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Research Article

Impact of Hepatitis B Viral Load and Liver Histopathology on the Decision to Treat Chronic Hepatitis B Patients with Persistent Normal Alanine Transaminases

Marwa khalaf[1], Mohamed A Mekky[2], Sherif Ibrahim Kamel[2], Mohamed El-Taher AbdelRahman[2] 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.

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Abstract

Background and aim: Despite of advancement in treatment of chronic hepatitis B (CHB), there is still ongoing debate in the management of (CHB) patients with persistent normal transaminases (PNALT). As most guidelines depend mainly on elevated enzymes as corner stone in making treatment decision in chronic HBV infection. In Egypt, there is a paucity of data on chronic hepatitis B virus (HBV) DNA levels and histologic lesions in CHB patients with (PNALT). There for, we here trail to evaluate HBV DNA viral load and histopathologic pattern in this particular subset of patients and their implication for therapy.

Methods: A total of 93 chronic hepatitis B patients with persistent normal transaminases mean age was 37 years (rang 20 - 58), (88.2%) eighty-two of them were males, (82.8%) 77 patients were HBe Ag negative. All patients were evaluated Clinical, biochemical, serological and underwent liver biopsy with histopathological evaluation according (METAVIR score). Serological diagnosis of HBV was done by ELISA and HBV DNA level was quantified by real time PCR.

Results: Of the 93 patients, (35.5%) had moderate-to-severe Inflammation ≥ A2 and (44.1%) had significant fibrosis ≥ F2, baseline characteristics showed that significant histology group was significantly older, (56.1%) of patients with age more than 40 years had moderate to severe fibrosis ≥ F2 ($p < 0.00$). HBV DNA levels were measured as baseline before performing liver biopsy ranged between 27 – 64× 10^6 IU/ML (median = 10.0 × 10^6 IU/ml), 24 patients (25.8%) had 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%) have more than 20.000 IU/ML.

Patients with higher HBV DNA viral load is significantly associated with necroinflammation ≥ A2 ($p = 0.034$), on other hand there was no relationship between HBV DNA and liver fibrosis. Also, significant fibrosis was predominant in HB eAg negative patients. Age and a platelet were independently associated with the risk for significant histological abnormalities by logistic regression analysis.

Conclusions: About half of the patients with PNALT had significant fibrosis. Therefore, liver biopsy should be considered in these patients especially with age older than 40 and higher HBV DNA viral load.

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 represents global problem, around two billion people are infected with HBV worldwide of which 400 million suffer chronic infection with HBV [1].

Patients with chronic hepatitis B are at risk of developing serious complications during life time; around 15% - 40% of these patients will suffer of cirrhosis, decompensated liver disease and hepatocellular carcinoma [2].

In Egypt, the prevalence of HBsAg is of intermediate endemicity (2 - 8%), 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].

ALT levels and HBV DNA level with cut off value >2000 for HBeAg –ve and > 20000 for HBeAg +ve, have been routinely used in assessment of chronic hepatitis B patients and making treatment decision [6].

Most international guidelines recommended treatment only for CHB patients with persistent elevated enzymes, while those with normal transaminases should not be treated and follow up is recommended by ALT evaluation every 3 months, liver biopsy may be my be advised but in especial situation [7,8].

However persistent normal enzymes is not always benign condition, several studies revealed that 12 - 40 %of patients with chronic HBV infection with PNALT have significant degree of fibrosis require treatment [9].

So, ALT levels may be not accurately reflect the presence or degree of hepatocellular injury and may be suboptimal in reflecting pathology at the hepatic tissue level, depending on ALT level in this setting may under estimate the proportion of patients with significant hepatic fibrosis who may benefit from anti-viral therapy [10,11].

Therefore, we aimed to assess the histopathological changes in chronic HBV patients with PNALT, and to evaluate the role of liver biopsy in implication for therapy in those patients.

Subjects and Methods

Study Design and Patients

This is a cross sectional study conducted at Assiut center for management of viral hepatitis, Egypt, during the period from June 2011 to June 2016.

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 histopathology of 93 patients with chronic hepatitis Patients, presented with PNALT from June 2011 to June 2016.

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 and 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).

