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Advances in Understanding the Progression of Non-Alcoholic Fatty Liver Disease to Non-Alcoholic Steatohepatitis: A Comprehensive Review

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Abstract:- The review article provides a comprehensive overview of the progression of nonalcoholic fatty liver disease (NAFLD) to nonalcoholic steatohepatitis (NASH). It begins by discussing the global prevalence of NAFLD and its two main components: NAFL and NASH. The article emphasizes the rising prevalence of NAFLD and its association with obesity and sedentary lifestyles. It also highlights the "Asian Paradox," where NAFL and NASH have been found in non-obese individuals in Asia. The distinction between NAFL and NASH is explained, along with the diagnostic methods used to differentiate between the two conditions. The article delves into the genetic factors associated with NAFLD, including the PNPLA3 gene and various genetic variants linked to fat infiltration. It also discusses the role of insulin resistance and fructose consumption in the progression of NAFLD to NASH. The review explores the impact of cytokines and innate immunity, as well as the role of gut microbiota in liver disease. In addition, the article provides insights into the conversion process from NAFLD to NASH, highlighting the polygenic nature of the disease and the influence of genetic variants. It also discusses the roles of genes, diet, immune signatures, cytokines, liver strains, and gut microbiota in understanding the progression from NAFL to NASH. The comprehensive review covers a wide range of topics, including genes present in NAFLD, insulin resistance, fructose consumption, cytokines, and innate immunity, and the role of gut microbiota in liver disease. It provides a detailed analysis of the various factors involved in the progression from NAFL to NASH.

➢ ***Key Message:***

The comprehensive review article on 'Advances in Understanding the Progression of Nonalcoholic Fatty Liver Disease to Nonalcoholic Steatohepatitis' delves into the global impact of NAFLD and NASH, highlighting the genetic influences, prevalence rates, diagnostic challenges, and disease progression. The review also examines the role of genes, insulin resistance, fructose consumption, cytokines, innate immunity, and gut microbiota in the pathogenesis of NAFLD and NASH, providing a comprehensive understanding of the complex factors involved in the progression of NAFL to NASH.

Keywords:- *Nonalcoholic Steatohepatitis (NASH), Global Prevalence, Genetic Factors, Insulin Resistance, Fructose Consumption, Liver Disease, Nonalcoholic Fatty Liver Disease (NAFLD).*

I. INTRODUCTION

A worldwide health issue, non-alcoholic fatty liver disease (NAFLD) primarily affects people in the West and is progressively making its way to east coast Asian nations[1]. Non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFL) are the two primary components of NAFLD. In people without a history of heavy alcohol use or an underlying steatosis cause, NAFL is defined by the presence of hepatic steatosis on imaging or histology[2]. However, NASH is defined by the co-occurrence of inflammation and steatosis, as well as indicators of hepatic cell injury in the form of inflammation, either with or without fibrosis1. About one-third of American adults are impacted by the progressively increasing consequences of non-alcoholic fatty liver disease (NAFLD)2. It is possible for the prevalence of obesity in individuals to reach 57,55–74 percent[3]. NAFL is found in 368.8% of adults in the Mediterranean region, 215.5% of Iranians, and 27% of adults in urban China, indicating the presence of global epidemics[4]. The degree of its dispersion varies amongst nations, though. That varies from 20 to 30 percent in Europe, 9 to 30 percent in Japan, and 5 to 24 percent in China[5]. Approximately 16–32% of people in India's urban areas and approximately 9% of people in its rural areas suffer from NAFLD[6].

