CLINICAL REVIEW

Clinical features of hepatocellular carcinoma associated with nonalcoholic fatty liver disease: a review of human studies

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Abstract Most cases of hepatocellular carcinoma (HCC) in Japan develop in the background of chronic liver disease caused by hepatitis C virus (HCV). Recently, however, HCV-associated HCC has been shown to be decreasing, while non-B and non-C HCC (NBNC-HCC), which is negative for HCV and hepatitis B virus infection, has increased. The main cause of NBNC-HCC is alcoholic liver disease, but the recent increase of NBNC-HCC is thought to be due to an increase in patients with nonalcoholic fatty liver disease (NAFLD). Approximately 10 % of NAFLD cases involve nonalcoholic steatohepatitis (NASH), and NASH can progress to liver cirrhosis and its associated complications such as HCC. There are no accurate data on the percentage of NASH-related HCC among all-cause HCC in Japan, because no large-scale investigation has been performed. However, the rate is thought to be about 3 % of overall HCC, which is lower than that in the United States. The incidence of HCC in patients with NASH-related cirrhosis is thought to be 2 % per year, which is lower than that in HCV-related cirrhosis. Risks for NASH-related HCC include advanced hepatic fibrosis, older age, and being male. NAFLD that includes NASH is associated with metabolic syndrome, which includes obesity and diabetes, and metabolic syndrome is a risk factor for HCC. Genetic factors and dietary patterns may also be related to NASH-related HCC. Thus, regular HCC surveillance, as performed for patients with viral chronic liver disease, is required for patients with NAFLD, and diagnostic markers are required for simple, rapid and specific detection of NASH-related HCC.

Keywords Hepatocellular carcinoma · Nonalcoholic steatohepatitis · Nonalcoholic fatty liver disease

Introduction

Nonalcoholic fatty liver disease (NAFLD) has liver histology similar to that of alcoholic liver disease, even in patients with a history of no or a small amount of alcohol intake (30 g/day for males and 20 g/day for females when converted to ethanol) [1]. NAFLD is divided into two types: nonalcoholic fatty liver (NAFL), in which only fatty deposition occurs; and nonalcoholic steatohepatitis (NASH), in which hepatocellular ballooning and advanced hepatic fibrosis occur. NASH can also progress to advanced liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Thus, NAFLD and NASH represent a wide-spectrum of disease that includes cirrhosis (NASH-related cirrhosis) and HCC (NASH-related HCC).

The prognosis in patients with NAFLD is mainly determined by liver disease-related death, including HCC, and cardiovascular disease; and the risks for complication with these diseases require evaluation in each patient to permit appropriate treatment [2–7]. Incidences of NAFLD have been reported worldwide at 6–35 % [8], and the incidence of NASH is estimated to be 3–5 % of the general population [8, 9]. NASH is currently the third most common indication for liver transplantation in the United States. However, the incidence of NAFLD is highest among chronic liver diseases in developed countries, and it has been predicted that NASH will be the most common underlying disease in patients requiring liver transplantation in 2020 [10]. In

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addition, the incidence of HCC is the third highest among malignant diseases [11–13], and an increase of NASH-related HCC is an important concern.

The number of NAFLD patients has been increasing yearly, along with increased consumption of a Western diet and an increase in the number of obese patients. The incidence of NAFLD in Japan is estimated to be 20–30 % [14–17]. Approximately 10 % of NAFLD cases are thought to be NASH and there are approximately two million patients with NASH in Japan [18]. Hepatitis C virus (HCV)-related chronic liver disease is currently the main cause of HCC in Japan, but a future increase of NASH-related HCC is a concern [19]. In this review, we focus on the diagnosis, characteristics and risk factors of NASH-related HCC, which will be increasingly important in the treatment of NAFLD.

Incidence of HCC in patients with NAFLD/NASH

NAFLD/NASH is a fatty liver disease that mainly develops due to obesity and insulin resistance. A focus has recently been placed on NASH-related HCC. HCC is one of the few cancers that are increasing in developed countries [20], and the incidence of HCC in males has risen threefold in the last 20 years in the United States [21]. This increase of HCC is partly due to the increased number of patients with NAFLD/NASH [22].