HBV DNA amplification and detection by real time PCR assay

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 as previously described [12]. HBV-DNA was determined quantitatively by real-time PCR assay using 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Oligonucleotide primers were selected from highly conserved regions of the HBV S gene; FOR 4 (5′-CCTATGGGAGTGGGCCTCA- 3′, nucleotides 639-657) and HBV REV 7 (5′-CCCCAATACCACATCATCCATATA-3′, nucleotides 761-738) (Invitrogen, USA) yielding a 123-bp. The probe sequence was selected within a conserved region of the 123 bp amplicon (5′ -CACTGAACAAATGGCACTAGTAAACTGAGCCA- 3′) [12]. The PCR reaction mix for real-time quantification contained 10 µl of the extracted DNA, 1×TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 45 pmol of each primer and 10 pmol of probe, in a final volume of 50 µl. Thermal cycling conditions were as as previously described [12].

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

Characteristics of patients

A total of 93 CHB patients with persistent normal enzymes who included in the study, 82 were male and 11 female, median age was 37, range (20 - 58 years). 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

Of HBe Ag negative patients (n -77 patients), 38 patients (49.4%) had moderate to severe fibrosis (F2-F4) comparing to HBe Ag positive patients (n-16 patients) 3 patients 18.2% had moderate to severe fibrosis. HBe Ag negative patients were significantly older in age than HBe Ag positive patients (P = 0.003) and had a lower HBV DNA levels (P = 0,000) (Table 2).

Item	Descriptive "N = 93"
Age "yrs.""**mean ±SD**" **< 40yrs.** **≥ 40yrs**	37.00 ± 9.62 60(64.5%) 33(35.5%)
Sex: **Male** **Female**	82(88.2%) 11(11.8%)
BMI (kg/m^2)	27.16 ± 2.99
Platelet count	228.60±65.48 range (96-411) Normal 86: abnormal 7
(INR)	1.05±0.08 range (1.0 - 1.3) Normal 85: abnormal 8
HBV DNA viral load (median) **< 2000** **2000-20000** **> 20000**	27- 64×10^6 (median 60560 IU/ML) 24(25.5%) 23(24.7%) 46(49.5%)
HBeAg status: **Negative** **positive**	77 (82.8%): 16 (50.5%)
Abdominal Ultrasound: **Normal** **Abnormal hepatic echotexture**	62(66.7%) 31(33.3%)
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: *Abbreviations: N: Number of patient; HBV: Hepatitis B Virus; ULN: Upper Limit of Norma; HBeAg: Hepatitis B e Antigen; BMI: Body Mass Index; INR: International Normalization Ratio; PCR: Polymerase Chain Reaction*

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						
< 2,000	24	23	95.8	1	4.2	0.000*
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.*

Relation between the HBV-DNA level and the severity of Fibrosis

Studying relationship between HBV DNA and liver histopathology in HBeAg-negative patients revealed a trend for increased significant fibrosis ≥ F2 with higher viremia > 20.000 IU/ML (52.2 %) versus lower HBV DNA < 2000 IU/mL (26.3%), however; it was with no significant P value (0.077). In case of HBeAg-positive patients, only three patients of with viral DNA load > 20.000 had significant fibrosis, so no relation was found between HBV DNA level and severity of fibrosis in HBeAg positive patients as shown in (Table 3).

	HBe Ag negative group (N 77/93)				
HBV-DNA viral load categorizations	**Fibrosis**				**P-value**
	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	
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	

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

On other hand, According to ASSLD, 2015 and APASL, 2016 guidelines. Standards, taking HBVDNA level of 2,000 IU/ml as a cut off value for HBe Ag-negative, and 20,000 IU/ml for HBe Ag positive patients our results have shown that 10 of 23 patients with serum HBV-DNA level below 2,000 IU/ml had significant fibrosis > F2 and became indicated for therapy.

Predictors of Significant Liver Disease

Univariate analysis comparing variants between significant and non-significant fibrosis revealed that significant histology group was older, (56.1%) of patients with age more than 40 years had moderate to severe fibrosis ≥ F2 with (p < 0.00) and more in HBe Ag negative patients (P value 0.025). Also, significant fibrosis group had lower mean platelet count and increased INR levels (p value 0.000* and 0.014) respectively as shown in (Table 4).

