Global Journal of Medicine & Public Health

Utilizing the association between Hepatitis B viral load and Hepatic failure biomarkers for guided antiviral therapy

*Manjula J Babariya[1], Niyati H Zaveri[2], Shivaji D Patil[3], Jitendrakumar S Parmar[*4], Varsha dhuliya [5], Bhavi Patel [6]*

ABSTRACT

Background

Hepatitis B virus infection is a global public health problem. Individuals with chronic Hepatitis B are at an increased risk of developing liver cirrhosis, hepatic failure and hepato cellular carcinoma. This study was undertaken in a tertiary care hospital with the aim to measure the Hepatitis B viral DNA and its association with hepatic biochemical markersso as to guide for better clinical management during therapy.

Methods and Material:

Blood samples from 94 Hepatitis B seropositive patients were collected and tested for HBV viral load by real time PCR.Patients were divided in two categories A & B (A= < 20000 IU/ml and B >20000 IU/ml) as based on the quantification of viral DNA. Biochemical tests were performed for assessing Serum ALT, AST, Platelet count, hemoglobin, Albumin, Bilirubin and Prothrombin time.

Results:

Total patients in category A (< = 20000 IU/ml) viral load were 51 (54%) Total patients in category B (>20000 IU/ml) were 43.Category B patients with >20000 IU/ml of HBV viral load had elevated levels of SGOT (AST) with statistical significance at P value 0.038. Moreover, Serum albumin and Platelet count were significantly noted on lower side in category B patients at P values 0.03 and 0.02 respectively.

Conclusion:

Viral load of Hepatitis B varies over time, depending on the phase of theinfection. The findings of this study points at strong correlation between HBV viral load and biochemical markers for hepatic failure.Thus timely and regular viral load monitoring along with hepatic biomarkers is crucial for the treatment of Hepatitis B.

Keywords: Hepatitis B virus, FIBROSIS-4 calculator, HBV viral load, Real time PCR

GJMEDPH 2025; Vol. 14, issue 1 | OPEN ACCESS

4*Corresponding author: JitendraKumar S Parmar, Associate Professor, Department of Pathology, NAMO MERI, Silvassa, DNH; 1.Manjula J Babariya, Professor, Department of Microbiology, NAMOMERI, Silvassa; 2.Niyati H Zaveri, Associate Professor, Department of Community medicine, Zydus medical college and hospital ,Dahod; 3.Shivaji D Patil, Assistant Professor, Department of General medicine, Zydus medical college and hospital ,Dahod;; 5. Varsha dhuliya ,Associate Professor, Department of Pathology, BMCRI, Palanpur; 6. Bhavi Patel , Assistant Professor ,Department of Pathology, BMCRI, Palanpur.

Conflict of Interest—none | Funding—none

INTRODUCTION

Hepatitis B virus is a DNA virus known to cause acute and chronic hepatitis. It chronically afflicts about 240 to 300 million humans worldwide, and about 600,000 deaths occur every year due to HBV-related liver pathologies.[1] Although sexual route of transmission is more common, it can also be transmitted by parenteral route like unscreened blood transfusion, injection drug use, infected body fluids and unsafe shaving practices. High risk group includes doctors, nurses, midwives, paramedical staff (healthcare staff and carers, among others), sexual workers and drug addicts. Chronic Hepatitis B is a known factor to cause life threatening complications like fulminant hepatitis, cirrhosis and liver cancer. Other hepatitis viruses (hepatitis E, coinfection with hepatitis B and D) as well as alcohol and drugs can also contribute to fulminant hepatitis and the other issues listed above.Serial measurement of hepatitis B virus (HBV) DNA levels in the serum and its relation with liver damage and are a guide to begin and/or end a treatment course.[2] In a developing country like India, the high cost of Anti HBV treatment and limited availability and accessibility for testing HBV viral load, Stigma linked with HepB infection, lack of education and knowledge, etc remains a challenge intreatment.[3] This study was undertaken in a tertiary care hospital catering to economically backward population with majority of patients from tribal and hilly regions with the aim to measure the Hepatitis B viral DNA and its association with Hepatic biochemical markers as well as use of non-invasive tools (Aspartase aminotransferase to platelet ratio index & FIBROSIS 4 Score) to guide for better clinical management during the therapy.

