Section A-Research paper

A REVIEW ON NAFLD AND CURRENT TREATMENT STUDIES

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Abstract

One of the most prevalent causes of liver illnesses is nonalcoholic fatty liver disease (NAFLD), and its incidence is rising globally. Over €35 billion in medical expenses are reportedly incurred each year in France, United Kingdom, Italy and Germany as a direct result of NAFLD and 100 billion dollars in the US. The origin and development of NAFLD are currently not fully understood, as hepatic inflammation is brought on by a variety of triggers, such as the buildup of cellular oxidative intermediates, stress in the endoplasmic reticulum (ER) and oxidative stress and tissue hypoxia and sinusoidal endothelium cell dysfunction development. Overconsumption of foodstuffs and a low physical activity cause hepatic steatosis. Numerous variables contribute to inflammation, NASH, and the development of scarring. Nevertheless, there is no treatment that is currently accepted for NAFLD or NASH at this time. There are four goals guide NAFLD management that is diet and lifestyle modification to facilitate weight loss; the management of risk factors for cardiometabolic disease; treating any and all modifiable risk factors associated with NAFLD's later stages; and protecting against liver and other organ problems. According to the World Health Organization (WHO), is a study that subjects people through one or maybe more therapies in order to assess how the treatments affect their health. As per the WHO's requirements, every trial must have been filed prior to participant recruiting may start. This review summarises the recent clinical trials that have been done with the goal of treating NAFLD and NASH. In order to examine prospective treatment strategies for NAFLD, preclinical investigations as well as clinical trials have now been carried out including things like synbiotics, probiotics, angiotensin-II receptor blockers, topogliflozin, CCR2/5 antagonists, and FXR agonists, amongst other things. It's possible that a combined treatment, like one that combines medical care and exercise, could shorten the duration of treatment while also improving the outcome.

Keywords: NAFLD, Clinical Trials, Oxidative stress, Lifestyle, Topogliflozin, CCR2/5 antagonists

1. Introduction

One of the most prevalent causes of liver illnesses is nonalcoholic illness of the fatty liver that is (NAFLD), Moreover, the number of cases is growing all across the world [1]. Over €35 billion in medical expenses are reportedly incurred each year in France, United Kingdom, Italy and Germany as a direct result of NAFLD and 100 billion dollars in the US [2]. A range of liver conditions known as NAFLD can develop when there are no other recognised reasons, also including excessive alcohol consumption. NAFLD has lately been called metabolic-associated fatty liver disease because it is a metabolic disorder (MAFLD) [3]. The disease is recognised as a distinct disease entity under the moniker MAFLD, which also removes the requirement for excessive alcohol consumption from the classification. We'll use the terminology NAFLD in this review to prevent nomenclature ambiguity. Hepatic steatosis is a component of NAFLD, and even more than 5% of the weight of the liver is made up of fat. The NAFLD with steatosis at a higher severity level, cellular destruction, additionally inflammation, nonalcoholic steatohepatitis (NASH), may develop from NAFL. One of the main causes of hepatocellular carcinoma (HCC) requiring liver transplantation is NAFLD [4]. The condition is connected to a number of extrahepatic conditions, including cardiovascular problems [5]. 24% of the general population is afflicted by NAFLD [6] and is on the rise in tandem with the obesity crisis. Type 2 diabetes and obesity are associated with NAFLD [6]. Up to 70% of those who are overweight and 90% of those who are extremely obese are affected by the condition [7]. Hepatic fibrosis is the best indicator of mortality in NAFL patients. [8]. Lean individuals can also develop NAFL and NASH [9]. Asians typically have higher levels of lobular inflammatory response and ballooning than people of other racial or ethnic groups [10]. Fat deposition at lower body masses is possible in the Asian population [9]. Additionally, differences in NAFLD frequency between ethnic groups have been noted [10,11,12]. The prevalence of NAFLD is rising among young children and adolescents as well [13]. For the treatment of NAFLD in particular, there is no pharmaceutical therapy available. The cause may be that NAFLD is a complex illness with incomplete knowledge of the underlying pathogenic pathways and a lack of reliable non-invasive diagnostic. This article reviews the pathophysiological mechanisms underlying NAFLD, available diagnostic tools, and prospective treatment targets.

2. Pathogenesis

The origin and development of NAFLD are currently not fully understood, as hepatic inflammation is brought on by a variety of triggers, such as the buildup of cellular oxidative intermediates, stress in the endoplasmic reticulum (ER) and oxidative stress and tissue hypoxia and sinusoidal endothelium cell dysfunction development [16]. Triglycerides (TGs) and cholesterol esters make up the majority of the hydrophobic neutral lipid core of LDs, which is encased in a phospholipid monolayer. LDs are metabolically active, dynamic organelles. Heterogeneous peptides or enzymes in charge of neutral lipid production or

metabolism are included into this monolayer. Adipose tissue, increased dietary intake of free fatty acids (FAs), and hepatocyte de-novo lipogenesis are all thought to be contributing factors to the liver's LD buildup. Intracellular lipid deposition and role in the pathogenesis activation processes that cause hepatocellular inflammation, steatosis, and fibrosis can result from dysregulation of LD biosynthesis and breakdown. [17]. Lipid metabolites and intermediates of TG production are created by the hydrolysis of TGs as well as other glycerides from LDs, which affects the homeostasis of cells and causes organelle failure, cell damage, cell malfunction, and cell death. [18].