Item	Non-Significant fibrosis group < F 2 (n 52)	Significant fibrosis group ≥ F2 (n 41)	P- value
Age (years) **< 40 (60 patients)** **≥ 40 (33 patients)**	42(80.76%) 10(19.23%)	18(43.90%) 23 (56.1%)	0.000*
Sex: **Male/female**	44(84.61%) /8(15.38%)	38(92.68%) /3(7.31%)	0.383
BMI (kg/m^2)	26.84 ± 2.59	27.57 ± 3.41	0.544
HBV DNA viral load by PCR **< 2000** **2000-20000** **>200,000**	14(58.3 %) 15(65.8%) 23 (44.2%)	10 (41.7 %) 8(34.8 %) 23(56%)	0.678
HBe Ag status:			
HBe Ag negative **HBe Ag positive**	39(75.0%) 13 (25.0%)	38 (92.68%) 3(7.31%)	0.025*
Platelet	251.79 ± 55.15	199.20 ± 66.32	0.000*
INR	1.04 ± 0.06	1.08 ± 0.09	0.014*
Abdominal U/S **Normal** **Abnormal texture**	49 (79.0) 3 (9.7)	13 (21%) 28 (90.3%)	0.00*

Table 4: *Univariate analysis comparing several variants between significant and non-significant fibrosis.*

The comparative study between significant and non-significant activity showed that, older age and higher HBV DNA viral load is associated with necroinflammation ≥ A2 (p value 0.000, 0.034 respectively) as shown in (Table 5).

Item	Total	Activity				
		A0/A1 (n = 60)		A2/ A3 (n = 33)		P-value *t test*
		No.	%	No.	%	
Age:						
< 40years	56	44	78.6	12	21.4	0.000*
≥ 40 years	37	16	43.2	21	56.8	
Sex:						
Male	82	52	63.4	30	36.6	0.787
Female	11	8	72.7	3	27.3	
HBe Ag status						
Negative	77	47	61.0	30	39.0	0.124
Positive	16	13	81.3	3	18.8	
HBV DNA						
< 2,000	24	17	70.8	7	29.2	0.034*
2,000 - < 20,000	23	19	82.6	4	17.4	
≥ 20,000	46	24	52.2	22	47.8	

Table 5: *Univariate analysis comparing significant and non-significant degree of activity according to demographic clinical and laboratory data.*

In multivariate logistic regression analysis, the significant histology was the dependent variable, while serum PLT, INR, HBe Ag negative levels and age at which patients entered the study used as independent predictor for significant histology. Older age and low platelet count were predictor for significant histology. The odds ratios were 2.93 (95% CI, 1.08 - 7.91) for patients aged > 40 years, and 0.99 (95% CI, 0.98 - 99) for low PLT level (p value was 0.034 and 0,033 respectively as shown in (Table 6).

	P-value	Exp(B)	95% C.I.	
			Lower	Upper
Age > (40 years)	0.034*	2.93	1.08	7.91
HBe Ag (negative)	0.209	2.59	0.57	11.42
PLT	0.033*	0.99	0.98	0.996
INR	0.277	53.14	0.04	168.53

Table 5: *Univariate analysis comparing significant and non-significant degree of activity according to demographic clinical and laboratory data.*

PLT: Platelet; INR: International Normalization Ratio; CI: Confidence Interval

Discussion

ALT is a surrogate marker of inflammation, in our study we found a considerable number of patients with PNALT that associated with a significant hepatic fibrosis. Near half of patients (44.1%) 41 patients had significant fibrosis (F2 - F4), and (35.5%) 33 patients were presented with moderate-to-severe Inflammation (A2 - A3).

In spite of the usual concept that, PNALT had a negligible liver pathology, our finding were also observed in two independent studies [9,13], they found significant fibrosis in 32.9% and 37% respectively. A finding that may raise an alarm to change the concept of ALT as a surrogate marker of inflammation [9,13].

One more add finding, moderate to severe fibrosis ≥ F2 was significantly more prevalent in HBeAg negative patients. Among the HBeAg negative patients (49.4%) had fibrosis ≥ F2, while in HBeAg positive patients (18.2%) had significant fibrosis. This finding to some extend was in agree with study done by Fatten et al., 2012[14] on 30 HBe Ag negative Egyptian patients, they founded that 20% of their patients had significant fibrosis ≥ F2, in comparison to 49.4% in our patients, this difference may be due to small number of included patients or selection of only patients had HBV DNA < 2000 IU/ML in their study.

