Effect of viral load on hepatic fibrosis in patients with chronic hepatitis B patients: assessed by fibroscan

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Abstract

Background: Hepatitis B virus (HBV) infection is a severe worldwide health problem and a primary cause of chronic hepatitis, hepatic fibrosis, cirrhosis, and hepatocellular cancer. In Egypt, the prevalence of HBsAg is of intermediate endemicity (2–8%). It has been known that the viral load and degree of hepatic fibrosis are considered independent factors that predict clinical outcomes after persistent HBV infection. However, the exact relationship between viral load and hepatic fibrosis is not well studied.

Objectives: Our objective was to investigate the clinical effects of viral load on the severity of hepatic fibrosis.

Patients and methods: Sixty patients with evident chronic HBV infection were enrolled. Using transient elastography, the patients were divided into two groups. Group 1: low fibrosis stage F1–2, and Group 2: high or significant fibrosis stage (F3–F4). Both groups were statistically compared for HBV-DNA viremia (PCR), clinical, and laboratory tests.

Results: Serum bilirubin (p = 0.048), international normalised ratio (p 0.0001), and albumin (p = 0.01) were significantly increased in patients with higher grades of liver fibrosis on top of CHB. In addition, the viral load was found to be considerably greater in individuals who had higher grades of liver fibrosis and cirrhosis (P = 0.03).

Conclusions: During follow-up, an obvious increase in the viraemia level may indicate significant hepatic fibrosis in patients with chronic HBV infection. Our results could influence the decision about liver biopsy or treatment at that point.

Keywords: Viral load; Hepatitis B; Transient elastography; Liver stiffness .

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Introduction

In the WHO's estimation, two billion people are infected with HBV. Around 350 million people are believed to be carriers of the virus. 600,000 people die each year from acute and chronic HBV infections **(Alter 2003)**. Almost 10% of HBV-infected young adults and 90% of HBV-infected children acquire a chronic infection **(Sugiyama et al., 2007)**. Egypt exhibits intermediate endemicity with a prevalence range of 3–11%, particularly concentrated in Upper Egypt and among the male population **(Ragheb 2010)**.

Persistent hepatitis B infection can result in latent carriage and chronic hepatitis B, which can develop into fibrosis, cirrhosis, or hepatocellular carcinoma. Numerous parameters, such as the HBV genotype, viral load, HBV genome mutations, serum hepatitis B e antigens (HBe Ag), and hepatitis B surface antigens (HBs Ag), have been used to predict the clinical outcome **(Kao et al., 2003).**

It is important to get a thorough assessment of the level of hepatic fibrosis for accurate staging and making decisions about treatment **(Sporea et al., 2008)**. The gold standard for assessing hepatic fibrosis is a liver biopsy, but this method is exceedingly intrusive and unpleasant and can result in life-threatening consequences **(Cadrenal et al., 2000)**.

Transient elastography (fibroscan) has been developed as a non-invasive approach for evaluating liver fibrosis **(Wilder and Patel 2014).** The elasticity (stiffness) of the liver is evaluated by measuring the velocity of low-frequency elastic waves within the liver **(Ziol et al., 2005; Marcellin et al., 2009)**. Basically, these waves propagate more rapidly in stiff tissues, meaning more stiff tissue means faster speed. The stiffness of the right lobe would be measured in the intercostal position for all patients by a single observer **(Reye et al., 2012)**. In FibroScan, the stiffness of the liver is measured in kPa. Normal values are between 2.5 and 7.5 kPa**(Castera 2011)**.

According to the literature, viral load and hepatic fibrosis are factors that independently predict clinical outcomes following persistent HBV infection. Despite this, it remains unclear how viral load correlates with hepatic fibrosis. In this study, we set out to explore the clinical effect of viral load on fibrosis severity and the extent of the disease.

Patients and methods

A total of 60 patients with CHB were included in this study. From June 2018 to December 2019, patients were sourced from the internal medicine outpatient clinic at Sohag University Hospital. The study has been approved by the ethical committee of our institute.

All included patients didnn't receive anti viral therapy (de novo patients). Based on the results of the fibroscan, the patients were split into two groups:

- Group 1 includes 30 patients with low fibrosis degree (F1-F2)
- Group 2 includes 30 patients with high fibrosis degree (F3-F4)

Inclusion criteria: Patients diagnosed with chronic hepatitis B who had positive HBsAg by ELISA and positive HBV DNA by PCR met the inclusion criteria. They ranged in age from 18 to 60.

