# INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is regarded as the most common liver disease in the 21st century ***(Younossi et al., 2015),*** a growing risk factor for hepatocellular carcinoma (HCC)***,*** a leading indication for liver transplantation ***(Wong et al., 2015),*** and a condition leaving individuals at increased risk of extra-hepatic morbidity and mortality ***(Angulo et al., 2015, Ekstedt et al., 2015)***.

Over the past 2 decades, NAFLD has grown from a relatively unknown disease to the most common cause of chronic liver disease in the world. In fact, 25% of the world's population is currently thought to have NAFLD ***(Younossi et al., 2018).*** The clinical spectrum of NAFLD ranges from relatively benign fatty infiltration to non-alcoholic steatohepatitis (NASH) that can progress to cirrhosis, liver failure or HCC ***(Charlton et al., 2011, Wong et al., 2014).*** NAFLD is also associated with increased risk of mortality due to liver disease and cardio-vascular disease. The distinction of different forms of NAFLD is important in the clinical management of patients due to very different prognoses ***(Vuppalanchi et al., 2018).*** Furthermore; fibrosis has emerged as the strongest predictor of long-term outcomes in patients with NAFLD ***(Angulo et al., 2015).***

The evaluation of liver fibrosis severity has become the main issue to verify the prognosis of NAFLD patients, and it

became mandatory to identify non-invasive tools to replace liver biopsy, which is regarded as an invasive procedure with potential life-threatening complications such as bleeding, hematoma and pain ***(Petta et al., 2017).*** Liver biopsy has long been regarded as the gold standard for diagnosis and prognostication of patients with NAFLD. However, histological interpretation of liver biopsy is subject to micro-inhomogeneity, sampling errors, presence of un-fragmented core and observer variability among pathologists ***(Vuppalanchi et al., 2018).***

Methods of non-invasive laboratory and radiological testing for the assessment of hepatic steatosis and fibrosis in NAFLD have evolved during the past decade, and these methods may be able to overcome the limitations of liver biopsy. These methods include scores such as: AST/Platelet Ratio Index (APRI) score, Fibrosis-4 (FIB-4) score, Fatty Liver Index (FLI), and NAFLD Fibrosis Score (NFS), in addition to radiological methods such as Transient Elastography (TE), which is an ultrasound-based technique, and considered as one of the most extensively used and well-validated non-invasive methods for the assessment of hepatic steatosis and fibrosis ***(Fallatah, 2014, Machado and Cortez-Pinto, 2014).***

Presently, non-invasive assessment of Steatosis and fibrosis may be conducted using both combined biochemical markers such as Cyto-Keratin 18 (CK18), and specific devices such as TE ***(Petta et al., 2015, EASL et al., 2016).*** Liver

stiffness measurement (LSM) by TE (Fibroscan, Echosens, Paris) uses ultrasound-based technology for quantitative assessment of hepatic fibrosis. It has been shown to be sufficiently accurate to predict the fibrosis stage in NAFLD patients ***(Petta et al., 2015, Sobhonslidsuk et al., 2015).***

Vibration controlled transient elastography (VCTE) measures the speed of a mechanically-induced shear wave using pulse-echo ultrasonic acquisitions in a much larger portion of the tissue, approximately 100 times more than a liver biopsy core. However, prior studies evaluating the performance of VCTE in NAFLD have been limited by medium (M) size probes with an ultrasound probe frequency of 3.5 MHz to measure LSM at a depth of 2.5 and 6.5 cm from the skin. Our study use also XL (large) probe for overweight patients with more sensitive ultrasound sensor. This large probe, specially designed for obese patients, has a central ultrasound frequency of 2.5 MHz and a measurement depth of 35–75 mm ***(Durango et al., 2013).*** LSM assessed by VCTE has been shown to be an easy to perform, non-invasive test to reliably estimate the degree of liver fibrosis in patients with NAFLD ***(Vuppalanchi et al., 2018).***

The newer version of VCTE has several features that not only overcomes its prior limitations, but also enhances its role as a diagnostic tool in the evaluation of patients with NAFLD. It is currently approved by the regulatory authorities to measure a 3.5MHz ultrasound coefficient of attenuation, known as the controlled attenuation parameter (CAP). CAP measures the

ultrasonic attenuation in the liver tissue depending on the viscosity [fat] of the medium [liver] and the distance of propagation of the ultrasonic signals into the liver ***(Sasso et al., 2016).***

