Document heading doi: 10.21276/apjhs.2019.6.1.26 Research Article

# Non-alcoholic fatty liver disease progression and current research

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**Received: 08-01-2019 / Revised: 28-02-2019 / Accepted: 18-03-2019**

**Abstract**

Non-alcoholic fatty liver disease (NAFLD) is clearly shown hepatic metabolic syndrome. It represents histopathological abnormalities which range from simple steatosis to non-alcoholic steatohepatitis (NASH) which eventually leads to fibrosis then cirrhosis and hepatocellular carcinoma. The buildup of toxic free fatty acids and mobilization of free fatty acid from adipose tissue and *de novo* hepatic fatty acid synthesis from glucose is denoted at the "first-hit"for NAFLD development. NAFLD progression leads to 'parallel, multiple fit' injuries such as oxidative-stress-induced mitochondrial dysfunction, ER stress, TLR-4 dependent release of inflammatory cytokines, these deleterious factors trigger the number of the signalling cascade that leads to inflammation, cell death& fibrosis, these are the hallmarks of NASH. It is such a chronic liver disease that leads to liver cirrhosis,liver cancer & ultimate death. This review identifies NAFLD progression and development screening method allows clinicians to isolate high-risk NAFLD patients that require early intensive intervention.

**Keywords:** NAFLD, NASH, HCC (hepatocellular carcinoma), FFAs, hepatocytes, ChREBPs, SREBP-1c.

## Introduction

Long chain fatty acids are a major source of energy and a necessary component of membrane lipids. They can be derived from food and synthesized from acetyl Co-A through a complex set of reactions, including glycolysis and TCA cycle, which together lead to the formation of backbone carbon of fatty acid and alongwith glycerols for the synthesis of lipids [1].The synthesis of fatty acid by FAS (Fatty Acid Synthase) requires NADPH, acetyl Co-A and malonyl Co-A. Malonyl Co-A is the carbon donor and help in the *denovo* synthesis of fatty acid and plays an important role as an inhibitor of CPTI (Carnitine/palmitoyl shuttle system) for fatty acid oxidation [2].

Survival of animals requires the availability of food Sources, in nature, the quality and quantity are highly variable. Once the animal locates the right meal, they eat as much as possible because they do not know when they will have the next, and survive.

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As animals consume the meals of carbohydrates, proteins and fats, through digestion, absorption and assimilation glucose, amino acid and fatty acid respectively are generated. Their metabolites provide the substrate for immediate energy needs in the form of glycogen, muscle protein and fats. When food is not available these stored glycogens, muscle proteins and fats get mobilized, degraded and consumed within a few days. Fat stores provide energy for longer times, in human 60 days, in polar bears for 6 months and in migratory birds for flying thousands of miles.For animals to exist for such a longer period of starvation, they needed synthesis and saving of the acquired fat and this special mechanism involve ACC1, ACC2 and CPTI.

Non-alcoholic fatty liver disease (NAFLD) is a different type of liver disease which is not affected by alcohol intake. It is caused by the high level of accumulated fat in the liver. Our normal liver is about 5-10% fat. It is defined as a clinicopathological syndrome which cannot be explained by excessive alcohol consumption. It was unidentified until the 1980s, but now it is recognized as the most common chronic liver disease in most parts of the world and western countries are in the first position. The disease increased its prevalence worldwide ranges from 6 to 35% in the general population from different countries. This high variability is due to differences in patients selection, diagnostic methods and dietary/lifestyle

customs. In the last 20 years, NAFLD among chronic liver disease increased from 47 to 75%.This high prevalence leads to NAFLD to become the leading cause of end-stage liver disease, liver transplantation and HCC(hepatocellular carcinoma) over the next decade. A reason for the high prevalence of NAFLD is that it is strongly linked to obesity, which is the global epidemic mainly associated with hypercaloric diet and low physical activity. Non-alcoholic fatty liver disease is the main hepatic manifestation of metabolic syndrome. It represents wide histopathological abnormalities ranging from simple steatosis to non-alcoholic steatohepatitis (NASH)[3].The progression of the disease is explained two decades ago by Day et al. by the "two-hit hypothesis".