With the lowest prevalence of non-alcoholic fatty liver disease (NAFLD) among Asian nations, Singapore has a startlingly low prevalence of just 5%. Overseas, sedentary lifestyles and obesity are frequently linked to non-alcoholic fatty liver disease. Remarkably, non-alcoholic steatohepatitis (NASH) and NAFL have also been found in non-obese people in Asia; this is known as the "Asian Paradox[7].". It's critical to keep in mind that fat in liver cells alone does not indicate a medical condition. The majority of NAFL patients do not progress to NASH, making the distinction between the two conditions crucial. Transabdominal ultrasound, with 100% sensitivity and 90% specificity[8], is frequently used when liver biopsy reveals more than 20 percent fat, even though liver histology remains the gold standard for diagnosing NAFL. Additional non-invasive diagnostic techniques include liver stiffness measurement using

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dynamic elastography (Fibroscan) and acoustic magnetic resonance imaging (ARFI), which correlate with the existence of fibrosis. Although magnetic resonance imaging spectroscopy is a quantitative technique for calculating liver fat, its lack of availability and high cost limit its clinical application. These randomized trials, however, are unable to differentiate between NASH and simple steatosis. Although increased levels of alanine aminotransferase and aspartate aminotransferase are thought to be indicative of NASH, many people with biopsy-verified NASH may have normal levels of these enzymes[9]. A Bayesian approach that integrates imaging, laboratory, and clinical data found an 81 percent probability of differentiating between NAFL and NASH in a recent study using a non-invasive tool[10]. Depending on the NAFL diagnostic standard, different diagnostic tests are used for this illness. For example, a liver biopsy-based study in India found a 53 percent prevalence of NASH, while an ultrasound-based study showed a 16 point 6 percent prevalence of NAFLD[11]. Stated differently, a study carried out on the east coast of India revealed that, according to a liver biopsy, one in four patients had NASH[12]. In a different Asian study with fifty-two patients, 32.6 percent had a diagnosis of NASH at the time of presentation, and 11% had a diagnosis of NAFL at the time of progression to NASH. On the other hand, 75% of patients in a recent Western study with a follow-up period of 6.6 years had NASH, and 44% had progressed from NAFL to NASH. Simple steatosis can progress to clinically significant fibrosis, as evidenced by liver biopsy findings in patients with the condition, which is more common in diabetic steatosis[13]. Similar data from the West shows that NASH patients can progress to cirrhosis at a rate of 21–26 percent in as little as 8–2 years. NAFLD raises the risk of all-cause mortality, as shown by meta-analyses[14]. Consequently, compared to patients with simple steatosis, patients with NASH need more strict control over their diet and lifestyle. There is still much to learn about the development of NAFL into NASH and cirrhosis. In order to obtain new insights into the progression of NAFL to NASH, this review examines the roles of genes, diet, immune signatures, cytokines, liver strains, and gut microbiota.

- *The Conversion Process from Nonalcoholic Fatty Liver Disease to Nonalcoholic Hepatosteatosis*

Simple steatosis to nonalcoholic steatohepatitis (NASH) are among the conditions that fall under the umbrella of nonalcoholic fatty liver disease (NAFLD). Liver cells need to be obese—beyond the normal physiological threshold of less than 5 percent—for NASH to manifest. Even though NAFL is common in obese people, not all of them go on to develop NASH. On the other hand, some people who are not obese can nevertheless get NASH. This implies that the disease is caused by variables other than obesity and insulin resistance. The original "two-hit theory" suggests that steatosis acts as the "first hit" in the development of NASH, resulting in damage to the liver, and that a "second hit" causes hepatocellular damage, inflammation, and fibrosis[15]. Recent data, however, suggests that the pathogenesis entails several interactions, with the host, environmental factors, gut microbiota, and host immunity all playing a role in how NASH progresses[16]. The various factors involved in the pathogenesis of NAFL to NASH will be discussed in detail in the following sections, as the transition is still not fully understood.