The number and percentage of patients with HCC induced by HCV has decreased, due to development of antiviral therapy and a decrease in new infection opportunities for hepatitis virus in Japan. In contrast, the number of cases of NBNC-HCC has increased [19], due to an increase in NASH-related HCC [23, 24]. In a study of 6,508 patients with NAFLD diagnosed by ultrasonography, the incidences of HCC were 0.19 and 0.51 % at 8 and 12 years, respectively [25]. Arase et al. [26] found an incidence of NASH-related HCC of 0.63 % over 8.2 years in 1,600 Japanese NAFLD patients aged 60 years old or older, and suggested that the high incidence was related to the older age of the subjects. The 5-year incidence of HCC in patients with NASH-related cirrhosis has been reported to be 11.3 %, which is lower than that of 30.5 % in patients with HCV-related cirrhosis [27] (Fig. 1).

In other countries, Adams et al. [2] found that the incidence of HCC in patients with NAFLD was 0.5 % over 7.6 years, and other reports also suggest an incidence of < 1 % [4, 28–30]. Ekstedt et al. [4] found an incidence of HCC in patients with NASH of 2.8 % over 13.7 years and the incidence of NASH-related HCC has been estimated to be 1–3 % over 10 years [29, 30]. In contrast, Ascha et al. [31] found a 3.2-year incidence of HCC of 12.8 % in patients with NASH-related cirrhosis, and Sanyal et al. [32]
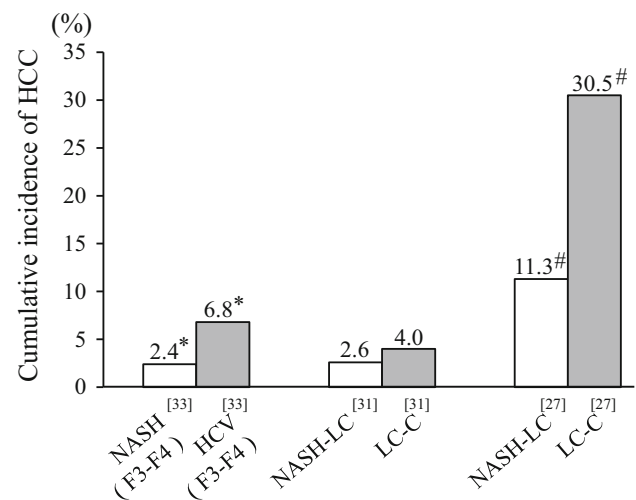

Fig. 1 Incidence of hepatocellular carcinoma in patients with nonalcoholic fatty liver disease or hepatitis C virus-related chronic liver disease. *Asterisk* 7.1-year cumulative incidence of HCC. *Hash symbol* 5-year cumulative incidence of HCC. *HCC* hepatocellular carcinoma, *NASH* nonalcoholic steatohepatitis, *HCV* hepatitis C virus-related chronic liver disease, *NASH-LC* nonalcoholic fatty liver disease-related liver cirrhosis, *LC-C* hepatitis C virus-related liver cirrhosis, *F3–F4* fibrosis stage 3–4 [reference number]

reported a 10-year incidence of 6.6 %. These results suggest that the incidence of NASH-related HCC is higher in patients in whom hepatic fibrosis has progressed to an advanced stage (Table 1).

The incidence of HCC in patients with NAFLD varies depending on patient background, but clearly increases when the disease is aggravated. HCC has a lower incidence in patients with NASH-related cirrhosis compared to those with HCV-related cirrhosis [31, 33, 34] (Fig. 1). However, HCC has been found to develop at a rate of approximately 2 % per year in patients with NASH-related cirrhosis, and thus regular screening tests are required for patients with NASH because these patients are at high risk for HCC.