While LSM is measured in kilopascals (KPa), CAP is measured in decibels per meter (dB/m), and reflects the decrease in the amplitude of ultrasound signal in the liver ***(Kwok et al., 2016).*** Therefore, a higher CAP is reflective of the higher degree of steatosis. CAP is displayed only when LSM is valid, as it is only computed from the ultrasound signals used for acquiring LSM. The shear wave speed with estimation of stiffness and CAP currently allows for simultaneous assessment of both liver fibrosis and steatosis ***(Tapper et al., 2016, Vuppalanchi et al., 2018).***

# AIM OF THE WORK

To assess hepatic steatosis and fibrosis by VCTE versus other non-invasive assessment scores in Egyptian patients with non-alcoholic fatty liver disease.

# Chapter 1

# NON ALCOHOLIC FATTY LIVER DISEASE

## Introduction

Over the past 2 decades, NAFLD has grown from a relatively unknown disease to the most common cause of chronic liver disease in the world. In fact, 25% of the world's population is currently thought to have NAFLD ***(Younossi et al., 2018).***

NAFLD is present if at least 5% of liver weight is fat without excess alcohol consumption or secondary causes of fat accumulation in the background ***(European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), & European Association for the Study of Obesity (EASO), 2016)***.

NAFLD is closely associated with the presence of metabolic diseases, such as obesity, type 2 diabetes, and insulin resistance. NAFLD comprises a spectrum of histopathological changes ranging from simple steatosis to NASH and fibrosis with subsequent development of cirrhosis and HCC ***(Younossi et al., 2018).*** Thus, the total mortality is increased, mostly due to cardiovascular reasons ***(Angulo et al., 2015; Ekstedt et al., 2015)***. The increase is, however, strongly associated with progressive NAFLD forms and may not be expanded to all

NAFLD patients ***(Angulo et al., 2015; Ekstedt et al., 2015).*** Additionally, the risk factors and risk profile of metabolic syndrome (Mets) and NAFLD are similar ***(Yki-Jarvinen, 2014),*** leading to lack of knowledge of the precise impact of each of these conditions on the mutual co-morbidity. The global disease burden and the costs of NAFLD are enormous. For instance, in the United States only the direct medical costs of NAFLD are about $100 billion ($1,600 per patient) annually. In Europe, the costs seem to be somewhat lower ***(Younossi et al., 2016).***

NAFLD is a heterogeneous disease. A great majority of subjects with NAFLD will not have a shortened life time or any hepatic event ***(Rinella & Charlton, 2016).*** On the other hand, those with progressive NAFLD are at risk of increased hepatic, extra-hepatic and overall morbidity and mortality ***(Angulo et al., 2015; Ekstedt et al., 2015).*** In recent years, a growing body of evidence has shown that the risk, clinical picture, and the disease burden of NAFLD is modified by genetic and epigenetic variations, many life-style and environmental factors, inflammatory status, the well-being of the gut microbiota and hormonal balance ***(Yki-Jarvinen et al., 2015; Buzzetti et al., 2016; Petaja and Yki-Jarvinen, 2016).***

## Definition, pathogenesis and spectrum of NAFLD

NAFLD is characterized by excessive fat accumulation in the liver as defined by the presence of steatosis > 5% of liver weight according to histological analysis or by a proton density fat fraction (PDFF) > 5.6% in proton magnetic resonance spectroscopy (1H-MRS) or magnetic resonance imaging (MRI) ***(EASL et al., 2016).*** Moreover, the definite diagnosis of NAFLD requires the exclusion of excess alcohol consumption (≥ 30g a day in men or ≥ 20g a day in women) ***(Nascimbeni et al., 2013; EASL et al., 2016).*** Notably, the methods used to exclude excess alcohol consumption (anamnesis, biochemical markers) are not fully reliable. Additionally, there is no threshold for harmful alcohol consumption as the alcohol-related hepatic injuries appear to increase uniformly with the amount of alcohol consumed ***(EASL 2012; Nascimbeni et al., 2013).*** Thus, the diagnostic thresholds are more or less arbitrary ***(Nascimbeni et al., 2013).*** The secondary causes to NAFLD, such as hepatotoxic medical history during the past six months, viral hepatitis, hemochromatosis and chronic autoimmune liver diseases, also need to be excluded ***(Nascimbeni et al., 2013).*** Of pharmacological agents, methotrexate, glucocorticoids, isoniazid, amiodarone and tamoxifen are known inducers of fatty liver ***(Williamson et al., 2011).***