Material & Method

The study was conducted at the central clinical laboratory of a tertiary care hospital in Silvassa in the Microbiology, Pathology and Biochemistry department after obtaining approval from the ethical committee. Samples were collected from 94 sero positive Hepatitis B patients (screened by Rapid Test and confirmed by Elisa method) over a period of 7 months from August 2021 to February 2022. Samples were tested for Hepatitis B quantitative viral load by real time PCR (Cartridge based nucleic acid amplification test). Patients were divided in two categories A & B based on the quantification of HBV DNA. Patients with viral load less than and equal to 20000 IU/ml were included in category A and more than 20000 IU/ml were included in category B (A = < 20000 IU/ml and B >20000 IU/ml). Samples were further tested for Hemoglobin, Serum Albumin, Bilirubin, Alanine transaminase (ALT) , Aspartate transaminase(AST) , Platelet count and Prothrombin time. Statistical analysis was done using unpaired T test and Pearson's correlation to determine the association between both categories of HBV viral load and biochemical hepatic markers. Hepatitis B viral load among various age groups and gender was analyzed. We have also calculated APRI score (Aspartase aminotransferase to platelet ratio index).

$$\text{APRI} = \frac{\dfrac{\text{AST Level}}{\text{AST (Upper Limit of Normal)}}}{\text{Platelet Count } (10^9/\text{L})} \times 100$$

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST (U/L)}}{\text{Platelet Count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$$

The Fibrosis-4 score helps to estimate the amount of scarring in the liver

Result

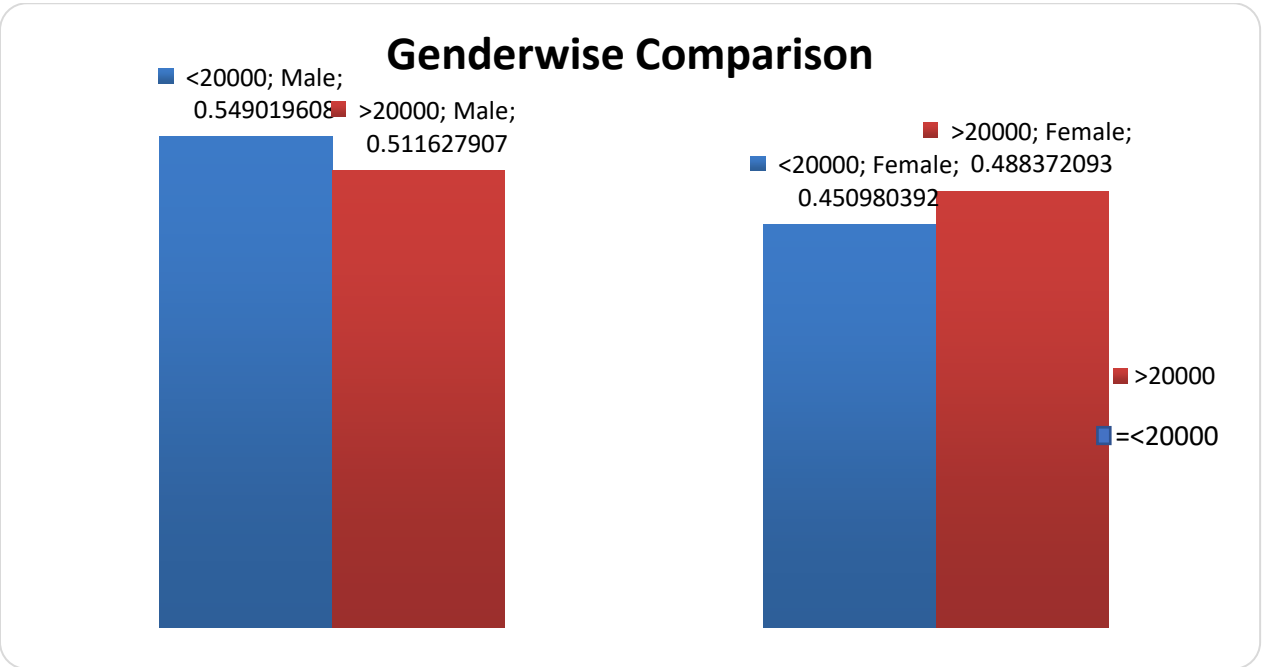
A total of 94 patients were enrolled in the study. Patients in category A (< = 20000 IU/ml) viload were 51 (54%) out of which male patients comprised 28 (54.9%) and female patients comprised 23 (45.10%).