The 'first hit' is by the lipid accumulation in the hepatocyte, a trait in which fat intake and IR (Insulin Resistance) play a key role. This condition enhances the vulnerability of the liver for damaging factors and this gives rise to 'second hit', for example, oxidative stress(OS),increased susceptibility to the disease, release of pro-inflammatory cytokines cause activation of inflammatory pathways by kupffer cells, adipokines from adipocyte, dysregulated hepatocyte apoptosis activation of hepatic stellate cells (HSC). This all constitutes the 'second hit' which when acts separately leading to macrovesicular steatosis to NASH [3].This progression leads to liver injury which associates hepatocyte apoptosis, liver inflammation, fibrogenesis, cirrhotic remodelling with liver failure and finally hepatocarcinogenesis.

**Physiopathological and Histological Mechanism of NAFLD**

NAFLD has a wide histological spectrum, in this pure triglyceride accumulate in droplets in the hepatocytes. If the inflammation occurs with or without fibrosis then it reaches to its next stage, a disorder referred to as NASH, this may eventually evolve to cirrhosis and hepatocellular carcinoma. The patients with early stage of NASH may progress to cirrhosis over a period of 5-10 years [4],and some may develop HCC. This is the chance that HCC may arise without cirrhosis[5, 6].

By the time after second hit theory, the concept has evolved and the progression of the disease explained more perfectly by a 'parallel multiple hit theory'. According to this theory, IR is an independent risk factor for NAFLD, it would be the 'first hit' that triggers the disease which leads to hepatocellular elevation of free fatty acids (FFAs),this molecule considered as the main pathogenic factor that makes the liver vulnerable for the above stated further hits. According to this model NASH as a condition which is preceded by IR (from a pathophysiological point of view) and by simple, macrovesicular steatosis (from a histological point of view).

Now,a more recent 'distinct-hit theory' has emerged which states the NASH and pure fatty liver are two independent twin conditions that are caused by IR, with steatosis in NASH is the beginning of epiphenomenon rather than a causal factor for inflammation and fibrosis, these are the two hallmarks for NASH[7].

**Metabolic basis of pathogenesis: NAFLD**

Dietary fat is the primary supply of adipocyte FFAs which goes to the liver and re-esterified there [8]. IR, lipolysis of peripheral fat which mobilizes to the liver in the form FFA and exacerbated hepatic lipogenesis, is the most important factor involved in hepatic FFA accumulation.[8],[9]. In NAFLD patients 80%of the total circulating FFA pool contributed by circulating FFA derived from dysregulated lipolysis in adipose tissue. Most of the hepatic fat comes from this FFA pool (59%)and other fraction comes from *de novo* lipogenesis (26%)and the remaining 15 % from the diet [10].

Anyone who is obese has IR and we can say both type-2 diabetes and obesity are interlinked with each other and are associated with IR. Obesity leads to chronically elevated FFAs in serum and IR can disturb different metabolic pathways and can induce IR in many tissues, like adipose one. Adipose tissue is the repository of excess energy derived from food intake and the unique physiological lipid depot. In obesity, there is massive accumulation of fat in adipose tissue that leads to dysfunctional adipocyte that causes abnormal release of adipokines[11], including inflammatory cytokines such as tumor necrosis factor-alpha(TNF-alpha), interleukin-6(IL-6), resistin, plasminogen activator and monocyte chemoattractant protein-1 (MCP-1) that impairs adipocyte response to insulin [12]; [13].

