# INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is now considered the most common chronic liver disease in the world ***(Angulo, 2002).***

It's characterized by presence of hepatic steatosis and comprises a clinical spectrum ranging from simple steatosis (fatty liver) to non alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis at last ***(Chalasani et al., 2012).***

NAFLD was traditionally thought to be a benign condition but now it has a great concern to prevent its complication and the progression to cirrhosis. There is also increasing evidence suggesting that NAFLD may play a significant role in the strong association between obesity and liver cancer ***(Ascha, 2010; Bugianesi, 2002).***

NAFLD is linked to metabolic syndrome and various cardiovascular risk factors like diabetes, hypertension, obesity and dyslipidemia ***(Targher et al., 2010; Marchesini et al., 2008; Hamaguchi et al., 2005; Adiels et al., 2008).***

The association of NAFLD with metabolic syndrome has lead to increased interest in its role in CVS disease causation and progression ***(Targher et al., 2010).***

In the United States, the burden of liver-related diseases is important. Over the last 2 decades, liver-related mortality ranked among the top 12 causes of death while among adults aged 45-54 years, it has been repeatedly listed as the forth leading cause of death ***(Hoyert and Xu, 2012).***

The prevalence of NAFLD in the general population of western countries is 20-30%. The prevalence of NAFLD is higher in males and increases with increasing age.

Although the "gold standard" for diagnosis and staging og NAFLD is histology, abdominal ultrasonography allows its detection ***(Ratziu et al., 2010; Sanyal, 2002).***

## Diagnosis of NAFLD is based on the following 3 criteria:

1. Non alcoholic patients (daily consumption of alcohol doesn't exceed 20 g in women and 30 g in males).
2. Detection of steatosis either by imaging or histology.
3. Appropriate exclusion of other liver disease ***(Hashimoto et al., 2013).***

Platelets producing vasoactive and prothromboticfactors like IL-1b and CD40L play an important role inatherothrombosis ***(Chu, 2010; Gawaz, 2005).***

The efficacy of antiplatelet drugs in reducing CVS events further strengthens the belief in the atherothrombotic role of platelets ***(Meadows, 2007).***

Mean platelet volume (MPV), a simple and inexpensive test, is commonly used to measure platelet size and is also a marker of platelet activity ***(Chu, 2010).***

# AIM OF THE WORK

In this study we want to measure the MPV in non alcoholic fatty liver disease (NAFLD) in both diabetic and non diabetic patients.

Mean platelet volume (MPV) is a biomarker of platelet activity and elevation in MPV has been observed in the setting of acute cardiovascular event.

# Chapter 1

# NON-ALCOHOLIC FATTY LIVER DISEASE

## Definition

The term Non-alcoholic fatty liver disease "NAFLD" is used to describe a spectrum best defined by liver biopsy findings ranging from deposition of triglycerides as lipid droplets in the cytoplasm of hepatocytes, namely simple steatosis, to the more aggressive form of non-alcoholic steatohepatitis (NASH), which is characterized by additional features of hepatocyte injury, inflammation, and variable degrees of fibrosis. Although simple steatosis appears to follow a non-progressive course, a subset of patients develop NASH, which is of concern owing to the potential progression to end-stage complications of liver cirrhosis and hepatocellular carcinoma (HCC) ***(Cohen et al., 2011).***

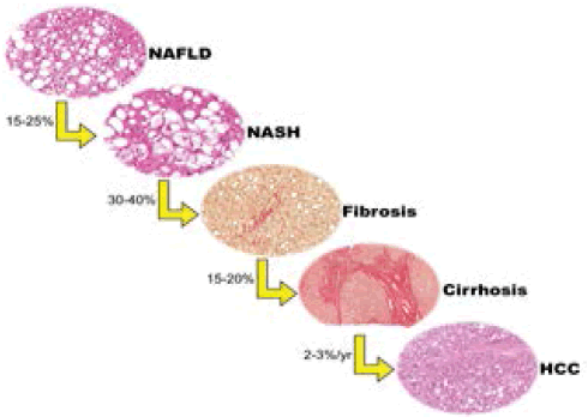


**Figure (1):** Spectrum of Non-Alcoholic Fatty Liver Disease ***(Wang et al., 2016)***.

## Epidemiology

The overall prevalence of chronic liver disease (CLD) has risen steadily over recent decades according to the National Health and Nutrition Examination Survey (NHANES) database ***(Vernon et al., 2011).***

