

Original Article

Risk factors for naturally-occurring early-onset hepatocellular carcinoma in patients with HBV-associated liver cirrhosis in China

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Abstract: Aims: Early onset of hepatocellular carcinoma (HCC) (males and females under the age of 40 or 50 years old, respectively) has a significant prevalence and poor prognosis; however, few studies have reported the risk factors and development of HCC in such cases. Methods: In this study, we retrospectively analyzed clinical, laboratory and demographic data from 588 treatment-naïve HCC patients with hepatitis B virus (HBV)-associated liver cirrhosis (LC) and 708 age-matched HBV-associated LC patients as control in Beijing 302 Hospital. Results: 15.1% (89/588) of the HCC patients and 36.7% (181/708) of the LC patients were classified as early onset. Compared with age-matched LC controls, male gender (odds ratio (OR) = 2.09, $P < 0.05$), family history of HBV infection (OR = 2.45, $P < 0.05$) and alpha-fetoprotein (AFP) > 200 ng/ml (OR = 30.8, $P < 0.05$) were independent risk factors for early-onset HCC. Comparing late-onset LC controls, male gender (OR = 1.92, $P < 0.05$), age (OR = 1.04, $P < 0.05$), family history of HCC (OR = 2.06, $P < 0.05$), history of smoking (OR = 1.68, $P < 0.05$) and AFP > 200 ng/ml (OR = 12.0, $P < 0.05$) were associated with the development of naturally occurring HCC. Overall, male gender and AFP >200 ng/ml is associated with HCC development across all ages, whereas a family history of HBV infection may identify younger HBV-associated LC patients at risk for HCC. Conclusion: Our data suggest that a family history of HBV infection is a unique risk factor for naturally-occurring early-onset HCC patients with HBV-associated LC, who should be considered for intensive screening programs.

Keywords: Risk factors, hepatocellular carcinoma, HBV-associated liver cirrhosis

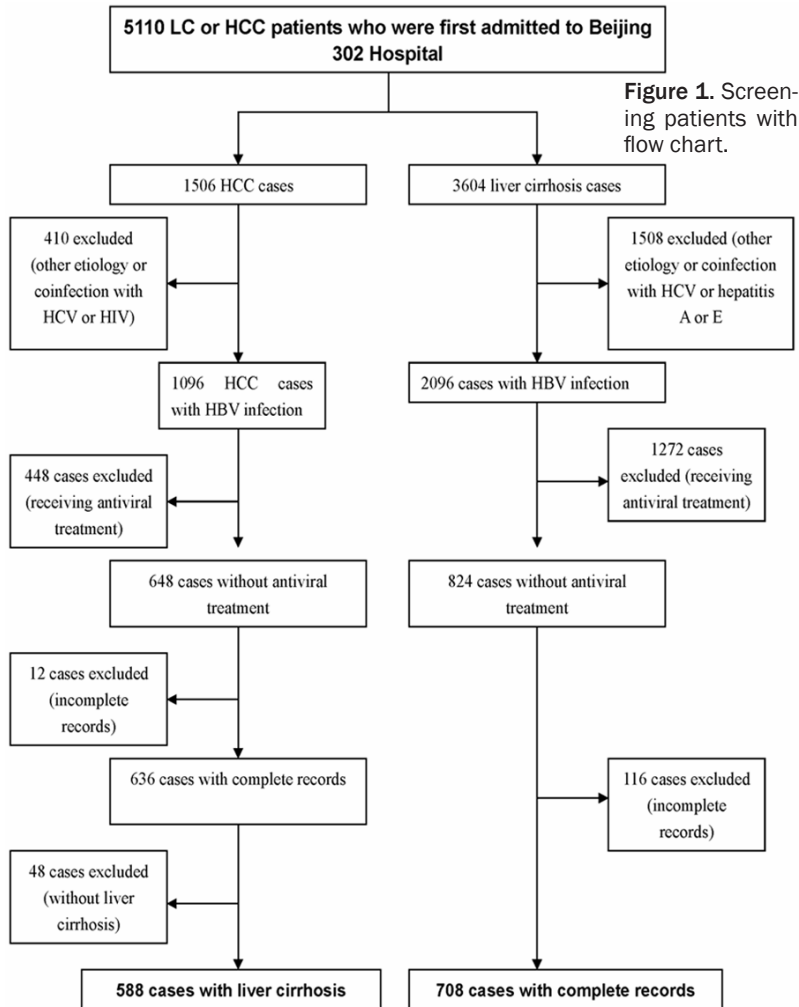
Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause for cancer-related death worldwide [1]; particular in China, over half of HCC is diagnosed and 70% attributable to chronic hepatitis B virus (HBV) infection [2]. The average age at onset of HBV-associated HCC is 50 years of age [3]. The patients with HCC usually present late and have mostly developed from serious liver cirrhosis. The life expectancy of patients with newly diagnosed HCC is measured in term of weeks to months with a mortality to incidence ratio close to 1 [4]. Therefore, it needs to know some risk factors that can be clinically applied for the prediction of this malignancy in the HBV-infected population. Many previous studies have identified greater age, male gender, family history of HCC, diabetes, obesity,

