

CHAPTER II

REVIEW OF LITERATURE

An exhaustive English language literature search was undertaken in Pubmed database and Cochrane library to collate information about the overall scenario of non-alcoholic fatty liver disease and *Tinospora cordifolia* for literature retrieval without any restriction over the time frame. Additionally, the references of the referred research papers were also looked into for more information. This section is presented under the following heads:

- Non-alcoholic fatty liver disease (NAFLD)
- Origin of NAFLD
- Global prevalence of NAFLD
- NAFLD from the gender and ethnicity perspective
- Natural history of progression of NAFLD
- Risk factors of NAFLD
- Type 2 diabetes as a major risk factor for NAFLD
- NAFLD: Metabolic syndrome from the hepatic dimension
- Diet in the pathogenesis of NAFLD
- Pathogenesis of NAFLD
- Diagnosis of NAFLD
- NAFLD as a risk factor for morbidity and mortality
- Management and treatment of NAFLD
- Lifestyle modification in the management of NAFLD
- Nutritional management of NAFLD
- Management of dyslipidemia for preventing NAFLD
- Botanical plants in the management of dyslipidemia
- *Tinospora Cordifolia*: An underutilised medicinal plant
- Botanical and structural profile
- Phytochemical profile and chemical constituents
- Physiological functions
- Traditional usage and *Tinospora cordifolia* in modern medicine
- Safety profile

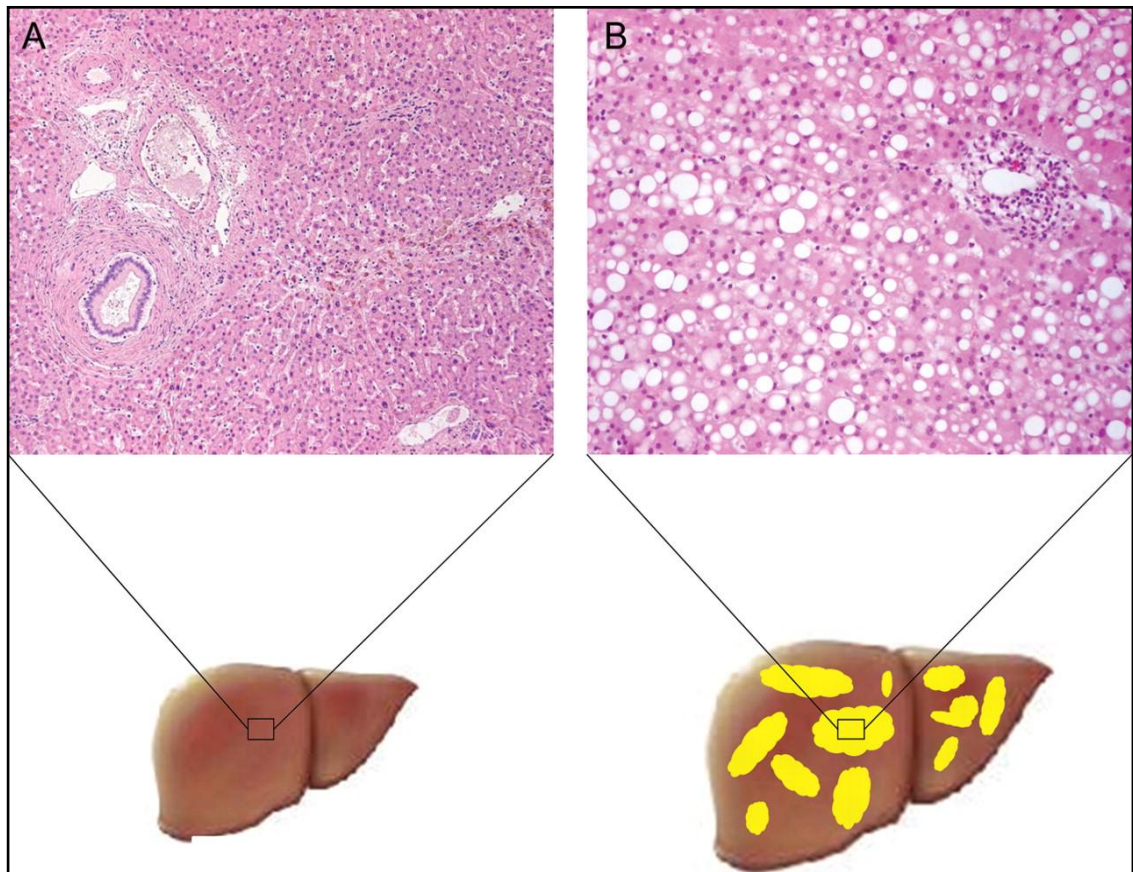
THE NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease (NAFLD) is defined as the entire spectrum of fatty liver disease in individuals without significant alcohol consumption, ranging from fatty liver to steatohepatitis and cirrhosis. The broad spectrum of NAFLD can be histologically categorised into two phenotypes (Matteoni et al., 1999); non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is defined as the presence of macrovesicular hepatic steatosis with no evidence of hepatocellular injury (Chalasani et al., 2012; Puri and Sanyal, 2012). It is a condition wherein triglyceride deposition occurs within the cytoplasm of hepatocytes, which exceeds 5% of the liver weight (Ratzliff et al., 2010; Angulo, 2007) or when more than 5% of the hepatocytes on histology (figure 2.1) have macrovesicular fat (Roden, 2006). NASH is defined as the presence of hepatic steatosis and inflammation (hepatitis) with evidence of hepatocyte ballooning with or without scarring or fibrosis (Chalasani et al., 2012; Puri and Sanyal, 2012; Clouston and Powell, 2004).

It is a state of ectopic fat accumulation in the hepatocytes (Byrne, 2012) which occurs as a natural corollary when adipocytes reach their saturation (Almeda-Valdés et al., 2009), marked by triglyceride infiltration (macro-vacuoles) and to some extent by fatty acids (micro-vacuoles) in the hepatocytes (Targher et al., 2010). It is an acquired metabolic stress related liver disease in the absence of ethanol intake, but has similar histological changes as that observed in alcoholic liver disease (AFLD) (Chalasani et al., 2012).

The spectrum of NAFLD is divided into four phases, namely, **type 1:** simple steatosis (no inflammation or fibrosis), that is non progressive in nature, **type 2:** steatosis with lobular inflammation but no fibrosis or balloon cells, maybe benign in nature, **type 3:** steatosis, inflammation and fibrosis of varying degrees (NASH), which may progress onto cirrhosis and **type 4:** steatosis, inflammation, ballooned cells, Mallory hyaline or fibrosis (NASH), which may progress onto cirrhosis and liver failure (Matteoni et al., 1999; Ahmed, 2015). Terms such as fatty-liver hepatitis, non-alcoholic Laennec's disease, diabetes hepatitis, alcohol-like liver disease and non-alcoholic steatohepatitis have been used to describe NAFLD (Angulo, 2002). Globally NAFLD is now the most common hepatic disease (Mavrogiannaki and Migdalis, 2013). However, it still

remains under-recognised and under-diagnosed (Mcavoy et al., 2006). It is postulated that NAFLD and NASH will affect the rich and the poor economies alike (LaBrecque, 2014).



Ref: Bhatia et al., 2012

FIG 2.1 LIVER HISTOLOGY IN NAFLD

A: Histological section of normal liver tissue

B: Simple steatosis, showing fat accumulation in hepatocytes

ORIGIN OF NON ALCOHOLIC FATTY LIVER DISEASE

In the 1960s, NAFLD was first described as a nutritional liver disease (MacDonald, 1962). A few years down the line, evidence of hepatic steatosis in the liver biopsy samples of obese patients undergoing or having undergone bariatric surgery was documented (Maxwell et al., 1977). Then, a couple of years later, the condition were described as obese diabetic liver disease (Adler and Schaffber, 1979). In 1980, the term NASH was coined after biopsy findings of 20 patients revealed histological changes similar to alcoholic hepatitis, but in the absence of alcohol intake (Ludwig et al., 1980). That time, it was a poorly understood condition. But, an inference was drawn that it was a disease associated with obesity, accompanied by diabetes mellitus, presence of hepatomegaly and mild abnormalities of liver enzymes (Firneisz, 2014). Then, in the late 1990s, the term NAFLD was coined to include the entire histological spectrum of the fatty liver disease (Matteoni et al., 1999).

GLOBAL SCENARIO OF NON-ALCOHOLIC FATTY LIVER DISEASE

The myth that NAFLD is a disease of the Western world is broken as NAFLD now spans countries globe across. Hence, it is not a disease only of the Western world anymore (Angulo, 2002; Williams, 2006). It is about time that NAFLD be treated as a global public health problem of a pandemic nature (Farrell, 2003).

Prevalence

Worldwide, the assumed prevalence of NAFLD in the general population ranges from 6.3% to 33%, with a 20% median and the estimated prevalence of NASH is notably lower, at 3% to 5%, based on different modes of assessment. There are issues with defining the correct prevalence of NAFLD as the definitions, diagnostics used and the population studied, varies widely (Vernon et al., 2011). NAFLD is a global problem accounting for 10% of the world's population (Oprea-Călin et al., 2014). In the past two decades, the prevalence of NAFLD has doubled whereas that of other chronic liver diseases has either remained stable or even decreased (LaBrecque, 2014).

Recent data highlights that in the Western population, the prevalence of NAFLD ranges from 20-30% (Everhart and Bambha, 2010), being the most common hepatic

disorder of the Western world (LaBrecque, 2014) and in the Eastern population the prevalence ranges from 10-20% (Loomba and Sanyal, 2013).

Owing to an epidemic of the underlying risk factors for NAFLD and NASH (discussed later), the prevalence of NAFLD and NASH is going to increase worldwide (Dharel and Fuchs, 2014; Caceasne, 2012). Though the prevalence of NAFLD is on a rise, most cases are unrecognised (Bhatia et al., 2012) owing to its asymptomatic nature (Papandreou and Andreou, 2015). The future public health costs on management of NAFLD holds a potentially huge burden (Bhatia et al., 2012).

Incidence

The data on incidence of NAFLD/ NASH varies widely and is often under-reported (Vernon et al., 2011). The global incidence of NAFLD is on the rise owing to a surge in the prevalence and incidence of metabolic syndrome (MS) (Koehler et al., 2012) and due to a rise in the incidence of the other predisposing factors for NAFLD, such as obesity and type 2 diabetes (Dharel and Fuchs, 2014). Even though NAFLD is now the most common hepatic disease (Mavrogiannaki and Migdalis, 2013), data is still lacking on its incidence.

NON-ALCOHOLIC FATTY LIVER DISEASE FROM THE GENDER AND ETHNICITY PERSPECTIVE

NAFLD/NASH has been observed in all the ethnic populations of the world and in both the genders (Lam and Younossi, 2010). However, according to preliminary evidence, Hispanics have increased prevalence of NAFLD even at lower degrees of obesity (Puri and Sanyal, 2012). They are found to be having a significantly higher prevalence of NAFLD as compared to non-Hispanic whites and non-Hispanic blacks (Browning et al., 2004; Kallwitz et al., 2008; Wagenknecht et al., 2009). The plausible association could be high prevalence of obesity and insulin resistance (IR) in cases of NAFLD amongst Hispanics (Browning et al., 2004).

In a study involving newly diagnosed NAFLD patients, the prevalence of NAFLD was the highest in the Hispanics (45%), followed by Asians (18%) and the African Americans (3%) (Weston et al., 2005). However, the prevalence of NAFLD appears

to be lower amongst the American-Indians and Alaskan native populations. But, it is thought to be an underestimated prevalence owing to lack of histological definition (Fischer et al., 2009; Bialek et al., 2008).

In a United States cohort, Hispanics followed by Caucasians and African Americans, had the highest risk of NAFLD and NASH. Gender also emerged to be risk factor for NAFLD with males having a higher predisposition (Williams et al., 2011). In the third National Health and Nutrition Examination Survey, Mexican-Americans had higher prevalence of NAFLD compared to the non-Hispanic whites and non-Hispanic blacks (Lazo et al., 2013).

Like Hispanics, Asians too have an increased prevalence of NAFLD even at lower degrees of obesity (Puri and Sanyal, 2012) with approximately 1.8 million Asians with NASH in the Asia-Pacific region (Chitturi et al., 2004). The alarming fact is that the clinical and the metabolic profile of NAFLD patients in Asia mirror the profile of Western NAFLD counterparts (Fan and Peng, 2007) though Asians have a lower cut off for obesity diagnosis than the latter. Of the Asians, those from the Indian subcontinent are at higher risk of developing NAFLD (Dharel and Fuchs, 2014). Asian Indians develop central obesity instead of general obesity (Sawant et al., 2011) hence are more susceptible to NAFLD. It is postulated that in the near future, Asian countries will harbour a major reservoir of the NAFLD pool (Farrell, 2003; Fan and Peng, 2007).

However, ethnicity has not been established as a predictor of advanced fibrosis in NAFLD (Campos et al., 2008; Harrison et al., 2008). More research is required to understand the incidence of NAFLD based on ethnicity and geographics (Chalasani et al., 2012).

There is still no surety about which gender has a greater predisposition to NAFLD or NASH as studies are documented in favour of either of the both (Harrison et al., 2008; Palekar et al., 2006; Ong et al., 2005) or have been unable to predict an association (Campos et al., 2008; Wanless and Lentz., 1990; Abrams et al., 2004). But, the male gender is said to be associated with a greater risk for NAFLD and its advanced forms at any given body mass index (BMI) (Loomba et al., 2012). Based on evidence, a few conclusions arrived at are; men among the NAFLD patients would be more prone to

having deranged liver enzymes, be histologically diagnosed with NASH, hepatic fibrosis and also be having a higher overall mortality (Ong et al., 2008; Kallwitz et al., 2008). However, in patients with MS, the female gender is an independent risk factor for NASH (Sorrentino et al., 2004). A few studies have also cited female gender to be a risk factor for advanced fibrosis (McCullough, 2004; Harrison et al., 2008). A recent editorial stated that NAFLD affects men and women alike, irrespective of the gender differences in prevalence of risk factors (Dharel and Fuchs, 2014).

NATURAL HISTORY OF PROGRESSION OF NON-ALCOHOLIC FATTY LIVER DISEASE

Not much is known about the true natural history of NAFLD (Mcavoy et al., 2006). Population based studies may provide an insight about disease progression (Angulo, 2002). Of the little evidence available from a few cohorts of prospective and retrospective nature, the scenario of natural history of NAFLD appears to be as discussed below (fig 2.2).

Hepatic steatosis is said to be of benign nature which may remain so for a prolonged period of time without causing any major hepatic damage (Li et al., 2002; McCullough, 2002). The histological progression, if any, is said to be slow (Vernon et al., 2011; Gambino et al., 2011). However, it may progress onto advanced NAFLD owing to overload of the hepatic mitochondrial beta (β) oxidation system (Westphal, 2008). Prolonged hyperinsulinemia may also predispose hepatic steatosis patients to develop steatohepatitis (Palekar et al., 2006; Ruhl and Everhart, 2004). Moreover, accepting steatosis to be a benign state is difficult (Chalasani, 2008) as the documented cohorts have been of a relatively shorter duration and their small sample size makes it difficult to arrive at a conclusive remark (Dam-Larsen et al., 2004; Teli et al., 1995). It is postulated that 40% of the patients with hepatic steatosis, progress onto NASH (Vanni and Bugianesi, 2014).

Patients with NASH may progress onto cirrhotic stage of the disease (Vernon et al., 2011; Gambino et al., 2011) with a likely possibility of 20% progression in NASH patients (Vanni and Bugianesi, 2014). It may occur in one fourth of the patients with NASH (Matteoni et al., 1999), within a span of ten years (Ratzu et al., 2010). Once cirrhosis develops, the natural history of NAFLD is perhaps similar to other causes of

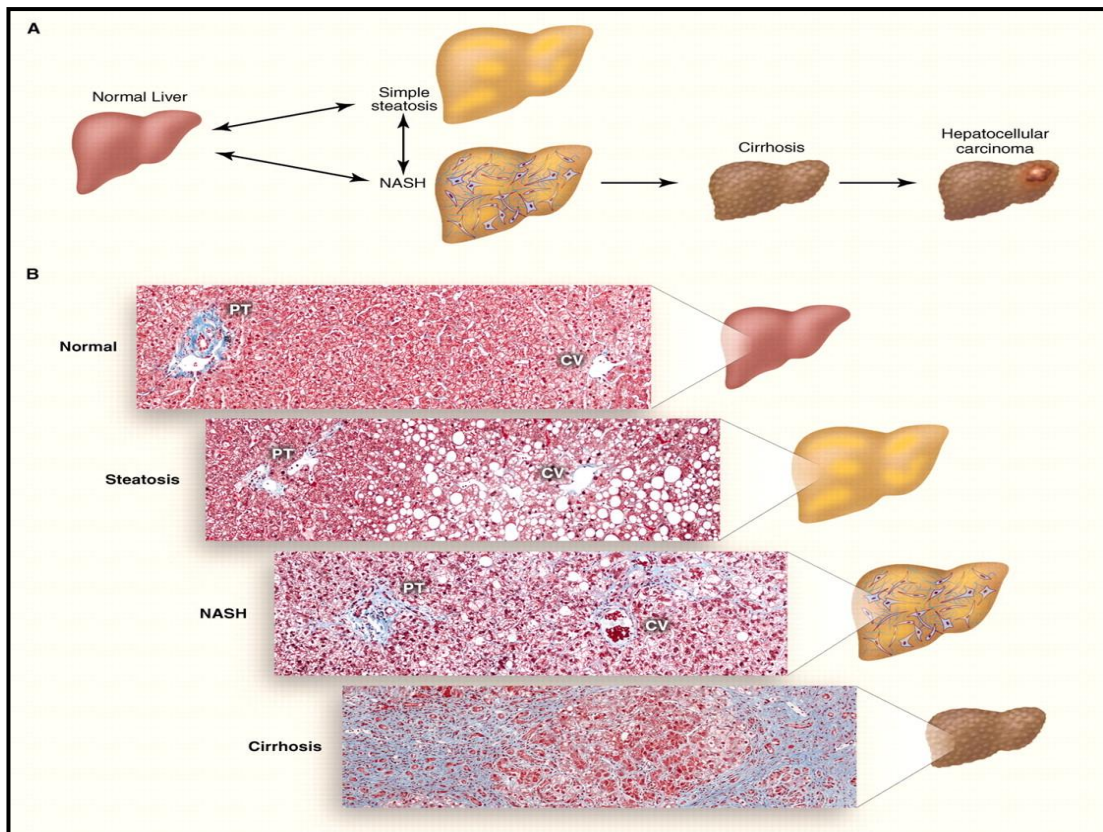
cirrhosis (Mcavoy et al., 2006). Cryptogenic cirrhosis maybe unidentified NAFLD in many of the patients as their clinical profile resembles much like that of the NASH patients (Tarantino et al., 2007) and many of the cases of cryptogenic cirrhosis might actually be cases of NAFLD/NASH (Yalamanchili et al., 2010). Burned out NASH is now used synonymously for cryptogenic cirrhosis (Caldwell and Crespo, 2004). The direct development from simple NAFL to cirrhosis has also been described (Ascha et al., 2010).

Of those with NASH, 30-40% develop liver fibrosis (Vanni and Bugianesi, 2014) and a previous review predicted a 40-50% probability of progression of NASH to fibrosis (Byrne, 2012). In a study on NAFLD patients, those with steatosis developed inflammation and ballooning and mild fibrotic changes, after five years of follow up (Pais et al., 2011).

NASH may also progress onto becoming a cause of liver failure in 3% of the NASH patients (Byrne, 2012). NASH related cirrhosis leads to either liver failure or liver related mortality in approximately one third of the cases (Ratziu et al., 2002; Hui et al., 2003).

An increased risk of hepatocellular carcinoma (HCC) is evident in NASH patients as they may progress to HCC without crossing the cirrhotic stage of liver disease (Vanni and Bugianesi, 2014). Though HCC maybe a rare complication in patients with NAFLD, it should not be under-estimated (Kikuchi et al., 2014). The risk of developing HCC is amongst those with advanced fibrosis and cirrhosis (Bugianesi et al., 2002; Hashimoto et al., 2009; Takuma and Nouse, 2010; Ascha et al., 2010). However, steatosis *per se* or the pathophysiological mechanisms of NAFLD have carcinogenic potential (Kikuchi et al., 2014).

HCC occurs in 11.3% of the cirrhotic NASH patients within five years (Yatsuji et al., 2009). About 15-50% of the cases of HCC are considered idiopathic, meaning, risk factors other than hepatitis C virus, chronic hepatitis B or alcoholic hepatitis maybe responsible for HCC development (Bruix and Sherman, 2005). Evidence has to it that these idiopathic causes are associated with NAFLD (Bugianesi et al., 2002). Alarminglly, in the coming decades, cases of HCC as a result of diabetesity will shoot up and those due to viral causes will roll down (Kikuchi et al., 2014).



Ref: Cohen et al., 2011

FIG 2.2: HISTOLOGICAL CHANGES ASSOCIATED WITH THE SPECTRUM OF NAFLD

(A) The accumulation of TG within lipid droplets in hepatocytes causes steatosis. Steatosis associated with inflammation, cell death, and fibrosis is referred to as NASH, which can progress to cirrhosis. Individuals with cirrhosis have an increased risk of hepatocellular carcinoma. (B) Histological sections illustrating normal liver, steatosis, NASH, and cirrhosis. Collagen fibers are stained blue with Masson's trichrome stain. The portal triad (PT), which consists of the hepatic artery, portal vein, and bile duct, and the central vein (CV) are shown.

SUMMARY

- NAFLD is an inclusive term for the entire spectrum of fatty liver disease, found in individuals not consuming significant amounts of alcohol.
- Histologically, NAFLD resembles much like the alcoholic fatty liver disease.

- It is an acquired metabolic stress related liver disease marked by ectopic fat deposition in the hepatocytes.
- NAFLD can be categorised into two major phenotypes; non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH).
- Despite being globally the most common hepatic disease, NAFLD remains under-recognised and under-diagnosed.
- The prevalence of NAFLD varies according to the definition and modality of diagnosis used and the population under consideration.
- About 20-30% Western world is affected by NAFLD vs. 10-20% in the Eastern population. Interestingly, both share similar NAFLD profiles.
- The prevalence of NAFLD has doubled whereas that of other chronic liver diseases has either stabilized or reduced in the past two decades.
- The surge in the incidence of risk factors for NAFLD poses a huge burden of the disease in the coming years and a major chunk of NAFLD patients will be from Asia.
- Asians have an increased prevalence of NAFLD at lower degrees of obesity.
- Those from the Indian subcontinent are at a higher risk of predisposition to NAFLD owing to development of central obesity than general obesity.
- Milder degrees of IR may trigger the causation of NAFLD, but not type 2 diabetes.
- Gender wise, there is still not much clarity about the predisposition to NAFLD.
- The natural history of NAFLD is unknown.
- Hepatic steatosis is taken to be benign, though uncertainty remains.
- Overloading of the mitochondrial beta oxidation system and prolonged hyperinsulinemia may predispose hepatic steatosis patients to advanced NAFLD.
- Many of the cases of cryptogenic cirrhosis maybe unidentified cases of NAFLD/NASH. Fibrosis and liver failure may also develop in those with NASH.
- There is also potential for the development of hepatocellular carcinoma in NAFLD cases, even without the development of cirrhosis.
- Idiopathic HCC has also been linked to NAFLD.

RISK FACTORS FOR NON-ALCOHOLIC FATTY LIVER DISEASE

Based on the causative factor, NAFLD can further be categorised into primary NAFLD and secondary NAFLD. Primary NAFLD has IR and metabolic derangements as the root cause of NAFLD (Kneeman et al., 2012). It is a term that is primarily reserved for the liver disease that is associated with obesity and the MS (Mehta and Younossi, 2012). The various risk factors that may contribute to primary NAFLD are; visceral obesity, atherogenic dyslipidemia, IR, type 2 diabetes, elevated triglycerides, low platelet count and the metabolic syndrome (Marchesini et al., 2003; Marchesini et al., 2001; Westphal, 2008). Secondary NAFLD occurs owing to non-insulin related factors (Kneeman et al., 2012; Nichols, 2013), which are;

- a. Nutritional / Intestinal: Jejunoileal bypass, gastroplasty for morbid obesity, extensive small bowel resection, total parenteral nutrition, rapid weight loss, bulimia, starvation and cachexia, marasmus, kwashiorkor, inflammatory bowel disease, jejunal diverticulosis with bacterial overgrowth.
- b. Drugs and toxins: Amiodarone, methorexate, tamoxifen, synthetic oestrogens, gluco-corticoids, nucleoside analogs, calcium channel blockers, perhexiline maleate, phosphorus, organic solvents, petrochemicals, dimethylformamide, rapeseed oil, copper toxicity.

The causes of primary NAFLD are discussed below as the focus of the current research was on primary NAFLD.

Insulin Resistance

IR is a condition wherein normal insulin secretions are unable to produce a normal glucose response. Thus, higher than normal insulin are required to attain the desired metabolic response (Ghamar-Chehreh et al., 2012). IR is seen as a universal phenomenon in NAFLD patients; be it in the obese, non-obese, diabetic or non-diabetic (Caceane, 2012). Though there is still not much clarity about the aetiology of NAFLD, IR is taken to be the major predisposing factor (Chitturi et al., 2002; McCullough, 2006; Bugianesi et al., 2005). However, the mist is yet to clear whether IR is a cause or a consequence of lipid accumulation/NAFLD (Conlon et al., 2013; Utzschneider and Kahn, 2006; Westphal, 2008) and which of the two occurs first, is still under debate (Mavrogiannaki and Migdalis, 2013).

Because IR is related to central obesity, there could be a plausible relationship between IR and NAFLD (Ahmed et al., 2012). IR at adipose and hepatic level along with reduced systemic insulin sensitivity is associated with hepatic steatosis (Gaggini et al., 2013) as it leads to enhanced gluconeogenesis, impaired response of insulin to suppress gluconeogenesis and impaired β oxidation of the fatty acids (Carey et al., 2005). IR is of two types; peripheral insulin resistance and hepatic insulin resistance (Fan and Peng, 2007).

Peripheral Insulin Resistance and Hepatic Insulin Resistance

Peripheral IR is defined as decreased insulin mediated glucose uptake by the peripheral tissues leading to decreased glucose utilisation by the skeletal muscles (Sanyal et al., 2001). Peripheral IR leads to steatosis and steatosis along with increased hepatic fatty acids leads to hepatic IR (Savage and Semple, 2010). Hepatic IR is defined as the inability of the insulin to retard endogenous glucose production in the hepatocytes (Fan and Peng, 2007). NAFLD occurs due to IR at the peripheral and the hepatic level that triggers triglyceride deposition by two pathways (Angulo, 2002);

a. Lipolysis: Peripheral IR leads to adipose tissue lipolysis that increases flux of free fatty acids (FFA) to the hepatocytes via the portal vein (Caceaux, 2012). The sensitivity of the insulin receptor is suppressed on the lipocyte membrane that leads to enhanced FFA in circulation (Angulo, 2002). IR down regulates insulin receptor substrate -1 (IRS-1) signalling by the excess FFA (Tarantino et al., 2007) and inhibits tyrosine phosphorylation of IRS-1 (Dey et al., 2005) which is a general mechanism for insulin action. Inhibition and accelerated dephosphorylation deactivates IRS-1 (Tarantino et al., 2007). Excess of FFA activates protein C kinase (PKC), which phosphorylates the serine residue on insulin receptor and IRS, which impairs tyrosine phosphorylation (Ruderman and Prentki, 2004), thus impairing insulin action.

b. Hyperinsulinemia: Prolonged IR triggers a state of hyperinsulinemia that induces hepatic lipogenesis by upregulating sterol regulatory element binding protein-1c (SREBP-1c) and blocks the mitochondrial β oxidation of fatty acids, subdues the production of apolipoprotein B-100 (Mavrogiannaki and Migdalis, 2013) and hence lowers the excretion rate of triglycerides as VLDL (Smith and Adams, 2011; Gaggini et al., 2013). This leads to an increase in the concentration of FFA (Angulo, 2002).

