ECRONICON OPEN ACCESS

EC GASTROENTEROLOGY AND DIGESTIVE SYSTEM

Research Article

Could we Depend on HBV DNA Level to Predict Significant Liver Fibrosis in Chronic Hepatitis B Patients with Persistently Normal Alanine Aminotransferase PNALT

Marwa khalaf[1], Mohamed A Mekky[2], Sherif Ibrahim Kamel[2], Mohamed El-Taher AbdelRahman[2], Mohamed O Abdelmalek[2], Hani I Sayed[1] and Helal F Hetta[3]*

[1]Assuit liver center for management of viral hepatitis, Assiut, Egypt

[2]Department of Tropical Medicine and gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt

[3]Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt

***Corresponding Author**: Helal F Hetta, Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Received: January 08, 2017; **Published:** February 04, 2017

Abstract

Background and Aim: One of the challenging issues facing hepatologist worldwide is the management of chronic hepatitis B patients with persistent normal enzymes, according international guidelines only patients with elevated enzymes were considered for therapy. Liver biopsy remains an integral part in determining disease severity in this group of patients; however it is an invasive procedure. So here we trail to evaluate the relationship between serum hepatitis B virus DNA and liver histopathology, which may guide clinicians for the beginning treatment depending on HBV DNA level in this particular subset of patients.

Methods: A total of 93 chronic hepatitis B patients with PNALT were enrolled in the cross sectional study. Serum viral load were determined using real time polymerase chain reaction method (RT-PCR) and liver biopsy with histopathological evaluation for activity and fibrosis according to (METAVIR score) were recorded. All patients were divided into two groups according to the HBe Ag status. The relationship between serum HBV DNA levels and liver histopathology was explored.

Results: A total of 93 CHB patients with PNALT were included in the study, 77 patients (82.8%) were HBe Ag negative and 16 patients (17.2%) were HBe Ag positive. HBV DNA levels ranged between 27 – 64× 106 IU/ML. Thirty three patients (35.5%) were presented with moderate-to-severe Inflammation (A2 – A3) and 41 (44.1%) had significant fibrosis (F2 – F4). The serum HBV DNA levels were not correlated to the histological stage of liver disease in either HBe Ag negative or HBe Ag positive patients (p value 0.077 and 0.625) respectively. HBe Ag negative patients with high viral load (> 20.000 iu/ml) had higher percentage of significant fibrosis when compared to those with low or moderate viraemia but the difference did not reach the statistical significance

Conclusion: No significant relationship between serum HBV DNA level and severity of liver fibrosis in CHB with persistent normal ALT.

Keywords: *Chronic Hepatitis B Virus; HBV DNA; ALT; Fibrosis; Necroinflammation; Persistent Normal ALT*

Abbreviations

CHB: Chronic Hepatitis B; PNALT: Persistent Normal Alanine Transaminases; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase

Introduction

Hepatitis B virus infection is a global problem and approximately 15 - 40% of patients with chronic HBV will develop cirrhosis, end-stage liver failure or hepatocellular carcinoma (HCC) in their lifetime [1,2].

In Egypt, nearly 2 - 3 million Egyptians are chronic carriers of HBV [3,4]. HBeAg-negative variant accounts for more than 80 % of CHB in Egypt [5]. Genotype D is the most predominant genotype in Egypt which is more associated with HBeAg-negative variants and more severe liver disease [6,7].

Current international guidelines recommended the use of Alanine transaminases level and serum HBV DNA to assess activity of liver disease and to identify patients for antiviral therapy. Patients with persistent elevated ALT were considered for therapy, while those with normal transaminases should not be treated and follow up is recommended by ALT evaluation every 3 months [8-10].

Recently, several studies reported that 12 - 40 % of patients with chronic HBV infection and persistently normal ALT (PNALT) levels have significant degree of fibrosis require treatment [11,12]. So, ALT levels may not accurately reflect the presence of hepatocellular injury and may be suboptimal in reflecting pathology at the hepatic tissue level [13,14].

Serum HBV DNA levels directly reflect the degree of HBV replication, and are strongly correlated with long-term mortality, The Risk Evaluation of Viral Load Elevation and Association Liver Disease (REVEAL-HBV) found that cumulative incidence of liver cirrhosis increase as function of HBV–DNA levels ranging from approximately 5% when a viral load undetectable < 300 copies/ml to 36%with > 10^6 copies/ml irrespective of ALT or hepatitis e Ag status [15].

Despite the foregoing, the relationship between HBV DNA levels with liver histopathology have not been clarified. So, the primary aim of this study was to evaluate if there is relationship between serum hepatitis B virus DNA levels and extend liver histopathology in chronic HBV patients with PNALT.