2.1. Stress on the endoplasmic reticulum and lipid droplet biogenesis

The ER controls the production of LDs (Fig.1). After being hydrolyzed by a lipoprotein enzyme lipase, dietary fatty acids or those produced from adipocyte to hepatocytes and change in the form of very low-density lipoprotein or in the form of lipid droplets [19]. De novo lipogenesis, another method for producing FAs in hepatocytes, is also possible [19]. This is a process of metabolism that is controlled by transcriptional elements such carbohydrate-responsive element binding protein, insulin-responsive sterol regulatory proteins that bind certain elements like SREBP1, but also Increased expression of X receptors in the liver as a result of cholesterol. Transcription factors promote transcription involved in lipogenesis once they are activated. Triglycerides and cholesterol ester called neutral lipids, that are created when FAs are delivered into the ER are then either stored as LDs or released as VLDL [19, 20]. Adipose tissue and liver metabolic functions are changed by the increased FA synthesis that results from the imbalance of lipid metabolism in obesity. For esterification, The ER is the destination for incoming FAs in the liver. Certain enzymes are responsible for the production of neutral lipids in the cytosol and on the side of lumen of the ER. [21]. The family of enzymes known as acyl-CoA synthetases induces the synthesis of fatty acyl-CoAs from long-chain fatty acids and CoA with help of ATP. As opposed to this, cholesterol is esterified by ACAT1 and 2 enzymes. TGs are created from cytosolic FAs by the enzymes diacylglycerol O-acyltransferases 1 and 2 (DGAT1 and DGAT2). To re-synthesise Triacylglycerols inside the lumen of ER, DGAT1 needs diacylglycerol (DAG), which is produced when TGs are lipolyzed within the cytoplasm. A key component in the creation of de novo TG from FAs is DGAT2, which is found on the lipid droplet's surface and inside dual layer of the ER. DGAT2 relocates to the surface of LD, when the Fatty acid concentration becomes more than normal level in cytoplasm, and creates TGs through synthesis that will get thrown in further entering the lipid droplets of the membrane [22,23]. Toll-like receptors DGAT1 as well as DGAT2 both limit the buildup of cellular oxidative lipids like FAs and DAG which, if not, would cause hepato-cyte inflammation by activating ER stress mechanism [22]. The concentration of Triacylglycerides in the double layer of endoplasmic reticulum (ER) bilayer membrane is related to the LD's ability to budding. ER membrane bilayer localization is caused by lipids at this concentration. [24,25]. When the membrane curvature leans towards the polar side of cytoplasm, it is positive; when it goes to the ER or away from the cytoplasm, it is negative [26,27]. Positive or negative curvature

depends on the type and structure of the lipids which constituted by the membrane of ER at the point of LD budding. Positive curvature is produced by lipids having inverted shape predominance of hydrophilic head that also promotes the development of LDs on the polar side of cytoplasm. As opposed to this, lipids having a shape of cone that predominate the lipolytic tale just like phosphatidylethylamine, and DAG exhibit a pessimistic inclination that encourages implantation of fat particles in that double layer of ER [28]. Importantly, under this condition, proteins as well as enzymes are unable to acquire LDs to either control the uptake, migration, or protein composition of neutral lipids[28]. Consequently, because implanted LDs are unable to clear unfolded and improperly folded proteins from the double coated layer of ER, causes ER stress and leads to inflammation [29,30].

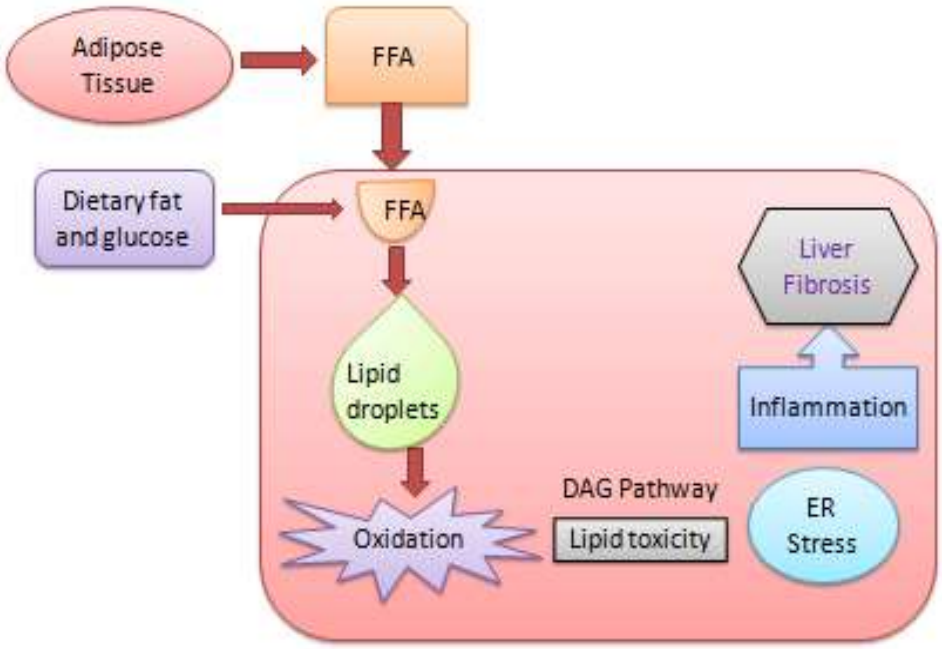

Fig. 1. ER stress in Pathogenesis of NAFLD

Overconsumption of foodstuffs and a low physical activity cause hepatic steatosis. Numerous variables contribute to inflammation, NASH, and the development of scarring. Mechanisms underlying the onset and development of NAFLD are shown in the bottom panel. Dietary fat, de novo lipogenesis or adipose tissue lipolysis, through carbohydrates and another dietary component all contribute to the reservoir of FAs in the liverFAs are converted within the liver into TG and very-low-density lipoprotein (VLDL), which are then released into the bloodstream [31] undergoes beta-oxidation in the mitochondria. [32] or retained in lipid droplets (LDs) up to 5% of the weight of the liver. When fasting, LDs

engage in lipid hydrolysis through lipolysis to produce FAs during β-oxidation. FAs for NAFLD patients who have persistent nutrient intake and diabetes mellitus or increased lipolysis of adipose tissue in NAFLD. FAs enter the liver in greater amounts than they are eliminated through β-oxidation or through VLDL secretion. Increased lipid buildup and Impaired lipolysis of LDs are brought on by lipotoxicity, causing oxidative damage, Stress on ER, and engagement of kupffer cells that create inflammatory mediators (cytokines) and produce inflammatory responses. Lipotoxicity also impairs the operation mitochondrial ETC, which results of formation of ROS. These reactive oxygen species (ROS) and inflammation trigger hepatic stellate cells (HSCs) that stimulate overproduction of extracellular matrix, which causes persistent fibrosis or scarring.

2.2. Mitochondrial Role in NAFLD

In the liver, there are 500–4000 mitochondria/hepatocytes, which make about 18% of the cell volume [33]. Mitochondria perform a number of crucial tasks in addition to β-oxidation and ATP synthesis, such as activation of Ca2+ communication and ROS production [34-36]. Studies suggest, mitochondria have been related to inflammasome activation and cell death [37]. Furthermore, mitochondria communicate with several cellular organelles, including the lysosomes and ER [38-40]. Hence, several metabolic disorders, such as NAFLD, Cirrhosis, NASH, and HCC, have been linked to dysfunction of mitochondria.

