Chapter 1

Introduction
1.1 Non-Alcoholic Fatty Liver Disease (NAFLD)

In 1980, Ludwig J [1] pronounced the term Non-Alcoholic SteatoHepatitis (NASH). Later on, the term ‘Non-Alcoholic Fatty Liver Disease’ (NAFLD) has been accepted which covers the full spectrum of fatty liver disease (Figure 1.1). It ranges from Non-Alcoholic Fatty Liver (NAFL, benign condition) to Non-Alcoholic SteatoHepatitis (NASH) which can result in fibrosis, liver cirrhosis, and, ultimately, hepatocellular carcinoma [2]. Overall, NAFLD is highly associated with risk factors such as obesity, insulin resistance, diabetes and hyperlipidemia [3]. However, the causes of steatohepatitis should be excluded such as alcohol induced steatohepatitis or diseased induced steatohepatitis (e.g. hepatitis C, Wilson’s disease, lipodystrophy, abetalipoproteinemia, Reye’s syndrome etc.) or drug-induced steatohepatitis such as antiviral drugs, amiodarone, corticosteroids, tamoxifen, and tetracycline [4-6].

1.1.1 Non-Alcoholic Fatty Liver (NAFL)

NAFLD starts with the accumulation of fat in the liver tissue often called simple hepatic steatosis or Non-Alcoholic Fatty Liver (NAFL). In this benign condition, liver functions are normal and the liver also looks normal under the microscope except excess amount of fat gets accumulated in the vesicles that displaces cytoplasm. NAFL is often associated with two conditions: macrovesicular steatosis (large fat vesicles) or microvesicular steatosis (small fat vesicles) in the liver. Generally, NAFL is defined as presence of hepatic steatosis (fat accumulation > 5% of hepatocytes) with no evidence of hepatocyte injury [5, 7].

1.1.2 Non-Alcoholic SteatoHepatitis (NASH)

NASH is the most severe form of NAFLD and is characterized by the presence of hepatic steatosis, oxidative stress, inflammation, and hepatocyte injury with or without fibrosis. The pathogenetic processes of NASH and its progression are
multifactorial in nature. NASH is highly prevalent in patients with diabetes, obesity, insulin resistance, hyperlipidemia and hypertension. Therefore, NASH can be considered as a hepatic manifestation of metabolic syndrome [7, 8].

1.1.3 NASH Associated with Fibrosis and Cirrhosis

NASH further progresses into hepatic fibrosis and its end stage i.e. Cirrhosis. Fibrosis is the initial stage of the formation of scar tissue in the liver. Due to chronic hepatic inflammation and injury, Hepatic Stellate Cells (HSCs) get activated and cause accumulation of Extra Cellular Matrix (ECM) rich in collagen particularly Type 1 and Type 3 collagen. Because of continuous accumulation of scar tissues causing loss of sinusoidal fenestrations, it further leads to perisinusoidal fibrosis [9]. As this cascade of processes continues, fibrous tissue bands separate the hepatocyte nodules ultimately producing liver dysfunction associated with portal hypertension, liver failure, and increased risk of hepatic carcinoma. Cirrhosis is an irreversible disease for which no treatment option is available except liver transplantation [10].

Figure 1.1 Full spectrum of Non-Alcoholic Fatty Liver Disease (NAFLD)
(Adapted from a web source [11], % indicates affected population)
1.1.4 Epidemiology

NAFLD affects up to 30% of the general population and is highly prevalent in 70-80% of individuals who are obese and diabetic and 50% of individuals with dyslipidemia [5]. In the United States, approximately 30% adults and ~10% of children are estimated to have NAFL and with biopsy proven NASH is 3 to 5% [12-14]. In India, the prevalence of NAFLD disease is around 9% to 32% of general population with higher incidence rate in obese and diabetic patients [15]. It has been estimated that 15 to 25% of NASH patients may develop liver cirrhosis over 10 to 20 years. Moreover, NAFLD is the third most common reason for liver transplants in the United States and it is believed that NASH will become the leading cause for liver transplantation by 2020 [16, 17].

1.1.5 Unmet Medical Need

Currently, there is no approved therapy for the treatment of NASH, although weight loss is recommended. Several pharmacotherapies have attempted to treat NASH, with limited benefit overall [18]. The global burden of NASH is increasing every year. Due to its high prevalence and lack of approved treatments, there remains a strong unmet medical need for therapeutic intervention. Therefore, the NASH market is currently deserted and awaits its first NASH-specific therapy in patients.

1.2 Pathophysiology of NASH

Initially, the “two hit” hypothesis theory has been proposed in the pathogenesis of NASH. The first hit includes the accumulation of fat into the hepatic tissues whereas the second hit includes oxidative stress and inflammation with hepatocyte injury. However, recently “multiple parallel hit” theory has been proposed in the development of NASH [3].

1.2.1 Hepatic Steatosis
NASH is more frequently associated with metabolic syndrome disorder consisting of diabetes, obesity, insulin resistance and dyslipidemia. The buildup of fat in hepatic tissues could be either due to increased uptake of fatty acids, increased \textit{de novo} lipogenesis and decreased fatty acid oxidation or decreased triglyceride secretion from the liver [19]. It has been reported that mostly free fatty acids (FFAs) which are released due to increased lipolysis of adipose tissues or dietary fat via chylomicron metabolism or \textit{de novo} lipogenesis lead to fat accumulation in the liver of NASH patients. Furthermore, in physiological conditions, the FFAs gets either oxidized at mitochondrial sites or exported from liver through very low density lipoprotein (VLDL) assembly into systemic circulation. But, the fatty acid oxidation and delivery of triglyceride to circulation has been reduced in NASH patients [19, 20].