Subjects and Methods

Study design and patient

After approval of the ethical committee of the faculty of Medicine, at Assuit University, we conducted a hospital based cross sectional study to evaluate the relationship between HBV DNA level and liver histopathology in patients with chronic hepatitis B presented with PNALT in the time period between December 2014 to October 2016 at Assiut center for management of viral hepatitis, Egypt.

Ninety three patients, hepatitis B patients have HBs Ag positive for more than 6 months, age >18 years old and, persistent normal transaminases confirmed on 3 consecutive liver enzymes reading over 9 months and detectable HBV DNA were included. Hepatitis C virus co-infection, clinical, serological evidence of cirrhosis was considered exclusion criteria from this study.

Liver Biopsy

For 93 patients who met the inclusion criteria, after written consent, liver biopsy guided abdominal ultrasound using 16-gauge true-cut needles was performed. (METAVIR) score for grade & stage is used. Significant histopathological abnormality was defined as fibrosis stage ≥ F2 or necroinflammation ≥ A2.

Serodiagnosis of HBV

Routine liver biochemical tests were performed using commercially available autoanalyzers. Hepatitis serological markers including HBs Ag, HBe Ag, Anti-HBs and total anti-HBc antibodies, were assayed using commercially available enzyme-linked immunoassays according to the instructions of the manufacturer (DiaSorin diagnostic kits,Italy).

Quantitative Real time PCR for detection and quantification of HBV DNA levels

HBV DNA was extracted from patients' serum samples by QIAamp DNA Mini Kit (Qiagen, Germany, Cat No. 51304) according to the manufacturer's instructions and HBV DNA level was determined quantitatively by real-time PCR assay using 7500 Fast Real-Time PCR System (Applied Biosystems, USA).as previously described [16,17].

Statistical analysis

Data entry and data analysis were done using SPSS version 19 (Statistical Package for Social Science). Data were presented as number, percentage, mean, standard deviation. Chi-square test was used to compare between qualitative variables. Independent samples t-test was used to compare between two quantitative variables in case of parametric data, and Mann-Whitney test for non-parametric. Spearman correlation was done to measure correlation between quantitative variables. P-value considered statistically significant when $P < 0.05$.

Result

Demographic data

Clinical and laboratory characterization of 93 patients analyzed in this study were summarized in (table 1).

Item	N = 93
Age "yrs." "mean ± SD" <40yrs. ≥40yrs	37.00 ± 9.62 60(64.5%) 33(35.5%)
Male: female	82(88.2%): 11(11.8%
Platelet count	228.60±65.48 range(96 - 411)
(INR)	1.05±0.08 range(1.0 - 1.3)
HBeAg status: Negative Positive	77(82.8%) 16 (17.2%)
HBV DNA level IU/ML < 2000 2000 - 20000 > 20000	27-64×10)median 60560 IU/ML) 24 (25.8%) 23 (24.7%) 46 (49.5%)
Histopathology (METAVIR) Activity of inflammation ≥ A2 degree of Fibrosis ≥ F2	33(35.5%) 41 (44.1%)

Table 1: *Demographic, laboratory and histopathologic evaluation of liver biopsy in studied patients.*

Abbreviations: *N: Number Of Patient; HBV: Hepatitis B Virus; ULN: Upper Limit of Normal; HBeAg: Hepatitis Be Antigen; INR: International Normalization Ratio; PCR: Polymerase Chain Reaction*

A total of 93 CHB patients with PNALT were included in the study, Seventy seven patients (82.8%) were HBeAg negative and 16 patients (17.3%) were HBeAg positive. HBV DNA levels ranged between 27 – 64× 10^6 IU/ML (median = 10.0 × 10^6 IU/ml). Twenty four patients (25.8%) had HBV DNA levels less than 2000 IU/mL, 23 cases (24.7%) with DNA between 2000 and 20,000 IU/ML and 46 patients (49.5%) had HBV DNA more than 20.000 IU/ML. thirty three patients (35.5%) were presented with moderate-to-severe Inflammation (A2 – A3) and 41 (44.1%) had significant fibrosis (F2 – F4) as shown in (table 1).

Comparative analysis between HBe Ag negative and HBe Ag positive patients:

The baseline characteristics between HBeAg negative group (n = 77 patients) and HBeAg positive groups (n = 16) were compared, the HBeAg –ve group were significantly older in age (p = 0.003), had a lower HBV DNA levels (P = 0.000). Significant fibrosis (F2 - F4) was present in 38 (49.4%) of HBeAg negative patients and in 3 (18.2%) HBeAg positive group (p value 0.025) as shown in table 2.