When mitochondria receive more resources than what they need to produce ATP, NAFLD develops. The response of biochemical functions promoting mitochondrial integrity is regulated by nutrient supply, which also affects cellular redox signalling, ATP demand, and mitochondrial function. [41]. In addition to the aforementioned processes, growing data suggests that alterations in mitochondrial function play a significant role in the pathogenesis of NAFLD [42-44]. NAFLD is caused by mitochondria-related variables include decreased β-oxidation, excessive levels of ROS production, a dysfunctional ETC in addition to ATP shortage, cellular dysfunction caused by oxidative stress, and mitochondrial ultra-structural shifts. The advancement of NAFLD are aided by these alterations in mitochondrial function and structure, which increase hepatic lipid buildup and set off inflammation and fibrogenesis [42,43,45]. At the outset of NAFLD, mitochondria appear to increase beta oxidation, cellular respiration and level of ketone bodies in humans and mice [44, 45]. When comparing with lean participants, obese people without MASH had higher cellular respiration, so that point to the adaptability of mitochondria present in liver at the initial phases of insulin resistance related to obesity [46]. An increase in reactive oxygen species due to a failure to maintain mitochondrial function has been linked to damage of mDNA, strain on the endoplasmic reticulum, cell apoptosis and inflammatory processes. By comparing individuals with uncluttered steatosis and those with NASH, Koliaki et al. found that patients of NASH had overproduction of ROS as compared to oxidative activities [46]. The production of inflammatory cytokines like tumour necrosis factor alpha and transforming growth factor beta are both upregulated in response to ROS activation of the NF-kappa B and the p38 MAPK signalling pathways [46]. As a result of inflammation,

stellate cells transform into collagen-secreted myofibroblasts then cause liver fibrosis Furthermore; mDNA damage triggers the inflammasome cause inflammation [47-50].

3. Current NAFLD treatment

There are four goals guide NAFLD management that is diet and lifestyle modification to facilitate weight loss; the management of risk factors for cardiometabolic disease; treating any and all modifiable risk factors associated with NAFLD's later stages; and protecting against liver and other organ problems [51-54]. An ideal treatment for NAFLD would theoretically lessen hepatotoxicity, and inflammation. Metabolic improvement is also necessary to avoid type 2 diabetes, heart disease, and malignancies outside of the liverIt's likely that a lone actor (or strategy) won't be enough to bring about these results. There are so a number of promising treatment strategies available (summarised in Fig.2.) and now being investigated in a variety of clinical trials [53].

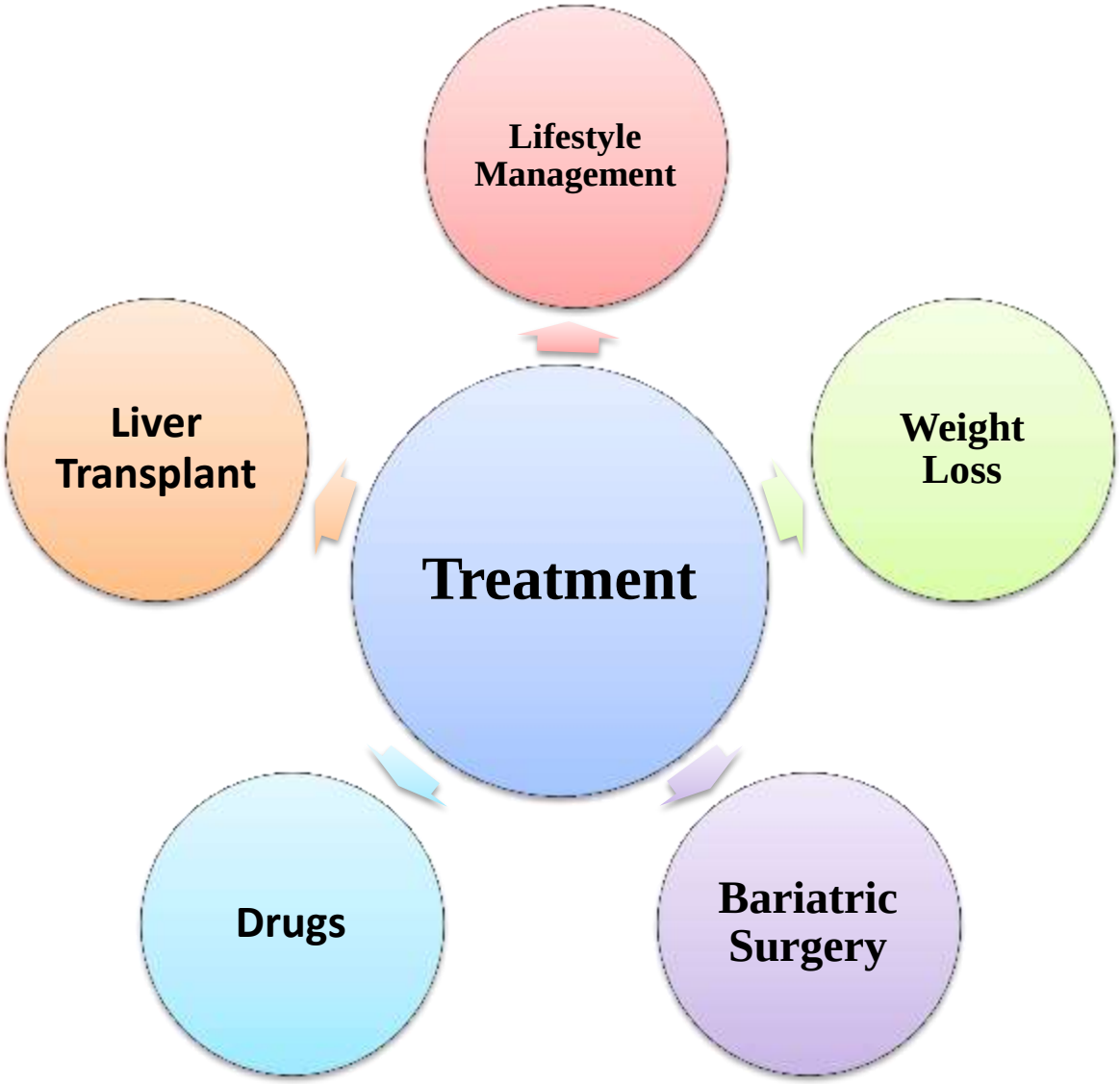

Fig.2. Current NAFLD Treatment

This narrative report reviews NAFLD treatments for people who have or do not have T2DM as well as suggests future treatments, ongoing trials, and outstanding problems.