alcohol consumption, cigarette use, aflatoxin exposure, elevated alanine transaminase, positive hepatitis B e antigen (HBeAg), high levels of HBV DNA, genotype C and various viral mutations as risk factors for HCC in HBV-infected patients [5-10]. In particular, LC is the single greatest risk factor for HBV-associated HCC (70-90%), though HBV can also cause HCC in the absence of LC [11, 12]. In China, more than 80% of HCCs develop from chronic HBV infection-associated LC [13].

Although frequently associated with a poor prognosis, HCC patients diagnosed at an early stage (within the Milan criteria) have the best chance for long-term survival. Thus, practice guidelines for HCC screening by the American Association for the Study of Liver Diseases (AASLD) have been developed to select at-risk patients who should be entered into screening

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HCC groups which may, to some extent, weaken the conclusion. Moreover, most cases of HCC develop from liver cirrhosis, therefore, HCC and cirrhosis share the same risk factors. A comprehensive analysis of naturally-occurring HCC cohorts with well-matched backgrounds is therefore warranted to identify the specific risk factors for HCC in younger patients.

The present study investigated risk factors for naturally-occurring HCC in younger and older patients with chronic HBV-associated LC, using age-matched LC patients as controls. Since the two groups of patients has same LC background, this study will allow to exclude LC factor and possibly identify some unique parameters that might merit earlier screening for HCC with LC background.

Methods

Patients and study design

programs [14]. The recommendations advise HCC screening for patients with chronic HBV infection should begin when they are cirrhotic or non-cirrhotic at age 40 or 50 years for Asian males and females, respectively. However, recent studies have reported that HCC has a significant prevalence and worse prognosis in younger individuals. These studies also suggest that early-onset HCC may have unique risk factors that distinguish it from late-onset HCC, but few researches have investigated this issue. Although a study of Asian immigrants with HBV infection indicated that patients with a history of smoking or a family history of HCC appeared to have an increased risk for younger-onset HCC [15], many possible risks were not included in the analysis, such as hepatitis B surface antigen (HBsAg) and HBeAg status, antiviral treatment and family history of HBV infection. In addition, the control populations in these studies were unmatched with according

Five thousand one hundred and ten patients, who were first admitted to Beijing 302 Hospital and diagnosed as LC (n = 3604) or HCC (n = 1506) during the period January-December, 2011, were enrolled for screening. The diagnosis of HBV was made by positive serology for HBsAg and a diagnosis of HCC was confirmed by either histology or established radiologic criteria [16]. Cirrhosis was defined either histologically or radiographically (i.e., nodularity, liver morphology, splenomegaly, presence of varices, ascites). HBV-associated HCC and LC control groups were categorized into early onset (males and females under the age of 40 or 50, respectively) or late onset (males and females of 40 or 50 years or older, respectively) in accordance with current AASLD guidelines. Patients were excluded if they had previously received any antiviral or antitumor treatment, or if they had chronic hepatitis C, acute hepatitis A, acute hepatitis E, HIV, autoimmune hepa-

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Table 1. Univariate analysis: associated risk factors compared between early-onset and late-onset HCC cases and age-matched controls