Peripheral IR worsens because of the ongoing production of FFAs (Sanyal et al., 2001). The FFA reduce the insulin signalling in a dose dependent manner and increase endogenous hepatic glucose and lipid production owing to stimulation of hepatic IR (Savage et al., 2007; Petersen et al., 2007). Moreover, the liver suffers further as hepatic IR leads to an uncontrolled production of endogenous glucose because glycogen synthesis is impaired and there is failure to suppress gluconeogenesis (Oprea-Călin et al., 2014). To add onto it, the impaired suppression of the endogenous hepatic glucose production and glucose uptake by the muscle tissue in conjunction with increased lipolysis in the adipose tissue contributes to storage of fatty acids in the hepatocytes (Caceane, 2012). This occurs because hepatic IR holds the key to determine the metabolic fate of triglycerides (Bugianesi et al., 2010). The increased IR sets up a vicious cycle between IR, increased fatty acids supply to the liver and increased hepatic steatosis and liver inflammation (Byrne, 2012). Eventually, the mitochondrial β oxidation system gets overloaded and the resultant detrimental effect is the production of free oxygen radicals that cause lipid peroxidation of the membranes of the hepatocytes (Angulo, 2002). End products of lipid peroxidation induce oxidative stress. This leads to hepatic inflammation owing to the cytokines. Inflammation acts as fertile ground for collagen formation that gives rise to fibrosis (Alavian, 2010).

Obesity

Obesity is the most commonly associated risk factor with the NAFLD having a significant contribution in its pathogenesis and its association with the entire spectrum of NAFLD (Chitturi et al., 2002). Hence, a rise in the prevalence of obesity is accompanied by a simultaneous rise in the prevalence of NAFLD as well (Sattar et al., 2014).

Prevalence of 58% NAFLD is observed amongst those who are overweight and it can shoot up to 98% amongst the obese who are not diabetic (Machado et al., 2006). Overall, the prevalence of NAFLD with respect to obesity varies between 30-100% (Ahmed et al., 2012). Obesity is associated with 4.6 times higher probability of having hepatic steatosis compared to those with a normal weight (Angulo, 2002; Larter et al., 2010). In obese subjects, 76% have NAFLD as compared to 16% with a normal BMI (Adams and Angulo, 2005). Morbid obesity is an even riskier condition

as 30% may have NASH and 12 – 25 % may have fibrosis (Gholam et al., 2007; Wong et al., 2010; Farrell et al., 2012). Severe obesity coupled with type 2 diabetes has shown a 100% prevalence of NAFLD (Angulo et al., 1999).

The distribution of fat mass maybe more relevant in the development of hepatic steatosis than the total adipose tissue *per se* (Zatloukal et al., 2004). For example, visceral fat increases the risk of developing NAFLD in obese as well as in non-obese individuals (van der Poorten et al., 2008). More of adipocytokines are released by the visceral adipose tissue (VAT) than the subcutaneous adipose tissue (Rector et al., 2008) and visceral or abdominal fat poses a greater risk for the development of IR and metabolic syndrome (MS) than the subcutaneous fat (Nseir et al., 2010). Moreover, visceral adiposity is a better predictor of deranged liver function tests and IR than general obesity (Kral et al., 1993). Additionally, visceral adipocytes are more resistant to insulin (Angulo, 2006). Lipolysis of the VAT owing to IR, leads to elevated FFAs flux into the portal vein for direct transport to the liver, known as the portal hypothesis, depicting the role of visceral adipocytes in the development of liver fat content (Bjorntorp, 1990). Visceral fat accumulation leads to decreased production of adiponectin, which is inversely related to IR and hepatic steatosis in NAFLD (Bugianesi et al., 2005). Moreover, a reduction in visceral adiposity is able to improve the IR (Huang et al., 2005).

To corroborate that, liver fat correlates with visceral adiposity, which is estimated through WC (Kotronen et al., 2008). Hence, visceral obesity is a major risk factor for NAFLD (Finelli and Tarantino, 2012). In a study conducted on 271 non-diabetic subjects, WC of both men and women showed the best correlation with hepatic fat (Kotronen et al., 2007). This stands valid also amongst those with a normal BMI but with abdominal obesity, in whom visceral adiposity or truncal adiposity contributes in the pathogenesis of NAFLD (Chitturi et al., 2002; Pagano et al., 2002).

Still, BMI is a predictor for hepatic steatosis, progression and pathogenesis of NASH (Caceane, 2012) and an independent predictor of the degree of fat infiltration (Angulo et al., 1999). There is a linear relationship between BMI and prevalence of NAFLD, resulting in higher prevalence of NAFLD among those with increasing BMI (Ahmed et al., 2012). Obesity is also an independent risk factor for the development of fibrosis (Festi et al., 2004) and HCC (Marrero et al., 2005). An increase in

adiposity is simultaneously accompanied by worsening of IR (Misra et al., 2007). Hepatic fat accumulation leads to hepatic IR (Marchesini et al., 2001; Marchesini et al., 2003; Marchesini et al., 1999; Petersen and Shulman, 2006).

Dyslipidemia

Dyslipidemia is defined as the presence of high triglycerides, low HDL-C, increase in small dense LDL particles, increased VLDL and elevated apolipoprotein B100 (Bhatia et al., 2012). NAFLD is associated with dyslipidemia (Chalasani et al., 2012) along with other features of the MS (Chatrath et al., 2012). The prevalence ranges from 20-92% and hypertriglyceridemia is the most common lipid abnormality (Angulo, 2002) observed in 64% of the NAFLD patients and low HDL-C in 30-42% (Day and James, 1998). In Indians, the prevalence of dyslipidemia is around 50% in NAFLD cases (Duseja et al., 2007).

The FFA are generated through three major sources; diet, endogenous synthesis and peripheral tissues. Metabolically, there are four possible pathways through which FFA may meet different fates; metabolization by β oxidation in the mitochondria, esterification and storage as triglycerides in lipid droplets, utilization to form other lipids or packaging with apoB into VLDL (Hooper et al., 2011). Hence, the lipid homeostasis in the liver is intricately maintained by a balance between lipid uptake, synthesis, catabolism and secretion (Enjoji et al., 2012). Hepatic IR holds the key to determine its metabolic fate (Bugianesi et al., 2010). Thus, any dysregulation or disturbance in this metabolism of lipid homeostasis leads to excessive accumulation of lipids via *de novo* lipogenesis (DNL) and retarded excretion of lipids out of the liver which gives rise to hepatic steatosis (Cohen, 2011; Enjoji et al., 2012) as depicted in fig 2.3. These stored hepatic triglycerides in turn contribute to visceral fat accumulation (Caceane, 2012). To further add onto the lipid burden, liver responds to the elevated FFA by enhancing cholesterol ester synthesis, VLDL production, and DNL that further promotes dyslipidemia (Sniderman et al., 2001; Rector et al., 2008). The pathogenesis needs more clarity in terms of which occurs first; increase in hepatic fat content or lipid aberrations (Caceane, 2012).

Though it is known that there is an up-regulation of FFA in the liver that leads to hepatic steatosis, whether down regulation of β oxidation of fatty acids occurs or not

is still under question (Browning and Horton; 2004; Cheung and Sanyal, 2009; Kotronen et al., 2009).

Postprandial hyperlipidemia is a risk factor for NAFLD and CVD (Ahmed et al., 2012). It correlates with high liver fat content and is a cause of concern as it can be one of the potent factors in the pathogenesis of CVD (Matikainen et al., 2007). This could be one of the plausible reasons why a few lean, overweight and obese NAFLD patients, though having a normal fasting lipid profile or inspite of being on lipid lowering drugs, end up having adverse cardiac events (Ahmed et al., 2012).

NAFLD is taken to be a lipogenic disorder owing to triglyceride accumulation in the hepatocytes (Enjoji and Nakamuta, 2010). The fatty acid overload as a result of increasing influx in the hepatocytes causes enhanced DNL and its uptake at the cellular level results in mitochondrial dysfunction, oxidative stress and impaired VLDL formation and all of these factors lead to disease progression (Browning and Horton, 2004; Cheung and Sanyal, 2009).

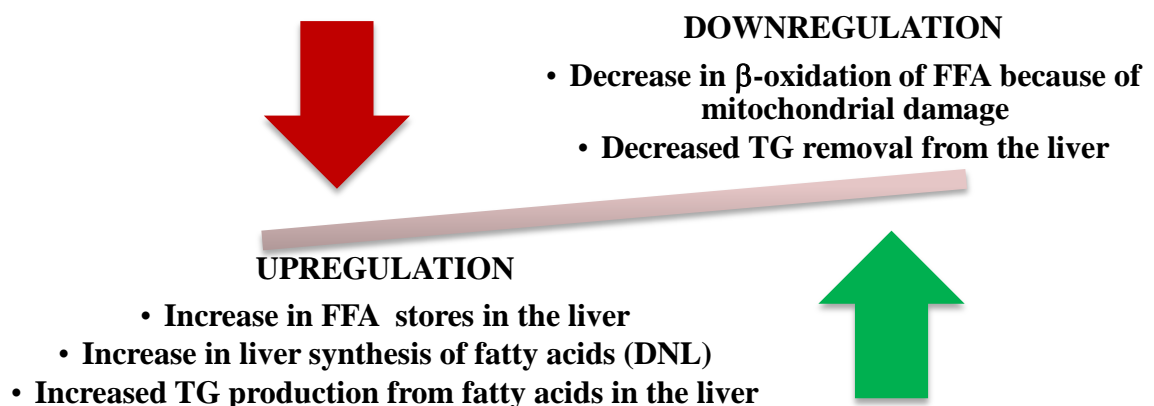


FIG 2.3: CHANGES IN LIPID HOMEOSTASIS LEADING TO NAFLD

Oxidative Stress and Inflammation

Adipose tissue is an active endocrine organ that produces pro-inflammatory cytokines in response to overload of the mitochondrial β oxidation system that contributes in the pathogenesis of NAFLD and its progression (Caceane, 2012). The major source of adipokines and cytokines are central obesity along with visceral fat. Adiponectin (protective), leptin (pro-fibrotic) and resistin (mediator of IR), are the various adipokines or peptides (Conlon et al., 2013) and tumour necrosis factor (TNF- α) and interleukin-6 (IL-6) are the pro-inflammatory cytokines released from the white

adipose tissue (Edmison and McCullough, 2007). Partly, lipid metabolism is regulated by the adipokines. TNF- α is pro-inflammatory and adiponectin is anti-inflammatory and thus both play opposite roles, TNF favours (Hui et al., 2004; Crespo et al., 2001) and adiponectin protects against NAFLD (Tolman et al., 2007). Any imbalance creates pro-steatotic and pro-inflammatory effect (Tolman et al., 2007).

NAFLD cases have significantly higher oxidative stress and inflammation in their system partly due to the existent diseased liver that may cause systemic inflammation and pro-thrombotic state (Chalasani et al., 2004; Targher et al., 2009). The inflammatory markers (CRP, IL-6, TNF- α , monocyte chemotactic protein 1), procoagulant factors (plasminogen activator inhibitor 1, fibrinogen, factor VII) and the oxidative stress markers (oxidised LDL, thiobarbituric acid reacting substances, nitrotyrosine) have been the highest in NASH, followed by hepatic steatosis and the least in those without steatosis. This association was established independent of obesity and other confounding factors (Targher et al., 2009).

The increased FFA concentration overloads the mitochondrial β oxidation system that generates reactive oxygen species (ROS) by lipid peroxidation, cytokine induction and induction of Fas ligand (Angulo, 2002; Mcavoy et al., 2006), increasing oxidative stress and inflammation (Lam and Younossi, 2010) that catalyses the disease progression (Angulo, 2002; Mcavoy et al., 2006). This is the second hit (Enjoji et al., 2012).

The ROS cause lipid peroxidation and release malondialdehyde and 4-hydroxynonema. The two products lead to apoptosis, cross link proteins causing Mallory's hyaline production, activation of stellate cells in response to oxidative stress and poor anti-oxidant defence systems of the body (Mcavoy et al., 2006) that produce collagen, paving the way for fibrosis (Mavrogiannaki and Migdalis, 2013). This is how the development of NASH from NAFLD is postulated to be, which concludes with end stage liver disease (Rolo et al., 2012).

TYPE 2 DIABETES AS A MAJOR RISK FACTOR FOR NON-ALCOHOLIC FATTY LIVER DISEASE

Way back in 1980, when NAFLD came into the picture, it was predicted that the presence of type 2 diabetes increased the risk and severity of NAFLD significantly (Ludwig et al., 1980) and till date this association stands true (Chalasani et al., 2012).

Usually, a very high prevalence of NAFLD amongst type 2 diabetics is observed (Vernon et al., 2011) which can be up to 70% (Chalasani et al., 2012; Ahmed, 2015). After matching the population for age and sex, the type 2 diabetics have 80% more fatty liver compared to the non-diabetics (Kotronen et al., 2008), being more than twice that of the prevalence observed amongst the non-diabetics (Targher et al., 2007; Leite et al., 2009). Moreover, type 2 diabetics are at a higher risk of developing NASH, cirrhosis and fibrosis compared to the non-diabetics. Infact, the presence of type 2 diabetes in NAFLD patients is taken to be a predictor for the development of fibrosis and eventual liver complications (Angulo et al., 1999; Younossi et al., 2004; Ong et al., 2005; Wanless and Lentz, 1990; Neuschwander-Tetri and Caldwell, 2003) such as more progressive forms of NASH (Oprea-Călin et al., 2014; Dyson et al., 2014). Majority of the cases of cryptogenic cirrhosis are diabetics (Maheshwari and Paul, 2006; Caldwell and Lee, 2008) and the lethal combination of type 2 diabetes and NASH may also predispose an individual to HCC (Bugianesi et al., 2007).

In type 2 diabetes, the amount of hepatic fat (intra-hepatic triglyceride content) influences the severity of IR. Thus, those with type 2 diabetes and NAFLD have higher hepatic and peripheral IR and poor glycemic control and are resistant to the peripheral action of insulin (Raz et al., 2005). Eventually they end up having higher HbA1c than those without NAFLD (Perseghin, 2009). Moreover, the quantum of hepatic fat is an important determinant of the amount of insulin required to achieve normal metabolic levels of glucose amongst those with type 2 diabetes, thus the insulin requirement correlates with the hepatic fat content (Ryysy et al., 2000; Juurinen et al., 2007).

The relationship between type 2 diabetes and NAFLD also emerges from the fact that insulin, after secretion, is delivered to the portal vein taking the path of the glucose absorption and the liver eliminates a large portion of portal insulin at first pass

(Firmeisz, 2014). Also, NAFLD is strongly associated with IR and hyperglycemia (Sattar et al., 2014). This link is said to be strong even beyond adiposity (Sung et al., 2012).

A deficiency of insulin promotes lipolysis in type 2 diabetics with poor glycemic control. Enhanced lipolysis mobilizes the FFA to the hepatocytes further leading to a favourable environment for triglyceride deposition. The damage caused along with inflammation may pave the way for fibrosis and hepatic apoptosis (Sharma et al., 2014).

Old age, duration of diabetes, prevalence of MS and hypertension are some of the known risk factors that may determine the presence of advanced NAFLD in type 2 diabetics (Pagadala et al., 2009). Also, those on exogenous insulin are predisposed to the risk of developing HCC because of severe hyperinsulinemia (Donadon et al., 2008). Thus, a close monitoring of the liver status is required for those with type 2 diabetes and NAFLD (Oprea-Călin et al., 2014).

PREVALENCE OF NON-ALCOHOLIC FATTY LIVER DISEASE AMONG TYPE 2 DIABETES PATIENTS

A prevalence of 82.9% of USG diagnosed NAFLD was reported in type 2 diabetic patients in Iran. BMI was found to be the only predictor variable for NAFLD in type 2 diabetics. The univariate analysis defined the ODDs ratio of 6.56 for the presence of NAFLD in those with MS. With the addition of each component of MS, the probability of having NAFLD also went up. No differences were observed in the HbA1c of either of the groups which may highlight the possible association between NAFLD and glycemic control of an indirect nature (Hosseiniapanah et al., 2007).

The prevalence of NAFLD was 69.5% among type 2 diabetics attending an outpatient clinic in Italy (Targher et al., 2007). An increase in the prevalence of NAFLD was observed with an increase in age. The study found NAFLD to be associated with prevalent CVD independent of classical risk factors, glycemic control, medications and the features of MS.

In a sample of type 2 diabetics, aged between 61-76 years, who underwent ultrasound for the diagnosis of NAFLD, ultrasonographic grading of the patients was compared

with magnetic resonance spectroscopy (MRS) in a sub-group. Around 56.9% prevalence of hepatic steatosis was observed. After ruling out the secondary causes of NAFLD, the final prevalence arrived at was 42.6%. The study found BMI, glycated hemoglobin and triglycerides as the independent predictors of NAFLD. The authors concluded that association with features of the MS could be used to target screening for NAFLD in the type 2 diabetics (Williamson et al., 2011).

In the Valpolicella Heart Study, a cohort of 2103 type 2 diabetics who were free of CVD at baseline, the prevalence of NAFLD was 75% among the type 2 diabetics. The study highlighted an independent association between NAFLD and the increased risk of future occurrence of CVD in these patients independent of the classical risk factors, liver enzymes and the MS (Targher et al., 2005).

In Iran, of the 172 type 2 diabetic patients enrolled from a tertiary referral centre, 55.8% were diagnosed as having NAFLD, with BMI and triglycerides being significantly higher in the NAFLD group as compared to the normal liver group. The prevalence was higher among females (60.1%) as compared to the males (44.8%) (Merat et al., 2009).

A study conducted on type 2 diabetic patients, 72 with NAFLD and 29 without NAFLD concluded that NAFLD is associated with visceral obesity and low HDL-C in type 2 diabetics. Glycated hemoglobin was similar in both the groups. Prevalence of overweight and obesity was significantly higher amongst those with NAFLD, along with ALT and low HDL-C. NAFLD was positively associated with above normal WC and above normal ALT. In another regression model, elevated WC and TC were positively associated with NAFLD whereas, it was negatively associated with HDL-C. BMI was found to be a significant predictor of NAFLD and WHR showed no relationship with NAFLD (Trojak et al., 2013).

In a study involving 180 type 2 diabetic patients, the prevalence of NAFLD was 69.4% based on ultrasonographic diagnosis. Abdominal obesity, hypertriglyceridemia were found to be associated with NAFLD. The study concluded that NAFLD's progression is independent of diabetes progression (Leite et al., 2009).

In another study involving type 2 diabetics, a prevalence of 78% NASH was diagnosed based on histology. Hypertriglyceridemia, low HDL-C and increased ALT

were independently associated with a higher risk NASH. High serum GGT levels, old age and the male gender correlated independently with the presence of NASH (Leite et al., 2011).

In a study involving type 2 diabetic health workers of Mexico, 40.1% females and 17.1% males had ultrasound diagnosed NAFLD. The NAFLD group of patients were more obese, had significantly higher HbA1c, triglycerides, body fat, fat mass and WC than the normal liver counterparts (Salas-Flores et al., 2012).

THE NON-ALCOHOLIC FATTY LIVER DISEASE: METABOLIC SYNDROME FROM THE HEPATIC DIMENSION

Metabolic syndrome is also known as Deadly Quartet, Syndrome X, Dysmetabolic syndrome and Insulin Resistance Syndrome (Fan and Peng, 2007). It is a cluster of metabolic derangements; impaired fasting glucose/previous type 2 diabetes, hypertiglyceridemia, low HDL-C levels, abdominal obesity and hypertension (IDF, 2006).

Little attention is being paid to the fact that, not only obesity, but the prevalence of MS is also on a rise as well in the developed as well as the developing countries. The primary causes of MS are; IR, glucose intolerance and hyperinsulinemia (Wu et al., 2014). The above mentioned causes also play a central pathogenic role in the causation of NAFLD. MS is gradually translating into a global epidemic and a common co-morbid condition with it is the NAFLD (de Wit et al., 2012). A rise in the prevalence of MS has fuelled the incidence of NAFLD as well (Koehler et al., 2012).

MS and NAFLD share the same pathophysiologic soil of IR (Marchesini et al., 2001; Kotronen et al., 2007; Bugianesi et al., 2005) and hyperinsulinemia (Pagano et al., 2002; Marchesini et al., 2001). IR occurs as a universal phenomenon in NAFLD as 95% NAFLD cases have IR (Ahmed et al., 2012). NAFLD occurs mostly owing to the impact of MS on the hepatic metabolism (Kneeman et al., 2012). It is now taken to be the hepatic expression of the MS, especially primary NAFLD, as the condition is related to type 2 diabetes, hypertension, hypertriglyceridemia, low HDL-C and abdominal obesity (Ahmed et al., 2012; Ahmed and Byrne, 2005; Bellentani et al., 2000; Ahmed and Byrne, 2007; Bedogni et al., 2005). The fact that 90% of the NAFLD patients have atleast one feature of the MS and 33% have a complete

diagnosis of MS, itself establishes NAFLD as the hepatic form of MS (Marchesini et al., 2003). Additionally, the plasma FFA released from the insulin resistant adipose tissue lipolysis appear to be the main source of hepatic triglycerides in NAFLD explaining the close association between NAFLD and MS (Bhatia et al., 2012).

Presence of MS confers a higher risk for NAFLD in both the genders (Hamaguchi et al., 2005). MS is prevalent in 60% females with NAFLD and 30% males with NAFLD (Caceane, 2012). The presence of MS defines a 4 to 11 fold risk of developing fatty liver (Hsiao et al., 2007) and it may precede the onset of NAFLD by several years (Hamaguchi et al., 2005). With the addition of each component of MS, the risk of having hepatic steatosis exponentially goes up (Caceane, 2012) and as the number of metabolic co-morbidities increase, the risk of advancement to more severe forms of NAFLD also increases (Medina-Santillan et al., 2013). Moreover, the presence of MS is an indicator of the severity of NAFLD in an individual, more likely to be having NASH (Fan and Peng, 2007; Marchesini et al., 2001; Marchesini et al., 2003; Marchesini et al., 1999) with a three times likelihood; or advanced fibrosis compared to individuals with fatty liver alone and no MS (Hamaguchi et al., 2005). The risk of progression to HCC also lurks in those with MS as depicted in fig 2.4. However, MS is not always accompanied by NAFLD and NAFLD is not always accompanied by MS (Lam and Younossi, 2010). Another think-tank states that liver should be considered as the hepatic trigger of the MS, rather than its target as liver plays a significant function in the regulation of carbohydrate and lipid metabolism (Oprea-Călin et al., 2014).

In the multi-ethnic study of atherosclerosis, the components of MS emerged to be strong predictors of fatty liver. The risk of NAFLD also increased with increasing number of the features of MS. The ODDs of having NAFLD were the highest among the diabetics, followed by those with MS (Zeb et al., 2013).

In a study comprising of 19 patients with NAFLD who were neither obese nor diabetic and had normal lipid profile, 47% of them were having features of the MS (Pagano et al., 2002). In The Dionysos nutrition and liver study, a prevalence of ~25% NAFLD was reported and it was found to be associated with most features of the MS (Bellentani et al., 2000).

In a study involving NAFLD patients in Italy, presence of MS was associated with a higher risk of development of NASH. About 16% of those with steatosis regressed to a normal liver even in the presence of MS. The regression was owing to a small weight loss of an average 2.3-2.5kgs (Marchesini et al., 2003). In a cross section study in Varanasi, 78.23% of the patients were found to be having MS and a strong association was found between NAFLD and MS as defined by the ATP III classification (Agrawal et al., 2011).

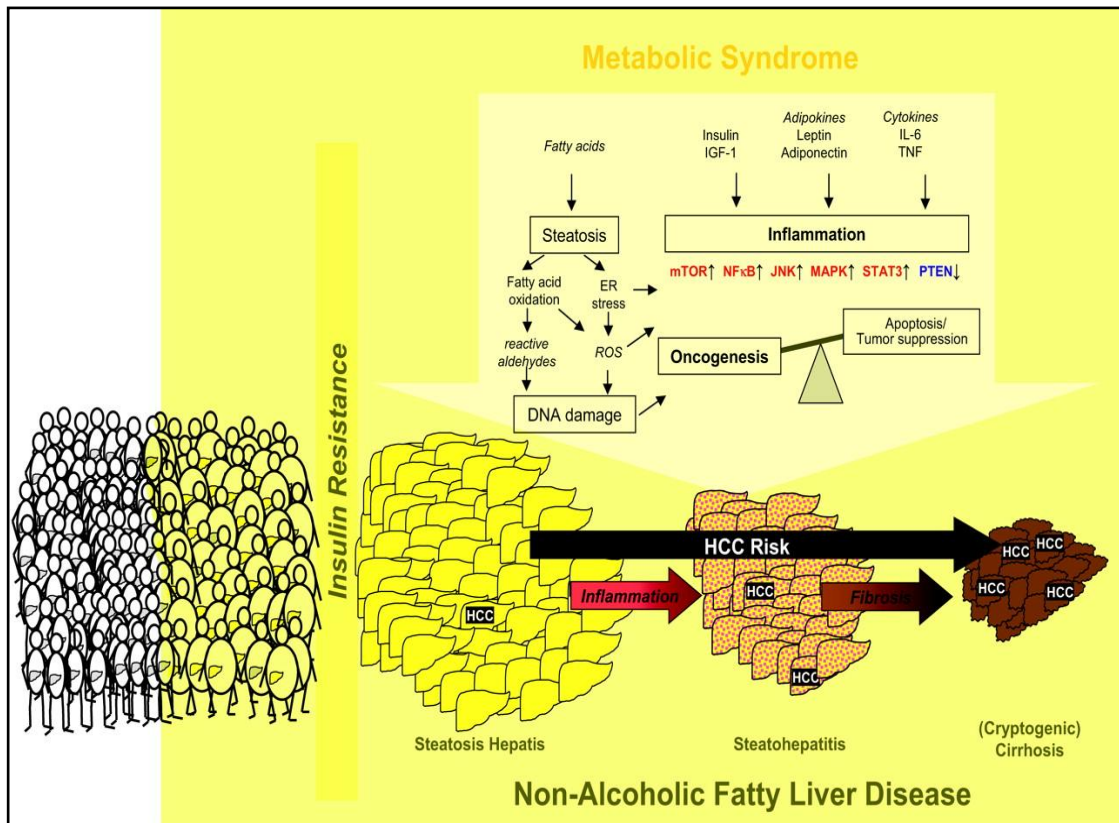


FIG 2.4: NAFLD AS A RISK FACTOR FOR HEPATOCELLULAR CARCINOMA: MECHANISMS AND IMPLICATIONS (Ref: Stickel and Hellerbrand, 2010)

SUMMARY

- NAFLD can be of two types; primary, owing to insulin resistance, and secondary, owing to non-insulin related factors.
- Insulin resistance is observed universally in NAFLD cases, which is said to be the major predisposing risk factor for NAFLD.
- Peripheral IR and hepatic IR retard insulin sensitivity at the adipose tissue and hepatic tissue, respectively, causing NAFLD through lipolysis and hyperinsulinemia.
- Obesity is associated with the entire spectrum of NAFLD.
- Visceral obesity is linked to the development of IR, MS and deranged liver enzymes. It is also associated with the development of NAFLD and also correlates with hepatic fat content.
- Dyslipidemia is commonly observed amongst those with NAFLD and NAFLD is taken to be a lipogenic disorder.
- Metabolic fate of the FFA is dependent on hepatic insulin resistance.
- Overload of the mitochondrial beta oxidation system leads to progression of NAFLD via release of adipokines and cytokines that cause oxidative stress and inflammation.
- Type 2 diabetics have a greater predisposition to NAFLD and advanced NAFLD than the non-diabetics.
- The quantum of insulin required to strive for normal glucose control is determined by the hepatic fat content.
- Enhanced lipolysis because of insulin deficiency in type 2 diabetics creates a favourable environment for triglyceride deposition in the hepatocytes.
- Type 2 diabetics with NAFLD require close monitoring of the hepatic status; requiring liver enzymes and lipid profile monitoring on a routine basis.
- Primary NAFLD is considered the hepatic manifestation of the MS.
- Ninety percent NAFLD patients have atleast one feature of MS and 33% have a confirmed diagnosis of NAFLD.
- With the addition of each component of MS, risk of NAFLD shoots up and presence of co-morbidities define an added risk of advanced NAFLD.