3.1. Modifications to One's Way of Life

Despite the absence of the reduction in weight, changes in lifestyle have been shown to have a favourable influence on NAFLD [55-57]. It is currently known that a 5% weight loss reduces liver fat and improves liver damage, whereas a more than 7% weight loss improves histology of NASH [55-58-61]. Interestingly, a correlation was seen between the percentage of weight lost and improvements in histologic markers in NASH [59]. Hepatic fibrosis is the most reliable predictor of mortality in individuals experiencing NASH, despite the fact that inflammation is necessary for the course of the illness [62, 63]. A greater impact on lowering liver fat can be achieved through lifestyle therapies that involve a perfect balance between exercise and dietary changes [64]. Nevertheless, more than half of the individuals who participated in the clinical studies were unable to accomplish this degree of weight loss [65]. Hence, lifestyle therapies improved NAFLD but are hard to maintain [66].

Nutritional therapies are effective in reducing NAFLD, whether or not they are combined with exercise, however the optimal diet and eating pattern remain controversial [67-70, 59, 61, 65, 71]. Most clinical and preclinical research show that NAFLD benefits from all type of exercise techniques and its intensities. With or without dietary changes, exercise lowers steatosis, biochemical parameters of liver, blood glucose level, as well as insulin level [72]. Exercise reduces oxidative stress, modulates liver structure and avoids damage of liver [73]. Exercise decreases liver fat through decreasing the expression FA but also increased expression of FA oxidation, minimizing both inflammation and oxidative stress, elevating antioxidant enzymes level [74]. Nevertheless, mitochondrial and gene regulation pathways associated with liver response toward changes in lifestyle need further study. Either Hypocaloric diet, or exercise, or both reduce liver steatosis, according to the 2018 ASSLD practise guidance. Steatosis improves with three to five percent weight loss, but fibrosis requires seven to ten percent weight loss. [75]. Guidelines from the National Institute for Health and Care Excellence (NICE) also included some recommendations [76] in Table 1. A small weight decrease of 3–10percentage points may help non-obese NAFLD clients recover from NAFLD, according to some research indicating that these patients are somewhat more inclined to keep weight loss adding normal liver tests throughout period, in comparison to obese NAFLD patients [77].

Drugs	Exercise	Diet	Guidelines
Pioglitazones in cases when type 2 diabetes is prevalent	3-5 intervals of aerobic activity at a moderate level of intensity or Ideally, should spend between 150 and 200 minutes exercising every week. Getting rid of 7–	diets that cut 500–1000 calories each day.	EASL-EASD-EASO[78]

	10% of your body weight is recommended as well as Limiting alcohol and coffee use are recommended.		
Vitamin E and pioglitazones with T2DM as well as NAFLD	a modest amount of physical activity that amounts to 150 minutes each week	Reduce the amount of alcohol you drink and focus on adopting a low-calorie diet with daily calorie cuts ranging from 500 to 1000 kcal	AASLD [79]
Patients with advanced fibrosis who do not have diabetes are given vitamin E, and patients who have type 2 diabetes are given pioglitazone in addition to vitamin E.	Aerobic exercise should last a minimal amount of 45 to 60 minutes each day to combat obesity	It is recommended that obese individuals adhere to a diet that results in a daily deficit of 600 calories; limit their use of alcoholic beverages; omega-3 fatty acid supplements are not recommended for long-term weight loss.	NICE [76]

Table 1. Management of NAFLD according to EASL-EASD-EASO, AASLD and NICE guidelines.

3.2. Surgery

When it comes to helping obese people lose weight, studies have shown that bariatric surgery is preferable to more traditional methods [80]. It can contribute about 40% reduction in the long term incidence of obesity [80] and treat the numerous ailments that are related to that as well [81 60]. Over through the previous ten years, there has been an estimated increase in surgical bariatric treatments of 761% worldwide [82 61]. Based on their method of action, surgeries can be classified into three general groups [83 62]:

(1) Surgically decreasing the stomach's size is one way that restrictive surgeries try to limit how much food a person can eat. The most frequently used restrictive techniques are included sleeve gastrectomy, laparoscopic adjustable gastric banding, and vertical banded gastroplasty;

(2) Because they require more technical skill to do than restrictive treatments, malabsorptive operations are less common. In order to reduce the amount of food absorbed, procedures try to

bypass a section of a small intestine. The most common site of operation is duodenal pouch at biliopancreatic diversion;

(3) The Rouxen-Y gastric bypass is a hybrid technique that attempts to reduce meal consumption by forming a narrow gastric pouch while simultaneously limiting absorption by omitting the proximal small bowel. In doing so, it prevents some of the undesirable consequences of malabsorptive processes, like nutritional deficits and diarrhoea, by maintaining 95% of the small intestines.

The following elements, which are clearly shown in the flowchart (Fig.3.) to contribute significantly to the aetiology of NAFLD, are likely to be improved in some way by bariatric surgery.

