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Review Article

Elevated Serum Alpha-fetoprotein Levels in Non-alcoholic Steatohepatitis: Possible Molecular Mechanisms and Potential Clinical Significance

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Abstract

With the improvement of living standards in recent years, up to 90% of obese patients have nonalcoholic fatty liver disease (NAFLD). The number of nonalcoholic steatohepatitis (NASH)-related deaths will gradually increase, and NASH is expected to be the most common cause of liver-related deaths in the future. Therefore, there is an urgent need to find effective and reliable serum biomarkers to distinguish simple hepatic steatosis (SS) from NASH. Liver cell regeneration, oxidative stress-induced DNA methylation, and biliary epithelial cell proliferation were reported to increase serum alpha-fetoprotein (AFP) levels. AFP has long been used as a tool to monitor liver cancer. However, the function of AFP in NAFLD, especially NASH, has not been clarified. Moreover, whether an elevated AFP level indicates the occurrence of NASH or serves as a serum biomarker remains to be elucidated. The miRNA-122 pathway, DNA methylation and DNA damage, and activation of resident stem cells and/or progenitor cells in the liver, as well as necrosis, regeneration, and repair of liver cells, may contribute to slight increases in AFP levels in the development of NASH in patients with NAFLD. Furthermore, mildly elevated AFP levels may indicate the development of NASH. This review explores the role of elevated AFP levels in the development of NASH, with a specific focus on the underlying molecular mechanisms and the clinical significance.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a disease in which excess fat is deposited in the liver cells that is not associated with alcohol consumption by those affected.[1] NAFLD includes simple hepatic steatosis (SS) and nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis or even hepatocellular carcinoma (HCC). NASH includes a range of histological lesions, including steatosis, lobular inflammation, hepatocyte balloon-like degeneration, and fibrosis.[2,3] Typical pathological features of NASH include fatty deposition in hepatocytes, infiltration of inflammatory cells (neutrophils and lymphocytes) in lobular cells, balloon-like degeneration of hepatocytes, Mallory Denk bodies, peri-sinusoid fibrosis, portal fibrosis, eosinophilic necrosis, and iron deposition.[4] The prevalence of NASH continues to rise with the increasing incidence of obesity, diabetes, and metabolic syndrome. In addition, research has shown that approximately 25% of adults have NAFLD, and approximately 25% will develop NASH during their lifetime, while another 25% will progress from NASH to cirrhosis. The prevalence of HCC is approximately 25% at 10 years after a diagnosis of cirrhosis.[5] Since the continuous development of antiviral therapy has reduced the incidence of viral hepatitis-associated HCC, NAFLD and related metabolic factors have become the most important risk factors for HCC. However, there is currently a lack of optimal treatment options for NASH. Thus, it

Keywords: Alpha-fetoprotein; Nonalcoholic steatohepatitis; Serum biomarkers; Molecular mechanisms; Clinical significance.

Abbreviations: AFP, alpha-fetoprotein; FLD, fatty liver disease; HCC, hepatocellular carcinoma; HPAFP, hereditary persistence of alpha-fetoprotein; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SS, steatosis.

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Table 1. Association between AFP and NASH

Authors	Research type	Subjects	Results of the study	Research conclusion
Babali *et al*.[18]	descriptive	84 subjects with NAFLD	The AFP level of grade 3 NAFLD patients (5.43 ± 1.51) was significantly greater than that of grade 1 (2.92 ± 1.06) and grade 2 (3.97 ± 1.45) patients.	The AFP level was positively correlated with the degree of hepatic steatosis
Xu *et al*.[19]	cross-sectional	9,800 subjects	The AFP level of FLD patients was significantly greater than that of non-FLD subjects ($P < 0.001$).	The serum AFP level was significantly correlated with FLD
Chen *et al*.[20]	cross-sectional	7,755 subjects	The AFP level of MS patients was significantly greater than that of non-MS patients ($P < 0.001$).	AFP was significantly associated with MS
M Kara *et al*.[21]	descriptive	103 subjects with NAFLD and 57 healthy controls	There was no difference in the serum AFP levels between NAFLD patients and healthy controls. AFP levels were similar in SS and NASH patients	AFP may not be involved in the pathogenesis of NAFLD
Kazuhiro Nouso *et al*.[22]	descriptive	115 cases of diabetes mellitus, 36 cases of NAFLD, 119 cases of NASH	The odds ratio of positive AFP-L3 for NASH was 9.81 (95%CI: 3.77–25.5).	There is abnormal fucosylation of serum AFP in NASH patients

AFP, alpha-fetoprotein; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; FLD, fatty liver disease; MS, metabolic syndrome.

is important to intervene early in the course of NAFLD to prevent disease progression.

