STUDY ON CHARACTERIZATION OF HEPATITIS B CORE - RELATED ANTIGEN (HBcrAg) AT MEDIC MEDICAL CENTER IN HO CHI MINH CITY

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Summary

Objectives: To describe some of the characteristics of hepatitis B core - related antigen (HBcrAg) and determine the correlation coefficient of HBcrAg and serum HBV - DNA concentration in chronic hepatitis B. *Subjects and methods:* This cross - sectional descriptive study and analysis included 106 cases at MEDIC Medical Center in Ho Chi Minh city from November 2019 to July 2020. *Results:* The rate of HbcrAg - positive patients in study was 71.7%, the mean HBcrAg concentration was 4.15 ± 1.63 log U/mL, distribution range was 2.0 - 7.0 log U/mL.The mean HBcrAg concentration was significantly difference by age group, enzyme ALT elevation, specific treatment, HBeAg status and serum HBV - DNA levels. The serum HBcrAg concentration correlated with the serum HBV - DNA levels in a positive and linear manner with correlation coefficient r = 0.75 in the treatment - naïve patients and r = 0.40 in the treatment - experienced patients. *Conclusions:* The study showed a significant difference in mean HBcrAg concentration in the ALT - increased group and in the normal ALT group; in the HBeAg - negative group and HBeAg - positive group; in the undetectable serum HBV - DNA group and detectable HBV - DNA group. The correlation coefficient between serum HBcrAg and HBV - DNA in the study sample was well correlated with r = 0.75 in the patients who were treatment - naïve and r = 0.40 in the patients who were treated with nucleos(t)ide analogues (NAs).

Key words: Hepatitis B core - related antigen, serum HBV - DNA, chronic hepatitis B.

INTRODUCTION

Hepatitis B virus (HBV) infection continues to be an important global health problem. Current data shows that 292 million people worldwide are carriers of HBV in a modelling study of HBsAg prevalence conducted in 120 countries in 2016[6]. Chronic hepatitis B progressing to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) is the main cause of death globally. The serum HBV - DNA levels have been shown to predict the risk of cirrhosis and HCC. Maintenance of HBV - DNA at high levels is associated with liver disease progression. Serum HBV - DNA levels is a prognostic factor in determining chronic HBV infection, indication for treatment and monitoring of the efficacy of antiviral drugs. Hepatitis B core - related antigen (HBcrAg) is the generic term for three types of antigen structural proteinwhich consists of 3 species of related protein sharing an identical 149 amino acid sequence: HBcAg, HBeAg and p22cr. In the treatment - naïve patients, HBcrAg levels correlated positively with the serum HBV - DNA levels in both HBeAg - positive and HBeAg - negative patients[3]. It has been reported that while HBV - DNA levels drop rapidly in patients undergoing nucleos(t)ide analogues (NAs) therapy, in many cases falling below the limit of detection, HBcrAg declines at a much slower rate. HBcrAg has been detected in samples below

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the limit of detection for HBV - DNA, with equal or better sensitivity than HBV - DNA[2]. The divergence between the two is thought to be attributable to the action of NAs in hindering reverse transcription and preventing HBV - DNA replication, while the HBV cccDNA remaining in the liver tissue continues to discharge HBcrAg. Therefore, the Japan Society for Liver Disease Research recommends HBcrAg as a useful serum marker for predicting flare - ups during therapy and determining when to conclude treatment[2]. In the 2017 European Association for Hepatitis B clinical practice guidelines, serum HBcrAg levels may be a potential new biomarker that partly reflect the amount of intrahepatic HBV - DNA and cccDNA in hepatocytes and it also be helpful in defining the phase of chronic HBV infection, especially in HBeAg - negative patients, as well as predicting the long-term HCC risk[1]. We conduct research to describe some characteristics of HBcrAg and determine the correlation coefficient between serum HBcrAg and HBV DNA levels in patients with chronic hepatitis B treated at Medic Medical Center in Ho Chi Minh City.

