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Non-Alcoholic Steatohepatitis (NASH): An Update

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Abstract

Non-alcoholic Steatohepatitis (NASH) is a globally rising, multifactorial disease which may be a result of obesity, diabetes (type-2), hypercholesteremia and gut dysbiosis. NASH is characterized by inflammation and fibrosis of liver tissues which further leads to hepatocellular carcinoma or liver failure. Patients with non-alcoholic fatty liver disease (NAFLD) show symptoms of NASH in which the fat accumulation in the liver exceeds 5-10%. Accumulation of this fat in the form of triglyceride in the hepatocytes is the key factor for the development of NASH. This may be caused due to increase in the flow of fatty acid and de novo lipogenesis. This abnormal retention of fat leads to lipotoxicity, which can cause apoptosis, necrosis, inflammation and an increase in oxidative stress. Since the pathological factors associated with NASH are unclear, the treatment to control the progression and management of the disease is cumbersome. Progress is being made in order to understand the cellular and molecular mechanisms associated with the pathogenesis of this condition. With studies reporting that NASH is the most common liver disease after hepatitis C, there is an increase in the demands for medical therapy and diagnosis of NASH. However, no treatment has been proven to be effective for long-term use. Gut dysbiosis, major pathways involved in NASH progression, experimental animal models and the current therapeutic strategies for NASH is discussed in the present review.

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INTRODUCTION

Non-alcoholic Steatohepatitis (NASH) is a chronic manifestation of liver disease caused in most cases due to over consumption of fatty foods rich in saturated fats, meats, refined sugars and a sedentary lifestyle [1]. Clinical displays of NASH include obesity, insulin resistance (IR), diabetes, dyslipidemia, and coronary artery disease. NASH is also a metabolic syndrome that may be developed through genetic, lifestyle and food trends. Diet and sedentary lifestyles are majorly involved with liver steatosis. A chronic fat rich diet leads to obesity and thereby

targets inflammatory reactions by infiltrating proinflammatory markers such as TNF-α and IL-6 and also reduces insulin sensitivity in adipocytes. This leads to an increase in lipogenesis by adipocytes resulting in the increase of Free fatty acids (FFA), which are further stored as triglycerides in hepatocytes. [2] explained the role of a chain of pro inflammatory markers involved in the inflammatory reactions, which is therefore considered as a therapeutic target for liver steatosis.

The earliest cellular response of the liver to over nutrition and obesity may be the activation of macrophages which are further responsible for the release of pro inflammatory mediators**,** TNF-α and IL-6, promoting hepatocyte lipogenesis. Increase in lipogenesis is involved in hepatocyte endoplasmic reticulum stress, the release of damage associated molecules and an increase in Kupffer cells which leads to monocyte infiltration in turn hepatocyte steatosis. Therefore, with obesity, macrophage infiltration increases a sequence of tissue remodeling and repair mechanisms through the production of pro inflammatory mediators [3, 4].

Hepatocyte stellate cells (HSCs) are identified to be the key factors responsible for the remodeling of the tissue matrix during acute and chronic liver damage. These cells undergo many structural changes identified by immune cells, such as transforming growth factor (TGF-β) and platelet derived growth factor, which triggers endoplasmic reticulum stress, autophagy and alterations in cholesterol metabolism [5].

Liver sinusoidal endothelial cells (LSECs)are the key cells that are involved in the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion protein-1 (VAP-1), which allows adhesion of leucocytes to the endothelium. They also show anti-inflammatory action by increasing the expression of cytokine IL-10 in Th1 cells via the notch pathway. So, any injury to LSECs leads to the appearance of Kupffer cells and HSCs, which further advance and deteriorate the pathological condition from simple steatosis to hepatic steatosis [6]. The present manuscript focuses on the role of gut dysbiosis in NASH, the key pathological mechanism involved in steatosis, current animal models and therapeutic strategies deployed in the management of NASH.

Role of Gut bacteria in NASH

Gut microbiota is the microbes residing in the gut lining which are useful to the host (human) in several ways. Majorly the bacteria exist in the lumen along with the other microorganisms in the microbial niche [7]. The microbiota environment may be disturbed by the change in the diet, flow of nutrients, immune function of the host, infections and occasional use of antibiotics during the diseased condition [8, 9].