Risk factors for HCC in patients with NAFLD/NASH

Progression of hepatic fibrosis

Progression of hepatic fibrosis is a major cause of liver disease-related death in patients with NASH [35], and is the most important risk factor for NASH-related HCC [34]. In a study of patients with NASH-related HCC, Hashimoto et al. [36] found advanced hepatic fibrosis in non-cancerous liver tissue in 88 % of the subjects, and showed that advanced hepatic fibrosis was the most important risk factor for HCC [odds ratio (OR) 4.232]. Kawamura et al. [25] identified a platelet count of ≤150,000/μL as a risk factor for development of HCC in patients with NAFLD

Table 1 Prevalence of HCC in patients with NAFLD or NASH

References	Type of NAFLD	Number	Age of cohort	HCC prevalence/years of follow up	Comment
Japan					
Kawamura et al. [25]	NAFLD	6508	49 (median)	0.02 %/4 years, 0.19 %/ 8 years, 0.51 %/12 years (0.043 %/1 year)	Diagnosed by US
Arase et al. [26]	NAFLD	1600	62.5 (9.5)[a]	0.63 %/8.2 years	Diagnosed by US
Yatsuji et al. [27]	NASH-LC	68	62.7 (13.2)[a]	11.3 %/5 years	Biopsy-proven
NAFLD/NASH patients outside Japan					
Adams et al. [2]	NAFLD	420	49 (15)[a]	0.48 %/7.6 (4)[a] years	Most cases were diagnosed by imaging
Ekstedt et al. [4]	NAFLD	58	46.7 (12.3)[a]	0 %/13.7 (1.3)[a] years	Biopsy-proven NAFLD
Ong et al. [28]	NAFLD	817	≤40 (50.4 %), 40–≤60 (34 %), >60 (15.6 %)	0 %/8.4 years	Analysis of cause of death
Rafiq et al. [29]	NAFLD	173 (NASH 72)	50.2 (14.5)[a]	0.58 %/10.5 years	Biopsy-proven NAFLD, death due to HCC
Starley et al. [30]	NAFLD	–	–	0.14 %	Review (overall HCC prevalence)
Ekstedt et al. [4]	NASH	71 (NASH-LC 4)	54.5 (12.4)[a]	2.8 %/13.7 (1.3)[a] years	Biopsy-proven NAFLD
Starley et al. [30]	NASH	–	–	1.6 %	Review (overall HCC prevalence)
Ascha et al. [31]	NASH-LC	195	56.6 (median)	12.8 %/3.2 years (2.6 %/1 year)	Biopsy and/or radiological evidence of cirrhosis
Sanyal et al. [32]	NASH-LC	152	55.3 (median)	6.6 %/10 years	Biopsy-proven
Bhala et al. [33]	NASH (F3–F4)	247	54.7 (11.6)[a]	2.4 %/7.1 years	Fibrosis stage 3 (47.7 %), 4 (52.2 %)

HCC hepatocellular carcinoma, *NAFLD* nonalcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *LC* liver cirrhosis, *US* ultrasonography

[a] Mean (SD)

[hazard ratio (HR) 7.19]. Thus, detection of advanced hepatic fibrosis in NASH cases is likely to be effective for early identification of patients at high risk for HCC.

In contrast, Sanyal et al. [37] found liver cirrhosis in non-cancerous liver tissue in 78 % of patients who developed HCV-related HCC, but in only 46 % of patients with NAFLD who developed HCC. Development of HCC in non-cirrhotic patients with NAFLD is also increasingly reported, and these patients have fatty changes and inflammatory findings in the portal region in the liver. Male patients with metabolic syndrome may also be at higher risk for HCC, even if they only have mild hepatic fibrosis of stage F0–F2 [22].

Age and gender differences

The prevalence of NASH is reported to be high in middle-aged males and elderly females [38–40]. An age ≥ 60 years is a risk factor (HR 4.27) for development of HCC [25], and thus HCC should be considered in treatment of older patients with NASH.

The incidence of NASH is high in young males compared to young females (66 vs. 24 %), but the incidence is higher in females ≥50 years of age compared to males ≥50 years of ages (76 vs. 34 %) [40]. This is thought to be because many females develop NASH due to rapid progression of hepatic fibrosis after menopause. However, despite the incidence of NASH-related cirrhosis being higher in females, the incidence of concomitant HCC is higher in males (62 vs. 38 %) [36], which suggests that males have a high risk of NASH-related HCC. Further, even young males with mild progression of hepatic fibrosis may be at higher risk of development of HCC compared to females [41]. Thus, male NAFLD patients with other risk factors should be especially carefully monitored for onset of HCC [42].

