# Diagnosis and Treatment of Non-alcoholic Fatty Liver Disease

By

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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

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# Approval

The thesis/project titled "Diagnosis and Treatments of Non-alcoholic Fatty Liver Disease" submitted by Sanjida Haque (ID – 15146031) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy (Hons) on 22nd August 2019.

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## Ethics Statement

The study does not involve any kind of animal trial and human trial.

## Abstract

Non-alcoholic fatty liver disease (NAFLD) is of a huge concern worldwide due to its current high prevalence. Globally the prevalence of NAFLD is 25.24%. It is a type of disease which has a large histologic spectrum ranging from just steatosis to its more severe form, non-alcoholic steatohepatitis (NASH). Some patients suffering from NAFLD are only limited to steatosis while in other patients the steatosis may progress and become NASH and then eventually turn into liver cirrhosis. NAFLD is a matter of concern because despite the large population of people already affected by the disease and its high prevalence, the pathogenesis and pathophysiology of NAFLD is still unclear and there are very few effective methods of diagnosis and treatment. The purpose of this review is to discuss all the methods of diagnosis and treatment currently available and mention some of the methods of diagnosis and treatment which have potential and should be further researched on.



**Keywords:** NAFLD; NASH; Steatosis; Cirrhosis

# Dedication

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*Dedicated to my parents*

## Acknowledgement

With utmost respect and appreciation, I would like to extend my heartfelt gratitude to all the people who have directly or indirectly helped me to accomplish the completion of this paper.

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# Chapter 1

# Introduction

Currently non-alcoholic fatty liver disease is a major concern worldwide among all liver diseases (Younossi et al., 2016). Due to its close association with obesity and type 2 diabetes mellitus, it is a highly prevalent form of chronic liver disease (Bertot & Adams, 2016). NAFLD expresses a histological spectrum which ranges from just steatosis to its more aggressive necro-inflammatory form, non-alcoholic steatohepatitis (NASH) and this eventually accumulates fibrosis and leads to cirrhosis, hepatocellular carcinoma and liver failure (Bertot & Adams, 2016; Hardy, Oakley, Anstee, & Day, 2016).

The global prevalence of NAFLD is 25.24 %, with the highest in Middle East and South America and the lowest in Africa (Younossi et al., 2016). In Asian countries, the prevalence of NAFLD is 16%-32% in the Indian sub-continent. Bangladesh falls within this region and it is observed that one third of the population of the country is affected by NAFLD (Hassan et al., 2018). The increasing prevalence of NAFLD and the large population already affected by NAFLD has led to the prediction that NALFD will become the most common indication for liver transplantation and the leading cause of liver related mortality and morbidity (Bertot & Adams, 2016; Chalasani et al., n.d.). As the history and progression of NAFLD is still incomplete and the treatment for NAFLD is still in a trial and error process, it has become very crucial to carry out further investigation into NAFLD in order to be able to manage the disease better and reduce the alarming rate of prevalence of NAFLD.

Over the last 15 years the clinical significance of NAFLD has increased notably but still there is apprehension surrounding this issue probably due to lack of time, information and experience in

addressing patients' needs to lifestyle changes and lack of adequate resources to approach as a comprehensive team. Due to these reason the treatment for this disease exclusively relies on drugs and the results have been poor thus far. This is why more research and studies should be conducted regarding NAFLD in order to be able to raise awareness of its importance and be able to treat the disease in a more effective manner (Marchesini, Petta, & Dalle Grave, 2016)

## 1.1 NAFLD and its types

Liver is the largest internal organ of the body. The basic functional units of liver are called lobules; they are composed of the functional cells of the liver called hepatocytes. The center of the lobule is the central vein and the periphery of the lobules is the portal triads. Functionally the liver is classified into three zones on the basis of oxygen supply. Zone 1 is located around the portal tracts, zone 3 is situated around the portal veins and zone 2 is located between zone 1 and zone 3. The hepatocytes perform a wide range of functions such as metabolic, secretory and endocrine. The liver carries out several functions such as carbohydrate, protein and fat metabolism, storage of vitamins and iron as ferritin, forms the substances used in blood coagulation and removes drugs, hormones and other substances from the body. Many of these functions are interrelated which becomes very evident in liver related diseases as several functions are stunted simultaneously.

Some of the liver related diseases that disrupt several liver functions are jaundice, hepatitis A, B and C, biliary cholangitis, hemochromatosis, non-alcoholic fatty liver diseases, Wilson's disease, cirrhosis etc. Among these, non-alcoholic liver disease is currently of utmost importance due to its sudden increase in prevalence and lack of knowledge and treatment.

### 1.1.1 Distinction between NAFL and NASH

Histologically NAFLD can be divided into two categories, non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) (Rinella, 2015). NAFL can be defined as the presence of 5% or more hepatic steatosis (accumulation of fat in the liver) devoid of any visible hepatocellular injury such as hepatocyte ballooning. On the other hand NASH is the presence of 5% or more hepatic steatosis along with hepatocyte injury (e.g., ballooning), with or without fibrosis (Chalasani et al., n.d.). Steatosis can further be divided into macrovesicular steatosis, which is the fatty degeneration caused by excessive lipid supply to the liver due to reasons such as obesity, insomnia, insulin resistance etc., and microvesicular steatosis which occurs due to the accumulation of small intracytoplasmic fat vacuoles in the hepatocytes. Macrovesicular steatosis is the more common kind of steatosis. Histologically NASH is undistinguishable from alcoholic steatohepatitis of the liver due to prolonged or excessive alcohol consumption. Hence NASH is also known as pseudo-alcoholic steatohepatitis (Bellentani, 2017). Patients with NASH have a greater chance of developing fibrosis, cirrhosis or hepatocellular carcinoma (Fazel, Koenig, Sayiner, Goodman, & Younossi, 2016).

### 1.1.2 Causes of NAFLD

A patient is considered to have NAFLD if, upon diagnosis by imaging or histology, evidence of hepatic steatosis is seen and this hepatic steatosis is not a by-product of any other changes being experienced by the body such as monogenic hereditary diseases, steatogenic medications, Wilson's disease etc. (Carr, Oranu, & Khungar, 2016). The diseases or complications which can lead to hepatic steatosis formation in the body as a by-product are known as the secondary causes (other secondary causes are given in Table 1 below). The primary causes or risk factors that are

usually associated NAFLD are obesity, diabetes mellitus and the metabolic syndromes which consist of dyslipidemia and hypertension (Kneeman, Misdraji, & Corey, 2012).

*Table 1: Known causes of secondary hepatic steatosis (Carr et al., 2016)*

| **Known causes of secondary hepatic steatosis** | |
|---|---|
| **Macrovesicular steatosis** | **Microvesicular steatosis** |
| Excessive alcohol consumption | Reye syndrome |
| Viral infection: hepatitis C | Viral infection delta hepatitis |
| Wilson disease | HELLP syndrome |
| Autoimmune hepatitis | Acute faty liver of pregnancy |
| Parenteral nutrition | Medications (eg. Valproate, tetracycline, antiretroviral) |
| Medications (e.g. Amiodarone, methotrexate, tamoxifen, corticosteroids, antiretrovirals) | Genetic anomalies and inborn errors of metabolism |
| Starvation: Kwashiorkor | Jamaican vomiting sickness |
| Lipodystrophy | |

