Non alcoholic fatty liver disease (NAFLD) is a fatty liver disease occurring in patients without alcohol consumption. It includes a broad spectrum of liver disease, from fatty infiltration, inflammation and fibrosis, to cirrhosis, usually having obesity, hyperlipidemia and diabetes mellitus as its etiology (Tang C P, et al. 2006). Fatty liver can be broadly classified as due to alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD). Due to its close relation with obesity, insulin resistance and diabetes mellitus, NAFLD is now considered as the hepatic manifestation of the metabolic syndrome (de Alwis NM, et al. 2008). Ludwig first described patients who had histological features identical to alcoholic hepatitis but who had no history of alcohol abuse and he added that they did not have AST higher than ALT. He introduced the term “nonalcoholic steatohepatitis” (NASH) to describe this entity that was more common in women than men and usually was associated with diabetes and/or obesity (Ludwig J, et al. 1980).

Non alcoholic fatty liver disease (NAFLD) also describes a clinicopathological condition that is characterized by significant lipid deposition in the hepatocytes of the liver parenchyma in patients with no history of excessive alcohol consumption. The spectrum of this disease is broad, ranging from a simple steatosis to non alcoholic steatohepatitis, fibrosis and cirrhosis. Obesity, insulin resistance and diabetes are well known
risk factors for the development of a fatty liver (Matteonica et al 1999; Kim H J, et al. 2004; Greenfield, et al. 2008). Non-alcoholic fatty liver disease is considered to be the hepatic manifestation of metabolic syndrome and includes number of abnormalities including insulin resistance, visceral obesity, dyslipidemia, diabetes, high blood pressure, plus several additional factors. It is the foremost cause of elevated liver function test (LFT) results after excluding other common causes (Angulo P, et al, 2002). Non-alcoholic fatty liver disease (NAFLD) is an entity that includes patients with simple steatosis (SS) and non-alcoholic steatohepatitis (NASH), which has the propensity of progressing to cirrhosis and hepatocellular carcinoma (Sanyal AJ, et al. 2002). The confirmation of NAFLD includes the presence of hepatic steatosis and absence of alcohol abuse i.e. regular consumption of >20 g ethanol per day (Neuschwander-Tetri BA, et al. 2003).

Normal liver fat content is <5% of the liver by weight, mostly in the form of triglycerides. The liver is said to be fatty when more than 5–10% of hepatocytes show steatosis histologically (Cairns SR, et al. 1983). The liver derives its fat from various sources, including diet, circulating free fatty acids (FFA), and de novo lipid synthesis in regulated proportions that vary with prandial status of the individual (Donnelly KL, et al. 2005). A “two-hit” model of pathogenesis has been proposed, encompassing two sequential events: (1) hepatic fat accumulation and, (2) hepatic oxidative stress. The oxidative
stress acts upon the accumulated hepatic lipids, resulting in lipid peroxidation and the release of lipid peroxides, which can produce reactive oxygen species (Farrell GC, et al. 2006). Moreover, the visceral adiposity index which uses BMI, waist circumference, and the levels of triglycerides and high-density lipoprotein cholesterol, is capable of indicating both fat distribution and function. This is associated with liver steatosis among chronic hepatitis C as well (Petia S, et al. 2010).

Steatosis with or without non-specific inflammation is generally a stable condition without significant clinical problems. In contrast, NASH is a condition in which there is hepatocyte injury that may progress to cirrhosis in 10% to 20% of cases. The main components of NASH are hepatocyte ballooning, lobular inflammation, and steatosis. The precise mechanisms of steatosis and hepatocellular damage in NAFLD are not entirely known, but genetics and environment play a role in the pathogenesis (Yeh MM, et al. 2007).

