

## Impact of Viral Load on Liver Damage in Bangladesh

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### ABSTRACT

**AIM:** In general, it is revealed that patients with chronic hepatitis B virus (HBV) infection with high viral load exhibit increased liver damages. More than 80% chronic HBV carriers live in the developing nations of the world, but little is known about relationship between HBV viral load and extent of liver damages in these countries. In this study, we addressed this issue to provide insights into this.

**METHODS:** A total of 306 chronic hepatitis B (CHB) patients from our pool of 561 Bangladeshi CHB patients were included. Viral load in CHB was measured by polymerase chain reaction (PCR) and all patients underwent per-cutaneous liver biopsy. A few were however evaluated for genotype due to economical constrains.

**RESULTS:** About 90% patients had high HBV DNA load. Of them 45% CHB patients with high HBV DNA load had non-significant hepatic necro-inflammation (HAI-NI <7). On the other hand, non-significant hepatic fibrosis (HAI-HF <3) was observed in 76% patient with high viral load. The study found no significant correlation between the high viral load and hepatic damage and also between serum alanine aminotransferase (ALT) and hepatic activity index (index for hepatic damage on the basis of cytopathology). The study found good correlation between the two hepatic activity index score ( $r=0.47$ ,  $p<0.001$ ).

**DISCUSSION:** It is generally assumed that CHB patients with high viral load develop more severe liver disease and the current guidelines also emphasize on reduction of viral load. However this may not be

the case as has been suggested in a number of studies. It is therefore important to look for other viral as well as host factors that may influence the course and outcome in CHB patients.

**CONCLUSION:** This study shows that a correlation could not be established between viral load and liver damage in patients with CHB in Bangladesh. A significant percentage of patients with CHB with high HBV DNA in Bangladesh do not have significant hepatic necro-inflammation and fibrosis.

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**Key words:** High viral load; Chronic hepatitis B; Hepatic necro-inflammation; Hepatic fibrosis; Bangladesh

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### INTRODUCTION

HBV infects nearly 350 million people worldwide. Of them 75-80% reside in Asia and Western Pacific. HBV is responsible for over 1 million deaths per year globally. The clinical manifestations vary widely with asymptomatic acute viral B hepatitis on one end and hepatocellular carcinoma (HCC) on the other end of the spectrum. It is a major cause of cirrhosis of liver and HCC worldwide<sup>[1]</sup>. Among factors known to influence the natural history of viral hepatitis are age at time of infection, duration of infection, serum alanine aminotransferase (ALT) levels, male sex, alcohol consumption and coinfections with hepatitis C or D viruses. In hepatitis B, serum HBV DNA concentration emerges as the key factor for predicting the development of liver disease<sup>[2]</sup>. The incidence of both HCC and cirrhosis were significantly associated with serum HBV DNA levels in a dose-response relationship from <300 (undetectable) to  $\geq 1000000$  copies/mL<sup>[3]</sup>.

The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) study carried out in Taiwan investigated natural history of chronic hepatitis B. The REVEAL-HBV study has established an HBV viral load paradigm in the natural history of chronic hepatitis B (CHB). Serum HBV

DNA level has been shown to be significantly and independently associated with incidence of hepatocellular carcinoma (HCC) and cirrhosis and liver-related mortality across a biological gradient<sup>[4]</sup>.

Bangladesh is in the intermediate prevalence region for HBV infection. The lifetime risk of acquiring this infection in Bangladesh is >40%. HBV results in a wide range of liver diseases with asymptomatic hepatitis B carrier state on one end and hepatocellular carcinoma (HCC) on the other end of the spectrum. It has been estimated that this virus is responsible for 10-35% cases of acute viral hepatitis, 35.7% cases of fulminant hepatic failure, 33.3-40.5% cases of chronic hepatitis and 46.8% cases of HCC in Bangladesh<sup>[5]</sup>.

With PCR now days, HBV DNA can be detected at levels as low as 20 copies/mL. It is important to know the pre-treatment HBV DNA level at baseline in order to monitor response to anti-viral agents and to look for drug resistance. The primary aim of anti-viral therapy is suppression of HBV DNA. In HBeAg positive CHB patients, anti-viral therapy is indicated if HBV DNA is >100000 copies/mL, subject to satisfaction of other criteria also. In case of HBeAg negative CHB however, treatment has to be started if HBV DNA is >10000 copies/mL<sup>[6]</sup>.

It is common in our practice to see patients having serum with >10<sup>5</sup> copies HBV DNA /mL. Various study showed that patients with high viral load are likely to develop more severe liver disease. Generally the guidelines also emphasize on the reduction of viral load in CHB patients. The aim of this study is to compare the correlation between high HBV DNA load and grade and stage of liver disease in patients with CHB.

