifestyle have resulted in a dramatic increase in the prevalence of obesity, which has paralleled the increase of fat deposition in hepatocytes resulting in fatty liver disease.

Nonalcoholic Fatty Liver Disease (NAFLD) occurs in every age group, but especially in people in their 4th and 5th decades, especially who have risk factors as hyperglycemia and hyperlipidemia in the presence of a high BMI. NAFLD is considered a multisystem disease affecting not only the liver, but also extrahepatic organs. It contributes to a higher risk of cardiovascular disease, chronic kidney disease and Type2 Diabetes Mellitus. For that reason, it increases mortality by 57% according to the result of several studies (*Byrne and Targher, 2015*).

NAFLD refers to the whole spectrum of fatty liver disease from simple steatosis to Non-alcoholic Steatohepatitis (NASH), advanced fibrosis and cirrhosis (*Kaneda et al., 2006*).

The common pathway leading to liver fibrosis and cirrhosis is growing deposition of extracellular matrix. The main structural role in the formation of extracellular matrix is played by Hyaluronic acid. It has been found that it is correlated with the histological stages of liver disease (*Orasan et al., 2016*).

Staging and diagnosing the severity of liver disease is done by either an invasive or a noninvasive approach. The invasive method is through taking a liver biopsy, which is so far the golden standard for diagnosing the severity of NAFLD. However, liver biopsy as an invasive technique carries risks of complications besides being expensive and not suitable for screening all NAFLD patients.

Several non-invasive markers have been evaluated for the diagnosis of NAFLD including both serological indices and imaging methods. A large number of algorithms have been developed for differentiating between simple steatosis and NASH including:

- APRI which is the Aspartate Aminotransferase to platelet ratio index (*Loaeza-del-Castillo et al., 2008*).
- Fibrosis-4 (FIB-4) score which is so far the most prominent scoring system for distinguishing NASH from simple steatosis. It includes (Age, AST, ALT, platelet count)
- Hepatic steatosis Index (gender, DM, BMI, ALT, AST)
- Bard score (BMI, AST/ALT ratio, DM)
- NAFLD Fibrosis score (Age, BMI, DM, platelet count, albumin, AST/ALT ratio)
- Nippon Score (gender, age, DM, Hypertension) (*Papagianni et al., 2015*).
- Fatty Liver Index (an algorithm based on BMI, waist circumference, triglycerides and GGT) (*Bedogni et al., 2010*)

However, these scores have some limitations. They have limited ability to detect earlier stages and they carry a high false positive rate in advanced fibrosis (*McPherson et al., 2017*).

According to the previous limitations there is a need for detecting a new non-invasive dependable biomarker for diagnosing severity of NAFLD.

Hyaluronic acid is a high molecular weight polysaccharide that is practically found in every tissue in the body and is synthesized in synovial lining cells and hepatic stellate cells by an enzyme called Hyaluronic acid synthase (*Gudowska et al., 2016*).

Production of Hyaluronic acid initially increases when collagen synthesis is accelerated under various inflammatory conditions and liver cell injury, resulting in raised serum levels of Hyaluronic acid.

Consequently, serum Hyaluronic acid concentration reflects the severity of liver damage which makes it a valuable non-expensive, simple and quick biomarker for staging of NAFLD (*Suzuki et al., 2005*).

AIM OF THE WORK

The Aim of this study was to evaluate the efficacy and reliability of Hyaluronic Acid as a potential noninvasive biomarker for diagnosis and prognosis of NAFLD and to compare it with the traditional noninvasive techniques and if it can replace liver biopsy.