	Early-onset cohort		Late-onset cohort		Early onset	Late onset	HCC	LC
	HCC N = 89 (%)	LC Controls N = 181 (%)	HCC N = 499 (%)	LC Controls N = 527 (%)	HCC vs. LC OR (P value)	HCC vs. LC OR (P value)	Early vs. Late OR (P value)	Early vs. Late OR (P value)
Mean age (years)	36.7	36.7	53.2	52.6				
Gender (Male)	63 (70.8)	105 (58.0)	439 (88.0)	430 (81.6)	1.75 (0.04)	1.65 (< 0.01)	0.30 (< 0.01)	0.31 (< 0.01)
Family history of HCC	9 (10.1)	10 (5.50)	63 (12.6)	32 (6.07)	1.92 (0.17)	2.24 (< 0.01)	1.30 (0.50)	9.10 (0.78)
Family history of HBV	57 (64.0)	80 (44.2)	233 (46.7)	234 (44.4)	2.25 (0.02)	1.09 (0.44)	1.04 (< 0.01)	1.00 (0.96)
Alcohol use	10 (11.2)	17 (9.39)	131 (26.3)	112 (21.3)	1.22 (0.64)	1.32 (0.06)	0.36 (< 0.01)	0.29 (< 0.01)
Smoking history	10 (11.2)	29 (16.0)	173 (34.7)	125 (23.7)	0.66 (0.30)	1.71 (< 0.01)	0.24 (< 0.01)	0.57 (0.03)
HBeAg (+)	23 (25.8)	73 (40.3)	113 (22.6)	203 (38.5)	0.52 (0.02)	0.47 (< 0.01)	1.20 (0.51)	0.99 (0.66)
HBeAb (+)	52 (58.4)	92 (50.8)	329 (65.9)	285 (54.1)	1.36 (0.24)	1.64 (< 0.01)	0.70 (0.17)	0.77 (0.45)
ALT > 80 IU/ml	16 (18.0)	75 (41.4)	79 (15.8)	180 (34.2)	0.31 (< 0.01)	0.36 (< 0.01)	1.17 (0.61)	1.36 (0.08)
TBil \geq 1.2 mg/dl	22 (24.7)	99 (54.7)	187 (37.4)	334 (63.4)	0.27 (< 0.01)	0.35 (< 0.01)	0.55 (0.02)	0.70 (0.04)
PLT < $100 \times 10^9/L$	26 (29.2)	105 (58)	185 (37)	368 (69.8)	0.29 (< 0.01)	0.25 (< 0.01)	0.70 (0.15)	0.60 (< 0.01)
PT > 14.3 s	15 (16.9)	87 (48.1)	71 (14.2)	256 (48.6)	0.22 (< 0.01)	0.18 (< 0.01)	1.22 (0.50)	0.98 (0.91)
ALB < 3.5 g/dl	21 (23.6)	106 (58.6)	195 (39.1)	384 (72.9)	0.22 (< 0.01)	0.24 (< 0.01)	0.48 (< 0.01)	0.53 (< 0.01)
AFP > 200 ng/ml	52 (58.4)	12 (6.60)	243 (48.7)	54 (10.3)	19.8 (< 0.01)	8.31 (< 0.01)	1.50 (0.09)	0.58 (0.15)
DNA > 20000 IU/ml	49 (55.1)	136 (75.1)	339 (67.9)	415 (78.8)	0.41 (< 0.01)	0.57 (< 0.01)	0.50 (0.02)	0.64 (0.31)
Child-Pugh A	69 (77.5)	78 (43.1)	360 (72.1)	188 (35.7)	(< 0.01)	(< 0.01)	(0.27)	(0.19)
Child-Pugh B	16 (18.0)	81 (44.8)	125 (25.1)	273 (51.8)				
Child-Pugh C	4 (4.5)	22 (12.2)	14 (2.81)	66 (12.5)				

titis, primary biliary cirrhosis or hemochromatosis. Additionally, if more than three items or variables were missing from their clinical data, they were excluded due to incomplete records. The study protocol has been approved by the Ethics Committees of Beijing 302 Hospital, and written informed consent was obtained from all participants.

Among the 1506 HCC cases identified, 1096 had HBV infection and 410 were excluded due to other etiologies or coinfection with HCV or HIV. Four hundred and forty-eight patients who had previously received antiviral treatment, 48 patients without LC and 12 patients with incomplete records were also excluded. The final group comprised 588 HCC patients, not by any treatment, with 89 early-onset and 499 late-onset cases (**Figure 1**).

In the LC control group, 1508 patients were excluded due to other etiologies or coinfection with HCV or acute hepatitis A or E. Two thousand and ninety-six patients with HBV infection were preliminarily identified; of these, 116 were excluded due to incomplete records and 1272 due to previous antiviral treatment. Thus, 708 patients were included in the final LC control group, which comprised 181 early-onset patients and 527 late-onset patients (**Figure 1**).