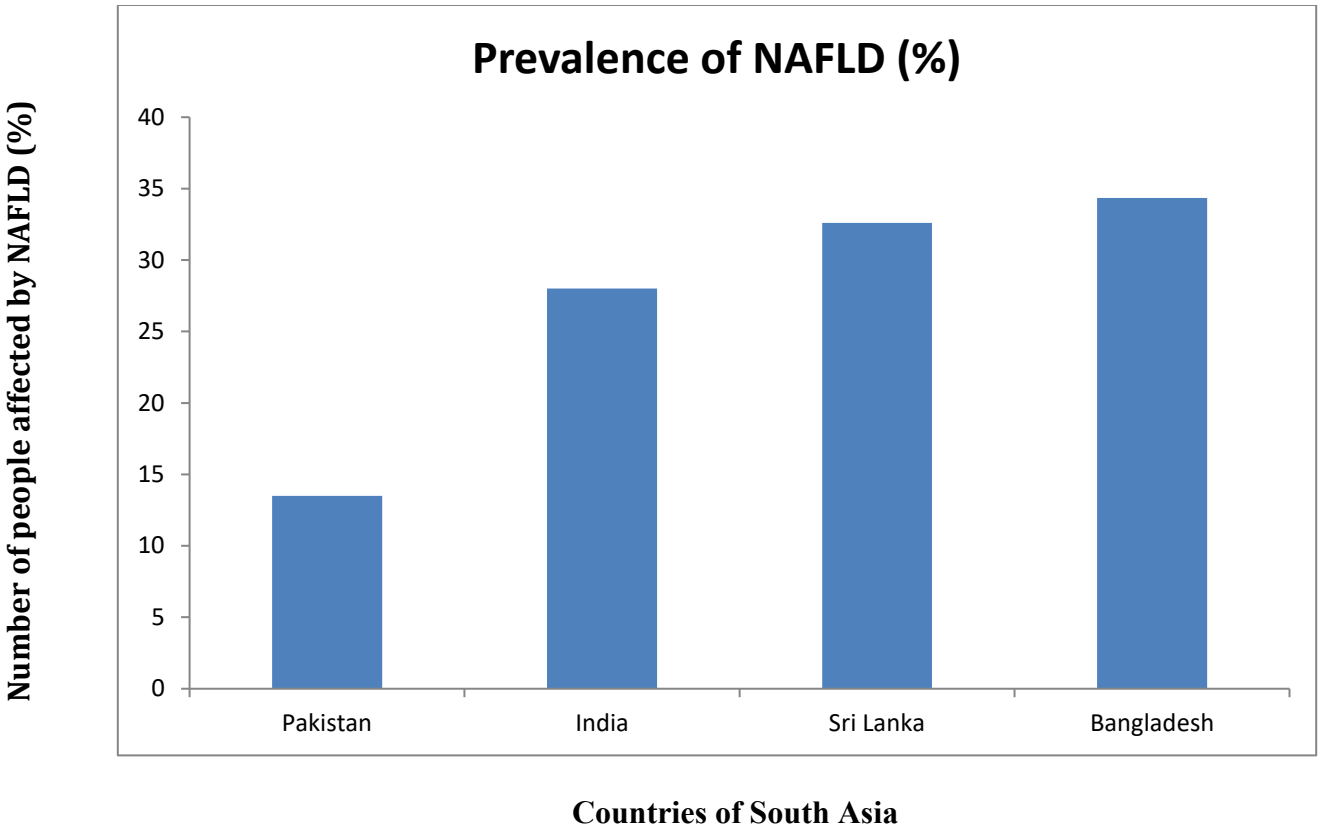
## 1.2 Prevalence of NAFLD

In the recent years the rapid rise in the prevalence of obesity and type II diabetes has been paralleled by the prevalence of NAFLD and is rapidly becoming a serious global health problem. According to a recent survey on prevalence of NAFLD it is estimated that NAFLD affects 13% Africans, 24% Europeans, 30% South Americans, 32% Middle Eastern, 30% of the United States population and 27% Asians. In the United States, African Americans have the lowest prevalence of NAFLD among all the racial and ethnic groups and compared to white Americans the Hispanic Americans have a greater prevalence. In the Hispanic population, Dominican republicans have the lowest prevalence and the Mexican heritage has the highest prevalence (Carr et al., 2016).

In May 2014 it was documented by WHO that 2.82% of the total deaths occurring in Bangladesh is due to liver diseases and is the 8th most common cause of death in Bangladesh(Hassan et al., 2018). Prevalence of NAFLD in the general population of Bangladesh has been estimated to be 4%-34.34% which jumps to 49.8% in diabetic patients. Among the South Asian countries Bangladesh is impacted the most due of NAFLD (Hassan et al., 2018).

*Table 2: Prevalence of NAFLD among Indian, Bangladeshi, Pakistani and Sri Lankan people (Hassan et al., 2018)*

| **Country** | **Population and Place** | **Sample Size (n)** | **Prevalence of NAFLD** |
|---|---|---|---|
| India | Selected population of Mumbai | 1168 | 16.6% |
| | General population of West Bengal (rural) | 1911 | 167 (8.2%) |
| | General population of Chennai (urban) | 541 | 173 (32%) |
| Bangladesh | General population nation wide | 2621 | 900 (34.34%) |
| | Selected population of Comilla ( rural) | 665 | 219 (33%) |
| Pakistan | General population (urban) | 2985 | 974 (32.6%) |
| Sri Lanka | Tertiary Care Hospital, Karachi | 952 | 129 (13.5%) |




*Graph 1: Prevalence of NAFLD in countries of South Asia (Hassan et al., 2018)*

NAFLD was once considered to be an occasional finding of no clinical significance but it is now among the top 3 conditions of terminal liver failure and will soon be among other reasons for liver transplantation (Hardy et al., 2016).

## 1.3 Pathophysiology of NAFLD

NAFLD being a spectrum of diseases tends to have very different rates of progression among individuals and different clinical manifestations. As the mechanism leading to NAFLD is unclear several proposed mechanisms are taken into consideration (Friedman, Neuschwander-Tetri, Rinella, & Sanyal, 2018). This makes it a very complex disease modulated by several aspects including metabolic, genetic, environmental and gut microbial factors. As most NAFLD patients

only have NAFL while some patients progress from NAFL to NASH, the delineation of this progression is still not properly established. The proposed pathophysiology of NAFLD suggests that the visceral adipose tissues induce several signals that cause an alteration in the metabolism of lipid and glucose. This causes hepatic fat to accumulate and forms a pro-inflammatory environment which leads to hepatocellular injury. Due to this injury, the inability to defend against harmful processes like oxidative stress, unfolded protein response dysregulation, lipotoxicity and apoptotic pathways promote liver damage and progressive fibrosis which may eventually cause cirrhosis leading hepatocellular cancer in a few patients (Cobbina & Akhlaghi, 2017; Rienella, 2015).

This review mainly focuses on the diagnosis and treatment of NAFLD. It summarizes the methods of diagnosis currently available and diagnostic methods which are being further researched and developed. The most relevant and effective method currently used for the diagnosis of NAFLD is looked into in this review. The types of treatments available for NAFLD up until 2018 are discussed in this review and the most effective ones are mentioned. The treatments which have potential and are being researched further are also briefly discussed in this review. The purpose of this review is to bring attention to the fact that there is lack of information and not enough research being done to find a proper and efficient diagnostic method and treatment for NAFLD despite the alarming rate at which the prevalence of NAFLD is increasing.

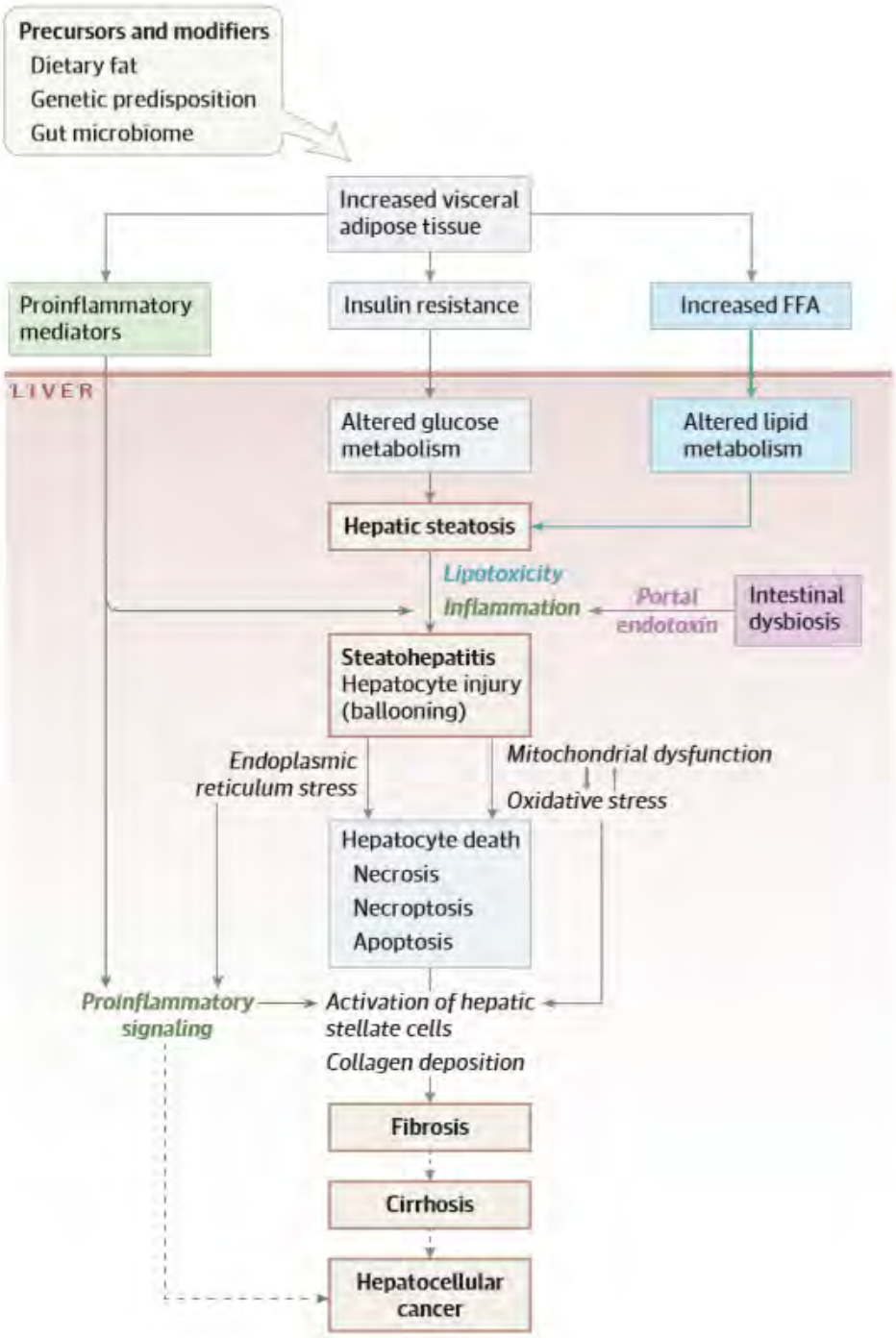



*Figure 1. The mechanisms involved in pathophysiology of NAFLD (Rienella, 2015).*

## 1.4 Methodology

All searches conducted for writing this review are based on articles and journals found in Google Scholars, Springer and Elsevier on the topic of NAFLD, its diagnosis and its treatment. All relevant articles were acquired in full text and all related relevant materials were evaluated and reviewed.

# Chapter 2

# Diagnosis of NAFLD

Increased enzyme activity seen in liver test results is commonly associated with NAFLD now (Rienella, 2015). Most of the affected patients are asymptomatic or possess non-specific symptoms like fatigue. In some cases the patient experiences pain in the upper quadrant; hence upon imaging of the liver for an unrelated condition NAFLD is incidentally diagnosed most of the time. Central obesity and hepatomegaly are often seen but there is no characteristic physical examination findings associated with NAFLD. Accurate diagnosis of NAFLD is required for its classification. Currently the available routine techniques are unable to distinguish between NAFL and NASH hence diagnosis of NAFLD is challenging (Cobbina & Akhlaghi, 2017). A combination of histologic information, clinical history, radiographic data and laboratory data are all required in order to diagnose NAFLD (Ahmed, Wong, & Harrison, 2015).