Liver biopsy remains the current gold standard for the diagnosis of progressive NASH. Nevertheless, it has many drawbacks, such as trauma, sampling error, risk of complications, high cost, and differences among pathological observers, which can cause economic, psychological, and physical distress to patients. Only some NAFLD patients can accept liver biopsy. Therefore, there is an urgent need to find serum biomarkers related to the development of NASH to guide clinical diagnosis and treatment.

Several NASH-related serum biomarkers have been reported, including those for apoptosis, inflammation, liver fibrosis, adipokines, and liver factors.[6] The most common biomarkers include cytokeratin-18, alanine transaminase, aspartate aminotransferase, interleukin-6, vascular cell adhesion molecule 1, serum alpha-2-macroglobulin, hyaluronic acid, tissue inhibitor of metalloproteinase 1, type IV collagen, adiponectin, adipocyte fatty acid binding protein, patatin-like phospholipase domain-containing protein 3, transmembrane 6 superfamily member 2, and microRNA (miRNA)-122, *etc*. However, there is still a lack of effective and reliable serum biomarkers to distinguish SS from NASH in clinical practice. Therefore, there is an urgent need to explore non-invasive, highly sensitive, highly specific, and clinically accessible biological markers to assess the risk of NASH early and to prevent further progression of NAFLD.

Alpha fetoprotein (AFP) is an embryogenic glycoprotein belonging to the serum albumin family. Its gene is located on chromosome 4 and synthesized by fetal liver cells and the yolk sac.[7] AFP expression decreases rapidly after two weeks of fetal life, and only trace amounts of AFP can be measured in adulthood, with AFP levels measuring less than 3 ng/mL for most of a human's lifetime.[8] Serum AFP is a commonly used and very important indicator for the diagnosis of liver cancer and the monitoring of disease progression. In addition, elevated AFP levels are often a widely used tumor marker in HCC patients.[9] According to its affinity with lentil lectin, it can be divided into AFP-L1, AFP-L2, and AFP-L3, from low to high. AFP-L1 is commonly associated with liver inflammation in chronic liver disease; AFP-L2 comes from the yolk sac and can be detected in the serum of pregnant women; and AFP-L3 is specifically expressed in HCC.[10] In most studies, 10 ng/mL is the optimal threshold for the normal range of AFP in adults. Serum AFP levels above 400 ng/mL are highly suggestive of HCC after the exclusion of pregnancy, chronic or active liver disease, embryo-derived gonad tumors, and digestive tract tumors.[11,12] Meanwhile, moderately elevated AFP levels (<150 ng/mL) usually indicate acute or chronic viral hepatitis and cirrhosis. Moreover, AFP concentrations may increase with hepatocyte regeneration and proliferation during liver disease progression.[13] The degree of elevated AFP levels also reflects the degree of liver destruction and subsequent liver regeneration.[14,15] Serum AFP levels have been reported to increase with the severity of liver histology over the course of disease progression from hepatitis to cirrhosis to HCC.[15,16] Furthermore, elevated AFP levels are occasionally detected in genetically susceptible individuals without a history of liver disease or an underlying malignancy. This condition, known as hereditary persistence of alpha-fetoprotein (HPAFP), is an extremely rare autosomal dominant disorder, but these patients all have underlying NAFLD.[17] Currently, the etiology, clinical features, and outcome of mildly elevated AFP levels to 200 ng/mL in asymptomatic individuals with NAFLD are unknown.[9] It remains to be elucidated if mildly elevated AFP levels in asymptomatic NAFLD patients is indicative of NASH after the exclusion of pregnancy, chronic or active liver disease, viral hepatitis, embryo-derived gonad tumors, and digestive tract tumors. This review explores the role of elevated AFP levels in the development of NASH, with a specific focus on the clinical significance and the underlying molecular mechanisms.