SUBJECTS AND METHODS

Subjects: Patients with chronic hepatitis B were defined by the American Association for the Study of Liver Disease 2018 diagnostic criteria have positive serum HBsAg for ≥ 6 months[5].

Exclusion criteria: Patient co - infected with hepatitis C and/or HIV co - infection; drug - induced hepatitis, alcoholism, fatty degenerative hepatitis, the patients did not agree to participate in the study.

Location and time of the study: Research conducted at the Department of Infectious disease, Medic Medical Center in Ho Chi Minh City; from November 2019 to July 2020.

Methods: Cross - sectional descriptive study and analysis.

Laboratory techniques applied in research:

Quantitation of serum HBcrAg: The specimen is mixed with anti - HbcrAg - coated particles and allowed to form antigen - antibody immunocomplexes. Alkalaine phosphatase - labelled anti - HBcrAg specifically binds to HBcrAg of the immunocomplexes on the particles, and additional immunocomplexes are formed. Luminescence is generated and the luminescent signal reflects the amount of HBcrAg. Quantitation of HBcrAg is made by comparing the chemiluminescence signal generated by known concentration of recombinant ProHBeAg. This assay is currently available in an automated format, using the Lumipulse G1200 CLEIA analyser (Fujirebio, Tokyo, Japan), with a lower limit of detection of 2.0 logU/mL, and a linear range of 3.0 logU/mL - 7.0 logU/mL (1 kU/mL is equal to 3 logU/mL).

Quantitation of serum HBV - DNA: HBV - DNA technique Taqman DNA extraction with Roche MN 96 machine; IDT Primer Probe and Mastermix, the HBV Taqman procedure runs on Realtime Agilent US in 80 minutes.

Statistical Analysis: Data were analyzed using SPSS version 20.0 software package (SPSS Inc, Chicago, IL).

RESULTS

From November 2019 to July 2020, 106 cases were eligible for inclusion in the study.

Demographic characteristics of the sample

Table1. Socio - demographic and geographic characteristics of the studied group (n = 106)

Demographic characteristics	Groups	Frequency (n)	Rate (%)
Age (year)	< 40	50	47.2
	40 - < 60	48	45.3
	≥ 60	8	7.5
Mean age (min - max) 41 ± 12 (17 - 77)			
Gender	Male	70	66.0
	Female	36	34.0
Anti - viral therapy	Yes	32	30.2
	No	74	69.8

Comment: Study sample included 106 chronic hepatitis B patients with mean age of 41 ± 12 years, the age group under 40 accounted for the highest percentage (47.2%), the age group from 40 -< 60 accounted for 45.3% and the age group ≥ 60 accounted for only 7.5%. The rate of males were higher than that of females (66.0% versus 34.0%). The frequency of patients under anti - viral treatment was 30.2%. The frequency of treatment - naïve patients was 69.8%.

Table 2. The clinical and laboratory features in the studied group (n = 106)

Characteristics			Frequency (n)	Rate (%)
Clinical	Jaundice		3	2.8
	Spiderangiomata		1	0.9
	Palmar erythema		1	0.9
	Ascites		2	1.8
	No symptoms		101	95.3
Ultrasound	Smooth liver parenchyma		48	45.3
	Crude liver parenchyma		52	49.0
	Cirrhosis		6	5.7
	HCC		0	0
Laboratory	ALT	< ULN	36	34.0
		≥ ULN	70	66.0
	AST	< ULN	82	77.4
		≥ ULN	24	22.6
	Blood platelet (cells/mm^3)	≤ 100,000	3	2.8
		> 100,000	103	97.2
	HBeAg	Negative	77	72.6
		Positive	29	27.4
	HBV - DNA (log IU/mL)	Negative	43	40.6
		Positive	63	59.4
		Mean (min - max)		4.86 ± 1.95 (2.44 - 9.27)

(ULN: upper limit of normal)