Although the prevalence rates of alcoholic liver disease and viral hepatitis have remained stable, NAFLD and its associated comorbidities (obesity, diabetes, and insulin resistance) have increased substantially. Prevalence estimates of NAFLD vary according to the diagnostic modality used to establish the diagnosis. Given that most patients are asymptomatic and do not undergo a liver biopsy, the reported prevalence rates are likely to underestimate the real problem ***(Younossi et al., 2011).***

In an autopsy series from Canada, the prevalence of NAFLD and NASH in lean subjects was 7% and 3%, respectively ***(Wanless and Lentz, 1990).*** Other autopsy studies have reported prevalence rates as high as 16% for NAFLD in the general population, but some of the cases were attributed to alcohol consumption ***(Amarapurkar and Ghansar, 2007).***

Non-invasive imaging modalities such as ultrasound and magnetic resonance spectroscopy (MRS) provide another estimate of the prevalence of NAFLD. Two population-based studies from Spain and Italy reported a prevalence of NAFLD

of 26% and 20%, respectively, based on ultrasound ***(Caballería et al., 2010).***

Similar numbers have been reported in India ***(Amarapurkar et al., 2007)*** (17%) and Japan ***(Kojima et al., 2003)*** (30%). As a more sensitive modality for detecting hepatic steatosis, MRS has been used investigationally to diagnose NAFLD. Initial studies from the Dallas Heart Study reported slightly higher prevalence rates of 33–34% based on MRS ***(Szczepaniak et al., 2005).***

**Table (1):** NAFLD Prevalence Stratified by Region ***(Younossi et al., 2016)***

| Region | N | Prevalence (%) | 95% CI (%) | $I^2$ (%) |
|---|---|---|---|---|
| Africa | 2 | 13.48 | (5.69-28.69) | 84.37 |
| Asia | 14 | 27.37 | (23.29-31.88) | 99.17 |
| Europe | 11 | 23.71 | (16.12-33.45) | 98.78 |
| Middle East | 3 | 31.79 | (13.48-58.23) | 99.14 |
| North America | 13 | 24.13 | (19.73-29.15) | 99.19 |
| South America | 2 | 30.45 | (22.74-39.44) | 69.10 |
| Overall | 45 | 25.24 | (22.1-28.65) | 99.07 |

## Pathogenesis:

### 1. *Evolution from the "two hit theory" to the "multiple hit model"*

During recent decades, the worldwide prevalence of obesity has increased in the pediatric population and the prevalence of NAFLD has more than doubled during the last 20 years in the United States ***(Chen et al., 2016).***

The development of NAFLD is strongly influenced by age, sex, and ethnicity, and appears twice as often in boys than in girls. NASH can progress to end-stage liver diseases such as hepatic cirrhosis or hepatocellular carcinoma ***(Giorgio et al., 2013).***

***Selvakumar et al.,*** analyzed the database and discovered that as the prevalence of NAFLD increased, the prevalence of NASH also increased, however, compared to adult the prevalence of liver fibrosis in children remained low, which indicated a possibly less aggressive NAFLD phenotype in children ***(Selvakumar et al., 2018).***

Although the prevalence of NAFLD is increasing, most affected patients present with isolated steatosis with only a minority of cases progressing to liver cirrhosis in children, and it is not clear whether pediatric and adult NAFLD are two different pathologic entities or just age-dependent manifestations of the same disease, which implies that the pathogenesis of NAFLD may be related to the interplay among

genetic, environmental, and individual factors. Early theories of the pathogenesis of NAFLD and NASH were described in terms of the "two hit hypothesis" ***(Dowman et al., 2009).***

At the onset of disease, the "first hit" is represented by an increase in liver fat, characterized by hepatic triglyceride accumulation and insulin resistance, and corresponding to hepatic steatosis once the accumulation of hepatic fat is more than 5%. Children, especially pre-pubertal boys, have a pattern of type 2 NAFLD characterized by a zone 1 distribution of steatosis, inflammation and fibrosis ***(Bush et al., 2017).***

