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Histological Differences in Naiive Chronic Hepatitis B Patients with Normal and Elevated ALT

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Abstract

Background and aims: HBV infection is a significant public health issue all over the world. Egypt shows marked variations of HBV prevalence by age, gender, and geographical distribution. Treatment guidelines for CHB are based on serum ALT, together with HBV viral load and liver inflammation or fibrosis. Our study aimed to compare histological findings between CHB patients with ALT levels >ULN and patients with normal levels.

Methods: Twenty-nine CHB adult Egyptian patients were included in our cross-sectional study. Liver biopsy was performed for all patients to detect fibrosis stage and inflammation grade using the METAVIR score, which was used to divide patients into two groups: patients with mild to moderate fibrosis (F0-1-2) and patients with advanced fibrosis (F3-4). Patients were also categorized into patients with ALT normal ALT; and Patients with ALT values > ULN.

Results: We found that moderate to severe inflammation was detected in 56.7% of cases with normal ALT compared to 28% of cases with raised ALT *(P = 0.015).* Advanced fibrosis was detected in 10.44% of cases with normal ALT and 20% of cases with raised ALT without a statistically significant difference between both groups.

Conclusions: Liver injury is present in a considerable proportion of patients with normal and those with mild ALT rise, so, these cases need careful evaluation in order not to miss a treatment indication.

Keywords: ALT, Hepatitis B, histopathology

Abbreviations

ALT: alanine transaminase, Anti HBe: hepatitis B e antibody, AST: aspartate transaminase, CHB: chronic hepatitis B, DNA: deoxyribonucleic acid, DNA: deoxyribonucleic acid, HB: hemoglobin, HBeAg: hepatitis B e antigen, HBsAg: hepatitis B surface antigen, HBV: hepatitis B virus, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, INR: international randomized ratio, PCR: polymerase chain reaction, PT: prothrombin time, ULN: upper limit of normal, vs.: versus.

1. Introduction

Hepatitis B virus (HBV) infection is a significant public health issue all over the world. Chronic hepatitis B virus (CHB) infection has many complications including hepatocellular carcinoma (HCC), end-stage liver cell failure, liver transplantation, and mortality. According to the World Health Organization, there are about 248 million people all over the world with CHB infection [1]. Egypt shows marked variations of HBV prevalence by age, gender and geographical distribution [2].

Treatment guidelines for CHB are based on serum alanine transferase (ALT), together with HBV viral load and liver inflammation or fibrosis [3]. Considering ALT levels alone without liver biopsy to determine the inactive carriers, a certain proportion of cases with significant inflammation or fibrosis may be missed. Therefore, ALT was excluded by many authors as an important factor for treatment decisions in CHB patients [4]. Multiple Asian studies found that a considerable percentage of HB e antigen (HBeAg) positive and negative patients with persistently normal ALT had significant fibrosis [5-7]. The current study aimed to detect and compare the histological findings in CHB cases with normal ALT and those with ALT higher than the upper limit of normal (ULN).

2. Patients and Methods

2.1.Selection of patients

We performed this cross-sectional research on 92 cases recruited from attendants to Tropical Medicine and Gastroenterology out-patient clinic, Sohag University Hospital from December 2016 to June 2018. We included patients with symptomatic or asymptomatic CHB infection. CHB infection was based on a positive hepatitis B surface antigen (HBsAg) for a duration longer than 6 months. We excluded patients with any of the following: serological evidence of hepatitis C virus (HCV) or human immunodeficiency virus infections, treated HBV patients, alcohol consumption, decompensated liver disease, patients with HCC, patients known to have other chronic liver condition such as autoimmune liver disease, primary biliary cirrhosis, Wilson's disease, Haemochromatosis, non-alcoholic fatty liver disease, or drug-induced chronic hepatitis). We also excluded patients with a contraindication to liver biopsy such as uncooperative patient, Prothrombin time (PT) >4 seconds more than control, international randomized ratio (INR) greater than 1.6[9], platelets count <100.000/mm [10]. According to the histological staging of fibrosis, cases were grouped into 2 categories: Group1: cases with mild to moderate fibrosis (F0-1-2 METAVIR score), Group 2: cases with advanced fibrosis (F3-4 METAVIR score). To compare histological findings in cases with raised ALT and cases with normal ALT, we categorized our cases into two categories: Category 1: cases with ALT levels ≤ULN (ULN= 40 IU/L) [3], Category 2: cases with ALT levels more than ULN.

2.2.Clinical assessment

Baseline patient characteristics including age, gender, and symptoms suggesting chronic liver disease or hepatic decompensation were collected from all participants.

2.3.Laboratory investigations

Peripheral venous blood sample of 5 mL was collected from each participant, and HBsAg, HBeAg, hepatitis B e antibody (Anti-HBe), polymerase chain reaction (PCR) for HBV deoxyribonucleic acid (DNA), HCV antibody, complete blood count, ALT, aspartate transaminase (AST), bilirubin, PT and INR were evaluated.