The bidirectional functional relationship between the liver and gastrointestinal tract, commonly called "Gut-Liver Axis", allows acceptance of the blood flow by the liver from the intestinal vascular structures and the bile from the liver to the gut for the proper digestion of food materials. In addition to the nutrients, the portal blood also contains other molecules that can actively or passively cross the gut liver axis, thus making the liver one of the most exposed organs to the bacteria and their derived metabolites [10].

Along with the blood, a fraction of gut microbiota travels to the liver, which is further eliminated by Kupffer cells. Abnormal alterations in the gut microbiota can alter the functional properties of the liver. The liver not just reacts to the microbiota and its metabolites yet effectively shape intestinal microbial creation and digestion through enterohepatic biliary flow [10].

The change in the composition of gut microbiota alters the amounts of metabolite components that reach the liver, thereby causing liver tissue damage [11]. Further, there are numerous ways that the structure of the microbial network is impacted in NASH. This incorporates the hereditary qualities of the host, diet, contamination, or clinical intercessions.

The cleanliness theory initially recommended that anti-toxin utilization and lifestyle changes limit microbial presentation inclining populaces of individuals to an immune system illness [12]. In this manner, it was proposed that, because of the failure to separate microorganism and commensal, antitoxins upset microbiota structure and, therefore, the co-developmental connection between resistant framework and the symbionts.

Dysbiosis is characterized as loss of gainful microbiota, expanded pathogenic microbiota or diminished microbiota assortment [13]. Gut microbiota in NASH patients comprises of *Proteobacteria, Enterobacteriaceae, Lachnospiraceae , Escherichia* and Bacteroidetes. Many researchers showed there is an increase in the levels of bacteriodates.

A study conducted by [14] on 57 liver fibrosis patients showed that bacteroides are independently associated with NASH and *ruminococcus* with significant fibrosis. Therefore, disruption of the composition of the gastrointestinal microbiota plays a role in increasing the development of FFA in the gut and increased permeability of the intestines [15, 16].

Faecal microbiota and metabolites in NAFLD

A study conducted by [17] on 60 NAFLD patient faecal samples showed the predominance of bacteriodes compared to Lactobacillus and Bifidobacteria Silva *et al.*, 2018, in their survey on the faeces of NAFLD patients, observed that *F. prausnitzii*, *Coprococcus* and *Ruminococcus* were less abundant. Both *Coprococcus* and *Ruminococcus* were found to produce single chain fatty acids whose absence leads to chronic inflammation, which is a key step in advancing early hepatosteatosis to chronic liver damage [18–20].

The proportions of propionate and isobutyric acid are higher in NAFLD patients when compared with healthy individuals. The remaining chemical constituents like butyrate, acetate, formate, and total short chain fatty acids (SCFA) remained almost the same. Increase in volatile compounds is observed in NAFLD patients [21].

Animal models for liver disease

A cascades of pathological changes takes place in the liver during the progression from simple steatosis to chronic liver injury, which is extensively studied in various animal models. Animal models are used not only to elucidate the pathophysiological events but also to find the potential biomarkers, thereby defining effective treatment(s) opportunities. The non-alcoholic related animal studies carried out so far that mimic liver disease is discussed below, along with their pros and cons.

Methionine and choline-deficient diet

A diet lacking methionine and cysteine results in the poor production of phosphatidylcholine, which results in decreased VLDL secretion, which in turn reduces triglyceride clearance. The process causes lipid accumulation in the liver, progressing to liver steatosis. According to [22], though methionine- and choline-deficient diet is able to show liver steatosis in 8 weeks, the body weight of the animals reduces to 40%, which cannot be entertained to elucidate the metabolic parameters of NAFLD. Other than the metabolic parameters, hepatocyte ballooning and Mallory Denk bodies are not observed in this model, hence it is not widely recommended.

High Fat Diet (HFD) and Modified High Fat Diet (MHFD)

HFD is a diet without nutrient deficiencies and 70% fat which elevates postprandial glucose levels, causing obesity and generating NASH in experimental animal models. The severity of HFD induced NASH depends on the species, gender and strain of the animals. Rats require a shorter period than mice because of their sensitivity [23]. In rats, male rats are more sensitive than females [24]. Sprague Dawley male rats significantly develop NASH easily compared with other strains [25]. The advantages of this model are less skill requirement, cost effective and low mortality rate, while the disadvantage includes the use of a large animal population due to inter-individual variability, duration, strain and sexual differences [26].