NON-ALCOHOLIC FATTY LIVER DISEASE IN INDIA

In India, the data on NAFLD has been sparse. There are many reasons for the neglect of NAFLD in India. The disease was recognised by the medical fraternity just a couple of decades ago. Moreover, upon its recognition, it was thought to be of benign nature. Our nation is already burdened much with the viral hepatitis. That has somewhere sidelined the attention that NAFLD was to receive (Duseja, 2010).

Based on evidence from various studies (Bajaj et al., 2009; Sharma et al., 2009; Petersen et al., 2006), it is presumed that one fourth of the Indian population living in the urban areas maybe having NAFLD (Misra and Shrivastava, 2013). In the general population, the prevalence of NAFLD ranges from 5% to 28% (Duseja et al., 2004; Madan et al., 2004). Owing to the westernization of the society, the culture of saturated fatty acid (SFA) rich and simple carbohydrate rich diet has percolated in. When it is coupled with a sedentary lifestyle, the condition becomes even more detrimental. Ethnically, Indians being more prone to type 2 diabetes, the condition in itself and its associated co-morbidities make the possibility of having NAFLD very high in the Indian population (Duseja et al., 2010). This can be substantiated with the data put up in fig 2.5 on the comparative prevalence of NAFLD in type 2 diabetics vs. the prevalence of NAFLD in type 2 diabetics of other countries, which clearly depicts similar prevalence for India and other countries.

In a general population study from Mumbai, involving residents of two railway colonies, the ultrasound diagnosed prevalence of NAFLD was 16.6%. Males had a higher prevalence compared to the females (24.6% vs. 13.6%). An age above 40 years, male gender, central obesity, BMI >25, elevated FBS, ALT and AST levels were identified as the risk factors associated with NAFLD (Amarapurkar et al., 2007).

A study from coastal eastern India reported 24.5% prevalence of NAFLD in the general population, with more males than females having NAFLD (27% vs. 14%). Those with NAFLD had a higher BMI. The authors were of the opinion that despite a lower prevalence of obesity, NAFLD may be as common in the developing countries as it is in the developed world (Singh et al., 2004).

In a study of urban South Indians, ultrasound based prevalence of NAFLD was 32%, with a higher prevalence in men than in women (35.1% vs. 29.1%). Prevalence of

NAFLD was highest in those with type 2 diabetes (54.5%), followed by those with pre-diabetes (33%), isolated impaired glucose tolerance (32.4%), isolated impaired fasting glucose (27.3%) and the least was in those who had normal glucose tolerance (22.5%). After adjusting for other variables, NAFLD was associated with diabetes and MS (Mohan et al., 2009).

In a prospective study conducted in India on type 2 diabetics attending an outpatient diabetic clinic, 87% were found to have NAFLD on histology. Age, duration of diabetes, glycemic control, BMI, WC and family history of diabetes did not predict the presence / severity of NAFLD or fibrosis. Alarmingly, all the patients with severe fibrosis were found to be having MS. Moreover, low HDL-C was found to be the only factor independently associated with fibrosis (Prashanth et al., 2009).

In a study from Mumbai involving type 2 diabetics, 49% prevalence of NAFLD based on ultrasound was diagnosed. However, no significant differences were observed in BMI, transaminase levels, cholesterol and triglycerides of those with and without NAFLD. A sub-sample of them underwent liver biopsy and 66% of them had mild NASH, 13% had moderate NASH, 9% had severe NASH and 22% showed the presence of fibrosis (Gupte et al., 2004).

To determine the anthropometric and metabolic profile of type 2 diabetes patients with NAFLD, 44 type 2 diabetics were enrolled from a diabetic clinic, 30 of them had NAFLD. A significantly higher BMI, WHR, AST/ALT >1 were observed amongst those with fatty liver as compared to the ones with normal liver (Pai et al., 2012).

In a study to assess the prevalence of NAFLD among type 2 diabetics in India, 60% of the females and 54.3% of the males had NAFLD based on elevated aminotransferase levels as defined by the NHANES III criteria. A prevalence of 44.1% was reported from Western India whereas the statistics rose to 72.4% in case of northern states (Kalra et al., 2013).

A total of 200 patients, 100 with obesity and type 2 diabetes and the other 100 non obese and non-diabetic were enrolled from an outpatient department. BMI and triglycerides were found to have a positive correlation with NAFLD and BMI, FBS, TC, TG, LDL, total bilirubin, ALT, ALP and GGT were significantly higher in the

NAFLD group and HDL-C was significantly lower in the NAFLD group (Sharma and Singh, 2014).

Fifty type 2 diabetics as cases and fifty non diabetics as controls were enrolled to study the prevalence of NAFLD from a tertiary care centre in southern India. A prevalence of 60% and 20% NAFLD was observed in the case and the controls, respectively. Obesity and duration of diabetes was significantly associated with NAFLD. Female gender was found to be a risk factor for the occurrence of NAFLD among the cases. Alkaline phosphatase and ALT were significantly higher in cases than controls (Jayarama and Sudha, 2012).

In a study in Gwalior, involving 400 obese and type 2 diabetic patients and 100 non-obese and non-diabetic participants, an increased BMI, FBS, TC, TG, LDL, VLDL, SGPT, ALP, GGT, fasting insulin, hs-CRP and decreased HDL were observed in NAFLD. Amongst those with NAFLD and type 2 diabetes, obesity, hyperglycemia, dyslipidemia, elevated liver enzymes, fasting insulin and CRP were observed in higher frequency (Sharma et al., 2014).

In a study conducted on type 2 diabetics at Nagpur, 56.6% were having USG diagnosed NAFLD. Elevated WC, BMI, SBP, DBP, HbA1c, AST, ALT, TC, TG and decreased HDL-C were observed in those with a fatty liver compared to those without fatty liver. Also, a higher prevalence of retinopathy, neuropathy and nephropathy was documented in the NAFLD group. The independent predictors of NAFLD were BMI, HbA1c, triglycerides and the presence of CAD (Somalwar and Raut, 2014).

In study involving type 2 diabetic patients, 64.7% had fatty liver on ultrasonography (USG). WHR, ALT, AST, total cholesterol (TC) and the triglycerides were found to be significantly higher in those with NAFLD. It was recommended that type 2 diabetics should routinely get their liver and lipid profile monitored (Krishnan and Venkataraman, 2011).

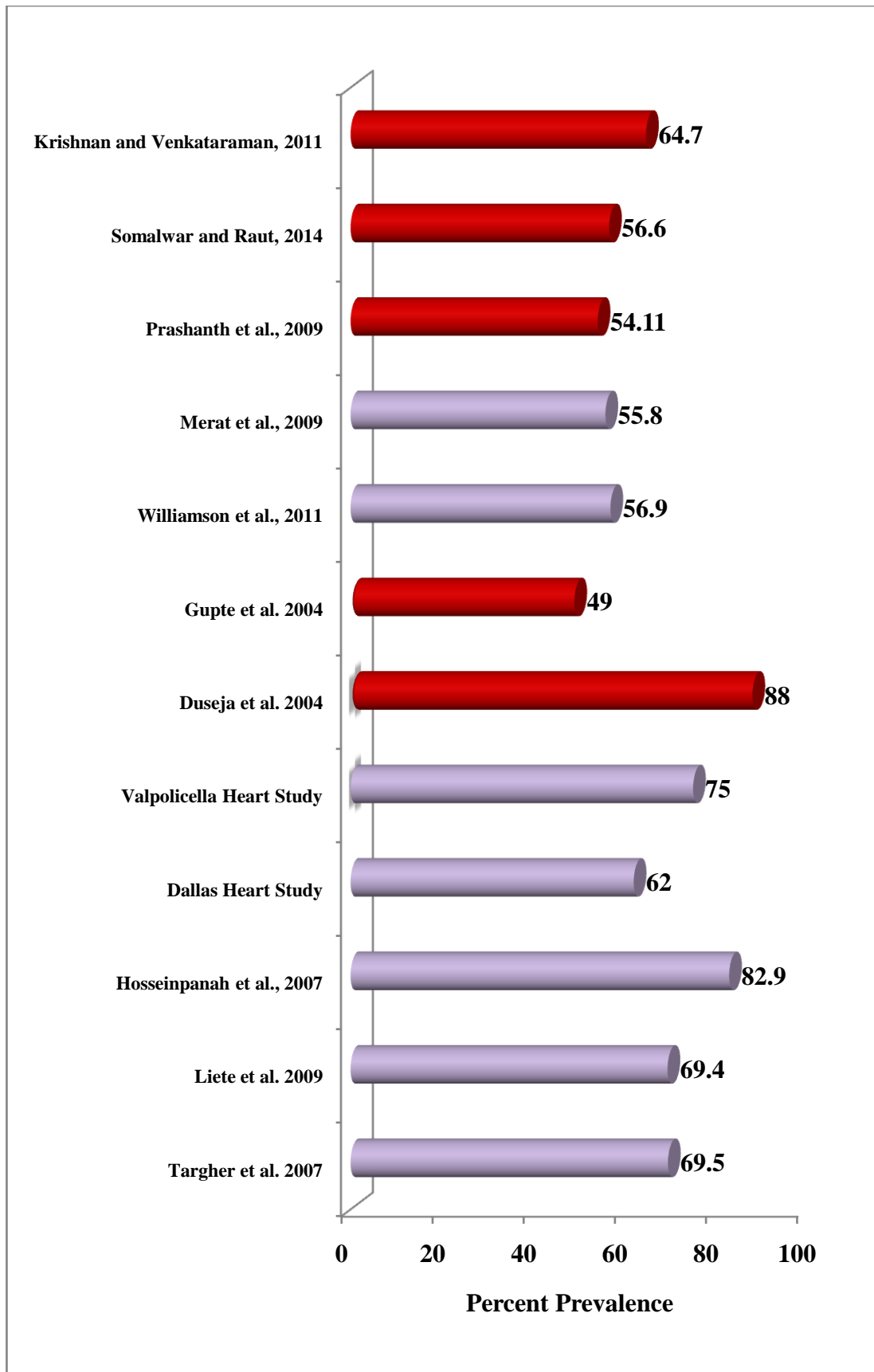


FIG 2.5: PREVALENCE OF NAFLD IN TYPE 2 DIABETICS IN INDIA AND OTHER COUNTRIES

DIET IN THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE

Diet has been implicated in the pathogenesis of NAFLD because excessive calorie intake, either from simple carbohydrates or fats leads to obesity. Obesity is an established risk factor in the causation of NAFLD as it increases the risk of NAFLD exponentially (Schwenger and Allard, 2014). The role of macronutrients, especially of carbohydrates and the lipids in the pathogenesis of NAFLD is poorly understood and requires further elucidation to bring in clarity (Scorletti et al., 2011). However, it is equally important to lay emphasis not only on the quantity, but also the quality of diet (Schwenger and Allard, 2014).

A high energy dense diet (Yasutake et al., 2014) and large meal volumes (Ledikwe et al., 2005) increase the intake of energy and such a pattern followed for a prolonged period of time results in obesity and NAFLD. As compared to healthy individuals, the NAFLD patients have been found to have increased energy intakes and higher calorie intakes (Capristo et al., 2005). Associations between; higher intake of saturated fat and cholesterol (Yasutake et al., 2012) and low PUFA (Musso et al., 2003), carbohydrate intake (Yasutake et al., 2012) and histological inflammation (Solga et al., 2004), total fat and elevated n-6/n-3 ratio (Cortez-Pinto et al., 2006) have been documented by various studies, which are the hallmarks of a NAFLD diet. However, independent of the total calorie intake, fructose, trans fats and saturated fats may contribute to intra-hepatic triglycerides (Sullivan, 2010). Dietary nutrients associated with NAFLD have been listed in table 2.1.

MACRONUTRIENTS

Carbohydrates

The type of carbohydrate or its quality may influence the development of NAFLD (McCarthy and Rinella, 2012). Simple carbohydrates are monosaccharides and disaccharides that raise blood sugar rapidly (Fan and Cao, 2013) and lead to subsequent hypoglycemia. This triggers hunger, an increase in appetite and hyperphagia (Melanson et al., 1999).

The simple sugars increase the circulating concentrations of insulin and triglycerides that cause hepatic DNL and decrease the hepatic insulin sensitivity (Maersk et al., 2012; Abdelmalek et al., 2010; Stanhope et al., 2009; Nomura and Yamanouchi, 2012; Ferolla et al., 2013). Within patients with NAFLD, NASH patients were found to be having higher rates of consumption of total as well as simple carbohydrates when compared to patients with only hepatic steatosis (Toshimitsu et al., 2007). Even in conditions wherein NAFLD patients do not always consume excess energy, a higher consumption of simple carbohydrates has been documented compared to healthy controls (Fan and Cao, 2013).

Because glucose uptake by the liver is not insulin dependent, any excess circulating glucose leads to an increasing uptake of glucose by the liver (Zivkovic et al., 2007). Insulin mediated DNL causes enhanced conversion of glucose to fatty acids, when the glycogen synthesis exceeds the capacity of the liver and when there is insulin mediated stimulation of DNL (Timlin and Parks, 2005).

Carbohydrate rich diets cause DNL by up-regulating sterol regulatory element binding protein (SREBP-1c) which are transcription factors involved in lipogenesis (Postic and Girard, 2008). In response to a high glucose intake, the pancreas produce increased quantities of insulin. Simultaneously, the liver also produces endogenous glucose inspite of the high glucose levels in the blood. This state of hyperglycemia along with elevated SREBP-1c levels causes increased DNL, eventually leading to fatty liver (Bosworth, 2007). A prolonged high carbohydrate diet triggers hepatic steatosis. A consequent post prandial stage of hyperglycemia raises the hepatic concentration of phosphorylated metabolites that activate carbohydrate regulatory element binding protein (ChREBP) and its target genes. The damage is even more far fetched as high carbohydrate diets as a protective response, induce IR in the hepatocytes to protect the liver from substrate overload (Agius, 2013).

Of all the monosaccharides, fructose is thought to be the most deleterious one in the pathogenesis of NAFLD. A diet high in fructose induces weight gain, hyperlipidemia and metabolic disturbances that contribute to hepatic fat accumulation (Byrne, 2012), inflammation and possibly fibrosis also (Vos and Lavine, 2013). Fructose also increases visceral adipose tissue (VAT) (Pollock et al., 2012) and plasma triglycerides (Lê et al., 2006). Unlike glucose, fructose does not lead to the secretion of leptin and

the feeling of satiety (Attar and Thiel, 2013) neither does it suppress ghrelin and nor does it stimulate insulin (Cook, 1969; Dencker et al., 1973). Hence, fructose is more lipogenic than the other two (Havel, 2005).

Glycemic Index

Consumption of high glycemic index (GI) foods eventually leads to obesity and development of IR (Liu et al., 2000). Infact, a close association has been observed between degree of hepatic steatosis and GI. It was independent of total energy intake and carbohydrate intake in 247 healthy individuals, wherein, a positive association between higher quartiles of GI with higher grades of hepatic steatosis was observed (Valtuna et al., 2006).

A Western diet pattern which includes simple and refined carbohydrates like candies, soft drinks which are high in GI, leads to the development of NAFLD (Zelber-Sagi et al., 2007; Oddy et al., 2013). High GI foods lead to hepatic DNL as there is rapid influx of glucose. When it overtakes the rate of capacity of glycogen synthesis in the liver, hepatic steatosis occurs (Bugianesi et al., 2010; Postic and Girard, 2008).

The impact of GI on the associated co-morbidities with NAFLD is suggestive of its potential role and care to be taken in diet consultations for NAFLD patients (York et al., 2009). Incorporation of lower GI foods in the diets of the NAFLD patients may help in the management of NAFLD (McCarthy and Rinella, 2012). However, the direct influence of GI on NAFLD is unknown. Moreover, a low GI diet only along with exercise aids in reducing post-prandial hyperinsulinemia thereby reducing hepatic IR (Solomon et al., 2010).

Artificial Sweeteners & Colouring Agents

No studies so far have documented the impact of artificial sweeteners on NAFLD status and hence their safety is unresolved (Kani et al., 2014). However, a study brought to light the possible contribution of aspartame as a risk factor for NAFLD. The postulated mechanism behind this association is thought to be due to mitochondrial dysfunction and depletion of ATP as a result of metabolism of aspartame (Trocho et al., 1998). Aspartame is 160 times sweeter than sugar and is

metabolized by the liver to form phenylalanine, aspartic acid and methanol (Nseir et al., 2010).

High fructose corn syrup (HFCS), containing 42-90% fructose, which is primarily used as a sweetener in soft drinks, is implicated in the causation of NAFLD (Ouyang et al., 2008; Montonen et al., 2007). An increased intake of the sweetener eventually leads to obesity and predisposes to NAFLD. Moreover, prolonged soft drink consumption is a strong predictor of NAFLD and is independent from MS (Abid et al., 2009). A high fructose intake leads to increased production of insulin and the hepatocytes too are engaged in the production of endogenous glucose production despite the high circulating glucose levels in the blood. This leads to fatty liver (Nichols, 2013). Fructose consumption is also significantly higher among the NAFLD patients as compared to those without NAFLD (Zelber-Sagi, 2007; Spruss and Bergheim, 2009; Abid et al., 2009). Fructose up-regulates SREBP-1c and ChREBP and hence causes fatty infiltration in the liver (Rutledge and Adeli, 2007). The aerated drinks also contain caramel colouring, which is quite rich in advanced glycation end products that can increase IR and also inflammation (Gaby, 2005).

Lipids

NAFLD/NASH patients ingest a higher percentage of energy from fat, which maybe an important nutritional risk factor for the development and progression of NAFLD (Sathiaraj et al., 2011; Vilar et al., 2008; Zelber-Sagi et al., 2007). In the NAFLD patients, a high SFA intake (Toshimitsu et al., 2007), high cholesterol coupled with poor PUFA intake, especially n-3 PUFA has been documented (Videla et al., 2006; Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012; Min et al., 2012; Parker et al., 2012; Bjermo et al., 2012; Musso et al., 2003).

An excess of lipid intake increases the caloric intake of food and causes accumulation of fat in the body. An increase in visceral fat increases the flow of fatty acids to the liver, thereby contributing to development of NAFLD (Pagano et al., 2002). Fatty acids cause impairment of insulin signalling and enhanced lipogenesis (Byrne, 2012) that promotes and activates the pro-inflammatory cytokines (Cusi, 2008). High saturated fat diet leads to IR and hepatic inflammation (Musso et al., 2003).

In a study conducted on morbidly obese patients who underwent bariatric surgery and liver biopsy estimation during the surgery, no significant associations were found between total calorie intake, protein intake and steatosis, fibrosis or inflammation. However, high carbohydrate intake was associated with inflammation and higher fat intake with lower odds of inflammation. A drawback of this study was reliance on a single 24 hour dietary recall and no differentiation between simple and complex carbohydrates (Solga et al., 2004).

Analysis of food frequency data of 43 NASH patients vs. 33 healthy controls found a negative correlation with total fat intake and saturated fat intake and with plasma glutathione/oxidised glutathione ratio. A positive correlation was established with carbohydrates, fibre, MUFA, PUFA and n-3 PUFA in specific (Machado et al., 2008).

The lipotoxic environment produces the pro-inflammatory cytokines that cause liver injury (Kirpich and McClain, 2012). The lipotoxicity is not restricted to the hepatocytes, but also impact the extra-hepatic tissues, eg: β cell dysfunction (Marchetti et al., 2012). This aberration owing to lipotoxicity affects the plasma glucose levels (Firmeisz, 2014). Thus, β cell dysfunction in association with increased hepatic IR and decreased suppression of hepatic glucose output causes hyperglycemia. This condition of glucotoxicity through direct and indirect means may further exacerbate the entire process (Miani et al., 2013; Ibrahim and Gores, 2012; Gurzov and Eizirik, 2011). Hepatic IR activates the hepatic stellate cells paving the way for fibrosis and cirrhosis (Trauner et al., 2010).

Fatty Acids

The different types of fats namely; saturated fat, monounsaturated fat, polyunsaturated fat, omega 3 fatty acids and trans fats contribute in their own way in NAFLD (Kani et al., 2014).

Saturated Fatty Acids (SFA)

A significantly high SFA intake has been documented in NAFLD patients, irrespective of excess energy consumption from lipids (Toshimitsu et al., 2007) or not (Fan and Cao, 2013) when compared to healthy individuals. In a seven day nutritional survey, the intake of saturated fats was found to be significantly higher in NAFLD

cases as to those free of NAFLD (Musso et al., 2003). A correlation between high saturated fat intake and impaired glutathione metabolism has also been observed in NASH patients (Machado et al., 2008).

Saturated FFA play a toxic role in the causation of NASH. When human hepatocytes were incubated with SFA, they (palmitate) caused stress on the endoplasmic reticulum (ER) and led to apoptosis as they poorly incorporated into the triglycerides (Gentile and Pagliassotti, 2008). SFA has detrimental effects because it alters the glucose and lipid homeostasis as it induces IR, which worsens the progression of MS and presumably of NAFLD as well (Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012).

Unsaturated Fatty Acids

When human hepatocytes were incubated with unsaturated fatty acids, they accumulated large amounts of triglycerides without causing any damage (Gentile and Pagliassotti, 2008). Unsaturated fatty acids have a protective role because of ease of esterification of unsaturated FFA into the neutral triglycerides (Li et al., 2009; Listenberger et al., 2003) and hence they prevent palmitate induced apoptosis by channelling it into less harmful triglyceride pools and away from the apoptotic pathways (Listenberger et al., 2003). Unsaturated fatty acids do not cause any stress to the ER and hence the question of apoptosis also does not arise (Li et al., 2009; Listenberger et al., 2003).

Monounsaturated Fatty Acids (MUFA)

MUFA has genes expression regulation potency of the genes involved in peripheral insulin sensitivity (Clark et al., 2001), anti-inflammation (Serrano-Martinez et al., 2005) and inhibitory effects on nuclear factor-KB (Madigan et al., 2000). Hence, diets low in MUFA decrease the PPAR- α in the background of IR (Assy et al., 2009). It is therefore postulated that diets rich in MUFA may play a pivotal role in the prevention and treatment of NAFLD (Conlon et al., 2013). However, role of MUFA or olive oil with respect to NAFLD is yet to be studied in humans (Zelber-Sagi et al., 2011).

Polyunsaturated Fatty Acids (PUFA)

Irrespective of excessive lipid intake, a low PUFA intake amongst NAFLD patients as compared to those without NAFLD is documented (Toshimitsu et al., 2007; Musso et al., 2003). Within the NAFLD patients, non-obese have a significantly lower intake of PUFA in comparison to the obese (Yasutake et al., 2009). This establishes the role of PUFA deficiency in the pathogenesis and progression of NAFLD (Yasutake et al., 2014).

The n-6/n-3 PUFA balance is capable of causing disease progression as n-6 has pro-inflammatory and prothrombotic action whereas, n-3 PUFA is anti-inflammatory in nature (Mouzaki and Allard, 2012). However, care needs to be taken as an excess of n-6 PUFA is implicated in the promotion of necro-inflammation (Cortez-Pinto et al., 2006).

IR may often be associated with deficiency of n-3 fatty acids (Capanni et al., 2006; Araya et al., 2004). A deficiency of n-3 fatty acids sets the perfect metabolic environment for NAFLD to occur as it is associated with higher lipogenesis owing to up-regulation of lipogenic transcription factors like SREBP-1c, greater hepatic uptake of FFA by the hepatocytes and decreased β oxidation of fatty acids and decreased synthesis of VLDL (Araya et al., 2004; Clarke, 2001; Clarke, 2001; Clarke, 2004). The deficiency impairs PPAR- α , which modulates lipid metabolism in the liver which leads to NAFLD (Reddy, 2001).

Trans fatty Acids (TFA)

The TFA content is not known many a times which creates a difficulty in establishing the role of TFA in NAFLD. Trans fats have not been studied so far with respect to their association with NAFLD in humans (Zelber-Sagi et al., 2011).

Cholesterol

In NAFLD, excessive intake of cholesterol is also implicated in the pathogenesis of NAFLD (Zelber-Sagi et al., 2011; Musso et al., 2012; Enjoji et al., 2012; Musso et al., 2003). Even though the total calorie intake maybe within the normal range, excess ingestion of cholesterol may be a strong catalyst for the pathogenesis of NAFLD. Cholesterol uptake in the form of LDL is limited by the intracellular accumulation of

fatty acid and cholesterol whereas fatty acid synthesis and cholesterol synthesis are upregulated in a NAFL (Enjoji et al., 2012). Alarming, excess intake of dietary cholesterol and decreased intake of PUFA may lead to pathogenesis of NAFLD even in the absence of obesity and IR (Enjoji and Nakamuta, 2010).

The supply of cholesterol and that of fatty acid synthesis via DNL is associated on a stream of the liver X receptor alpha (LXR- α)-SREBP-1c pathway. Hence, LXR- α is a key regulator of cholesterol and fatty acid metabolism in the hepatocytes. It also regulates the endogenous agonistic ligands, oxysterols, which are metabolites of cholesterol. Hence, an excessive intake of dietary cholesterol will produce more of oxysterols, which will activate the LXR- α SREBP-1c pathway (Zelcer and Tontonoz, 2006; Yasutake et al., 2009). This will lead to up-regulation of fatty acid synthesis in the hepatocytes (Enjoji and Nakamuta, 2010). The LXR- α expression has been documented to be significantly higher and up regulated in NAFLD patients (Zelcer and Tontonoz, 2006) and also in non-obese NAFLD patients (Nakamuta et al., 2008). Evidence of cholesterol dysregulation emerges from DNL of cholesterol activation in the NAFL, even in conditions of cholesterol overload in the hepatocytes (Nakamuta et al., 2009). However, a recent review concluded that more studies are required to assess the dietary cholesterol intake and its restriction on large populations to gain greater insights into the role of cholesterol in causation of NAFLD (Yasutake et al., 2014).

Protein

Excess as well as deficit protein intake is linked to NAFLD (Kani et al., 2014). Protein deficiency and malnutrition is a risk factor for NASH (Colak et al., 2012). There is no evidence to elucidate on the role of dietary proteins in NAFLD. The Western diet is not lacking in protein so a deficiency of the same is a highly unlikely pathogenic factor (Fan and Cao, 2013).

Increased consumption of red meat is implicated to cause IR and also increase the risk of CVD (Linn et al., 2000; Schulze et al., 2003). Increased meat consumption was found to be a risk factor for the increased risk of developing NAFLD in the population based study in Israel (Zelber-Sagi et al., 2007). A high protein intake aids in reducing intra-hepatocellular lipids (IHCL) was proven in a study wherein healthy

subjects lost 22% of IHCLs on a high protein-high fat diet compared to a high fat and control diet. The only drawback of the study was its shorter duration of conduction (Bortolotti et al., 2009). However, a high protein intake has been found to be associated with IR and glucose intolerance (Linn et al., 1996; Linn et al., 2000).