## METHODS

In this retrospective study, we reviewed the records of 306 chronic hepatitis B (CHB) patients from our pool of 561 Bangladeshi CHB patients. They were all HBsAg positive for at least 6 months and attended our Out Patient Departments in Dhaka, Bangladesh between July 2008 and October 2011. Written informed consent was obtained from each patient. The patients had to be negative for anti-HCV antibody and positive for serum HBV DNA by quantitative analysis of HBV in human serum. First HBV DNA was extracted from the patient's serum. It was then amplified by polymerase chain reaction (PCR) and detected using probes specific for HBV by the Rotor Gene-3000 (Corbett Research).

All patients underwent percutaneous liver biopsy. Biopsies were done using trucut biopsy needle under local anaesthesia. We checked prothrombin time and platelet count in every patient, within a week before the procedure. Liver biopsies were done if baseline prothrombin time was not prolonged more than 3 seconds beyond control value and platelet count was not less than 100000/mm<sup>3</sup>. Biopsies were scored using histologic activity index (HAI) scoring system<sup>[7,8]</sup>.

Statistical analysis was done by a biostatistician and Statistical Package for Social Science V-11 software was used for analysis. The categorical variables were expressed in percentage and were compared by  $\chi^2$  test, while the scale variable tested by independent 't' test, correlation and linear regression.

## RESULTS

Patient characteristics are shown in table 1. Out of 306 participants under analysis, most were male (78%) having over all mean ( $\pm$ SEM) age 25.24 $\pm$ 0.53 (range=8-55) years with homogenous age distribution between gender ( $t=2.55$ ,  $p=0.11$ ). Except HBV DNA per mL of blood, the plasma level of ALT, HAI-NI, HAI-HF score did not vary

between both sexes. On average females had three times more HBV DNA than that of male counterparts ( $t=4.795$ ,  $p=0.03$ ).

**Table 1** General characteristics of the study participants.

Parameters	Mean $\pm$ SEM			t value	p <sup>1</sup> value
	Total	Male	Female		
Age in year	25.24 $\pm$ 0.53	24.8 $\pm$ 0.49	26.84 $\pm$ 1.63	2.55	0.111
Serum ALT (U/L)	62.72 $\pm$ 5.4	67.97 $\pm$ 6.88	43.72 $\pm$ 2.88	3.374	0.067
HBV DNA (copies/mL)	1.7 $\times$ 10 <sup>11</sup>	1.06 $\times$ 10 <sup>11</sup>	4.1 $\times$ 10 <sup>11</sup>	4.795	0.029
HAI-NI	6.13 $\pm$ 0.15	6.10 $\pm$ 0.17	6.2 $\pm$ 0.32	0.160	0.689
HAI-HF	1.7 $\pm$ 0.05	1.44 $\pm$ 0.06	1.5 $\pm$ 0.13	0.665	0.415

p<sup>1</sup> value of  $p < 0.05$  was taken as significant.

HBV DNA >10<sup>5</sup> copies/mL by PCR was considered as high viral load. In this study nearly 90% (275/306) patient had high viral load (Table 2) without significant distribution between the sexes ( $\chi^2=2.88$ ,  $df=1$ ,  $p=0.09$ ).

About 45% (118/263) CHB patients with high HBV DNA load had non-significant hepatic necro-inflammation (HAI-NI <7). On the other hand non-significant hepatic fibrosis (HAI-HF <3) was observed in 76% (205/268) patient with high viral load (Table 2).

The study found no significant differences in viral load with hepatic damage indicated by HAI-NI ( $\chi^2=0.286$ ,  $df=1$ ,  $p=0.59$ ) and HAI-HF ( $\chi^2=0.148$ ,  $df=1$ ,  $p=0.70$ ) (Table 2).

**Table 2** Cross-tabulation between categorical histological activity score and categorical HBV DNA copies in blood.

Categorical activity score	Histologic activity score	Categorical HBV DNA copies in blood		$\chi^2$ value	p <sup>1</sup> value
		Number of patients with low viral load	Number of patients with high viral load		
Sex	Male	28	212	2.88	0.09
	Female	3	63		
	Total	31	275		
HAI-NI score	HAI-NI <7	15	118	0.286	0.596
	HAI-NI $\geq$ 7	15	145		
HAI-HF	HAI-HF <3	22	205	0.148	0.700
	HAI-HF $\geq$ 3	8	63		

p<sup>1</sup> value of  $p < 0.05$  was taken as significant.