Lipase, is the rate-limiting enzyme that catalyzes TG lipolysis in adipose tissue are inhibited by insulin (hormone sensitive to lipase),IR in this tissue leads to failure in the inhibition of the enzyme involved in adipocyte lipolysis[14].This leads to massive mobilization of FFAs from adipose tissue to other non-adipose tissue and causes ectopic intracellular accumulation of FFAs. This fact with the distance action of cytokines {fat derived) leads to generalized IR [15].The liver receives an overload of glucose i.e. not internalized by peripheral tissues and insulin (due to compensatory hyperinsulinemia)when there is impairment of insulin uptake which is IR induced, predominates in extrahepatic tissue[16]. Under this condition, both glucose, through activation of the transcription factor carbohydrate response element binding protein (ChREBP) and insulin, through activation of the transcription factor sterol regulatory

binding protein-1c(SREBP-1c)induces the liver synthesis of FFAs [17].

SREBP-1c activates the transcription of the gene that codifies enzymeor transporters involved in FFA metabolism, FFA synthesis (e.g. ACC (acetyl-co-A Carboxylase and FFA synthase) and TG synthesis (e.g.glycerol-3-phosphate acyltransferase)[17, 18]. ChREBP also stimulates transcription of many lipogenic genes. It also induces the expression of liver-type pyruvate kinase (L-PK),hasa key role in the regulation of glycolysis and glucose-6-phosphate (G6P),it is the critical enzyme that is involved in gluconeogenesis [17, 19-21].LXR(Liver X receptor) is the ligand-activated nuclear receptor, its activation leads to induction of SREBP -1C by dietary cholesterol. SREBP-1C is the transcription factor which binds to oxysterols, intermediate metabolites in the bile acid biosynthetic pathways from cholesterol[22].The kind of fatty acid we consume in our diet also impact on the SREBP-1C expression, saturated one upregulates while polyunsaturated ones down regulate the transcription factor [23]. Fructose intake also activates both SREBP-1C and ChREBP[24, 25]

Apolipoprotein(apo) B synthesis, the main protein cofactor of very low-density lipoprotein (VLDL) repressed by insulin and that resulting in impair exportation of hepatic lipid through this lipoprotein [26].Another impairment in the VLDL exportation is the shortage of the main human methyl donor, S-adenosylmethionine (SAMe)[27, 28].In advanced NAFLD there is an impairment in mitochondrial β-oxidation,in this metabolic process FFAs is converted into ketone bodies. This impairment is caused due to; SREBP-1C mediated induction of acetyl-co-A carboxylase2 (ACC2)which leads to the overproduction of malonyl-co-A, a metabolite inhibitory β-oxidation regulatory enzyme carnitine palmitoyltransferase1(CPT1)[29]. Accumulation of atypical toxic lipids in hepatocyte that inhibit mitochondrial β-oxidation, like oxidized cardiolipin [30, 31] and ceramides [32, 33].

The OS due to a metabolic process developed within mitochondria, it can damage complexes of the mitochondrial respiratory chain. This event eventually impairs β-oxidation, also hampers the re-oxidation of & $FADH_2$into $NAD^+$ and FAD,it leads to inhibition of mitochondrial β-oxidation andthe tricarboxylic acid cycle that requires $NAD^+$ and FAD to occur [34].FFA β-oxidation in the later stages of NASH can be augmented and treated as a compensatory mechanism to the increased uptake and synthesis of FFAs [35], this involves both activations of PPAR-α(peroxisome proliferator-activated receptor-α),and consequent enhancement of CPT1activity and loss of affinity of CPT1 for its inhibitor, malonyl-CoA[36]. Other cellular FFAoxidation mechanism, including peroxisomes (β-oxidation) & microsomal (ω-oxidation), are also upregulated to decrease FFA accumulation, with the former increase in the $H_2O_2$ production in peroxisome [37]. However, these processes might not overcome the increasing rate of liver FFA synthesis.

