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NON-ALCOHOLIC FATTY LIVER DISEASE: INTROSPECTION

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Summary

Non-alcoholic fatty liver disease (NAFLD) has become one of the top concerns for the practicing hepatogastroenterologist due to the obesity epidemic and it's potential to progress to advanced liver disease which significantly impacts on overall and liver-related mortality. NAFLD may cause the liver to swell (steatohepatitis). An inflammation in liver may cause scarring (cirrhosis) over time and may even lead to liver cancer or liver failure.

Review deals with pathophysiology, diagnosis and treatment and animal model for the NAFLD. It also focuses on the different kind of the animal models used in the preclinical study of the NAFLD. The effect of the individual diet component on the progression of the NAFLD is explained in details. Review also consist the recent development in the treatment of the NAFLD. In this review we have discussed the futuristic approach in the research of the NAFLD like, development of the animal model for preclinical studies, treatment for the NAFLD.

Key Word: NAFLD, Pathophysiology, obesity, liver biopsy, animal models, treatment

Introduction

Non Alcoholic Fatty Liver Disease (NAFLD) emerges as one of the most common liver diseases in Asian and Western countries. The number of patients is increasing rapidly, and more than one-fifth of the population is thought to suffer from NAFLD. [1, 2] Because of the rapid rise of obesity in children globally, NAFLD is now recognized as the most common cause of liver abnormality also in the pediatric population and a leading cause of referrals to liver clinics in many Western countries. [3]

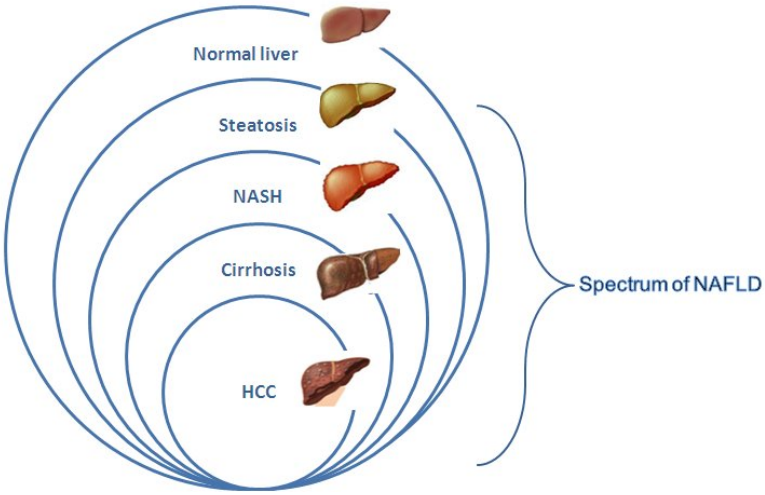

Figure 1: Progress of patients to the liver disease
NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; CH, cirrhosis; HCC, hepatocellular carcinoma

Non-alcoholic fatty liver disease (NAFLD) affects 10–25% of the population and its prevalence increases steadily to ~70–90% in people with obesity or type-2 diabetes. [4]

NON-alcoholic steatohepatitis (NASH) is a distinct hepatic disorder that histologically resembles alcohol induced liver damage, observed in patients without a history of significant alcohol consumption. It was first described in obese and diabetic women. [5]

NASH is considered to be part of the spectrum of non-alcoholic fatty liver disorders (NAFLD), ranging from bland steatosis to steatohepatitis and cirrhosis. Progression of the disease depends on the presence of hepatocellular damage, inflammation and fibrogenesis. [5]

NAFLD has four histological stages. [2] Fatty infiltration of the liver; Fatty infiltration plus inflammation (<33% of hepatocytes affected); Fatty infiltration with ballooning degeneration (33 to 66% of hepatocytes affected); Fatty infiltration with lesions similar to alcoholic hepatitis and sinusoidal fibrosis, polymorphonuclear infiltration with or without Mallory hyaline (>66% of hepatocytes affected).[2]

Coexisting pathological conditions frequently associated with NAFLD are abdominal obesity, Type 2 diabetes, Insulin resistance, Hypertension Dyslipidemia – the typical components of the metabolic syndrome (MetS). Their coexistence within the same individual increases the likelihood of having more advanced forms of NAFLD. [6]

