



Inflammation and IL 6: A possible role in NAFLD

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Abstract

A review was carried out to check where IL6-174G/C gene polymorphisms is associated with NAFLD and number of studies were reviewed and found that the 174C genotype is almost absent in African and Asian populations where as it appears that the majority of Asian populations carry the GG genotypes, ranging from 75.0 to 100.0%, whereas Caucasians in the West had a higher frequency of CC genotypes, ranging from 18.0 to 32.0% and it was found that IL-6 -174C polymorphism is associated NAFLD and can be used as a predictor for the metabolic abnormalities in NAFLD and possible progression of the disease and IL6 gene polymorphism could be helpful in identifying individuals at increased risk for developing NAFLD in obese adolescents.

Introduction

Nonalcoholic liver disease diseases (NAFLD) may be a complicated spectrum of diseases starting from easy steatosis to soft steatohepatitis (NASH), liver pathology, cirrhosis, and malignant hepatoma caused by accumulation of excess fat in the liver of people who are nonalcoholic that exceeds 5% of liver weight. Excessive lipid accumulates in the liver generally when the influx of lipids (increased fatty acids import or denovo fatty acid synthesis) exceeds the ability of hepatic lipid clearance by fatty acid oxidation or triglycerides export^[1], by this process only 2% of people with NAFLD may develop NASH and advanced liver diseases. NASH is a condition in which steatosis is combined with inflammation and fibrosis as it is a progressive disease, the most extreme form of NAFLD. So NAFLD leads to NASH which in

turn leads to cirrhosis and cirrhosis in turn leads to liver failure. People with NAFLD diagnosed by abnormal liver function test during routine blood tests or Ultra sonography (USG have few or no symptoms usually). The emergence and development of NAFLD which depends on a number of interrelated factors: genetic polymorphisms, diet, and lifestyle^[2]. It has been suggested that genetic factors predispose to nonalcoholic fatty liver disease (NAFLD)^[3] and that these might explain the difference in NAFLD progression between individuals, this led to research focusing on genetic factors that may possibly have a role in NAFLD etiology, and genetic variability which is now believed to be one of the most important determinants of disease phenotype and progression in patients^[3]. Pathogenesis of fatty liver is multifactorial. They are not only represented by the agents causing the

injury, but also by the character of the lesions, the mechanism of liver toxicity and the associated risk factors^[4, 5].

Factors causing NAFLD

a. Genetic factors

Genetic factors, having role in pathogenesis of NAFLD are either the number of mutational changes at hot spot positions or the polymorphisms in the gene PNPLA3, APOC3, GSKR, MBOAT7, TM6SF2 and TRIB1

b. Adipokines and Cytokines

Cytokines and adipocytokines (mediators mainly derived from adipose tissue) plays a main role in inflammation process throughout the body. The proinflammatory cytokines TNF and IL6 are involved in the pathophysiology of NAFLD.

c. Other factors

NAFLD is usually triggered by Hepatotropic viruses, toxins and alcohol, metabolic liver disease or autoimmunity which on acting chronically in the liver activate cellular pathways involving transcription factors of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) family and signal transducer and activator of transcription 3 (STAT3), as well as cytokines such as interleukin-6 (IL-6) and IL-1α, etc.

Origin of NAFLD

NAFLD first being considered to be a benign disease has now got recognized as a leading cause of liver-related mortality and morbidity in the Western countries. Initially the consideration of NAFLD as a benign disease can be explained by the fact that simple actions such as lifestyle interventions, i.e. dietary changes and physical activity, can reverse simple hepatic steatosis but however, when disease-promoting factors such as eating an unhealthy diet persist, simple steatosis progresses to NASH, which is characterized by inflammation and fibrosis, and can then evolve into cirrhosis and to a lesser extent to hepatocellular carcinoma^[6].

Inflammation and its role in NAFLD

Inflammation being first line of defense mechanism in the body, the immune system of the body recognizes the damaged cells, irritants and pathogens and it begins the healing process. Inflammation does not necessarily mean that there is infection, but an infection can cause inflammation as infection, wounds and any damage to tissues would not be able to heal without an inflammatory response. Inflammation will be classified as either acute or chronic: Acute inflammation is that the initial response of the body to harmful stimuli and is achieved by the inflated movement of plasma and leukocytes from the blood to the injured tissues, prolonged inflammation, known as chronic inflammation, usually is productive or proliferative. Cells in the chronic inflammation process tend to produce substances that add new tissues, such as collagen and new blood vessels, in general, chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once. The primary risk factors involved in NAFLD are diet, obesity, type II diabetes, and the metabolic syndrome including dyslipidemia and hypertension which causes inflammation and thus risk of the disease. However, diseases other than metabolic syndromes can be associated with hepatic fat which might enter into the differential diagnosis of fatty liver disease. Other causes like Hepatitis C virus, celiac disease (systemic immune related disorder is related to gluten sensitivity) in which patients have elevated transaminase levels, and they carry a increased risk of developing fatty liver, hepatitis, fibrosis and cirrhosis.

