Original Article

Relationship between Hepatitis B Viral DNA Load and Hepatocellular Carcinoma

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Abstract

Background: Hepatitis B virus (HBV) infection, an established cause of hepatocellular carcinoma (HCC) and is associated with poor prognosis. It is the fifth most frequent cause of cancer related death in men and the ninth most frequent cause of cancer related death in women. High HBV DNA load has been identified as the most important predictor of the development of HCC in HBsAg patients. HBV DNA>10^5 copies/ml increases HCC risk associated with Hazard Ratio of 8.6(3.7-20.1) ($p<0.0001$), compared to HBV DNA<300 copies /ml (undeletable). Chronic hepatitis B virus carriers have a 100-fold relative risk of developing HCC comparing with non-carriers. Therefore we need to establish HBV DNA level in patients with HCC and to see the relationship between HBV DNA load and HCC was considered. **Methods:** This cross sectional, analytical study was done to measure HBV DNA load to evaluate its significance in the causation of HCC. This study was done in the Department of Hepatology BSMMU, Dhaka, Bangladesh from January 2006 to December 2007. After considering inclusion and exclusion criteria, 30 HBV and FNAC/liver biopsy proved HCC patients were taken as case group. 30 HBV related liver disease patients without HCC were enrolled as control group. **Results:** In this study 30 HBV related HCC patients had a high viral load (>10^5-copies/ml) while all of the control had low (<10^5-copies/ml) viral load. Comparison between groups was done by student's t-test and chi-square (χ^2). Here the p-value was <0.001 which was less than 0.05 and was statistically significant. This study showed that HBV related HCC was associated with high HBV DNA load. **Conclusion:** From this study it was concluded that HBV DNA (PCR) levels were higher in patients with HCC compared with control subjects. This cross sectional study revealed that high viral load was associated with the development of HCC.

Indexing words: Hepatitis B, HBsAg, Hepatocellular carcinoma (HCC), HBV DNA (PCR)

Introduction:

Hepatitis B Virus (HBV) infection is an established cause of hepatocellular carcinoma (HCC) and is associated with poor prognosis[1]. Despite decades of experimental and epidemiological investigations and widespread acceptance of its carcinogenicity, the specific mechanisms by which this virus lead to HCC remain poorly understood.

It has been estimated that about 350 million people around the world are chronically infected with hepatitis B virus[1] accounting for around 1 million deaths each year related to chronic liver disease, cirrhosis and HCC[2]. Vast majority of this disease burden occurs in developing countries.

Geography plays an important role, whereby variation in the epidemiologic patterns of infection and the corresponding HBV prevalence crudely reflects HCC incidence patterns[2]. The greatest burden of HCC is in sub-Saharan Africa and parts of Asia, where HCC is the most frequent cause of cancer related death among men. Chronic HBV infection is highly prevalent and is the predominant risk factor for HCC in this high-incidence regions[3].

It is the fifth most frequent cause of cancer related death in men and the ninth most frequent cause of death in women[4].

Regardless of vaccination, large numbers of persons are infected with HBV or will be infected; several studies strongly supported the relation between the viral replication and the risk of

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HCC[4,5]. Relative risk of HCC among hepatitis B "e" antigen (HBeAg) positive patients is much higher than among inactive hepatitis B surface antigen (HBsAg) carriers. Furthermore, high HBV DNA load has been identified as the most important predictor of the development of HCC in HBsAg positive subjects with Hazard Ratio of 8.6 (3.7- 20.1) ($p<0.0001$), compared to HBV DNA <300 copies / ml (undetectable). HBV DNA 100,000 to 1 million associated with Hazard Ratio of 7.3 (3.3 – 16.4) ($p<0.0001$). HBV DNA 10,000 to 100,000 associated with Hazard Ratio of 2.8 (1.2 – 6.6) ($p<0.0173$). HBV DNA 300 – 10,000 associated with Hazard Ratio of 1.6 (0.7 – 3.9) (p 0. 2675)[6].

HBV DNA level- a measure of viral loads and can be measured by polymerase chain reaction (PCR)[7]. The HBV Rotor Gene-6000 (HBV RG) PCR kit, a HBV RG master contain all the reagents and enzymes (with the exception of MG^{2+}) for the specific amplification of a 110 b p sequence of the HBV genome and for the direct detection of the PCR product in cycling AFAM of the Rotor Gene-6000 instrument. In order to detect potential PCR inhibition in the absence of a HBV PCR product, the kit also contains an inhibition control monitor for the amplification efficiency of the system. The amplification – detection is carried out in Rotor–Gene 6000 sequence detector (Corbett Research).

Viral load is divided into three categories[6]:

Undetected- $<1.6\times 10^3$ copies/ ml, low titer- $< 10^5$ / ml and high titer- > or $=10^5$ copies/ ml. HBV DNA > 1 million copies/ml increases HCC risk.

