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Role of Liver Biopsy in HBV Infected Egyptian Patients: A New Insight

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EAR and MS designed the study. Authors KG and MME wrote the protocol, wrote the first draft of the manuscript, managed the literature searches, analyses of the studied groups, comprehensive systematic review of their relation to international guidelines and writing the whole manuscript with refined revision. Authors KG, MME and MN were responsible for the clinical part of the study. Authors NE and DA performed the pathological examination and analysis of liver biopsies. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: In chronic hepatitis B (HBV) Egyptian patients; international guidelines showed a wide discrepancy in validity of liver biopsy as a prerequisite to solve the dilemma of whether to start treatment or not.
Aim: Evaluating role of liver biopsy in deciding to treat or not Egyptian patients with chronic HBV irrespective of HBV-DNA and/or ALT levels.
Methods: This prospective study was carried out on four equal groups of chronic HBV Egyptian patients, selected from viral hepatitis clinic, National Liver Institute, Menofia University. They were classified according to their HBV DNA and ALT levels. All patients were HBsAg positive for at least 6 months, detectable HBV-DNA by PCR, with no prior submission for antiviral regimens. Liver biopsy was performed and analyzed according to Metavir scoring systems.

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Results: Patients were 32.2 years old with 87.3% male prevalence. 88% of patients were HBeAg negative with 22% showing significant pathology {fibrosis (F) ≥2 and/or necroinflamatory activity (A) ≥2}. Patients eligible for treatment according to EASL 2009 guidelines were 39%, decreased to 29% with implementation of high ALT versus 22% when treatment decision was based on liver biopsy. While only 10.7% were eligible for treatment according to ASSLD 2009 guidelines, decreased to 4.3% with implementation of high ALT levels versus 22.3% when treatment decision was based on liver biopsy findings.

Conclusion: In spite of the discrepancy between treatment indications using either EASL 2009 or ASSLD 2009 guidelines, liver biopsy was more reliable in deciding which chronic HBV Egyptian patients to be treated, irrespective of HBV-DNA and/ or ALT level.

Keywords: HBV; liver biopsy; HBV PCR DNA; treatment.

1. INTRODUCTION

More than 400 million people are infected with hepatitis B virus (HBV) [1]. The prevalence of HBsAg in Egypt is of intermediate endemicity (2–8%). Nearly 2-3 million Egyptians are chronic carriers of HBV [2]. The prognosis and management of chronic liver diseases depend strongly on the degree of liver fibrosis. Until recently, liver biopsy (LB) examination was the only way of evaluating liver fibrosis [3], the lack of correlation of HBV DNA load and ALT level with the severity of liver damage would favor liver biopsy to be the most useful primary criterion for deciding to treat or not the Egyptian patients [3]. Liver biopsy is not routinely needed to diagnose hepatitis B, just used for monitoring the progression of liver damage in people with chronic hepatitis and helping to choose or evaluate treatment options [4]. However, biopsy is a costly procedure associated with side effects and some risks [5]. It also has limitations in underestimating liver fibrosis with small samples and is prone to intra- and inter-observer variation [6]. Moreover, several studies suggested that liver biopsy is far away from being a perfect gold standard since its performance is size-dependent [6,7-8]. Some studies would suggest that an adequate liver biopsy sample should contain more than 5 portal tracts and be at least 15 mm in length [9,10]. In a critical review of the literature concerning the use of liver biopsy in chronic viral hepatitis, Guido and Rugge suggest that in an era of evidence-based medicine the use and interpretation of liver biopsy is very often flawed by unacceptable methodological limits and that a biopsy sample of 20 mm or more containing at least 11 complete portal tracts should be considered reliable for adequate grading and staging [8]. Application of international guidelines on Egyptian patients meets some limitations as HBV infection is a dynamic process with replicative and non-replicative phases. Also, HBeAg status is negative in more than 80% of cases which may necessitate lifelong therapy [2]. Furthermore, these international guidelines necessitate long term follow up with frequent monitoring pre, on- and post-treatment that is not feasible among Egyptian patients. Accordingly, we have to develop guidelines just tailored to our local condition [2].

1.1 The Aim of the Work

Evaluation of the role of liver biopsy in treatment decision of chronic HBV Egyptian patients with chronic hepatitis B at initial presentation irrespective of HBV-DNA and/or ALT levels focusing on those with low viremia and/or normal ALT levels.