Obesity and metabolic syndrome

Obesity is a strong risk factor for HCC [43]. A cohort study of 900,000 people living in North America showed high mortality in patients with HCC with BMI >35 kg/m^2 (HR 4.52 in males and 1.68 in females) [44]. In another cohort study with 578,700 subjects in Northern Europe, 266 subjects developed HCC during the 12-year observation period, and a high BMI [relative risk (RR) 1.39] and metabolic

syndrome (RR 1.35) were confirmed as risk factors for HCC [45]. In a multicenter collaborative study in Europe with 359,525 subjects, a weight increase in adulthood was found to be a risk factor for HCC (RR 2.48) [46]. Meta-analyses in the United States, Europe and Asia have also suggested that an overweight status is a risk factor for HCC (OR 1.89) [47]. In a study of 3,649 HCC patients and 743 patients with intrahepatic cholangiocarcinoma in the United States, metabolic syndrome was found to be a risk factor for development of HCC (OR 2.13) and intrahepatic cholangiocarcinoma (OR 1.56) [48]. These findings clearly suggest that obesity and metabolic syndrome are closely related to HCC.

In a review article, Starley et al. [30] found that 34–54 %, 18–29 %, 27–37 %, and 9 % of NASH patients showed no progression, improvement, aggravation of hepatic fibrosis, and development of liver cirrhosis, respectively, over 5.6 years. These authors also suggested that obesity and high BMI are independent risk factors for progression of hepatic fibrosis [30]. A study of NAFLD/NASH patients who underwent liver biopsy several times also showed that decreases in BMI and waist size were independent factors associated with no progression of hepatic fibrosis [49], and a 7 % weight decrease improves the NAFLD activity score [50]. Thus, weight control is important in treatment of NAFLD/NASH, and hepatic fibrosis progression and the high incidence of HCC may be ameliorated by decreasing weight in NAFLD/NASH patients.

Diabetes

In a large-scale cohort study, the risk of malignant disease was found to be higher in patients with diabetes compared to those without diabetes (1.27 times in males and 1.21 times in females). Diabetes also led to a higher risk of HCC in males and females (HRs of 2.24 and 1.94, respectively), and was more strongly associated with development of HCC among malignant tumors [51]. A meta-analysis also suggested that diabetes increases the risk of HCC (RR 2.38) [52] and diabetes is a risk factor for HCC in patients with NAFLD (HR 3.21) [25].

In studies outside Japan, diabetes has also been shown to be an independent risk factor for HCC [53, 54]. A study in the United States found that 74 and 22.2 % of type II diabetes patients had NAFLD and NASH, respectively [9], and patients with a complication of diabetes are at high risk for NAFLD (HR 1.98) and HCC (HR 2.16) [55]. A meta-analysis also showed that many patients with diabetes develop HCC concomitantly (HR 2.01) [56].

These findings show that onset of HCC should be monitored in NAFLD/NASH patients, and especially in those with diabetes. The incidence of future complication of diabetes is also high in NAFLD [14], and diabetes should be viewed as an important complication in the treatment of NAFLD/NASH.

Hyperlipidemia

Excess intake of cholesterol [57] and saturated fatty acids [58] are both risk factors for NAFLD, and total and low-density lipoprotein (LDL) cholesterol may be increased in patients with NAFLD [59]. Fatty liver is also an independent risk factor for an increase in small dense LDL [60]. Thus, hyperlipidemia is associated with NAFLD [17], but there is no direct evidence from human studies that shows that hyperlipidemia contributes to onset of HCC.

Genetic factors

Genome-wide association studies (GWAS) have suggested that patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a genetic factor that contributes to the development and progression of NASH [61–63]. PNPLA3 is present on chromosome 22 and is involved in metabolism of triglyceride. A single nucleotide polymorphism (SNP) (rs738409) of PNPLA3 is closely related to fatty liver, and the fat level in the liver increases by approximately 100 % in patients who are homozygous for this risk allele of PNPLA3. This SNP is also involved in progression of fibrosis in NAFLD [64, 65], and is significantly correlated with NAFLD of Matteoni classification type 4 [63]. The C<G variation in SNP rs738409 of PNPLA3 also increases the risk of HCC in patients with NAFLD [66]. Compared to the CC allele, the GC and GG alleles have risks for HCC with ORs of 2.52 and 12.19, respectively. Thus, SNP rs738409 of PNPLA3 appears to be related to development of HCC in patients with NAFLD/NASH.