## 2.1 Types of Diagnosis

### 2.1.1 Abdominal Imaging

There are several ways of performing an abdominal imaging. Ultrasounds are widely available and most preferred by patients. However NAFLD patients that appear to have less than 30% steatosis in liver biopsy are often not detected by abdominal screening, in addition to this, the process is operator dependent which is also considered to be a drawback (Bohte & Werven, 2011).

Computed tomography (CT) has low sensitivity to fat mapping and is expensive, in addition to this it imposes radiation hazard and introduces color contrast related risk. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have the highest precision

(sensitivity and specificity) in mapping fat in the liver and quantifying steatosis (Schwenzer, Springer, Schraml, & Stefan, 2009). Unfortunately, MRI and MRS are currently costly and have limited availability but in the future they may become the standard in the diagnosis and management of NAFLD given the precision of their data. In NAFLD patients, MR elastography (MRE) compared to MRI is the superior method for the measurement of hepatic stiffness in terms of non-invasive diagnosis of cirrhosis and liver fibrosis (Le, Kroeker, Kipfer, & Lin, 2012; Noureddin et al., n.d.). MRE may also be able to help identify individuals with steatohepatitis before the onset of significant amount of fibrosis, even though further studies are required to validate this finding. Patients suffering from NAFLD with inflammation and no fibrosis have greater hepatic stiffness compared to isolated steatosis and a lower mean stiffness in NAFLD patients with fibrosis. Currently, abdominal imaging is unreliable in the diagnosis of NASH and MRI and MRS are not cost effective neither easily accessible, hence these are not preferred or usually recommended for the diagnosis of NAFLD (Loomba et al., n.d.).

### 2.1.2 Liver Biopsy

In order to validate the characteristic histologic features of NASH, a liver biopsy is done. In the U.S because of the burden NASH inflicts, liver biopsy is selectively recommended only to those NAFLD patients that have a greater risk of progression to NASH. Prior evaluation such as the medical history and age of the patient is required while considering the benefits and risks of diagnostic liver biopsy due to its invasive nature. However a biopsy is currently considered to be the gold standard in the diagnosis NAFLD due to its accuracy in differentiating the isolated steatosis from NASH, ability to identify the severity of the fibrosis which impacts the progression and management of the disease, cost –effectiveness and easy accessibility (Cobbina & Akhlaghi, 2017; Yki-Järvinen, 2016).

However steatosis may not be present in case of advanced fibrosis or cirrhosis which may lead to NAFLD not being detected. In addition to this, a liver biopsy sample of hepatocellular ballooning histologically may be interpreted differently by different experienced pathologists (Gawrieh, Knoedler, Saeian, Wallace, & Komorowski, 2011; Yki-Järvinen, 2016). Due to this difference in point of view between pathologists surrounding hepatocellular ballooning or sampling error can lead to fewer patients qualifying for the clinical trials (Noureddin et al., 2013). Hence, liver biopsy also has various drawbacks as a means of diagnosis for NASH patients.

On a liver biopsy index (a reference limit which helps indicate severity of necro-inflammation), patients that appear to have isolated hepatic steatosis with a certain degree of necro-inflammation are diagnosed as being at a greater risk of encountering progressive histologic damage, but still at a comparatively lower risk than those suffering from NASH. Along with that, patients that are clinically detected to have metabolic syndrome or individual components of metabolic syndrome and also diagnosed with isolated hepatic steatosis and inflammation on liver biopsy are diagnosed to be at a risk of swiftly progressing histologic damage (R Pais et al., 2011; Raluca Pais et al., 2013). Suspected NAFLD patients suffering from consistent high levels of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) levels along with fatty liver observed in abdominal imaging, fall within the age range greater than or equal to 65 years, speculated to have co-existing liver disease, have components of metabolic syndrome or metabolic syndrome are all recommended to have a liver biopsy. The severity of the baseline risk factors have an impact on whether liver biopsy is recommended or not, despite the age and other clinical indications. Even though one of the main objectives of liver biopsy tends to be staging the severity of fibrosis, being able to detect NASH in the initial stage in order to differentiate it

from simple steatosis can be significant due to the progressive nature of the disease and its adverse effects related to NASH, and the possibilities of assisting current and future therapies (Cecilia & Hultcrantz, 2009; Noureddin et al., 2013; Rafiq et al., 2009).

Predictors of histologic evidence of NASH on an index liver biopsy (Ahmed et al., 2015):

- Body mass index greater than 30kg/m²
- Persistently abnormal liver enzymes with AST (alanine aminotransferase) and ALT (aspartate aminotransferase)
- Diabetes mellitus (type 2) with elevated AST and ALT
- Metabolic syndrome with elevated AST and ALT
- Hypertension
- High triglycerides and low high-density lipoprotein

### 2.1.3 Non-invasive biomarkers

The high prevalence of NAFLD, the conflict surrounding the clinical predictors of NASH and the limitations of liver biopsy in differentiating NAFL from NASH has led to the urgent need to create non-invasive biomarkers (Chalasani et al., n.d.). Several noninvasive clinical and laboratory markers have been scrutinized to help detect NASH in patients suffering from NAFLD. Absence of fibrosis or mild fibrosis can be distinguished from advanced bridging fibrosis or cirrhosis using non-invasive markers of liver fibrosis. Nevertheless, non-invasive tests are unreliable when detecting intermediate or moderate grade and in staging of the hepatic injury. Along with this, as mentioned before NAFLD consisting less than 30% hepatocyte fat content cannot be detected using routine abdominal imaging (Nascimbeni et al., 2013). Biomarkers such as keratin 8/18 (antibody) immunostaining to detect cytokeratin 18 (filament

protein) and others may be available in the future. Presence of keratin is correlated to the levels of cytokeratin 18 based on initial data, however, it is unable to provide the histologic details and sensitivity shown by liver biopsy (Cusi et al., 2014; Wieckowska et al., n.d.). The abundantly available clinical markers are used for the evaluation of advanced fibrosis, which helps derive the NAFLD fibrosis score. The apparent accessibility of non-invasive biomarkers has the potential to provide an alternative to biopsy even though, liver biopsy is currently the most accurate method for the evaluation of the histologic stage and grading of the disease (Lichtinghagen, Pietsch, Bantel, Manns, & Bahr, 2013).

## 2.2 Grading and Staging of NAFLD

In the 1999 classification of Matteoni et al., NAFLD patients were divided into 4 types; type 1- steatosis alone, type 2- steatosis with lobular inflammation, type 3- steatosis with ballooning degeneration and type 4- steatosis with ballooning degeneration along with fibrosis and/or Mallory-Denk bodies. After comparing the clinical traits and results, the authors concluded that type 3 and type 4 patients showed cirrhosis and liver – related deaths exclusively hence were termed as NASH while type 1 and 2 patients were considered to be non-NASH. This classification did not take the severity fibrosis or the degree of steatosis/inflammation or the location of these changes into consideration, it only aided in clearly distinguishing between NASH and non-NASH. After Matteoni's classification was published, a semi-quantitative grading and staging system for NASH was proposed by Brunt et al., ballooning degeneration, steatosis and inflammation was graded by the authors and the degree of fibrosis was used to establish the staging (Hashimoto, Tokushige, & Ludwig, 2015).

*Table 3: Brunt scheme for grading nonalcoholic steatohepatitis (Abd El-Kader & El-Den Ashmawy, 2015)*

| | **Mild (grade 1)** | **Moderate (grade 2)** | **Severe (grade 3)** |
|---|---|---|---|
| Steatosis | Predominantly macrovesicular, involves < 33%-66% of the lobules | Any degree and usually mixed macrovesicular and microvesicular | Typically > 66% (pan acinar); commonly mixed steatosis |
| Ballooning | Occasionally observed; zone 3 hepatocytes | Obvious and present in zone 3 | Predominantly zone 3; marked |
| Lobular inflammation | Scattered and mild acute (polymorphs) inflammation and occasional chronic inflammation (mononuclear cells) | Polymorphs may be noted associated with ballooned hepatocytes, peri-cellular fibrosis; mild chronic inflammation maybe seen | Scattered acute and chronic inflammation; polymorphs may appear concentrated in zone 3 areas of ballooning and peri-sinusoidal fibrosis |
| Portal inflammation | None or mild | Mild to moderate | Mild or moderate |

Since this classification was only valid for NASH, the NASH Clinical Research Network Pathology Committee formulated a valid histological grading score for NAFLD known as NAFLD activity score (NAS) in 2005 based on Brunt's classification. It was created for the purpose of addressing the whole scale of lesions of NAFLD. The histological aspects taken into consideration for NAS were classified into five broad groups each with its own scoring scale. The aspects, which were independently correlated with NASH, include steatosis (0-3), hepatocellular injury (0-2), lobular inflammation (0-3) and miscellaneous features such as Mallory-Dank bodies and glycogenated nuclei. The NAS is the unweighted summation of

steatosis, lobular inflammation and hepatocellular ballooning scores. Diagnosis of NASH is associated with a NAS of ≥5% and NAS < 3% activity is considered to be 'not NASH '(Brunt, Kleiner, Wilson, Belt, & Neuschwander-tetri, n.d.; Cobbina & Akhlaghi, 2017).

