Predicting risk of hepatocellular carcinoma for patients with chronic hepatitis B using serum levels of hepatitis B virus DNA splicing

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Background and Aims

In chronic hepatitis B (CHB) patients, serum levels of hepatitis B virus (HBV) splice variant (spHBV) DNA have been shown to be significantly higher in patients with hepatocellular carcinoma (HCC) than in non-HCC patients. We have now investigated the predictability of spHBV DNA levels on HCC development using samples collected well before HCC diagnosis from a large community-based treatment-naïve cohort, and assessed the risk adjusted for and combined with other recognized risk factors.

Materials and Methods

Subjects

In REVEAL cohort, Pre-diagnosed samples closest to HCC diagnosis of 152 incident HCC cases and matching samples from 378 age- and sex-matched non-HCC controls

Laboratory Methods

- spHBV DNA levels and wtHBV DNA levels were determined by Real-time PCR
- spHBV DNA levels= spHBV/(spHBV+wtHBV) x 100%

External validation

A published data was used for external validation (Bayliss et al., J. Hepatol. 2013)

Results

- Compared to patients with spHBV <20%, the adjusted OR (95% CI) of HCC development was 23.3 (6.9-79.3), P<.0001 for those with spHBV ≥20% (Table 1).
- Risk of HCC for patients with spHBV ≥20% raised to extremely high levels when combined with male, elevated levels of HBV DNA and α-fetoprotein, HBV genotype C, and liver cirrhosis (Table2).
- A subset of 77 HCC cases and 28 non-HCC controls had repeated measurements of spHBV DNA levels prior to HCC development or censored . spHBV DNA levels increased with time approaching HCC development in individual HCC cases, while remained stably low in non-HCC controls (Figure 1).
- Within 5 years prior to HCC diagnosis, patients with spHBV ≥20% had a 32.8-fold higher risk for HCC (adjusted OR (95% CI)= 32.8 (10.2-105.9), P<.0001) (Table 1).
- In the external validation analysis, the proportion of spHBV ≥20% among non-HCC controls was similar to our finding (1.6% vs. 1.7%). In addition, the patients with spHBV ≥20% had a 4.5-folds risk for HCC compared to patients with spHBV <20%; and 5.3-folds higher risk for HCC within 5 years. Although calculated relative risks were crude ORs, the trends were similar to results using the REVEAL data (Table3).

Table1. Predictability of spHBV levels on HCC development

spHBV DNA levels (%)	No (%) of Non-HCC (n=411)	No (%) of HCC (n=152)	HCC vs. Non-HCC Adjusted odds ratio	P-value*	<5 years prior to HCC (n=103) No. (%)	Adjusted odds ratio (95%CI)	P-value*
The cut-off determined by Youden Index							
<20	404 (98.3)	138 (90.8)	1.0		89 (86.4)	1.0	
≥ 20	7 (1.7)	14 (9.2)	23.3 (6.9-79.3)	0.0002	14 (13.6)	32.8 (10.2-105.9)	<.0001

*Adjusted for gender, HBV DNA levels, α-fetoprotein, HBV genotype, and liver cirrhosis

Table3. External validation

spHBV DNA levels (%)	No (%) of Non-HCC (n=125)	No (%) of HCC (n=74)	HCC vs. Non-HCC Crude odds ratio (95%CI)	P-value	<5 years prior to HCC (n=103) No. (%)	Crude odds ratio (95%CI)	P-value
<20	123 (98.4)	69 (93.2)	1.0		58 (92.1)	1.0	
≥ 20	2 (1.6)	5 (6.8)	4.5 (0.8-23.6)	0.0788	5 (7.9)	5.3 (1.0-28.1)	0.05

Figure 1. Trajectories of spHBV levels prior to HCC development

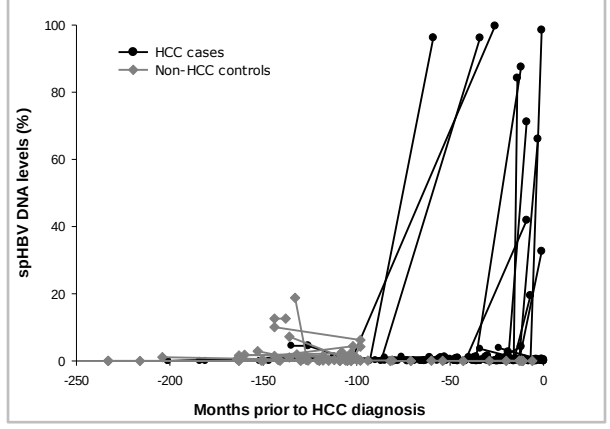

Table 2. Combined effects of spHBV levels and other risk factors on HCC risk

Combined Variables	HCC (n=152) No. (%)	Non-HCC (n=411) No. (%)	HCC vs. non-HCC Adjusted odds ratio* (95%CI)	P value	for interactio
Gender / spHBV levels (%)					
Female / <20	25 (16.5)	93 (22.6)	1.0		0.786
Female / ≥ 20	2 (1.3)	2 (0.5)	21.8 (2.6-181.4)	0.004	
Male / <20	113 (74.3)	311 (75.7)	2.5 (1.4-4.6)	0.003	
Male / ≥ 20	12 (7.9)	5 (1.2)	39.4 (10.3-150.5)	<.0001	
HBV DNA level (copies/mL) / spHBV levels (%)					
10^4 - 99,999 / <20	14 (9.2)	102 (24.8)	1.0		0.572
10^4 - 99,999 / ≥ 20	7 (4.6)	4 (1)	22.6 (5.0-102.0)	0.0001	
≥ 100,000 / <20	124 (81.6)	302 (73.5)	2.9 (1.4-5.8)	0.004	
≥ 100,000 / ≥ 20	7 (4.6)	3 (0.7)	34.9 (6.7-183.0)	<.0001	
α-fetoprotein (ng/mL) / spHBV levels (%)					
<10 / <20	85 (55.9)	344 (83.7)	1.0		0.989
<10 / ≥ 20	8 (5.3)	7 (1.7)	14.4 (4.5-45.9)	<.0001	
≥ 10 / <20	53 (34.9)	60 (14.6)	4.4 (2.5-7.5)	<.0001	
≥ 10 / ≥ 20	6 (4)	0 (0)	∞	0.986	
HBV genotype / spHBV levels (%)					
B+BC / <20	46 (31.9)	243 (64.5)	1.0		0.986
B+BC / ≥ 20	11 (7.6)	7 (1.9)	13.6 (4.3-42.8)	<.0001	
C / <20	84 (58.3)	127 (33.7)	3.2 (2.0-5.1)	<.0001	
C / ≥ 20	3 (2.1)	0 (0)	∞	0.982	
Liver cirrhosis / spHBV levels (%)					
No / <20	67 (44.1)	299 (72.8)	1.0		0.985
No / ≥ 20	9 (5.9)	7 (1.7)	14.6 (4.6-46.5)	<.0001	
Yes / <20	71 (46.7)	105 (25.6)	2.2 (1.4-3.5)	0.001	
Yes / ≥ 20	5 (3.3)	0 (0)	∞	0.981	

*Adjusted for gender, HBV DNA levels, α-fetoprotein, HBV genotype, and liver cirrhosis

Conclusions

- Serum spHBV DNA levels could be a useful biomarker for predicting HCC development over the 5-year period leading up to diagnosis.
- Patients who have serum spHBV DNA levels above 20% could benefit from more detailed assessment of HCC risk, especially in those individuals who are male, with high levels of HBV DNA and α-fetoprotein, HBV genotype C infection, or cirrhosis.