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# The Role of Microbiota in the Occurrence of Non-Alcoholic Fatty Liver Disease

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## Abstract:

Non- alcohol fatty liver disease (NAFLD) is the world's most common liver disease. In Western countries, the prevalence between 20 and 30 % is reported among the adult population. Nutrition, excess intake of saturated fats, and high caloric food, along with low intake of vegetables, fruits, proteins, grains, and 3-Fatty Acids are key causes of NAFLD growth. Human beings have nearly one thousand bacterial organisms and several millions of bacteria, with 150-time more genes than the human genome colonizing in the human intestinal tract. Firmicutes (Lactobacillus, Peptoniphilus, Ruminococcus, Clostridium, and Eubacteria), and Bacteroidetes (Bacteroides, Prevotella) are the two main phyla in the human intestines. However, the components and presence of gut microbiota vary due to a high heterogeneity among people due to several factors as age, sex, general conditions, pregnancy, hormonal changes, traveling, infection, and drugs as chemotherapeutic agents or proton pump inhibitors. We aim to demonstrate the effect of gut microbiota in the development of NAFLD.

**Keywords:** NAFLD, Gut Microbiota, Dysbiosis, Probiotics.

## Introduction:

Non- alcohol fatty liver disease (NAFLD) is the world's most common liver disease [(1)]. In Western countries, the prevalence between 20 and 30 % is reported among the adult population. This disease has a lower prevalence in Eastern societies, but some recent studies indicate that it is increasing due to variations in Eastern food habits, together with a decline in exercise sedentary lifestyle ("Westernized Society"). Nutrition, improper and excessive intake of saturated fats and excess caloric diet, along with a decrease in eating vegetables, fruits, proteins, grains, and 3-Fatty Acids are the main causes of NAFLD growth. [(2)]

NAFLD means a high accumulation of fat in the liver cells (hepatic steatosis). This is closely related to several risk factors like obesity, resistance to leptin, and insulin (IR), dyslipidemia, and metabolic syndrome. Nonalcoholic steatohepatitis (NASH) is the most severe form of the disease, which leads to cirrhosis in 20 % of people. NAFLD is a rising cause of end-stage liver disease. NASH patients are at risk for progression to

liver cirrhosis and/or hepatocellular carcinoma (HCC). The risk of end-stage liver disease due to NAFLD is expected to increase three times by 2030[3]. NAFLD's initiation and progression are generally assumed to be related to oxidetive stress, inflammation, dyslipidemia, insulin resistance, and obesity. This mechanism is affected by the interaction of several factors like diet, genetic background through biochemical and immune-biological changes in lipid and glucose metabolism. Despite several years of research on NAFLD's, the pathogenesis, still is uncertain, with several pathways. One possible mechanism is the direct link of the intestines to the liver through the portal vein. Microbiota colonized in the intestine can modulate metabolic processes that affect metabolic syndrome and its associated co-morbidities directly or indirectly [4].

## The gut microbiota (GM):

Human beings are known to have nearly one thousand bacterial organisms and several millions of bacteria, with 150-time more genes than the human genome colonizing in the human GIT. Firmicutes (Lactobacillus, Peptoniphilus, Ruminococcus, Clostridium, and Eubacteria), and Bacteroidetes (Bacteroides, Prevotella) are the two main phyla in the human intestines. Some phyla less common are Actinobacteria, (Bifidobacterium), Proteobacteria, and Verrumicrobia. The components and presence of GM vary due to a high heterogeneity among individuals due to several factors as age, sex, general conditions, hormonal changes, pregnancy, traveling, infection, and the effect of drugs as chemotherapeutic agents or proton pump inhibitors. [5]

## Dysbiosis:

Dysbiosis can be identified as an imbalance between healthy and pathogenic microorganisms; it is manifested as changes in diversity and variations in the presence of specific microorganisms. The homeostasis of GM is critical for maintaining health and protecting against diseases in the host. The homeostasis of GM is essential for maintaining health and protection against several diseases in humans [6]. Several studies have demonstrated the association of GM dysbiosis with metabolic disease, obesity, DM type2, and NAFLD. Zhu et al. 2013 used 16S rRNA sequencing and concluded that NASH patients have a unique phylum, population, and ratio of firmicutes, bacteroidetes, and actinobacteria in comparison to healthy individuals. NASH patients have an elevated number of alcohol-producing bacteria that would elevate serum alcohol levels and oxidetive stress, resulting in liver disease [7].

Del Chierico et al 2017. compared NAFLD, NASH, and obese children with normal controls and found that NAFLD patients experienced high numbers of Anaerococcus (Actinobacteria), Ruminococcus (Firmicutes), Peptoniphilus (Firmicutes), Dorea (Firmicutes), Bradyrhizobium (Proteobacteria), and Propionibacterium acnes (Actinobacteria) but a reduced number of Rikenellaceae (Bacteroidetes) and Oscillospira (Firmicutes). There are minor changes in the composition of microbiomes between the NAFLD, NASH, and obese groups [8]. GM can be used as an indicator of the incidence and progression of NAFLD. Bacteroides number was excess in patients with both NASH and fibrosis, and ruminococcus was in excess number in patients with fibrosis. Although these studies demonstrated a relation between GM dysbiosis and NAFLD, gut dysbiosis remains unclear as a risk factor that causes NAFLD [9].