Parameter		HBe Ag status				P-value
	Total number	Negative (n= 77)		Positive (n = 16)		
		No.	%	No.	%	
Age:						0.003*
<40 years	56	43	76.8	13	23.2	
≥ 40 years	37	34	91.9	3	8.1	
Sex:						0.927
Male	82	68	82.9	14	17.1	
Female	11	9	81.8	2	18.2	
BMI:						0.819
Normal	18	14	77.8	4	22.2	
Overweight	62	52	83.9	10	16.1	
Obese	13	11	84.6	2	15.4	
Degree of fibrosis METAVIR:						0.025*
F0 - F1	52	39	50.6	13	81.2	
F2 - F4	41	38	49.4	3	18.8	
Degree of activity METAVIR						0.124
A0 - A1	60	47	61.0	13	81.3	
A2 - A3	33	30	39.0	3	18.8	
HBV-DNA Viral load						0.000*
< 2,000	24	23	95.8	1	4.2	
2,000 - < 20,000	23	23	100.0	0	0.0	
≥ 20,000	26	13	50.0	13	50.0	

Table 2: *Comparative study between HBeAg negative and HeAg positive patients.*

Correlation between the HBV-DNA level and the severity of Fibrosis in HBe Ag negative group (N 77/93)

Our data revealed that HBeAg negative patients with high viral load (> 20.000 iu/ml) had higher percentage of significant fibrosis when compared to those with low or moderate viraemia but the difference did not reach the statistical significance (P value 0.077).

HBV-DNA viral load categorizations	HBe Ag negative group (N 77/93)				P-value
	Fibrosis				
	F0/F1 (n = 39)		F2/ F3/ F4 (n= 38)		
	No.	%	No.	%	
< 2,000	13	33.3	10	26.3	0.077
2,000 - < 20,000	15	38.5	8	21.1	
≥ 20,000	11	28.2	20	52.6	

Table 3: *Correlation between the degree of fibrosis and viral load in HBe Ag negative group (N 77/93) and HBe Ag positive group (N 16/ 77).*

Correlation between the HBV-DNA level and the severity of Fibrosis in HBe Ag positive group (N 16/93)

Results of our work showed that no statistical significant difference between viral load and stage of fibrosis among in case of HBeAg-positive group. Only three patients of with viral DNA load > 20.000 had significant fibrosis as shown in table 4.

	HBe Ag positive group (N 16/ 93).				
HBV-DNA viral load categorization	Fibrosis				P-value
	F0/ F1 (n = 13)		F2/ F3/ F4 (n = 3)		
	No.	%	No.	%	
< 2,000	1	7.7	0	0.0	0.625
2,000 - < 20,000	0	0.0	0	0.0	
≥ 20,000	12	92.3	3	100.0	

Discussion

Liver biopsy is a widely used clinical practice to verify the diagnosis of CHB and evaluate the efficacy of antiviral therapy. Histotpathological diagnosis of CHB is the "gold standard" for evaluating the severity of hepatic inflammation and fibrosis. HBV DNA level has an essential role in making treatment decision, however the relationship between HBV DNA viral load and effect on liver did not clarified till now. In present study we investigated the relationship of serum HBV DNA with liver histology of chronic hepatitis B patients with PNALT. in Egypt we have few studies evaluating liver histopathology in patients with HBeAg positive (immune tolerant) or HBeAg negative (inactive carrier state) and most of data we have from studies done on Asian and European patients who have different HBV genotype [18].

In our study, no correlation was found between HBV DNA level and liver fibrosis in HBeAg negative state with (P = 0.077) and HBeAg positive (P value = 0.625). This results were in agree with Shao., *et al.* [19] who reported that serum HBV DNA level was uncorrelated with histological grading or staging of liver diseases in CHB patients, Yuen et al also founded that, no association between HBV DNA levels and liver histology (inflammation or fibrosis) in HBeAg-positive patients [20].

In contrast to our finding, a number of investigators have demonstrated a close correlation between serum HBV DNA level and the severity of hepatic inflammation and fibrosis, for example, in a study done by Bai., *et al.* on 215 chronic hepatitis B patients, they reported a positive correlation between serum HBV DNA level and hepatic inflammation in both HBeAg-positive and HBeAg-negative patients aged ≥ 35 years, but in patients aged < 35 years positive correlation was only observed in HBeAg-negative patients [2], also the same result noticed by Wong., *et al.* who reported that patients with serum HBV DNA > 4 log10 copies/ml had a higher risk of cirrhosis [21].

In present study, HBeAg negative patients were older in age, had lower serum HBV DNA levels and more degree of significant fibrosis than HBeAg positive patients. This high percentage of advanced fibrosis in our HBeAg negative patients may be explained by vertically or early childhood transmission of HBV which is mainly characterized by prolonged duration of infection with continuous replication. Another explanation is predominate genotype D in Egypt which is more associated with HBeAg-negative variants which is characterized by higher incidence of spontaneous mutation in the core or core promoter region of the viral genome [22], these mutations allow HBV replication in the absence of HBeAg. The hallmark of this state is its fluctuating course and more severe liver disease [23].