2.4.Ultrasound-guided percutaneous liver biopsy

The current research included 92 liver biopsy specimens. The obtained tissue cores (15 mm in length each) were fixed in 10 % formaldehyde, processed, as usual, embedded in paraffin. Hematoxylin and eosin stain was used to stain the sections to assess both the grade and the stage of chronic viral hepatitis using METAVIR staging systems [11]. A single pathologist examined the biopsy specimens.

2.5.Statistical analysis:

Data were analyzed using IBM SPSS Statistics for Windows version 20 and Medcalc version 15.8.0. Means ± standard deviation was used to express normally distributed quantitative data, median and IQR were used for skewed data. Number and percentage were used for qualitative data. Shapiro–Wilk test was used to test the normality of quantitative data. Mann–Whitney U test and Spearman's correlation were used for skewed data. The qualitative variables were compared using the Chi-square ($\chi 2$) test and Fisher's Exact Test. In all statistical tests used in our research, a level of five percent was selected as a level of significance.

3. Ethical considerations

The study protocol was approved by the Ethical Committee of Sohag Faculty of Medicine, Sohag University, Egypt. Written informed consent was taken from each patient before enrollment in this study.

4. Results

From December 2016 to June 2018, 92 patients (73 males and 19 females) were enrolled in this research. The mean age of the participants was 36.41 ± 11.03. Based on METAVIR score, we categorized the cases into cases with advanced fibrosis (12 patients (13%), mean age 44.83 ± 11.74, 10 males (83.3%)), and cases with mild to moderate fibrosis (80 patients (87%), mean age 35.15 ± 10.42, 63 males (78.8%)). Baseline characteristics of the studied groups are presented in **Table 1**which shows statistically significant differences for age *(P = 0.006)* and AST *(P = 0.042)*.

Eighty-eight percent of the studied patients (81/92) were HBeAg negative without a significant difference between the studied groups. The median ALT level was 24.85 (IQR 18.93-41) without significant difference between both groups.

Liver fibrosis stage and inflammation grade by METAVIR score in studied patients are summarized in **Table 2.** Among the 92 patients, 50 patients (54.3%) showed significant fibrosis (F2-F3-F4) and 45 (48.9%) showed significant inflammation (A2-A3).

As shown in **Table 3**, no significant relation was detected between ALT values and liver fibrosis and inflammation based on the METAVIR score. However, ALT value tends to increase with increasing stages of fibrosis.

Table 4 demonstrates a comparison between the histological changes in patients with normal ALT and patients with high ALT (>ULN). Moderate to severe inflammation was found in 56.7% of patients with normal ALT compared to 28% of patients with raised ALT *(P = 0.015).* Advanced fibrosis was found in 10.5% of patients with normal ALT and 20% of patients with raised ALT without a statistically significant difference between both groups.

Table 5 shows the HBeAg state in cases with normal ALT and cases with high ALT. HBeAg negativity was more prevalent in cases with normal ALT compared to cases with high ALT (92.5% vs. 76%) *(P = 0.04).*

Variables	Total (N=92)	Group (fibrosis)		P-value
		Mild to moderate (N= 80)	advanced (N= 12)	
Age Mean± S.D.	36.41 ± 11.03	35.15 ± 10.42	44.83 ± 11.74	**0.006**
Gender				
Female	19 (20.7%)	17 (21.2%)	2 (16.7%)	1
Male	73 (79.3%)	63 (78.8%)	10 (83.3%)	
HBV DNA Mean± S.D.	6426399.64±30583121.65	5244005.06±27051969.14	14309030.17±49030971.99	0.114
HBeAg				1
Negative	81 (88%)	70 (87.5%)	11 (91.7%)	
Positive	11 (12%)	10 (12.5%)	1 (8.3%)	
Anti-HB e				0.502
Negative	25 (27.2%)	23 (28.8%)	2 (16.7%)	
Positive	67 (72.8%)	57 (71.2%)	10 (83.3%)	
ALT(IU/l) Median (IQR)	24.85 (18.93 – 41)	24.6 (18.93 – 40.5)	38 (16 – 47)	0.378
AST (IU/l) Mean± S.D.	27.08 ± 12.09	25.65 ± 10.26	36.63 ± 18.44	**0.042**
Total bilirubin (mg/dl) Mean± S.D.	0.74 ± 0.24	0.73 ± 0.24	0.78 ± 0.19	0.498
Prothrombin time (seconds) Mean± S.D.	12.88 ± 1.02	12.85 ± 1.01	13.03 ± 1.08	0.223
INR Mean± S.D.	1.05 ± 0.11	1.04 ± 0.11	1.07 ± 0.1	0.286
HB (g/dl) Mean± S.D.	13.98 ± 1.73	14.08 ± 1.69	13.25 ± 1.83	0.16
Platelets (x1,000/mm^3) Mean± S.D.	227.74 ± 67.45	232.74 ± 64.45	194.42 ± 80.05	0.056

table 1: Baseline characters of the studied patients' categories

HBV DNA: hepatitis B virus deoxyribonucleic acid, HBeAg: hepatitis B e antigen, Anti- HBe: hepatitis B e antibody, ALT: alanine transaminase, AST: aspartate transaminase, INR: international randomized ratio, HB: hemoglobin