Chapter 1

NON ALCOHOLIC FATTY LIVER DISEASE

1.1 Natural history

NAFLD is a hepatic disease that can be diagnosed by histology, imaging, or both. The patient shows proof of hepatic fat tissue accumulation within the absence of chronic alcohol consumption, or use of drug causing steatosis, or hereditary disorders. NAFLD may be histologically distinguished from alcoholic steatohepatitis (ASH) by the absence of alcohol consumption according to the history of the patient, and on histological findings: hepatocytes ballooning, lobular inflammation, portal granulocytic inflammation, Mallory-Denk bodies, satellitosis, acute cholestasis, perisinusoidal fibrosis, sclerosing hyaline necrosis, and veno-occlusive disease (*Dumitrascu and Neuman, 2018*).

NAFLD includes a worldwide prevalence of about 25%. Incidence is expanding with rising levels of obesity, type 2 Diabetes and metabolic syndrome. Non-alcoholic fatty liver disease is predicted to become the leading reason for cirrhosis requiring liver transplantation within the next decade. It envelops a spectrum of disease starting from non-alcoholic fatty liver (NAFL) through to NASH, fibrosis and cirrhosis (*Maurice and Manousou, 2018*).

This disease spectrum is described in table 1 (*Kim et al., 2019*).

Table (1): Disease Spectrum of NAFLD

Type	Definition
Non alcoholic fatty liver (NAFL)	Presence of hepatic steatosis without hepatocyte ballooning degeneration, or fibrosis. The chances of progression in cirrhosis or hepatocellular carcinoma are minimal.
Non alcoholic steatohepatitis (NASH)	Presence of hepatic steatosis with histological manifestation of either lobular inflammation and/or hepatocyte ballooning degeneration, with or without fibrosis.
NASH cirrhosis	Presence of cirrhosis with evidence of steatosis or NASH diagnosed via histology
Cryptogenic cirrhosis	Unclear etiology of cirrhosis which is usually enriched with metabolic abnormalities after serological, clinical and pathological assessment has been performed. Progression of NAFLD to cirrhosis may cause difficulty in diagnosing NASH cirrhosis due to reduced hepatic steatosis.

Since it was first described in 1980, NAFLD is defined as the accumulation of hepatic fat, as proven by radiologic or histologic examination, in the absence of a coexisting cause of chronic liver disease or secondary cause of steatosis (including drugs, significant alcohol consumption, or inherited or acquired metabolic states). The spectrum of NAFLD includes two subtypes: NAFL and NASH. Isolated NAFL is characterized by steatosis (which may be accompanied with mild chronic inflammation) in at least 5% of hepatocytes.

On the other end of the spectrum, NASH is explained by a pattern of characteristics that involve steatosis, lobular and portal inflammation, and liver cell injury in the form of hepatocyte ballooning. Lobular inflammation is classically mild, characterized by a mixed inflammatory cell infiltrate. Other histological findings include Mallory–Denk bodies, iron deposition, periportal hepatocytes with vacuolated nuclei, ductular reaction, megamitochondria, lobular lipogranulomas, periodic acid–Schiff–diastase–resistant Kupffer cells, and acinar zone 3 perisinusoidal/ pericellular fibrosis, which may be hard to distinguish from alcoholic steatohepatitis (*Lindenmeyer and McCullough, 2018*).

Figure 1 shows a simplified diagram explaining liver damage in NAFLD (*Silent liver disease — non-alcoholic fatty liver disease | University of Iowa Hospitals & Clinics, 2020*).

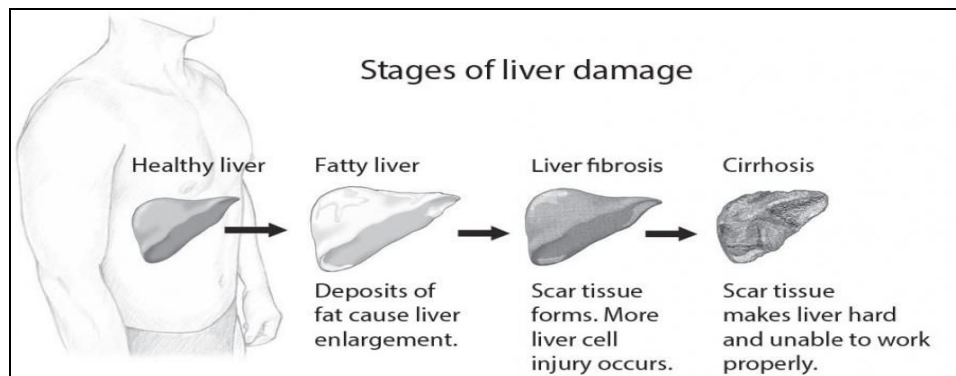


Figure (1): Stages of liver damage in NAFLD.