To meet the limitations of HFD, many modified models of HFD were introduced. These include choline deficient (CD) HFD, fructose induced HFD, Trans fats added HFD and chemical based HFD. CD-HFD resembles human NASH in developing ballooned hepatocytes, infiltration of immune cells, satellitosis, Mallory Denk bodies and glycogenated nuclei. Also, CD-HFD fed closely resembles the human condition by developing tumours [27]. In this model, NASH develops in 24 weeks in rats.

Fructose enriched HFD fed animals are well-known for generating metabolic syndrome. Fructose not only blocks hepatic β-fatty acid oxidation leading to fat accumulation in hepatic cells but also contributes to intestinal dysbiosis, impaired intestinal barrier function and increased translocation of bacterial metabolites in NAFLD [28]. Mice fed with high fructose through the water along with HFD developed obesity, insulin resistance, hepatic injury, oxidative stress, macrophage infiltration, and fibrosing NASH over a period of 16 weeks [24].

[29] proposed that the wild C57BL/6 mice fed with a western-type diet rich in soya bean oil and cholesterol developed liver steatosis that mimics features of NASH in humans with metabolic syndrome within 16-20 weeks. A diet enriched with both fructose and cholesterol, commonly called ”fast food diet” or ”western diet”, was followed by many scientists for developing NASH, which mimics human metabolic syndrome.

Addition of Trans fats to the western diet increased insulin resistance, hepatic lipogenesis and oxidative stress in mice models of NASH. Also, mice fed with HFD enriched with transfats and fructose developed obesity, hepatic insulin resistance, hyperinsulinemia, marked steatosis, moderate lobular inflammation, and mild-stage hepatocellular ballooning within 26–30 weeks. After a period of 52 weeks, the mice developed hepatocellular carcinoma, the pattern which relates to human NAFLD and features as a metabolic syndrome.

[30] showed after 12 weeks of CCL4 administration along with HFD induced hepatic steatosis, inflammatory cell accumulation, hepatocellular ballooning, fibrosis, and increased serum aminotransferase levels. Though the model induces NASH symptoms,

it attenuated the increase in body weight, cholesterol and insulin/ glucose levels. Administration of CCL4 subcutaneously for longer periods causes chronic liver damage.

Therapeutics and non-therapeutic strategies in the management of NASH

Non-therapeutic approaches

Nutritional interventions

Hyper caloric diets play a very crucial role in the progression of NASH. Products with high sugar content and high fat lead to the progression of simple steatosis to acute and chronic conditions of NASH. According to many scientists, NASH not only leads to liver damage but also is an intermediary for many metabolic disorders, including obesity, cardiovascular diseases, diabetes mellitus, hepatocellular carcinoma and chronic kidney diseases.

Reports also indicate that NASH is a risk factor in the development of colorectal cancer, metabolic bone disease and rare metabolic diseases like lipodystrophies and glycogen storage diseases (EASL–EASD–EASO, 2016). Therefore, a diet that compromises low sugar, low fat and low calories along with physical activities is a prominent non-therapeutic approach for NASH [31]. EASL–EASD–EASO Clinical Practice Guidelines recommends the Mediterranean diet, a diet that opts for low sugar and fructose intake, high intake of olive oil that is rich in monounsaturated fats, vegetables, and fruits. A diet containing polyunsaturated fatty acids reduces the risk of steatohepatosis and intra-hepatic triglycerides content.

Physical activity

Physical activity, on the other hand, is the best approach for NAFLD patients who are accustomed to a sedentary lifestyle. According to [32], the severity of physical exercises is directly related to a lower risk of the disease. An increase in the production of AMP-activated protein kinase (AMPK) by exercise leads to the phosphorylation of acetyl coenzyme A carboxylase by inactivating the transcription factor SERB1, thereby inhibiting the conversion of acetyl co-A to FFA. Studies conducted by [33] showed that exercise increases the expression of PPARα, a receptor that stimulates β-oxidation in the liver by increasing the oxidation of mitochondrial fatty acids, a method similar to thiazolidinediones. [34] demonstrated a reduction in TLR4, TLR5, CD11b, and CD14 expression on PBMCs with increased aerobic exercise programmes resulting in the improvement of innate immunity.