The importance of NAFLD is increasing as a clinical entity from the following facts. [7]

It is next to alcoholic liver disease and chronic hepatitis C as most common reason for referral to a hepatology clinic. It plays a definite role in the progress and pathogenesis of diseases including chronic hepatitis C and alcoholic cirrhosis. It causes raised hepatic amino transferase levels persisting for 6 months or more in an asymptomatic individual. It is a potential cause of cirrhosis leading to end stage liver disease and rarely even hepatic carcinoma. [7]

Pathophysiology

Insulin resistance is an important driving force, which promotes lipolysis of peripheral adipose tissue which in turn increases free fatty acid (FFA) influx into the liver. Accumulation of hepatic triglyceride results when lipid influx and *de novo* synthesis exceeds hepatic lipid export and utilization Hyperinsulinemia and hyperglycemia also increases

the de novo lipogenesis as well as indirectly inhibits FFA oxidation. [8] Because of defective incorporation of triglyceride into apolipoprotein carrier proteins in the NAFLD lipid export from the liver gets impaired. [9] The steatotic liver appears to be susceptible to further hepatotoxic insults, which may lead to hepatocyte injury, inflammation, and fibrosis. [10] As shown in the fig 2. overgrowth of Gram-negative organisms in small intestine could promote insulin resistance. Adipose tissue inflammation leads to obesity, insulin resistance and development of type 2 diabetes. The gut micro biota is a rich source of molecules such as lipopolysaccharide and peptidoglycan that may cause inflammation in peripheral tissues of the body. These findings suggest that the gut micro biota may affect host metabolism by altering adipose tissue inflammation.[11] Secretion of the proinflammatory factor likes cytokines leads to the secretion of the Interferon á (INF α), interleukin β (IL α) and interleukin 1 (IL 1) which causes the free fatty oxidation and lipid peroxidation. [12]

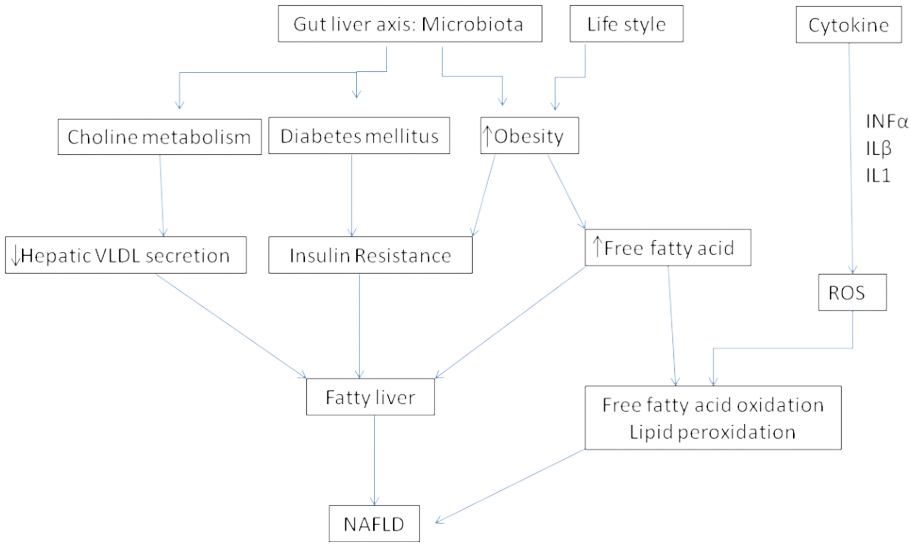

Figure 2: Pathophysiology of the NAFLD

The exact mechanisms promoting progressive liver injury are not well defined, although lipid peroxidation, cytokine induction, and mitochondrial dysfunction may appear due to oxidative stress. Microvascular insufficiency has also been implicated in the exacerbation of liver injury as shown in the figure 2. [11, 12]

Gender and prevalence of the NAFLD

It has been reported the significant effect of the gender exist in the prevalence of NAFLD. Earlier studies NAFLD found more common in women than in men. [13] But recent studies identified that NAFLD is as frequent in men as women [13]. In Japan prevalence of NAFLD or fatty liver disease was found approximately two fold higher in men than in women. [14]