Total patients in category B (>20000 IU/ml) were 43 (45.7%) out of which male patients were 22 (55.16%) and female patients were 21 (48.84%) as shown in table 1 and graph 1.

Table 1

Gender-wise Distribution						
Viral Load	**Male**		**Female**		Total	
	N	**%**	**N**	**%**	**N**	%
< =20000	28	54.90%	23	45.10%	51	**54.26%**
>20000	22	51.16%	21	48.84%	43	**45.74%**
Total	**50**	**53.19%**	**44**	**46.81%**	**94**	**100.00%**

Graph 1 :



Mean age in both the categories of patients were 33.84 (A) and 33.95 (B) respectively as shown in table 2.Category B patients with >20000 IU/ml of HBV viral load had elevated levels of AST with statistical significance at P value 0.032. 51 patients in category A showed mean AST level 42.55 unit/L and 37 patients of category B showed mean AST level 87.68 unit/L. Serum albumin levels in 37 patients of category B was 3.36 g/dL compared to 3.70 g/dL in 51 patients of category A. Thus, albumin level was significantly reduced in patients of category B at P value 0.03. Mean average platelet count in 51 patients of category A was 407058/uL compared to 187711/uL in 38 patients of category B. Thus, platelet count was significantly reduced in patients of category B at P value 0.02 as shown in table 2. Mean total bilirubin in 37 patients of category B was 0.85 mg/dL as compared to 0.60 mg/dL in 51 patients of category A. Mean Creatinine in 37 patients of category B was 1.55 mg/dL compared to 1.40 mg/dL

in 51 patients of category A. Mean Prothrombin time in 19 patients of category B was 19.5 seconds compared to 11.2 seconds in 22 patients in category A. Mean EGFR in 19 patients of category B was 59.92 ml/min/1.73m^2 compared to 95.23 ml/min/1.73m^2 in 22 patients in category A. Mean Fibrosis 4 score was noted to be 1.28 in 27 patients of category A compared to 1.11 in 18 patients of category B. In this study it was observed that 27 patients with category A had mean APRI 0.40 and 18 patients in category B had APRI 0.65.

Table 2 Unpaired T test

Comparison of study parameters between both categories using T test							
Group		**N**	**Mean**	**SD**	**SEM**	**t-stat**	**p-value**
Age	<20000	51	33.84	13.96	1.96	-0.346	0.7301
	>20000	43	34.95	17.15	2.62		
Haemoglobin	<20000	51	12.08	2.24	0.31	-0.4895	0.6257
	>20000	38	12.3	1.98	0.32		
S. Albumin	<20000	51	3.7	0.67	0.09	2.194	0.0309*
	>20000	37	3.36	0.78	0.13		
Serum Bilirubin Total (mg/dL)	<20000	51	0.6	0.49	0.07	-1.1794	0.2414
	>20000	37	0.85	1.38	0.23		
SGPT/ ALT(IU/L)	<20000	51	46.62	96.96	13.32	-1.4482	0.1511
	>20000	37	90.7	189.13	31.09		
SGOT/ AST(IU/L)	<20000	51	42.55	44.32	6.09	-1.8158	0.0328*
	>20000	37	87.68	173.38	28.5		
Platelet Count (permicrolitre)	<20000	51	407058	1221670	169415	1.1019	0.02735*
	>20000	38	187711	109191	17713.1		
S.Creatinine	<20000	51	1.4	2.39	0.33	-0.2959	0.768
(mg/dL)	>20000	37	1.55	2.43	0.4		
PT time	<20000	22	11.2	15.9	1.37	1.399	0.170
	>20000	19	19.5	19.4	2.24		
eGFR (Estimated glomeral filtration rate (ml/min/1.73m^2))	<20000	22	95.23	94.92	20.24	1.399	0.170
	>20000	19	59.92	59.71	13.7		
APRI	<20000	27	0.4	0.27	0.05	-1.42	0.170