Exclusion criteria: Patients with HCC, HCV, other hepatic viral infections, autoimmune hepatitis, alcoholic liver disease, or who were receiving antiviral medication for chronic hepatitis B.

All recruited patients were subjected to the following procedures:

1- Full medical history (eig, clinical presentation, and relevant past and family histories), as well as risk factors (such as blood transfusion , surgical operations, medical staff, … etc) for infection with HBV.
2- Clinical examination and the severity of liver disease was defined according to Child-Pugh scoring system into (A, B, and C representing mild, moderate, and severe, respectively).
3- In-lab investigations, such as:
- Complete blood count
- Complete liver function test (AST, ALT, Bilrubin, Serum Albumin, INR).
- Alpha fetoprotein (AFP) (Tosoh Bioscience AIA-360 automated immunoassay analyzer , Japan)
- An enzyme-linked immunosorbent assay (ELISA) for HBsAg (Inc. Stat Fax 4700 Microstrip Reader, USA)
- Quantitative Polymerase Chain Reaction (PCR) test for the detection of HBV viremia (COBAS TaqMan 48 Real-Time PCR System, Roche Molecular Systems, Inc., Branchburg, NJ)

4- Abdominal ultrasound to measure the size of the liver, its echogenicity, the size of the spleen, and the presence of ascites in the abdominal cavity (Toshiba Xario XG Ultrasound Machine, Japan).
5- FibroScan® (Touch 502, Echosens, Paris, France) is a non-invasive procedure using transient elastography to assess liver stiffness. It measures the velocity of a vibration wave generated through the skin, with a normal range of 2-7 kPa. FibroScan data categorizes hepatic fibrosis into F0 to F1: no or mild hepatic scarring. F2: moderate liver scarring. F3: severe liver scarring, and F4: advanced liver scarring (Cirrhosis) (**Afdhal, 2012**).

Statistical analysis

The data were analyzed using STATA 14.2 (Stata Statistical Software: Release 14.2; College Station, TX: Stata Corp.). Statistical data were represented as the mean, standard deviation, median, and interquartile range (IQR). For comparing two groups' means, the student t-test was used, and for comparing three or more groups' means, the ANOVA was used. Spearman's correlation test was used to determine PCR's correlation with various factors. P value less than 0.05 was considered significant.

Results

Two groups of 60 patients were included in the study; 30 patients each. Group1 had 15 females and 15 males, with a mean age of 37.83±10.60 years. While Group 2 has 17 females and 13 males with a mean age of 43.87±13.54 years.

In terms of clinical findings and laboratory results, both groups were compared. We found that higher grades of liver fibrosis on top of CHB were linked to a significant increase in ascites ($p = 0.01$), serum bilirubin ($p = 0.048$), INR (p 0.0001), and a significant drop in serum albumin ($p = 0.01$).

However, neither group differed in pallor, jaundice, presence of splenomegaly, Child-Pugh score, leucocytic count, hemoglobin level, platelet count, or liver enzymes.(**Tables 1 and 2**).

Table 1. A comparison of clinical examinations between groups 1 and 2

Variables	Group 1 (F1-F2) N=30	Group 2(F3-F4) N=30	Test	P value
Pallor				
Yes, *n.* (%)	3 (10.00%)	7 (23.33%)	$\chi^2 = 1.92$	0.17
No, *n.* (%)	27 (90.00%)	23 (76.67%)		
Jaundice				
Yes, *n.* (%)	1 (3.33%)	7 (23.33%)	$\chi^2 = 5.19$	0.052
No, *n.* (%)	29 (96.67%)	23 (76.67%)		
Splenomegaly				
Yes, *n.* (%)	4 (13.33%)	8 (26.67%)	$\chi^2 = 1.67$	0.20
No, *n.* (%)	26 (86.67%)	22 (73.33%)		
Ascites				
Yes, *n.* (%)	0	7 (23.33%)	$\chi^2 = 7.92$	**0.01**
No, *n.* (%)	30 (100%)	23 (76.67%)		
Child-Pugh score				
A	30 (100%)	23 (76.67%)	$\chi^2 = 5.19$	0.062
B	0%	7 (23.33%)		
C	0%	0%		

Fisher's exact test, Bold value denotes a significal P value (less than 0.05); *n.*; Number of patients