Association between AFP and NASH

The significance of elevated AFP levels in patients with NASH is not consistent among current studies (Table 1).[18–22] Babali *et al*. have reported that the AFP level of NAFLD patients was significantly greater than that of healthy controls in a cohort of 84 NAFLD patients diagnosed using ultrasound for the first time.[18] In addition, the AFP level in patients with grade 3 NAFLD was sig-

Table 2. Perspectives on the study of elevated AFP

Authors	The AFP Research Perspective
Caviglia *et al*.[23]	the AFP levels increased significantly from advanced fibrosis without HCC to the progression of HCC.
Farber *et al*.[24]	The high levels of AFP were produced by hepatic progenitor cells in the periportal vein area.
Seung *et al*.[26]	An elevated serum AFP level often indicates active liver regeneration.
Johannie *et al*.[27]	The AFP level increased in the presence of steatosis and liver regeneration.
Kuhlmann *et al*.[8] and Assimakopoulos *et al*.[28]	An increase in AFP expression during hepatocyte regeneration.
Nishida *et al*.[2]	The serum AFP level correlated with the accumulation of oxidative DNA damage in hepatocytes.
Goldstein *et al*.[29]	Alterations in hepatocellular interactions and loss of the normal structural arrangement led to an elevated serum AFP level.

HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein.

nificantly greater than that in patients with grade 1 or 2 NAFLD, and the AFP level in patients with grade 2 NAFLD was significantly greater than that in patients with grade 1 NAFLD. These findings suggested that AFP levels gradually increase with an increase in the degree of SS and that AFP levels are positively correlated with the grade of SS.[18] This study also found that persistent liver inflammation, regeneration, and/or fibrosis may be responsible for elevated serum AFP levels in patients with severe fatty liver disease (FLD), which was most likely secondary to cell necrosis or cytokine stimulation leading to AFP production.[18] Subsequently, Xu *et al*. conducted a large cross-sectional study involving 9,800 subjects, 2,601 of whom had FLD.[19] The results showed that the level of AFP in FLD patients was significantly greater than that in non-FLD subjects. This finding suggested that serum AFP levels significantly correlated with FLD and that AFP is not an independent risk factor for the pathogenesis of FLD.[19] Furthermore, Chen *et al*. reported that SS and subsequent liver regeneration may be responsible for elevated serum AFP levels in patients with metabolic syndrome (MS).[20] However, Kara *et al*. reported results that are inconsistent with those described previously; in their study, 103 male patients with NAFLD (confirmed by liver histology) and 57 male healthy controls were recruited, and there was no significant difference in the AFP levels between the NAFLD patients and the healthy controls.[21] Subgroup analysis showed that the AFP levels were similar between the NASH and SS patients, suggesting that AFP may not be involved in the pathogenesis of NAFLD. In the latest study, Kazuhiro *et al*. found abnormalities in AFP-L3 in NASH patients, indicating that AFP-L3 may be a useful biomarker for the diagnosis of NASH. However, the mechanism of AFP-L3 elevation in NASH patients remained unclear.[22] In conclusion, the correlation between AFP and NASH remains controversial, and more studies are needed to further clarify their relationship.

Possible role of AFP in the development of NASH: Hypothesis on the underlying molecular mechanisms

Studies have shown that there are three main possible mechanisms for increased AFP levels: hepatocyte regeneration, oxidative stress-induced DNA methylation and DNA damage, and proliferation of biliary epithelial cells.[20] Among traditional HCC, AFP is a biomarker for early detection in patients with cirrhosis of viral etiology. However, NAFLD-related HCC data are scant. Caviglia *et al*. found that AFP levels increased significantly from advanced fibrosis without HCC to the progression of HCC ($P < 0.001$), with a moderate performance for AFP (AUC = 0.763).[23] Therefore, a slight increase in AFP levels may indicate hepatocyte regeneration and repair caused by inflammatory injury, and may have predictive significance for NASH, but further studies are needed. In addition, Farber *et al*. reported that high AFP levels were produced by hepatic progenitor cells in the periportal vein area and that the expression of AFP during hepatocyte regeneration was directly correlated with the degree of liver fibrosis.[24] Liver fibrosis is a pathological repair of chronic liver injury, where hepatocytes are repeatedly destroyed and then regenerated. The correlation between AFP and the degree of liver fibrosis means that hepatocyte destruction, regeneration, and repair may be a mechanism that leads to elevated AFP levels. Another study reported that an elevated serum AFP level was independently associated with advanced liver fibrosis.[25] Moreover, Seung *et al*. found that an elevated serum AFP level (>10 ng/mL) may indicate liver regeneration and repair in patients with acute hepatitis A. In various types of acute liver injury, elevated serum AFP levels often indicate active liver regeneration.[26] For example, Johannie *et al*. reported increased AFP levels in the presence of steatosis and liver regeneration.[27] Kuhlmann *et al*. and Assimakopoulos *et al*. also showed that the proliferation of oval cells was accompanied by an increase in AFP expression during hepatocyte regeneration, and the mechanism was different from that of AFP expression in hepatocytes.[8,28] Furthermore, it has been found that oxidative stress and oval cell proliferation are responsible for elevated serum AFP levels in patients with MS.[20] Additionally, Nishida *et al*. reported that patients with high serum AFP levels, hepatocyte ballooning, and liver inflammatory cell infiltration had significant oxidative DNA damage and suggested that serum AFP levels and the degree of hepatocyte ballooning were independently correlated with the accumulation of oxidative DNA damage in hepatocytes.[2] Finally, Goldstein *et al*. demonstrated that alterations in hepatocellular interactions and loss of normal structural arrangement led to elevated serum AFP levels in patients with chronic hepatitis (Table 2).[2,8,23,24,26–29]