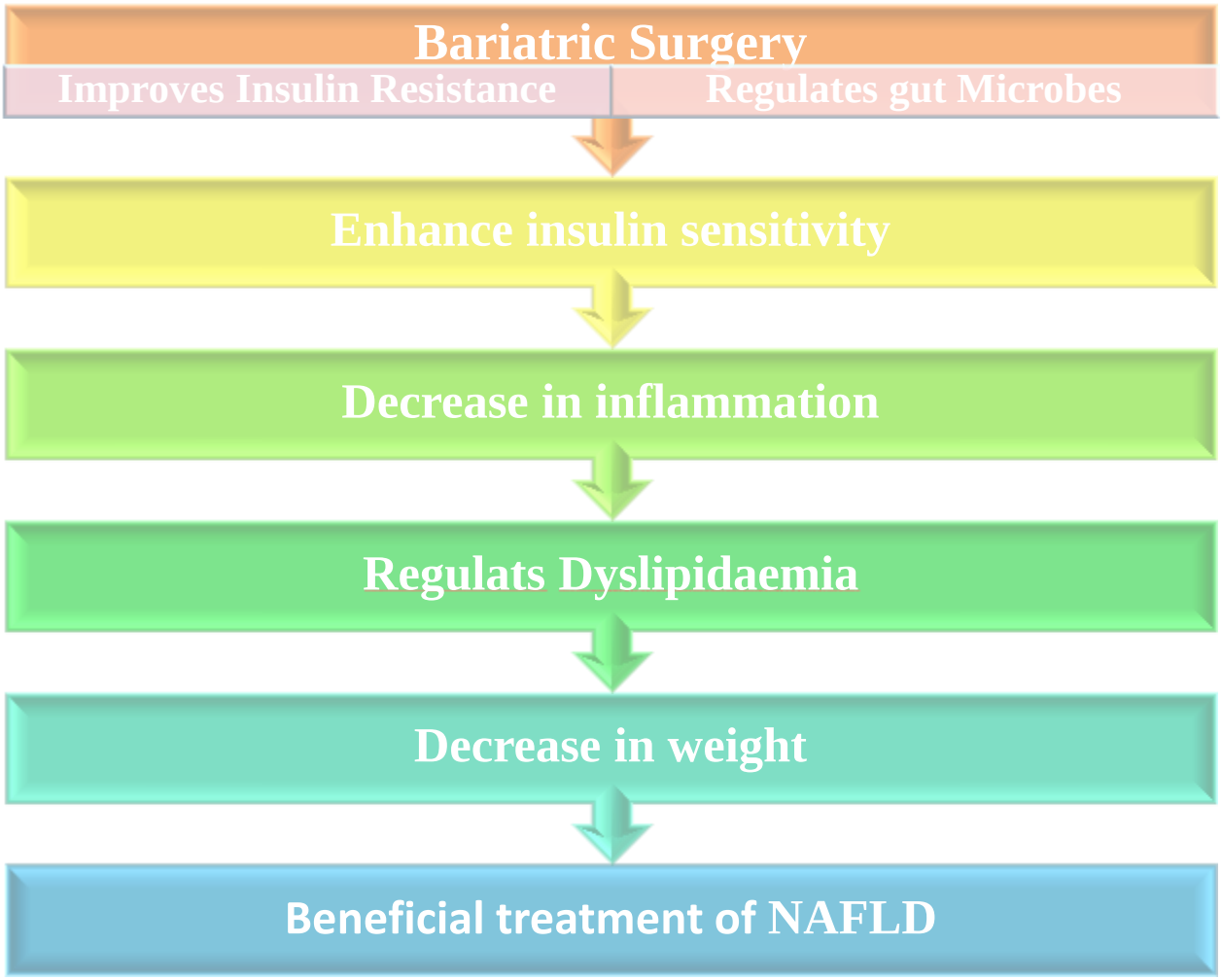

Fig.3. Bariatric Surgery

3.3. Modification of Gut Microbiota

NAFLD is caused by dysbiosis of the gut microbiota, which results from alterations in gut hormones, inflammatory markers and metabolites. The development of liver disease is linked to the prevalence of certain types of bacteria. In non-obese patients with NAFLD, changes in liver

fibrosis severity or the number of Eubacterium are negatively correlated [84] and in contrast, posively correlated with bacteria named *Ruminococcaceae* and *Veillonellaceae* [85]. Liver disease can be improved by altering the gut microbiota using a variety of methods, such as faecal microbiota transplantation, lifestyle adjustment (managing diet), antibiotics, and others [86]. Livers of NAFLD patients can be improved by probiotic supplementation that has been shown to decrease the expression of C-reactive protein and tumour necrosis factor (inflammatory factors) [87]. After six weeks of treatment, allogenic faecal microbiota transplantation (FMT) from thin, healthy individuals to NAFLD patients can lower small bowel permeability [88]. FMT does not have much of an impact on metabolic syndrome or liver dysfunction, though. Additionally, a phase 2 double-blind trial revealed that only a yearlong course of synbiotics, the combination of the two of prebiotic and probiotic merely altered the gut microbiome, whereas it had no effect on the liver's fat levels or inflammatory reaction indicators [89].

Additionally, BS modifies the microbial makeup of the GIT. At instance, having a laparoscopic gastric bypass, the prevalent Bacteroides bacteria decreased [90]. Patients are impacted by BS in part due to changed bacterial metabolism and bacterial composition. [91].

3.4. Drug-based strategy

What population will receive NAFLD/NASH pharmacologic treatment is the most challenging topic to answer? According to the 2016 European practise recommendation, For Nafld individuals who have fibrosis of grade 2 or above plus initial scarring who are at high risk of cirrhosis, pharmacotherapies must be taken into consideration [92]. The prevalence of severe fibrosis (stage 2 or higher) is the good determinant of deaths in people experiencing NAFLD, as found in a meta analysis that looked into five adult having NAFLD cohorts. [93]. Here the AASLD statement further advises that individuals have nafld and cirrhosis should be the only ones eligible for pharmacotherapies with a primary focus on improving liver problems [94]. According to AASLD guidelines non aggressive type nafld has no need of pharmacological therapy, but even they strategically need to protect renal or cardiac diseases.

4. Clinical trials:

An interventional clinical trial, according to the World Health Organization (WHO), is a study that subjects people through one or maybe more therapies in order to assess how the treatments affect their health. As per the WHO's requirements, every trial must have been filed prior to participant recruiting may start. The clinical trials that have been done with the goal of treating NAFLD and NASH are summarised in this study.

In RCTs using resveratrol treatments for obesity featuring weight loss is the endpoint, as listed in ClinicalTrials.gov, Hillsley et al. 2022 assessed unique features across the board. A polyphenol called resveratrol has been shown to help obese people lose weight. However, the variety in RCT designs has made it difficult to compare the efficacy of resveratrol consumption encourages the weight loss in obese patients. The researchers discovered differences in the criteria used to include participants that are age ranges of participants, sample size, BMI, sex of participants, and their medical history, with design of an intervention like doses, delivery methods, and time frames and key results assessed by means of biochemical methods. They found five delivery

methods for therapeutic resveratrol that design for the periods varying between2 weeks to up to 6 months. They found that the study sample size varied by over three times, the minimum inclusion age varied by twice. Only three out of the seven trials that were reviewed listed weight loss as the major outcome. As a result, the variation in resveratrol study design raises the possibility that results linked to weight loss are challenging rotation estimation and comprehend. The results of human investigations have, in fact, yielded conflicting results. It could be explained by the study of heterogeneous design, which comprises significant variations in the sex of sample population, its age, BMI, their basic health problems, and assessments of end-point or outcomes (95).