MICRONUTRIENTS

Vitamin D

Vitamin D plays a significant role in causing inflammation as well as in autoimmunity (Yasutake et al., 2014). A deficiency of vitamin D may lead to IR, MS as well as its hepatic manifestation, NAFLD (Alvarez and Ashraf, 2010). A recent review suggested that an excess of energy intake and presence of vitamin D deficiency (VDD) may enhance the onset as well as the progression of NAFLD and NASH (Yasutake et al., 2014).

In patients with NAFLD who had a biopsy proven diagnosis, decreased concentrations of 25-hydroxyvitamin D3 were associated significantly as well as independently with the increased severity of NAFLD; be it hepatic steatosis or fibrosis (Targher et al., 2007). VDD has shown to correlate with the severity of NAFLD activity score and hepatic fibrosis (Manco et al., 2010). The possible mechanism behind this association is postulated to be because of greater oxidative stress conferred by deficiency of vitamin D (Wu et al., 2011).

A meta-analysis concluded that the ODDs of having VDD is 1.26 times in those with NAFLD (Eliades et al., 2013) Further, the authors concluded that low Vitamin D levels maybe a part of pathogenesis of NAFLD. The possible pathogenic role of VDD in NAFLD may arise from its subdued anti-inflammatory and immuno-modulatory effect which may contribute towards severity of NAFLD.

Vitamin E

A study revealed lower consumption of vitamin E among the NASH patients as compared to the controls (Sullivan, 2010). Studies have demonstrated that NAFLD and NASH patients have a deficient intake of vitamin E as compared to those without NAFLD and NASH (Musso et al., 2003; Erhardt et al., 2011).

Choline

As choline is metabolized and stored in the liver, a deficiency of choline is implicated in the pathogenesis of NAFLD. In a state of deficiency, there is an increased phospholipid synthesis, defect in secretion of lipoproteins, oxidative damage due to mitochondrial dysfunction and ER stress. Moreover, the bioavailability of choline may be influenced by the gut microbiota (Corbin and Zeisel, 2012). However, the dietary requirement of choline is modulated by oestrogen and by the single nucleotide polymorphisms (SNPs) in the genes concerned with choline and folate metabolism (Fan and Cao, 2013).

TABLE 2.1: DIETARY NUTRIENTS ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE

RISK	UNCERTAIN	PROTECTIVE
High fat diet	Total caloric intake	Light alcohol drinking
More SFAs (butter, coconut oil, palm oil)	Total protein intake	Coffee drinking
More TFAs (fast foods, baked foods, deep fried foods)	Excessive n-6 fatty acids	Green tea
Lower intake of PUFA	Dietary antioxidants (vitamin C or E, ginger, genistein)	MUFA (Olive oil, nuts, avocados, peanut butter, peanut oil)
High cholesterol intake	Betaine	n-3 fatty acids (fish oil, walnuts, salmon, shellfish)
High carbohydrates intake	Probiotic rich foods (yogurt, kefir)	Low GI foods (oats, soya, linseed, whole grains)
High simple carbohydrate intake		Soy protein and whey
High fructose and sucrose intake		Dietary fibre (whole grains, fruits, vegetables)
Decreased fruit intake		
Decreased choline intake		

Ref: Adapted from Fan and Cao, 2013

SUMMARY

- Data on NAFLD is lacking in the Indian perspective.
- Every one in four urban Indian maybe having NAFLD.
- Owing to genetic predisposition to type 2 diabetes, the risk of having NAFLD also runs high amongst Indians.
- In the general population, prevalence of NAFLD in India ranges from 5-28%, higher among males than females.
- Indian data on prevalence of NAFLD in type 2 diabetics no different from those of the Western countries.
- Role of diet as a pathogenic factor for NAFLD does not hold much clarity.
- Quantity and the quality of diet are crux factors that may determine the contribution of diet in causation of NAFLD.
- A NAFLD diet is marked by higher intakes of; energy, carbohydrate, total fat, saturated fat, cholesterol, elevated n-6/n-3 ratio and low PUFA.
- Quality of carbohydrates holds a greater relevance in the determination of NAFLD.
- Excess glucose in circulation is taken up by the liver leading to DNL.
- Fructose is the most lipogenic monosaccharide.
- An association between degree of hepatic steatosis and GI has been observed.
- High GI foods trigger hepatic DNL.
- Consumption of artificial sweeteners and colouring agents is debatable for NAFLD cases.
- SFA have a toxic impact in NAFLD, whereas MUFA may play a protective role in NAFLD.
- Protein deficit and surplus may lead to NAFLD.
- Vitamin D deficiency and vitamin E deficiency may have a pathogenic role in NAFLD.

PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE: THE COMPLEX INTERPLAY OF RISK FACTORS

NAFLD surfaces out of a complex web of genetics, diet and lifestyle. The interactions together are labelled as the NAFLD phenotype (Finelli and Tarantino, 2014). Under normal conditions, hepatocytes are empowered to bind, transform, catabolize and export the FFA (Tarantino et al., 2007). However, repeated insults or the multiple hits through genetic predisposition and environmental factors, favours the development of NAFLD.

Genetics

In 26-35% of the NAFLD patients, genetic factors may influence the development of NAFLD (Attar and Thiel, 2013). Genetic defects have been identified that obstruct the removal of triglycerides from the liver, thereby leading to hepatic steatosis. Defect of the enzyme hydroxy acyl-CoA transferase, which is required for the β oxidation of the fatty acids in the mitochondria, also leads to hepatic steatosis (Hooper et al., 2011). Deficits in oxidative phosphorylation and impaired mitochondrial function (Lowell and Shulman, 2005; Sanyal et al., 2001) have also been observed. Genetic defect such as familial hypo β lipoproteinemia is implicated with development of NAFLD (Nichols, 2013). A family history of steatohepatitis or of cryptogenic cirrhosis is also a risk factor for NAFLD (Wanless and Lentz, 1990; Silverman et al., 1990) and in Asian Indians, NAFLD has been genetically associated with SREBP-2 1784 G>C genotype (Bhatt et al., 2011) and peroxisome proliferators activated receptor- γ (Bhatt et al., 2011). Recent research in genetics has identified patatin-like phospholipase domain-containing protein 3 (adiponutrin) and ApoC3 genetic polymorphisms to be associated with NAFLD. The Apo C3 gene mutations identified in Asians correlate with hepatic triglyceride accumulation and NAFLD (Tabibian et al., 2011).

Environment

Popularly known as the two-hit hypothesis, which was proposed by Day and James in 1998 elaborates on how two major hits give rise to hepatic steatosis and NASH. The first hit is adipose IR mediated triglyceride deposition in the hepatocytes and prolonged deposition giving rise to lipotoxicity (fig 2.6). This gives rise to oxidative stress that produces inflammatory cytokines that gives birth to stellate cells (Cusi,

2009). Hepatocyte death is labelled as the third hit now, which is accompanied by impaired hepatocyte regeneration (Ahmed, 2015). To provide a better clarity of the flow of hepatic events, the pathogenesis has been truncated into 4 steps, which are as follows:

Step 1: Adipose Tissue Insulin Resistance Leading to Hepatic Steatosis

Peripheral IR causes adipose tissue lipolysis that increases the concentration of FFA in circulation. These FFA are released into the portal vein that carries it directly to the hepatocytes (Bjorntorp, 1990; Caceane, 2012). Also, IR impairs suppression of adipose tissue lipolysis which in turn increases the release of FFA from the adipocytes that are once again delivered to and taken up by the hepatocytes (Donnelly et al., 2005; Collantes et al., 2004; Marchesini et al., 1999; Westphal, 2008, Cusi, 2008). A prolonged state of peripheral IR up-regulates SREBP-1c, which is a transcription factor for DNL (Mavrogiannaki and Migdalis, 2013). A state of hyperinsulinemia also blocks the mitochondrial β oxidation of fatty acids leading to reduced removal of triglycerides from the liver (Smith and Adams, 2011; Gaggini et al., 2013). The net effect is an increased concentration of FFA in the hepatocytes owing to enhanced DNL and retarded β oxidation of fatty acids. This imbalance between liver's uptake, synthesis and disposal of fatty acids leads to hepatic steatosis (Marchesini et al., 1999; Li et al., 2002). Adipose tissue IR and subsequent lipid accumulation in the hepatocytes is the first metabolically driven first hit (Novo and Parola, 2008) and is a major biochemical event in NAFLD (Firneisz, 2014).

Adipose tissue lipolysis accounts for 60% of the triglyceride deposition in the hepatocytes, followed by up-regulated DNL accounting for 30% of the triglyceride deposition in hepatocytes. The remaining 10% fat accumulation in the hepatocytes is through increased nutritional intake (Neuschwander-Tetri and Caldwell, 2003; Byrne et al., 2009).

Step 2: Prolonged Triglyceride Deposition Giving Rise to Lipotoxicity

Prolonged increased concentration of insulin owing to peripheral IR triggers a state of hyperinsulinemia (Mavrogiannaki and Migdalis, 2013). It favours energy accumulation in the form of fat as it induces SREBP and ChREBP in the liver, activating the genes required for hepatic DNL (Firneisz, 2014), and is capable of

causing a reduction in VLDL secretion which further adds on to triglyceride storage in the hepatocytes (Jacobs et al., 2008). The FFA reduce the insulin signalling in a dose dependent manner and increases endogenous hepatic glucose owing to stimulation of hepatic IR (Wei et al., 2007; Savage et al., 2007; Petersen et al., 2007) and lipid production (Belforte et al., 2005; Wei et al., 2007). The liver suffers further insults owing to an uncontrolled production of endogenous glucose because glycogen synthesis is impaired and there is failure to suppress gluconeogenesis (Oprea-Călin et al., 2014). To add onto it, the impaired suppression of the endogenous hepatic glucose production and glucose uptake by the muscle tissue in conjunction with increased lipolysis in the adipose tissue contributes to more storage of fatty acids in the hepatocytes (Caceane, 2012). The increased IR sets up a vicious cycle between IR, increased FFA supply to the liver, increased hepatic steatosis and liver inflammation (Byrne, 2012). Necroinflammation sets in if the liver fails to adapt to prolonged triglyceride accumulation (Cusi, 2009).

Step 3: Lipotoxicity Induced Oxidative Stress and Pro-Inflammatory Cytokines

The subsequent overload of the hepatic mitochondrial β oxidation system causes lipotoxicity and the lipotoxic metabolites cause oxidative processes that generate reactive oxygen species (ROS), free electrons and hydrogen peroxide. These products are capable of damaging the mitochondrial DNA and may lead to impaired mitochondrial function (Perlemuter et al., 2007; McCullough, 2002). The mitochondrial dysfunction alters the hepatic energy metabolism owing to lipotoxicity, oxidative stress and inflammatory mediator effect (Koliaki and Roden, 2013). The cytokines synthesis thus stimulated by ROS causes activation of the inflammatory pathways (Perlemuter et al., 2007; McCullough, 2002), which comprises the second hit (Cusi, 2009). It further interferes with insulin signalling (Wieckowska and Feldstein, 2008; Crespo et al., 2001). Presence of advanced NAFLD is dependent on presence of FFA, inflammatory cytokines, adipokines, oxidative stress and mitochondrial dysfunction, the key factors in the pathogenesis of NASH from hepatic steatosis (Dowman et al., 2010).

Step 4: Birth of Hepatic Stellate Cells

The increased production of pro-inflammatory cytokines causes the inflammatory cells to migrate to the liver, thus causing the shift from hepatic steatosis to hepatic steatohepatitis (Li et al., 2002). This leads to the activation of the stellate cells that are responsible for collagen synthesis and fibrosis development (Perlemuter et al., 2007; Angulo, 2002; McCullough, 2002). Moreover, β oxidation of fatty acids is up-regulated in NASH and it fails to overcome or exceed the rate of hepatic DNL. The resultant non-esterified fatty acids oxidation leads to increased oxidative stress thereby providing the substrates for disease progression (Bugianesi et al., 2002; Bugianesi et al., 2010). The degree of fibrosis and the probability of progression to cirrhosis depend on the degree of cross talk between the hepatocytes, macrophages and the hepatic stellate cells (Cusi, 2009).

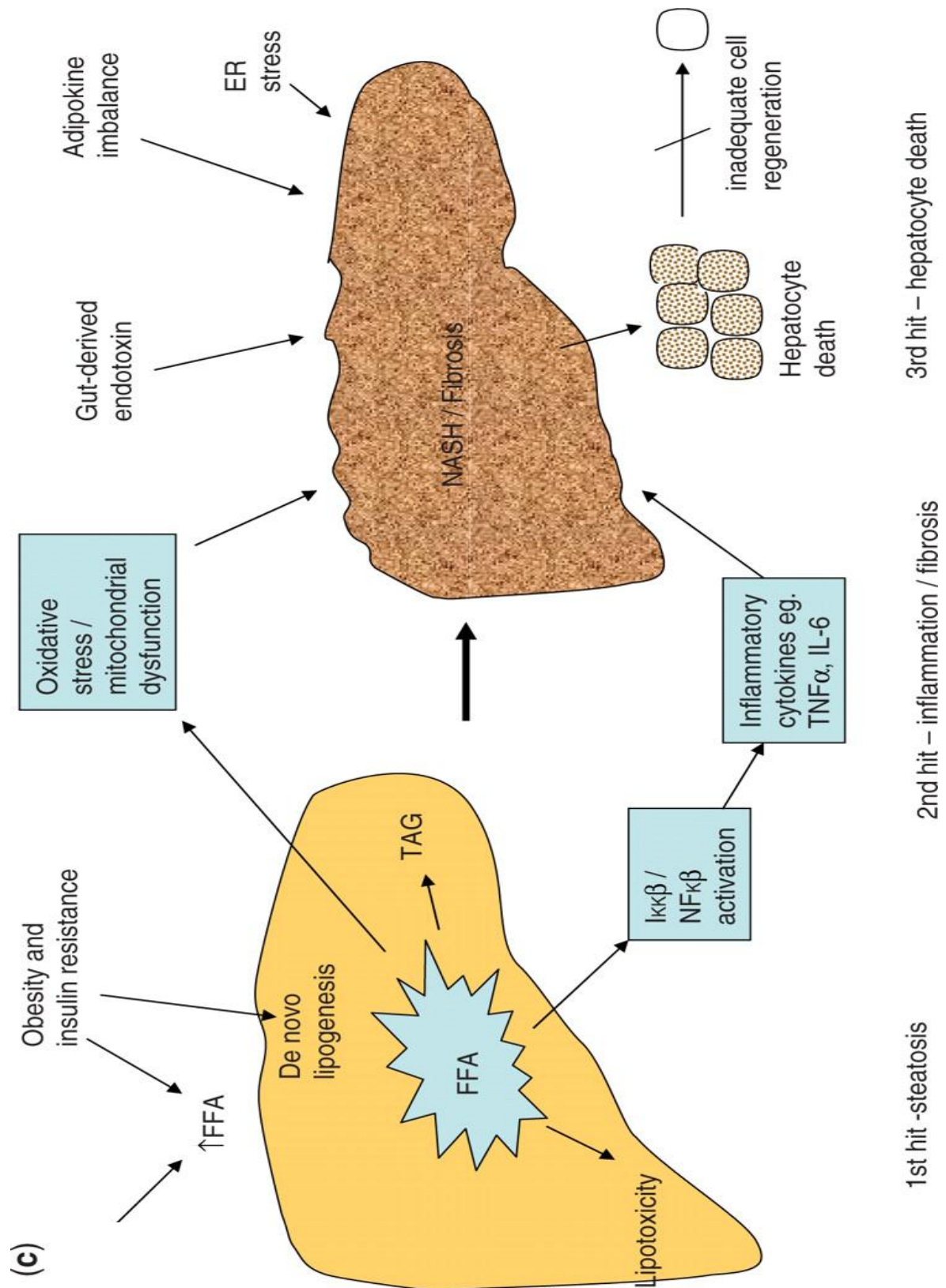


FIG 2.6: PATHOGENESIS OF NAFLD (Ref: Dowman et al., 2010)

SUMMARY

- The interaction of the genetics, lifestyle and diet are together labelled as NAFLD phenotype.
- Genetic defects in hydroxy acyl-CoA transferase, triglyceride removal, oxidative phosphorylation, impaired mitochondrial function, familial hypo β lipoproteinemia, family history of steatohepatitis and cryptogenic cirrhosis are risk factors for NAFLD.
- Certain genotypes and genetic mutations have been identified in Asian Indians with NAFLD.
- The two-hit hypothesis best describes the flow of hepatic events that translate into NAFLD and subsequent NASH.
- The first hit comprises of peripheral insulin resistance mediated adipose tissue lipolysis that directs the FFA into hepatocytes.
- Prolonged peripheral insulin resistance leads to DNL that is activated by SREBP-1c.
- The imbalance between liver's uptake, synthesis and removal of fatty acids leads to hepatic steatosis.
- Hyperinsulinemia triggers a lipotoxic environment in the liver as hepatic insulin resistance sets in.
- Free fatty acids reduce insulin signalling in a dose dependent manner.
- Hepatic insulin resistance increases glucose concentration in the liver.
- A vicious cycle sets in between insulin resistance, FFA delivery to the liver, existing state of steatosis, unabated endogenous glucose production that gives rise to inflammation.
- Lipotoxicity occurs owing to the overload of the mitochondrial beta oxidation system.
- Inflammation and lipotoxic environment stimulate cytokine production generated by ROS.
- These cytokines give rise to hepatic stellate cells that produce collagen and lead to fibrosis which is the second hit.

DIAGNOSIS OF NON-ALCOHOLIC FATTY LIVER DISEASE

For diagnosis of NAFLD, the prerequisites are to; a) rule out ethanol intake or an intake less than 21 drinks/week in case of men and less than 14 drinks in case of women to correctly diagnose fatty liver of non-alcoholic nature (Sanyal et al., 2011), b) evidence of fat deposition in the triglycerides through imaging or histology, c) rule out other competing aetiologies for fatty liver and d) absence of co-existing causes of chronic disease (Nichols, 2013; Chalasani et al., 2012). The World Gastroenterology Organisation (WGO) global guidelines recommended that the alcohol consumption threshold should be < 30 g/day (3.75 units) in men and < 20 g/day (2.5 units) in women.

There are differences in features of the profile of those with NAFLD with alcoholic fatty liver disease. The details of the same have been elucidated in the following table (table 2.2).

TABLE 2.2: TYPICAL FEATURES OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND ALCOHOLIC FATTY LIVER DISEASE (AFLD)

FEATURES	NAFLD	AFLD
Body weight	Increased	Variable
Fasting blood glucose or glycated hemoglobin	Increased	Normal
Reported daily alcohol intake	< 20 g for women and < 30 g for men	> 20 g for women and > 30 g for men
ALT	Increased or normal	Increased or normal
AST	Normal	Increased
AST / ALT ratio	< 0.8	> 1.5
GGT	Increased or normal	Considerably increased
Triglycerides	Increased	Variable, may be considerably increased
HDL – C	Low	Increased
Mean corpuscular volume	Normal	Increased

Ref: Adapted from Sattar et al., 2014

Clinical Signs and Symptoms

The disease in majority of the patients is of a primarily asymptomatic nature (Puri and Sanyal, 2012; Lewis and Mohanty, 2010; Smith and Adams, 2011; WGO, 2014). Hence, its impact remains highly underestimated (Kim and Younossi, 2008). If at all the symptoms are present, they often confound with the symptoms of other morbidities, or other non-specific symptoms of the discomfort in the gastrointestinal tract. Fatigue may be reported by few; however, it correlates poorly with the histological stage of the disease (Tarantino et al., 2007). Hepatomegaly is a common physical finding upon examination (Puri and Sanyal, 2012) and mild hepatomegaly may be evident in half of the NAFLD patients (Duseja, 2010). However, signs of liver failure maybe visible only when the NAFLD patient may have progressed onto cirrhosis or HCC (Duseja, 2010).

Biochemical Aberrations

A perfect biomarker for diagnosing a disease should be simple, reproducible, reliable, valid and readily available (Mavrogiannaki and Migdalis, 2013). Unfortunately, there is no such biomarker for diagnosis of NAFLD. However, in the routine clinical practice, liver enzymes are estimated as they are relatively cheap and easy to measure (Alavian, 2010).

Mild or a moderate increase in ALT or the AST levels are observed in NAFLD patients (Angulo, 2007), which may be 1-2 times the upper limit of normal (Sanyal, 2002; Neuschwander-Tetri and Caldwell, 2003). Lipid deposition in the hepatocytes swells the mitochondria that increase the lysosomal fragility causing release of enzymes from the hepatocytes (Angulo et al., 1999). ALT is more sensitive to hepatic triglyceride deposition and it correlates with hepatic fat independent of obesity (Kotronen et al., 2007). AST levels also rise when there is progression from simple steatosis to NASH (Marchesini et al., 2008). However, aminotransferase levels can be within the normal range in individuals with NAFLD (Vernon et al., 2011) with upto 50% (Sanyal et al., 2011) of them having normal liver enzymes concentration and also in those with advanced disease (Mofrad et al., 2003; Ruhl and Everhart, 2003; Gupte et al., 2004; Ahmed, 2015). Hence, normal aminotransferases do not guarantee absence of NAFLD or of advanced liver disease (Szczepaniak et al., 2005). The entire

spectrum of NAFLD may be present even without elevation of the transaminases (Mofrad P et. al, 2003). Moreover, aminotransferases need not necessarily correlate with the severity of NAFLD or with liver histology (Byrne, 2012).

GGT maybe considered a reliable marker of visceral and hepatic fat and thereby, of IR (Gohel and Chacko, 2013) and it maybe >35 U/L in NAFLD (Mcavoy et al., 2006). GGT elevation has also been used to predict advanced fibrosis in NAFLD patients using a cut off value of 96.5 U/L with 83% sensitivity and 69% specificity (Tahan et al., 2008). Alkaline phosphatase is sometimes elevated (Pantsari and Harrison, 2006).

Mildly elevated serum ferritin is thought to be common in patients with NAFLD and it need not necessarily indicate increased iron stores (Vuppalachchi and Chalasani, 2009; Kowdley, 2010). Since ferritin is an acute phase protein, the elevation is seen owing to systemic inflammation or increase in iron stores or both (Kowdley et al., 2012). Also, the pro-inflammatory cytokines like TNF- α , up regulate ferritin (Smirnov et al., 1999; Kwak et al., 1995; Pham et al., 2004). However, the role of elevated ferritin in the contribution of NAFLD and its progression requires further elucidation (Sumida et al., 2009). But, NAFLD patients with elevated ferritin and transferrin saturation should be screened for genetic hemochromatosis (Chalasani et al., 2012).

Hypoalbuminemia (low albumin count), prolonged prothrombin time, thrombocytopenia (low platelet count) and hyperbilirubinemia (elevated bilirubin) are not so commonly seen and are suggestive of NAFLD in advanced stage (Collantes et al., 2004; Perlemuter et al., 2007), the cirrhotic stage of NAFLD (Angulo, 2002). This is especially so in case of portal hypertension present in conjunction with cirrhosis (Smith and Adams, 2011).

The novel biomarker of hepatic apoptosis, cytokeratin-18 (CK-18) is emerging to be a marker of NASH (Dowman et al., 2011). It is found to be significantly elevated in those with NASH and had an AUC of 0.93 in predicting NASH (Wieckowska et al., 2006). It was not only found to be an independent predictor of NASH but also of its severity (Feldstein et al., 2009). CK-18 can be used to assess the impact of therapy on NASH cases (Obika and Noguchi, 2012). Type IV collagen 7S domain and haluronic

acid have shown promising results to be used as biomarkers for the diagnosis and staging of fibrosis (Obika and Noguchi, 2012). Markers of oxidative stress have been investigated with special interest in vitamin E, copper to zinc superoxide dismutase activity, glutathione peroxidase activity, showing mixed results (Wieckowska et al., 2007; Bonnefont-Rousselot et al., 2006, Chalasani et al., 2004).

Imaging

Ultrasonography (USG)

Ultrasound is recommended as the first line of investigation to confirm the presence of fatty liver owing to its availability (Ratzu et al., 2010). USG is the most preferred modality for the diagnosis of NAFLD as it is non-invasive, radiation free, has no side effects and is relatively cheaper than other modalities (Singh et al., 2013; Roldan-Valadez et al., 2008). USG has a good sensitivity and specificity in detecting moderate and severe steatosis (Bhatia et al., 2012). A recent meta-analysis concluded that ultrasound is an accurate and a reliable imaging technique for the diagnosis of NAFLD (Hernaez et al., 2011). In detecting hepatic steatosis, ultrasound has 89% sensitivity and 93% specificity and in detecting fibrosis, ultrasound has 77% sensitivity and 89% specificity (Angulo, 2002).

The liver appears brighter than the kidney and the spleen owing to fatty infiltration (Valls et al., 2006) and this contrast in brightness aids in detecting fatty liver (Sanyal, 2002). A significant disadvantage of the ultrasound technique is that the sensitivity goes down when fatty infiltration is less than 30% (Ryan et al., 2002) and 40% in case of those with obesity (Mottin et al., 2004). Its accuracy is dependent upon the prevalence of steatosis and is also operator dependent (Roldan-Valadez et al., 2008).

Computed Tomography Scans (CT Scans)

CT can detect focal lesions (Roldan-Valadez et al., 2008) and can diagnose hepatic steatosis $\geq 30\%$ with 82% sensitivity and 100% specificity (Park et al., 2006). High resolutions CT scans that do not make use of the contrasting agents is considered to be the best method for assessing the hepatic fat. It allows for the quantitative measurement of the attenuation of the liver in Hounsfield units (HU) (Santos et al., 2010). Fatty infiltration is diagnosed when liver attenuation is < 48 HUs (Kadama et

al., 2007). The advantage with this technique is that it is quick and non-operator dependent (Singh et al., 2013). However, the information about the liver attenuation varies between the radiologists (Obika and Noguchi, 2012). Also, it is unable to diagnose mild steatosis owing to limited sensitivity. Then there is cost factor and risk of radiation exposure as well (Bohte et al., 2011).

Magnetic Resonance Imaging (MRI) or Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance imaging is able to detect even less than 33% of fat in the hepatocytes (Roldan-Valadez et al., 2008) and is most accurate technique in detecting steatosis at the low levels (Obika and Noguchi, 2012). It makes use of two techniques to assess the liver fat content, namely, chemical displacement and spectroscopy (Santos et al., 2010). It is expensive, time consuming, and is not accessible to many and the software required for the post processing of the data is also not easily available, even though the MRI scanner would be (Roldan-Valadez et al., 2008).

Magnetic resonance spectroscopy allows the direct measurement of the area under the lipid resonance peak, providing quantitative evaluation of the fatty infiltration in the hepatocytes (Roldan-Valadez et al., 2008). MRS helps to detect hepatic fatty infiltration even in microscopic quantities (Singh et al., 2013). It has 91% sensitivity and 87% specificity in detecting hepatic fat (van Werven et al., 2010). The proton MRS holds the best accuracy amongst all the imaging modalities to diagnose NAFLD and quantify the intra-hepatic fat content. It also correlates better with the biochemical profile even when fat deposition is in small quantities. It also holds the advantage that it takes into consideration a larger mass of hepatic tissue for analysis than the biopsy (Schwenzer et al., 2008; Szczepaniak et al., 1999). However, it is not available for routine usage in a clinical set up (Longo et al., 1995). The summary of non-invasive methods of detecting hepatic fat content has been listed in table 2.3.

New Imaging Technologies

The newer technological concept such as sonoelastography is a finer technique that is now being used to detect fatty liver. Sonoelastography aids in the estimation of liver stiffness which is affected by fatty infiltration in the liver and it includes transient elastography/fibroscan and acoustic radiation force pulse (ARFI) elastography (Singh et al., 2013). It aids in assessing liver fibrosis by measuring the liver stiffness (Yoneda

et al., 2008). It was originally developed for patients with hepatitis C (Ziol et al., 2005) but is now being used in those with NAFLD as well (Yoneda et al., 2007).