Comment: The majority of patients in the study group had no clinical symptoms (95.3%). On abdominal ultrasound images, 45.3% of patients had smooth liver parenchyma, 49.0% had crude liver parenchyma, and 5.7% showed cirrhosis findings. The rate of normal ALT (< ULN) in both males and females was 34.0%, the rate of increased ALT (≥ ULN) was 66.0%. The rate of normal AST (< ULN) in both males and females was 77.4%, the rate of increased AST (≥ ULN) was 22.6%. The majority of patients hadblood platelets over 100,000 cells/mm^3 (97.2%). For HBeAg status, there was 72.6% HBeAg(-) and 27.4% HBeAg(+). The rate of serum positive HBV - DNA was 59.4% and negative is 40.6%. In the positive HBV - DNA group, the mean viral load was 4.86 ± 1.95 logIU/mL.

Table 3. The HBcrAg concentration in the studied group (n = 106)

HBcrAg	Distribution range	Frequency (n)	Rate (%)
Negative	2.0 -< 3.0 (log U/mL)	30	28.3
Positive	3.0 -> 7.0 (log U/mL)	76	71.7
Mean (log U/mL)		*4.15 ± 1.63*	

Comment: With a positive HBcrAg classification threshold ≥ 3.0 logU/mL as specified by the manufacturer, the study included 28.3% of HbcrAg - negative (< 3.0 log U/mL) and 71.7% patients. HbcrAg - positive patients with a distribution range of 3.0 - 7.0 logU/mL. The mean concentration of HBcrAg in the study sample was 4.15 ± 1.63 logU/mL.

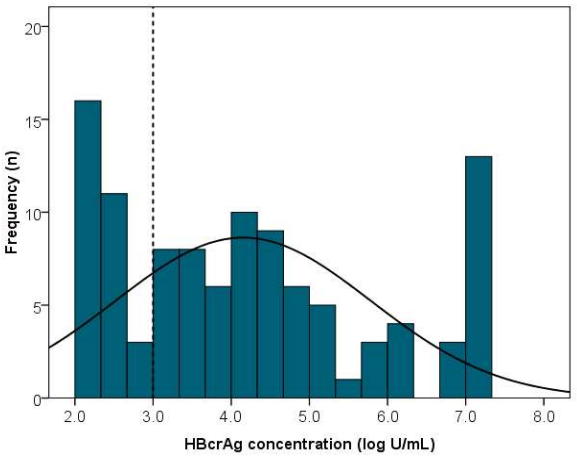

Figure 1. Distribution of HBcrAg concentration in the study sample (n = 106)

Comment: Serum HBcrAg concentration in research samples distributed in the range of 2.0 - 7.0 logU/mL. Observing the graph, we can see that the concentration of HBcrAg distributed in bell shape as standard distribution with mean value of 4.15 ± 1.63 logU/mL (sknewness index = 0.44).

Table 4. The mean HBcrAg concentrations in population groups (n = 106)

Characteristics	Groups	Mean HBcrAg log (U/mL)	p (t - test)
Age (year)	< 40 (n = 50)	4.54 ± 1.70	0.031[a]
	40 - < 60 (n = 48)	3.92 ± 1.44	
	≥ 60 (n = 8)	3.14 ± 1.74	
Gender	Male (n = 70)	4.22 ± 1.55	0.577
	Female (n = 36)	4.03 ± 1.79	

Characteristics	Groups	Mean HBcrAg log (U/mL)	p (t - test)
Anti - viral therapy	Yes (n = 32)	4.78 ± 1.54	0.008
	No (n = 74)	3.88 ± 1.61	
Clinical symptoms	Yes (n = 5)	4.33 ± 1.47	0.662
	No (n = 101)	4.54 ± 1.85	
Ultrasound	Smooth liver parenchyma (n = 48)	4.17 ± 1.61	0.923
	Crude liver parenchyma and/ or cirrhosis (n = 58)	4.14 ± 1.67	
ALT (U/L)	< ULN (n = 36)	3.64 ± 1.42	0.021
	≥ ULN (n = 70)	4.41 ± 1.68	
Blood platelet (cells/mm^3)	≤ 100,000 (n = 3)	4.37 ± 1.12	0.583
	> 100,000 (n = 103)	4,55 ± 1,84	
HBeAg	Negative (n = 77)	3.46 ± 1.18	< 0.001
	Positive (n = 29)	6.0 ± 1.15	
HBV DNA	Negative (n = 43)	3.54 ± 1.39	0.026
	Positive (n = 63)	4.36 ± 1.76	