A clinical trial on the bispecific antibody BFKB8488A receptor, which targets both Klothoβ and fibroblast growth factor receptor 1c, was designed by Wong C et al. in 2022. NAFLD patient having type 2 diabetic mellitus (T2DM) were evaluated for the tolerability, safety, pharmacokinetics as well as pharmacodynamics, and immunogenicity of BFKB8488A in this phase 1b study. For 12 weeks, individuals were allocated to take a placebo and maybe the drug BFKB8488A at various doses in different intervals of dosing That's once a week, twice a month, or four times a month. The safety of BFKB8488A was the main result. A total of 153 patients were enrolled in which 62 patients have only nafld and 91 have nafld with T2DM.they all were prescribed at least one dose of medication. With exposure increments that were more than the proportion of dose, BFKB8488A did not show a linear pharmacokinetics. There is 22.7% incidence of Anti-drug antibody was occur related to treatment. High-density lipoprotein (HDL) levels often increased in response to drug exposure, while triglyceride levels decreased. Low-exposure tertiles saw decreases in both enzymes alanine aminotransferase as well as enzyme aspartate aminotransferase, while high-dose tertiles had decreases of 7.3% and 11.2%, when compared to gains respectively, in those receiving placebos on the day of 85. Patients who have non-alcoholic fatty liver disease at Day 85 experienced a reduction in fat of liver from baseline of 13.0%, 34.5%, and 49.0%, respectively, compared to 0.1% with placebo (96). When dealing with patients who have T2DM with NAFLD, BFKB8488A was well tolerated and reduced triglycerides and improved HDL level in blood. Both populations' liver health indices show BFKB8488A patterns of improvement, and patients with NAFLD have a notable decrease in liver fat.

The purpose of the research conducted by Amrousy DE et al. in 2022 was to examine the effects of vitamin D supplementation on the development of NAFLD and the accumulation of fat in the livers of children. Vitamin D has been shown to have anti-inflammatory and insulin-sensitizing effects. Unfortunately, data on vitamin D's effect on fat levels in children having nonalcoholic fatty liver disorder is limited (NAFLD). Out of 109 children with histological NAFLD who started the randomised controlled trial, only 100 completed it. Patients in the treatment group were given Vitamin D3 at a dose of 2000 international units per day over a period of six months, while those in The placebo was administered to the control group. At the first step and end of the study, biochemical parameters such as aspartate aminotransferase (AST), vitamin D, alanine, and total cholesterol (TC), LDL, TG, HDL, FBG, HOMA-IR, and FBG are measured (FBI). Liver

biopsies were performed at the beginning, the middle, and the end of the study on each and every child who participated in the clinical research. The results of a liver biopsy performed after therapy revealed that those in the treatment group who had steatosis of the liver with inflammation throughout the lobules had greatly improved. However, there was no appreciable change in hepatocyte ballooning or fibrosis. The therapy group had higher HDL and vitamin D levels and lower ALT, LDL, TG, AST, FBI, FBG, and HOMA-IR, than the placebo group. It has been established that giving children vitamin D supplements can effectively heal paediatric conditions of NAFLD (97).

In 2022, a randomised controlled clinical experiment carried out by Khodami B et al. discovered the repercussions of sugar-free diets leading to fewer deaths. Those people who had NAFLD evidence confirmed by FibroScan were given a random assignment for a dietary intervention that would last for 12 weeks. The most important result was a change in the hepatic steatosis assessment from the beginning to 12 weeks. Alterations in glycemic indices, anthropometric characteristics, inflammatory markers, lipid profiles, and liver enzymes were some of the secondary outcomes that occurred as a result of this study. Despite the fact that it is common knowledge that consuming a large amount of saccharose in addition to fructose promotes to the development of even more NAFLD. Responses that adult individuals with non-alcoholic fatty liver disease have not yet been investigated in relation to a diet low in free sugar in terms of how they affect disease management. As a consequence of this, it is essential to investigate how a diet low in free sugar impacts the primary characteristics of NAFLD. Patients with morbid obesity and NAFLD who follow a diet that is low in sugar or sugar-free may experience a reduction in hepatic fibrosis and steatosis, as well as a lowering of their total cholesterol and triglyceride levels, an improvement in their glycemic indices, and a reduction in inflammation (98).

Miriam B. Vos et al. conducted a clinical investigation on the use of losartan to treat juvenile NAFLD in the year 2022. There is currently no treatment for paediatric NAFLD that is known to exist. Because of the anti-fibrotic qualities that losartan possesses, it has already been proposed as a treatment option. losartan is a receptor blocker for angiotensin II. A multicenter, placebo-controlled, double-masked, randomized control trial in children with histologically proven NAFLD was done at 10 sites that picked from Clinical Research Network from Sept. 2018 to Apr. 2020. Age between years of 8-17, a histological evaluation of NAFLD of at least 3 score, also ALT level of at least 50 U/l were the inclusion criteria. Children were given either losartan (100 mg) or a placebo (placebo) orally once daily for a period of six months. The major comparison was made between the losartan group and the placebo group based on the change in ALT from the beginning of the study to the end. The wide variation in GGT, AST, CRP, HOMA-IR, serum lipids, anthropometric measures, and adverse events were secondary outcomes. An unanticipated interim study conducted while an enrollment halt was being carried out as a result of the coronavirus pandemic 2019 found a modest possibility about seven percent of considerable group variance. The Data and Safety Monitoring Board suggested that the trial be stopped earlier than planned. Losartan was safe to use for the treatment of paediatric NAFLD for a period of 6 months; on the other hand, GGT and ALT levels did not improve, despite the

fact that these two biomarkers are strongly linked to histopathological improvement in case of NAFLD (99).