FIB 4	>20000	18	0.65	0.71	0.16		
	<20000	27	1.28	1.42	0.27	0.51	0.641
	>20000	18	1.11	0.84	0.19		

Table 3 Pearsons' correlation coefficient

		Viral_load	Hemoglobin	S_Albumin	Serum Bilirubin Total (mg/dL)	SGPT/ ALT(IU/L)	SGOT/ AST(IU/L)	PlateletCount (permicrolitre	S.Creatinine (mg/dL)	PT	APRI
Haemoglobin	Corr	-.131									
	p-value	.434									
	N	38									
S. Albumin	Corr	-.217	.311								
	p-value	.197	.061								
	N	37	37								
Serum Bilirubin Total (mg/dL)	Corr	-.071	-.125	-.321							
	p-value	.678	.460	.053							
	N	37	37	37							
SGPT/ ALT(IU/L)	Corr	-.137	-.370*	-.112	.012						
	p-value	.418	.024	.510	.944						
	N	37	37	37	37						
SGOT/ AST(IU/L)	Corr	-.136	-.366*	-.164	.119	.957**					
	p-value	.423	.026	.331	.482	.000					
	N	37	37	37	37	37					
Platelet Count (permicrolitre)	Corr	-.055	.244	.388*	.122	-.270	-.254				
	p-value	.743	.140	.018	.470	.106	.129				
	N	38	38	37	37	37	37				
S.Creatinine (mg/dL)	Corr	.039	-.259	-.133	-.019	.173	.289	.028			
	p-value	.820	.122	.433	.911	.306	.083	.869			
	N	37	37	37	37	37	37	37			
PT time	Corr	-.322	-.071	.553*	-.056	-.282	-.280	.408	-.299		
	p-value	.179	.771	.017	.827	.257	.260	.083	.227		

APRI	N	19	19	18	18	18	18	19	18		
	Corr	-.114	-.330	-.108	-.017	-.018	-.016	-.068	-.059	.010	
	p-value	.652	.181	.679	.948	.947	.951	.789	.821	.972	
FIB 4	N	18	18	17	17	17	17	18	17	15	
	Corr	.154	-.352	-.476	.161	.426	.439	-.250	.748**	-.351	-.001
	p-value	.542	.152	.053	.537	.088	.078	.318	.001	.200	.998
	N	18	18	17	17	17	17	18	17	15	18

***. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).**

Note: **All biochemical, hematological and hepatic markers for all 94 patients were not performed due to rare unavailability of reagents and kit in laboratory.**

suggesting that sex hormones have a role in complication risk. Several ALT levels >40 IU/L, contrary to AST, had a weak correlation with viral load. A Study done by et al shows Nine patients (24%) developed resistance to lamivudine, all after 12 months of treatment. Among them, the mean serum albumin concentration had increased from 39.6 +/- 1.2 to 42.9 +/- 0.8 g/L before resistance emerged, but then decreased to 39.3 +/- 1.7 g/L (p = 0.01) at the time of reappearance of HBV DNA[16]. According to the American Association for the Study of Liver Disease (AASLD Practice Guidelines) treatment has to be administered if ALT is > 2 ULN (Upper limit of normal) or moderate / severe hepatitis observed in biopsy and Hepatitis B viral load is > 20,000 IU/mL.[15]

In adults showing normal ALT (SGPT) level with no evidence of or undetectable / <2000 IU/ml viral load treatment is not required.[5] It is necessary to observe the intra hepatic or plasma cytokine levels in chronic hepatitis patients to explore the disease prognosis as well as to monitor the outcome of antiviral therapy[15]. In our study we couldn't test intrahepatic or plasma cytokine levels because of unavailability of test reagent. Treatment is recommended, if HBV Viral load > 20000 IU/mL irrespective of age and HBeAg status.Treatment is also recommended, if APRI >1.5 OR FIB -4 >1.45 in non-cirrhotic & persistently elevated ALT level with HBV viral load >20000 IU/mL and particularly if the age of patients is > 30 years. Treatment is also recommended, if APRI >1.5 OR FIB -4 >1.45 in non-cirrhotic & normal ALT level with HBV viral load >2000 IU/mL.[17] On the basis of these criteria 45 patients are eligible for treatment in our study.

Limitations of this study

This is because of sexual behaviour of males and hetrosexual activities are more common in males.