Prevalence

Worldwide Clinical studies have revealed dramatically high prevalence of NAFLD^[7] High prevalence of NAFLD in children's and adolescence were also revealed (He F.2001). In obese child and adolescence, NAFLD affect about 20-74%, indicating that this disease might start early during life, providing more time for its deleterious effect^[8]. The prevalence of NAFLD,

primarily caused by obesity and alcohol consumption is approx 20% in general population of developed countries and is expected to increase further due to increase in obesity in the general population^[9]. Several studies indicate that only 28-55% of diabetes^[10] and 30-40% of obese persons have NAFLD^[11]. In addition, only 7% of patients with metabolic syndrome have increased ALT^[12] but not all patients with NAFLD are insulin resistant. NAFLD is commonly associated with obesity and diabetes and is characterized by the insulin resistant.

Two hit hypothesis in relation with NAFLD

The 'two hit hypothesis' has become a very important theoretical framework for understanding the pathologic process of NAFLD. The 'first hit' is fat accumulation within the liver, a process that is closely linked with insulin resistance. In the majority of patients with excess liver fat there will be no or only scarce hepatic inflammation, and the condition will be termed simple steatosis. However, a 'second hit' may trigger the necro inflammatory response characterizing NASH. A "two hit" mechanism is proposed to drive NAFLD/NASH pathogenesis^[13]. In first hit, hepatic steatosis, is closely associated with lipotoxicity-induced mitochondrial abnormalities that sensitize the liver to additional pro-inflammatory insults. These second hits include enhanced lipid peroxidation and increased generation of reactive oxygen species (ROS)^[2]. Inflammasomes are cytoplasmic, multi-protein complexes composed of one of several NLR and PYHIN proteins, including NLRP1, NLRP3, NLRC4 and AIM2. Inflammasomes are sensors of endogenous or exogenous pathogen associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)^[14]. that govern cleavage of effect or proinflammatory cytokines such as pro-IL-1b and pro-IL-18^{[15],[16]}. Most DAMPs trigger the generation of ROS, which are known to activate the NLRP3 inflammasome^[17] and IL 6. Therefore, inflammasome-dependent processing of IL-1b, IL-

18 and IL 6 may have an important role in the progression of NAFLD.

Adipokines and its role in NAFLD

The pathologic process of NAFLD/NASH and, in particular, the mechanisms responsible for liver injury and disease progression remain still incompletely understood. Recent studies have centered on the adipokines, bioactive proteins secreted by adipose tissue, including adiponectin and IL-6. Adipokines which are central factors in the development and progression of NAFLD and inflammation have been investigated. Increasing evidence indicates that they might play important roles in the NASH pathogenesis^[18]. A number of studies have demonstrated the association between hypo adiponectinemia and NAFLD^[18]. In study done by^[19] observed significantly lower serum concentration of adiponectin in patients with NAFLD than in healthy subjects, this means that high levels of adiponectin are associated with a protective effect against fatty liver. Their findings are in accordance with a report by^{[20],[21]} and^[22] reported that serum adiponectin level was significantly lower in patients with NAFLD than in the control group. Moreover,^[23] observed that lower serum adiponectin level in patients was associated with more extensive necro inflammation. In above study, they have also evaluated that the levels of IL-6 were found to be higher in NAFLD patients compared to healthy controls but not significant. This agrees with the study of,^[24] reported that Adipocytokines and other recognized cytokines produced partially by inflammatory cells infiltrating adipose tissue, play an important role in the pathogenesis of IR (Insulin resistance) and NAFLD, through complex and interactive paracrine and endocrine mechanisms. Some adipocytokines, including adiponectin and leptin decrease IR, while others, including tumor necrosis factor (TNF)-alpha, IL-6 and resistin enhance IR. Also^[25] reported that NAFLD patients with abnormal ALT had the highest plasma levels of IL-6 but it did not reach

the statistical differences as compared with other group. Another issue in this research was that they have observed a positive significant correlation between the concentration of IL-6 with concentration of TG and cholesterol in NAFLD patient group. These current results agree with those reported by a study of^[25] as after lifestyle changes and vitamin E administration it has been demonstrated that patients with NAFLD have a dysregulated cytokine metabolism at baseline and a significant decrease in IL-6 levels.