Also 3.5- fold higher incidence of the disease was found in men than in women in Asian US-based citizens. In women study showed that the prevalence increased by age and further, the possibility of postmenopausal condition might affect the development of NAFLD. [15]

NAFLD and diets

Whole-body metabolism and its regulation are getting affected because of diet via effects on hormones, transcription factors, and lipid metabolic pathways are considered to play a central role in NAFLD. Inappropriate diet or nutrition is thought to lead to chronically elevated glucose, insulin, and free fatty acid (FFA) concentrations in the blood in many cases. [16]

Use of diet to decrease body weight, and improve glycemic control, dyslipidemia, and cardiovascular risks, as well as to treat fatty liver is major diet management strategy in such patients. There is a bewildering array of diets that have been recommended for the prevention and treatment of all of the components of the metabolic syndrome. It is also important to note that cognitive-behavioral approaches, in addition to dietary modification, are necessary for the long-term success of dietary and lifestyle

interventions. [17]

The diet comprising of fatty acids like saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acids and omega -3- fatty acids have the impact on the liver metabolism.

Carbohydrates

As previously known that dietary carbohydrate is more steatogenic than fat the results of dietary fat intake, carbohydrate overfeeding in humans results in excessive weight gain and hepatic steatosis in a short period of time. High consumption of the carbohydrates was reported in the patients with NASH. Low-carbohydrate diets have been showing good results with weight loss, decrease intrahepatic triglyceride content, and improvement of metabolic parameters of patients with obesity. But long term use these ketogenic low carbohydrate diet stimulate the development of the NAFLD and glucose intolerance. Understanding the long-term systemic effects of low-carbohydrate diets is crucial to the development of efficacious and safe dietary interventions. [18]

Omega-3 Fatty acids

It has the beneficial effects in the NAFLD patients Omega-3 fatty acids have been suggested as a treatment for NAFLD. They have several potential mechanisms of action, decreased plasma triacylglycerol, FFAs, glucose, and insulin; prevention of peripheral insulin resistance; decreased triacylglycerol concentrations, VLDL secretion, and lipogenesis in the liver and the most important being to alter hepatic gene expression, thereby switching intracellular metabolism from lipogenesis and storage to fatty acid oxidation and catabolism. [19]

Cholesterol

High intake of the cholesterol initiates the development of NAFLD. The progression from simple steatosis to steatohepatitis (NASH) usually involves the second hit, such as oxidative stress and inflammation. Extra dietary cholesterol and decreased dietary PUFA intake are responsible for the NAFLD development without the presence of obesity or insulin resistance. [19]

Saturated fatty acids (SFA)

SFAs have following impact; promote endoplasmic reticulum stress as well as hepatocyte injury. Accumulation of SFAs in the liver due to high-SFA or high-fructose diets led to an increase in markers associated with endoplasmic reticulum stress and liver dysfunction. SFA intakes <7% and >10% of energy may be suboptimal for NAFLD patients. [20]

Polyunsaturated fatty acids (PUFA)

Experimental evidence suggesting that polyunsaturated fats may impair the export of fats from the liver by facilitating oxidative damage of the proteins involved. It also shows the effects to decrease the risk of heart disease when consumed in place of SFAs in both epidemiologic. Replacement of n-6 PUFAs with α-linolenic acid improved peripheral insulin sensitivity and lowered cholesterol concentrations in rats with fructose-induced insulin resistance. [21]

Diagnostic Approach

Biopsy of the liver

The only method for differentiating NASH from steatosis with or without inflammation is a liver biopsy and still considered to be first choice technique for diagnosis of the NAFLD. [22] Exclusion of other causes of liver disease, distinguishes steatosis from NASH, estimate prognosis, and determination of progression of fibrosis over time are the key point for the popularity of the liver biopsy. But some limitations in performing a liver biopsy include: the risks and costs associated with a liver biopsy. [23] Therefore alternative noninvasive methods for diagnosis of NAFLD; radiologic and serum chemistries have been investigated.