1) Several parameters which are critical for clinical decision making with regards to treatment could not be done like HBeAg for infectivity and liver biopsy which is required to assess the stage ofliver damage.

2) Continous viral load monitoring was not done during the therapy for escalation or de escalation of antiviral drugs.

3) Lower limit of detection of this assay is 40 IU/ml. Values below 40 IU/ml do not exclude the possibility of infection. It may reflect a viral load below the detection limit of assay.

Conclusion

Viral load of Hepatitis B varies over time depending on the phase of the infection. The findings of this study point at a strong relationship between HBV viral load and biochemical markers for hepatic failure. Thus timely and regular viral load monitoring along with hepatic biomarkers is crucial for the treatment of Hepatitis B. Supportive treatment for hepatic failure is indicated in all patients with high viral load.

REFERENCES

1) World Health Organisation. Epidemiology of hepatitis B virus.Division of Health and Development. WHO; 2013. [Google Scholar]

2) Biazar, T. and Yahyapour, Y. Relationship between Hepatitis B DNA viral load in the liver and its histology in patients with chronic hepatitis B. Caspian Journal of Internal Medicine 2015;6(4) 209-212.

3) Tiwari V, Balasundaram P, Sherin Raj T. Cost of treatment and consequences for chronic hepatitis B and C virus infection at a tertiary care hospital in Delhi. Indian Journal of Public Health. 2020;64:409.

4) Iregbu K, Nwajiobi-Princewill P. Viral load pattern among hepatitis B surface antigenpositive patients: Laboratory perspective and implications for therapy. Annals of Medical and Health Sciences Research. 2016;6:95.

5) Chen Q, Liu J, He Y, Yang L, Luo H, Wang Y, Zhang X, Li N. Prevalence of HBsAg among reproductive age couples in Chongqing: A population-based, cross-sectional study.PLOS ONE. 2021;16:e0260028.

6) Khan F, Shams S, Qureshi I, Israr M, Khan H, Sarwar M, Ilyas M. Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. Virology Journal. 2011;8.

7) Anna S F Lok and Brian J McMahon. Chronic hepatitis B. Hepatology2007 Feb;45(2):507-39. doi: 10.1002/hep.21513.

8) Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, et al. EASL INTERNATIONAL CONSENSUS CONFERENCE ON HEPATITIS B13–14 September, 2002Geneva, Switzerland. Journal of Hepatology. 2003;38(4):533-540.

9) Liaw YF, Leung N, Guan R, Lau GK, Merican I, McCaughan G, Gane E et al. Asian- Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. Liver Int. 2005;25 472–489.

10) Keeffe E, Dieterich D, Han S, Jacobson I, Martin P, Schiff E et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States.Clinical Gastroenterology and Hepatology. 2004;2(2):87-106.

11) Arroyo V. [Presentation.] Gastroenterol Hepatol. 2006;29 Suppl 2:1.

12) Marugán R, Garzóno S. DNA-guided hepatitis B treatment, viral load is essential, but not sufficient. World Journal of Gastroenterology. 2009;15:423.

13) Mbaye P, Sarr A, Sire J, Evra M, Ba A, Daveiga J et al. Liver Stiffness Measurement and Biochemical Markers in Senegalese Chronic Hepatitis B Patients with Normal ALT and High Viral Load. PLoS ONE. 2011;6:e22291.

14) Esmaeelzadeh A, Saadatnia H, Memar B, Mokhtari Amirmajdi E, Ganji A, Goshayeshi L et al. Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative hepatitis B patients. Gastroenterol Hepatol Bed Bench. 2017;10:39-43.

15) Mohasin M, Nila T, Tushy TS, Keya K, Haque ME. Correlation of serum alanine aminotransferase and hepatitis C viral RNA levels in Bangladeshi hepatitis patients. Bioresearch Communications-(BRC). 2019 Jan 1;5:678-83.

16) J Hui, J George, C Liddle, R Lin, D Samarasinghe, E Crewe et al. Changes in serum albumin during treatment of chronic hepatitis B with lamivudine: effects of response and emergence of drug resistance. The American Journal of Gastroenterology 2002;97,1003-1009,

17) Viral hepatitis silent disease facts & treatment guidelines, NCDC , ministry of health & family welfare – GOI