*Table 4: Non-alcoholic fatty liver disease activity score (NAS) (Abd El-Kader & El-Den Ashmawy, 2015)*

| **Steatosis** | **Score (NAS)** |
|---|---|
| 0 | < 5% |
| 1 | 5% - 33% |
| 2 | > 33% - 66% |
| 3 | >66% |
| **Lobular inflammation (counted in 20 × fields)** | |
| 1 | < 2 foci |
| 2 | 2-4 foci |
| 3 | >4 foci |
| **Hepatocellular Ballooning** | |
| 1 | Few |
| 2 | Many |

However since the specificity and sensitivity of NAS in the diagnosis of NASH is not very high, it cannot replace the histopathological classification of NAFLD types. On the other hand FLIP (European Fatty Liver Inhibition of Progression) formulated another scoring system for NAFLD in order to reduce and standardize the variation among observers of histologic diagnosis of NASH. This system is based on the NASH features, Steatosis (S), Activity (A) and Fibrosis (F). This SAF scoring originated from the NAFLD grading and staging system of patients with morbid obesity and undergoing bariatric surgery (Burt, Lackner, & Tiniakos, 2015). Steatosis, lobular inflammation and ballooning are taken into consideration by SAF while distinguishing NAFL and NASH. The activity can be described as the summation of the grades of ballooning

and lobular inflammation which ranges from 0-4. In order to distinguish NAFL and NASH presence of steatosis ranging from 0 to 3 (S = 0-3) is required and varying degree of activity ranging from 0 to 4 (A = 0-4) along with a fibrosis range of 0 to 4 (F = 0-4 ) (Brunt et al., n.d.; Raluca Pais et al., 2013).

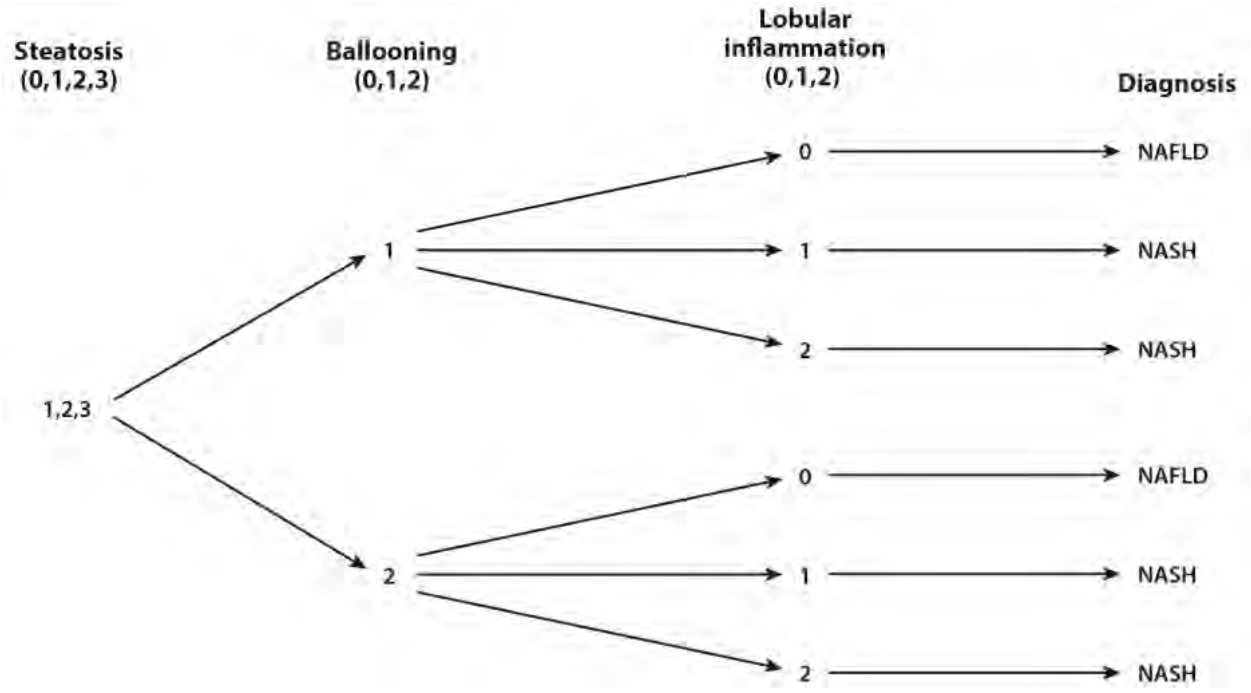



*Figure 2. SAF (steatosis-activity-fibrosis) score system (Hardy et al., 2016)*

# Chapter 3

# Treatment of NAFLD

Despite the constantly increasing knowledge about NAFLD, there is still no definite pharmacotherapy available which is why the treatment of NAFLD stirs up a lot of controversies. The existing limitations in the ability to differentiate between the different stages of NAFLD make it difficult to develop any sort of targeted therapy. The proposed beneficial treatments currently available are bariatric surgery or changing the lifestyle by incorporating regular exercise and dietary changes for patients that are extremely obese. This helps to lose weight gradually. Among other evidently beneficial treatments are anti-inflammatory or antioxidant agents which have to be used along with modifications to the lifestyle (Hossain, Kanwar, & Mohanty, 2016). The following are the available treatments for NAFLD:

## 3.1 Weight Reduction

Two of the most essential risk factors associated with NAFLD are obesity and metabolic syndrome. These two factors highly contribute to the prevalence of NAFLD. This is because the majority of the people now lead a life which involves overconsumption of food rich in saturated fats and complex carbohydrates and a sedentary lifestyle which has led to higher prevalence of metabolic syndrome and obesity, this in turn has increased the number of cases of NAFLD. This is why weight loss is speculated to be a therapeutic option for NAFLD. At least 7% reduction in weight is needed in order to see any histologic improvement and for maximum improvement 10% or more weight loss is required (Juluri et al., 2011). However losing weight in order to reach a healthier and beneficial weight is a challenge because there are many variables which

play a major role hence only 50% of the patients are able to lose weight (Le et al., 2012; Schwenzer et al., 2009). Although reduction of weight is considered to be an ideal strategy for treating NAFLD, a swift drop in weight can lead to worsening of the liver disease (Sanyal et al., 2004; Talwalkar, Yin, Glaser, Sanderson, & Ehman, 2011). Several studies have shown that losing weight has helped normalize metabolic disturbances and improved steatosis, fibrosis, necro-inflammatory changes and other liver functions (Lutchman et al., 2007). Aside from losing weight, a healthy diet and physical activity alone may also be beneficial in the treatment of NAFLD (Hossain et al., 2016).

## 3.2 Dietary Improvements

The development and progression of NAFLD is sometimes associated with the imbalance in the nutritional intake which is why the balance between proteins and carbohydrates along with saturated and unsaturated fats are closely monitored by doctors. According to several studies a diet deficient in polyunsaturated fatty acids (PUFA) may cause NAFLD. It is also noticed that NASH patients tend to have low levels of PUFA in their diet (Byrne, Olufadi, Bruce, Cagampang, & Ahmed, 2009). Fructose is another ingredient which is associated with steatosis; it is a commonly used sweetener (Abid et al., 2009; Ouyang et al., 2008). In a study conducted on 427 NAFLD patients, it was observed that there was a correlation between greater intake of fructose and an advance hepatic fibrosis stage. In patients above the age of 48, high fructose intake seemed to increase the rate of hepatic inflammation and ballooning (Abdelmalek et al., 2010). Aside from this, among other nutrients associated with NAFLD is protein. A diet deficient in protein and malnourishment has been considered to be a cause of NASH (Abdelmalek et al., 2010). In summary, a diet should consist of 15-20% protein of total energy intake, a carbohydrate intake of 40-50%, less than 30% fat intake. This should include less than

10% intake of saturated fatty acids of the total energy intake and consumption of large amounts of mono and polysaturated fatty acids. Aside from this consumption of other additives such as monosodium glutamate and fructose must be prohibited (Kargulewicz, Stankowiak-Kulpa, & Grzymisałwski, 2014).

Caloric restrictions along with fat and carbohydrate restrictions can help improve hepatic enzymes, hepatic steatosis and metabolic risk factors. There is no unanimity on a fixed amount of calorie intake limitation in order to reach a goal weight for effective response on NAFLD (Cusi et al., 2014). Caloric restriction may lead to histologic improvement because limiting the amount of calorie intake decreases the liver size and fat content which can reverse hepatic steatosis. Even though hypocaloric diet is considered to help treat NAFLD, the conducted studies on the effect of this diet and histological analysis done are not enough, hence the benefits of this approach cannot be guaranteed (M. C. Lewis et al., 2006; Sevastianova et al., 2012).

There are some studies which have evaluated the effect of losing weight through dietary restriction focusing on limiting carbohydrate and fat components. A study conducted on 170 obese people, randomly limited either fat or carbohydrate content in their diet along with calorie restriction in all the participants for 6 months. The study concluded that both groups appeared to have lost weight, intrahepatic lipid, visceral fat and body fat content and between the two groups no significant difference was seen (Haufe et al., 2011). A similar study conducted on 59 women also randomly placed them under either a low fat or low carbohydrate diet for 6 months. This study also concluded that there was no apparent difference between the two groups and both groups showed a decrease in weight and liver enzyme activity (Rodríguez-Hernández, Cervantes-Huerta, Rodríguez-Moran, & Guerrero-Romero, 2011). Based on these studies it can be assumed that hypocaloric diet along with fat or carbohydrate component restriction can help

improve liver enzymes and intra hepatic lipid content (Rodríguez-Hernández et al., 2011).