Fine needle aspiration cytology (FNAC) is a useful technique, for assessing liver mass cytology both in inpatient and outpatient settings[8].

It allows a minimally invasive and rapid diagnosis of tissue. Aspiration is carried by a clinician or by a cytopathologist but cytological interpretation is done by an experienced cytopathologist.

More than 80% of HCC cases occur in individuals with cirrhosis of liver.

Therefore, treatment for the underlying liver disease may be best approach for prevention of HCC.

Methods:

This was a cross sectional analytical study carried in the department of Hepatology, BSMMU during the period of January 2006 to December 2007.

Enrolled patients for the study were divided into two Groups:

Group-1: Thirty patients of HBV related HCC were taken as case.

Group-2: Thirty chronic hepatitis B patients without HCC were taken as control

Patients Selections

Group-1:

Inclusion Criteria:

Positive serological test for HBsAg
Age: 15- 70 years
Alpha- fetoprotein: $\geq$ 400ng/ml
USG/CT evidence of liver SOL suggestive of HCC
FNAC/Biopsy proved HCC
Quantitative HBV DNA (PCR): HBV detected

Exclusion criteria:

Anti- HCV positive
Causes of CLD other than HBV
Bleeding disorder
Pregnant women
Metastatic liver Patient refuses FNAC/ liver biopsy

Group-2:0

Inclusion Criteria:

Positive serological test for HBsAg
Age: 15 to 70 year
Quantitative HBV DNA (PCR): HBV detected

Exclusion criteria:

Anti- HCV positive patients
Causes of CLD other than HBV
Raised α- fetoprotein ($\geq$ 50 ng / ml)
USG/CT evidence of liver SOL suggestive of HCC

Study Procedure:

Patients presenting with HBsAg positivity at outpatient or inpatient department of Hepatology, BSMMU Dhaka, were purposively provisionally selected for study. HBsAg was detected by ELISA method using the kits "DiaSorin S.p.A. (Italy)".

Then detail history and physical examination were done. Ultrasonography/CT and alpha fetoprotein (AFP) were done in all patients.

After considering the inclusion and exclusion criteria, all patients were divided into two groups:

Group-1:
Thirty HBV patients presenting with AFP 400 ng/ml or more and ultrasound/CT evidence of SOL in the liver suggestive of HCC were included in group 1.

FNAC/ liver biopsy was done in all patients for the confirmation of HCC. After confirmation of HCC, HBV DNA (PCR) was done to see the viral load. HBV DNA was extracted from 200nL of each plasma sample by using HBV Real-TM Quant, Sacace Biotechnologies srl., Italy, following the manufacturer's instructions. The TaqMan chemistry is based for DNA analysis. The PCR primers are designed against a conserved region of the HBV genome, which includes the genes encoding the X-protein and DNA polymerase. The Rotor Gene-6000 (Corbett Research) was used for quantification of amplification at each cycle. The limit of detection for the assay was $5x10^2$ to $1x10^8$ copies/ml (without dilution).

HBV DNA (PCR) positive patients were included in the study as cases.

Group-2:
Thirty HBV patients with no evidence of SOL in the liver and AFP <50 ng/ ml. HBV DNA (PCR) was done in all patients and HBV DNA (PCR) positive patients were taken as control.
Individual patient had a code number. Data was collected from each patient by a questionnaire and was entered into a personal computer. It was thoroughly checked for any possible error and was analyzed by SPSS programmed. Significance of the test was tested by student's t-test and Chi-squared test (χ^2). Result of test was statistically significant if p- value < 0.05.

Results:

Age distribution:
Table 1 shows the age distribution between groups. The highest (36.7%) age incidence of cases (HCC) was found at 50 or above 50 years of age followed by 33.3% between 40 – 50 years, 16.7% between 30 – 40 years and 13.3% between 20 – 30 years. None was found below 20 years. In the control group (CLD), 10% were below 20 years, 30% between 20 – 30 years, 13.3% between 30 – 40 years, 16.7% between 40 – 50 years and 30% 50 or above 50 years of age. The mean of the cases was higher (44.5 ± 12.4 years) than that of their control counterpart (37.7 ± 15.1 years), although the difference between the two groups was not statistically significant (p = 0.064).

Table 1: Age distribution between case and control (n = 60)

Age (yrs)	Group		p values
	Case(n=30)	Control(n=30)	
< 20	00	3(10.0)	
20 – 30	4(13.3)	9(30.0)	
30 – 40	5(16.7)	4(13.3)	
40 – 50	10(33.3)	5(16.7)	
≥ 50	11(36.7)	9(30.0)	
Mean ± SD	44.5 ± 12.4	37.7 ± 15.1	0.064

Data were analysed using Chi-square (χ^2) Test; level of significance was 0.05.