Most early studies had emphasized that NAFLD was more common in women. Female hormones seem to have a protective effect as the disease is more prevalent in post-menopausal as compared to premenopausal females. (Carulli L, et al. 2006). Although NAFLD can occur at all ages the prevalence increases with age. Most Asian studies have shown the condition to be more common in the 4th and 5th decades of life. A bimodal age distribution is also
observed with the peak prevalence in men occurring earlier (40–49 years) than in women (over 50 years), explaining the male predominance in younger populations. Development of insulin resistance with increasing age could be one of the etiological factors (Chen CH, et al. 2006; Tsang SW, et al. 2006; Kaneda H, et al. 2006; Duseja A, et al. 2005; Khurram M, et al. 2007). American studies have shown a higher prevalence of NAFLD among East Asians and those from the Indian subcontinent as compared to Caucasians (Browning JD, et al. 2004).

The confirmation of NAFLD includes the presence of hepatic steatosis and absence of alcohol abuse i.e. regular consumption of >20 g ethanol per day. Majority of NAFLD subjects are symptomatic, although mostly these are non-specific and unreliable for accurate diagnosis (Smith BW, et al. 2011; Lewis JR, et al. 2010). However, these include hepatomegaly, general malaise, abdominal discomfort, vague right upper quadrant abdominal pain, nausea, and other non-specific symptoms referred to the gastrointestinal tract. Few patients' presents with steatohepatitis-related cirrhosis may present with ascites, splenomegaly, spider angiomas, palmar erythema, caput medusae, and jaundice etc (Smith BW, et al. 2011; Lewis JR, et al. 2010).

Non-alcoholic fatty liver disease (NAFLD), accounting for asymptomatic elevation of aminotransferase levels in up to 90 per cent of cases, is the most frequent cause of abnormal liver function tests results
Introduction

Mild to moderate elevation of serum aminotransferases (ALT and AST) is the most common and often the only laboratory abnormality found in patients with NAFLD (Angulo P, et al. 2002). Serum alkaline phosphatase may also be slightly elevated in about one-third of patients. Hyperbilirubinemia, hypoalbuminemia and prolongation of the prothrombin time are noted infrequently and generally only seen once liver failure has become established. Elevated serum lipid profiles and glucose concentrations are also common in NAFLD patients, reported in 25 to 75% of cases (Sheth SG, et al. 1997). Serum levels of gamma-glutamyltransferase (GGT) are frequently elevated in patients with NAFLD and correlates with increased risk for mortality (Haring R, et al. 2009). This is also an excellent predictor of advanced fibrosis in NAFLD patients; with a sensitivity of 83% and specificity of 69% (Tahan V, et al. 2008).

Patients with type II diabetes mellitus (T2DM) who have non-alcoholic fatty liver disease (NAFLD) often have an atherogenic lipoprotein profile of high triglycerides (TG) and lower high-density lipoprotein cholesterol (HDL-C) (Kelley DE, et al. 2003). Non-alcoholic fatty liver disease is very common in patients with type II diabetes mellitus (DM) with majority of patients with DM having evidence of fatty liver on imaging. Prevalence of NAFLD in diabetics was 74% in a North American study and 70% in an Italian study (Targher G, et al. 2007; Williams CD, et al. 2011). It is found that 35 out of 40 (88%) non
alcoholic patients with DM in India had evidence of fatty liver on ultrasound (Duseja A, et al. 2004). Prashanth and colleagues studied 204 type II diabetic patients in Mumbai, India in 2009 and reported that 87% of them had NAFLD on histologic findings (Prashanth M, et al. 2009). In a study from India, the prevalence of fatty liver in patients with prediabetes and diabetes was 33% and 55%, respectively (Mohan V, et al. 2009). Other studies from India have shown the prevalence of NAFLD in diabetics to vary from 57% to 64% (Sanyal D, et al. 2009; Agarwal AK, et al. 2011). Recently, it was reported that there is a near-universal association of nonalcoholic steatohepatitis (NASH) and insulin resistance, irrespective of obesity (Chitturi S, et al. 2002).