The following figure (Figure 2) shows the histological difference between NAFL, NASH and Cirrhosis respectively.

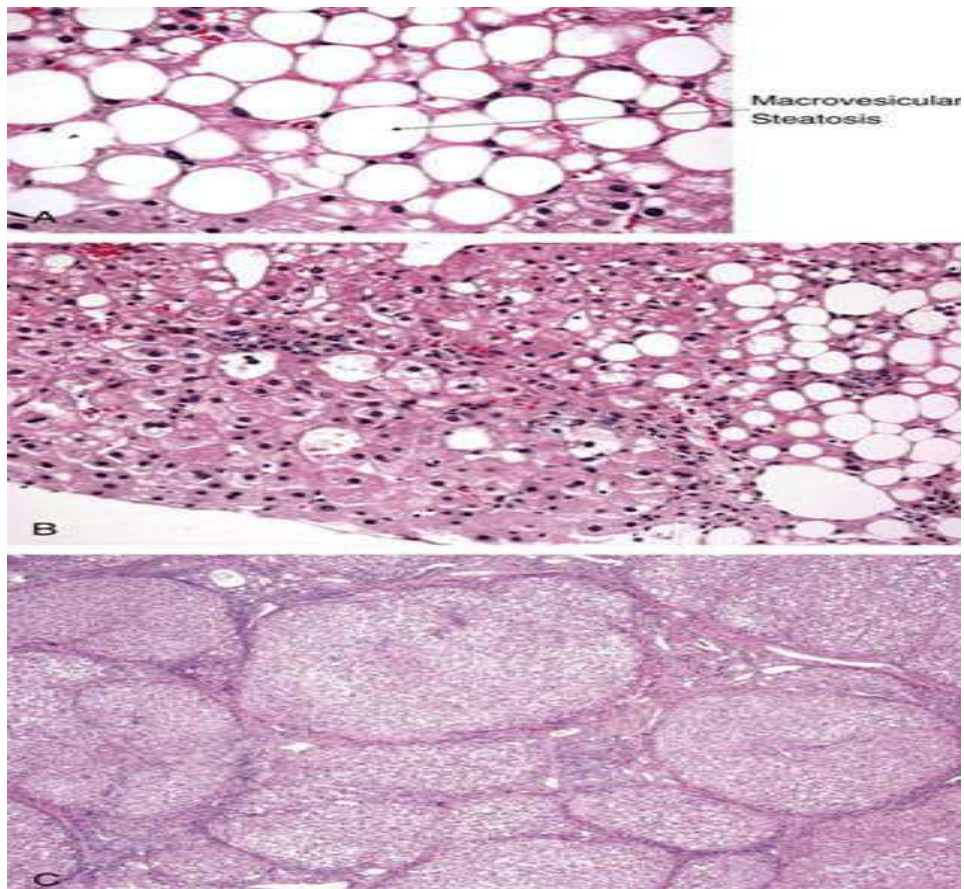


Figure (2): Spectrum of disease in NAFLD. A: Non-Alcoholic Fatty Liver (NAFL), B: Non-Alcoholic Steatohepatitis (NASH), C: Cirrhosis (*Image Courtesy of Lisa M. Yerian, Carey, 2020*).

1.2: Prevalence and Epidemiology

In the last years, NAFLD is common in developed countries as well as in developing countries and is therefore a worldwide, rather than regional, public health issue. NAFLD is highly widespread in all countries, but the highest rates are

reported from South America (31%) and the Middle East (32%), followed by Asia (27%), the USA (24%) and Europe (23%), whereas NAFLD is less common in Africa (14%).