Liver fat accumulation is associated with a hypercaloric diet, sedentary lifestyle, and is perhaps genetically predisposed. It successfully established an *in vivo* NAFLD animal model induced by a high-fat diet, and reported that lifestyle interventions have an effect on NAFLD in obese children ***(Clemente et al., 2016).***

Subsequently, the "second hit" emerges, which includes inflammatory cytokines, adipokines, mitochondrial dysfunction, and oxidative stress. As the fatty liver is more susceptible to this "second hit", necroinflammation and fibrosis can develop and ultimately lead to cirrhosis. However, with the development of new technology and further research, this view appears too simplistic for recapitulating the complexity of human NAFLD ***(Berardis and Sokal, 2014).***

Now, the widely accepted theory is the "multiple-hit model", involving more widespread metabolic dysfunction because of the interaction of genetic and environmental factors as well as changes in crosstalk between different organs and tissues, including adipose tissue, the pancreas, gut, and liver. However, liver fat accumulation, caused by obesity and insulin resistance, still seem to represent the "first hits" ***(Ayonrinde et al., 2015).***

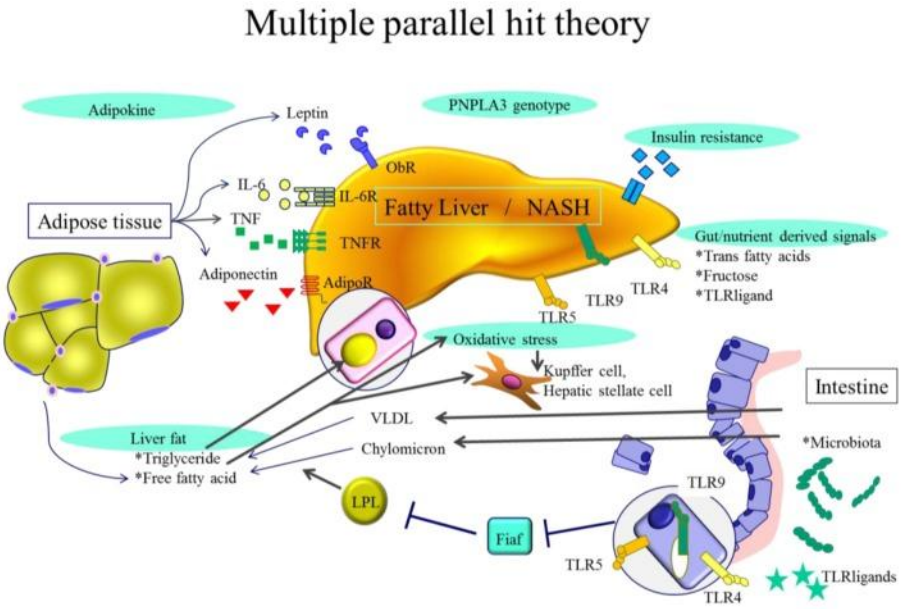


**Figure (2): Multiple parallel hit theory.** Genome-wide association studies have confirmed importance of *patatin-like phospholipase 3* (*PNPLA3*) gene polymorphism in NAFLD. This genetic polymorphism can differentiate simple steatosis with or without minimal inflammation and fibrosis progressing to NASH. In some instances, inflammation could precede steatosis and anti-tumor necrosis factor (TNF)-α antibody improves steatosis in ob/ob mice. Obesity and diabetes induce insulin resistance, adipocyte proliferation and changes in intestinal flora. Adipokines such as IL-6 and TNF-α produced by adipocytes affect hepatocyte fat content and liver inflammatory environment. Gut-derived signals could be affected by ingested trans fatty acids, fructose, or TLR ligands. Ingested free fatty acids and free cholesterol induce ER stress and oxidative stress resulting in hepatic inflammation and fibrogenesis ***(Takaki et al., 2013).***

## *2. Fat accumulation and insulin resistance*

Fat accumulates in the liver of patients with NAFLD mainly in the form of triglycerides, which derive from the esterification of glycerol and free fatty acids (FFAs) ***(Musso et al., 2013b).***

Triglyceride accumulation is not hepatotoxic, in contrast with the excess of FFAs that undergo acetyl coenzyme A (acyl-CoA) synthase activity and form fatty acyl-CoAs which may trigger esterification or β-oxidation pathways ***(Ferramosca and Zara, 2014).***