In contrast to our finding, study done by Kumar., *et al*. who reported (13, 8%) HBe Ag negative patients had ≥ F2 and (37.9%) of HBeAg positive had significant fibrosis ≥ F2 [10]. Moreover, in another study done by Alam., *et al*. 2011[15], he reported that (10.8%) of HBeAg negative and (9.7%) of HBeAg-positive chronic hepatitis B patients with normal ALT had significant fibrosis.

This high percentage of advanced fibrosis in our HBeAg negative patients may be attributed to predominate genotype D in Egypt, which is characterized by high incidence of spontaneous mutation in the core or core promoter region of the viral genome[16], the precore mutation produces a stop codon in a region of the HBV genome that prevents the formation of HBeAg, whereas the basal core promoter (BCP) mutation affects HBeAg transcription, these mutations permit HBV replication in the absence of HBeAg. The hallmark of this state is its fluctuating course and ongoing progression [17].

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 the current study, relation between the HBV-DNA level and significant liver pathology was evaluated, we found that high HBV DNA viral load is significantly associated with necroinflamation ≥ A2 (p value 0.034), This results was in agree with, Bai., *et al*. who reported positive correlation between serum HBV DNA level and hepatic inflammation in both HBeAg-positive and HBe Ag-negative patients aged ≥ 35 years, but in patients aged < 35 years, positive correlation was only observed in HBe Ag-negative patients [18]. This finding may be supported by, The Risk Evaluation of Viral Load Elevation and Association Liver Disease (REVEAL-HBV) the study found the 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 HBe Ag status [19]. Conversely, Yuen reported that there was no association between HBV DNA levels and liver histology (inflammation or fibrosis) in HBe Ag-positive patients [20].

In the current study, age was associated with significant liver disease, thirty-two (56.09.7%) of patients with age more than 40 had moderate to severe fibrosis ≥ F2 (p < 0.00), several longitudinal studies have demonstrated that the incidence of cirrhosis and HCC increases with age, especially in patients > 40 years [21]. Previous studies have reported that age >45 years is an independent predictor of significant histology [22], which may be explained by that most patients generally in Mediterranean region including Egypt may acquire HBV infection perinatally or during early childhood. Patients may continue to experience fluctuating levels of viral replication with recurrent flares and remission, which are believed to contribute over time to the progression to cirrhosis and HCC through chronic necroinflammation and regenerative proliferation over many years. In the current study, age above 40 and low platelet count at the time of liver biopsy were predictors of significant liver disease. These data support findings from other studies showing that ALT is not an accurate predictor of liver histology [23].

Although advances in treatment of hepatitis B patients, however area of ongoing debate in management of CHB patients with PNALT still present. In Egypt, this problem have many aspects; first most international Guidelines (APASL, ASSLD, EASL) recommend follow up in these group of patients with PNALT, hesitating decision of liver biopsy unless, older age or family history of HCC. The process of Follow up in our patients may be impractical because many patients lose follow up, as most of them not suffering appearing healthy individual

with no awareness about possibility of disease reactivation and progression, and lastly financial aspects may play a role [5,13,24]. Second, 80% of Egyptian patients are HBe Ag negative in which it is sometimes difficult to differentiate between truly low replicative phase with favorable outcome on long term follow up from HBeAg negative chronic hepatitis that characterized by intermittent or persistent increase in alanine aminotransterase (ALT), diagnosed as HBeAg-negative variants [5]. Third, in Egypt genotype D is the most predominant genotype characterized by more HBe Ag negative and more progressive liver disease [13]. So, liver biopsy is more informative on liver stage of disease at time of presentation, and introduce more patients for therapy who may miss treatment [5,13].

Conclusion

A fair proportion of patients with CHBV infection with PNALT have significant histologic fibrosis, so liver biopsy should be considered in those patients with persistent normal ALT. Also, age above 40 years and low platelet count could predict extensive liver damage.

It is recommended, more attention should be directed to future research for alternative noninvasive markers of liver damage.

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