Concluding, in addition to extrahepatic IR, it is well recognized that IR at the hepatic level also plays a major role in NAFLD/NASH [38]. Because of impaired insulin degradation, NAFLD itself can promote hyperinsulinemia[39]. Excessive FFA accumulation in liver triggers pro-oxidizing, pro-fibrotic,pro-inflammatory and pro-apoptotic signal pathwaythat leads to characteristic features of NASH i.e. OS, inflammation, fibrosis, and apoptosis.FFA accumulation may induce: OS, due to mitochondrial over function [40], and further mitochondrial dysfunction [36], as well as it leads to induction of cytochrome P450 family 2 subfamily E member 1 (CYP2E1)[41, 42]. Systemic and hepatic inflammation, with upregulation of pro-inflammatory cytokines, such as TNF α and different interleukins, they are pro-steatotic, pro-inflammatory, profibrotic& pro-apoptotic in nature [43]. Production of TGF-β (transforming growth factor-β)by kupffer cells, that activates HSCs that produce collagen and it leads to fibrosis [44]. The upregulation of hepatocyte apoptosis via OS and cytokine-mediated mechanisms[45].All these events are intertwined and a vicious cycle occurs between them.

**Pathogenesis**

Disease development is complicated and has been designated as "multiple hit and organ theory"[46].

**Mechanism of steatogenesis**

The initial step of NAFLD onset is the accumulation of triacylglycerol in hepatocytes. FA is usually absorbed from the circulation into the hepatocyte and other from glucose in the liver by the process of lipogenesis. FA β-oxidation is mainly regulated by the nuclear receptor peroxisome proliferator-activated receptor-α (PPAR-α).Its down-regulation leads to NAFLD/NASH. Hyperglycemia enhances *de novo* lipogenesis that is strongly regulated by insulin by activation of transcriptional factor sterol regulatory element binding protein-1c (SREBP-1c). This mechanism partially explains the very close relationship between NAFLD/NASH and IR (Insulin Resistance). The changes in hepatocyte lipid droplets are important considerations in the mechanism of hepatic steatosis.

Approximately 60% of fatty acid originates in the liver from white adipose tissue[10], and its dysfunction will

lead to the FA overflow and resultant NAFLD/NASH.Humans having CIDEC mutation exhibit lipodystrophy, additionally, insulin resistance and it give rise to NASH with advanced fibrosis [47]. It has been diagnosed that adipocyte-specific FSP27 disrupted mice because of impaired fat storage and due to enhanced lipolysis in white adipose tissue aggravate high-fat diet-induced hepatic steatosis[48]. This enhanced white adipose lipolysis induced by a choline-deficient dietof steatotic mice promotes FA mobilization from adipose to liver that increases hepatic oxidative stress and that leads to the development of steatohepatitis.

**Mechanism of promoting hepatocyte injury and inflammation**

TAG stored as lipids are not toxic to hepatocytes. TAG precursors and intermediates,like palmitate, DAG (diacylglycerol) and ceramide are causing harm to hepatocyte. Palmitate is responsible for oxidative stress and ER stress, leading to c-jun N–terminal kinase (JNK) activation and lipoapoptosis. [49-51]. Diacylglycerol activates protein kinase C and also leads to the disruption of insulin signalling. Ceramide is taking part in upregulation and expression of SREBP -1c and promotes the production of palmitate[52]. Fat rich cells are prone to lipid peroxidation, which leads to mitochondrial and ER dysfunction.If cholesterol is freely circulating it also causes mitochondrial dysfunction andinflammasome activation [53]. Injury in NASH is cytotoxicities andlipotoxicities mediated and it is the major cause.

**Diagnosis**

NAFLD is detected by abnormal liver function, often asymptomatic,imaging results in health checkups and can be detected during follow up for other diseases. Patients can be suspected having NAFLD with an elevation of serum aspartate aminotransferase (AST) & alanine aminotransferase(ALT) and fat change on ultrasonography (US) or computed tomography (CT) without having a history of habitual drug or ethanol intake or positive HBV or autoantibodies. NAFLD/NASH can also develop after gastrointestinal surgery,involving pancreaticoduodenectomy and intestinal bypass[54, 55].