NAFLD is an umbrella term covering simple non-alcoholic fatty liver (NAFL), in which there is pure hepato-steatosis only (or steatosis with either mild inflammation or ballooning but not

both), and NASH. The co-existence of all these three histopathological features, i.e., steatosis, inflammation and ballooning (swollen hepatocytes), is required for NASH, which also covers the most progressive forms of NAFLD: fibrosis, cirrhosis and HCC ***(EASL et al., 2016).***

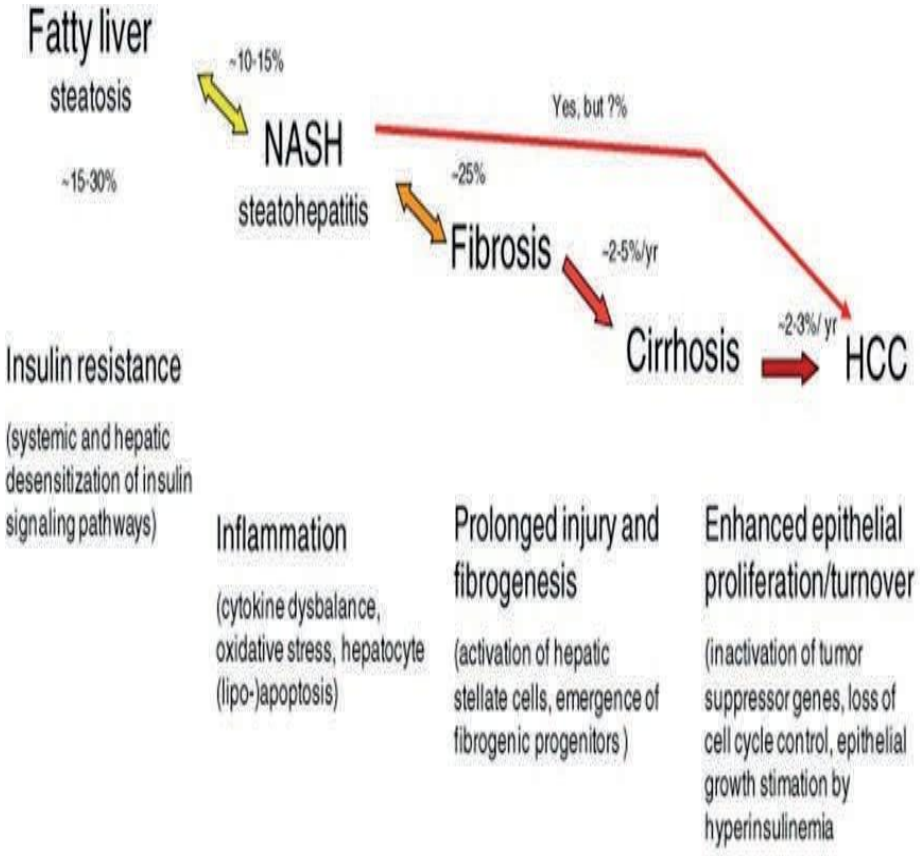


**Figure (1): The spectrum of NAFLD.** NAFLD comprises different stages with stage-specific risk factors and pathomechanisms. Estimated risks of progression are displayed. HCC, NASH ***(Schuppan & Schatten, 2013).***