Alcohol intake and smoking

Alcohol intake may play a protective role against fatty liver [67], but persistent alcohol abuse is a well-established etiologic factor in HCC. A prospective study of NASH-related cirrhosis also showed that the risk of HCC in subjects with a small amount of alcohol intake (occasional drinking) was higher (HR 3.6) than that for subjects who did not drink alcohol [31]. Furthermore, the incidence of advanced hepatic fibrosis is high in smokers [68], and the percentage of patients with a smoking history has been found to be higher among those with newly developed NAFLD compared to those without NAFLD over a 10-year study period [15]. Tobacco smoking is a significant risk factor for development of HCC, and significant dose-dependent and duration-dependent relationships between

tobacco use and HCC risk have been found [69]. After exclusion of subjects who were daily drinkers and were positive for hepatitis B virus (HBV) or HCV infection, the risk of HCC associated with long-term smoking was 1.85-fold higher than that in subjects who had never smoked (HR = 1.85, 95 % CI 0.66–5.18) [69]. Thus, alcohol intake and smoking may promote development of NASH-related HCC.

Inflammatory cytokines

The "two hit" theory [70] and the "multiple parallel hits" hypothesis [71] have been proposed for the pathogenesis of NASH. The multiple parallel hits hypothesis suggests that multiple equilibrium hits from the intestinal tract and fat tissues are involved in progression of NASH, and that changes such as increased inflammatory cytokines (TNFα, IL-6 and resistin) and decreased adiponectin, an anti-inflammatory cytokine, may promote hepatocarcinogenesis [72–76]. Changes in intestinal bacteria associated with obesity might also promote development of HCC [77], although further studies in humans are needed.

Fructose

The increased intake of fructose in recent years is related to weight increases and development of obesity in childhood and adolescence [78–80]. Excessive intake of fructose may aggravate NAFLD [81–84]. Patients with histologically diagnosed NAFLD have increased progression of hepatic fibrosis if their fructose level is high, despite having a mild level of fatty liver. Excessive fructose intake also appears to aggravate the clinical conditions of NAFLD/NASH [85]. These findings suggest that excess fructose may contribute to development of NAFLD and progression of hepatic fibrosis, and may be related to onset of HCC.

Iron metabolism

In patients with NAFLD, the ferritin level is closely correlated with the degree of hepatic fibrosis [86–88]. Iron deposition in liver tissues is higher in NASH than in NAFL, and excess iron produces reactive oxygen species and aggravates hepatic disorder through oxidative stress. The iron level in liver tissues in NASH patients with HCC is also significantly higher than that in NASH patients without HCC [89]. Excess iron is thought to be a risk for progression of hepatic fibrosis and hepatocarcinogenesis in NASH, and thus patients with a high serum ferritin level, which suggests excess iron, should be screened carefully for the development of HCC.

Characteristics and diagnosis of HCC in patients with NAFLD/NASH

Appropriate selection of patients with advanced hepatic fibrosis is important in screening for NAFLD/NASH-related HCC. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (γ-GTP) levels do not reflect the severity of NAFLD, and such liver function test results are occasionally within normal limits in patients with NAFLD/NASH [90, 91]. Therefore, longitudinal evaluation with tumor markers and hepatic fibrosis markers is important for identification of patients at high risk for NASH-related HCC.

Tumor markers

Of 19 NASH patients who developed HCC during an observation period, abnormal levels of α-fetoprotein (AFP, >10 ng/mL) and protein induced by Vitamin K absence or antagonists (PIVKA)-II [also known as des-γ-carboxy prothrombin (DCP), >40 mAU/mL] were found in ten (53 %) and nine (47 %), respectively, upon diagnosis of HCC, suggesting that the positive rates for these tumor markers are not particularly high in patients with NASH-related HCC [92]. Also, the increase in average AFP from before to after development of HCC (6.0–10.5 ng/mL) was not significant, but the increase of PIVKA-II (18–34 mAU/mL) was significant. These findings suggest that an increase in PIVKA-II may be useful to detect NASH-related HCC, but that screening for NASH-related HCC using only tumor markers is insufficient [92].