Mitochondrial dysfunction, which consists of oxidative stress and production of reactive oxygen species and endoplasmic reticulum stress-associated mechanisms, also results from NAFLD ***(Buzzetti et al., 2016).***

Physiologically, insulin controls hepatic glucose production by regulating lipolysis of adipocytes, leading to decreased fatty acid flux in the liver. Consequently, the availability of hepatic acetyl coenzyme A (acyl-CoA) concentrations and the activity of pyruvate carboxylase are reduced, resulting in the decreased conversion of pyruvate to glucose ***(Edgerton et al., 2017).***

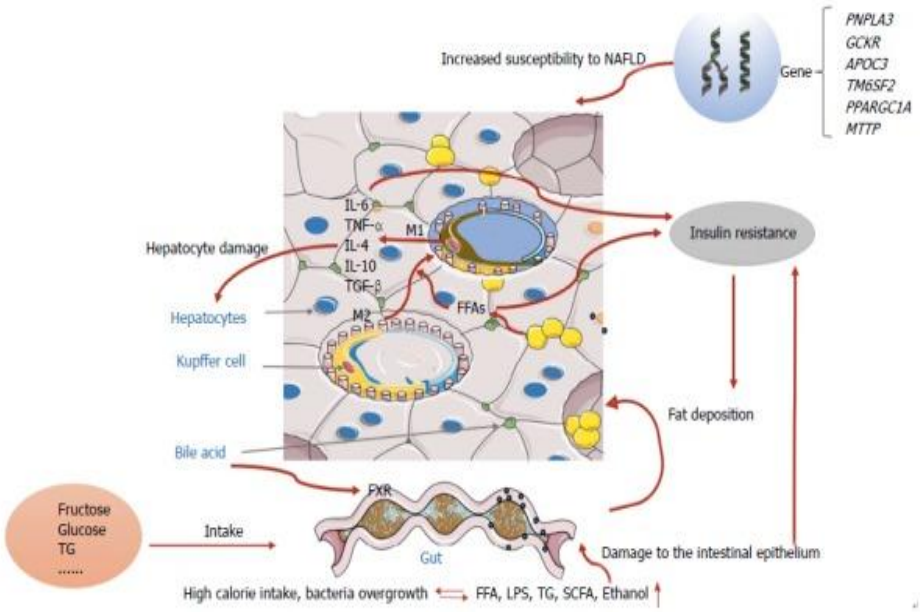


**Figure (3):** Schematic mechanistic diagram of the "multiple hit model". NAFLD: Nonalcoholic fatty liver disease ***(Fang et al., 2018).***

Insulin resistance (IR) refers to a defective metabolic response to the effect of the hormone in the target cell (*e.g*., muscle cell, hepatocyte, and adipocyte) or at the whole organism level. Systemic IR means that the ability of insulin to lower the serum glucose concentration to the appropriate level is hampered due to disrupted translocation of the GLUT4 receptor at the surface membrane of the muscle cell. As a result, glucose uptake (which depends on insulin) decreases. Hepatic IR consists of disturbed insulin mediated suppression of hepatic glucose production, but in the presence of preserved stimulation of lipogenesis ***(Petersen and Shulman, 2017).***

In the adipose system, insulin resistance means that insulin is unable to suppress lipolysis. In humans, when the availability of lipids exceeds the lipid accumulation capacity, systemic IR and hepatic IR are likely to progress ***(Shulman, 2014).***

## *3. Inflammatory pathways*

Increased FFA levels can cause lipotoxicity and insulin resistance, and together with other factors (such as gut-derived endotoxins), activate the release of proinflammatory cytokines systemically and also locally in the liver. There are two main classical pathways involved in the process of NAFLD inflammation: JNK-AP-1 and IKK-NF-κBD ***(Hotamisligil, 2017).***

JNK-AP-1 is a mitogen-activated protein kinase associated with apoptosis and NASH; IKK-NF-κB is a transcription factor regulating inflammatory activation. Previous studies have shown that persistent activation of NF-κB was found in NAFLD animal models as well as in humans with NASH ***(Ribeiro et al., 2004).***

Animal models demonstrated that hepatic exposure to high levels of proinflammatory cytokines could lead to histological changes mimicking NASH ***(Tomita et al., 2006).***