Careful exclusion of some disorders like Wilson's disease, citrin deficiency[56-58] and cholesteryl esters storage disease[59]exhibits hepatic steatosis and it mimics NAFLD. In cirrhotic NASH, AST/ALT levels in serum and hepatic TAG accumulation in hepatocyte are markedly reduced, which may be diagnosed or cryptogenic liver cirrhosis[60, 61]) Hereby, should be noted that normal ALT levels cannot exclude the happening of NASH with advanced fibrosis, for an accurate diagnosis combination of liver function testing with fibrosis markers should be done.

**Borderline between non-alcoholic &alcoholic status**

Because the impact of ethanol on the liver differs that's why threshold of ethanol consumption between NAFLD and alcoholic liver disease is problematic as it differs among individual with regards to race, sex, aldehyde dehydrogenase 2 gene polymorphism, mode of drinking and lifestyle. It is hard to estimate the precise amount of ethanol intake. According to some reports have shown that mild drinking reduces hepatic steatosis.The occurrence rate of HCC is higher in those patients which had mild drinking habit (<20 g/d) having higher male prevalence, increased gamma-glutamyltranspeptidase, and more frequent liver cirrhosis[62].

**Evaluation of hepatic steatosis using imaging modalities**

Ultrasonography is a method to detect fatty liver, but its quantitative performance guidelines cause interobserver differences that complicate the monitoring of fat accumulation changes during interventions [63, 64].CT is calculated by Hounsfield unit (HU) abdominal CT liver/spleen scores of <40 or ratio of liver/spleen <0.9 indicates the presence of hepatic steatosis.As CT has greater quantitative performance, objectivity and reproducibility as compared with the US, it is having some disadvantages like radiation exposure, cost, HU variability which depends on device set-up and inaccuracy is iron/copper depositions [63, 64]. MRI (Magnetic Resonance Imaging) perfectly quantify the hepatic fat accumulation.

CAP (Controlled Attenuation Parameter) and E value is quantified through recently introduced Fibroscan, it quantifies the degree of hepatic fat accumulation and fibrosis. CAP values are correlated with the area of lipid droplets in liver histology only in NAFLD patients having BMI < 28 kg/m2. Now, further improvements in diagnostic performance are needed in this area as there are large number of obese patients.

**Clinical Significance of Liver Biopsy and Detection Of Ballooned Hepatocytes**

Repeated liver biopsy is somewhat invasive, costly and ultimately unrealistic. There is a problem in sampling error and its diagnosis[65-67].In order to search for less invasive and more accurate methods to asses NAFLD pathology, several serum biomarkers have been detected to see the presence of ballooned hepatocytes[68-72]. For example, cytokeratin 18 (CK 18) accumulates with Mallory-Denk body-like inclusion bodies. CK18 circulating fragment is significantly increased in NASH compared to NAFLD. Prognosis of NASH is poorer than NAFLD.

NASH is steatosis without ballooned hepatocytes. NASH may transform into NAFLD as ballooned hepatocytes may sometimes disappear and vice versa. NAFLD is not always benign as it is described by one case, one patient with NAFLD at first liver biopsy gradually preferred to cirrhosis and HCC over 20 years and he underwent careful for 27 years follow up [60]. HCC may develop from NAFLD regardless of the absence of advanced fibrosis, past HBV infection, or regular ethanol consumption [73].Recent studies have detected the presence of advanced fibrosis, but not ballooned hepatocytes, was a clear picture of poor prognosis in NAFLD patients.If we take all together, it seems that the clinical significance of ballooned hepatocytes has given the way to that of fibrosis in NAFLD/NASH.

**Evaluation of liver fibrosis**

Platelet count and serum levels of hyaluronic acid, Mac2-binding protein, autotoxin, type 4 collagen 7S are relevant biomarkers that predict advanced fibrosis (Table 1)[74-78]. Results are emphasised by some conditions like co-existing collagen disease, systemic inflammation and renal dysfunction. AST-to- platelet ratio index (APRI), NAFLD fibrosis score, FIB-4 index, BARD, CA index ELF, and fibro test have indicators to predict advanced fibrosis in NAFLD patients (Table 2)[79-82].