Conclusion

We could not depend on HBV DNA Level to Predict Significant Liver Fibrosis in CHB Patients with PNALT. HBe Ag negative patients have different clinical features, so longer and closer monitor is needed for those patients with PNALT. Although advances in treatment of hepatitis B patients, an area of ongoing debate in management of CHB patients with PNALT still present.

Bibliography

1. Ott JJ., *et al*. "Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity". *Vaccine* 30.12 (2012): 2212-2219.

2. Bai H., *et al*. "Influence of age and HBeAg status on the correlation between HBV DNA and hepatic inflammation and fibrosis in chronic hepatitis B patients". *Digestive Diseases and Sciences* 58.5 (2013): 1355-1362.

3. Attia MA. "Prevalence of hepatitis B and C in Egypt and Africa". *Antiviral Therapy* 3.3 (1998): 1-9.

4. Shalaby S., *et al*. "Hepatitis B and C viral infection: prevalence, knowledge, attitude and practice among barbers and clients in Gharbia governorate, Egypt". *Eastern Mediterranean Health Journa*l 16.1 (2010): 10-17.

5. El-Zayadi AR., *et al*. "Evaluation of liver biopsy in Egyptian HBeAg-negative chronic hepatitis B patients at initial presentation: implications for therapy". *American Journal of Gastroenterology* 104.4 (2009): 906-911.

6. Zaky S., *et al*. "Clinicopathologic features and genotyping of patients with chronic HBV infection in the Upper Egypt". *Cellular Immunology* 265.2 (2010): 97-104.

7. Khaled IA., *et al*. "Prevalence of HBV genotypes among Egyptian hepatitis patients". *Molecular Biology Reports* 38.7 (2011): 4353-4357.

8. "EASL clinical practice guidelines: Management of chronic hepatitis B virus infection". *Journal of Hepatology* 57.1 (2012): 167-185.

9. Terrault NA., *et al*. "AASLD guidelines for treatment of chronic hepatitis B". *Hepatology* 63.1 (2016): 261-283.

10. Sarin SK., *et al*. "Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update". *Hepatology International* 10.1 (2016): 1-98.

11. Lai M., *et al*. "The clinical significance of persistently normal ALT in chronic hepatitis B infection". *Journal of Hepatology* 47.6 (2007): 760-767.

12. Gobel T., *et al*. "High prevalence of significant liver fibrosis and cirrhosis in chronic hepatitis B patients with normal ALT in central Europe". *Journal of Medical Virology* 83.6 (2011): 968-973.

13. Kumar M., *et al*. "Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT". *Gastroenterology* 134.5 (2008): 1376-1384.

14. Chen JD., *et al*. "Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death". *Gastroenterology* 138.5 (2010): 1747-1754.

15. Iloeje UH., *et al*. "Predicting cirrhosis risk based on the level of circulating hepatitis B viral load". *Gastroenterology* 130.3 (2006): 678-686.

16. Zanella I., *et al*. "Quantitative analysis of hepatitis B virus DNA by real-lime amplification". *European Journal of Clinical Microbiology and Infectious Diseases* 21.1 (2002): 22-26.

17. Marwa khalaf MAM., *et al*. "Impact of Hepatitis B Viral Load and Liver Histopathology on the Decision to Treat Chronic Hepatitis B Patients with Persistent Normal Alanine Transaminases". *EC MICROBIOLOGY* 4.1 (2016): 647-656.

18. Liu CJ and JH Kao. "Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J". *Seminars in Liver Disease* 33.2 (2013): 97-102.

19. Shao J., *et al*. "Relationship between hepatitis B virus DNA levels and liver histology in patients with chronic hepatitis B". *World Journal of Gastroenterology* 13.14 (2007): 2104-2107.

20. Yuen MF., *et al*. "Significance of HBV DNA levels in liver histology of HBeAg and Anti-HBe positive patients with chronic hepatitis B". *American Journal of Gastroenterology* 99.10 (2004): 2032-2037.

21. Wang CC., *et al*. "Factors predictive of significant hepatic fibrosis in adults with chronic hepatitis B and normal serum ALT". *Journal of Clinical Gastroenterology* 42.7 (2008): 820-826.

22. Hadziyannis SJ. "Natural history of chronic hepatitis B in Euro-Mediterranean and African countries". *Journal of Hepatology* 55.1 (2011): 183-191.

23. Sharma S., *et al*. "Clinical significance of genotypes and precore/basal core promoter mutations in HBV related chronic liver disease patients in North India". *Digestive Diseases and Sciences* 55.3 (2010): 794-802.

Volume 2 Issue 1 February 2017