Table 2. Laboratory investigations comparison between groups 1 and 2

Variables	Group 1 (F1-F2) N=30	Group 2(F3-F4) N=30	Test	P value
WBCs (10^3/mL)				
Mean ± SD	6.6±2.51	6.54±1.75	Z = 1.40	0.16
Median (IQR)	5.8(4.8:8.6)	6.55(5.9:8.5)		
Hb (g/dL)				
Mean ± SD	13.45 2.05	13.24 2.51	t= 0.35	0.72
Median (IQR)	13.65(12.1:15)	13.7(11.7:15)		
PLTs (10^3/ml)				
Mean ± SD	244.97±62.57	247.43±115.04	Z = 0.24	0.81
Median (IQR)	224.5(201:278)	236.5(155:300)		
AST (U/L)				
Mean ± SD	28.2± 12.34	33.3± 16.05	Z = 1.25	0.21
Median (IQR)	22.5(19:33)	28.5(25:42)		
ALT(U/L)				
Mean ± SD	26.23±12.96	32.83±16.42	Z = 1.92	0.06
Median (IQR)	22(16:33)	31(20:39)		
Albumin(g/dL)				
Mean ± SD	3.99±0.32	3.55±0.60	t= 3.47	**0.01**
Median (IQR)	4(3.9:4.1)	3.75(3.2:4.0)		
Bilirubin(mg/dL)				
Mean ± SD	0.70±0.27	0.98±0.47	Z = 2.61	**0.048**
Median (IQR)	0.7(0.6:0.8)	0.9(0.6:1.1)		

INR Mean ± SD Median (IQR)	1.05±0.10 1.04(1:1.09)	1.16±0.21 1.09(1.04:1.1)	t= 2.67	**<0.0001**

Fisher's exact test, **IQR**; Interquartile range , Bold values denote significal P values (less than 0.05). **WBCs;** While Blood cell count, **Hb;** Haemoglobin level,**PLTs**; Platelet count**, AST;** Aspartate Aminotransferase**, ALT**; Alanine Aminotransferase**, INR**; International Normalization Ratio**, SD**; Standard Deviation

Using the Spearman correlation test: there was a significant positive correlation between the level of viremia on PCR in both (F1-F2) and (F2). **(Table 3).**

Table 3. Correlation between HBV-DNA PCR assays and other laboratory variables of study groups in various stages of hepatic fibrosis

Variables	**F1-F2 N=30**	**F3-F4 N=30**	**F1 N=21**	**F2 N=9**	**F3 N=17**	**F4 N=13**
WBCs (10^3/mL)						
r	-0.09	0.10	-0.15	-0.07	0.05	0.03
P	0.62	0.52	0.52	0.86	0.84	0.93
Hb (g/dL)						
r	0.08	0.09	0.20	0.67	0.16	-0.06
P	0.67	0.63	0.40	0.05	0.55	0.85
PLTs (10^3/ml)						
r	-0.13	-0.14	-0.11	-0.15	-0.03	-0.35
P	0.46	0.47	0.65	0.70	0.90	0.23
AST (U/L)						
r	0.48	0.29	0.32	0.76	0.09	0.49
P	**0.008**	0.13	0.15	**0.02**	0.73	0.09
ALT (U/L)						
r	0.43	0.19	0.25	0.65	0.05	0.50
P	**0.02**	0.32	0.28	**0.06**	0.89	0.08
Albumin (g/dL)						
r	-0.21	-0.09	-0.20	0.13	0.02	-0.15
P	0.26	0.65	0.39	0.73	0.95	0.61
Bilirubin (mg/dL)						
r	-0.01	0.05	-0.07	0.009	-0.09	0.15
P	0.95	0.78	0.76	0.98	0.72	0.63
INR						
r	0.13	0.19	0.24	-0.29	0.19	0.22
P	0.48	0.32	0.30	0.45	0.46	0.47

Spearman correlation test, Bold values denote significant positive high correlation. **WBCs;** While Blood cell count, **Hb;** Haemoglobin level,**PLTs**; Platelet count**, AST;** Aspartate Aminotransferase**, ALT**; Alanine Aminotransferase**, INR**; International Normalization Ratio**, SD**; Standard Deviation

There was no difference in the levels of viral load on PCR between groups, however, it was observed that viral load was significantly higher in patients having cirrhosis and liver fibrosis at higher grades (P = 0.03), (**Tables 4 and 5).**

Table 4. Diagnostic performance of HBV polymerase chain reaction (PCR) in differentiating between fibrosis degrees

Stages	AUC (S.E.)	P value	95% (confidence interval)
F3-F4 from F1-F2	0.62 (0.07)	0.12	0.47:0.76
F4 from F1-F3	0.59 (0.09)	0.32	0.41:0.77
F3 from F1-F2	0.60 (0.09)	0.29	0.42: 0.78
F2 from F1	0.81 (0.09)	**0.0005**	0.63: 0.98

AUC; area under the curve, SE; Sample entropy.