Radiologic imaging

Contrast-enhanced ultrasound (US) using Levovist is the first imaging technique to demonstrate efficacy in distinguishing between simple Steatosis and NASH. [24] The accumulation of

Levovist micro bubbles in the liver parenchyma decreases in NASH but not in NAFLD or chronic viral hepatitis, this decrease seen in NASH is correlated with fibrosis rather than Steatosis. Although this remains an experimental technique at present, evolving imaging modalities may thus soon permit the non-invasive differentiation between steatosis and NASH. [25]

CT scan also yields the similar diagnosis to the US for NAFLD. In the unenhanced CT liver: spleen (L/S) attenuation ratio is considered to show the severity of the steatosis. A CT L/S cut-off of value of 0.8 yielded 100% specificity and 82% sensitivity for diagnosing macro vesicular steatosis of 30% or greater. [26]

Using a novel attenuation parameter termed 'Controlled Attenuation Parameter' (CAP), Fibroscan has also showed utility in detecting and quantifying steatosis, which by using a process based on Vibration Control Transient Elastography (VCTE, Echosens, Paris, France) targets the liver. Ability to quantify and detect steatosis from only 10% of liver infiltration, and being non-ionizing, relatively cheap and non-operator dependent makes this technique preferable over other imaging techniques. [27]

Clinical and blood serum Parameters

Apart from the imaging some clinical scoring systems based on simple clinical or laboratory indices can be used to identify advanced fibrosis in patients with NAFLD and other liver diseases. Aspartate aminotransferase (AST)-to-platelet ratio index (APRI), the AST/alanine aminotransferase (ALT) ratio, the BARD score, the FIB-4 score and the NAFLD fibrosis score are the recently studied techniques in the developing the non-invasive diagnostic tools to NAFLD. [28]

see Table 1.

In recent studies, Type – IV collagen levels were significantly increased in patients with NASH among NAFLD patients as compared to controls. By comparing the liver function tests and lipid profile, NASH was found to have predictive negative and positive values among the NAFLD. [33]

Animal Models to Study NAFLD

Non-alcoholic fatty liver disease (NAFLD) represents a histological spectrum of liver disease associated with obesity, diabetes and insulin resistance that extends from isolated steatosis to steatohepatitis and cirrhosis. As well as being a potential cause of progressive liver disease in its own right, steatosis has been shown to be an important cofactor in the pathogenesis of many other liver diseases. [34] Animal models of NAFLD may be divided into two broad categories: those caused by genetic mutation and those with an acquired phenotype produced by dietary or pharmacological manipulation.

The literature consist numerous different mouse models that exhibit histological evidence of hepatic steatosis or, more variably, steatohepatitis; however, few replicate the entire human phenotype. [34] The genetic leptin-deficient (*ob*/*ob*) or leptin-resistant (*db*/*db*) mouse and the dietary methionine/choline-deficient model are used in the majority of published research. Existing model are not demonstrate the entire NAFLD phenotype as encountered in clinical practice, and many differ from the human disease. Because of these inconsistencies and the lack of a reliable model of progressive fibrosingsteatohepatitis the research on the NAFLD has not progressed. [35]

see Table 2.

In the recent studies, use of targeted gene disruption and supra-nutritional diets to induce NAFLD have gained greater prominence as researchers have attempted to bridge the phenotype gap between the available models and the human disease.

Treatments of NAFLD

Major targets for the treatment of the NAFLD are obesity, insulin sensitivity and weight loss by lifestyle change, pharmacological treatment or by surgical treatment. Also the lipid lowering agents, antioxidants cytoprotective agents, anti TNF agents play an important role in the treatment of NAFLD.[40] Patients with NAFLD or metabolic syndrome are encouraged to adopt a program of diet and exercise with the goal of weight loss as a first step in their treatment.The pharmacological drugs like orlistat and sibutramine are used for the treatment of obesity and found to be effective in the NAFLD.[41] In the surgical treatment "Biliopancreatic diversion" with or without duodenal switch is the only form of bariatric surgery still in use that aims at effecting weight loss through malabsorption of macronutrients. [42]