\textbf{1.2.2 Insulin Resistance}

Insulin resistance plays a central role in the progression of NAFL to NASH. It is well known that reduced insulin sensitivity was observed in muscle, adipose tissue and in liver of NASH patients [7]. In obese patients, insulin is unable to show anti-lipolytic effect in adipose tissues and further leads to release of FFA which then deposit in the hepatic tissues. Due to continuous release of FFA from lipolysis of adipose tissue, this causes accumulation of lipid in the liver finally manifesting as NAFL i.e. a condition called as non-alcoholic fatty liver. Moreover, this accumulated lipid generates lipo-toxic metabolites such as diacyl glycerol (DAG) and ceramide which in turn cause hepatic insulin resistance [21].

\textbf{1.2.3 Inflammation and Oxidative Stress}

The second hit includes oxidative stress induced lipid peroxidation and activation of inflammatory pathways involved in the progression of NAFL to NASH. It has been reported that FFAs cause downstream activation of JNK and IKK complex via TLR
receptor activation and further lead to activation of NF-κB, where it expresses multiple cytokines such as TNF-α, MCP-1 and adhesion molecules. JNK activation can also lead to hepatic insulin resistance via phosphorylation of IRS-1 and IRS-2 [8]. In the context of obesity, adipocytes secrete adipokines such as TNF-α and IL-6 that further plays a critical role in inflammation and insulin resistance. In addition, some of the intracellular lipo-toxic metabolites cause generation of ROS through β-oxidation of FFAs. Multiple pro-oxidants have been proposed such as uncoupling of oxidative phosphorylation to release reactive oxygen species at mitochondrial sites or from endoplasmic reticulum (induction of cytochromes P450 [CYP] 2E1 and 4A) or from peroxisomes and inflammatory cells (NADPH oxidase) in the progression of NASH [8, 22].

1.2.4 Multiple Parallel Hit Theory

The pathogenesis of NASH was explained using the “two hit theory” for a number of years [21]. According to this theory, in the setting of steatosis alone (i.e. NAFL), a second hit from other factors such as oxidative stress, was required for the development of NASH. This theory is now outdated and the multiple hit theory has been propounded to explain the progression of NASH which suggests that a myriad collection of insults potentially contribute to the development of this disease. Recently a genome-wide association study (GWAS) identified a novel triglyceride metabolism gene i.e. patatin-like phospholipase 3(PNPLA3) to be associated with development of NASH [23]. Another gene polymorphism for e.g. variant in glucokinase regulatory protein GCKR rs780094 C>T has been associated with the severity of liver fibrosis and with higher serum triglyceride levels [24]. Additionally, the role of gut microbiome has also been proposed in the progression of NASH. Enteric bacteria inhibited the synthesis of fasting-induced adipocyte factor (Fiaf) amplifying
lipoprotein lipase (LPL) activity which further promoted the accumulation of triglyceride in hepatic tissues. Macrophages also played an important role in the induction of inflammation and insulin resistance [3]. It has been reported that, in obesity, due to increased gut permeability and bacterial overgrowth, it may lead to excess production of LPS or fatty acids or danger signals that ultimately may contribute to hepatic steatosis and inflammation. Furthermore, a variety of pattern associated molecular patterns and danger-associated molecular patterns are involved in the pathogenesis of NASH [25]. Therefore, the multiple parallel hit theory explains the pathogenesis of progression of NAFL to NASH, considering numerous simultaneous hits derived from different sources that promote hepatic injury, oxidative stress, inflammation, fibrosis and finally end stage liver disease as shown in Fig. 1.2.4.

![Multiple Parallel Hit Theory](image)

Figure 1.2.4 Multiple Parallel Hit Theory. Ref: Takaki A. et al. 2014 [3]
1.2.5 Fibrosis

It has been reported that lipotoxicity leads to hepatocyte cellular injury resulting in cell death and necrosis (necro-inflammation) in the progression of NASH. Chronic necro-inflammation in liver disease often caused induction of fibrosis considered as “third hit” in the pathogenesis of NASH associated fibrosis. Fibrosis is mediated by activated hepatic stellate cells (HSCs) and myofibrillar cell infiltration of the liver. These cells are involved in the excessive production of extra cellular matrix (ECM), mostly in the form of collagen which is a principle component of scar tissues [26]. It is well known that hepatic steatosis and lipid peroxidation products have been involved in the production of ECM via TGF-β signaling in the development of hepatic fibrosis [27].

1.2.6 Diagnosis of NASH

Most patients with NASH are asymptomatic; although some common symptoms were observed such as right upper quadrant pain, fatigue, malaise and hepatomegaly on physical examination. Various non-invasive tests based on laboratory, clinical and radiological tests have been developed for the identification of steatosis and fibrosis in NASH patients. Liver function tests (LFT) were most commonly used which measure Alanine Transaminase (ALT), Aspartate Transaminase (AST) and γ-glutamyltranspeptidase (γGT) levels indicating presence of hepatic injury in NASH patients. In addition, some imaging techniques such as ultrasonography, Magnetic Resonance Imaging (MRI), and Computed Tomography (CT) have been used for fatty liver imaging in NASH patients. However, liver biopsy remains the gold standard method for the diagnosis of severity of NASH [5, 28].
1.3 References