Data collection

Data were extracted from electronic medical records and included age, sex, family history of HCC, family history of HBV infection (defined as positive if at least one parent had been diagnosed as HBsAg positive), timing of HCC or LC diagnosis, and clinical and laboratory test results. Smoking tobacco (more than 10 pieces per day for more than 1 year) or regular alcohol use (i.e. more than one drink per day) was considered to be positive in the smoking history and drinking history. Baseline laboratory values recorded upon initial visit included total bilirubin, platelets, prothrombin time, ALT, HBV viral load, alpha-fetoprotein (AFP), HBeAb, HBeAg status and Child-Pugh score.

Statistical analysis

The clinical characteristics of the early-onset and late-onset groups were analyzed using descriptive techniques. Univariate analysis employing the chi-square test for categorical variables was used to assess differences between HCC cases and age-matched LC controls. Additional comparisons were made directly between early-onset and late-onset HCC and LC cases. Multivariate logistic regression analysis was applied to investigate differ-

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Table 2. Multivariate analysis: associated risk factors compared among early- and late-onset HCC

	B	P	OR	95% CI
Early-onset HCC: associated risk factors compared between early-onset HCC cases and age-matched LC controls				
Male gender	0.74	0.049	2.09	0.99-4.40
Family history of HBV	0.89	0.012	2.45	1.22-4.95
AFP > 200 ng/ml	3.43	< 0.001	30.8	12.7-74.8
Late-onset HCC: associated risk factors compared between late-onset HCC cases and age-matched LC controls				
Male gender	0.65	0.006	1.92	1.20-3.05
Age	0.03	0.001	1.04	1.01-1.06
Family history of HCC	0.72	0.01	2.06	1.19-3.59
History of smoking	0.52	0.003	1.68	1.19-2.36
AFP > 200 ng/ml	2.49	< 0.001	12.0	8.12-17.8

ences between the clinical characteristics of the groups. All statistical tests were two-tailed and statistical significance was determined at $P < 0.05$. All analyses were performed using SPSS version 13.0.

Results

Patient characteristics

One thousand two hundred and ninety-six patients met the inclusion criteria. Of these, 588 were HCC patients with HBV-associated LC and 708 were LC patients who had not been diagnosed with HCC. Diagnoses of HCC and LC were made at 18-82 years and 20-82 years, respectively. The mean age of diagnosis of early-onset HCC was 36.7 years, compared with 36.7 years for the LC controls; the mean age of diagnosis of late-onset cases was 53.2 years for the HCC patients versus 52.6 years for the LC controls (**Table 1**). Fifteen percent (89/588) of the HCC patients and 36.7% (181/708) of the LC patients were in the early-onset category.

All 588 HCC patients and 708 LC patients were compared for gender, family history of HCC, family history of HBV infection, alcohol use and smoking history, HBeAg and hepatitis B e antibody (HBeAb) status, AFP, HBV viral load, Child-Pugh score and liver function markers such as ALT, TBIL, PLT, PT and ALB levels in early-onset and late-onset cases (**Table 1**). Among the HCC patients, 63 of 89 (70.8%) early-onset and 439 of 499 (88%) late-onset cases were male. Nine of the 89 (10.1%) early-onset patients had a family history of HCC; in the late-onset group, 63 of 499 (12.6%) had a family history. Ten (11.2%) early-onset patients had a positive

alcohol or smoking history, whereas in the late-onset group 131 (26.2%) and 173 (34.7%) had a positive alcohol or smoking history, respectively. More than 15% early-onset and late-onset HCC cases had abnormal liver functions. Similarly to the HCC patients, early-onset LC patients were less likely to be male and fewer had histories of alcohol use or smoking compared with the late-onset LC patients. Notably, 57 of the 89 (64%) early-onset HCC patients had a positive family history of HBV infection, whereas in the late-onset HCC group, 233 of 499 (46.7%) had a positive family history, indicating a greater possibility of having a family history of HBV infection in early-onset HCC than in late-onset cases. More than 1/3 early-onset and late-onset LC cases had abnormal liver functions. Thus, the chance of having a family history of HBV infection was similar between early-onset and late-onset LC.