Doppler perfusion index (DPI) is a ratio of hepatic arterial blood flow to total liver blood flow. It is also an ultrasound based technique. Under condition of NAFLD, the DPI will be altered owing to changes in the liver parenchyma because of fat deposition (Leen et al., 2000; Kakkos et al., 2000; Dugoni et al., 2008).

TABLE 2.3: NON-INVASIVE METHODS OF DETECTING HEPATIC FAT

Factors	Imaging techniques		
	Ultrasound	Computed Tomography	Magnetic Resonance Imaging
Assessment	Qualitative	Qualitative and quantitative	Qualitative and quantitative
Cost	Low	Medium	Expensive
Method of assessment	Visual comparison	Liver attenuation, liver-spleen attenuation ratio	Spectroscopy, in and out of phase
Guide for liver biopsy	Yes	Yes	Yes
Accuracy	Operator dependent and steatosis features dependent	Not operator dependent	Not operator dependent
Acquisition time	Variable, 5-20 minutes, operator dependent	Less than 5 minutes	10-15 minutes
Availability	Equipment present in most hospitals	Equipment present in most hospitals	Software not easily available
Use of radiation	None	Ionizing radiation	None
Nephrotoxic contrast agent	None	Some methods use iodide contrast agents	None
Threshold of detection	Patients with more than 30% steatosis	Patients with more than 30% steatosis	Detects grade 1 steatosis (5-30%)
Distinguishes NASH from NAFLD	No	No	No (some sequences can quantify fibrosis)

Ref: Adapted from Roldan-Valadez et al., 2008

Liver Biopsy

Liver biopsy is the gold standard for the diagnosis and staging of NAFLD (Rinella et al., 2014; Nouredin and Loomba, 2012). It aids in diagnosing the grade i.e. the lesions of steatosis and inflammation and the type or stage of fibrosis (Attar and Thiel, 2013). It can exclude drug induced hepatotoxicity, Wilson disease and autoimmune hepatitis (Angulo, 2002; van Ness and Diehl, 1989). Biopsy requires quantifying the accumulated fat, peri-lobular inflammation if any and to what extent, ballooning degeneration, Mallory hyaline and acidophil bodies with or without fibrosis and its staging (Caceane, 2012; Schwenger and Allard, 2014). For grading and staging of NAFLD, four parameters are used; steatosis, inflammation, cellular ballooning and fibrosis (Rinella et al., 2014).

The global guidelines for NAFLD/NASH recommend that liver biopsy should be conducted on those having risk factors for NASH and/or other liver diseases (WGO, 2014). It is also recommended for those whose liver enzymes remain persistently abnormal inspite of having incorporated lifestyle changes (Collantes et al., 2004) or wherein persistent liver enzymes elevation is seen without its known origin (Firmeisz, 2014). Simultaneously, there has to be assessment of the presence of the following risk factors; age grater than 45 years, presence of obesity, type 2 diabetes, AST/ALT ratio ≥ 1 (Angulo, 2002).

Liver biopsy is a painful invasive procedure (Castera et al., 1999) with infection, biliary leakage (Roldan-Valadez et al., 2008), bleeding, and risk of mortality as significant disadvantages (Ryan et al., 2002). It requires the patient to undergo atleast 6-8 hours of bed rest (Grant and Neuberger, 1999). Hence, there is not much acceptance for this procedure and it is difficult to be repeated (Mavrogiannaki and Migdalis, 2013). The possibility of sampling error is high as less than 1/50,000th of the liver is available for histological analysis (Mehta et al., 2008) and may lead to misdiagnosis or staging inaccuracies (Merriman et al., 2006) owing to patchy nature of the disease (Byrne, 2012), especially fibrosis (Ratziu et al., 2005; Janiec et al., 2005). Intra and inter observer variability can occur in the analysis of liver biopsy samples (Kleiner et al., 2005; Younossi et al., 1998). Moreover, the procedure related morbidity and mortality does not warrant its use in the routine clinical practice (Chalasani et al., 2012). Hence, it is not a viable screening tool for NAFLD.

Therefore, it should be performed sparingly (Weiß et al., 2014). Moreover, it is futile to conduct liver biopsy in the absence of evidence based guidelines for the treatment of NAFLD (WGO, 2014), nor any available evidence to alter the natural history of the disease (Santos et al., 2010).

SUMMARY

- Diagnosis of NAFLD requires to rule out; significant ethanol intake, other competing aetiologies for fatty liver, co-existing causes of other chronic disease and imaging or histological evidence of fat deposition in the hepatocytes.
- NAFLD is asymptomatic. However, mild hepatomegaly upon examination and GI tract disturbances may be present in a few NAFLD cases.
- There is no biomarker available till date to accurately diagnose NAFLD.
- Reliance often is on liver enzymes that are poor surrogate markers of NAFLD.
- The entire spectrum of NAFLD maybe present in the presence of normal liver enzymes.
- GGT maybe a close correlate of IR, visceral fat and hepatic fat.
- Ferritin as a biomarker of NAFLD is still debatable.
- Hypoalbuminemia, prolonged prothrombin time, thrombocytopenia and hyperbilirubinemia are suggestive of cirrhotic stage of NAFLD, especially in case of portal hypertension.
- Cytokeratin-18 is emerging as a novel biomarker of NASH and of its severity.
- Ultrasound is recommended as the first line of confirmatory diagnosis of NAFLD. It scores over other imaging modalities and biopsy owing to its manifold advantages over other techniques.
- High resolutions CT scans are considered to be the best method for quantitative hepatic fat assessment.
- Magnetic resonance scores over other techniques in terms of accuracy in NAFLD diagnosis but is not an easily available technology.
- New techniques such as transient elastography and Doppler perfusion index are being explored as new modalities for the assessment of NAFLD.

- Liver biopsy is the gold standard for the diagnosis and staging of NAFLD.
- Liver biopsy is recommended for those with prevalent risk factors for NASH, abnormal liver enzyme concentration of unknown origin or after lifestyle incorporation.
- The technique has more disadvantages to its credit rather than merits, hence, liver biopsy is not a viable screening tool for NAFLD.

THE REPERCUSSIONS OF NEGLECT OF NON-ALCOHOLIC FATTY LIVER DISEASE

Liver is a metabolic organ that is involved in energy and lipid metabolism (Fan and Cao, 2013). It plays a fundamental role in protein synthesis, glycogen storage and detoxification (Yasutake et al., 2012). Any dysregulation in this machinery gives rise to several metabolic derangements (fig 2.7). Consequently, NAFLD has relevant hepatic and cardio metabolic repercussions (Scorletti et al., 2011; Byrne et al., 2009; Bhatia et al., 2012). It is associated with an increased risk of all cause mortality (Ahmed and Byrne, 2007; Adams et al., 2005). Alarming, the mortality in those with NAFLD has increased significantly, especially in the general population (Hamaguchi et al., 2007; Santoliquido et al., 2005).

It is postulated that NAFLD and NASH will have its impact by becoming an increasingly common liver disease worldwide, with all the economies suffering alike. Moreover, it will affect the global public health systems and the health care costs (WGO, 2014). With respect to type 2 diabetics, the liver disease is now recognised as a major complication (Tolman et al., 2004 and Marco et al., 1999) because the presence of NAFLD is associated with increase in total mortality, independent of the classical risk factors (Söderberg et al., 2010).

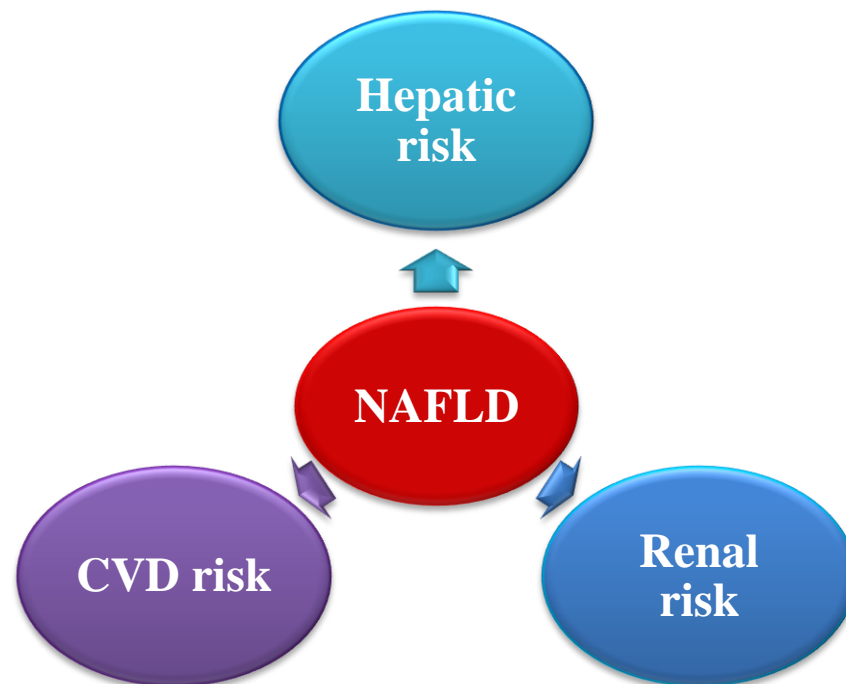


FIG 2.7: MAJOR REPERCUSSIONS OF NAFLD

Hepatic Risk

Hepatic steatosis is said to be of benign nature which may remain so for a prolonged period of time without causing any major hepatic damage (Li et al., 2002; McCullough, 2002). However, it may progress onto advanced NAFLD owing to the risk factors (Westphal, 2008; Palekar et al., 2006; Ruhl and Everhart, 2004; Dixon et al., 2001) as depicted in fig 2.8. Steatosis *per se* or the pathophysiological mechanisms of NAFLD have carcinogenic potential as well (Kikuchi et al., 2014). The risk is highest amongst those with advanced fibrosis and cirrhosis (Bugianesi et al., 2002; Hashimoto et al., 2009; Smedile and Bugianesi, 2005; Takuma and Noso, 2010; Ascha et al., 2010; Yasui et al., 2011; Afendy et al., 2009). Danger lurks for those with non-cirrhotic NASH as they can develop NASH related HCC without cirrhosis (Vernon et al., 2011). Though HCC maybe a rare complication in patients with NAFLD, it should not be under-estimated (Kikuchi et al., 2014).

Of those with simple steatosis, 40% may progress onto NASH (Vanni and Bugianesi, 2014). Those with NASH have a higher liver related mortality as compared to those with hepatic steatosis (Musso et al., 2011) and the general population (Adams et al., 2005; Rafiq et al., 2009; Ong et al., 2008; Bellentani and Marino, 2009; Sanyal et al., 2006; Wong et al., 2010). NASH can progress onto cirrhosis, HCC and also liver failure (Serfaty and Lemoine, 2008; Pais et al., 2011; Vernon et al., 2011; Musso et al., 2011), arising the need for liver transplantation as well (Marrero et al., 2002). Now, NASH is becoming a major reason for liver transplantation (Byrne, 2012). The problem becomes even more acute as only one in ten would get a liver and of them 30% would have a relapse of fatty liver after the liver transplantation (Nichols, 2013). Moreover, if an allograft has biopsy proven NASH, it is deemed unfit for liver transplantation (Tevar et al., 2010).

There is a greater risk for liver related mortality in NAFLD patients with type 2 diabetes (Rafiq et al., 2009). A risk ratio of 3.3 for overall mortality and 22.8 for liver disease related mortality was reported in diabetic patients with NAFLD as compared to the non-diabetic patients with NAFLD (Younossi et al., 2004). Type 2 diabetics are at a higher risk of developing cirrhosis as compared to the non-diabetics (Angulo et al., 1999; Younossi et al., 2004). Type 2 diabetes is known to cause more severe NASH (Oprea-Călin et al., 2014). Moreover, majority of the cases of cryptogenic

cirrhosis are diabetics (Maheshwari and Paul, 2006; Caldwell and Lee, 2008). NASH related cirrhosis is now the second most common cause of age related mortality in type 2 diabetics (Das et al., 2006). T2DM and NASH together may predispose an individual to HCC (Bugianesi et al., 2007). The presence of type 2 diabetes in NAFLD patients is taken to be a predictor for the development of fibrosis and eventual liver complications (Wanless and Lentz, 1990; Neuschwander-Tetri and Caldwell, 2003). Though cardiac disease maybe the leading cause of mortality in type 2 diabetics, liver failure is also a potent threat, that is unrealised and neglected (Bugianesi et al., 2007; Younossi et al., 2004) and hepatic causes of mortality are actually masked by cardiovascular events (Gaede et al., 2003).

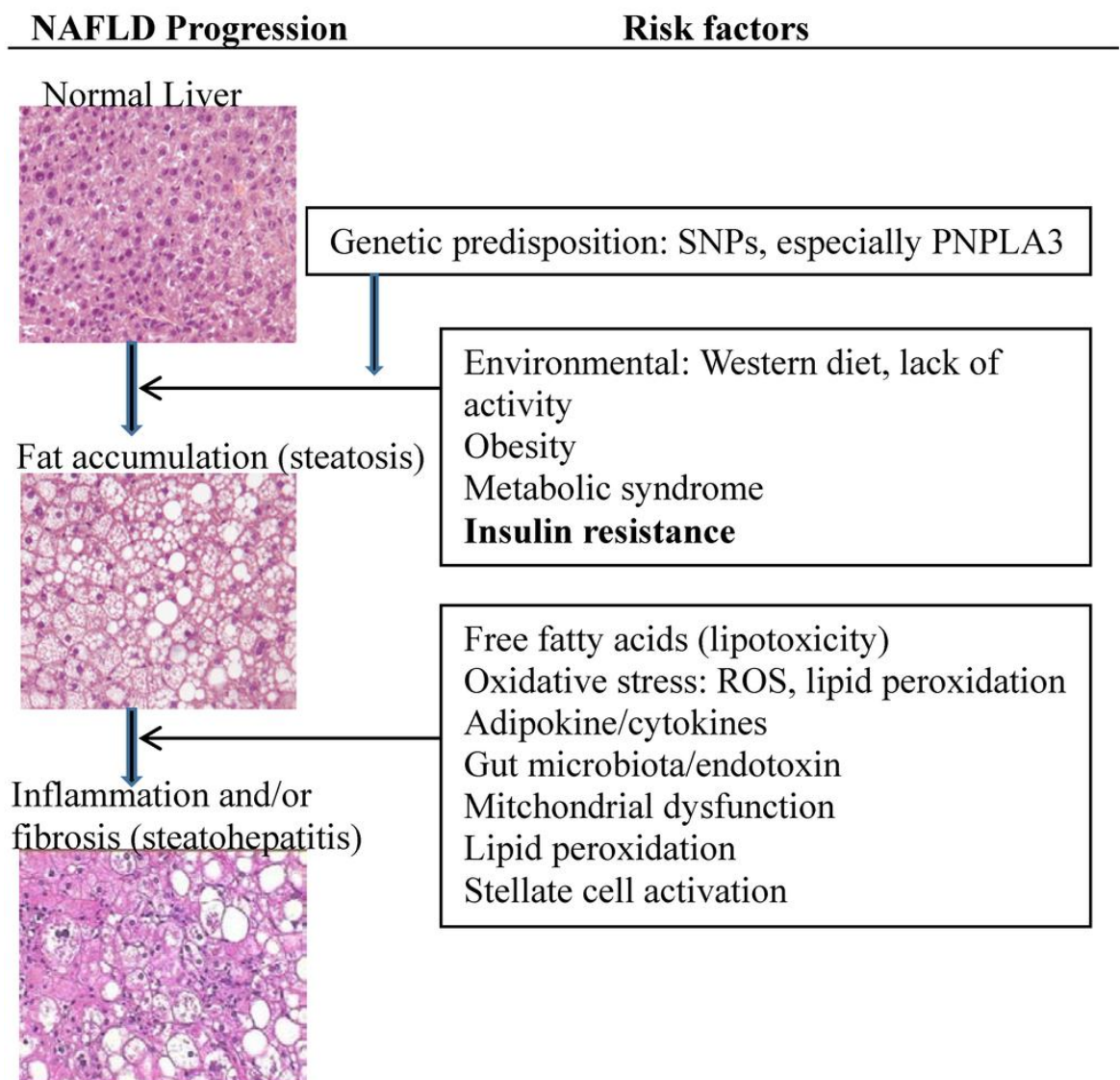


FIG 2.8: PROGRESSION OF NAFLD (Ref: Yang et al., 2014)

Cardiovascular Disease Risk

A significantly higher carotid intima-media thickness (CIMT), impaired endothelial function (Villanova et al., 2005), increased prevalence of carotid atherosclerotic plaques (Sookoian and Pirola, 2008) and lower concentrations of adiponectin have been documented in cases of fatty liver compared to those with a non-fatty liver (Caserta et al., 2010; Treeprasertsuk et al., 2011).

Reduced survival in patients with bland steatosis owing to cardiovascular events in a median follow up of 24 years has been observed (Soderberg et al., 2010). Alarming, simple steatosis is associated with silent carotid atherosclerosis (Ramilli et al., 2009). This may suggest the possible role of NAFLD in cerebrovascular disease causation (Byrne, 2012).

NAFLD is associated with an increased risk of mortality due to cardiovascular disease (CVD) and it also predicts future CVD events (Takeuchi et al., 2012; Bhala et al., 2011) over and above the classical risk factors (Ahmed and Byrne, 2007; Kim et al., 2012), confirmed by a recent systematic review and meta-analysis as well (Lu et al., 2013). Patients with NAFLD have CVD as the most common cause of mortality (Chalasani et al., 2012; Targher et al., 2010). NAFLD is now considered to be an independent risk factor for the occurrence of CVD (Targher et al., 2010). Recently, an increased incidence of CVD in NAFLD patients was found to be associated with reduced insulin sensitivity (Gastaldelli et al., 2009). Those with NASH are at a greater risk of mortality due to CVD than liver related mortality compared to patients with simple steatosis (Ekstedt et al., 2006).

In an approximately 13 years (Rafiq et al., 2009) and 21 years (Dam-Larsen et al., 2009) cohort on cases of biopsy proven NAFLD, CVD and malignancy were the main causes of mortality. In a cohort of mean 28 years involving Swedish NAFLD patients, mortality risks was highest in the NASH followed by NAFLD and least in the general population. CVD was the most common cause of mortality (Soderberg et al., 2010).

With respect to diabetics, significantly higher age and sex adjusted prevalence of cardiovascular, cerebrovascular and peripheral vascular disease was reported amongst the type 2 diabetic NAFLD patients as compared to those without NAFLD (Targher et al., 2007). Independent of the components of MS and the classical risk factors for

CVD, the combination of type 2 diabetes and NAFLD maybe linked to incident CVD (Targher et al., 2005; Targher et al., 2006; Targher et al., 2007) as depicted in fig 2.9.

In a community based cohort of NAFLD type 2 diabetics followed up for 9 years, overall mortality was significantly associated with diagnosis of NAFLD, presence of ischemic heart disease and the duration of diabetes (Adams et al., 2010). Early features of LV diastolic dysfunction maybe detected in type 2 diabetics who have NAFLD (Bonapace et al., 2012).

Though prospective studies have documented an increased incidence of CVD among the NAFLD patients, it is yet unclear whether NAFLD is simply a risk factor that coexists in people at high risk of CVD or is an independent risk factor in itself for the occurrence of NAFLD (Ekstedt et al., 2006; Targher et al., 2007; Soderberg et al., 2010; Haring et al., 2009; Adams et al., 2005). A weak link between NAFLD and CVD has also been established (Perseghin, 2010).

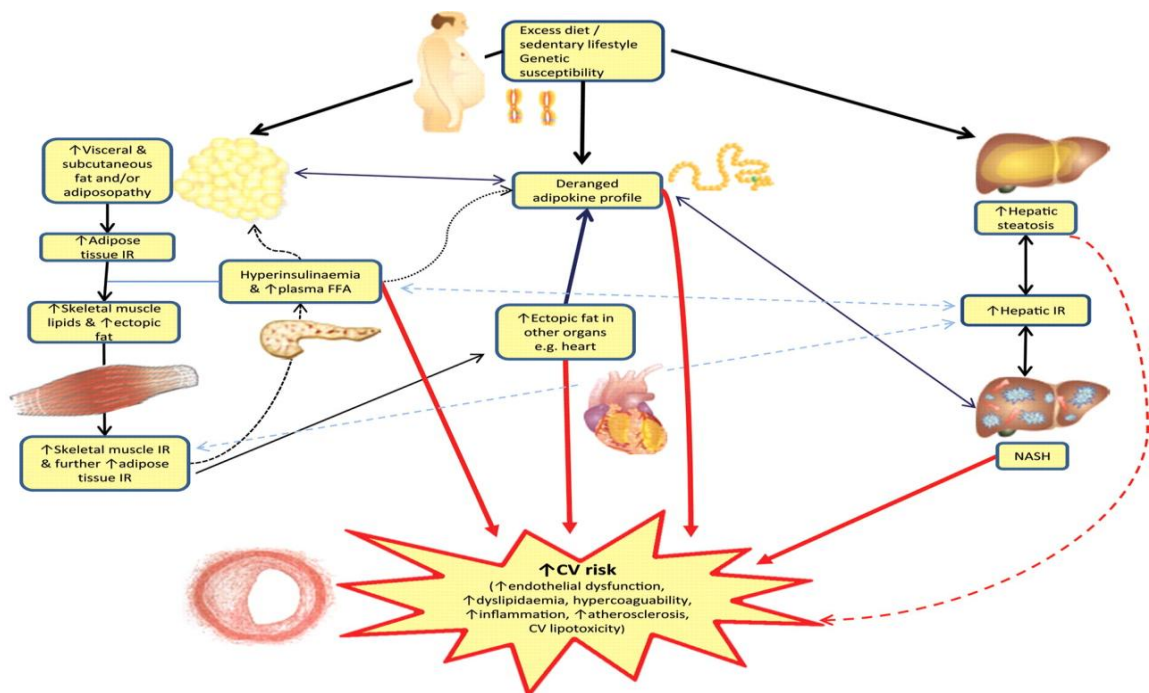


FIG 2.9: SCHEMATIC DIAGRAM OF THE PATHOPHYSIOLOGICAL PROCESSES INVOLVED IN NAFLD LEADING TO INCREASED CV RISK, HIGHLIGHTING THE COMPLEX INTER-RELATIONSHIPS BETWEEN VISCERAL ADIPOSE TISSUE, ADIPOCYTOKINES, INSULIN RESISTANCE, ECTOPIC FAT ACCUMULATION AND NAFLD (Ref: Bhatia et al., 2012).

Renal Risk

A recent systematic review and a meta-analysis revealed that NAFLD was associated with an increased risk of prevalent and incident chronic kidney disease (CKD). NASH was associated with a higher prevalence and incidence of CKD than simple steatosis. Advanced fibrosis was associated with a higher prevalence and incidence of CKD than non-advanced fibrosis. The presence and severity of NAFLD are associated with an increased risk and severity of CKD (Musso et al., 2014). In type 2 diabetics with NAFLD as well, prevalent and incident CKD has been observed (Targher et al., 2013). NAFLD has also been found to be independently associated with an increased prevalence of CKD as well as retinopathy in type 2 diabetics (Targher et al., 2008).

SUMMARY

- NAFLD is associated with detrimental hepatic, cardio-metabolic and renal repercussions.
- Hepatic steatosis is thought to be of benign nature but it may progress onto advanced NAFLD under the presence of risk factors.
- Steatosis, advanced fibrosis, cirrhosis, non-cirrhotic NASH confer carcinogenic risk as it may translate onto hepatocellular carcinoma.
- NASH is becoming a major reason for liver transplantation.
- For type 2 diabetics, there is a risk ratio of 3.3 for overall mortality and 22.8 for liver disease related mortality and they also have a higher risk of developing cirrhosis.
- Type 2 diabetes causes more severe NASH.
- NASH related cirrhosis is the second most common cause of age related mortality in type 2 diabetic.
- Simple steatosis is associated with silent carotid atherosclerosis.
- CVD is the most common cause of mortality in NAFLD patients.
- Type 2 diabetes and NAFLD together maybe linked to incident CVD, irrespective of classical risk factors and MS.
- Whether NAFLD is a co-existing or an independent CVD risk factor is unclear.

- NAFLD is associated with an increased risk of prevalent and incident CKD and NASH has an even higher association.
- In type 2 diabetics, NAFLD is independently associated with an increased prevalence of CKD and retinopathy.

IS NON-ALCOHOLIC FATTY LIVER DISEASE REVERSIBLE?

When NAFLD is caught in its initial stages, the condition can be reversed if the underlying metabolic aberrations are corrected or interrupted from further progression (Santos et al., 2010). For the same, incorporating lifestyle changes is critical for reversing the course of NAFLD and NASH (WGO, 2014) and to decrease the hepatic fat (Byrne, 2012).

NAFLD may also be reversible if BMI and the components of the MS are addressed for management, wherein weight may not essentially normalise (Powell et al., 2005). A recent review concluded that NAFLD is reversible under conditions wherein at least 3-5% of weight loss is documented (Weiß et al., 2014).

An 8kg weight loss in type 2 diabetics who had poor control, reversed their hepatic steatosis. The fasting plasma glucose concentrations, rate of endogenous hepatic glucose production and the hepatic insulin responsiveness also normalized in them (Petersen et al., 2005).

Fatty liver was resolved in 35% of the NAFLD with hypertriglyceridemia after 6 months of 15ml/day n-3 PUFA supplementation (Hatzitolios et al., 2004).

A study wherein lifestyle intervention in NAFLD cases comprised of 10 sessions with a dietician and moderate intensity physical activity (PA) of 3hours/week, a significant decrease in body fat and liver fat was observed along with an increase in fitness levels. The condition of NAFL resolved in 20 patients at the end of the intervention (Kantartzis et al., 2009).

In a randomized controlled trial involving 31 sedentary type 2 diabetic patients with NAFLD of Caucasian race, the effects of four months of aerobic training and resistance revealed that after the intervention, hepatic fat content saw a similar reduction in both the groups, also one quarter of patients in both the arms became free of hepatic steatosis. An improvement in insulin sensitivity along with reduction in total body fat mass, VAT and superficial and deep subcutaneous fat and glycated hemoglobin reduced comparably in both the arms. The study concluded that both the forms of training were equally effective in reducing the hepatic fat content in the study group (Bacchi et al., 2013).

MANAGEMENT AND TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

There are no established therapies nor any evidence based guidelines for the treatment of NAFLD owing to dearth of prospective, double blind, controlled trials (WGO, 2014). The current treatment and management modalities revolve around correcting the underlying metabolic abnormalities associated with NAFLD and discontinuing hepatotoxic drugs, if any (Lewis and Mohanty, 2010; Than and Newsome, 2015). The WGO guidelines emphasize on proper control of diabetes, hyperlipidemia and cardiovascular risks in the treatment of NAFLD/NASH (WGO, 2014).

The primary aim of management of NAFLD is to improve steatosis and prevent progression to fibrosis (Schwenger and Allard, 2014). There are two primary goals while treating type 2 diabetic NAFLD patients; 1) to improve their quality of life and 2) to prolong their survival. The primary target is to change their lifestyle and strengthen self monitoring through patient education and the secondary target is to reduce the liver fat content and avoid further disease progression (Shams et al., 2011).

Weight loss and lifestyle modification should be propagated and used as the first line therapy for the treatment of NAFLD (Schwenger and Allard, 2014; Mavrogiannaki and Migdalis, 2013) and whatever be the treatment modality, lifestyle intervention has to be inclusive of it (Rinella et al., 2014). The various management modalities of NAFLD have been discussed below (fig 2.10).