([a]: Anova test)

Comment: The mean serum HBcrAg levels were not significantly different in sex distribution, clinical symptoms, ultrasound liver damages, and blood platelet levels. The differences in HBcrAg concentration by age groups were significant (p = 0.031). Mean HBcrAg concentration in the age group <40 years was higher than that in the age group 40 -< 60 and age group ≥ 60. Mean HBcrAg concentration varied according to anti - viral therapy status, HBcrAg was significantly higher in the patients who treated with NAs compared to the treatment - naïve group (p = 0.008). Mean HBcrAg concentration in the group with increased ALT (≥ ULN) was significantly higher than in the group with normal ALT (p = 0.021). Similarly, HBcrAg concentrations varied according to serum HBeAg and HBV - DNA, significantly higher in HBeAg - positive and HBV - DNA - positive groups compared to the HBeAg - negative and HBV - DNA - negative groups with p <0.001 and p = 0.026, respectively.

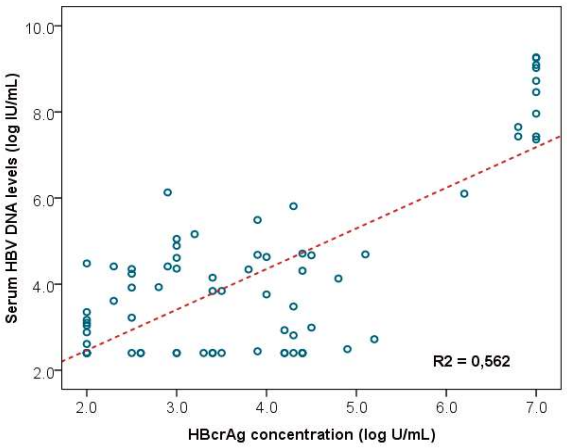

Figure 2. Correlation between serum HBcrAg concentration and HBV - DNA levels in the treatment - naïve patients (n = 74)

Comment: In the group of patients not treated with NAs, the serum HBcrAg concentration was positively and strongly correlated with serum HBV - DNA concentration with Pearson correlation coefficient r = 0.75 (p = 0.01).

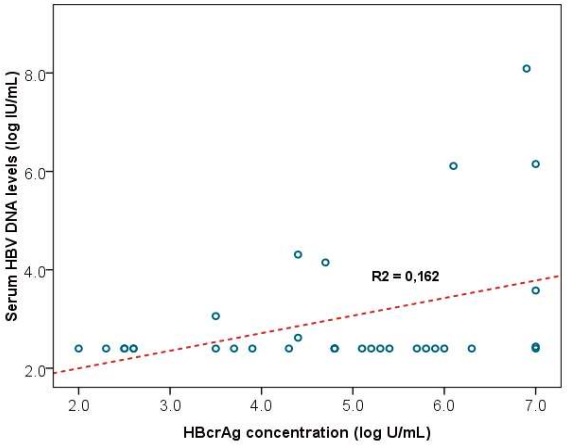

Figure 3. Correlation between serum HBcrAg concentration and HBV - DNA levels in the patients treated with NAs (n = 32)

Comment: In the group of patients on antiviral therapy, HBcrAg concentration has a positive and weak correlation with serum HBV - DNA concentration with Pearson correlation coefficient r = 0.40 (p = 0.05).

DISCUSSION

The characteristics of serum HBcrAg in the study sample (Table 3, Table 4 and Figure 1)

HBcrAg consists of three species of related proteins