Metabolic co morbidities associated with NAFLD include obesity (51.34%), type 2 diabetes (22.51%), hyperlipidemia (69.16%), hypertension (39.34%) and metabolic syndrome (42.54%). As the global spread of obesity fuels metabolic conditions, the clinical and economic burden of NAFLD will become enormous (*Younossi et al., 2016*).

With the increasing prevalence of NAFLD and the development of curative options for hepatitis C viral infection, NAFLD is expected to become the leading cause of HCC in developed countries.

Figure 3 Describes the global burden of NAFLD and its worldwide prevalence (*Global burden of NAFLD and NASH, 2019; Younossi et al., 2018*).

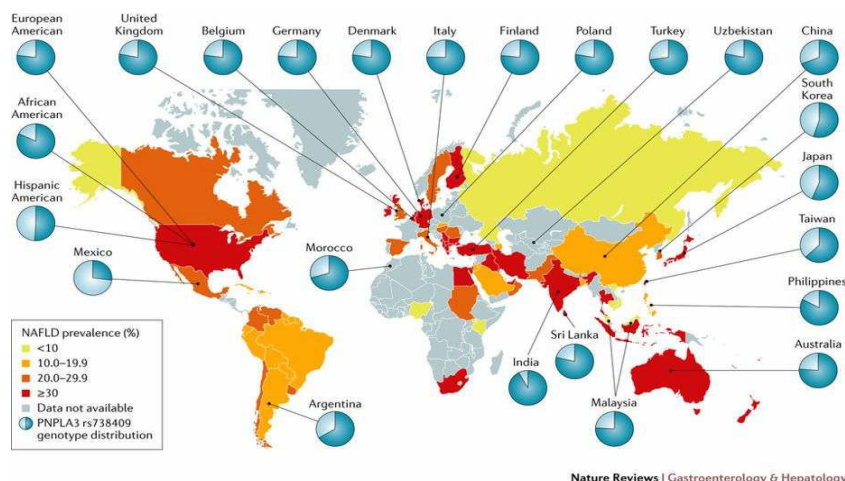


Figure (3): Worldwide estimated Prevalence of NAFLD.

Some researchers noticed that males are more susceptible to NAFLD. But in these studies, the males were compared to premenopausal women, who have a high level of estrogen which protects them from NAFLD. When age was considered in a South China study, the occurrence of fatty liver disease in women over 50 years old are higher than that in men, because women are no longer protected by estrogen as age advances. Furthermore, it was found that obese women are at higher risk of NAFLD. Generally people with a higher Body Mass Index (BMI) have a higher incidence to develop NAFLD. This is not affected by age or sex. It was shown that high Diastolic Blood Pressure (DBP) and Low-density lipoprotein cholesterol (LDL-C) have different effects on the risk of NAFLD in different age groups. We observed that among individuals under 50 years old, high DBP increases the likelihood of having NAFLD. Another interesting finding is that high LDL-C is a risk factor for NAFLD among younger people even if non-obese. Individuals whose BMI is under 24 kg/m² are at increased risk with increasing levels of LDL-C. Higher LDL-C level increases the incidence rate of NAFLD. Overweight and obese individuals with low HDL-C or high DBP have greater possibilities of having NAFLD compared to individuals whose BMI is under 24. Although the mechanism by which this occurs remains to be further explored, it suggests that obese people should pay more attention to the impact of changes in levels of HDL-C and DBP (*Tang et al., 2019*).