Therefore, elevated AFP levels could be due to the strong regenerative capacity of hepatocytes, and AFP levels increase during hepatocyte injury, repair, and regeneration.[30] On the other hand, oxidative stress-induced DNA damage causes transcription factor activation, which induces proto-oncogene expression through DNA methylation, leading to HCC development. In addition, in cases of severe liver injury, hepatocyte regeneration is blocked.[8] The liver rebuilds through biliary epithelial cells, and the proliferative cells of the bile duct system reach differentiation, leading to an increase in AFP-specific immune cell expression and subsequent

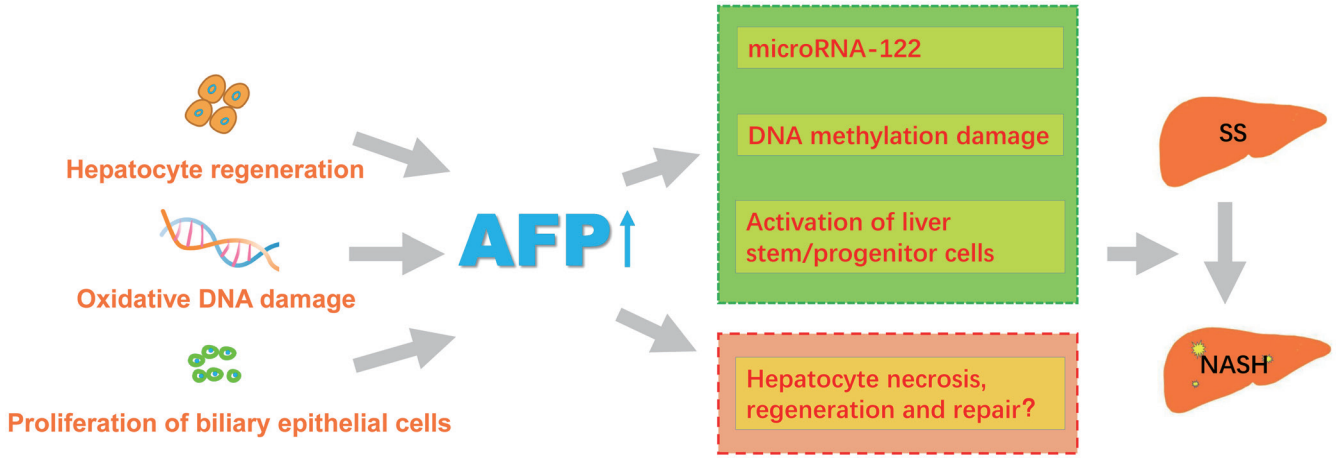

Fig. 1. The pathogenic role of AFP in NASH. AFP, alpha-fetoprotein; NASH, nonalcoholic steatohepatitis; SS, simple hepatic steatosis.

increases in AFP levels (Fig. 1).

The mechanism of AFP elevation in NASH may be due to necrosis, regeneration, and repair of hepatocytes; however, the precise mechanism(s) remains unclear.[31] It has been reported that in the development of NASH, adipose tissue secretion of cytokines leads to cell destruction, inflammation, and/or fibrosis, and activated macrophage-driven adipose tissue inflammation is of great significance. The decreased expression of certain miRNAs in adipose tissue may lead to increased miRNAs that encode soluble pro-inflammatory molecules.[32] For example, miRNA-122 levels have been found to be closely related to liver fibrosis stage and liver inflammatory cell activity.[33] In addition, it has been reported that the miRNA-122 pathway is a possible mechanism of AFP overexpression in HCC.[34] Moreover, studies have shown that AFP expression can be detected in white adipose tissue.[32] Therefore, a mild increase in AFP levels in NASH patients may be related to the miRNA-122 pathway; however, the specific mechanism and this correlation need to be further studied.