Fibrotest and ELF are direct markers of collagen synthesis & degradation, but these measurements are not common in clinical situations.These test like NAFLD fibrosis score, APRI and FIB-4 exploit the biochemical test components of age, ALT, AST,glucose,BMI, platelets and albumin, they are routinely obtained in clinical practice. This is also unclear that changes in AST, ALT and BMI are correlated with the degree of actual fibrosis.

**Table 1:Biomarkers predicting ≥ F3 fibrosis in Japanese non-alcoholic fatty liver disease patients**

| Biomarker | Fibrosis stage | Cut-off value | AUC | Reference |
|---|---|---|---|---|
| Platelet count | F4 | $15.3\times10^4/\mu L$ | 0.92 | [74] |
| | F4 | $16\times10^4/\mu L$ | 0.98 | [75] |
| Hyaluronic acid | ≥F3 | 42ng/mL | 0.97 | [75] |
| Type 4 collagen 7S | ≥F3 | 6.0ng/mL | 0.88 | [76] |
| Mac2-binding protein | ≥F3 | 2.24 µg/mL | 0.78 | [83] |
| $WFA^+$ Mac2-binding protein | ≥F3 | 1.23 COI | 0.83 | [77] |
| Autotaxin | ≥F3 | 1.19mg/L | 0.75 | [78] |
| | F4 | 1.20mg/L | o.87 | |

AUC: Area under the receiver operating characteristic curve

**Table 2.Representative indices predicting ≥F3 fibrosis in non-alcoholic fatty liver disease patients**

| Score/index | Formula | Reference |
|---|---|---|
| NAFLD Fibrosis curve | 1.675+0.037×age +0.094×BMI +1.13X IFG/DM (with =1,without=0)+0.99×AST/ALT-0.013×PLT-0.66×Alb | [78] |
| APRI | [(AST/upper limit of normal AST) PLT]×100 | [80] |
| FIB-4 Index | [age×AST]/[PLT×$ALT^{1/2}$] | [81] |
| BARD score | BMI≥28(1 point) AST/ALT≥0.8(2 point) The presence of DM (1 point) | [82] |
| ELF score | 2.494 + ln (hyaluronic acid)+In (P-III-P)+In(TIMP-1) | [84] |
| CA index –fibrosis | 1.5 ×4C7S + 0.0264×AST | [85] |

BMI-Body Mass Index; IFG: Impaired Fasting Glucose;ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PLT: Platelet count; Alb:Albumin; 4C7S:Type 4 collagen 7S; P-III-P:Procollagen type III amino-terminal peptide;TIMP-1: tissue inhibitor of metalloproteinase-1;NAFLD: Non-alcoholic fatty liver disease.

**Treatment**

Treatment from liver fibrosis is body weight reduction.

**Body weight Reduction**

The early stage of NAFLD/ NASH is resolved by weight loss, lifestyle modifications that will reduce weight are routinely prescribed. NASH patients who received repeated biopsy and having weight loss, absence of diabetes, ALT normalization, young age & baseline NAS <5 are predictions for NASH resolution without fibrosis after 1 year of lifestyle management (Vilar Gomez et al ([86]. Multi-disciplinary cooperation of doctors (gastroenterologist, cardiologist, endocrinologist etc.), nurses, dietitians and exercise therapist are needed for facilitating the exercise regimens to achieve 10% weight loss. For unsuccessful weight reduction for morbid obesity, bariatric surgery is a promising option. It is proven that bariatric surgery can significantly improve NASH [87]. It gives long term safety and its effectiveness is always under debate.

**Pharmacological interventions for underlying disorders:-**

NAFLD/NASH diseases are accompanied by dyslipidemia, hyperglycemia and insulin resistance for disease management correction of this disorder is beneficial.