Platelet count

Platelet count is a useful index for stage of hepatic fibrosis in NAFLD. Compared to NAFL patients, NASH patients have lower platelet counts, and progression of NASH should be suspected in patients with a decrease in platelet count over time. However, this requires careful confirmation, because the decrease in platelet count is minor in many NAFLD/NASH patients compared to patients with viral hepatitis, such as chronic hepatitis C. A platelet count of 150,000/μL suggests hepatic fibrosis of stage F2 in patients with chronic hepatitis C, whereas platelet counts of 192,000/μL and 153,000/μL suggest hepatic fibrosis of stages F3 (sensitivity 62.7 %, specificity 76.3 %) and F4 (sensitivity 80.5 %, specificity 88.8 %), respectively, in NAFLD patients [93]. A platelet count of ≤150,000/μL may be a risk factor for the development of HCC (HR 7.19) [25]. NAFLD/NASH patients with a platelet count of ≤150,000/μL may have liver cirrhosis and may be at high risk for HCC.

Blood biomarkers

Serum concentrations of cytokeratin 18 fragment [94], adiponectin [95], high sensitivity CRP [96], thioredoxin [97], and MnSOD [98] are useful for diagnosis of NASH. In a large-scale study, cytokeratin 18 fragment was shown to a useful single diagnostic marker for NASH [99]. A glycosylation marker has also been suggested to be effective for evaluating the progression of hepatic fibrosis and the disease stages of viral hepatitis and NAFLD [100, 101]. Further accumulation of evidence is required to determine which marker is most useful for screening of NASH-related HCC.

Hepatic fibrosis scoring systems

Scoring systems using multiple factors are useful for determination of the disease stage of NAFLD/NASH. The NAFLD fibrosis score (NFS) [102], FIB-4 index [103, 104], and NAFIC score [87] increase with progression of NASH, and the NFS and FIB-4 index are useful as prognostic markers [105]. Kawamura et al. [25] found an incidence of HCC in patients with NAFLD of 0.043 %/year, and showed that the incidence of HCC in patients diagnosed with advanced hepatic fibrosis based on an AST-to-platelet ratio index (APRI) of >1.5 was significantly higher than that in patients without advanced hepatic fibrosis (APRI ≤1.5). Using the FIB-4 index, Yoneda et al. [106] found advanced hepatic fibrosis (F3–F4) in 16.1 % of NAFLD patients, even when ALT levels were normal. These findings suggest that these scoring systems are useful for prediction of hepatic fibrosis and HCC.

Imaging tests

Transient elastography is useful for noninvasive evaluation of hepatic fibrosis based on measurement of the stiffness of the liver by ultrasonography [107, 108]. NASH can be diagnosed by detection of dysfunction of Kupffer cells using contrast-enhanced ultrasonography [109]. Magnetic resonance imaging (MRI) is also useful for evaluation of progression of hepatic fibrosis in NAFLD [110]. Since it is difficult to perform liver biopsy for all NAFLD patients, scoring systems and imaging tests that enable estimation of the degree of hepatic fibrosis are useful to identify patients at high risk for HCC.

Conclusion

The increase in the number of NAFLD/NASH patients suggests that these liver diseases will be more important in the future. The risk of HCC in NAFLD/NASH patients is lower than that in patients with HCV-related chronic liver disease, but the number of patients with NAFLD/NASH is large, and thus the percentage of patients with NASH-related HCC among all HCC patients is likely to increase. However, there are no diagnostic markers for simple, rapid and specific detection of NAFLD/NASH-related HCC. Furthermore, the frequency of HCC surveillance for NAFLD patients is low compared to patients with HCV-related chronic liver disease and alcoholic liver disease [111]. This emphasizes the importance of development of accurate blood tests and imaging tests for HCC surveillance in NAFLD/NASH patients.

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