Role of IL 6 in NAFLD

In liver pathology, the role of IL6 is very complex, the inflammatory cytokine, IL-6, is a multifunctional cytokine that plays a major role in the response of hepatic epithelia to inflammation Fig 1. IL6 is produced by a variety of cell types and can act both as a pro and anti-inflammatory cytokinin. Cross-sectional and prospective studies have shown that increased levels of systemic inflammatory markers such as interleukin (IL)-6, a major proinflammatory cytokine expressed in several tissues including leukocytes, adipocytes, and endothelial cells, which are associated with type 2 diabetes and glucose disorders^{[26][27][28]}. Furthermore, IL-6 is considered as a predictor marker of insulin resistance and cardiovascular diseases. In patients undergoing bariatric surgery, decreased IL-6 concentrations were associated with weight loss and insulin resistance improvement^[29]. Serum IL-6 levels are higher in animal models and patients with NAFLD^[12, 30] Interleukin 6 (IL-6) is elevated in the plasma and peripheral blood monocytes of patients with fatty diseases, including alcoholic liver disease and nonalcoholic steatohepatitis, and elevation of IL-6 correlates with the progression and severity of liver disease,^{[31][11,12,32]} M.2003) suggesting that IL-6 may be involved in the pathogenesis of fatty liver disease (Table 1). IL-6 is a four helical cytokine of 184 amino acids^[33], present at chromosome no 7. IL6 activates several cells, like immune cells, hepatocytes, haematopoietic stem cells, and osteoclasts^[33]. Further, it has a wide range of biological function, including induction

of inflammation and oncogenesis, regulation of immune response and support of hematopoiesis^[33]. All IL-6 kind cytokines share the membrane compound protein gp130 as a typical receptor and signal electrical device monetary unit. IL-6 and IL-11 initially bind to the membrane bound α receptors IL-6 receptor (IL-6R) or IL-11R, respectively. Subsequently, IL-6/IL-6R or IL-11/IL-11R complexes associate with gp130, leading to gp130-homodimer formation and signal initiation, after auto phosphorylation, JAK1 phosphorylates five tyrosine residues within the cytoplasmic portion of gp130, this results in the activation of many living thing communication pathways as well as the MAP enzyme and PI3 enzyme pathway and therefore the signal electrical device and substance of transcription (STAT)1 and STAT3 pathway. Subsequently, STAT3 is able to up regulate suppressor of cytokine signaling (SOCS)3, which leads to a down regulation of gp130 signals and thereby represents a negative feed-back loop^[33,34]. Whereas gp130 is expressed on all cells of the body, the IL-6R is only expressed on few cell types such as hepatocytes, some leukocytes and some epithelial (e.g. biliary epithelial cells) and non-epithelial cells (e.g. hepatic stellate cells). Therefore, only IL-6R expressing cells can directly respond to the cytokine IL-6^[33]. IL6 is a key element in APR, synthesis several APP (C-reactive protein and serum amyloid A)^[35]. Its important for hepatocyte hemostasis and is a potent hepatocyte mitogen. Synthesis of IL6 in visceral fat exceeds production in hypodermic fat and IL-6 in vena portae is concerning 20-30% high compared to vein blood and general levels. Visceral adiposity bears a high risk to develop NAFLD^[36]. Interleukin 6 (IL 6), as a cytokine, has pleiotropic features, takes part in regulation, proliferation, differentiation and various activities of cell types. It also has an important part in neuro-endocrine and homeostasis of immune system^[34]. Dysregulated IL 6 production is implicated in the

pathology of several disease processes such as multiple myeloma, arthritis, diabetes, atherosclerosis and hepatocellular carcinoma (HCC) in patients with chronic hepatitis B [37][38][39, 40]. Interleukin 6 (IL-6) is elevated in the plasma and peripheral blood monocytes of patients with fatty diseases, including alcoholic liver disease and nonalcoholic steatohepatitis, and elevation of IL-6 correlates with the progression and severity of liver disease,^{[11][31, 32][12]} M.2003) suggesting that IL-6 may be involved in the pathogenesis of fatty liver disease. Thus, though IL-6 might improve internal organ regeneration and repair, it might conjointly sensitize the liver to injury, stimulate hepatocyte necrobiosis induce internal secretion resistance, and participate in NASH development. However, increasing evidence indicates that IL-6 is an important hepatoprotective cytokine for promoting liver regeneration^{[41][42, 43]} and protecting against liver injury caused by various insults in lean animals.^{[44][45][46][47][48][49]} The molecular etiology of NAFLD involves multiple genetic and nongenetic mechanisms contributing to the final phenotype. In the liver, IL-6 is a very important inducer of the acute phase response and infection defense. IL-6 is what is more crucial for hepatocyte homeostasis and could be a potent hepatocyte agent. It is not solely concerned in liver regeneration, however conjointly in metabolic perform of the liver. However, persistent activation of the IL-6 communication pathway is prejudicious to the liver and may ultimately end in the event of liver tumors. So IL-6 acts as a key element in the acute phase response, mediating the synthesis of several acute phase proteins (such as C-reactive protein and serum amyloid A)^[35]. Thus, we have a tendency to cannot exclude the likelihood that IL-6 may also play Associate in Nursing indirect injurious role in NAFLD pathologic process. Serum IL-6 levels are higher in animal models and patients with NAFLD^{[30][12]}. Polymorphism – 174G/C is the most widely studied polymorphism