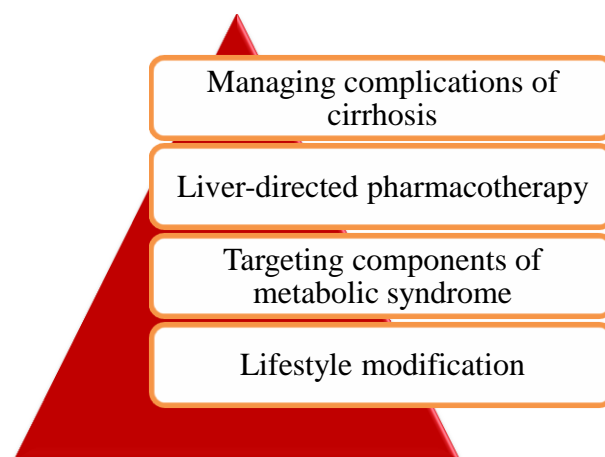


FIG 2.10: MANAGEMENT STRATEGIES IN NAFLD

Ref: Adapted from Dyson et al., 2014

Insulin Sensitizing Agents

Biguanides increase the hepatic insulin sensitivity and reverse IR induced by TNF- α . Glitazones activate the nuclear transcription factor, peroxisome proliferators-activated receptor (PPAR) γ (Kim and Younossi, 2008). However, sulfonylureas lead to weight gain that may hinder with the effective management of NAFLD (Dyson et al., 2014).

Metformin

Metformin is used in the treatment of type 2 diabetes as it decreases gluconeogenesis in the liver and decreases intestinal glucose absorption (Rector et al., 2008). This stimulates glucose uptake in the muscle and increases fatty acid oxidation (Zhou et al., 2001) and leads to an improvement in insulin sensitivity (Mazza et al., 2012).

In non-diabetic individuals with NAFLD, metformin reduces hepatic fat by half and aids in reducing inflammation and necrosis (Bugianesi et al., 2005). Metformin has shown to channelize the fatty acids towards β oxidation rather than favouring triglyceride production (Tarantino et al., 2007).

Metformin trials for improvement in liver histology have reduced aminotransferase levels and IR but with no changes in liver parenchyma (Marchesini et al., 2001; Nair et al., 2004), possibly because of limited anti-steatogenic effect and inability to increase the adiponectin levels (Tiikkainen et al., 2004). Thus, metformin is not recommended as a specific treatment for those with NASH (Chalasani et al., 2012).

In a study to determine the efficacy and safety of metformin in NAFLD, after three months of treatment with 20mg/kg/day, a reduction in aminotransferase levels was observed because of improvement in insulin sensitivity. But, this improvement could not be maintained during the entire tenure of the treatment which was for one year. This brought about the conclusion by the authors that metformin should not be used for the treatment of NAFLD (Nair et al., 2004).

Thiazolinidiones (TZDs)

TZDs are peroxisomal proliferator activated receptor- γ (PPAR- γ) agonists that are used for the treatment of diabetes that aid in improving insulin sensitivity within the liver, muscle and adipose tissue, promote hepatic fatty acid oxidation and decrease

hepatic lipogenesis (Oh et al., 2008; Van Wagner and Rinella, 2011). TZDs promote adipocyte maturation by regulating lipid metabolism at the hepatic level so as to improve insulin sensitivity at the adipose tissue, muscle and the liver (Rinella et al., 2014). They also aid in increasing the adiponectin levels and promote the β oxidation of fatty acids (Bajaj et al., 2004; Coletta et al., 2009).

They have shown to improve insulin sensitivity and bring down hepatic fat in type 2 diabetics (Belfort et al., 2006; Juurinen et al., 2008). A meta-analysis of type 2 diabetics treated with pioglitazone found an 18% reduction in death, myocardial infarction and stroke (Lincoff et al., 2007). However, the safety aspect of TZDs is not established in type 2 diabetics yet (Chalasani et al, 2012).

Rosiglitazone has shown to improve aminotransferases and hepatic steatosis but not necroinflammation or fibrosis (Neuschwander-Tetri et al., 2003; Ratziu et al., 2008, Ratziu et al., 2010). With pioglitazones as well, improvements in steatosis and inflammation have been observed minus significant improvement in fibrosis (Vernon et al., 2011). However, concerns remain over the long term usage of pioglitazones owing to congestive heart failure (Lago et al., 2007), bladder cancer (Piccinni et al., 2011), reduced bone density (Lecka-Czernik, 2010) as side effects.

The EASL and the AASLD recommend that TZDs could be used to treat biopsy proven NASH (Chalasani et al., 2012; Ratziu et al., 2010). However, they have also been associated with weight gain (Caldwell et al., 2006) of 3-5kgs (Musso et al., 2010) in 60-70% of the patients. This appears to be due to an increase in the adipose tissue than the water weight (Balas et al., 2007). Additionally, TZD therapy has often failed to show its effectiveness in the absence of lifestyle modification (Ratzu et al., 2008; Lutchman et al., 2007).

Exogenous Insulin

Initiation of insulin therapy with metformin at the point of diagnosis of type 2 diabetes has shown to normalise the aminotransferase and reduce hepatic steatosis. With the rationale that at the point of onset of type 2 diabetes, the patients were bound to have poor glycemic control, insulin therapy was taken as a treatment modality. This insulin deficiency was curbed by the exogenous insulin that may have perhaps activated peripheral lipogenic pathways that deposited fat in the extrahepatic adipose

tissues rather than the liver (Lingvay et al., 2007). Also, intensive insulin therapy at the basal and pre-meal stage in NAFLD patients with type 2 diabetes has shown to reduce hepatic steatosis (Mathew et al., 2009).

Lipid Lowering Drugs

Correction of dyslipidemia is necessary for NAFLD patients to reduce the risk of cardiovascular disease (Dyson et al., 2014). Hence, NAFLD patients with dyslipidemia are put on lipid lowering drugs.

Statins

Currently, there is a lack of evidence regarding risk of statin usage by NAFLD patients and hence the practice guidelines state that statins can be used only for the treatment of dyslipidemia in NAFLD and NASH patients and not for the condition of NAFLD and NASH *per se* (Chalasani et al., 2012). However, serious hepatotoxicity caused from statins (statin induced idiosyncratic hepatotoxicity) is rare and there is a dearth of evidence with data to statin usage in those with pre-existing NAFLD as well (Browning, 2006). Statin treatment even at high dosage is considered to be safe in NAFLD, even in conditions wherein transaminases maybe upto three times the upper limit of normal (Eslami et al., 2013).

Statins have also shown to be anti-inflammatory and immuno-modulatory in nature with inhibition of downstreaming the production of mediators of cell growth (Demierre et al., 2005) and promote cell programmed cell apoptosis (Wu et al., 2004) and anti-carcinogenic effect (Dyson et al., 2014). However, a limitation with the statins is that they may lead to IR indirectly (Bhatia and Byrne, 2010).

Ezetimibe

Targeting cholesterol reduction may also hold the key to treat NAFLD (Enjoji et al., 2012). Ezetimibe is a cholesterol absorption inhibitor (Ahmed and Byrne, 2010) or a Niemann-Pick C1-like 1 (NPC1LI) specific inhibitor capable of blocking 54% of cholesterol absorption from the intestine (Sudhop et al., 2002). It has a half life of 24 hours and is quickly absorbed into the blood and enters the enterohepatic circulation (Enjoji et al., 2012). The liver plays a significant role in maintaining cholesterol homeostasis. The liver receives most of the cholesterol absorbed by the small intestine

and then excretes it out as bile. The rate at which the liver synthesizes cholesterol is dependent on the amount of cholesterol being delivered to it by the small intestine. Any intervention in this bio process is capable of changing rates of cholesterol synthesis, conversion to bile acids, incorporation into VLDL-C, cholesterol esterification or release of unesterified cholesterol into the bile. Thus, ezetimibe may hold promise to be a potential treatment modality for NAFLD by correct lipid aberrations (Byrne, 2012) through downregulation or inactivation of the LXR- α SREBP-1c pathway (Enjoji and Nakamuta, 2010). However, more clinical evidence is required to establish cholesterol management as a mainstay therapy for the management of NAFLD (Enjoji et al., 2012).

Fibrates

Fibrates alter lipoprotein metabolism through the PPAR- α receptor (Tarantino et al., 2007). They activate PPAR- α that decreases TG, LDL, VLDL and increases the HDL, thereby correcting dyslipidemia (Athysos et al., 2006; Nakamuta et al., 2005). But, no significant improvements were observed in IR, hepatic steatosis, necroinflammation or fibrosis (Fernandez-Miranda et al., 2008). However, there is no recommendation with regard to fibrates in NAFLD.

Antioxidants, Cyto-Protective Agents, Hepato-Protective Agents

Ursodeoxycholic Acid (UDCA)- nor-Ursodeoxycholic Acid (nor-UDCA)

UDCA is a naturally occurring secondary bile acid (Schwenger and Allard, 2014) which is cytoprotective (Kim and Younossi, 2008). It has also been explored as a therapy for the treatment of NAFLD/NASH (Lindor et al., 2004). In a randomized controlled trial of a double blind nature, obese NAFLD patients benefited from UDCA given at 10mg/kg/day over a three month period. It was able to reduce liver enzymes but it failed to show an impact on liver fat content (Santos et al., 2003). However, it is not recommended for the treatment of NAFLD and NASH (Chalasani et al., 2012) as it has been found to lead to development of cirrhosis, need for liver transplantation or even death in patients with primary sclerosing cholangitis (Lindor et al., 2009).

Betaine

Betaine may help in controlling the oxidative stress as it is a methyl group donor that increases the hepatic S-adenosyl-methionine levels. However, a randomized placebo controlled trial failed to show its effect on liver enzymes and liver histology of NASH patients (Abdelmalek et al., 2009).

Obeticholic Acid

It is a farnenoid X receptor agonist. A recent conducted study on its efficacy and safety found that it improves insulin sensitivity in patients with type 2 diabetes and fatty liver (Mudaliar et al., 2013). Hence, it may hold promise as a future therapy for the management of NAFLD.

Bariatric Surgery

Bariatric surgery is not to be considered as the first line of treatment for NASH. The emphasis has to be on other non-invasive measures to correct the metabolic profile rather than directly resorting to bariatric surgery (Chavez-Tapia et al., 2010). It should be considered in those in whom all the above mentioned approaches may have failed, but care needs to be taken that the surgery be performed prior to the liver turning cirrhotic (WGO, 2014), especially in those with portal hypertension as the risk of hepatic decompensation in such patients remains very high owing to the weight loss after surgery (D' Albuquerque et al., 2008). However, the challenge is the correct type of bariatric surgery for the treatment of NASH has not been identified yet (Dyson et al., 2014). Hence, it is not recommended for NAFLD patients given the dearth of evidence regarding the benefits and risks involved with such a procedure (Mummadi et al., 2008; Chavez-Tapia et al., 2010).

Liver Transplantation

NASH is becoming a major reason for liver transplantation (Byrne, 2012). For patients who have decompensated cirrhosis with NASH, liver transplantation is recommended, provided the accompanying co-morbidities permit (Angulo, 2002). If obesity and diabetes are present (Tarantino et al., 2007), or in morbid obesity, transplantation is not favoured (WGO, 2014). Liver transplantation is successful in those who meet the criteria for liver failure. However, NASH may reoccur after liver

transplantation (Browning et al., 2004; Ahmed, 2015) in 4-25% (Dyson et al., 2014) even in those with cryptogenic cirrhosis (Tarantino et al., 2007). Some of the risks for the development of *de novo* NAFLD and NASH after the liver transplantation are; post transplant obesity, medical history of type 2 diabetes, hyperlipidemia and/or portal hypertension (Dumortier et al., 2010; Ong and Younossi, 2010).

SUMMARY

- NAFLD can be reversed if caught in its initial stages and if the underlying metabolic aberrations are corrected, for which incorporating lifestyle changes is critical.
- There are no evidence based guidelines for the treatment of NAFLD. The focus remains on correcting the underlying metabolic abnormalities.
- The primary goals while treating type 2 diabetic NAFLD patients are; 1) to improve their quality of life through propagating lifestyle changes and 2) to prolong their survival by reducing hepatic fat.
- Weight loss and lifestyle modification should be propagated and used as the first line therapy for the treatment of NAFLD.
- Metformin improves liver histology, reduces aminotransferase levels and IR but with no changes in liver parenchyma. Metformin is not recommended as a specific treatment for those with NAFLD/ NASH.
- The safety aspect of TZDs is not established in type 2 diabetics.
- TZDs can be used to treat biopsy proven NASH. TZD therapy often fails to show its effectiveness in the absence of lifestyle modification.
- Initiation of insulin therapy with metformin at the point of diagnosis of type 2 diabetes and intensive insulin therapy at the basal and pre-meal stage in type 2 diabetic NAFLD patients reduces hepatic steatosis.
- Statins can be used only for the treatment of dyslipidemia in NAFLD and NASH patients and not for the condition of NAFLD and NASH *per se*.
- Ezetimibe may correct lipid aberrations through downregulation of the LXR- α SREBP-1c pathway.

- Fibrates correct dyslipidemia but there is no recommendation for it in NAFLD.
- UDCA which is a naturally occurring cytoprotective secondary bile acid is not recommended for the treatment of NAFLD and NASH.
- Betaine is a farsenoid X receptor agonist which may hold promise as a future therapy for the management of NAFLD.
- The correct type of bariatric surgery for the treatment of NASH is not known.
- Liver transplantation is successful in those who meet the criteria for liver failure.
- NASH can reoccur after liver transplantation also owing to post transplant obesity, medical history of type 2 diabetes, hyperlipidemia and/or portal hypertension.

BEHAVIOURAL THERAPY FOR LIFESTYLE MODIFICATION IN THE MANAGEMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

Behavioural Therapy

Behavioural therapy is about providing the patients with a set of principles and techniques to modify their eating and activity habits (Wadden et al., 2004; Fabricatore, 2007). It is based on learning theory, or what may be called as behaviourism (Bellentani et al., 2008). It states that behaviours behind a certain health condition have a strong educational component, which can be modified and relearned (Wadden et al., 2004). The theory further propagates that positive changes in behaviours can only be achieved by modifying the environmental cues and reinforcing these behaviours (Wing, 2002). Thus, lifestyle modification is a combination of diet, exercise and positive behaviours (Wadden et al., 2004), which is the mainstay of NAFLD management now (Than and Newsome, 2015).

Behavioural therapy is an area of research that has been extensively applied in the treatment of overnutrition and its associated metabolic derangements (Bellentani et al., 2008). Lifestyle modification through behavioural therapy helps to reduce aminotransferases and improve hepatic steatosis as confirmed by ultrasound (Andersen et al., 1991; Park et al., 1995; Ueno et al., 1997; Kugelmas et al., 2003; Sreenivasa et al., 2006; Hickman et al., 2004; Suzuki et al., 2005).

Appropriate counselling programs with a cognitive behavioural approach are effective in NAFLD/NASH patients as they improve insulin sensitivity, liver enzymes, hepatic fat content as well as the grade and stage of hepatic inflammation and fibrosis (Bellentani et al., 2008) and they are also effective in the long run (Greaves et al., 2011). It is only a behavioural approach which can empower the patients with a practical instrument to achieve lifestyle changes via diet and exercise and aid in maintenance (McCarthy and Rinella, 2012).

Lifestyle Modification

Lifestyle interventions are considered as the cornerstone of management of NAFLD (Bacchi et al., 2013). A recent review on clinical approaches to NAFLD concluded that lifestyle modification results in weight loss or increased physical activity (PA), is

capable of reducing liver enzymes and inflammation; improve liver histology, glucose control, insulin sensitivity and lipid oxidation. Thus, while developing a NAFLD treatment plan, lifestyle modification is to be used as a first step in clinical setting (Schwenger and Allard, 2014).

The fundamental goals of lifestyle modification through weight loss by diet and/or PA (Fan and Cao, 2013) are increase in muscle mass, peripheral insulin sensitivity (Conlon et al., 2013; Kim and Younossi, 2008; Bhat et al., 2012), improvement in lipemic status (Colak et al., 2012; Fan and Cao, 2013), IL-6 reduction (Monzillo et al., 2003); hepatic insulin sensitivity (Conlon et al., 2013) and improved liver histology (Bhat et al., 2012).

In a systematic review which evaluated the effect of diet or PA or a combination of both in adult NAFLD population, found that lifestyle modification brings about a reduction in hepatic fat, improves glucose control and insulin sensitivity owing to weight reduction and/or increased PA (Thoma et al., 2012).

Weight Loss

For the ideal treatment of NAFLD and MS, managing body weight is the crux of it (Rector et al., 2008). For weight loss; weight management, dietary macronutrient composition, PA and behaviour therapy are the key elements (McCarthy and Rinella, 2012). Diet and PA can together contribute towards weight management that is known to improve liver histology and delay the progression of NAFLD to more advanced stages (McCarthy and Rinella, 2012).

The EASL recommends a 7% weight loss (Ratzliff et al., 2010). A 3-5% weight loss improves steatosis (Attar and Thiel, 2013) and average of 4% is also able to reduce steatosis in 56% of the NAFLD patients (Zelber-Sagi et al., 2012). A 5% weight loss aids to bring down the ALT (Daly et al., 2006; Suzuki et al., 2005; Kugelmas et al., 2003) by intensive behavioural therapy. Westphal, 2008 concluded that a modest weight loss of <10% in obese with NAFLD may help to improve insulin sensitivity. However, care needs to be taken that the weight loss should not be beyond 1.6 kilograms/week as it may trigger worsening of fatty liver as it increases VAT lipolysis and delivers the FFA generated directly to the liver via the portal vein leading to

further damage (Andersen et al., 1991; Luyckx et al., 1998; Tolman et al., 2007; Almeda-Valdes et al., 2009; Clark, 2006).

What Research Says About the Role of Lifestyle Modification in Non Alcoholic Fatty Liver Disease

In a study involving 23 biopsy proven NASH patients of whom 16 completed one year of nutrition counselling, a non-significant reduction in mean WC, visceral fat, fasting glucose, IR, triglycerides, AST, ALT and no significant changes in the macronutrient composition of the responders and the non responders were observed. Not all of the NAFLD patients in the experimental arm were able to achieve the target weight loss. Nine out of fifteen patients showed histological improvement. The authors recommended that a less intense but more structured lifestyle modification intervention could be more effective and translatable for general practice for the treatment of NAFLD (Huang et al., 2005).

A significant decrease in BMI, weight, WC, percent body fat and glycated hemoglobin and hepatic steatosis was observed after a one year intervention in type 2 diabetic patients who had hepatic steatosis (Lazo et al., 2010). However, no reductions in AST and ALT were observed. The intervention comprised of a combination of moderate calorie restriction (1200-1500 kcal/day) and increased moderate PA (175 minutes/week).

In a randomized controlled trial involving obese NASH patients, a combination of diet (1000-1500 kcal/day), exercise (10000 steps per day and 200 minutes/week of moderate PA) and behaviour modification, brought about a significant improvement in NASH and reduced weight by 9.3% in the intervention arm. Amongst those having greater than 7% weight loss, a significant improvement in steatosis was also observed (Promrat et al., 2010).

NAFLD patients with elevated liver enzymes and central obesity were enrolled in a study to measure the effectiveness of lifestyle intervention, which had the components of dietary guidance, PA and behaviour modification. Patients were randomly allocated into two groups; low PA intensity group (3 sessions/4 weeks) and moderate intensity PA group (6 sessions/10 weeks) and were compared with controls. A reduction in

liver enzymes was observed in the moderate intensity PA group as compared to the control group (St George et al., 2009).

A study demonstrated that following a trend of deduction of 500kcal out of the total day's intake along with PA for six months, brought down intra-hepatic lipids by 40%. The resultant impact was observed due to nutrition counselling that involved behaviour therapy (Thomas et al., 2006). The table below summarises the therapeutic weight loss techniques in NAFLD (table 2.4).

A study evaluated 65 patients with NAFLD over a minimum of 3 months who were placed on an aerobic exercise regimen and a specific diet. The exercise regimen consisted of brisk walking, jogging or rhythmic aerobic exercises for a minimum of 45 min, 5 days per week, to achieve a target heart rate of 60–70% of their maximal heart rate. The dietary regimen was predicated on a total of 25 kcal/kg per day containing 60% carbohydrate, 20% fat, 20% protein and 200 mg of cholesterol. A total of 44 patients complied with the exercise programme and were included in the analysis. There was a significant improvement in BMI, WC, WHR and serum aminotransferases in the patients adherent to the diet and exercise regimen (Baba et al., 2006).

Tamura et al, 2005 randomised 14 type 2 diabetic patients to either 27.9 kcal/kg/day diet or a similar diet plus exercise (walking 2–3 times/day for 30 min, 5–6 days per week) for 2 weeks. Diet plus exercise resulted in improved muscle insulin sensitivity as measured by a decrease in intramyocellular lipid content and improved glucose infusion rates, an effect not seen with diet alone. However, both groups decreased intrahepatic lipid concentrations by 27%.

In a study involving cognitive behavioural therapy (CBT) for weight loss and increasing physical activity in NAFLD subjects, CBT was associated with a higher probability of weight loss, normalization of liver enzymes, improvement in insulin sensitivity and reduction in the MS score. A weight loss $\geq 7\%$ was found to be beneficial in improving liver biochemistry and histology (Moscatiello et al., 2011).

Overweight and obese NAFLD patients who underwent three months of diet therapy (50% carbohydrates, 30% fat and 20% protein with a deficit of 500 calories), of the 23 enrolled, fifteen of them decreased one grade of fatty liver and eight others decreased by two grades. A significant correlation was observed between decrease in grade of fatty liver and a decrease in weight and BMI (Tahaei et al., 2010).

Hickman et al., 2004 reported an improvement in steatosis and liver histology following 12 wk of diet and exercise, with a weight loss of 6.6%.

TABLE 2.4: THERAPEUTIC WEIGHT LOSS TECHNIQUES IN NAFLD

Categories	Description or Recommendation
Dietary modification	At least 3-5% weight loss achieved by hypocaloric diet with or without exercise, generally reduces hepatic steatosis, and 10% weight loss may be needed to improve necro-inflammation.
Increased physical activity	A regular exercise program with 200 min/week of moderate-intensity. Exercise alone in adults with NAFLD may only reduce hepatic steatosis.
Behavioural interventions	Included self-monitoring, setting weight loss goals, addressing barriers to change, and strategizing about maintaining long-term changes in lifestyle.
Pharmacological agents	Aim to decrease appetite, block fat absorption, or reduce stomach volume, only be used under the strict supervision of a specialist.
Bariatric surgery	Foregut bariatric surgery is not contraindicated in otherwise eligible obese individuals with NAFLD/NASH; it is premature to consider it as an established option to specifically treat NASH.
Dietary supplements	Although a wide array of dietary supplements available to the public, very few are effective in long term and are not considered a healthy option for weight loss.

Ref: Adapted from Fan and Cao, 2013

NUTRITIONAL MANAGEMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

Nutrition therapy is the basic form of treatment for NAFLD patients (Yasutake et al., 2014). Referral of NAFLD patients to medical nutrition therapy may aid in suitable dietary modifications and promote behavioural changes (Spahn et al., 2010). Currently, there is a lack of evidence based guidelines for the nutritional management of NAFLD (Conlon et al., 2013). In wake of widespread prevalence of NAFLD, diet therapy seems to be a cost effective option for its treatment (Phillips and Barton, 2014).

General Recommendations and Type of Diet

The general recommendations are to have more of home cooked food than eating outside, a low energy dense diet and higher quantities of vegetables (Yasutake et al., 2014). As eating hastily is related to lesser feeling of satiety (Andrade et al., 2008) and is associated with a higher mean BMI (Sasaki et al., 2003; Otsuka et al., 2006) than those who chew their food properly, more than 20 chews per mouthful is recommended for NAFLD patients in order to prevent overeating. In starting nutrition therapy for NAFLD patients, the recommendation is to enhance energy intake during breakfast and restrict energy intake during evening time and dinner (Yasutake et al., 2014).

The optimum diet for correction and management of NAFLD is not known yet (Dyson et al., 2014). Any change in the proportion of one macronutrient is likely to bring a change in the proportions of other macronutrients as well and hence attribution of dietary changes becomes difficult with single macronutrient of interest. Thus, defining low-carbohydrate, high carbohydrate diet becomes challenging owing to the variability (Wylie-Rosett et al., 2012).

The various diets aimed at weight loss or maintaining weight are primarily divided into four major categories, namely; low fat diets, low carbohydrate diets, low calorie diets and very low calorie diets (Sacks et al., 2009; Moyer, 2012). A meta-analysis of a few randomized controlled trials revealed that there was no difference between low calorie, low carbohydrate and low fat diets, with a weight loss ranging from 2-4 kgs in a span of 12 months to 18 months (Sacks et al., 2009). Usually very low calorie diets

are detrimental as they exacerbate liver injury and may lead to liver failure in NASH patients (Chalasani et al., 2012; Fan and Cao, 2013; Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012). Low carbohydrate diets are popular for treating NAFLD and its associated co-morbid condition of obesity as these diets have shown to decrease intra-hepatic fat triglycerides and improve various metabolic parameters (Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012; Sacks et al., 2009; Schugar et al., 2012). However, though they are low in carbohydrates and high in protein, they can also be termed as low carbohydrate high fat diets (Gill and Wu, 2006). Importantly, the long term effects of low carbohydrate diets are unknown (Sullivan, 2010). On the other hand, low fat diets are safe, cardio-protective and effective in weight loss. Hence, it is the most recommended form of diet by the medical professionals (Gill and Wu, 2006). The WGO 2014 global guidelines for NAFLD/NASH recommend that a moderately calorie restricted diet will produce better results compared to a very low calorie diet (WGO, 2014) as a hypocaloric diet (<500kcal/day), tends to aggravate NAFLD because of starvation (Drenick et al., 1970).

Classification of weight loss diets with respect to NAFLD

1. Low-fat diets: The reduction of the percentage of fat in diet (25% fat), without intentional restriction of caloric intake.
2. Low carbohydrate diets: The reduction of the percentage of carbohydrates in diet, that is, Atkins and Protein Power, relatively high in protein and fats, sometimes lead to *ketogenic*.
3. Low calorie diets: Provide about 1000–1200 calories a day for women and 1500 to 1800 calories for men.
4. Very low calorie diets: Provide 200–800 calories per day, maintaining protein intake but limiting calories from both fat and carbohydrates. Must be undertaken with medical supervision to prevent adverse side-effects, such as loss of lean muscle mass, increased risks of gout, gallbladder stone, and electrolyte imbalances (Fan and Cao, 2013).

Altering Macronutrient Composition

Changing dietary macronutrient composition without necessarily reducing the caloric intake may offer a more realistic and feasible alternative to treat NAFLD patients (Zelber-Sagi et al., 2011). This recommendation came up because adherence to long term weight reduction and weight maintenance may be a challenging issue (Katan, 2009). Moreover, sustainable changes in lifestyle should be the mainstay for natural weight loss that would promote it rather than promoting weight loss through specific dietary intervention (McCarthy and Rinella, 2012). Other than that, an alteration of the macronutrient composition might be beneficial as well in the management of NAFLD (Lewis and Mohnaty, 2010).

Overweight and obese NAFLD patients who underwent three months of diet therapy (50% carbohydrates, 30% fat and 20% protein with a deficit of 500 calories), of the 23 enrolled, fifteen of them decreased one grade of fatty liver and eight others decreased by two grades. A significant correlation was observed between decrease in grade of fatty liver and a decrease in weight and BMI. The authors concluded that USG should be used as a guide to weight loss and those with greater severity would respond more to weight loss (Tahaei et al., 2010).

Fifteen biopsy proven NASH patients who were put on a 40% carbohydrates/fibre, 35-40% fat and 15-20% protein diet registered 2.9kgs of weight loss, 60% of the patients demonstrated improvement in histology (Huang et al., 2005). Thus, weight loss even of marginal difference is capable of bringing about hepatic healthy changes (Colak et al., 2012).