Aside from hypocaloric diet, other diets composed of balanced macronutrients have also been studied for their impact on weight loss, lipid profile and insulin resistance. A diet that outlines consumption of nuts, legumes, fruits, vegetables, whole grains and fish in large amounts and low consumption of red meat is known as a Mediterranean diet. The lipid content of this diet is mostly composed of monounsaturated fatty acids which are provided by extra virgin olive oil (EVOO). This diet is known to help decrease the risk of major cardiovascular events, mortality rate and metabolic syndrome (Trovato, Castrogiovanni, Malatino, & Musumeci, 2019). A study was conducted using 58 healthy individuals as control and 73 NAFLD patients in order to assess the correlation between NAFLD severity and Mediterranean diet. The study showed a negative correlation between the adherence to Mediterranean diet and insulin levels, stage of fibrosis, ALT, severity of steatosis and the possibility of having NASH (Kontogianni et al., 2014). Another study compared a diet similar to Mediterranean diet with another diet composed of 15-20% protein, 50-60% carbohydrate and less than 30% fat over a period of two years. The results of the study show that there was lower resistance to insulin in the Mediterranean style diet group hence we can assume that Mediterranean diet helps to control the metabolic risk factors associated with NAFLD and may assist in reversing steatosis (Esposito et al., 2004).

## 3.3 Only Exercise

Improvement in steatosis has been linked to physical activity, especially aerobic exercise and the reduction in metabolic risk factors is directly proportional to the intensity of physical activity. A study involving 23 adults suffering from obesity with a sedentary lifestyle were randomly given aerobic exercise (cycling) and regular stretching (considered to be the placebo group) for 4

weeks. According to the results of the study, compared to the placebo group, the aerobic exercise group showed notable decrease in mean visceral adipose tissue, hepatic triglyceride content and plasma free fatty acids (Clouston & Powell, 2004). In another study the effects of aerobic exercise was studied for a longer period (12 weeks). The similarity in the results of both the studies was that a decrease in visceral and hepatic lipids was observed without the reduction of weight. Aerobic exercise has also proven to have more impact than resistance training when it comes to decreasing visceral fat (Van Der Heijden et al., 2010).

Recent studies have come to the conclusion that both consistency and intensity of exercise is required to assist in reversing the histology of NASH patients. While analyzing 813 biopsy proven NAFLD patients based on their self-reported time spent in physical activity, the patients were divided into the activity groups – inactive, moderate or vigorous. In this histological analysis it was observed that neither moderate intensity nor complete consistency of exercise per week seemed to decrease the risk of having NASH rather vigorous exercise seemed to reduces the risk of contracting NASH and in the US guidelines as well it was stated the there is a correlation between reduced risk of liver fibrosis and vigorous exercise (Kistler et al., 2011).

## 3.4 Combination of Diet and Exercise

A combination of the two prior mentioned lifestyle changes has been evaluated in several studies and has proven to give better results. A potential study consisting of 261 biopsy proven NASH patients exhibited >5% reduction in weight related to decrease of NAS and a resolution of NASH was observed in 90% patients that had >10% reduction in weight due to a combination of average intensity exercise and hypocaloric diet (Bohte & Werven, 2011). In addition to this there was a distinct correlation between weight reduction and betterment of many metabolic risk

factors and liver enzyme activity (Hossain et al., 2016). In an unbiased trial of 56 patients inclusive of a control group, patients were randomly categorized into 4 groups, moderate exercise along with low fat diet, moderate exercise plus average fat/low carbohydrate diet, standard care (only physicians' advice) or only moderate exercise for 6 months. The patients underwent liver biopsy prior to the trial and after the trial. 41 patients completed the study of which 36 have borderline NASH. In conclusion to the trial it was observed that all groups showed visible reduction in NAS and a rise in aminotransferases and there seemed to be no difference between the different groups. However most of the patients did not appear to have any reduction in weight instead they gained weight and experienced histological improvement (Eckard et al., 2013). On the other hand a similar study was conducted on 1006 NAFLD patients, where the patients were randomly designated a modified lifestyle (combination of diet and exercise) or a control group for 12 months. The patients in the modified lifestyle group showed evident decrease in waistline, ALT level, BMI and other metabolic markers(Hossain et al., 2016). Therefore it can be concluded that the combination of diet and exercise is more beneficial than each of them on their own and a minimum of 5% to 10% weight reduction is necessary to impact liver histology, insulin resistance and serum transaminases.

## 3.5 Bariatric Surgery

Currently the most effective method of weight reduction in people with morbid obesity is bariatric surgery. Due to the strong association between NASH and obesity, it is expected that bariatric surgery will be beneficial to NASH to some extent. It is also a very cost-effective option for NASH patients with different fibrosis stage. In a French study conducted on 381 severely obese patients, histology and some metabolic markers were observed prior to bariatric surgery and 1 and 5 years after bariatric surgery. The result showed that improvement was seen in

ballooning, steatosis and over all NAS, there was also a reduction in the percentage of NASH patients after 5 years. However after 5 years some of the patients were experiencing worsening fibrosis, the reason for this was unclear but most of the patients had a high BMI (Mathurin et al., 2009). In another study gastric bypass was performed on 78 very obese patients and a liver biopsy was done prior to the surgery and after the surgery. In the prior biopsy the patients were seen to have steatosis and 44.8% of them had borderline fibrosis. In the biopsy after the bypass it was observed that there was a reduction in the number of NASH patients and the prevalence of hepatic fibrosis had decreased. Even though bariatric surgery has proven to be very beneficial the fact that some of the studies reported worsening of liver fibrosis can't be ignored (Moretto, Kupski, Da Silva, Padoin, & Mottin, 2012).

## 3.6 Weight loss medication

Among weight loss medications the two drugs which have been studied for the purpose of treating NAFLD are orlistat and sibutramine. The studies thus far have not provided convincing results. Orlistat works by preventing the absorption of free fatty acids in the pancreas by acting as an inhibitor. On the other hand a serotonin-norepinephrine reuptake inhibitor, sibutramine, helps prolong the feeling of stomach/gastric fullness (Neuschwander-tetri et al., 2014). An unbiased trial was conducted on 50 biopsy proven NASH patients where they were either provided with a 1400 Cal/day diet along with vitamin E 800 IU only or in combination with orlistat for 9 months. The decrease in necro-inflammation, ballooning, steatosis and NAS along with weight loss was observed in both groups (Harrison, Fecht, Brunt, & Neuschwander-Tetri, 2009; Neuschwander-tetri et al., 2014). The trial concluded that patient who successfully lost more than 5% weight showed better insulin sensitivity and decreased steatosis and the patients that were able to lose more than 9% weight showed enhanced histological improvements but

there was no apparent difference between the two groups in the context of histological and metabolic markers so the improvement was due to the weight loss rather than the medication. According to another study which was placebo controlled, unbiased and double-blinded, conducted on 52 NAFLD patients that were randomly given placebo or orlistat for 6 months showed that 50% improvement in steatosis occurred in both groups and no change occurred in the state of fibrosis and inflammation in either groups (Zelber-Sagi et al., 2006).

On the contrary, 11.4% nonfatal strokes and myocardial infarctions were linked to the use of sibutramine when compared to placebo in SCOUT trials (Sibutramine Cardiovascular Outcomes Trial) which took place in England and involved 10744 overweight patients hence the FDA extracted sibutramine from the US market. All the studies were also terminated due to the drug's side effects. Hence even though orlistat is not very effective in the treatment of fibrosis, it is used in the reduction of steatosis (Johansson, Neovius, Desantis, Rössner, & Neovius, 2009).

## 3.7 Probiotics

It is assumed that the proportion of intestinal bacteria may have an impact on the NASH pathogenesis because of increased permeability of the intestine, absorption of endotoxins and stimulation of inflammatory cytokines. Excessive bacterial growth is observed in 50% of NASH patients (Miele et al., 2009; Szabo, Bala, Petrasek, & Gattu, 2010). So probiotics are evaluated as an option in the treatment of NASH. However, a few studies have concluded that probiotics further deteriorate steatosis (Hossain et al., 2016). A placebo controlled double blinded trial including 30 biopsy diagnosed NAFLD patients was conducted and the patients were randomly administered *Lactobacillus bulgaricus* and *Streptococcus thermophiles* or placebo. The group receiving the treatment experienced a considerable change in liver enzymes. Another study

involving 66 patients randomly assigned *Bifidobacterium longum* with *fructooligosaccharides* and lifestyle changes or life style changes alone for 24 weeks. The treatment group showed a decrease in CRP, AST, HOMA-IR, TNF-alpha, NAS and steatosis (Hossain et al., 2016).

## 3.8 Anti-lipidemic Agents

### 3.8.1 Fibrates

The function of these drugs is to inhibit apolipoprotein (proteins that bind lipid soluble substances to form lipoprotein) and initiate PPAR alpha and lipoprotein lipase. There is very little information on the advantages of using fibrates to treat NAFLD. In a study on 40 biopsy proven NASH patients that were administered 200 mg fenofibrate per day for 48 weeks, it was observed that there was a reduction in the markers of NAFLD; gammaglutamyl transpeptidase, alkaline phosphatase and triglycerides and an improvement in the grade of ballooning degeneration but no significant change was seen in transaminases, hepatic steatosis, insulin resistance, necro-inflammation or fibrosis (Fernández-Miranda et al., 2008). According to another trial using 46 NASH patients consuming either 600 mg/day of gemfibrozil or placebo, it was observed that the gemfibrozil group showed considerably less liver enzymes and no histological changes (Basaranoglu, Acbay, & Sonsuz, 1999). According to another study on fenofibrate, the results showed an increase in death rate from cardiovascular disease in comparison to the placebo group. Hence there is an inconsistency in the results of different studies on the therapeutic value of fenofibrate to treat NAFLD so further research needs to be conducted on fenofibrates to be able to properly evaluate its effectiveness and assess its risk factors.