Table 5. A comparison of HBV-DNA PCR assays between the two groups

Stages	Number	HBV PCR (IU/ml)		
		Mean ± SD	Median (IQR)	Range
F1-F2	30	594850±1752074	16883 (1950:89000)	(424:7700000)
F3-F4	30	1270749±2380497	198500 (2440:751000)	(440:7950000)
Mann–Whitney test (z-value) =-1.54 P value =0.12				
F1	21	85026±213435	13500 (1331:22700)	(424:840000)
F2	9	1784441±2956297	143000 (18750:1130000)	(1950:7700000)
F3	17	1514554±2836027	201000 (2440:751000)	(440:7950000)
F4	13	951928±1666222	196000 (6900:739000)	(512:5700000)
Kruskal–Wallis test (χ^2 value) = 8.79 P value= 0.03				

IQR; Interquartile range, **SD**; Standard Deviation

Discussion

Persistent hepatitis B infection can result in latent carriage and chronic hepatitis B, which can cause fibrosis, cirrhosis, or hepatocellular carcinoma. Several factors, such as HBV genotype, viral load, HBV genome changes, blood hepatitis B e antigens (HBe Ag), and hepatitis B surface antigens (HBs Ag), have been used to predict clinical results after persistent infection (**Kao et al., 2003**).

The efficacy of transient elastography for chronic hepatitis C virus (HCV) disease assessment has been demonstrated (**Regev et al., 2002**). In the same way, it seems to be a safe way to find fibrosis or cirrhosis in HBV patients, and its limit values are slightly different from those described in HCV patients (**Marcellin et al., 2009**).

A detailed assessment of the degree of fibrosis is required for proper

staging and therapy decision-making (**Sporea et al., 2008**).

In the current study, there was no significant difference between the groups in terms of pallor, jaundice, splenomegaly, Child-Pugh score, platelet count, or AST level. Patients with higher grades of liver fibrosis, however, had lower serum albumin levels, whereas their INR and total bilirubin levels were significantly higher.

Demir et al. (2014) found that AST and ALT were higher in patients with higher stages of fibrosis than in those with lower stages. In their study, there was a positive relationship between liver fibrosis and liver enzymes and a negative relationship between the fibrosis score and serum albumin. **Mohamad Nejad et al. (2006)** also found that CHB patients with severe fibrosis had lower serum albumin levels.

The current study showed that HBV PCR showed significant differences between different hepatic fibrosis stages rather than between the groups themselves. A similar strong connection between HB viral load and the histological staging and grading of liver disease was discovered by **Biazar et al. (2015).** According to our hypothesis, viral load positively correlates with cirrhosis and fibrosis of the liver.

In their investigation, **Demir et al. (2014)** discovered the same result—higher HBV-DNA levels in patients with advanced fibrosis than in those with non-advanced fibrosis—and found no connection between the severity of the fibrosis and viral load. Additionally, **Calvaruso and Crax (2011)** wrote that cirrhosis was strongly correlated with circulating virus levels, with patients with higher viral levels being more prone to developing it.

Wang et al. (2008) also reported that the serum HBV-DNA level was independently correlated with stage 2 fibrosis or more on liver biopsy.

Contrary to our study, most of the previous studies relied on liver biopsy as an indicator of hepatic fibrosis, which is an invasive method. Besides its well-known complications and limitations, liver biopsy is considered an "imperfect gold standard" **(Wilder , 2014).** As a result, we used the fibroscan as a non-invasive and reliable method for hepatic fibrosis assessment.

The small number of patients, especially those with higher grades of liver fibrosis (Child Scores B and C), was the major limitation of our study.

Conclusion

A high viral load of CHB may play an important role in predicting hepatic fibrosis and hepatic function in people with chronic hepatitis B.

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