NAFLD progresses slowly ***(EASL et al., 2016).*** Traditionally, NAFL has been thought to be a benign disease,

but an accumulating body of evidence has shown that NAFL may progress to NASH ***(Schuppan and Schattenberg, 2013; Buzzetti et al., 2016; Bertot and Adams, 2016; EASL et al., 2016).*** It is estimated that over time about 30–40% of subjects with NAFL and elevated liver enzymes will develop NASH and 40–50% of those with NASH will have fibrosis ***(Ekstedt et al., 2006);*** in all NAFLD subjects, these numbers are thought to be about 10–15% and 25%, respectively ***(Schuppan and Schattenberg, 2013).*** NAFL may even leap directly to fibrosis ***(Pais et al., 2013),*** in which there may still exist mild inflammation but without other mandatory NASH criteria ***(Singh et al., 2015),*** or prior NASH may has been missed. In addition, NASH and even NAFL may progress to non-cirrhotic HCC ***(Torres and Harrison, 2015; Buzzetti et al., 2016)*** and, thus, cirrhosis is not a mandatory step in the malignant disease progression as was previously thought ***(Bertot and Adams, 2016).***

Sedentary lifestyle accompanied by excess caloric intake leads to accumulating visceral adipose tissue, insulin resistance and the release of pro-inflammatory factors. These promote the lipolysis of free fatty acids (FFA) from visceral adipose tissue to the liver ***(Asrih and Jornayvaz, 2015).*** Simple hepato-steatosis is formed when hepatic FFA input is greater than output, both of which are altered in subjects with NAFLD ***(Fabbrini et al., 2010).***

The progression of NAFL to NASH and fibrosis requires a complex and multifactorial interplay of genetic, epigenetic, environmental, inflammatory, adipose tissue-derived factors and intrinsic microbial factors ***(Baran and Akyuz, 2014; Singh et al., 2015; Buzzetti et al., 2016)***. The high levels of FFAs and many other lipid metabolites in a hepatocyte are lipotoxic. As a consequence, mitochondrial dysfunction with oxidative stress and production of reactive oxygen species and endoplasmic reticulum stress-associated mechanisms are activated. Moreover, the pro-inflammatory factors from visceral adipose tissue are present in the liver ***(Buzzetti et al., 2016).*** The inflamed environment leads to chronic hepatic inflammation, which is further amplified by unfavorable genetic and epigenetic modifications and alterations in the gut flora ***(Buzzetti et al., 2016).*** The alterations in the gut flora are derived from an unbalanced diet (high fat, high sugar/fructose, low fiber, nutrient/vitamin deficiency), which causes gut microbiota dysbiosis, mild inflammation and alteration in gut barrier function leading to increased translocation of microbial components into splanchnic veins which takes part in the formation of NAFLD progression ***(Delarue and Lalles, 2016).***

Once NASH and the chronic inflammation have developed, hepatocyte necrosis and apoptosis is promoted. Apoptotic bodies from the damaged hepatocytes can activate hepatic stellate cells and Kuppfer cells, which drive the formation of liver fibrosis by inflammatory and fibrogenic

responses. Thereby, transformation of hepatic stellate cells into myofibroblasts takes place and this results in the accumulation of collagen, proteoglycans and glycoproteins and, thereby, changes in the extracellular matrix composition ***(Liang et al., 2016).*** Activated hepatic stellate cells also enhance the pro-inflammatory responses and the formation of the vicious circle between inflammation and the profibrotic processes. The transition between hepatic stellate cells to myofibroblasts involves signaling pathways, which in NASH-driven fibrosis seems to be dominated by Hedgehog signaling ***(Liang et al., 2016).***

This theory of the progression of NAFLD is called the multiple-hits theory and it has replaced the outdated two-hits theory ***(Buzzetti et al., 2016)*** and distinct hit theory ***(Yilmaz, 2012).*** However, it is known that the development of progressive NAFLD occurs over such a long time course that progressive histological follow up is difficult. To date, no biochemical marker specific for NASH has been found that could point to the distinct pathophysiological routes.