Up to September 23, 2021, Denfenge Ren et al. 2022 combed ClinicalTrials.gov for trials including stem cell therapy for liver disorders. The team conducted an investigation into the type of study, origin of stem cells, and type of liver illnesses that were present in the trials that were included, and the SPSS18.0 programme was used to assess these features. 92 of 559 ClinicalTrials.gov studies were selected. Eight (8.70%) were observational and 84 were interventional. There were a total of 44 trials, 24 (28.57%) of which were designed to test the efficacy, 16 (19.05%) were designed to examine the safety, and the remaining 44 (52.38%) were designed to evaluate both. 81.52 percent of the clinical trials used mesenchymal stem cells (MSCs) as their source of stem cells. Of these 73 trials, 26 used stem cells derived from the bone marrow from mesoderm, 22 used stem cells (MSCs) derived from placenta, 20 used unclassified mesenchymal stem cells, and five used stem cells derived from adipose tissue. Sixty trials classified cirrhosis, 16 liver failure, and eight autoimmune liver disease. About 22 studies were accomplished while four already had findings on internet. According to the findings of the study, more clinical trials of stem cell therapy for liver illnesses are required, and trial sponsors are urged to disclose their findings (100).

El-Kady et al. 2022 designed a randomised clinical trial on nicotinamide supplementation in diabetics with fatty liver disease. In animal models of nonalcoholic fatty liver disease (NAFLD), nicotinamide prevents liver steatosis but also metabolic abnormalities. Seventy patients with diabetes and non-alcoholic liver disease (NAFLD) were split into two different groups, then given either nicotinamide (n = 35) or a placebo (n = 35) in addition to their antidiabetic treatment for 12 weeks. The improvement in score of liver steatosis was the primary outcome, and secondary outcomes were level of liver enzymes, assessment of liver stiffness, insulin resistance, lipid profile, serum adiponectin, serum malondialdehyde, and also patients' quality of life. The research was only carried out on 61 individuals in all, with 30 participants receiving nicotinamide and 31 serving as controls. Both between-group and within-group comparisons found that differences in steatosis and fibrosis scores were not statistically significant. Nicotinamide was well tolerated at a dose of 1000mg once in a day, improved metabolic anomalies and enhances quality of life in diabetic NAFLD patients, but had little effect on liver steatosis or fibrosis (101).

NAFLD patients were studied by Zhang Y et al. 2022, who looked at the consequences of GLS in terms of both the clinical effects they had and the quantities of FGF-21 found in their serum.Evodiae Fructus and Coptidis Rhizoma both have the ability for NAFLD treatment. An essential self-care strategy in NAFLD is increased fibroblast growth factor (FGF) 21 secretion by liver cells. The 126 patients with NAFLD who participated in the 3 months, exploratory drug trial were split evenly between the GLS group and the polyene phosphatidylcholine (PPC) group. Allocation anonymity was ensured by using DPS-generated random numbers in conjunction with sealed, opaque envelopes. Anthropometric variables, liver enzymes, blood sugar, lipids, and fibroblast growth factor 21 were measured at the beginning and conclusion of the trial, and two

software named ultrasonography (US) and US-based controlled attenuation parameter (CAP) were used to evaluate hepatic fat deposition. The Value of the CAP at 3 months was key indicator of success in this trial. Improvements inlipid profile, anthropometric parameters, FGF 21, glucose, biochemical parameters, hepatic fatty deposition and as measured by ultrasound were secondary outcomes. In this investigation, an exploratory clinical trial with an open-label was used. It took a lot of effort to go blind either the Those receiving care and those providing it in the study due to the specifics of the investigation, such as the multiple dose forms of PPC and GLS. As a result, we decided to construct open trial or experiment instead. Clinical trials with an open endpoint on medications with clinical experience advise and confirmation clinical trials that will either start up or be halted in a climate Given a low proportion of new drugs being approved for use, and worrying research and development money waste. Without an adequate treatment currently on the market, NAFLD might benefit from GLS, as shown by medical findings (102).

Deoxyribonucleic acid from chum salmon milt has been studied for its potential to enhance hepatic functions, its safety and efficacy have been assessed by Takahashi Y et al., 2022. The growing prevalence of liver damage caused by diet a common cause for concern in terms of public health. In animals, hepatosteatosis is improved when treated with DNA derived by chum salmon, It is a waste product from making the medicinal ingredient protamine. SM DNA was tested on healthy Japanese patients with slightly impaired liver function, normal body mass index but high alanine aminotransferase levels, in the parallel, double-blind, randomised investigation. The study involved 50 people in which SM DNA (530 mg day−1) and placebo (dextrin) were given to 24 and 26 subjects, respectively. SM DNA did not improve primary like liver-to-spleen ratio and hepatic functions or secondary outcomes like NAFLD blood glucose, fibrosis score, blood lipids, serum protein levels, inflammatory markers like adipokines, cytokines. Specific gender group findings indicates that guys who ingested SM DNA had better primary and secondary results than placebo-treated males. On the other hand, researchers did not find any evidence of this effect in females. Altogether, the results of this clinical investigation revealed that SM DNA may be able to improve hepatic function in males and demonstrated that SM DNA possesses the ability to combat obesity (103).

Therapeutic effects of rosemary leaf on liver function as well as biomarkers have been the subject of experimental clinical trials, which Akbari S. et al. 2022 have explored. The purpose of this research was to determine whether or not individuals having NAFLD benefited from combining a diet low in calories and high in rosemary leaf powder. One hundred ten participants were involved randomised research study, with half receiving 4 g powder of leaf of rosemary other half receiving powder of starch as placebo, for 2months. All of the participants were also given advice on how to lose weight by changing their eating habits and increasing their exercise. After 2 months, Of the several ways beta-cell dysfunction was evaluated, just the homeostasis model demonstrated a statistically significant distinction between both the placebo and rosemary groups. Results were better in the rosemary group, yet, there was no discernible improvement over what was achieved by the diet and exercise regimen simply, according to the present clinical trial study (104).

The clinical experiment of hydrogen/oxygen inhalation by Tao et al. 2022 (China Clinical Trial Registry) involved 43 participants and lasted for 13 weeks; some received placebos during the trial. They discovered that serum lipid and hepatic enzyme levels improved due to hydrogen/oxygen inhalation. In extreme cases, CT scans and ultrasonography showed a marked reduction in liver fat content after inhalation of hydrogen/oxygen. The team also conducted an animal study using a mouse model of NASH caused caused by an MCD diet (low in methionine, low in choline). The liver histopathology and systemic inflammation were both enhanced by inhalation of hydrogen/oxygen. Mice exposed to hydrogen/oxygen by breathing experienced increased autophagy, however this effect was reversed after being treated with chloroquine. As an added bonus, AML-12 cells exposed to molecular hydrogen showed a marked reduction in lipid formation. Palmitic acid (PA) incubation caused autophagy, and additional incubation with 20% hydrogen boosted this process. The suppressive impact of hydrogen on lipid accumulation within cells might be reversed, to a lesser extent, by adding 3-methyladenine. One possible mechanism by which hydrogen affords protection is via stimulating hepatic autophagy. As a whole, patients with moderate to severe NAFLD reported improvement after using hydrogen/oxygen inhalation (105).