Carbohydrates

The source of carbohydrates, fibre and the GI are the important aspects to be kept in mind while planning a meal for a NAFLD patient (Conlon et al., 2013). A moderate carbohydrate intake diet which comprises of 40 – 65% of the energy seems reasonable for a NAFLD patient keeping the ADA recommendations in mind (ADA, 2013). For individuals with diabetes, the total carbohydrate intake should not be less than 130g/day (Bantle et al., 2008).

Another recommendation is to avoid HFCS (Nazarenko et al., 2012) and restrict fructose intake (Colak et al., 2012). A recent review (Yasutake et al., 2014) concluded that complex carbohydrates found in whole grains may aid in preventing the development of NAFLD as well as help to conquer its progression as they contain antioxidants and dietary fibre (Ross et al., 2013). Whole grains also help to decrease visceral fat and thereby improves obesity, dyslipidemia and MS (McKeown et al., 2009; Katcher et al., 2008).

Low GI foods tend to decrease calorie intake and keep the glucose and TC under control (Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012; Sacks et al., 2009; Schugar et al., 2012). CHO high in indigestible and fermentable fibre, low in GI may aid in maintaining glucose concentrations, insulin and FFA in those with IR and NASH (Zivkovic et al., 2007). Thus, GI should be given emphasis while chalking out the recommendations for NAFLD patients; however, so far there are no studies available that establish associations between GI and NAFLD (Fan and Cao, 2013).

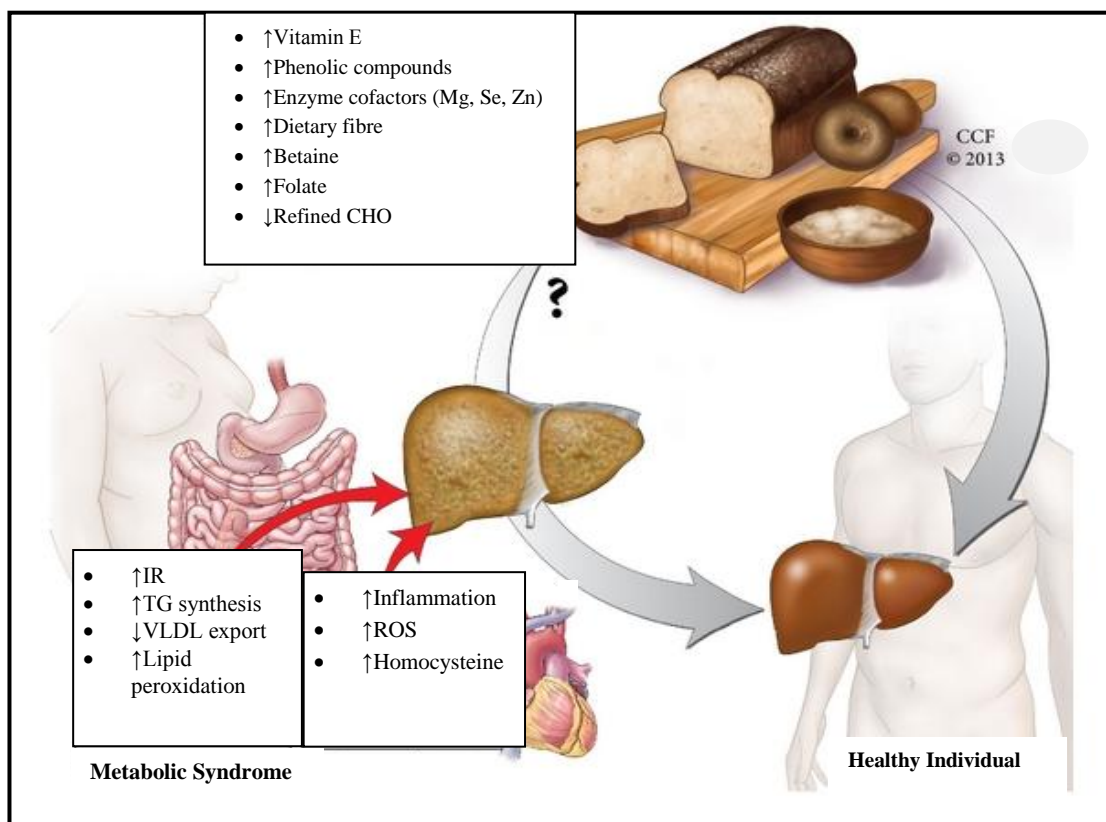


FIG 2.11: THE ROLE OF WHOLE GRAINS IN THE PREVENTION OF NAFLD Whole grains may have an impact on NAFLD through many complementary

mechanisms. Choosing more whole grains over refined carbohydrate sources will increase the intake of many nutrients that are known to, or suggested to, play a role in preventing NAFLD and related comorbidities. While yet to be studied directly, it is probable that a diet rich in whole grains would play a role in the prevention of NAFLD. Whether they could be a biologically active part of a diet to treat NAFLD remains to be investigated. (Ref: Ross et al., 2013)

Protein

Dietary protein may aid in the management of NAFLD as catabolism of amino acids requires energy and a high protein intake may trigger increased β oxidation of fatty acids via an increase in the energy expenditure of the hepatocytes (de Wit et al., 2012). A high protein intake may aid in weight loss and improve glucose homeostasis in those with IR and nullify the impact of a high fat diet on the intra-hepatic lipids (Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012; Bortolotti et al., 2011; Tovar and Torres, 2010). It is postulated that high protein intake leads to a higher metabolic rate of amino acids in the liver and consumes large energy. The excess energy consumption increases lipid oxidation and hence aids in preventing fat accumulation in the hepatocytes (Leidy et al., 2007).

As nitrogen balance improves when meals are divided into 4-6/day (Swart et al., 1989), NAFLD patients with deficient protein intake should chunk their meals into fragments to have a good nitrogen balance. Lipogenesis is also inhibited by the bile acids produced as a result of protein consumption (Duran-Sandoval et al., 2005). Ketogenesis is also stimulated in the liver because of increased day long release of glucagon (Longuet et al., 2008).

Whey proteins may be another potential therapy for the nutritional management of NAFLD. Owing to its high branched chain amino acids content, whey modifies the gene expression causing more β oxidation of fatty acids and also protects against AMP activated kinase mediated apoptosis of the pancreatic β cells (Cai et al., 2008).

However, the data is insufficient to provide evidence with regard to recommended protein intake for the NAFLD patients (Conlon et al., 2013). But, substitution of red

and processed meat with lean meat and fish is recommended for NAFLD patients (Zelber-Sagi et al., 2011).

Fats

SFAs have a detrimental effect and aid in the development of NAFLD whereas PUFAs have a positive effect in retarding the onset and progression of NAFLD (Yasutake et al., 2014). Hence, the recommendation is to reduce the intake of SFAs, but increase the intake of PUFA, especially n-3 PUFA (Zelber-Sagi et al., 2011). Trans-fats are to be avoided by NAFLD patients (Micha and Mozaffarian, 2009; Zelber-Sagi et al., 2011). A recent review recommended that SFA intake between 6% to 10% maybe most beneficial for NAFLD patients, along with an intake of up to 25% of MUFA and increased intake of n-3 PUFA (McCarthy and Rinella, 2012). Moreover, a reduced SFA intake improves NAFLD pathophysiology (de Wit et al., 2012).

A review opined that increasing the ratio of MUFA in the diet may benefit the NAFLD patients. Inclusion of nuts, like hazelnuts and walnuts will help as they are also good sources of vitamin E (Colak et al., 2012). Though MUFA has beneficial effects in NAFLD, further research is required to ascertain this association for recommending MUFA and olive oil (Assy et al., 2009). A Mediterranean diet that is rich in MUFA has shown to reduce hepatic steatosis and improve insulin sensitivity in non-diabetic patients with NAFLD (Ryan et al., 2013). MUFA induce a beneficial impact on the lipemic profile marked by low oxidized LDL, LDL, triglycerides, and a low TC/HDL ratio (Assy et al., 2009). It is postulated that diets rich in n-3 PUFA and MUFA may play a pivotal role in the prevention and treatment of NAFLD (Conlon et al., 2013). A study recommended that substitution of SFA with MUFA and n-3 PUFA may aid in anti-inflammation and yield cardiovascular benefits (McCarthy and Rinella, 2012). Whether SAFA in the diet be substituted for improvement in lipemic status with MUFA is still under debate (de Wit et al., 2012).

To reduce the risk of CVD in NAFLD patients, the targets of lipid control remain the ingestion of TC and that of SFA (Carvalhana et al., 2012; Mouzaki and Allard, 2012; McCarthy and Rinella, 2012). Keeping cholesterol under control can also help to control the C-reactive protein levels as well as insulin sensitivity (Fernandez et al.,

2008). A review (Yasutake et al., 2014) concluded that deliberate supplementation of n-3 PUFA may help to prevent CVD in NAFLD patients (Iso et al., 2006; Yamagishi et al., 2008) and may hence be an effective component of the nutritional management of NAFLD patients.

In non-obese NAFLD patients, it is recommended that their ingestion of dietary cholesterol rich foods like eggs, liver and cakes should be estimated as there maybe a possibility of over ingestion. The authors recommended that reducing cholesterol intake should also be a mainstay of nutritional management in NAFLD patients (Yasutake et al., 2014) as it may prevent the development of NAFLD, irrespective of obesity (Teramoto et al., 2013).

Supplementation with n-3 PUFA in NAFLD patients for 6 months ranging between 2 – 6 grams/day (Zhu et al., 2008; Spadaro et al., 2008) to up to one year with 1gram/day (Capanni et al., 2006) has demonstrated beneficial effects such as improvements in NAFLD, glycemic status as well as lipid profile. Owing to its anti-oxidant and anti-inflammatory properties, eicosapentanoic acid (EPA) has shown to improve NASH (Tanaka et al., 2008). PUFA aids in the management of NAFLD as it is capable of decreasing hepatic TNF- α (Calder, 2008; Riediger et al., 2009) and repressing fatty acid synthesis by negatively controlling the SREBP-1c whose inhibition decreases the expression of genes which are involved in hepatic DNL and hence aid in reducing fatty liver (Masterton et al., 2010), enhancing β oxidation by positively controlling PPAR- α (Zuniga et al., 2011; Pettinelli et al., 2009). The processes aid in improving insulin sensitivity (Yasutake et al., 2014; Masterton et al., 2010) and reduce inflammation (Byrne, 2012; Masterton et al., 2010). However, according to a systematic review and meta-analysis, n-3 PUFA supplementation does not reduce the ALT levels (Parker et al., 2012).

Fatty liver was resolved in 35% of the NAFLD with hypertriglyceridemia after 6 months of 15ml/day n-3 PUFA supplementation (Hatzitolios et al., 2004). A 53% reduction in fatty liver was observed in an RCT wherein the intervention arm was supplemented with 2grams of thrice daily n-3 PUFA, whereas the control arm that was on diet plus placebo saw a reduction of fatty liver in only 35% (Vega et al., 2008). Omega 3 PUFA supplementation for a period of 8 weeks at 4g per day in a

double blind cross over trial reduced hepatic content in the supplementation group compared to placebo (Cussons et al., 2009).

Those NAFLD patients who have an excessive intake of carbohydrates or a shortage of PUFA may benefit the most with n-3 supplementation therapy, rather than those NAFLD patients with excessive fat intake (Yasutake et al., 2012).

However, more trials of adequate size and duration, with histological endpoints are required to establish the efficacy of n-3 PUFA as a prophylactic and treatment measure for NAFLD, with core evidence of stipulated dosage for different target groups (Conlon et al., 2012). The current recommendation states it is very early to establish omega 3 fatty acid therapy to treat NAFLD or NASH, but they can be considered as the first line agents to treat hypertriglyceridemia in NAFLD cases (Chalasani et al., 2012).

For the non-vegetarian population, the recommendation is to substitute saturated fat laden meat with increased frequency of consuming n-3 rich fish (Yasutake et al., 2014). N-3 rich fish and n-3 supplements (EPA and DHA), are recommended for nutrition therapy for non-obese NAFLD patients (Yasutake et al., 2012).

MICRONUTRIENTS

A review came up with the conclusion that there is dearth of evidence in standing for or against use of micronutrient supplementation in those with NAFLD (Lirussi et al., 2007). However, since oxidative stress is implicated in the pathogenesis of advanced NAFLD, the various anti-oxidants may benefit those with NAFLD by preventing the progression to NASH (Conlon et al., 2013).

Vitamin D

The data remains insufficient to draw evidence based conclusions to recommend vitamin D supplementation over and above its RDA which is 600 IU/day for 19-70 year old adults, 800 IU/day for adults aged above 70 years (Conlon et al., 2013). However, a review recommended that prior to beginning the nutritional therapy, the vitamin D status of the NAFLD patient should be assessed and consumption of food sources of vitamin D, like mushrooms and fishes, should be propagated (Yasutake et al., 2014).

Vitamin E

Alpha tocopherol inhibits oxidative stress by scavenging the free radicals and reducing hepatic pro-fibrotic activity (Attar and Thiel, 2013), stabilize mitochondrial function, inhibit lipid peroxidation and the subsequent free radical reactions (Kim and Younossi, 2008) and hence reduce hepatic steatosis (Attar and Thiel, 2013). However, there is limited data on vitamin E's role in the management and treatment of NAFLD (Chalasani et al., 2012).

In the Pioglitazone vs Vitamin E vs. Placebo for Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) clinical trial, 800IU of Vitamin E/day given to non-diabetic NASH patients for 96 days, brought about a reduction in hepatocellular inflammation, hepatic steatosis, improvement in liver enzymes. The study concluded that vitamin E effectively treated NASH in patients without diabetes (Sanyal et al., 2010).

The controversy with vitamin E arises because of its association with increase in all cause mortality in a dose dependent manner with high dosage or a dose ≥ 400 IU /day (Miller et al., 2005; Sesso et al., 2008) though a few other studies failed to establish this association (Berry et al., 2009; Gerss and Kopcke, 2009; Dietrich et al., 2009). High dosage is also associated with haemorrhagic stroke (Schurks et al., 2010) and prostate cancer (Lippman et al., 2009). Thus, till further data establishes the benefits or the risks, the practice guidelines recommend vitamin E is not to be used for treating NASH in type 2 diabetics, NAFLD without liver biopsy, NASH cirrhosis, cryptogenic cirrhosis (Chalasani et al., 2012). NAFLD or NASH patients on high dosage vitamin E should be closely monitored (Yasutake et al., 2014). Based on research (Sumida et al., 2013; Han et al., 2014), it was concluded that 300mg of vitamin E per day is presumed to be safe and effective for patients with fibrosis and/or impaired fasting glucose (Yasutake et al., 2014).

The recommendation for NAFLD patients with vitamin E deficiency is to consume higher quantities of yellow and green vegetables as increased amounts of vitamin E over and above the recommended intake maybe required to manage the oxidative stress (Yasutake et al., 2014). The dosage and duration of treatment require further research (Dyson et al., 2014).

NUTRITIONAL THERAPIES UNDER EXPLORATION

Pro-Biotics

Probiotics are foods with live bacteria that are capable of regulating the intestinal microbiota of the host (Yasutake et al, 2014). There are no randomized clinical trials on impact of probiotics on NAFLD (McCarthy and Rinella, 2012). However, they are known to alter the enteral environment, improving the pathology of NAFLD (Iacono et al., 2011; Kelishadi et al., 2013). They exert protective effect by control of epithelial cell proliferation and differentiation, production of short chain fatty acids and amino acids, preventing overgrowth of pathogenic organisms, stimulation of intestinal immunity (Nichols, 2013) and have shown promising results in NASH (Wong et al., 2013). However, another study found that probiotics had no significant impact in improvement of liver disease (Solga et al., 2008). There is a need for trials with a good sample size to arrive at generalization of probiotics for the treatment of NAFLD (Kelishadi et al., 2013; Schwenger and Allard, 2014).

Pre-Biotics

Prebiotics are non-digestible carbohydrates that stimulate the proliferation of colonic bacteria. They have also shown potential for their treatment of NAFLD however; further validation is required (Parnell et al., 2012). In a very small study comprising of seven NASH patients, oligofructose supplementation for 8 weeks with 16 grams/day, AST and ALT decreased in the intervention arm and insulin levels came down in the 4th week itself. The conclusion arrived at was that prebiotics can be a potential treatment therapy for NAFLD (Daubioul et al., 2005). To further validate these findings, there is a need for studying the effects of prebiotics with histological end points (Schwenger and Allard, 2014).

Isoflavones

Isoflavones are found in good quantities in soya protein. In soya protein the two major isoflavones are genistin and daidzin, which can cause a reduction in hepatic fat content (Yang et al., 2011).

Green Tea

The polyphenolic catechins of green tea that exhibit antioxidant, hypolipemic and anti-inflammatory effects may help to protect against the onset and progression of NAFLD (Masterjohn and Bruno, 2012).

Coffee

Caffeine, which is the bioactive component present in coffee, has been documented to be associated with a lower risk of NAFLD and is also associated with bringing about a significant risk reduction of fibrosis in patients with NASH. Thus, these beverages hold the potential for being a prophylactic as well as a management modality for NAFLD (Birerdinc et al., 2012; Molloy et al., 2012). How coffee leads to hepatic protection, the mechanism is still not well understood (McCarthy and Rinella, 2012).

PHYSICAL ACTIVITY RECOMMENDATIONS

In a study of sedentary NAFLD patients, resistant exercises (8 weeks; 3 times/week lasting 45-60 minutes) brought about a reduction in liver lipids, improvements of lipid oxidation, glucose control and IR and about a 13% relative reduction in hepatic fat content in the absence of changes in body weight, whole fat body mass or VAT (Hallsworth et al., 2011).

Physical activity (PA) up-regulates insulin receptors in the muscle increasing delivery of glucose and insulin to the muscles (Goodyear and Kahn, 1998). PA in the form of exercise training in the type 2 diabetics improves body fat distribution, insulin sensitivity, glycemic control and the cardio-metabolic risk factors (Boule et al., 2001; Umpierre et al., 2011).

PA should be a core component of behavioural therapy for NAFLD patients, as minor changes in fitness levels can confer major health benefits (Zelber-Sagi et al., 2011) as compared to a completely sedentary lifestyle (Frith et al., 2010). An exercise regime of 3 to 4 times a week to achieve an age based 60-75% of the heart rate is recommended for NAFLD patients (WGO, 2014). As per the Asia Pacific guidelines for the management of NAFLD, an hour of PA/day for atleast three days a week is recommended. However, the frequency should be increased to five times a week gradually (Asia Working Party on NAFLD, 2007).

SUMMARY

- Behavioural therapy empowers the patients with a practical instrument to achieve lifestyle changes via diet and exercise.
- Lifestyle modification is to be used as the first line of treatment therapy for NAFLD.
- Cognitive behavioural approach improves insulin sensitivity, liver enzymes, hepatic fat content, grade and stage of hepatic inflammation and fibrosis and they are also effective in the long run.
- For the ideal treatment of NAFLD and MS weight loss through dietary macronutrient composition, physical activity and behaviour therapy is recommended.
- The EASL recommends a 7% weight loss but it should not be beyond 1.6 kilograms/week as it may trigger worsening of fatty liver.
- Nutrition therapy is the most cost effective treatment for NAFLD patients that provides suitable dietary modifications and promotes behavioural changes.
- Having more of home cooked food, low energy dense diet and higher quantities of vegetables, more than 20 chews per mouthful, enhancing energy intake in breakfast and retarding the intake at dinner is recommended for NAFLD patients.
- The optimum diet for correction and management of NAFLD is not known yet.
- Low fat diets are safe, cardio-protective and effective in weight loss. Hence, it is the most recommended form of diet by the medical professionals.
- Changing dietary macronutrient composition without necessarily reducing the caloric intake is a more realistic and feasible alternative to treat NAFLD patients.
- USG should be used as a guide to weight loss and those with greater severity would respond more to weight loss.
- Weight loss even of marginal difference is capable of bringing about hepatic healthy changes.

- The source of carbohydrates, fibre and the GI are to be kept in mind and a moderate carbohydrate intake diet (40-65% of the energy) seems reasonable for a NAFLD patient.
- Avoid HFCS and restrict fructose intake. Complex carbohydrates may aid in preventing the development and progression of NAFLD.
- CHO high in indigestible and fermentable fibre, low in GI may aid in maintaining glucose concentrations, insulin and FFA in those with IR and NASH.
- Dietary protein may aid in the management of NAFLD as high protein intake leads to a higher metabolic rate of amino acids in the liver and consumes large energy.
- NAFLD patients with deficient protein intake should chunk their meals into fragments to have a good nitrogen balance.
- Whey proteins hold promise in nutritional management of NAFLD.
- Data is insufficient to provide evidence with regard to recommended protein intake for the NAFLD patients.
- The recommendation is to reduce the intake of SFAs (between 6% to 10%), increase the intake of PUFA, especially n-3 PUFA. Trans-fats are to be avoided by NAFLD patients, along with an intake of up to 25% of MUFA.
- NAFLD patients with excessive intake of carbohydrates or a shortage of PUFA may benefit the most with n-3 supplementation therapy.
- Omega 3 fatty acid therapy can be considered as the first line agents to treat hypertriglyceridemia in NAFLD cases.
- Substitution of saturated fat laden meat with increased frequency of consuming n-3 rich fish is propagated.
- There is dearth of evidence in standing for or against use of micronutrient supplementation in NAFLD.
- Vitamin D assessment of NAFLD patients is necessary and its increased consumption through food sources should be propagated.
- Vitamin E is not to be used for treating NASH in type 2 diabetics, NAFLD without liver biopsy, NASH cirrhosis, cryptogenic cirrhosis.

- NAFLD or NASH patients on high dosage vitamin E should be closely monitored. 300mg of vitamin E per day is presumed to be safe and effective for patients with fibrosis and/or impaired fasting glucose.
- Prebiotics and probiotics can be potential treatments for NAFLD. But, there is a need for studying their impact on NAFLD with histological end points.
- Genistin and daidzin may cause a reduction in hepatic fat content.
- Green tea and coffee hold promise in the management of NAFLD.
- Physical activity in type 2 diabetics improves body fat distribution, insulin sensitivity, glycemic control and the cardio-metabolic risk factors.
- According to the Asia Pacific guidelines for the management of NAFLD, an hour of physical activity per day for atleast three days a week is recommended.

TRADITIONAL MEDICINE IN THE TREATMENT OF DYSLIPIDEMIA AND DYSGLYCEMIA

Plant products have been part of phytomedicines as therapeutic agents since time immemorial (Criagg and David, 2001; Sharma et al., 2010). These plant products can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg and David, 2001). India is the largest producer of medicinal herbs (Pandey et al., 2013) and is called as botanical garden of the world (Seth and Sharma, 2004; Joseph and Jini, 2011). To date 20,000 medicinal plants have been recorded (Pandey et al., 2013), of which over 400 traditional plant treatments for diabetes have been reported. Only a few of these have received scientific and medical evaluation to assess their efficacy (Modak et al., 2007), even though they maybe more potent than the commercial drugs that are available (Konda et al., 2013). This is so because there is hardly any clinical data available to establish the efficacy of these medicinal plants. Hence, the need of the hour is to establish the bioactive compounds present in the traditional medicinal plants and conduct clinical trials to generate scientific data (Konda et al., 2013).

The therapeutic use of herbal medicine is gaining considerable momentum in the world during the past decade (Nasreen et al., 2010). According to the statistics, 40% of the people in the developed world (Pandey et al., 2013) and 80% of the people in the developing world are utilising the traditional medicine for the prevention and treatment of various ailments (Tsay and Agrawal, 2005). Needless to say, in the last few years there has been an exponential growth in the field of herbal medicine because of their natural origin and less side effects (Modak et al., 2007; Pandey et al., 2013), easy availability, less expense and better efficiency (Yadav and Agarwala, 2011; Pandey et al., 2013). The advantage of a botanical extract is that if they are shown to be effective, at the clinical level, these remedies can be mainstreamed for public health (Cefalu et al., 2011). Through scientific evidence via clinical studies, these medicinal plants may prove to be valuable resources for innovative, evidence-based and effective treatment solutions for the disease (Campbell-Tofte et al., 2012). So far, the reported efficacy data for many of the natural products are only in the form of uncontrolled studies and anecdotal reports. Currently, there is a paucity of consistent and reproducible efficacy data in humans to suggest any recommendations for most botanical or bioactive supplements as adjunct treatments. However, owing to

dearth of clinical research, traditional medicinal plants with established efficacy are far from being labelled as modern medicine (Wadkar et al., 2008).

POSTULATED MECHANISMS OF BOTANICAL ACTION

An essential challenge that blocks medicinal plants from being a part of modern medicine is that for most of the botanicals, the mechanism of action is not known (Cefalu et al., 2011) or rather there is little biological knowledge on the specific modes of action of these plants (Malviya et al., 2010). From the very little that is known, it is hypothesized that glycosides, alkaloids, phenolics, flavonoids, terpenoids and the glycosides maybe imparting the anti-diabetic effect (Malviya et al., 2010; Grover et al., 2002). It is postulated that the medicinal plants may impart a physiological effect on metabolism by the following mechanisms (Cefalu et al., 2011; Cefalu and Ribnicky, 2009; Mamun-or-Rashid et al., 2014);

- Modulating adipocyte function, thereby regulating endocrine secretions
- Regulating hepatic gluconeogenesis and inhibiting endogenous hepatic glucose production
- Enhancing pancreatic β -cell function in terms of synthesis and release
- Increased insulin sensitivity or insulin-like activity
- Inhibition of glucose absorption from the intestine
- Direct regulation of insulin action in peripheral tissues such as skeletal muscle and adipose tissue, enhancing glucose uptake

All of these actions may be responsible for the reduction or abolition of diabetic complications (Mamun-or-Rashid et al., 2014). However, evidence is lacking whether any of the above mentioned postulated mechanisms are consistent for the medicinal plants which are currently available as supplements in the market. Hence there is a need for delving into in-depth research on the botanical modes of action (Mamun-or-Rashid et al., 2014) and establishing their efficacy through scientific data. Some of the commonly utilised medicinal plants with anti-diabetic and other medicinal properties are listed below.

Bitter Gourd (*Momordica charantia*)

It is a plant of Asian origin. Bioactive compounds such as polypeptide-p, cucurbitane-type triterpenoids, steroidal saponins called “charantins,” insulin like peptides, and alkaloids are postulated to favourably alter carbohydrate metabolism (Saeed et al., 2010; Grover and Yadav, 2004; Budrat and Shotipruk, 2008; Leung et al., 2009). Hence, it is used in the treatment of diabetes (Singh et al., 2011) owing to its anti-diabetic effect. In an intervention study comprising of 100 type 2 diabetic patients who underwent three days of washout of OHAs, fresh bitter melon decreased fasting as well as postprandial glucose (Ahmad et al, 1999). It has also been documented to benefit patients with renal disease, neuropathy, GI tract disturbances, cataract and dyslipidemia (Grover et al., 2002; Grover et al., 2012; Fernandes et al., 2007).

Fenugreek (*Trigonella foenum-graecum*)

Right from the historical times, fenugreek has been documented as an anti diabetic agent (Basch et al., 2003; Evans, 2003). The seeds are rich in protein, vitamin A and fibre that impart hypoglycemic effect by decreasing gastric emptying that reduces postprandial glucose rise, hypocholesterolemic effect (Mullaicharam et al., 2013; Srinivasan, 2006). The seeds also contain fiber fenugreekine, a component that may have hypoglycemic activity (Mullaicharam et al., 2013). The gel-forming property of fenugreek fibre reduces glucose absorption and insulin response (Broca et al., 2000). Additionally, the seeds contain 4-hydroxyleucine which increases the glucose stimulated insulin release by isolated islet cells (Sauvaire et al., 1998) and exhibits insulintropic activity (Broca et al., 2000). Thus, it favourably alters glucose tolerance (Gopalpura et al., 2009). Supplementing diabetic subjects with fenugreek for a period of 8 weeks has shown to improve fasting glucose as well as dyslipidemia (Kassaian et al., 2009). Supplementing at 10-20 grams per day of fenugreek has provided more consistent results (Srinivasan, 2006). In a randomised, cross-over trial in type 2 diabetics, fenugreek supplementation for ten days brought about a significant reduction in blood glucose and improved glucose tolerance (Sharma and Raghuram, 1990). About 25 grams of fenugreek seeds may serve as a good adjunct therapy in the management of diabetes (Broca et al., 2000).