of the IL-6 gene In humans with NASH, a positive correlation between IL-6 expression in hepatocytes and the severity of NAFLD has been observed^[50]. Also IL-6 correlates significantly with ALT, however, a study done by^[51] found that the IL-6 -174C variant, is significantly more prevalent in NAFLD than in healthy subjects and is associated with increased fasting insulin and homeostasis model assessment of insulin resistance, and is an independent predictor of NAFLD and NASH. These finding are in contrast with other studies which showed that the IL-6 -174G variant was associated with lipid abnormalities^[52] and with diabetes in Caucasians as well as Pima Indians^{[53][54]} and that the C allele at -174 position was unlikely to play a role in the development of type 2 diabetes mellitus in a Taiwanese population^[25]. Study done by^[55] investigated IL6 gene polymorphisms in NASH patients and healthy controls and attempted to define their roles in the pathogenesis and severity of disease. The results indicated that polymorphisms in IL 6 genes had no effect on the identification of NASH as there was no difference compared to healthy controls.. Besides, polymorphisms of -174 C/G polymorphism in the IL 6 gene had no roles in the progression of liver fibrosis. The poorly understood pathogenesis of NASH seems to be multifactorial^[13]. In their study, it was confirmed that higher BMIs in NASH patients. In a Japanese study, it was concluded that obesity is a substantial variable in the progression of NASH^[56]. In that study, the role of IL 6 polymorphism in the “second hit” pathophysiology of NASH., but no statistically remarkable difference was found between NASH patients and healthy controls. Some studies concluded that IL6 polymorphisms affect histopathological progression of HCV, hepatic insulin resistance, inflammation and occurrence and development of HCC^{[39][40][57][58]}. In the study, among NASH patients, there was not a difference between the fibrosis groups regarding IL6 and IL8 gene polymorphisms. NASH may progress to liver fibrosis, liver failure and

eventually death, they showed that IL6 had no effect on NASH pathogenesis and progression of liver fibrosis. Another study done by^[56], the genotype analyses of IL-6 – 174G/C in 70 obese Egyptian adolescents with NAFLD were studied, and 70 healthy controls were included for comparison. There study showed that the distribution of genotypes of the IL-6 – 174G/C polymorphisms was significantly different in NAFLD adolescents as compared with healthy controls and the frequency of the Callele was more prevalent in NAFLD patients than in controls [69 (67.0%) vs. 34 (33.0%)], supporting role of IL 6 in NAFLD^{[59][60]}. The genotype distribution of the IL-6 polymorphism has been described in Americans and Spanish Caucasian patients by^[59]. It was reported by them that the frequency of the –174C allele is much lower in Japanese, Africans, and Asian Indians compared

with European Caucasians^[61]. It appears that the majority of Asian populations carry the GG genotypes, ranging from 75.0 to 100.0%, whereas Caucasians in the West had a higher frequency of CC genotypes, ranging from 18.0 to 32.0%^[62]. **The allele – 174C is almost absent in African and Asian populations**, whereas its frequency is more than 10% in Latin Americans and 40% in Caucasians^{[61][63][64]}. The main finding of the study was that the IL-6 – 174 Cvariant was associated with several metabolic risk factors in obese Egyptian adolescents with NAFLD. Their study concluded that IL-6 – 174C polymorphism is associated NAFLD and can be used as a predictor for the metabolic abnormalities in NAFLD and possible progression of the disease. These findings could be helpful in identifying individuals at increased risk for developing NAFLD in obese adolescents.