Vitamin E, pioglitazone (for NAFLD/NASH with diabetes), statins (for NAFLD/NASH with dyslipidemia) are recommended therapeutic agents by the Japan Society of Gastroenterology (JSG) and Japan Society of Hepatology (JSH) [88]. And American Association for Study of Liver Disease (AASLD) has also included these substances. Vitamin E is very beneficial as it scavenges the free radicals to reduce oxidative stress in NAFLD/NASH livers. It significantly improved the NASH histology in non-diabetic & non-cirrhotic adult NASH patients compared to placebo [89].

Vitamin E treatment has not been confirmed on the safety of long term & its high dose. Insulin resistance, steatosis, lobular inflammation and fibrosis in diabetic/pre-diabetic NASH patients are attenuated by the PPAR-g activator, pioglitazone which increases circulating adiponectin (clinicaltrials.gov identifier: NCT00994682) [90].HCC prevalence in diabetic patients also reduced by the PPAR-γ agonist, but it also causes some adverse effects like fluid retention (edema, heart failure) and osteoporosis. For pharmacological agents, it is the constant challenges to minimize the adverse effect with benefits and risks along with improvements.

**Novel agents under clinical trials**

Some of the trials are undergoing clinical trials, among them, obeticholic acid, elafibranor, selonsertib, and cenicriviroc are phase 3rd trials [91]. These trials evaluate not only histological improvement of NASH but also helpful in the prevention of progression into cirrhosis, hepatic decompensation and death.

Obeticholic acid is a potent whole body Farnesoid X Receptor (FXR) agonist [92]. This significantly increases blood triglyceride and low-density lipoprotein cholesterol levels and decreases high-density–lipoprotein-cholesterol concentrations which havethe chance for the risk of cardiovascular diseases.

Elafibranor (GFT-505) acts as a dual agonist for PPAR-α/δ. PPARα activation attenuates hepatic steatosis and inflammation. While that of PPAR-α stimulation ameliorates hepatic inflammation and fibrosis [92]. Selonsertib (GS-4997) is very important apoptosis signal-regulating kinase 1 (ASK1) inhibitor.ASK1 is activated through stimulus like hyperglycemia, transforming growth factor β and oxidative stimulus that induces apoptosis and fibrosis by p38 and JNK. Upregulation of ASK1-JNK1 resulting insulin resistance, steatosis, and inflammation and further activation of ASK1, resulting in a vicious cycle.

ASK1 inhibition reduces body weight along with hepatic fat and fibrosisand improves insulin resistance in an animal NASH model [93]. In the phase 2 study, selonsertib makes better the NASH activity and fibrosis[94]. For stage 3 fibrosis and cirrhotic patient, an international phase 3rd trial is on working. (STELLAR 3 study, NCT03053050) and STELLAR4 study, NCT03053063, respectively. Ultimately cenicriviroc, the antagonist for C-C motif Chemokine Receptor (CCR) 2/5. Currently, a NASH patient with stage 2/3 fibrosis is underway for phase 3 clinical trial of effect of cenicriviroc evaluation (Table 3)[95-99].

**Conclusion**

NAFLD consists of hepatic disorders share common features, that includes hepatic fat infiltration from the histological point of view while from the pathogenic point of view IR is the feature that affects NAFLD. The spread of NAFLD is increasing at an alarming rate, and it is the most frequent hepatocyte nowadays. In the past few years, the information that has been accumulated has led to the identification of targets for therapeutics. Recently, the trends in diet and nowadays' lifestyle increased the prevalence of NAFLD worldwide. Some work is needed to checkup individuals with low fibrosis stage and at the risk of disease progression. New line drugs should be optimized for maximum benefit and few of the adverse events.

**Acknowledgement**

Malkhey Verma thanks to the Central University of Punjab, Bathinda, India for seed grant funding in 2016.

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**Conflict of Interest: None**
**Source of Support: Nil**