Sex distribution:
A male preponderance was observed in both cases and controls. Majority (96.7%) of the subjects in the case group was male, while 76.7% of the controls were male (Fig. 1).

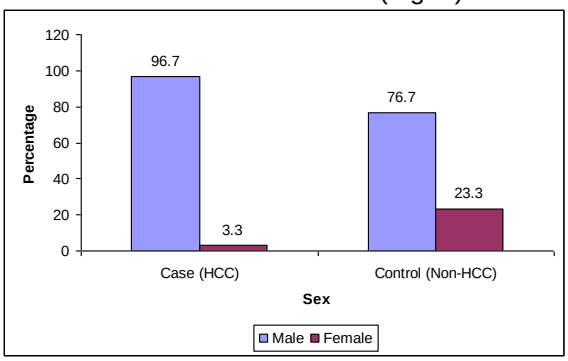


Figure 1: Age distribution between case and control.

HBV DNA load:
From the Table 2 it is seen that all of the subjects in the case group had HBV DNA load > 10^5 copies/ml, while the entire control group had HBV DNA load ≤10^5 copies/ml (p <0.001).

Table 2: Comparison of HBV DNA load between groups (n = 60)

HBV DNA (copies/ml)	Group		p value
	Case(n=30)	Control(n=30)	
<= 10^5	00	30(100.0)	< 0.001
> 10^5	30(100.0)	00	

Figures in the parentheses denote corresponding percentage
Data were analyzed using χ^2 Test.

Discussion:

To identify the relationship of HCC with HBV-related liver disease, we investigated HCC patients infected with hepatitis B virus and assessed the associated high HBV DNA load as a risk factor for HCC.

The study included thirty (30) HBV related HCC as cases (29 men and one women); mean age was 44.5±12.4 years (Table 1).
Thirty (30) HBV related liver disease without HCC were enrolled as control group (25 men and 5 women); mean age was 37.7±15.1 years (Table 2).The mean age of cases was higher (44.5±12.4 years) than that of their control counterpart (37.7±15.1 years). The highest age incidence of cases (HCC) was found at 50 or above 50 years of age. The mean age of our study population was similar to the study done by Ohata et al (2004)[9]. The hepatocellular carcinoma was seen predominantly in men and their 50s.

A male preponderance was observed in both cases and controls (in case 29 were male and one was female; in control 25 were male and 5 were female). Majority (96.7%) of the subjects in the case group was male, while 76.7% of the controls were male (Fig. 1).

In this study, among 30 HBV related HCC patients had a high viral load (>10^5) while all of the control had low (<10^5) viral load (Table 3). In HBV related liver disease patient, HBV-DNA was the strongest predictive factor for the development of HCC. Thus it was conceivable that patients with a high viral load may have a high potential for hepatocarcinogenesis

The results of this study were compatible with those of other study done earlier by Chen et al (2006)[6]. He did this study on 2763 HBsAg positive patients.

Another study done by Ohata et al (2004)[9] on 73 patients who were diagnosed with chronic HBV infection at Nagasaki University Hospital (Nagasaki, Japan) between January 1980 and December 1999. The significance of age, sex, habitual drinking, serum ALT level, HBV viral load, interferon treatment, hepatic fibrosis and hepatic inflammation on the development of HCC was examined using univariate and multivariate analyses. Multivariate analysis identified high viral load was an independent and significant risk factor (p=0.05) for HCC.

Comparison between groups was done by student's t-test and chi-squared test (χ2). Here the p-value was <0.001, which was less than 0.05 and statistically significant (Table 3). So hypothesis is accepted and mull hypothesis is rejected.

High viral load is a risk factor for HCC in patients with chronic hepatitis B viral infection.

Conclusion:

Individual with hepatitis B infection are at an increased risk of developing hepatitis B virus related liver complications including HCC. In some areas of Asia especially in Bangladesh, HCC is one of the most important cause of death due to cancer.

Worldwide more than 350 million individuals have hepatitis B virus infection and in endemic areas (including Bangladesh), the carrier rate is upto 10 to 20% of the population. Chronic hepatitis B virus carriers have a 100-fold relative risk of developing HCC comparing with non-carriers.

This study was done in the department of Hepatology, BSMMU, Dhaka from January 2006 to December 2007. HBV DNA measurement was done in HBV rel ated 30 HCC patients and also in 30 hepatitis B patients without HCC. It was found that among 30 HCC patients HBV DNA load was >105 copies/ml while all of the control had hepatitis B viral load <105 copies /ml.

This cross-sectional study that high viral load was associated with HCC. A growing number of studies support the clinical significance of quantitative HBV viral load measurement. High viral load was specially significant in subjects older age. Thus it is conceivable that patient with a high hepatitis B viral load have a high potential for hepatocarcinogenesis.

In conclusion, serum level of HBV DNA may be considered as a major risk factor for HCC.

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