Comparison of clinical and laboratory characteristics: univariate analysis

The results of univariate analyses of the younger and older patient groups are shown in **Table 1**. In both early-onset and late-onset HCC groups, compared with LC control groups, AFP > 200 ng/ml and Child-Pugh score A were positively associated with HCC development, whereas HBeAg positivity, ALT > 80 IU/L, TBIL ≥ 1.2 mg/dL, PLT counts $< 100 \times 10^9/L$, PT > 14.3 second, ALB < 3.5 g/dL and serum HBV load > 20,000 IU/ml were negatively associated with HCC development. In addition, in the early-onset HCC group compared with age-matched LC controls, family history of HBV infection were uniquely positively associated with HCC development; and in the late-onset HCC group compared with according LC con-

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trols, male gender, family history of HCC, history of smoking and HBeAb positivity were significantly positively associated factors.

We further compared these factors between early-onset and late-onset patients. Among the HCC cases, univariate analysis showed that late-onset patients, compared with early-onset patients, were more likely to be male, to have a history of alcohol use or smoking, to have TBIL ≥ 1.2 mg/dL, ALB < 3.5 g/dL and HBV load $> 20,000$ IU/ml, but fewer had a family history of HBV infection (all $P < 0.05$). Among the LC patients, direct comparison between early-onset and late-onset cases by univariate analysis showed that late-onset patients, compared with early-onset patients, were more likely to be male and to have a history of alcohol use or smoking, to have TBIL ≥ 1.2 mg/dL, PLT counts $< 100 \times 10^9/L$ and ALB < 3.5 g/dL (all $P < 0.05$). No other variables were found to be statistically significant between early-onset and late-onset patients. Because gender, alcohol use and smoking history, TBIL ≥ 1.2 mg/dL, PLT counts $< 100 \times 10^9/L$ and ALB < 3.5 g/dL were all risk factors for both early-onset and late-onset HCC and LC patients, a family history of HBV infection may be uniquely associated with early-onset HCC.

The Barcelona Clinic Liver Cancer (BCLC) staging system is generally used to classify the severity of HCC [14]. In the present study, the early-onset HCC cases were classified as stage 0 (1.1%; 1/89), A (38.2%; 34/89), B (29.2%; 26/89), C (27%; 24/89) or D (4.5%; 4/89). The late-onset HCC cases were classified as stage 0 (1.2%; 6/499), A (36.3%; 181/499), B (30.1%; 150/499), C (29.7%; 148/499) or D (2.8%; 14/499). BCLC stage at presentation did not differ significantly between the early-onset and late-onset HCC cases.

We also staged all cases using the Child-Pugh score system [17]. The early-onset HCC cases were classified as stage A (77.5%; 69/89), B (18%; 16/89) or C (4.5%; 4/89) and the LC cases as stage A (43%; 78/181), B (44.8%; 81/181) or C (12.2%; 22/181). The late-onset HCC cases were classified as stage A (72.1%; 360/499), B (25.1%; 125/499) or C (2.8%; 14/499) and the LC cases as stage A (35.7%; 188/527), B (51.8%; 273/527) or C (12.5%; 66/527). The difference in Child-Pugh score was statistically significant between both early-

onset and late-onset HCC patients and the corresponding LC patients.

Multivariate analysis

On logistic regression multivariate analysis, statistically significantly associated factors in early-onset HCC were family history of HBV infection (odds ratio (OR) = 2.454), male gender (OR = 2.092) and AFP > 200 ng/ml (OR = 30.797). For late-onset HCC, statistically significantly associated factors were male gender (OR = 1.917), age (OR = 1.035), family history of HCC (OR = 2.063), history of smoking (OR = 1.675) and AFP > 200 ng/ml (OR = 12.035). These findings suggest that a family history of HBV infection is a specific risk factor for early-onset HCC (Table 2).

Discussion

Beijing 302 Hospital is the largest hospital specialized in liver diseases that receives almost 600, 000 out-patient visits and 35, 000 in-patients annually in China. More than 95% of the inpatients are with liver diseases, in which HBV-associated chronic hepatitis, LC and HCC are the most common conditions. Because most HCCs develop from LC and more than 90% (588/636) of the HCC patients in the present study had a LC background, we excluded 48 HCC patients without LC. Thus, this study allows us to find some unique risk factors of HCC patients with LC background. We also excluded some patients who had received previous antiviral treatment, because antiviral therapy can slow the development of HCC and/or LC in patients with CHB [18, 19]. By comparing treatment-naïve HCC patients with well-matched LC controls, we identified a family history of HBV infection as a unique risk factor for early-onset naturally-occurring HCC with LC.