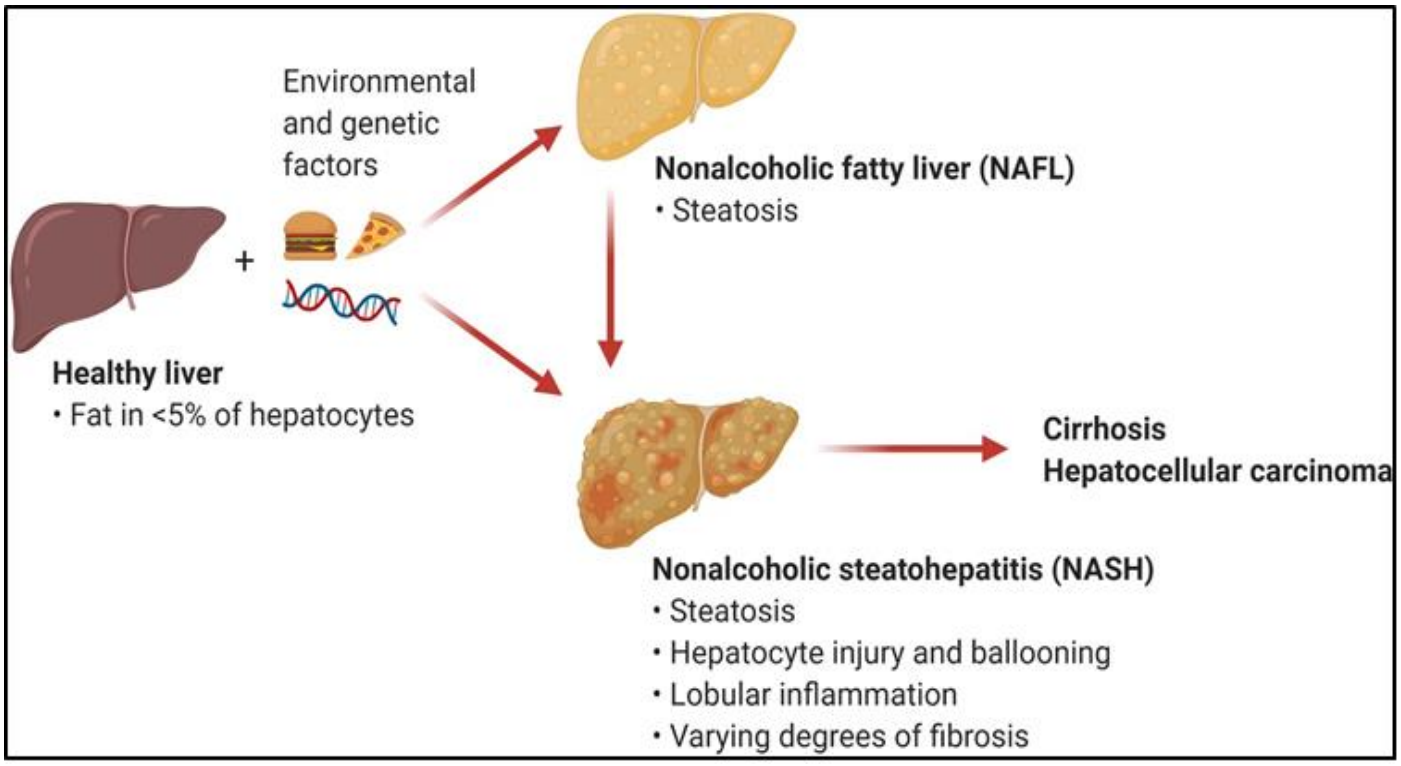

Fig 1 The Conversion of Nonalcoholic Fatty Liver Disease to Nonalcoholic Hepatosteatosis