Therapeutic approaches

Thiazolidines

Pioglitazone is a drug used to treat type 2 diabetes patients with high blood sugar levels. Pioglitazone showed an effect on NASH and related metabolic diseases and found it to be effective since it helps reversal of advanced fibrosis condition when treated for 24 months [35]. A possible mechanism underlying this observation is linked to activation of adenosine monophosphate-activated protein kinase and induction of hepatic stellate cell senescence [36]. Though pioglitazone was known to control NASH, adverse effects like weight gain and lower limb oedema were its major adverse effects. Hence, its use is restricted in patients with a higher risk of liver fibrosis and liver related deaths [35].

Exenatide

Exenatide therapy is a human GLP1 based therapy that decreases fat accumulation in the liver by modulating fatty acid oxidation, lipogenesis, insulin secretion from β-cells and hepatic glucose metabolism. A study showed the effect of exendin-4 in improving the fatty acid metabolism in the liver by activating AMPK and its downstream target genes involving fatty acid oxidation [37]. By activating the Sirt-1 signalling cascade, it exceeds in attenuated fatty acid accumulation and improved glucose metabolism, proving which reveals its therapeutic potential in metabolic diseases associated with fatty liver diseases.

Saroglitazar

In a mice model of choline deficient high-fat diet-induced NASH, saroglitazar reduced alanine aminotransferase (ALT), hepatic steatosis, inflammation, ballooning and prevented fibrosis development [38]. A study conducted on diabetic dyslipidemia by [39], showed that saroglitazar significantly reduced lipid profile parameters like TG, TC, LDL-C and increased HDL-C and glycemic parameters—HbA1c. PPARs are known to be the key regulators of liver homeostasis by inhibiting intrahepatic lipid accumulation (PPARα) and by increasing insulin sensitivity in adipocytes (PPARγ). Since Saroglitazor has dual agonist activity for PPARα and PPARγ, it shows a strong antisteatotic effect [40].

Metformin

Metformin is an adenosine monophosphate (AMP)-activated protein kinase (AMPK) activator used to treat prediabetes and type 2 diabetes patients, which is also an effective regimen in decreasing apoptosis in myofibroblasts. Metformin is also reported to reduce the risk of mortality and liver transplant in a study conducted on 191 patients with type 2 diabetes and NASH [41].

Faecal Microbiota Transplantation (FMT)

Choline is an important component in liver metabolism and its conversion produces phosphatidylcholine and other phospholipids. Deficiency of choline causes disturbance in liver metabolism, thereby causing NASH. An imbalance in the gut bacteria causes the conversion of choline in intestinal cells to trimethylamine, which is further absorbed by intestinal epithelial cells leading to a choline-deficient stage in the liver [42]. A simple route to modify the gut microbiome is through pre and probiotics. Pre and probiotics improve lipid metabolism by modifying gut dysbiosis. Faecal microbiota transplantation (FMT) was found to be a promising therapy to modify the gut microbiome and aim at treatment of many diseases like colitis, inflammatory bowel disease, autism and acute graft-versus-host disease. According to a short study conducted by [43] on ceftriaxone-treated mice, among Bacterial consortia transplantation (BCT) and FMT, BCT was found to be the safe transplantation.

In contrast, FMT caused aggravation of inflammatory responses because of significant inter-individual variation and the possible risk of disease transmission. But as per recent evidence made by [44], FMT reportedly reduced necro-inflammation and steatosis in liver histology by increasing erine dehydratase, an enzyme required to convert serine to pyruvate and ammonia, which predominantly resides in the perivenous region of hepatocytes [44]. Current approaches in microbiome transplantation are the use of bacteriophages in combination with CRISPR as tools to eliminate pathogenic bacteria from the gut that produces toxic metabolites.

CONCLUSIONS

Though many drugs developed established pathways in the management of NASH, physical activity and controlled diet might help control the disease progression in the initial stages of liver steatosis. Many researchers proved the efficiency of saroglitazar and pioglitazone, the PPAR agonists, in treating critical stages of hepatitis. Advanced treatments included BCT, FMT, pathogenic bacteria specific bacteriophages and CRISPR technologies in NASH.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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