The liver consists of parenchymal cells and nonparenchymal cells (NPCs); NPCs include sinusoidal endothelial cells. Kupffer cells (KCs) and hepatic stellate cells are less numerous than hepatocytes but play a key role in the

immune regulation of the liver, especially through substances released from KCs, which act as antigen presenting cells. The hypothesis is that when the flow of FFAs or other pathogenic factors (such as endotoxins) from the gut into liver are excessive, KCs phagocytose the factors and present them through pattern recognition receptors (PRRs)***(Thomson and Knolle, 2010).***

PRRs include toll-like receptors (TLRS) such TLR4, TLR9, and nucleotide oligomerization domain-like receptors (NLRs). Inflammasomes, through NLR, activate the cascade events which finally generate mature IL-1, IL-8, and IL-1, contributing to regulate the activation of the transcription factor NF-κB ***(Szabo and Csak, 2012).***

KCs *per se* will differentiate into either the M1 or M2 phenotype, depending on the environmental inducer; the former releasing cytokines like TNF-α, IL-1, and IL-12 and the latter, more heterogeneous, being able to stimulate the secretion of IL-4, IL-10, and TGF-β according to different triggers ***(Soysa and Crispe, 2016).***

IL-6 and TNF-α are the cytokines responsible for NASH progression. Patients with NASH have higher serum TNF-α levels, which play an important role in hepatic fibrosis through KC activation. Therefore, TLR suppression is thought to block the immune response, thereby alleviating liver inflammation. However, to date, despite some animal experiments aiming to

reveal the links between TLRs and NAFLD pathogenesis, no investigations on TLR agonists have yet been conducted in humans ***(Szabo and Csak, 2012).***

## *4. Gut-liver axis*

In recent years, many studies have been carried out on gut-liver axis (GLA) dysfunction (including intestinal dysbiosis, bacterial overgrowth, and alteration of mucosa permeability) intending to find the possible therapeutic target of NAFLD ***(Rotman and Sanyal, 2017).***

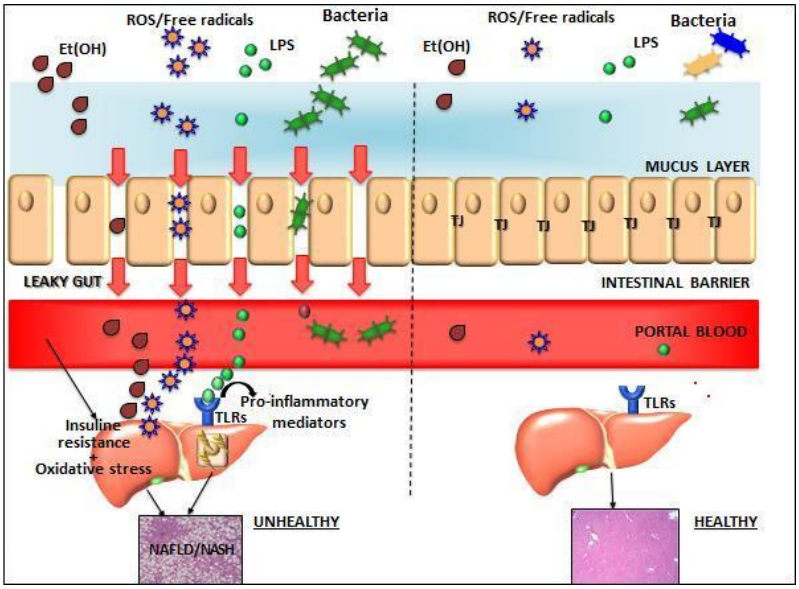


**Figure (4):** Gut–liver axis components in normal conditions (right part) and in non-alcoholic fatty liver disease (NAFLD)(left part).The presence of the dysbiotic microbiome and an altered intestinal barrier influenced by bacterial ethanol (EtOH), possibly associated with disruption of tight-junctions (TJs) (" leaky gut "), promotes the translocation of several bacterial products into the portal circulation. The interaction of bacterial products with toll-like receptors (TLRs) on the hepatic cell surface promotes the progression from simple steatosis to inflammation and fibrosis of the liver. ROS: reactive species of oxygen; NASH: non-alcoholic steatohepatitis; LPS: lipopolysaccharide; TLR: toll-like receptor.