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II. GENES PRESENT IN NON-ALCOHOLIC FATTY LIVER DISEASE

When a disease's growth rate and severity differ noticeably, it suggests that genetics may be involved in the disease's development. This illness is polygenic, which means that it interacts with the environment and diet in addition to having several genetic origins. Genetics is thought to be the cause of 25–35 percent of cases[17]. Numerous genetic variations found in various places have been connected to the accumulation of fat. Even at lower body weights, research indicates that Asian Indians are genetically predisposed to obesity[18]. The PNPLA-3 gene is located at 22q13.31, the primary genetic location linked to this disease. Numerous genome-wide association studies carried out in diverse cultures have demonstrated a correlation between nonalcoholic fatty liver disease (NAFLD) and a particular variation in the gene known as rs738409[19]. This variant, which is mostly expressed in the liver and adipose tissue, is found in the third exon of the gene. The amino acid shift that it brings about is the loss of triglyceride hydrolyzing activity, from isoleucine to methionine (I148M)[20]. Severe liver disease, steatohepatitis, and elevated necrotizing inflammation are associated with the PNPLA-I148M variant. higher levels of fasting insulin, HOMA-IR, alanine aminotransferase, and aspartate aminotransferase in NAFLD patients in India have been linked to the prevalence of the C/G and G/G genotypes of the rs738409 variation[21]. Furthermore, hepatocellular carcinoma risk is five times higher in people with the PNPLA3 rs738409 GG homozygous genotype, albeit larger-scale research is required to validate these results[22]. Furthermore, African Americans have the lowest risk of NAFLD due to the PNPLA3 S453 variation's correlation with lowered cholesterol levels[23]. Further research on populations in Europe has found more genetic variants, including those around LYPLAL, GKCR, PPP1R3B, and NCAN, which are linked to fibrosis, metabolic characteristics, and nonalcoholic steatohepatitis (NASH) in addition to steatosis[24]. The Japanese population has also been associated with the rs780094 variation to have a lower HOMA-IR, elevated triglyceride levels, a lower risk of type 2 diabetes, and a lower ability to fast. According to results from another Japanese study, SAMM50, PARVB, and PNPLA3 are risk factors linked to NAFLD. FDFT1, COL13A1, EFCAB4B, and PZP were found to be causal loci for steatosis, NAFLD functional score, degree of fibrosis, lobular inflammation, and serum levels of alanine aminotransferase (ALT) in a genome-wide consortium study that involved Caucasian individuals[25]. Using the same genetic approach as the previously mentioned studies, PNPLA3, SAMM50, PARVB, PZP, NCAN, and PZP level were found to be risk factors for NAFLD in an analysis of Indian NAFLD patients. Research based on single hypotheses or gene candidates has found a number of loci that either prevent or inhibit NAFLD, steatosis, or obesity[26]. Although the exact cause and mechanism are still unknown, the SCAP rs2101247 "A" allele appears to reduce the risk of NAFLD, especially in women with metabolic syndrome[27]. In people who are Indian in origin, the variants rs3772627, rs3772630, and rs2276736 appear to prevent NAFLD specifically[28]. While the PPAR-α gene's Val277Ala mutation has been linked to non-alcoholic fatty liver disease (NAFLD), it has also been linked to reduced waist circumference and waist-hip ratio, which may indicate a protective effect against obesity. On the other hand, differences in genes that affect nicotinic N-methyltransferase (NNMT), uncoupling protein 3 (UCP3), sterol regulatory element binding protein 1C (SREBP-1C), adapter proteins (APPL1 and 2), and mitochondrial superoxide dismutase 2 (SOD2) heighten susceptibility to NAFLD[29]. Another gene linked to NAFLD is the apolipoprotein 3 (APOC3) gene. People who are not Asians carryin[30].