Insulin resistance (IR) plays the central pathogenetic role in both type II diabetes mellitus and NAFLD with the being considered as the hepatic manifestation of the metabolic syndrome (Marchesini G, et al. 2001). Recent studies have pointed to hyperinsulinemia and insulin resistance as pathogenic factors in NAFLD using the homeostasis model assessment (HOMA) method. Insulin resistance was the laboratory finding most closely associated with the presence of NAFLD in a large series of patients, irrespective of BMI, fat distribution, or glucose tolerance. Accordingly, NAFLD might represent another feature of the metabolic syndrome, with decreased insulin sensitivity being the common factor (Marchesini G, et al. 1999; De Fronzo RA, et al. 1991). The strong association of NAFLD with other features
of the metabolic syndrome (obesity, central fat distribution, diabetes, dyslipidemia, hypertension, and atherosclerotic cardiovascular disease) supports this hypothesis (Cortez-Pinto H, et al. 1999). Other non-invasive markers such as Homeostasis Model Assessment-Insulin (HOMA) Resistance level ≥3.0 and type IV collagen 7S ≥5.0 ng/ml were found to be more sensitive and specific for predicting the relevant diagnosis (Shimada M, et al. 2007). NAFLD covers a wide spectrum of hepatic lesions including simple fatty infiltration of the liver, steatohepatitis with necroinflammatory changes and a variable degree of fibrosis which may finally progress to liver cirrhosis. Fatty liver is now believed to be an integral part of the metabolic syndrome, since it has been shown to be independently related to insulin resistance independent of obesity and abdominal adiposity (Seppala-Lindroos A, et al. 2002). The pathogenesis of NAFLD is multi-factorial and it has been suggested that the presence of insulin resistance (IR) is essential for the accumulation of hepatocellular fat (Neuschwander-Tetri BA, et al. 2003).

A recent multicenter study from Japan showed that the prevalence of NAFLD increased linearly with increasing BMI, with BMI < 23 Kg/m2 having a prevalence of 10.5% while BMI > 28 Kg/m2 had prevalence of 84.2% (Eguchi Y, et al. 2012). In obese individuals, the increased supply of FFA to the liver from the diet, from adipose tissue, and through increased de novo lipogenesis all serve to promote hepatic steatosis. Obesity also predisposes to IR. The
mechanism behind this phenomenon is complex and is related to an excess of circulating FFA, an abundance of pro-inflammatory cytokines with a relative or absolute deficiency of anti-inflammatory cytokines, which is governed by the hormonally active adipose tissue, as discussed in subsequent sections.

The role of obesity in the pathogenesis of NAFLD is supported by results of interventional trials that have shown that weight loss, whether by lifestyle interventions or pharmaceuticals, improves hepatic steatosis and inflammation in obese individuals (Promrat K, et al. 2010; Lazo M, et al. 2008; Harrison SA, et al. 2009; Zelber-Sagi S, et al. 2006).

Obesity might be seen in approximately 71% of patients with NAFLD. Obesity and insulin resistance are crucial for the pathogenesis of NAFLD (Wanless IR, et al. 1990; Marceau P, et al. 1999). NAFLD is more prevalent among obese subjects and also in patients with type II diabetes independently of degree of obesity (Gastaldelli A, et al. 2007). The prevalence increases to 57% in obese subjects, 70% in diabetic subjects and 90% in morbidly obese people. On the other hand NASH may be present in up to 3% of the general population and in up to two thirds of individuals with morbid obesity and/or type II diabetes (Bellentani S, et al. 2000; Chalasani N, et al. 2012). Majority (57–74%) of obese individuals have NAFLD and 15–20% have NASH compared to 10–24% and 3–4%, respectively in the general population. Using Asia Pacific criteria in more than 100 Indian patients with NAFLD, we found
overweight and obesity in 20% and 68% of patients, respectively. Indian patients with nonalcoholic fatty liver disease presenting with raised transaminases are different at presentation (Duseja A et al 2007). Differences in insulin resistance patterns, body mass index and also the presence of HFE gene mutation could explain this disparity (Duseja A, et al. 2005). Non-alcoholic fatty liver disease is considered a disease of modernization and affluence and has been linked to the epidemic of diabetes and obesity (Prashanth M, et al. 2009; Ong JP, et al. 2005). Globally, the prevalence of NAFLD varies in the general adult population between 10% and 24.0%, with higher estimates (up to 74.0%) among those who are obese (Angulo P, et al. 2002).