Gymnema (*Gymnema sylvestre*)

It is also known as gurmar and its leaf part has been documented for its usage in the treatment of diabetes (Grover et al., 2002). It imparts anti-diabetic effect through the following bio-active compounds; oleanane triterpenoid saponins (gymnemic acids), dammarane saponins called gymnemosides, and a polypeptide called gurmarin (Porchezian and Dobriyal, 2003). The gymnemic acid, other than anti-diabetic properties, also possesses anti-obesity properties that aid in weight loss (Shanmugasundaram et al., 1990; Baskaran et al., 1990; Bunyapraphatsara et al., 1996). It improves glucose uptake by the peripheral tissues, enhances insulin secretion, increases the beta cells in the islet of Langerhans (Dey et al., 2002) and regenerates and revitalizes the remaining beta cells owing to the presence of dihydroxy gymnemic triacetate (Rawat and Parmar, 2013). Moreover, gymnema is postulated to decrease absorption of glucose from the small intestine, thereby improving glucose metabolism and improving insulin secretion, decrease glycosylated hemoglobin in the long run and improve dyslipidemia (Baskaran et al., 1990; Porchezian and Dobriyal, 2003). It also holds the potential to reduce insulin dosage in diabetics (Shanmugasundaram et al., 1990; Baskaran et al., 1990; Bunyapraphatsara et al., 1996). Additionally, it possesses sweet inactivation property owing to the presence of triterpene saponins (Tiwari et al., 2014). In a quasi-experimental study on impact of 500 mg of gymnema sylvestre for a period of 3 months on type 2 diabetics, the supplementation reduced polyphagia, fatigue, blood glucose, glycated hemoglobin and favourably altered the lipid profile (Nandakumar et al., 2010). However, there is still a dearth of literature to provide evidence of gymnema's efficacy and safety (Leach, 2007).

Curry leaves (*Murraya koenigii*)

It is commonly used as a spice in India, especially in the Southern states. In Ayurvedic system, it has been used to treat diabetes, especially in conditions associated with lipid and cardiovascular abnormalities (Dineshkumar et al., 2010). The plant is rich source of carbazole alkaloid (Jain et al., 2012). The hypoglycaemic effect of curry leaves has also been established in type 2 diabetics (Iyer and Mani, 1990; Felicia et al., 1993).

Costus pictus (Insulin plant)

Insulin plant grows in tropical and sub-tropical regions of South and Central America and has been recently introduced in India (Jose and Reddy, 2010), where diabetic people consume its leaves to keep their blood glucose in control (Devi and Urooj, 2010). It holds potential as an anti-diabetic agent. In type 2 diabetics, it has shown favourable impact on glycemic control (Shetty et al., 2010). The plant is known to contain phytochemicals; triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids and steroids (Hegde et al., 2014) besides containing ascorbic acid, α -tocopherol and β -carotene (Devi and Urooj, 2010; Shankarappa et al., 2011).

Eugenia jambolana (Indian gooseberry, jamun)

A decoction of jamun kernels has been used for the treatment of diabetes as mentioned in Ayurveda (Chopra et al., 1958; Bhowmik et al., 2013). In type 2 diabetics, supplementation for 60 days with aqueous and alcoholic extract of jamun and its lyophilized powder imparted a hypoglycemic effect by reducing the blood glucose levels. In case of mild, moderate and severe diabetes, it brought about 73.51%, 55.62% and 17.72% reduction in blood glucose, respectively (Sheela and Augusti, 1992). This maybe due to the low GI of jamun (Chopra et al., 1958; Bhowmik et al., 2013).

Coccinia indica (Ivy gourd)

It belongs to the Cucurbitaceae family. It imparts anti-diabetic effect owing to the presence of triterpenes that may perhaps act like insulin (Kurpad and Raj, 2002). In a supplementation study on untreated type 2 diabetics given dried extracts of *C. indica* at 500mg/kg body weight for six weeks, it restored the activity of lipoprotein lipase and reduced glucose -6-phosphatase and lactate dehydrogenase (Kamble et al., 1998). In a double-blind, randomised placebo-controlled trial on newly detected type 2 diabetics, an alcoholic extract of 1g of leaves and fruits brought about a hypoglycaemic effect in a period of 90 days. A drop in fasting and post-prandial blood glucose was observed along with lowering of glycosylated Hb. However,

anthropometric parameters or blood lipids remained unaltered (Kurpad and Raj, 2002).

Cinnamon (*Cinnamomum cassia*)

Cinnamon has been documented since historical times for its use in the treatment of diabetes. Though the precise anti-diabetic bioactives are not known, it is postulated that polyphenol type-A may have insulin mimicking effect (Jarvill-Taylor et al., 2001; Anderson et al., 2004). Infact, a substance isolated from cinnamon has been termed as insulin potentiating factor (Khan et al., 1990). In a study on type 2 diabetics who were previously treated with lifestyle intervention, a 1g/day of cinnamon for a period of 90 days significantly lowered HbA1c in the intervention arm (Crawford, 2009). There is evidence that points towards cinnamon's role in improving lipemic status in addition to glycemic status as well (Khan et al., 2003; Anderson, 2008; Crawford, 2009). In small clinical trials, cinnamon has demonstrated anti-hypertensive potential as well amongst the cases of MS (Ziegenfuss et al., 2006). However, inspite of the growing body of evidence, inconsistency in findings remain owing to the selection criteria, clinical end points, dosage, and the fraction of plant part chosen for supplementation (Dugoua et al., 2007).

Garlic (*Allium sativum*)

Garlic finds it way into the traditional system of Indian medicine owing to its anti-thrombotic, anti-hypertensive, hypocholesterolemic, anti-oxidant, anti-mutagenic and anti-microbial effects (Cefalu et al., 2011). Though the precise anti-hypertensive mechanism of garlic is not known, it is postulated that it modulates endothelial production of nitric oxide, inhibits angiotensin-converting enzyme activity, decreases the production of vasoconstrictive agents thromboxane-B2 and prostaglandin-E2 and has potent free-radical scavenging activity (Medina-Campos et al., 2007). A meta-analysis of various clinical trials with garlic revealed that hypertensive individuals benefited more from garlic compared to the placebo group (Ried et al., 2008). Garlic is used as prophylactic as well as treatment food in atherosclerosis, hypertension and diabetes (Augusti, 1997). Allicin, the sulphur containing compound has a significant hypoglycemic activity by producing active nitrogen compounds (Sheela and Augusti, 1992; Lawson and Bauer, 1998). The postulated physiological mechanism is thought

to be due to increased insulin release and/or insulin sparing effect (Bever and Zahnd, 1979).

Onion (*Allium cepa*)

Onions are known to have antioxidant properties and act as a hypolipemic agent (Joseph and Jini, 2011). In type 2 diabetic patients supplemented with 50 grams of onion juice, it brought about a significant control over the post prandial glucose levels (Mathew and Augusti, 1975). Infact, onion as a dietary supplement has shown its potential in the management of type 1 and type 2 diabetes (Eldin et al., 2010).

Aloe (*Aloe vera*)

The aloe vera gel is postulated to contain water soluble fibre glucomannan which has hypoglycaemic potential and imparts insulin sensitizing action demonstrated by a randomized controlled trial on type 2 diabetics and those with MS, respectively (Vuksan et al., 1999; Vuksan et al., 2000). Fasting glucose levels have improved with aloe vera juice along with glibenclamide in type 2 diabetics (Bunyapraphatsara et al., 1996) and in newly diagnosed type 2 diabetics (Yongchaiyudha et al., 1996). Hypoglycemic and hypolipidemic effects have also been reported in long-term dietary supplementation of the gel with 20 g of psyllium seed husks (Agarwal, 1985). However, a systematic review concluded that though potential hypoglycaemic properties of aloe vera have been documented, yet further validation is required (Yeh et al., 2003).

Barley

Barley reduces the risk of CVD through cholesterol lowering effect (Behall et al., 2004; Keenan et al., 2007; Shimizu et al., 2008). It has also shown to improve glucose tolerance (Behall et al., 2006). The active ingredient thought to provide barley its health benefits is b-glucan. A meta-analysis on barley concluded that increased consumption of barely products should be considered as a dietary approach to reduce LDL cholesterol concentrations (AbuMweis et al., 2010).

Panchratna juice

It is a juice that is packed with the goodness of amla, tulsi, ginger, mint and turmeric. Its impact was studied in the management of type 2 diabetes for which a total of 55 stable type 2 diabetics were enrolled and were given either fresh panchratna juice for 45 days or processed panchratna juice for 90 days and compared with diabetic controls. Fresh panchratna juice or processed panchratna juice supplementation for long-term did not have any significant impact on the glycemic and lipemic status of diabetic subjects (Iyer et al., 2010).

Amla (*Euphorbiaceae officinalis*)

Amla is a rich source of vitamin C, amino acids, and minerals. The fruit is used either alone or in combination with other plants to treat common cold, fever; as a diuretic, laxative, liver tonic, stomachic, restorative, antipyretic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive (Bhandari and Kamdod, 2012). It also imparts anti-hyperglycemic and hypolipidemic effect in normal as well as in diabetic subjects (Akhtar et al., 2011).

TINOSPORA CORDIFOLIA: THE MEAGRELY EXPLORED PLANT

A very meagrely explored medicinal plant with immense pharmacological potential is *Tinospora cordifolia* (Sharma et al., 2010). It belongs to the family *Menispermaceae* (Sankhala et al., 2012) which consists of about 70 genera and 450 species which primarily thrive in tropical lowland regions and are a rich source of alkaloid and terpenes (Sharma et al., 2010). *Tinospora* is one of the important genera of the family, consisting of about 15 species. Some medicinally important species includes *T. Cordifolia*, *T. Malabarica*, *T. Tementosa*, *T. Crispa*, *T. Uliginosa* (Sharma et al., 2010). It is an important medicinal plant of the Indian Systems of Medicine as Rasayana (Sinha et al., 2004; Devprakash et al., 2011) especially for a stronger immune system (Khare, 2007). It is known to have hepatoprotective, antioxidant (Onkar et al., 2012), immunostimulatory (Devprakash et al., 2011; Onkar et al., 2012), hyperlipidemic (Devprakash et al., 2011), anticancer and antidiabetic properties (Onkar et al., 2012).

गुडूची कटुका तिक्ता स्वादुपाका रसायनी ।
संग्राहिणी कषायोष्णा लघ्वी बल्याग्निदापनी ॥
दोषत्रयामतृड्दाहमेहकासांश्च पाण्डुताम् ।
कामलाकुष्ठवातास्रज्वरकृमिवमीर्हरेत् ॥ (Bhavaprakasha)

In ancient Indian literature, the plant is termed as Amrit, Guduchi, Madhuparni, Chinnaruha, Vatsadaani, Tantrika, Kundalini and Chakralakshanika in Sanskrit and Abb-e-Hyat (Urdu) meaning water of life. In Hindi, the plant is commonly known as Giloya or Guduchi, a mythological term that refers to the heavenly elixir having saved celestial beings from old age and kept them eternally young (Singh et al., 2003). It is known as *Amrita* (Krishna and Patel, 2009) because it imparts youth, vitality and longevity to its patron (Sankhala et al., 2012). *Tinospora cordifolia* is a Latin name and is commonly known as heart leaf moonseed or Indian *Tinospora* in English, Gulvel in Marathi, Galo in Gujarati, Gulancha in Bengali, Amrita balli in Kannada (Khare, 2007; Sharma et al., 2010), Tippaatigo in Telugu and Shindilakodi in Tamil (Sharma et al., 2010).

It has been used to strengthen the immune system as it holds biological potential to conquer the impurities of various organs of the body (Srivastava, 2011). Right from the Vedic era, *Tinospora cordifolia* has been considered to be one of the most rejuvenating herbs impacting the seven dhatus that aid in keeping the body free from all types of ailments. In today's era of modern medicine, *Tinospora cordifolia* is called 'the magical rejuvenating herb' (Singh et al., 2003) owing to its properties to cure many diseases (Srivastava, 2011).

Botanical and Structural Profile

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: *Tinospora*

Species: *T. cordifolia*

FIG 2.12: MORPHOLOGY OF DIFFERENT PARTS OF TINOSPORA CORDIFOLIA



Ref: Mittal et al., 2014

It is a large climbing shrub and is found in higher altitudes (Rana et al., 2012; Parthipan et al., 2011), however it is widely distributed in India (Sinha et al., 2004) and also in neighbouring tropical countries such as Pakistan, Sri Lanka, Myanmar, Bangladesh and China (Sharma et al., 2010; Sankhala et al., 2012) and also in Malaysia, Indonesia, Philippines and Thailand (Sharma et al., 2010).

It is a deciduous plant that is large and glabrous (Sharma et al., 2010) that grows upto 1 meter high (3.3 feet) by 0.5 meters (1.65 feet) wide. It can grow in condition of moderate soil moisture in acidic as well as in alkaline soil with partial sun exposure to

full sun exposure (Sharma et al., 2010). The bark is thin, greyish or creamy white. It attains a great height and often climbs the neem tree trunk. The leaves are juicy and heart shaped. The flowers are greenish that bloom in summer. Male flowers occur in clusters and the female flowers occur single. Fruits occur in winter and are shiny, fleshy and single seeded. The seeds are curved and pea sized (Sankhala et al., 2012). The stem is succulent having fleshy aerial roots that arise from the branches (Sankhala et al., 2012) and is bitter in taste (Kirtikar et al., 1987). The starch that is obtained from the stem of *Tinospora cordifolia* is known as '*Guduchi-satva*.' It is considered to be highly nutritive and digestive and hence is used in treating many of the ailments (Sinha et al., 2004).

Phytochemical Profile and Chemical Constituents

A variety of chemical constituents/bioactive compounds such as; alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides (Sankhala et al., 2012; Upadhyay et al., 2010; Grover and Bansal, 2012; Singh et al., 2003; Yadav and Agarwala, 2011) lignans (Grover and Bansal, 2012), proteins, flavanoids, saponins (Yadav and Agarwala, 2011) have been isolated from the various fractions of *tinospora cordifolia* (table 2.5). An indepth analysis has revealed the presence of the following phytoconstituents; tinosporone, tinosporic acid, cordifolisides A to E, syringen, berberine, giloin, gilenin, crude giloininand, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F, amritosides, cordioside, tinosponone, ecdysterone, makisterone A, hydroxyecdysone, magnoflorine, tembetarine, syringine, glucan polysaccharide, syringine apiosylglycoside, isocolumbin, palmatine, tetrahydropalmatine, jatrorrhizine, respectively (Singh et al., 2003).

With particular regard to the stem, the following bioactive compounds have been derived; berberine, palmatine, tembetarine, magnoflorine, tinosporin, tinocordifolin (Kumar et al., 2000; Maurya et al., 1997). Further, the dried stem bits packed in polyethylene lined gunny bag retain the highest alkaloid content (0.042%) as compared to storage under ambient conditions (Padmapriya et al., 2009).

TABLE 2.5: PHYTOCONSTITUENTS CHARACTERIZED IN DIFFERENT PARTS OF TINOSPORA CORDIFOLIA

Chemical class	Phytoconstituents	Plant Part
Alkaloids	Berberine, Tembetarine	Stem
	Choline, Tinosporin, Isocolumbin, Tetrahydropalmatine, Jatrorrhizine	Root
	Palmatine, Magnoflorine	Stem, root
Glycosides	18-norclerodane glucoside, furanoid diterpene glucoside, tinocordiside, tinocordifolioside, cordioside, cordifolioside A, cordifolioside B, syringin-apiosylglycoside, Palmatosides C, Palmatosides P1, cordifolioside C, cordifolioside D, cordifolioside E	Stem
Diterpenoid lactones	Clerodane derivatives, tinosporon, tinosporides, jateirine, columbin	Whole plant
Sesquiterpenoids	Tinocordifolin	Stem
Steroids	β -sitosterol, δ -sitosterol, 20 β -hydroxy-ecdysone	Aerial plant
	Ecdysterone, Makisterone A, Giloinsterol	Stem
Aliphatic compounds	Octacosanol, heptacosanol, nonacosan-15-one	Whole plant

Ref: Adapted from Grover and Bansal, 2012

PHYSIOLOGICAL FUNCTIONS OF TINOSPORA CORDIFOLIA

Research over the years have documented that the plant possesses anti-oxidant, anti-hyperglycemic, anti-neoplastic, anti-stress, anti-dote, anti-spasmodic, anti-pyretic, anti-allergic, anti-leprotic, anti-inflammatory, anti-hyperlipidaemia, analgesic, CNS depressant, cardio-protective, hepatoprotective, anti-osteoporotic and immunomodulatory properties. Various parts of the plant contain immense medicinal properties (Sharma et al., 2010; Singh, 2004; Singh et al., 2003; Upadhyay et al., 2010; Saha and Ghosh, 2012; Sharma et al., 2012).

Traditional Usage of *Tinospora cordifolia*

Traditionally, the plant has been documented to possess anti-spasmodic, anti-inflammatory, expectorant, digestive, anti-stress and aphrodisiac properties. It has been used in the treatment of jaundice, diabetes, urinary tract infections, fever, general debility and skin diseases (Farooqi et al., 2001).

The dry stem crude extract of *Tinospora cordifolia* has shown to increase the leucocytes and phagocytic cells (Manjrekar et al., 2000; Dikshit et al., 2000). The stem's crude aqueous extract also produced significant anti-inflammatory effect in both acute and sub-acute models of inflammation (Rai and Gupta; 1966; Pendse et al., 1977) and also shown to be effective in treating skin diseases (Aiyer and Kolammal, 1963; Raghunathan and Mittra, 1982).

The powdered root and stem bark is used along with milk for treatment of cancer by the tribals of North Gujarat (Bhatt and Sabnis, 1987). The tribals also make use of the root decoction in diarrhoea and dysentery and the stem decoction in fever (Bhatt and Sabnis, 1987). Oral administration of decoction of stem is used for treatment of various skin diseases while the decoction with cold or hot water in morning on empty stomach is used as a tonic in general debility by the tribals of Maharashtra (Shah et al., 1983). The tribals staying in and around Mumbai and especially the fishermen community make use of *Tinospora cordifolia* in the treatment of fever, jaundice, chronic diarrhoea and dysentery (Shah, 1984).

The Muslim, Gujjar, and Backwal tribals of Jammu make use of the plant in the treatment of bone fractures (Jee et al., 1984). The paste and juice of the leaves is used for application over burn wounds to relieve the burn sensation. Juice of the stem along with honey is used for the treatment of asthma (Sinha et al., 2004).

***Tinospora Cordifolia* in Modern Medicine**

In a double blind, randomized placebo controlled trial of 21 days involving 30 healthy individuals aged 18-30 years, *Tinospora cordifolia* enhanced memory and learning without any significant side-effects (Bairya et al., 2004).

A randomized controlled study on investigation of the immunomodulatory role of *Tinospora cordifolia* as an adjuvant in surgical treatment of diabetic foot ulcers indicated that the extract is beneficial in immuno-modulation for ulcer healing (Purandare and Supe, 2007).

A clinical evaluation of the plant extract for immunostimulant action against the symptoms of HIV suggested the herb can be used in HIV/AIDS management. HIV positive participants were randomly assigned to receive either *Tinospora cordifolia* extracts or placebo for six months. Treatment led to decreased recurrent resistance of HIV virus, reduced eosinophil count, stimulation of B lymphocytes, macrophages, polymorph-nuclear leucocytes and Hb percentage (Kalikar et al., 2008).

The effects of the alcoholic *Tinospora cordifolia* extracts on the proliferation, differentiation and mineralization of bone like matrix on osteoblast model systems in vitro revealed stimulation of the growth of osteoblasts, increased differentiation of cells, increased cell numbers into osteoblastic lineage and increased mineralization of bone like matrix on the osteoblast model systems. Their finding suggests that 20-OH beta ecdysone isolated from *Tinospora cordifolia* extract could be explored as a potential anti-osteoporotic agent (Abiramasundari et al., 2012).

When patients with infective hepatitis were administered three tablets of *Tinospora cordifolia* of 500 mg potency, after four weeks, it relieved the symptoms and corrected the altered liver enzymes profile (Prakash and Rai, 1996).

In post menopausal women, supplementation with minofil, a non-hormonal drug which contains *Tinospora cordifolia*, showed to be a better sustained therapy with no side effects and was also cost effective. However, further validation of the findings is required before the drug can be universally used to treat post menopausal syndrome (Nandaa, 1997).

In a randomized double blind placebo controlled trial on impact of *Tinospora cordifolia* extract on those with allergic rhinitis, the experimental arm reported 100% relief from sneezing in 83% patients, 69% from nasal discharge, 61% from nasal obstruction and 71% from nasal pruritus. The difference between the two groups was

highly significant. TC significantly decreased all symptoms of allergic rhinitis (Simons, 1988).

The effect of *Tinospora cordifolia* on blood sugar level in obese and non diabetic females was studied by giving 2gm of *Tinospora cordifolia* leaf powder orally twice a day for 30 days. The blood sugar level decreased significantly in all the subjects only in 30 days of oral consumption of *Tinospora cordifolia* leaf powder (Tripathi et al., 2014).

Role of *Tinospora Cordifolia* in Management of Dyslipidemia and Dysglycemia

Streptozotocin diabetic albino rats benefited from different dosages (200 and 400 mg/kg b.w.) of *Tinospora cordifolia* stem extracts (both aqueous and alcoholic) as it had significant anti-diabetic activity in diabetic animals and had an efficacy of 40% to 80% compared to insulin. Because the extract did not cause any increase in serum insulin levels or regeneration of pancreatic β cells but caused increased hepatic glycogen synthase and decreased glycogen phosphorylase activity, it was postulated by the authors that *tinospora cordifolia* maybe acting as an anti-hyperglycemic drug through some peripheral mechanisms, such as increasing the glycogen storage in the liver or by decreasing the activity of glycogen phosphorylase, thereby retarding or preventing glucose release from the liver (Puranik et al., 2010).

The antidyslipidemic activity of *tinospora cordifolia* stem extract has been demonstrated in alloxan-induced (150 mg/kg body wt.) diabetic rats who were (dyslipidemic) orally fed stem extract (500 mg/kg body weight) for 15 days that resulted in significant decrease in plasma glucose, HbA1c lipid peroxide, total lipid and FFA. The extract also had an anti-diabetic effect but it was lesser in intensity than glibenclamide. Antidiabetic and antidyslipidemic activities of *T. cordifolia* stem may be partly due to presence of the alkaloids (Mahdi et al., 2013).

Further evidence comes from a study wherein hexane, ethyl acetate and methanol *Tinospora cordifolia* stem extract at a dose of 250 mg/kg b.w. for a period of 100 days had an antidiabetic effect which reduced blood sugar level in streptozotocin induced diabetic rats. The supplementation also significantly reversed reduced glucokinase

and increased glucose-6-phosphatase activity and decreased the HbA1c (Rajalakshmi et al., 2009).

Another evidence of antidyslipidemic activity of the *Tinospora cordifolia* stem extract comes from a study on alloxan induced diabetic rats who were orally administered 500 mg/kg bw. p.o. stem extract for 30 days. Blood glucose, plasma lipids reduced significantly and post heparin lipoprotein lipase activity was reactivated. Importantly, the extract inhibited the generation of super oxide anions and hydroxyl radicals, in both enzymic and non-enzymic systems in vitro (Kumar, 2015).

There was a significant decrease in total cholesterol, triglycerides, LDL and VLDL and HDL remained unaffected in Sprague dawley rats who were induced with hyperlipidemia but were treated with methanolic extract of *Tinospora cordifolia* stem at 400 mg kg⁻¹ dose. The reduction was found to be significant when compared with atorvastatin, though of lesser intensity (Thahera and Nyamathulla, 2011).

Safety Profile

It is a misconception that traditional medicines are always safe. They may also pose health risks either in the form of adverse reactions or drug-drug interactions. The safety of *Tinospora cordifolia* was established through a randomized, double-blind placebo controlled study on healthy volunteers using a battery of haematological, biochemical tests and open questionnaire method. The study concluded that it is safe at a dose of 500mg/ day for a period 21 days in healthy volunteers for the parameters studied (Karkal and Bairy, 2007). It has shown not to exert any remarkable adverse effects on the cardiovascular (Dhar et al., 1968; Singh et al., 1975), renal (Singh et al., 1975), central nervous (Kundnani et al., 1985; Bairy et al., 2004) and gastrointestinal system (Spelman, 2001; Chandrasekaran et al., 2009). However, the potent curative effects of the plant against particular human ailments need to be verified by more controlled and exhaustive clinical trials (Grover and Bansal, 2012).

Since the stem contains higher alkaloid content than the leaves, it is only the stem part of the plant that is approved for medicinal usage by the Ayurvedic Pharmacopoeia of India (MOHFW, 2001). *Tinospora cordifolia* remains unexplored in the arena of

management of diabetic dyslipidemia. With a host of phytochemical properties present in the stem, the plant may hold potential to manage dyslipidemia as well as dysglycemia in patients with type 2 diabetes.

SUMMARY

- India is known as botanical garden of the world.
- About 40% of the people in the developed world and double the figure in developing world make use of medicinal plants.
- Medicinal plants are known to have fewer side effects, are easily available, less expensive and demonstrate better efficiency.
- Of the 400 traditional medicinal plants with anti-diabetic effect, hardly any clinical data is available to establish their efficacy.
- *Tinospora cordifolia* (Willd.) Hook.f. & Thomson is an important medicinal plant of the Indian Systems of Medicine as Rasayana for a stronger immune system.
- According to the ancient Indian literature, the plant is heavenly elixir as it imparts youth, vitality and longevity to its patron.
- In modern medicine, *Tinospora cordifolia* is called ‘the magical rejuvenating herb’ owing to its properties to cure many diseases.
- It is a large deciduous climbing shrub widely distributed in India.
- The starch obtained from the stem of *Tinospora cordifolia* is known as ‘Guduchi-satva’ and is used for treating a variety of ailments.
- *Tinospora cordifolia* contains; alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides, lignans, proteins, flavanoids and saponins.
- The stem contains berberine, palmatine, tembetarine, magnoflorine, tinosporin, tinocordifolin.
- Traditionally, the plant has been used as an anti-spasmodic, anti-inflammatory, expectorant, digestive, anti-stress and aphrodisiac agent.
- *Tinospora cordifolia* enhances visual memory, logical memory, verbal memory, attention span, and concentration without any significant side-effects.
- It can be used as an adjuvant in surgical treatment of diabetic foot ulcers.

- The plant extract can be used in HIV/AIDS management.
- *Tinospora cordifolia* also holds potential as an anti-osteoporotic agent.
- It relieves the symptoms of infective hepatitis and corrects the liver enzymes profile.
- The plant also holds promise in the treatment of post menopausal syndrome.
- The stem of *Tinospora cordifolia* is approved for medicinal usage by the Ayurvedic Pharmacopoeia of India.
- The plant extracts have been found to effectively reduce symptoms of allergic rhinitis.
- *Tinospora cordifolia* leaves have demonstrated positive impact on the glycemic profile of obese but non-diabetic women.
- *Tinospora cordifolia* is safe at a dose of 500mg/ day for a period 21 days in healthy volunteers. However, the need of the hour is to establish the plant's efficacy through clinical studies.
- There are no studies on impact of *Tinospora cordifolia* in the management of dyslipidemia, especially amongst those with type 2 diabetes. Moreover, though the anti-diabetic potential of the plant has been established through pre-clinical studies, there are no clinical studies to corroborate the same.