## The pathogenesis of NAFLD and microbiome:

NAFLD / NASH development pathophysiology is complex, and the concept of a "multiple hit" suggests the theory that several attacks function together to promote disease progression. Insulin resist-ance, genetic and epigenetic causes, dietary supplements, and intestinal microbiota are some of these threats. The liver receives portal venous circulation and is exposed from the gut system to both food supply and GM dependent metabolites. Because of its similar anatomy, the gut-liver axis is distinguished by a functional, bidirectional relationship between the GIT and the liver. GM and liver have interconnected relationships that are very complicated and mediated by a complex metabolic and immuneologic network. Initial mechanism can be demonstrated as modifying energy harvest mode, inflammatory cytokines and several signaling pathways, altered biochemical changes, and GM-derived metabolites (bile acid, short-chain fatty acids, aromatic AA derivatives, branched-chain AAs, and ethanol [(9)].

## Intestinal Microbiota and the Host Immune System:

Several factors as obesity, diet, infection with alcohol intake, and drugs may affect the microbiome, resulting in a defect in the intestinal integrity, bacterial overgrowth, and release of LPS. The endotoxemia enters the liver through the portal circulation, which stimulates the release of inflammatory cytokines, which results in a liver injury that may contribute to NAFLD. Patients with NAFLD also had higher rates of small intestinal bacterial overgrowth (SIBO), associated with the disease severity [(10)].

Defective gut barrier, bacterial overgrowth, and bacterial translocation may enable the passage of bacterial endotoxin into the liver. Lipopolysaccharides activete Kupffer cells by toll-like receptor activation (TLRs). These TLRs are pattern recognition receptors that identify pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) associated with damage and are inactive in healthy liver cells. If the intestinal barrier is defective and endotoxemia reaches the liver-gut through the gut-liver axis. This will lead to increased levels of PAMPs and DAMPs binding with TLRs (TLR2, TLR4, TLR5, TLR9, etc.), the release of inflammatory cytokines (TNFα, IL-8, IL1β) could be initiated and the aggregation of lipids and liver cell death could be induced leading to the release of NAFLD, NASH, and cirrhosis. In a NAFLD host, dysbiosis plays an important role in compromising the local immunity of the mucosa. In dysbiosis, the numbers of CD4 and CD8 are reduced in the propria of duodenal mucosa lamina, and levels of TNFα, IL-6, and IFN γ are increased in the NAFLD patients [(11)].

## 2- Different effects of Microbiota on metabolites:

### a- Bile acids:

GM controls homeostasis of the bile acid. Through regulation of the expression of bile acid synthesis enzymes, the microbiome is involved in the synthesis of primary bile acids, cholic acid, and chenodeoxycholic acid. GM influences other processes of bile acid metabolism, as liver conjugation, terminal Ileum reabsorption, small intestine deconjugation, colon conversion to secondary bile acids (lithocholic acid and deoxycholic acid), and enterohepatic circulation, by influencing related enzymes or activity.

Altered metabolism of the bile acids resulting in metabolic and immunological changes leading to NAFLD. Bile acids by farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor 5 (TGR5) can regulate metabolism and inflammation. The primary bile acids activate FXR while the secondary bile acids activate TGR5 [(12)].

Bile acids help ensure the integrity of the intestinal barrier, guarding against inflammatory cascades in the liver that are related to GM. Bile acids may reduce hepatic inflammation and fibrosis through the signaling FXR and TGR5 Pathways. FXR prevents denovo lipogenesis, increases oxidation of fatty acids, and controls gene expression involving homeostasis of triglycerides, which decreases steatosis and controls gluconeogenesis downwards. FXR also regulates the synthesis of glycogen in liver cells and regulates the expression of glucose transporter 4 (GLUT-4) and glucagon-like peptide 1 (GLP-1) which influence insulin sensitivity associated with NAFLD. TGR5 influences glucose balance by inducing GLP-1 secretion, which enhances energy intake and decreases dietary obesity [(13)].

## b- Short-Chain Fatty Acids (SCFAs):

SCFAs are composed mainly of acetate, propionate, and butyrate [(6)].SCFAs minimize the synthesis of hepatic cholesterol and fatty acids while increasing the oxidation of lipids. SCFAs primarily function on the G-protein - coupled GPR-41 and GPR-43 receptors, which are widely distributed in the intestinal enteroendocrine L cells, liver, white adipose tissue, and skeletal muscles. These L cells synthesize glucagon-like peptides that act on hepatocytes by activating fatty acid oxidation genes and insulin sensitivity that are associated with NAFLD. SCFAs have anti-inflammatory effects by decreasing immune cell migration and proliferation (T cells, neutrophils, macrophages, monocyte cells), by decreasing several forms of pro-inflammatory cytokines (monocyte chemotactic protein-1, tumour necrosis factor-alpha, etc.), and by increasing anti-inflammatory cytokine PG E2 [(14)].