Previous studies have found that male gender [15], older age [20, 21], a family history of HCC [22] and smoking history [23, 24] were risk factors for HCC. Our study, similar to these previous studies, further identified these risk factors only in late-onset HCC but not in early-onset HCC through univariate analysis and logistic regression multivariate analysis. The reasons for this difference between early-onset and late-onset patients is elusive, and may be associated with genetic and hormonal differences [16] or age-dependent accumulating effects for

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smoking and alcohol use [24, 25]. In addition, the precise age of patients at HCC diagnosis or other risk factors may also have influenced the results [15, 26]. Future studies are needed to determine the relative risks of these factors in early-onset and late-onset HCC.

AFP is also well documented as an HCC risk factor. Generally, healthy people have low AFP levels, but it may elevate in certain diseases. Serum AFP has long been used as a diagnostic marker for HCC, though with controversies [27]. In particular, a recent study indicated that AFP is a strong independent predictor of HBV-associated HCC, but it remained controversial whether it could serve as a risk marker for the development of HCC in patients with HBV-associated LC [28]. The present study showed that AFP > 200 ng/ml was an independent risk factor for both early-onset and late-onset HCC. As an AFP test is both convenient to perform in the clinic and inexpensive, many more patients with HBV-related disease should receive this test, especially younger patients.

One novel finding of our study is that a family history of HBV infection may be a risk factor specific to early-onset HCC. It is somewhat surprising that this factor was significant only in the early-onset HCC cases, and the underlying reasons remain unclear. Because HBV infection time may be associated with HCC development [25], the family history of HBV infection may suggest longer infection time than those without family history of HBV infection. While in late-onset group, the HBV infection time may reach the ceiling effects, thus leading to a similar distribution of the family history of HBV infection between HCC and LC groups. A family history of HBV infection may suggest a genetic predisposition that is more influential at an earlier age. Future studies are needed to elucidate whether host genetic susceptibility to HBV infection differs between younger and older individuals.

Interestingly, our univariate analysis indicated that Child-Pugh scores are negatively related with HCC occurrence, which is further supported by the finding that HCC development is reflected by better liver functions than LC controls. This finding is different two previous studies [20, 29], in which Child-Pugh class B/C cirrhosis were independent prognostic factors for HCC. The reasons underlying this difference

remain unclear and may be related with differing cohort control, host genetics or environmental exposure. Another possible explanation is that different immune surveillance exists in various phase of HCC development. The host immune responses have been widely demonstrated to play key roles in liver pathogenesis [30]. In patients with Child-Pugh class B/C, more strong immune responses may, on some extent, suppress HCC occurrence; by contrast, in patients with Child-Pugh class A, the weak immune responses are likely to fail to control HCC development. In addition, we found that HBV DNA > 20,000 IU/ml is more frequently occurred in LC patients as compared with HCC patients in our univariate analysis, which is consistent with a previous study [31] but contradictory to another study [13]. One possible explanation is that HBV replication-dependent normal hepatocytes are largely reduced in HCC cases than that in LC patients. Anyway, other risk factors may also have influenced the results and the precise mechanisms need to be elucidated in future.

Our study has limitations. First, given its retrospective nature, it is subject to recall bias. Second, many HCC and HBV-associated LC patients were excluded due to incomplete records, and this may introduce a selection bias. Third, some patients with incomplete records were included; therefore, the missing of information on detailed alcohol and smoking quantity, HBV genotype, viral mutations and aflatoxin exposure hindered the investigation of the relevance of these risk factors. However, we did analyze important data such as family history of HBV infection, HBsAg and HBeAg/Ab status, serum HBV DNA level and liver function markers.

In conclusion, a significant proportion of HBV-associated LC patients developed naturally into HCC at ages younger than the recommended cutting-age for screening according to current guidelines. This early-onset of HCC among treatment-naïve patients is significantly associated with male gender, a family history of HBV infection and elevated serum AFP levels. Our results suggest that younger LC patients with HBV infection, especially those male patients with high AFP or a family history of HBV infection, appear to be at increased risk for early screening regardless of their age.

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Disclosure of conflict of interest

None.

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