Table 1 Genes Present in Non-Alcoholic Fatty Liver Disease

Genes Present in Non-Alcoholic Fatty Liver Disease
PNPLA3
LYPLAL1
PPP1R3B
NCAN
GCKR
SAMM50
PARVB
FDFT1
COL13A1
EFCAB4B
PZP
SCAP
PPAR-α
UCP3
SREBP-1C
APPL1
APPL2
NNMT
SOD2
APOC3

➢ *Insulin Resistance and Fructose in Non-Alcoholic Fatty Liver Disease*

- *Insulin Resistance*

In Western countries, obesity and metabolic syndrome are associated with an increased risk of insulin resistance for non-alcoholic steatohepatitis (NASH)[31] and non-fatty liver disease (NAFLD)[32]. Remarkably, research has revealed that severe NASH and insulin resistance can occur in people without metabolic syndrome, especially in Asian populations. While research from India revealed that both subcutaneous and total fat tissue were positively associated with disease severity in patients with biopsy-proven NAFLD, previous beliefs suggested that visceral fat was a major factor in the development of NASH in Asians. It has been discovered that between 21 and 41 percent of Indian NAFLD patients have metabolic syndrome[33]. A recent Indian study, however, found that one-third of patients with severe fibrosis and 50% of Indian patients with non-alcoholic fatty liver disease (NAFLD) lacked insulin resistance11. Patients with Indian non-alcoholic fatty liver disease (NAFLD) showed varying insulin levels; those with

metabolic syndrome characteristics had higher levels, while those with less severe NAFLD had lower levels.

- *Fructose*

Owing to its cheaper cost than sucrose and its capacity to improve satisfaction when used as a sweetener, fructose consumption has grown throughout time. Phosphorylation of fructose occurs in the liver following absorption in the small intestine via a fructose-specific glucose transporter[34]. Unfortunately, by lowering HDL cholesterol, raising triglyceride levels, and indirectly producing hepatotoxicity, fructose exacerbates metabolic syndrome. Metabolic stress arises from its metabolism, which causes ATP depletion. NASH is triggered by low-grade endotoxemia, gastric hyperpermeability, and intestinal dysbiosis, all of which are linked to high fructose consumption[35]. Iron overload and copper deficiency, both of which are connected to NASH, are also directly correlated with fructose consumption. Research has shown that while total body fat remains unchanged, fructose consumption increases visceral adipose tissue and waist circumference. Advanced glycation end products (AGEs) are formed 17 times faster with fructose than with glucose, and this is how fructose is toxic. Because of oxidative stress, hepatic stellate cell activation, cytokine synthesis (TNF-α and IL-6), and other mechanisms, the high levels of AGEs in the Western diet lead to liver damage, inflammation, and fibrosis[36]. So, consuming too much fructose can result in dyslipidemia, obesity, and glucose intolerance, which can then cause AGEs to be produced, which are harmful proteins. Therefore, AGEs may be potent, as well as their receptor pathways[37].

➢ *Cytokines and Innate Immunity*

- *Innate Immunity*

The functions and phenotypes of the lymphocytes in the liver are different from those of the other organs. These include normal lymphocytes such as B cells, CD4+ and CD8+ T cells, and natural killer cells, as well as aberrant lymphocytes such as gd TCR+ T cells and natural killer T cells. A distinct subset of specialized T cells found in the liver's vascular network, mucosal-associated invariant T cells are crucial for immune activation and function. Hepatocytes and cholangiocytes are not the only cell types in the liver that help maintain the body's homeostasis[38].

Important immune cells in the liver are dendritic and kupffer cells, which are both members of the myeloid lineage. Although dendritic cells are the main immune cells, cholangiocytes can also be immune cells and contribute to the immune system of the liver. Dendritic cells in NAFLD patients exhibit a functional phenotype that is unknown when stimulated by lipopolysaccharide, which results in the release of inflammatory cytokines and exacerbates the disease. Natural killer (NK) cells, neutrophils, macrophages (Kupffer cells), and natural killer T (NKT) cells are among the body's immune system components implicated in liver diseases like NASH[39].