In a 2022 study, Alami F. et al. looked into how a fruit-rich diet (FRD) affected lipid profile, liver steatosis, hepatic enzymes, Insulin resistance, and in people having NAFLD. About eighty NAFLD adults participated in this randomised controlled experiment. There were two groups of participants: those with FRD who ate at least four fruit servings each day, and those with the control group who ate only about two servings. The grade of steatosis, blood levels of liver enzymes lipid and glucose level at the starting point and at the end point of the study. According to the findings of the current study, consuming more than four servings of fruits per day can make steatosis, dyslipidemia, and glycemic control worse in NAFLD patients. Further research is needed to understand how exactly fruits affect NAFLD (106).

Chavez-Tapia NC, et al. 2022 assessed how disseminating more diagnostic data might affect patients' decisions to seek treatment after receiving a diagnosis of NAFLD. There are significant barriers to diagnosis and treatment adherence for patients with chronic diseases like NAFLD. Prevalence of Nonalcoholic Fatty Liver Disease is rising, which means there is a greater demand for incentives to encourage individuals to seek medical care. There are currently no scientific proof therapies that can fill this gap. Researchers in Mexico conducted a study in which they divided persons who had been given a sonographic assessment of NAFLD into one of five groups using a random assignment process. Every single group was provided with medical advice. In first group, there were no further therapies, second group got multimedia educational material (MEM); third group received MEM as well as NAFLD fibrosis score (NFS); then fourth group got with transient elastography (TE); and the last fifth group received MEM with NFS and also TE. 1209 randomised participants with 91% follow-up, 82% male, and a BMI of 30.5 4 kg/m2 were studied. Those in groups that were well informed were more likely to seek out specialised healthcare services. More diagnostic information appeared to encourage patients to

seek medical attention. If patients with chronic diseases were given more specific information about their diagnosis, it might motivate them to get health care (107).

The purpose of the review by Yu L et al. 2022, which comprised five clinical research and nine preclinical investigations, was to determine the clinical effectiveness of garlic supplementation against non-alcoholic fatty liver disease (NAFLD). The published dates of the clinical studies spanned from 2016 to 2020; all were double-blind randomized clinical trials conducted in Iran; and 470 patients were enrolled across the investigations. Biomarkers of oxidative stress, markers of metabolic health, markers of liver function, and dietary intake were all measured. The evaluation of side effects was provided in one of the included studies. Garlic powder was swallowed by each patient, however the length of the therapy as well as the amount taken by each patient were different. Preclinical research suggests that balancing lipid metabolism and preventing oxidative stress and also hepatic steatosis with garlic supplementation may have beneficial effects. Evidence from a pooled analysis of five randomised controlled trials suggests that using garlic powder might improve oxidative stress, insulin resistance, and dyslipidemia. Meta-analysis was done by filtering data redundancy because all RCTs in this study exhibited multiple publication bias. Due to a paucity of evidence, additional clinical data supporting that supplementation of garlic has significant effect on NAFLD is required (108).

Clinical trials were conducted by Sara et al. in 2022 to determine the impact of probiotic yoghurt on NAFLD-related liver enzymes, steatosis, and fibrosis. Axis of the intestine to the liver is a potential target for probiotics within the context of NAFLD therapy. To participate Whilst on trial, 68 people having NAFLD would be enrolled. Patients were randomly allocated to consume either a probiotic yoghurt in 300gram per day which contains lactic stain like acidophilus, Bifdobacterium lactis and also Lactobacillus in the quantity of 106 cfu/g or a plain yoghurt (300 g/d) for 3 months, according to age, sex, and their BMI as block factors. At the beginning of the trial as well as after the intervention, participants had their height, weight, and size of waist measured. Liver markers, Plasma glucose, lipid profile and also serum insulin were measured at beginning and conclusion. QUICKI and HOMA-IR were used to measure insulin resistance and insulin sensitivity. Liver fibrosis and steatosis were examined with help of software called fibroscan (109).

Tofogliflozin and pioglitazone were examined by Yoneda et al. in 2022 for the treatment of hepatic steatosis in patients who have the combination of type 2 diabetes and nonalcoholic fatty liver disease (NAFLD). This study is the continuation of their earlier efforts and is part of the ToPiND research project. Individuals having T2DM with NAFLD and also have liver fat fraction of 10% who were examined under MRI was going through in this clinical trial. Patients who qualified for the study were chosen at random either to receive 15-30 milligram of pioglitazone or 20 mg of tofogliflozin by oral route per day for 6 months; afterward, they received both drugs for a further 6 months. Both single as well as combination therapy for diabetes mellitus and liver steatosis were evaluated before and after treatment. Thirty-two patients who met the criteria for the study were given the concoction

of pioglitazone as well as tofogliflozin both drugs. Compared to each monotherapy group, the combination therapy improved liver steatosis and its stiffness, glycated haemoglobin, Lipid profile, and levels of biochemical markers (110).

5. Conclusion

Metabolic disorders are increasing NAFLD and NASH rates. NAFLD accelerates HCC, the main liver malignancy. Nevertheless, there is no treatment that is currently accepted for NAFLD or NASH at this time. Hepatic steatosis and nonalcoholic fatty liver disease (NAFLD) are both conditions whose pathophysiologies have been better understood. How NAFLD starts and then advances to fibrosis, the condition that is the best predictor of death in NAFLD patients, is an issue that has recently gained significant importance. NAFLD can be effectively treated by a variety of lifestyle modifications. In order to examine prospective treatment strategies for NAFLD, preclinical investigations as well as clinical trials have now been carried out including things like synbiotics, probiotics, angiotensin-II receptor blockers, topogliflozin, CCR2/5 antagonists, and FXR agonists, amongst other things. It's possible that a combined treatment, like one that combines medical care and exercise, could shorten the duration of treatment while also improving the outcome.

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