It has recently been demonstrated that the risk of hepatic steatosis increases exponentially by the addition of each component of metabolic syndrome: type II DM, hyperlipidemia, visceral obesity and hypertension (Garcia-Monzon C, et al. 2000; Ratziu V, et al. 2010). NAFLD is strongly associated with obesity, type II diabetes mellitus and hyperlipidema. Many studies show that it is hepatic component of metabolic syndrome (Angulo P, et al 2002; Hanley A, et al. 2005). The central features of NAFLD are peripheral insulin resistance, obesity, hyperinsulinemia, hypertriglyceridemia and hypertension. It has been reported that fatty liver influences the severity of hepatic insulin resistance in type II diabetes mellitus (Angelico F, et al.
The prevalence is reported higher among patients with diabetes mellitus and obesity ranging from 35% to 75% in various studies (Gupte P, et al. 2004).

The NAFLD liver fat score is one such panel marker that is a combination of many variables: Presence of metabolic syndrome and type II diabetes mellitus (T2DM), fasting serum insulin, serum AST, and the AST/ALT ratio. Using these variables, a value of area under curve (AUC) between 0.86 and 0.87 is sufficient to predict NAFLD. An addition of presence of a genetic variant in the PNPLA3 gene (rs738409) increases accuracy of the prediction. Another marker includes a liver fat equation that estimates the percentage of liver fat content to predict liver fat (Kotronen A, et al. 2009). NASH affects men and women equally and the condition is strongly associated with obesity and the other components of the metabolic syndrome, such as dyslipidemia, hyperinsulinemia and insulin resistance. It is estimated that more than 70% of obese individuals have some form of NAFLD. It is the most common cause of so-called cryptogenic cirrhosis, namely cirrhosis of “unknown” origin. NAFLD contributes to the progression of other liver diseases such as HCV infection and HCC. The epidemic of obesity in the United States heightens concern that NAFLD will increase in prevalence. Obesity, type II (non-insulin dependent) diabetes mellitus and hyperlipidemia, alone or in combination, are co-morbid conditions frequently associated with NAFLD (James O, et al.
1999). There is a direct correlation between the degree of obesity and prevalence and severity of NAFLD. The prevalence of NAFLD increases by 4.6 fold in obese people. With any degree of obesity, type II diabetes mellitus significantly increases the prevalence and severity of NAFLD (Silverman JF, et al. 1990; Wanless IR, et al. 1990; Ballentani S, et al. 2000).

Another inflammatory marker, High-sensitivity C-reactive protein (hs-CRP) has given mixed results as a biomarker while interleukin-6 (IL-6) has been shown to distinguish differing disease states and independently correlates with fibrosis (Tarantino G, et al. 2009; Lemoine M, et al. 2009). Higher levels of leptin, a hormone secreted from adipose tissue, has also been shown to be correlated with liver histology. This in combination with HOMA distinguished differing disease states such as NAFLD and NASH (Lemoine M, et al. 2009). High-sensitivity C-reactive protein (hs-CRP) is an acute-phase reactant and a non-specific marker of low-grade inflammation. It has been associated with conditions related to the metabolic syndrome and arteriosclerosis (Ridker PM, et al. 2003; Duncan BB, et al. 2003). Serum levels of High-sensitivity C-reactive protein (hs-CRP) are usually elevated in obesity, dyslipidaemia and hyperglycaemia, all features of the metabolic syndrome (Frohlich M, et al. 2000). High-sensitivity C-reactive protein (hs-CRP) is one of the major acute phase proteins and is a marker of systemic inflammation. In contrast to regular hs-CRP assays, a high sensitivity CRP (hs-CRP) assay
enables the diagnosis of even low grade inflammation. It has important clinical and prognostic implications in cardiovascular disease. However, hs-CRP could be a promising biomarker for screening of NAFLD asymptomatic subjects independently of other metabolic disturbances associated with metabolic syndrome and cardiovascular risk (Lizardi-Cervera J, et al. 2007).