### 3.8.2 Niacin

It has been proposed that niacin helps in the prevention of steatosis in experimental mice models, but according to a double-blinded unbiased trial including 27 obese NAFLD patients it was seen that niacin was unable to reduce the intrahepatic triglycerides (Fabbrini et al., 2010). In addition to this, another data showed that niacin has hepatotoxic side effects starting from mild increase in aminotransferase levels to complete hepatic failure (Hossain et al., 2016). Therefore it is currently not being used to for the treatment NAFLD as further evaluation is required on its risk factors.

### 3.8.3 Statins

For NAFLD patients suffering from hyperlipidemia, lipid lowering drugs such as statins, niacin, fibric acid derivatives, ezetimibe and n-3 PUFA have been considered as a treatment option. Dyslipidemia contributes to metabolic syndrome and cardiovascular diseases which are risk factors of NAFLD (Chatrath, Vuppalanchi, & Chalasani, 2012). Even though several studies involving statins and NAFLD have been conducted, majority of them did not include histological evaluation (Heatth, 2004). Due to statin's anti-inflammatory, antioxidant and anti-fibrinogenic properties it is considered to be beneficial for NAFLD (Heatth, 2004). In a cross-sectional study conducted in 2014, the data analysis of 2578 patients who had a liver ultrasound showed that hepatic steatosis was present in 35.3% of them among which 990 subjects were currently using statins or had used statins in the past. Patients that had been using statin for over 2 years and had a BMI greater than 27.5 $kg/m^2$ were seen to have three times lower prevalence of steatosis(de Keyser et al., 2014).

An inconsistency was seen in the results of studies conducted on the efficacy of simvastatin, while one study showed improvement in ALT levels, the other did not have any histological improvements (Abel, Fehér, Dinya, Eldin, & Kovács, 2009). A trial that was placebo-controlled, multicenter, double-blinded and randomized involved 326 patients suffering from chronic liver disease and hyperlipidemia (hepatitis C – 117 and NAFLD – 209). The subjects were randomly given placebo or 80 mg pravastatin for 36 weeks. Pravastatin was seen to decrease the lipid panel without changing the ALT levels compared to the placebo group (J. H. Lewis et al., 2007). Studies conducted using atorvastatin showed considerable reduction in steatosis and ALT and lipid levels along with slowing the progression of NAFL to NASH (Athyros et al., 2006). In another trial conducted on patients with ultra-sonographic proof of hyperlipidemia or NAFLD, 62 patients were prescribed 200 mg a day fenofibrate, sixty three patients were prescribed 20 mg a day of atorvastatin and 61 patients were prescribed a combination of fenofibrate and atorvastatin. The combination and atorvastatin alone group showed greater degree for biochemical improvement and regression in the ultra-sonogram of NAFLD patients. Hence majority of the studies show improvement of transaminase levels and some show the possibility of slowing down progression of NAFL to NASH and reversing of steatosis. Therefore, it can be assumed that statins help improve the condition of NAFLD and hyperlipidemia patients (Athyros et al., 2006).

### 3.8.4 PUFA (Polyunsaturated Fatty Acids)

Omega – 3 is a fatty acid which belongs to this group. It has been thoroughly studied as a therapeutic option in the treatment of NAFLD. Some studies have concluded that reduction in levels of hepatic PUFA is related to NAFLD. For a study on 144 NAFLD patients with hyperlipidemia, the participants were either assigned AHA (American Heart Association) diet

with PUFA or placebo along with AHA diet for 24 weeks(Zhu, Liu, Chen, Huang, & Zhang, 2008). The results of the study showed that 19.7% patients had a total lapse in steatosis and 53.03% of the patients showed overall decrease in steatosis as well as higher ALT levels in the PUFA group compared to the placebo group (Zhu et al., 2008).

Another double-blinded and placebo-controlled study was carried out on 37 biopsy proven NASH patients with diabetes where the patients were randomly prescribed placebo or PUFA for 48 weeks (S. et al., 2015). Liver biopsy was performed before and after the study. The study concluded that a significant improvement was observed in NAS and hepatic steatosis in the placebo group but deterioration in the lobular inflammation was seen, whereas in the PUFA group a deterioration of insulin resistance was seen and no histological change was experienced (S. et al., 2015). Hence PUFA is not a plausible option for the treatment of NASH patients with diabetes. In another similar trial conducted on 34 patients with NASH, placebo or n-3 fish oil 3000mg / day was randomly assigned to the patients for a year. The results of the study showed no significant difference between the two groups. 4 patients from the treatment group and 3 patients from the placebo group experienced a reduction in NAS by 2 points (Argo et al., 2015). PUFA needs to be evaluated further in order to consider it as a therapeutic option for the treatment of NAFLD.

### 3.8.5 Neimann-Pick C1-Like 1 (NPC1L1) Inhibitor

Several studies have proved that ezetimibe improves the histological markers and biochemical parameters of NAFLD. However most of the studies conducted on ezetimibe did not have a control group or the sample sizes were small. 32, biopsy proven, NAFLD patients were either assigned placebo or 10 mg/day of ezetimibe for 6 months in a recent study. The ezetimibe group

experienced a considerable improvement in the fibrosis stage and the ballooning score in comparison to the control group but it had no effect on inflammation or steatosis (Takeshita et al., 2014). The study also shows a correlation between ezetimibe and increased hepatic long chain fatty acids, insulin resistance, HbA1c and oxidative stress which contribute in the progression of NAFLD.

## 3.9 Antidiabetic Medications

### 3.9.1 Metformin

This drug decreases the hepatic gluconeogenesis (the metabolic pathway which helps generate glucose) and fatty acid oxidation, improves the peripheral and hepatic insulin sensitivity, reduces the absorption of glucose in the intestine and lowers serum lipid concentration, all these in turn increases insulin resistance (Viollet et al., 2011). According to current studies performed on animals, it is observed that metformin possibly assists in hepatocellular carcinoma prevention (Bhalla et al., 2012; Zheng et al., 2013). In several studies the effect of metformin on NAFLD patients has been evaluated but the results have not been promising. In some studies metformin has increased ALT levels and improved the liver histology possibly due to its ability to reduce weight, increase insulin sensitivity and help improve other metabolic syndrome markers.

In a study conducted on 110 NAFLD patients with diabetes for 12 months consisted of three groups- vitamin E, prescribed diet and metformin. According to the results the metformin treatment appeared to be more effective in increasing aminotransferase levels compared to the other two groups (Bugianesi et al., 2005). Improvement in the hepatic steatosis and inflammation was also observed during the histological analysis. However other similar trials have not been able to show similar advantageous impacts of metformin on the liver histology. Since most

studies show that metformin has no added benefits other than its assistance in reducing weight and increasing insulin sensitivity, it is not prescribed as a first line of treatment of NAFLD but since it has shown some potential in the treatment of NAFLD, further research should be done.

### 3.9.2 Thiazolidinediones (TZD)

These drugs assist in enhancing hepatic fatty acid oxidation by acting as a peroxisomal proliferator activated receptor (PPAR)-gamma-agonist. This in turn enhances hepatic lipogenesis and insulin sensitivity (Chiarelli & Di Marzio, 2008). According to several studies a marked difference in the biochemical efficacy was observed in NAFLD patients who used TZDs. Some of the studies portrayed improvement in the histology like reduced inflammation and steatosis. However the impacts of TZDs are not irreversible, in some studies it was noticed that suspending the use of TZDs led to the reappearance of NASH. More importantly several side effects have been associated with TZD use such as heart failure, edema, osteoporosis and increase in weight which have to be considered (Lutchman et al., 2007).

The most commonly studied TZDs are rosiglitazone and pioglitazone. Sixty-three, biopsy proven, NASH patients were enlisted for a trial called FLIRT (fatty liver improvement with rosiglitazone therapy). Here the patients received rosiglitazone or placebo for the duration of 1 year. The drug seemed to help improve steatosis, enhance ALT levels and insulin sensitivity and adiponectin despite of causing an increase in weight gain. However NAS and fibrosis were unaffected (Ratziu et al., 2008). In a study of 137 biopsy proven NASH patients, either rosiglitazone was administered alone or in combination with metformin or in combination with losartan randomly for 48 weeks. All the 3 groups experienced a decrease in steatosis, fibrosis, ballooning degeneration, inflammation and also decrease in ALT levels but the difference

between the groups was not significant (Torres et al., 2011).

Upon extensive studying pioglitazone appeared to be more effective on the liver enzymes and histological changes (Belfort et al., 2006). 55 glucose intolerant/diabetic biopsy proven patients were enlisted in a study conducted by Belfort et al. Here the patients were provided a hypocaloric diet along with pioglitazone or a hypocaloric diet along with a placebo for 6 months. The group which was using pioglitazone experienced a considerable improvement in their insulin sensitivity, ALT levels, steatosis, ballooning and inflammation. However there was no apparent decrease in fibrosis in either of the groups (Belfort et al., 2006). A trial was carried out on a large group of nondiabetic patients with NASH called the PIVENS trial. The trial was conducted on 247 patients and was a multicenter, double-blinded, random and placebo-controlled. The participants were divided into 3 groups; one group was assigned to take pioglitazone 30mg daily, another group was assigned to take vitamin E 800IU daily and the 3rd group was assigned placebo. The initial end-point of the trial was to reduce ballooning by 1 point and reduce NAS by 2 points which the pioglitazone group failed to achieve. The vitamin E group on the other hand appeared to have a greater percentage of patients with improved NASH condition however both the groups experienced an enhancement in ALT levels, decrease in steatosis and lobular inflammation and no effect on fibrosis (Sanyal et al., 2004). Even though most studies show that there is no effect on fibrosis, thiazolidinediones may be able to retard the advances of fibrosis in NASH patients. Hence the overall data suggests that thiazolidinediones can assist in treatment of NASH by improving the histological markers and biomarkers like inflammation, steatosis, ballooning and retardation of fibrosis. Use of TZDs have drawbacks as well such as cardiovascular diseases and the impacts of TZDs are reversible once the medication is stopped so it should be used with caution (Hossain et al., 2016).