The correlation of HBV DNA with HAI-NI and HAI-HF showed no correlation (Table 3). The correlation coefficients were 0.02 ( $p=0.737$ ) and 0.075 (0.196) for HAI-NI and HAI-HF respectively. The study found significant correlation between viral load and serum ALT ( $r=0.03$ ,  $p=0.60$ ). The study also found good correlation between the two hepatic activity index score, HAI-NI and HAI-HF ( $r=0.47$ ,  $p<0.001$ ) (Table 3).

**Table 3** The correlation table.

Variables correlated	Correlation coefficient	p <sup>1</sup> value
HBV DNA vs. ALT	-0.030	0.607
HBV DNA vs. HAI-NI	0.023	0.688
HBV DNA vs. HAI-HF	-0.075	0.193
ALT vs. HAI-NI	-0.026	0.650
ALT vs. HAI-HF	0.063	0.279
HAI-NI vs. HAI-HF	0.473	<0.001

p<sup>1</sup> value of  $p < 0.05$  was taken as significant.

Among 306 participants, 29 were typed genetically. The distribution of serum ALT and HBV DNA showed no difference in level of ALT ( $F=0.925$ ,  $p=0.44$  and DNA copies ( $F=2.728$ ,  $p=0.06$ ) among the genotypes A, B, C and D (Table 4).

The distribution of histological activity score among the genotypes are shown in table 5. Our study showed that genotype had association with the histological outcome in regard to necro-inflammation ( $\chi^2=1.85$ ,  $df=3$ ,  $p=0.60$ ) and hepatic fibrosis ( $\chi^2=6.008$ ,  $df=3$ ,  $p=0.112$ ).

**Table 4** Viral load and enzyme among genotypes.

Parameter	Viral group	Number	Mean	Std. Error	F value	$p^1$ value
Serum ALT	HAV	2	200.50	128.50	0.925	0.443
	HBV	1	49.00	-----		
	HCV	12	43.75	5.25		
	HDV	14	141.00	63.00		
	Total	29	101.68	32.22		
HBV DNA (copies/mL)	HAV	2	673558400	476945800	2.728	0.065
	HBV	1	1608387800	---		
	HCV	12	436843219	103489163		
	HDV	14	344426165	128804611		
	Total	29	448951363	89388148		

$p^1$  value of  $p < 0.05$  was taken as significant.

**Table 5** The histological outcome in genotypes.

Categories of histological activity index	HAV	HBV	HCV	HDV	$\chi^2$ value	$p^1$ value
CAT_NI	HAI-NI >7	1	0	6	1.85	0.602
	HAI-NI $\geq$ 7	1	1	6		
	Total	2	1	12		
CAT_HF	HAI-HF <3	1	0	11	6.002	0.112
	HAI-HF $\geq$ 3	1	1	3		
	Total	2	1	12		

$p^1$  value of  $p < 0.05$  was taken as significant.

## DISCUSSION

The aim of this study was to assess whether patients with high HBV DNA load necessarily develop significant ongoing necro-inflammation or fibrosis in the liver in case of CHB. Contrary to the usual belief, we observed that majority patients with CHB have non-significant necro-inflammation and/or fibrosis. Similar studies are sparse in the world literature.

However there are several studies that have failed to show any correlation between HBV DNA load and severity of liver damage in CHB patients. These include study from Iran with a sample size of 200 patients<sup>[9]</sup>. There are at least two more papers in the literature having similar observations<sup>[10,11]</sup>. From Bangladesh also, our group as well as our colleagues have reported similar experience in the recent past<sup>[12,13]</sup>. Two more recent studies have rather reported that high HBV DNA load is associated with less severe histologic liver disease<sup>[14,15]</sup>, which is similar to our present observation.

Several studies have failed to show correlation between HBV DNA and serum aminotransferases, which is additional evidence that HBV DNA does not correlate with severity of liver disease<sup>[16,17]</sup>. In our present study, we did not find any significant correlation between viral load and serum ALT.

HBV DNA load in CHB patients may be influenced by host factors like immune response and alcohol consumption and viral factors like genotype and pre-core/core promoter mutations<sup>[17]</sup>. CHB patients may have fluctuating HBV DNA levels, which may also explain such observation. The pathogenesis of liver damage is immune-mediated where the role of cytokines is crucial.

## CONCLUSION

This study shows that a correlation could not be established between viral load and liver damage in patients with CHB in Bangladesh. Majority patients with high HBV DNA have non-significant hepatic necro-inflammation and fibrosis. Further study may be needed to find out the influence of other factors on liver damages in CHB patients in developing nations like Bangladesh. Most of these patients have not been characterized and treatment modalities have not been defined for them.

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