The majority of the body's mononuclear phagocytes, known as kupffer cells, are found in the liver sinusoids and are the first to respond to endogenous signals or immunoreactive substances. Phagocytosis, antigen processing, antigen presentation, and the release of different pro-inflammatory mediators such prostaglandins, nitric oxide, reactive oxygen intermediates, and cytokines are among the functions they carry out[40]. A high-fat diet causes the Kupffer cells to proliferate and become more inflammatory, which in turn causes NKT cell hyperactivation, cell death, and NKT cell deficiency in the NASH developmental stage[41]. Kupffer cells that are exposed to low concentrations of gut-derived lipopolysaccharide (LPS) release IL-10, which inhibits cytokines that promote inflammation. Nonetheless, activation of Kupffer cells' toll-like receptor 4 (TLR4) stimulates the synthesis of several chemokines and cytokines[42]. Adiponectin has been demonstrated to change the polarization of Kupffer cells into an anti-inflammatory phenotype, halting the development of NASH in mice. Furthermore, Kupffer cells play a major role in metabolic diseases because they have metabolic processes and cytokine release mechanisms to adapt to increased caloric intake[43].

- *Cytokines*

TNF-α and IL-6 are two examples of cytokines that contribute to the inflammation and metabolic alterations that signal the early stages of liver damage. These cytokines trigger the production of additional cytokines, which starts the healing process, including fibrosis, and causes cell migration. TNF-α levels and the extent of fibrosis are correlated in NASH patients. TNF-α or its receptor gene expression is elevated in the liver and adipose tissue of NAFLD patients[44]. Similarly, increased serum levels of tumor necrosis factor (TNF) receptor and polymorphisms in the TNF-α promoter are linked to the progression of NAFLD[45]. By controlling suppressor of cytokine signaling 3 (SOCS3), IL-6 derived from adipose tissue contributes to the modulation of insulin resistance (IR). Sterol regulatory element binding protein (SREBP-1c), which regulates fatty acid synthesis, and IR are both elevated in mouse livers where the SOCS3 gene is expressed more frequently. On the other hand, reducing SOCS3 in obese mice results in increased insulin sensitivity and restored SREBP-1c expression. There is a strong correlation between IL-6, IR, elevated oxidative stress, and histological lesions in NAFLD patients. When comparing NAFLD patients to those with chronic liver disease and healthy controls, higher levels of oxidative stress and cytokines have been found. Moreover, oxidative stress is elevated in both diabetic and non-diabetic Indian NAFLD patients[46].

Recent research has demonstrated a connection between elevated IL-1b production and NASH, potentially due to inflammatory activation. Saturated fatty acids (SFAs) raise the susceptibility of primary hepatocytes to LPS and cause gastroenteritis in vitro, which raises IL-1b levels and causes inflammation in steatohepatitis. SFA-induced apoptosis in hepatocytes further activates inflammatory cells and IL-1b, suggesting that SFAs "prime" LPS-induced inflammatory activation in liver disease[47].

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III. ROLE OF GUT MICROBIOTA IN NON-ALCOHOLIC LIVER DISEASE

With over 10^4 species of microorganisms and a secondary genome 100–400 times larger than that of humans, the gut microbiota is now recognized as a major contributor to metabolic diseases. Yeasts, viruses, bacteria, and archaea make up the gut microbiome. Recent research suggests a connection between the gut microbiota and non-alcoholic fatty liver disease (NAFLD), which can lead to non-alcoholic steatohepatitis (NASH). With the use of culture-based techniques centered on 16S ribosomal RNA gene sequencing, the gut microbiota and obesity-related non-alcoholic fatty liver disease (NAFLD) in both human and animal models are linked by the gut-liver axis. Through the portal vein, substances found in the intestines—such as bacterial DNA, peptidoglycan, and lipopolysaccharide—are transported to the liver. They stimulate cell surface receptors, which sets off a chain reaction that results in fibrosis and inflammation of the liver[48]. The microbiome composition of obese people is different from that of normal weight people. The abundance of Firmicutes-Bacteroidetes is higher in NASH than Bacteroidetes. Recent research, however, indicates that regardless of dietary intake and body mass index, the prevalence of Bacteroidetes in NASH is low. Neither the healthy controls nor the cases of simple steatosis showed this trend. Gut microbial products are kept out of the bloodstream by the intestinal mucosal barrier, which functions as a filter[49].