Variables	Summary statistics
Fibrosis	
F0	**6 (6.5%)**
F1	**36 (39.1%)**
F2	**38 (41.3%)**
F3	**4 (4.3%)**
F4	**8 (8.7%)**
Inflammation	
A0	**4 (4.3%)**
A1	**43 (46.8%)**
A2	**39 (42.4%)**
A3	**6 (6.5%)**

table 2: Liver fibrosis stage and inflammation grade by METAVIR score in studied patients (No. = 92)

METAVIR		Mean ALT	Kruskal Wallis test	P-value
Fibrosis	**0**	**23.92±12.66**	**4.819**	**0.306**
	1	25.69±12.49		
	2	**32.81±18.90**		
	3	37.75±12.99		
	4	**38.05±30.67**		
Inflammation	**0**	32.18±21.54	4.828	0.185
	1	**33.32±17.97**		
	2	25.35±15.33		
	3	**36.70±24.56**		

Table 3: Relation between ALT level and liver fibrosis and inflammation by METAVIR score
ALT: alanine transaminase

Histological changes	Group		P-value
	Normal ALT (N= 67)	ALT >ULN (N= 25)	
Fibrosis			**0.051**
0	**5 (7.4%)**	**1 (4%)**	
1	**32 (47.8%)**	**4 (16%)**	
2	**23 (34.3%)**	**15 (60%)**	
3	**2 (3%)**	**2 (8%)**	
4	**5 (7.5%)**	**3 (12%)**	
Mild to moderate fibrosis (F0-F1-F2)	**60 (89.5%)**	**20 (80%)**	**0.297**
Advanced fibrosis (F3-F4)	**7 (10.5%)**	**5 (20%)**	
Inflammation			**0.015**
0	**2 (3%)**	**2 (8%)**	
1	**27 (40.3%)**	**16 (64%)**	
2	**35 (52.2%)**	**4 (16%)**	
3	**3 (4.5%)**	**3 (12%)**	

Table4: Comparison between histological changes in cases with normal ALT and cases with high ALT.
ALT: alanine transaminase.

		ALT		P-value
		Normal ALT	High ALT	
HBeAg	**Negative**	62(92.5%)	19(76%)	**0.040**
	Positive	5(7.5%)	6(24%)	

Table 5: HBeAg state in cases with normal ALT and cases with high

ALT: alanine transaminase, HBeAg: hepatitis B e antigen.

Discussion

Our study found an unexpected inverse relation between ALT level and inflammation grade as we detected moderate to severe inflammation in 56.7% of cases with normal ALT and only 28% of patients with raised ALT. As regard fibrosis, although no statistically significant difference was found between cases with normal ALT and those with raised ALT, advanced fibrosis was noticed in about 10% of cases with normal ALT. ***Liao et al*** [7] reported that 4.3% of cases with normal ALT had moderate to severe inflammation, while 40.7% of cases with raised ALT had moderate to severe inflammation. He also reported that significant fibrosis was detected in 42.1% of cases with normal ALT and 78.2% of cases with raised ALT.

Wang et al [12] reported that both inflammation and fibrosis were significantly higher in patients with mild raised ALT. They found that moderate to severe inflammation was detected in 25% of cases with normal ALT and 51% of cases with mild raised ALT, while significant fibrosis was detected in 18.8% of cases with normal ALT and 37% of cases with raised ALT.

Seto et al [8] found that ALT level did not correlate with pathological changes of the liver in both HBeAg positive

and negative cases. Moreover, ***Prati et al*** [13] reported that ALT could not predict histopathological changes of the liver, as many patients with hepatic injury had normal ALT.

However, serum ALT in CHB patients is fluctuating [3], and this could explain the conflicting results as our study is a cross-sectional study that evaluated ALT at one point.

The appropriate approach to CHB cases with active high HBV DNA and normal ALT should be individualized [14]. As regard cases with normal ALT, the decision to carry out a liver biopsy must balance the costs and risks of the maneuver with the probability of not detecting cases with risk of disease progression without treatment.

To conclude, liver injury is detectable in a considerable percentage of CHB cases with normal and mild raised ALT, so, these cases need careful evaluation in order not to miss a treatment indication.

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