## c- Aromatic Amino Acid Derivatives and Branched-Chain Amino Acids:

A new category of bacterial metabolites derived from aromatic amino acids (AAA) has recently received attention including tryptophan, phenylalanine, and tyrosine, and has been considered to be factored in NAFLD development. Bacterial metabolites derived from tryptophan consist of indole, indole-3 propionic acid, tryptamine,indole-3 aldehyde, indole-3 acetic acid, and 3-methylindole. These compounds keep bowel integrity, limit bacterial translocation, prevent microbiota- products from being released, and reduce inflammatory cascades [(15)].

Bacterial metabolites derived from phenylalanine consist of phenylacetic acid, phenyl propionic acid, and benzoic acid. The levels of plasma phenylacetic acid (PAA) were found to be positively associated with the severity of steatosis. Branched-chain amino acids (BCAA), as valine, leucine, and isoleucine, are also involved in hepatic steatosis. There is also a strong relationship between the levels of hepatic steatosis and BCAA in plasma and urine. PAA can substantially elevate the use of hepatic BCAA that can synergistically promote the production of hepatic lipids [(16)].

## d-Choline:

In dysbiosis, the choline synthesis is impaired, and choline deficiency was associated with NAFLD. Dysbiosis is capable of metabolizing trimethylamine choline (TMA), which is oxidized by monooxygenase-containing hepatic flavin and converted to trimethylamine-N-oxide (TMAO). The benefits of this process: (1) Reducing the choline levels. (2) Increasing the TMAO levels. Choline plays a vital role in VLDL synthesis of VLDL and promotes the production of hepatic lipids and choline deficiency contributes to the accumulation of triglycerides in hepatocytes. TMAO also increases resistance to insulin and induces inflammation and oxidative stress [(17)].

## e- Microbial Synthesis of Ethanol:

Patients with NAFLD showed an excess serum level of ethanol, despite no alcohol intake. Patients with NASH displayed marked elevated levels of blood ethanol relative to their non-NASH lean or obese counterparts, together with a high number of ethanol-producing bacteria. Patients with NAFLD had increased endogenous ethanol production in the dysbiosis context [(18)]. Subsequently, the accumulation of endogenous alcohol from an increased number of alcohol-producing bacteria results in excess free radicals and reactive oxygen, which cause mitochondrial dysfunction, hepatic cellular inflammation, and damage. It also induces up-regulating de novo lipogenesis, decreases the oxidation of fatty acids, and decreases the lipoprotein (VLDL) exportation from the liver [(6)].

## Effect of Probiotics and Symbiotics in NAFLD/NASH Patients:

Probiotics are living microorganisms, non-pathogenic, which provide the host with a health benefit by modifying GM when supplied in sufficient quantities. Lactobacilli, Streptococci, and Bifidobacteria are the most commonly known probiotics in recent clinical trials. Prebiotics are non-digested carbohydrates that can be produced by bacteria, and then adjust the structure and dynamics of GM to facilitate health benefits. Synbiotics refers to Prebiotics and Probiotics combinations. Probiotics and synbiotics can normalize GM and reverse dysbiosis, which would help patients with NAFLD [(19)].

There are several mechanisms of their protective functions. This involves decreesing hepatic lipid deposition, decreesing endotoxemia, minimizing oxidative stress, anti-inflammatory effects by modulating the nuclear factor kappa B (NF-B), tumor necrosis factor ( TNF), and antifibrotic effects by decreasing (TGFβ) and collagen expression. These studies indicate probiotic therapy may be a possible pharmacological intervention for patients with NAFLD. Most clinical trials concluded that probiotics in NAFLD patients significantly enhance AST and ALT as compared to the placebo community. They used capsules of probiotics (Lactobacillus acidophilus, Lactobacillus case, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium long, and Streptococcus thermophiles), and 1000 mg of metformin per day [(20)].

## Conclusion:

With NAFLD's increasing incidence and prevalence, and the lack of appropriate pharmacological intervention, there is an urgent need to develop new treatment drugs. The restoration of dysbiosis may therefore be a possible therapeutic target

for NAFLD, by the use of probiotics and synbiotics in NAFLD and NASH. The literature to date demonstrates that probiotics/synbiotics may enhance liver enzymes, hepatic steatosis, and NAFLD activity score. Probiotics/synbiotics can also reduce proinflammatory cytokines such as TNFβ and (IL-1, IL-6, and IL-8). In the end, probiotics/synbiotics have a safe effect, furthermore, several studies can be done in the future.

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