Tight junction disruption in NAFLD permits the stomach and liver to communicate. The metabolism of insufficient choline intake, the secretion of lipopolysaccharide, and the release of endogenous ethanol are all facilitated by the microbiota. Lipopolysaccharide, unmethylated DNA, and lipopeptides are examples of pathogen-associated molecular patterns (PAMPs) that are recognized by toll-like receptors in hepatocytes. The nod-like receptor (NLR) family is activated and pro-inflammatory genes are expressed as a result of this recognition. Zhu along with others. examined the gut microbial composition of children with non-alcoholic steatohepatitis (NASH), children who were obese, and children who were healthy using 16S ribosomal RNA amplicon sequencing. Different gut microbial populations were found in each of the three individual groups, according to the results. Additionally, alcohol-induced bacteria are more prevalent in the NASH microbiome, which increases inflammation and oxidative stress[50]. One area of active research is the gut microbiome in non-obese NASH patients. Finding metagenomic genes linked to NAFL or NASH and incorporating them into non-invasive diagnostic instruments is essential for determining the risk of NASH progression stratification. One developing trend in the search for novel therapeutic targets is the modification of the microbiota to prevent or treat NAFL/NASH[51].

IV. CONCLUSION

Non-alcoholic fatty liver disease (NAFLD) is a major global health concern that affects a significant portion of the population in Western countries and is slowly spreading to east Asian countries. Non-alcoholic fatty liver disease (NAFL) and non-alcoholic steatohepatitis (NASH) are its two main components. The prevalence of nonalcoholic fatty liver disease (NAFLD), which affects about one-third of adult Americans, is rising over time. Obesity rates can vary from 57 to 74 percent of the population. Furthermore, there are epidemics around the world: 368.8% of adults in the Mediterranean region, 215.5% of adults in Iran, and 27% of adults in urban China have NAFL. However, the extent to which it has dispersed varies by country. It could range from 5 to 24 percent in China, 9 to 30 percent in Japan, and 20 to 30 percent in Europe. NAFLD affects roughly 16–32% of people in India's urban areas and 9% of people in its rural areas. Singapore has the lowest prevalence of non-alcoholic fatty liver disease (NAFLD) among Asian countries, at a startlingly low 5 percent. Sedentary lifestyles, obesity, and non-alcoholic fatty liver disease are commonly associated with living overseas. Notably, non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver (NAFL) have also been detected in non-obese individuals in Asia; this finding is referred to as the "Asian Paradox.". ".". It's important to remember that liver cell fat does not always signify a medical problem.

A number of genetic factors are involved in the transition from nonalcoholic fatty liver disease to nonalcoholic steatohepatosis. Notably, 25–35 percent of cases are thought to be genetically based, suggesting that genetics play a major role in the development of the disease. Fat accumulation has been connected to numerous genetic variations at various locations. Asian Indians may be genetically predisposed to obesity even at lower body weights, according to research. Nonalcoholic fatty liver disease (NAFLD) has been linked to specific gene variations, according to a number of genome-wide association studies carried out in various cultures. Elevated inflammation, steatohepatitis, and severe liver disease are associated with these variations, which are primarily expressed in the liver and adipose tissue. Moreover, it has been determined that the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is significantly influenced by insulin resistance and fructose consumption. According to studies, insulin resistance increases the risk of NAFLD and NASH, especially in obese or metabolic syndrome-afflicted people. Consuming fructose has been demonstrated to worsen metabolic syndrome and increase hepatotoxicity, which results in NASH. Innate immunity and cytokines also have a major part in the pathogenesis of NASH. TNF-α and IL-6 are two examples of cytokines that contribute to the inflammation and metabolic alterations that characterize the early stages.

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