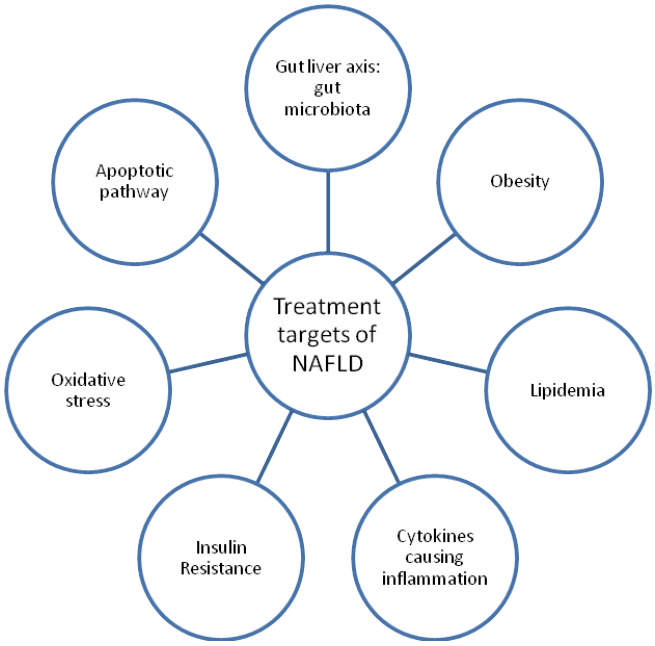

Figure 3: Target sites for the treatment of the NAFLD

Thiazolidinedione (TZDs): pioglitazone and rosiglitazone; metformin are among the most studied insulin sensitizing agents used for the treatment of NASH shows favorable results.TZDs acts by increasing fatty acid oxidation and decreasing fatty acid production within the liver. Insulin sensitivity is improved both peripherally and within the liver. [43] Role of dyslipidemia in metabolic syndrome and its association with NAFLD has increased the interest in the use of anti-hyperlipidemic agents for NAFLD. [44] Statins, Fibrates, Omega-3 fatty acids are efficient anti-hyperlipidemic agents.

Mechanism of action of the stanins is to inhibit hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, thereby decreasing cholesterol production and reducing serum cholesterol. Some fibrates, such as clofibrate, gemfibrozil, and fenofibrate may have some benefit in NAFLD treatment. [45] Oxidative stress is considered a major contributor as the "second hit" in the pathogenesis of NAFLD and NASH, [46] An antioxidant agents Alpha-tocopherol, the form of vitamin E used in the treatment of the NAFLD which is preferentially metabolized in humans, inhibits transforming growth factor beta1, which is thought to contribute to fibrosis progression. Ursodeoxycholic acid (UDCA), a naturally occurring bile acid with multiple hepatoprotective activities improves liver condition in patients with a wide range of chronic liver diseases and hepatobiliary diseases. Betaine and N-acetyl-cysteine (NAC) are among the other antioxidant agents used in the NAFLD. [47]

Agents improving necrosis, inflammation, and fibrogenesis caused by a number of pro-inflammatory adipocytokines, including tumor necrosis factor alpha (TNF-α).[48] can target the "second hit" in the pathogenesis of NAFLD e.g. Pentoxifylline. Reduction in the collagen accumulation by pioglitazone or rosiglitazone showes that drugs should be indicated also to reduce the progression of liver damage in patients with NAFLD. [49]Cytoprotective agents like Lecithin, Silymarin and Beta-carotene are found effective in the NAFLD and NASH. [50] Silybin the main component of the flavonoid silymarin which is a radical scavenger, stimulates hepatocite RNA synthesis and suppresses the proliferation of hepatic stellate cells and the collagen deposition in vitro. Conjugated with a phytosome and vitamin E, Silibyn improves liver steatosis, insulin resistance and plasma markers of liver

fibrosis in patients with NAFLD. [49]

Recent studies on lifestyle interventions provide consistent evidence to reduce energy in take and/or increase physical activity reduces intrahepatic triacylglycerol concentration (IHTAG) and improve insulin sensitivity in patients with NAFLD. Increased physical activity and/or cardiorespiratory fitness, as well as macronutrient composition, may also act independently to prevent or reverse disease progression. [51]