1.3: Etiology and Pathogenesis of NAFLD

A ‘two-hit’ theory was predicted for several years to explain NASH pathogenesis. The first hit results in fat accumulation as lipid droplets in the cytoplasm of hepatocytes and causes simple steatosis. This condition is reversible, and is associated with abnormal triglyceride storage. Triglycerides are produced from free fatty acids (FFAs). This theory suggests that in the setting of steatosis alone (i.e., NAFL), a second ‘hit’ from other factors (for example, oxidant stress) was required for the development of NASH; however, this view is now considered outdated. There are many molecular pathways that lead to the development of NASH, and it is not even certain whether NASH is always preceded by NAFL. Moreover, pathogenic drivers are not likely to be identical among all patients. Thus, both the mechanisms leading to disease and their clinical manifestations are highly heterogeneous (*Kupčová et al., 2019*).

The following figure (figure 4) summarizes the two hit theory in pathogenesis of NAFLD.

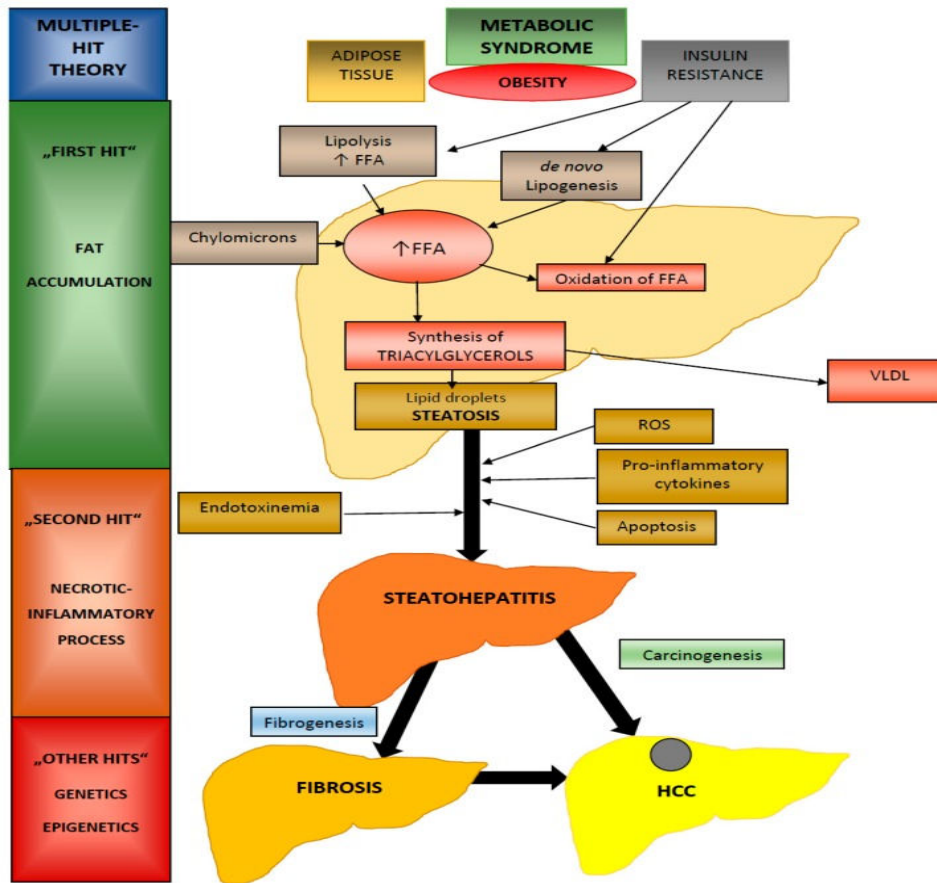


Figure (4): Pathogenesis of NAFLD. FFA—free fatty acids, VLDL—very low density lipoproteins, ROS—reactive oxygen species, HCC—hepatocellular carcinoma (Kupčová et al., 2019).

1.3.1 Response to lipotoxic lipids

In describing pathogenic drivers of NAFL and NASH it can be said that the liver's capacity to deal with the primary metabolic energy substrates, carbohydrates and fatty acids, is overwhelmed, resulting in accumulation of toxic lipid species. These metabolites cause hepatocellular stress, injury and death, leading to fibrogenesis and genomic instability that causes