### 3.9.3 Other Antidiabetic Agents

Meglitinides and incretin mimetics (Glucagon-like peptide-1 analogs) like liraglutide and taspoglutide have been studied to evaluate their impact on fatty liver. The information on the use of meglitinide to treat NAFLD is very little. Meglitinide is used to stimulate the release of insulin in the pancreas and enhance the growth of pancreatic beta cells. Even though some of the studies provided evidence of improved histology and metabolic markers, the sample sizes used for the studies were very small (Cuthbertson et al., 2012; Kenny et al., 2010).

A GLP-1 agonist called liraglutide is used for the treatment of obesity and type 2 diabetes. Glucagon-like peptide-1 (GLP-1), an incretin, is released from the L cells of the intestine and causes central, endocrine and gastrointestinal effects. Liraglutide also helps in weight loss by prolonging gastric emptying and decreasing the appetite (Cuthbertson et al., 2012; Kenny et al., 2010). In a study conducted on 52 over weight participants with NASH were given liraglutide or placebo randomly for 48 weeks. The results showed that there was no effect or advances in fibrosis for duration of the study and an improvement in NASH in 9 of the 23 patients that were administered liraglutide and 2 of the patients that used placebo (Armstrong et al., 2016). Even though it was concluded that liraglutide has no effect on fibrosis, it deliberated that liraglutide may be able to retard the advances of fibrosis (Nascimbeni et al., 2013).

## 3.10 Antioxidants

### 3.10.1 Vitamin E

Vitamin E appears to have the ability to improve some of the histological markers such as transpeptidase, alkaline phosphatase and triglycerides and aminotransferases in NASH patients.

A few studies have also come to the conclusion that vitamin E may also be responsible for the complete removal of hepatic steatosis, but there is an inconsistency in the results of the studies evaluating the effect of vitamin E on liver fibrosis. PIVENS trial (elaborated in the pioglitazone section) revealed that in comparison to the placebo group, vitamin E was able to exhibit a greater extent of improvement in necro-inflammation (43% versus 19%) but had no effect in fibrosis. Hence vitamin E can be used to treat diabetic patients, however it should be prescribed with caution since it is known to increase the risk of cardiovascular mortality and prostate cancer (Sanyal et al., 2004).

In several combination therapies the impact of vitamin E on NAFLD has been studied. A placebo controlled double blinded study consisted of 45 NASH patients and the patients were randomly assigned vitamin E 1000 IU along with vitamin C 1000 mg or placebo for duration of 6 months (Harrison, Torgerson, Hayashi, Ward, & Schenker, 2003). According to the results, the treatment group showed a considerable reduction in fibrosis but had no effect on ALT or necro-inflammation. Similar results were obtained in a combination therapy study conducted on 48 NASH patients (Dufour et al., 2006). The patients were either prescribed ursodeoxycholic (UDCA) and vitamin E or just UDCA or placebo for 2 years. The combination group expressed greater improvement in steatosis and serum aminotransferases compared to the other two groups. Hence vitamin E has a positive impact on non-diabetic NASH patients but when used in combination with other agents like insulin sensitizing agents, anti-inflammatory agents and anti-oxidants it may have greater beneficial effects (Dufour et al., 2006).

### 3.10.2 Other Antioxidants

A factor that may contribute to the advancement of steatosis is change in metabolism of folate/methionine as it helps improve the histological markers of NAFLD. Anti-apoptotic, cytoprotective, antisteatogenic activity and anti-TNF (anti-tumor necrosis factor) alpha activity are the properties expressed by some nutritional supplements like betanin and S-adenosyl methionine (SAM), they may also be able to reverse insulin resistance (Abdelmalek, Angulo, Jorgensen, Sylvestre, & Lindor, 2001; Kathirvel et al., 2010). NAC (N-acetyl cysteine), a glutathinonine precursor that helps elevate glutathionine level in hepatocytes and restricts oxygen species that are reactive and cause hepatocellular injury. Majority of the studies conducted on antioxidants either have a small sample size or the duration of the study was not long enough. More large scale studies should be conducted in order to evaluate the impacts of antioxidants on NAFL/NASH.

### 3.10.3 Antiapoptotic and Cytoprotective Agents

UDCA (ursodeoxycholic acid) helps improve hepatobiliary diseases due to its cytoprotective, immunomodulatory and antiapoptotic characteristics (Lazaridis, Gores, & Lindor, 2001). There has been an inconsistency in the results of the studies done to see the effect of UDCA on NAFLD, where some studies show beneficial effects on ALT and other histological factors while other trials have not shown any significant difference in comparison to placebo. According to a 2013 review of 12 unbiased clinical trials, treatment with only UDCA had improved steatosis, fibrosis and liver enzymes in a few studies whereas a combination therapy of UDCA with other drugs has proven to given better results in steatosis and inflammation (Xiang et al., 2013). The variation in the outcome of the results of the studies may have been due to the use of different

doses for different studies. Either way more studies need to be conducted to reach a solid conclusion about the effects of UDCA.

### 3.10.4 Agents with anti-inflammatory properties

Pentoxifylline is a xanthine derivative which prevents TNF-alpha which helps increase anti-inflammatory activity in the body. TNF-alpha is a proinflammatory cytokine which can cause lipid peroxidation which in turn causes activation of reactive oxygen species and initiates fibrogenesis, necroinflammation, apoptosis and hepatic insulin resistance. A placebo-controlled study was conducted on 49 NASH patients where they were either prescribed pentoxyfylline or placebo randomly (Zein et al., 2011). The subjects in the treatment group either experienced an improvement in their NASH or no change. The treatment group showed considerable improvement in inflammation, steatosis, ALT and fibrosis but ballooning was unaffected. 25% of the patients that received the treatment experienced a reduction in their NASH at the end of the study (L.B. et al., 2011). On the other hand in another study involving 30 biopsy proven NASH patients did not appear to have any considerable change in their histology and aminotransferases compared to placebo but an overall improvement was observed in both groups (L.B. et al., 2011). In a meta-analysis pentoxifylline appeared to decrease the BMI, steatosis, lobular inflammation, fasting glucose, fibrosis and aminotransferases, however further evaluation of pentoxifylline is required to confirm its effects (Zeng, Zhang, Zhao, & Xie, 2014).

## 3.11 Other Therapeutic Agents

### 3.11.1 Vitamin D

Currently the amount of data available in the evaluation of the effect of vitamin D supplementation on NAFLD is not enough, even though several studies have associated progression of NAFLD to deficiency of vitamin D (M. Eliades et al., 2013; Myrto Eliades & Spyrou, 2015). A study involving 60 biopsy proven NAFLD patients and 60 healthy individuals as control showed that majority of the NAFLD patients had low levels of vitamin D in comparison to the healthy individuals from the control group. The low level of vitamin D was also associated with an increase in histological severity of necro-inflammation, steatosis and fibrosis independent of other variables (Targher et al., 2007). In a placebo controlled, randomized, double-blinded trial involving 53 patients suffering from NAFLD was prescribed 50,000 IU vitamin D every two weeks or a placebo for 4 months. The results showed no change in aminotransferases or TNF-alpha levels or in insulin resistance. Hence the effectiveness of vitamin D in treating NAFLD cannot be fully determined yet (Hossain et al., 2016).

### 3.11.2 Phlebotomy of Ferritin

Phlebotomy of ferritin is the removal of iron in blood. This method is of interest in NAFLD due to the correlation seen between high levels of iron and patients encountering NAFLD. Despite this correlation, the studies that have been done on the role of iron in NAFLD show conflicting results. One study showed that NAFLD patients had high levels of iron in the body and greater hemoglobin level (Bai, 2015). In another study conducted on 1201 biopsy proven NAFLD patients, the data showed that high ferritin levels can be related to fibrosis and steatosis but it is not a good predictor of fibrosis (Yoneda et al., 2014). A multi-centered study involving 1014

NALD patients also had similar results (Angulo et al., 2014). A phase two clinical trial which consisted of 31 biopsy proven NAFLD patients, all the participants did phlebotomy to attain ferritin level ≤50 $\mu$g/liter. Six months after the phlebotomy a biopsy was done, where it was observed that there was a correlation between iron reduction and the overall NAS even though no significant improvement was observed in the histological features of inflammation, steatosis, ballooning or fibrosis(Beaton et al., 2013). Hence it can be concluded that phlebotomy of ferritin has potential in the treat of NAFLD but there isn't sufficient evidence to be considered a therapeutic option.

### 3.11.3 Herbal Medication

Recently herbal therapy has been as a treatment for NAFLD. Some animal models have projected positive effects of improving markers of inflammation, transaminases, metabolic factors and histological markers like steatosis. However prior to being considered for NAFLD treatment, there arises a need for further investigation to find adverse effects and a need for cautious use of herbal supplements.

Milk thistles also called *Silybum marianum* or silymarin, have antifibrogenic, anti-inflammatory, and antioxidant activity along with the ability to help with liver cell regeneration (Schrieber et al., 1909; Trappoliere et al., 2009). A double-blinded, randomized, controlled trial showed significant improvement in steatosis through the use of silybin (key active constituent of silymarin) in combination with vitamin E and phospholipids (Hossain et al., 2016). Another double-blinded, multicenter clinical trial consisting of 138 patients were randomly given either phosphatidylcholine and silybin or a placebo for a duration of 12 months. Drastic improvement in liver histology, enzymes and HOMA-IR was seen in patients receiving the combination

treatment (Loguercio et al., 2012). Silybin as a combination treatment seems to have potential therapeutic ability, however more studies need to be conducted to assess its beneficial effect on NAFLD/NASH.