Conclusion

Non-alcoholic fatty liver disease is the commonest cause of abnormal liver biochemistry in many developed countries affecting both adults and children especially those with obesity, type 2 diabetes, insulin resistance, and dyslipidemia. It is estimated to affect 40-70% of people with type 2 diabetes. Lack of the sensitive non- invasive diagnostic approaches leaves the population in dark about the NAFLD. Prognosis is also uncertain, and since there is no specific treatment to offer patients, but in recent studies life style intervention, Thiazolidinedione (TZDs), metformin, lecithin, silymarin and beta-carotene are found effective in the treatment of NAFLD.

Inconsistent and less reliable the animal model limits the study of the NAFLD hence development of the reliable animal model play important role in the advancement of the preclinical studies. Future research should be on new insights into its progression, particularly in terms of identifying the initiating mechanisms and patients at risk, developing innovative diagnostic methods adapted for large-scalescreening and prognostic evaluation. Also characterizing the key pathways and molecular targetsamenable to pharmacological therapy, and theimprovement of implementation of lifestyle changes are key factors in the future research. Further development in the rational approach of therapeutics of NAFLD has to be made.

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Test	Parameters required	How to calculate	Range
AST-PLT Ratio Index(APRI)	AST value and Platelets count	$\frac{\left(\frac{\text{AST (IU/l)}}{\text{ULN of AST}}\right)}{\text{Platelets count } (X10^9/L)} \times 100$	≤0.3 and ≤0.5 : Not significant fibrosis and cirrhosis ≥1.5 : Significant fibrosis
AST- ALT Ratio	AST value, ALT value	$\frac{\text{AST}}{\text{ALT}}$	> 0.8 associated with higher risk of advanced fibrosis
BARD Score	BMI, AST/ALT ratio (AAR) and the presence of diabetes(DM)	BMI ≥28 = 1 point, AAR of ≥0.8 = 2 points, DM = 1 point, to generate a score from 0 to 4	A score of 2–4 was shown to be associated with an odds ratio for advanced fibrosis
FIB- 4 Score	Age, AST(IU/L), ALT(IU/L),	$\frac{\text{Age (years)} \times \text{AST [IU/L]}}{\text{Platelets } [10^9/L] \times (\text{ALT [IU/L]})^{1/2}}$	<1.45 moderate fibrosis >3.25 significant fibrosis
NAFLD fibrosis score(NFS)	Age, AST(IU/L), ALT(IU/L), BMI, Platelets count and albumin count	NFS = - 1.675 + 0.037 × Age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×10^9/L) - 0.66 × albumin (g/dl)	The low cut-off score (<-1.455) has a negative predictive value (NPV) of 88–93% and the high cut-off score (> +0.676) has a positive predictive value (PPV) of 79–90% for the presence of advanced fibrosis in NAFLD

Table 1: Test to evaluate the NAFLD.[29-32]

Animal Models		Characteristics
Genetic Model	**Ob/ob mice model**	A spontaneous mutation in the leptin gene (leptin deficient), mice are hyperphagic, inactive, extremely obese and severely diabetic, with marked hyper-insulinemia and hyperglycemia, require other stimuli such as an MCD diet or a high fat diet to trigger progression to steatohepatitis.
	Db/db mice model	A natural mutation in the leptin receptor (Ob-Rb) gene, The mice are obese and Insulin Resistant, readily develop symptoms of NASH upon induction with a second hit, such as feeding with an MCD diet.
Dietary Model	**MCD diets model**	Impaired VLDL secretion due to lack of phosphatidyl choline synthesis results in steatosis, lose weight (due to a vastly lower caloric intake) and do not become insulin resistant.
	CD diets model	CD diets increase body weight, induce dyslipidemia and cause insulin resistance; choline deficiency in the context of a high fat diet can improve glucose tolerance in mice.
	High-fat diets model	High-fat diets (HFD) increases body weight, body fat and induce insulin resistance in rodent models, also increase liver fat levels quite rapidly (within days) and is associated with hepatic insulin resistance.

Table 2: Different types of animal models used to study the NAFLD. [34-39]