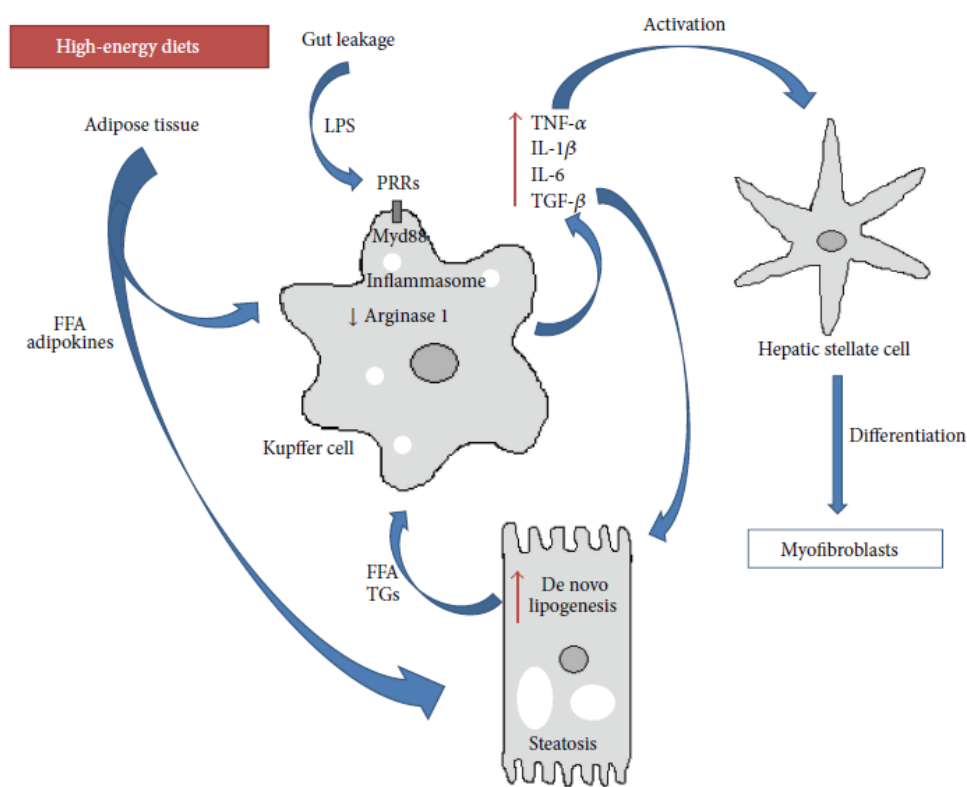


Fig: Development of Steatosis^[65].

Table 1. IL 6 and its association with NAFLD

Ref.	Country Ethnicity	Cases Controls	Genotyping method	Association
[51]	Italy Caucasian	79 114	PCR-RFLP	Positive significant (NAFLD)
[66]	Spain Caucasian	32	PCR-RFLP	Positive significant (diabetes and lipid abnormalities)
[67]	Spain Caucasian	258 101	TaqMan genotyping	NS (alcoholic liver disease)
[68]	China Asian	77	PCR-RFLP	Positive significant (PBC)
[54]	Rome Italy	275	PCR-RFLP	Positive significant (insulin resistance)
[69]	Section on Liver Biology	Animal Study	Administration of IL 6	Positive significant
[55]	Turkey	38	PCR-RFLP	NS for NASH
[19]	[55][55][54][53]Egyptian	104, 21	ELISA KIT	Positive significant
[50]	American	50 patients with suspected NALFD	Immunohistochemistry, ELISA	Positive significant for NASH and IR.
[70]	Norway	47 patients	EIA	Positive significant
[71]	Egypt	70	PCR- RFLP	PS

Conclusion

NAFLD is a rising concern in our population which led us to go for review in order to get know how of genetic analysis and polymorphisms of IL 6 that goes together with the increasing prevalence and incidence of NAFLD. Risk factors involved in NAFLD pathogenesis are insulin resistance, adipose tissue distribution, dietary and genetic factors. From this review, it is clear how IL 6 is involved with the pathogenesis of the disease. The lack of knowledge in relation with NAFLD to its pathogenesis may become a hurdle in the path towards novel approaches for the prevention and treatment of the disease. In this review, it is clear how IL 6 -174G/C polymorphism is associated with NAFLD. Prevention of NAFLD can only be based on calorie restriction and favorable dietary composition as well as exercise. Lifestyles and therapeutic methods should be addressed not only to prevent fat deposition, but primarily to avoid subclinical inflammation which in turn prevent development of NAFLD to NASH. We conclude from this review that that IL-6 – 174C polymorphism is associated NAFLD and can be used as a predictor for the metabolic abnormalities in NAFLD and possible progression of the disease.

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