Nishida *et al*. analyzed oxidative DNA damage in 65 NAFLD patients using immunohistochemistry with 8-hydroxydeoxyguanosine.[2] The results showed that patients with elevated serum AFP levels and highly balloon-like liver lesions showed oxidative DNA damage. The increased serum AFP level reflected the accumulation of 8-OHFG in hepatocytes, and a high level of 8-hydroxydeoxyguanosine was the result of persistent oxidative stress and cell damage. Additionally, patients with NASH exhibited higher levels of oxidative DNA damage compared to those with other liver diseases. Previous studies have shown that oxidative DNA damage and the resulting silencing repressor may underlie AFP overexpression. Therefore, a slight increase in AFP level may be associated with DNA methylation and DNA damage during NASH development.

Activation of hepatic resident stem and/or progenitor cells is thought to be part of the hepatic response in NASH.[35] Persistent inflammation also leads to destruction of hepatocytes, which are repaired by activating liver resident stem and/or progenitor cells,[36,37] which leads to liver regeneration and repair. Some studies also suggested this process was a healing response to liver trauma caused by lipotoxicity. Of note, AFP is mainly produced by naive hepatocytes. Therefore, activation of hepatic stem and/or progenitor cells during NASH development is considered one of the mechanisms responsible for elevated AFP levels in adults. For example, it has been shown that AFP can stimulate the expression of epithelial cell adhesion molecule[38,39] and that inhibition of epithelial cell adhesion molecule can inhibit liver fibrosis and hepatic stellate cell proliferation in a mouse model.[38] Thus, in NASH patients, the destruction of hepatocytes due to persistent inflammation may contribute to elevated AFP levels through activation of hepatic resident stem and/or progenitor cells. While in SS patients, an elevated AFP level may stimulate inflammatory factors, leading to NASH.

Clinical significance of AFP in NASH

Chen *et al*. found that a continuous increase in AFP levels was a positive predictor of tumor development, while a single or recurrent elevation may not be associated.[40] With the increase in global prevalence of metabolic diseases, the prevalence of NASH is also increasing. Clinically, AFP levels in NASH patients are less than 150 ng/mL. After excluding a history of liver disease, gonadal disease, and rare HPAFP, NASH may occur in patients with stable and low levels of AFP or repetitive mild elevation of AFP. With the development of antiviral drugs, which significantly reduce the incidence of viral hepatitis progressing to HCC, NASH will gradually become the most important cause of HCC. Therefore, asymptomatic NAFLD patients may have a persistent low level of AFP or a reproducible increase in AFP during follow-up, which is likely due to the ongoing necrosis, regeneration, and repair of hepatocytes. Pathological liver biopsy should be recommended to rule out the development of NASH for these patients, along with lifestyle modifications such as a reduced-calorie diet, moderate-intensity exercise, treatment of coexisting metabolic conditions, and smoking and alcohol abstinence.[41] However, the specific mechanism and effect of AFP at a stable and sustained low level or repeated mild elevation remain unclear. The significance of AFP in the occurrence and progression of NASH needs to be further determined through clinical research. In the future, whether a mildly elevated AFP level can be used as a tool to monitor NASH needs to be confirmed with a large-scale study of clinical data.

Conclusion

In the development of NASH, AFP levels may be slightly increased through the miRNA-122 pathway, DNA methylation and

DNA damage, and activation of resident stem cells and/or progenitor cells in the liver, as well as necrosis, regeneration, and repair of liver cells. However, the specific underlying molecular mechanisms of elevated AFP need to be further elucidated. A mildly elevated AFP level in patients with NAFLD may indicate the development of NASH. Further investigation is required to confirm the clinical significance of AFP in NASH and determine whether AFP can serve as a serum biomarker for NASH or provide guidance for clinical diagnosis and treatment.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (CLZ and XYN), analysis and interpretation of data (WYR), drafting of the manuscript (WYR), and critical revision of the manuscript for important intellectual content (CXH, ZHY, DXQ, HDD, SYW, CLZ, and XYN). All authors have made a significant contribution to this study and have approved the final manuscript.

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