## 3.12 Agents in Phase 3 Development

### 3.12.1 Obeticholic Acid

It is a very strong farnesoid X receptor (FXR) agonist. FXR is a bile acid nuclear receptor which is frequently seen in kidney, liver, intestine and adipose tissues. FXR's basic role is to metabolize bile, prevent the expression of sterol regulatory element-binding protein 1c (SREBP-1c) in the liver and decrease hepatic lipogenesis (Xiong et al., 2014). It also regulates several lipoproteins and enhances the removal of triglycerides from the liver.

In a study called FLINT ( Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis) the results initially showed a reduction in the NAFLD score by 2 points and no deterioration of fibrosis was observed in the duration of the 72 weeks in 45% of the participants that were prescribed obeticholic acid and in 21% of the patients taking placebo. 35% of the treatment group expressed improvement in fibrosis and 19% of the placebo group showed improvement (Neuschwander-tetri et al., 2014). However it is observed that obeticholic acid causes a rise in the LDL levels and decrease in the HDL levels and it is also linked to pruritus (Neuschwander-tetri et al., 2014). Given that cardiovascular diseases and dyslipidemia are frequently seen in NASH patients, the safety associated with using obeticholic acid should be evaluated. Presently obeticholic acid is being used for the primary treatment of biliary cholangitis and is being studied in phase 3 REGENERATE study (Nevens et al., 2016).

Due to the presence of FXR in several tissues, the use of organ specific FXR has ben of interest recently. Lately GS-9674, a non-steroidal intestine selective FXR agonist, has been tested on cynomolgus monkeys consuming high cholesterol and fat diet to study its effects. GS-9674 does not decrease the overall cholesterol level or hinder with the LDL and HDL levels and serum apolipoprotein B like obeticholic acid (Adorini, Pruzanski, & Shapiro, 2012). The effect of GS-9674 on NASH is yet to be seen.

### 3.12.2 Elafibranor

Elafibranor is a peroxisome proliferator-activated receptors alpha and delta agonist. Activation of these nuclear receptors has given beneficial metabolic and anti-inflammatory effects in the pre-clinical studies. In a study called GOLDEN – 505, reduction in NASH was seen in 19% of the participants that were prescribed elafibranor 120 mg/day for a year and 12% lapse was seen in the placebo group. Elafibranor also helps increase insulin sensitivity and improve lipid profile (Ratziu et al., 2016).

### 3.12.3 Cenicriviroc

Cenicriviroc acts as a dual antagonist for type 2 and 5 chemokine receptors found on the surface of the cytokine. These receptors are present on the Kupffer cells and hepatic stellate cells of the liver. According to the preclinical studies cenicriviroc has shown anti-inflammatory and anti - fibrotic activity (Lefebvre et al., 2016; Puengel et al., 2016). According to the CENTAUR study, cenicriviroc was unable to reach the target outcome of decreasing activity of NAFLD by 2 points along with no deterioration of fibrosis in phase 2b (Ratziu et al., 2012). However, the main secondary outcome of reducing liver fibrosis without affecting NASH was achieved by cenicriviroc in 20% of the participants from the treatment group and 10% reduction was seen in

the placebo group. It also decreased the serum levels of interleukin-6, C-reactive protein and fibrinogen which indicates that its effects on inflammation cannot be judged completely on the basis of crude histology, hence phase 3 trials will be done due to its potential of improving inflammation (Friedman et al., 2018).

### 3.12.4 GS-4997

GS-4997 acts as an inhibitor for apoptosis signal-regulating kinase 1 (ASK1) which beneficial in case of NAFLD patients as NAFL slowly leads to liver cell apoptosis. ASK1 belongs to the mitogen-activated kinase family and is associated with endoplasmic reticulum stress and apoptosis. After 24 weeks of treatment in a phase 2 study, GS-4997 decreased liver fibrosis in NASH patients. These results were replicated consistently while measuring the liver stiffness by magnetic resonance elastography and while using hepatic collagen by morphometry. Hence GS-4497 is being further studied in phase 3(Rédei, 2008).

In the table below a summary of all the proposed treatments and its effectiveness in treating NAFLD is given.

*Table 5: Summary for treatment options of NAFL/NASH(Hossain et al., 2016)*

| Treatment | Medication/Mediation | Effectiveness |
|---|---|---|
| Lifestyle Changes | Weight loss | For histological and biochemical improvements a reduction of 5-10% in weight is required |
| | Hypocaloric diet only | Decrease of fat and carbohydrate consumption along with exercise is more effective |
| | Constituents of the diet | An improvement is seen in the histological markers such as ALT and |

| | | |
|---|---|---|
| | | insulin levels when Mediterranean diet is followed.<br>A diet should also consist of PUFA and less saturated and trans fatty acids |
| | Only exercise | Effective with and without weight loss |
| | Combination of diet and exercise | Most effective |
| | Bariatric surgery | Maybe effective in reversing NASH; however deterioration of fibrosis may occur in some cases |
| | Sibutramine | It has been removed from the market by FDA due to its side effects |
| | Orlistat | Not enough proof to consider it effective |
| Probiotic | | Anti-inflammatory markers, liver enzymes and insulin resistance can be improved with the use of probiotics |
| Antilipidemic agents | Fibrates | Suspected to increase mortality risk in patients of NAFLD |
| | Niacin | Could be hepatoxic |
| | Statins | May help delay the progression of NAFL to NASH and reduce transaminases; NAFL and NASH patients can safely use it |
| | n-3 polyunsaturated fatty acids | According to some studies it could assist in reversing NAFLD |
| | NPC1L1 inhibitor | Not enough proof to consider it effective |
| Antidiabetic drugs | Metformin | Does not show any significant biological or histological improvements |

| | | |
|---|---|---|
| | Thiazolidinediones | Helps improve biomarkers, insulin resistance, inflammation and steatosis in NASH patients; it is associated with cardiovascular risks |
| | Other antidiabetic drugs | Phase 2 trials of GLP-1 analogues showed histological improvements |
| Antioxidants | Vitamin E | Has shown improvement in histological markers of non-diabetic NASH patients; associated with cardiovascular risks |
| | Other antioxidants | Not enough proof to consider them effective |
| Cytoprotective and antiapoptotic agents | Ursodeoxycholic acid | Improvement of liver enzymes and histology may be seen but more research needs to be conducted |
| Anti-inflammatory agents | Pentoxifylline | Assumed to improve histology and liver enzymes |
| Other therapeutic strategies | Vitamin D | No improvement seen upon replacement |
| | Phlebotomy of ferritin | Not enough proof to consider it effective |
| | Herbal medicines | Milk thistle is considered to improve histology and liver enzymes; more effective when used in combination with other agents such as phospholipids and vitamin E |
| Agents in phase 3 development | Obeticholic acid | Bile acid receptor (FXR); improves insulin resistance and aminotransferases; may worsen lipid panel |
| | Elafibranor | A PPAR alpha/delta agonist; may lead |

| | | |
|---|---|---|
| | | to resolution of NASH and help improve metabolic risk factors |
| | Cenicriviroc | Chemokine receptor agonist; assumed to improve c-reactive proteins and interleukin-6 serum levels and decrease fibrosis |
| | GS-4997 | ASK1 inhibitor; helps prevent liver cell apoptosis |

ASK1: apoptosis signal-regulating kinase 1; FXR: farnesoid receptor; FDA: Food and Drug Administration; NAFL: non-alcoholic fatty liver; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NPC1L1: Niemann-Pick C1-like 1; ALT: alanine amino transferase.

# Chapter 4

# Conclusion

This review inspected all the available methods for the diagnosis of NAFLD and NASH. It appears that even though biopsy is an invasive method, till date it is the most accurate and cost-effective method of diagnosis. Other diagnosis methods such as MR elastography (MRE) has shown great potential in successfully diagnosing NAFLD and it is also non-invasive. However MRE is not cost-effective method yet. The review also explored the most recent treatment options available for NAFLD/NASH including pharmaceutical, dietary, nutritional and herbal approaches. Thus far lifestyle modification, i.e., reduction in weight with a proper prescribed diet along with exercise appears to be the first-line of treatment for NAFLD as it has shown the most effective results. In terms of diet, Mediterranean diet appeared to be very effective in losing weight and when paired with regular exercise, it has been very effective in treating NAFLD. Even though bariatric surgery is very invasive, it is currently the most cost-effective and guaranteed way of losing weight which in turn helps treat NAFLD. Some of the pharmacological agents like vitamin E and pioglitazone have shown great potential in treating NASH. However several pharmacological drugs have shown adverse effects such as cardiovascular diseases. Many agents are currently in phase 2 and 3 trials that have expressed great potential as future therapeutic agents for the treatment of NAFLD.

# Chapter 5

# Future Directions

Non-invasive biomarkers are considered to be more accurate in differentiating between the different degrees of fibrosis and helps predict whether NAFL will progress into NASH in a patient. In addition to that it is also a cheaper and easily accessible method of diagnosis hence further research should be carried out to find more non-invasive biomarkers in order to make it the standard method of diagnosis.

Therapeutic agents such as orlistat, NPC1L1 inhibitor, PUFA, Vitamin E, GS-4997 etc., have shown potential in treating NAFLD but lack sufficient data to properly evaluate its effectiveness and its risk factors. Hence more clinical trials and studies can be carried out of the proper evaluation of these treatment strategies.

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