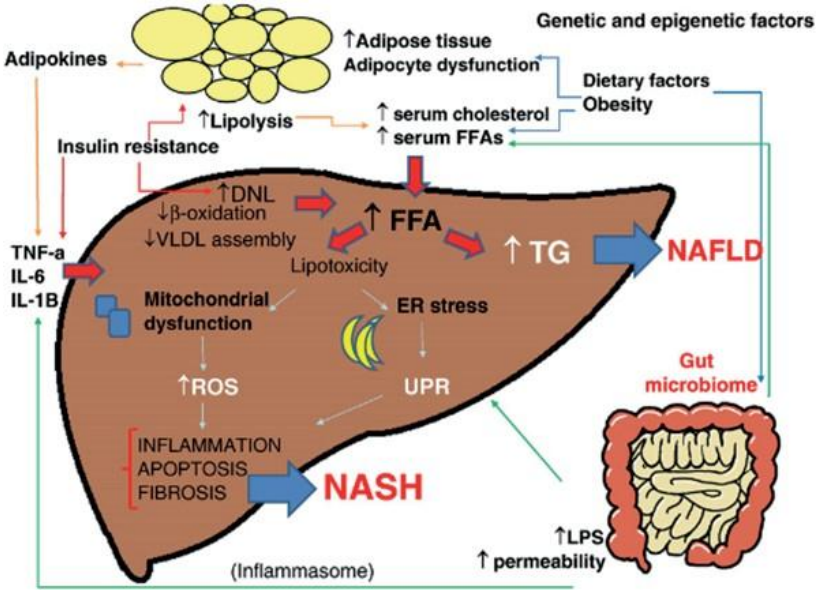


**Figure (2): Multiple hit hypothesis for the development of NAFLD**. Abbreviations: FFAs, free fatty acids; DNL, de novo lipogenesis; VLDL, very low density lipoproteins; CH, choles- terol; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; TG, triglycerides; ROS, reac- tive oxygen species; ER, endoplasmic reticulum; UPR, unfolded protein response; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic stea- tohepatitis. ***(Buzzetti et al., 2016).***

## Hepatic complications of NAFLD

The average risk of cirrhosis in subjects with simple steatosis is under 4% in 20 years, but in NASH subjects the risk reaches up to 25% in nine years Moreover, the amount of NAFLD-related cirrhosis is likely to be underestimated because the histological signs of steatohepatitis may no longer exist at the cirrhotic stage, leading the cirrhosis to be labeled as 'cryptogenic', and because there is a lack of systematic evaluation in community-based studies ***(Bertot and Adams, 2016).*** Indeed, given the high prevalence, NAFLD is the

leading cause of cryptogenic cirrhosis and the second or third most common cause of liver transplantation ***(Wong et al., 2015; Bertot and Adams, 2016; EASL et al., 2016).*** NAFLD is predicted to be the most common cause of liver transplantation in the near future ***(Charlton et al., 2011; Bertot and Adams, 2016).*** Looking back, there has been a 5-fold increase in the transplantations for NASH during this millennium ***(Agopian et al., 2012).***

Liver-related deaths (cirrhosis or HCC) accounted for up to 9–28% of all deaths in three long-term longitudinal studies of biopsied NAFLD patients ***(Younossi et al., 2011; Angulo et al., 2015; Ekstedt et al., 2015),*** although Cardio-Vascular Diseases (CVDs) remain the most common cause of death in NAFLD subjects ***(Ekstedt et al., 2015; EASL et al., 2016).*** Liver death is the third most common cause of death after CVDs and non-gastrointestinal malignancies among biopsied NAFLD patients ***(Angulo et al., 2015; Ekstedt et al., 2015).*** Among all NAFLD subjects, the liver-related mortality is estimated to be around 2% worldwide ***(Rinella and Charlton, 2016; Younossi et al., 2016).*** However, as the global prevalence of NAFLD is reported to be 25%, which equals about 1 billion adult NAFLD subjects, this will lead to 20,000,000 liver-deaths among patients with NAFLD who are currently alive ***(Rinella and Charlton, 2016).*** This exceeds hepatitis C as a cause of liver-related death ***(Rinella and Charlton, 2016).*** Nonetheless, only the presence of fibrosis

seems to predict the liver-related mortality, along with CVD-related and all-cause mortalities, and the more severe the fibrosis stage is, the greater the risk ***(Angulo et al., 2015; Ekstedt et al., 2015; Dulai et al., 2017).***

Once at cirrhosis stage, the overall prognosis is still relatively good as 10-year survival has been reported to be around 81–84% in all NASH-related cirrhotics. However, nearly half of Child- Pugh A cirrhotics (45%) develops decompensated cirrhosis during 10 years**,** which is, nonetheless, a lower proportion than in hepatitis C -related cirrhosis (60%)**.** After decompensation, there are no differences in mortality between NASH or hepatitis C related cirrhotics and the survival is reduced as nearly every subject with Child-Pugh B or C dies within 2–3 years ***(Sanyal et al., 2006).***