2. PATIENTS AND METHODS

This interventional hospital based study was prospectively carried on four equal groups of Egyptian patients with chronic hepatitis B according to their HBV DNA and ALT levels at the initial presentation:

Group I: 25 patients with HBV PCR ≥ 2000 IU/ml and elevated ALT level > ULN.
Group II: 25 patients with HBV PCR ≥ 2000 IU/ml and normal ALT level.
Group III: 25 patients with HBV PCR < 2000 IU/ml and elevated ALT level > ULN.
Group IV: 25 patients with HBV PCR < 2000 IU/ml and normal ALT level.

* Normal ranges for ALT will be 30 IU/L for males and 19 IU/L for females [3].

Patients were selected from the outpatient clinics of Hepatology department, National Liver Institute (NLI), Menoufia University, from April 2010 to April 2012. All patients had positive HBsAg for at least 6 months, detectable HBV-DNA by PCR, with no prior submission for antiviral regimens. Patients co-infected with

hepatitis C virus, hepatitis D virus or human immunodeficiency virus, patients with concomitant autoimmune disorders, patients with clinical evidence of cirrhosis (gastroesophageal varices, ascites or hepatic encephalopathy) or hepatocellular carcinoma and pregnant ladies were absolutely excluded. All patients were subjected to the following: Careful medical history, thorough clinical examination, Laboratory tests.

2.1 Liver Function Tests

Serum bilirubin (total and direct), albumin (ALb), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). All were measured using Cobas Integra 800 Auto analyzer (Roche Diagnostics Ltd – Germany. Catalogue number; M, 87432). Prothrombin test was done using BFT II Analyzer (Dade Behring Marburg GmbH, D-35041 Marburg, Germany).

2.2 Complete Blood Picture: Serological Testing for HBV Markers

HBsAg, HBeAg, anti-HBe, Anti-HBc IgM and total anti-HBc. They were done using commercially available enzyme-linked immunosorbent assays (International Reagents Co., Kobe, Japan).

2.3 HCV and HDV Antibodys

They were detected by means of a third generation enzyme immunoassay (Ortho HCV version 3.0 ELISA; Ortho- Clinical Diagnostics INC., Raritan. NJ, USA).

2.4 Testing for HBV DNA by Polymerase Chain Reaction (PCR)

The Abbott real-time technique (Abbott molecular Inc.Desplaines; IL 60018, USA) were performed with a lower limit of detection of the kit was 10 IU/ml. After an informed consent, percutaneous ultrasonography guided liver biopsy was performed for all patients who met the inclusion criteria. A Tru-cut needle (14-gauge) was used to obtain an enough hepatic tissue. A core of liver tissue containing from 5-11 portal tracts is considered fair enough. All liver biopsies were interpreted by a single well experienced pathologist. The evaluation of necroinflammatory changes (grades) and architectural changes (stages) recorded as the histological activity index (HAI) and fibrosis score, respectively, were performed according to Metavir scoring systems. Metavir scoring system was also used for both inflammation and fibrosis. Fibrosis stage was classified as F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; or F4, cirrhosis. Activity was graded as A0, none; A1, mild; A2, moderate; and A3, severe. Patients graded ≥ A2 and / or ≥ F2 were considered to have significant pathology [11].

All patients were furtherly stratified according to the EASL practice guidelines (2009) based on ALT, HBV-DNA levels and liver histology for management of chronic hepatitis B [3].

2.5 Statistical Procedure

Data were statistically analyzed using SPSS (statistical package for social science) program version 13 for windows and for all the analysis a p value < 0.05 was considered statistically significant. Data were shown as mean, range or value and 95% confidence interval (95% CI) and frequency and percent. Chi square test, ANOVA test, Kruskal-Wallis test, Tamhane test, Sensitivity: Specificity: Accuracy: Roc curve (Receiver operating characteristic curve): Spearman's correlation; all were used in data analysis.

3. RESULTS

3.1 Demographic Variables of the Four Studied Groups Included in the Study

Age and body mass index were found to be statistically not significant between the four studied groups ($P>0.05$). Regarding age, the mean was 30.20± 8.98, 33.20±9.05, 34.68±6.77 and 30.76±8.54 years in the four studied groups respectively. Body mass index average values in the four groups were 24.58 ±4.85, 26.15±3.82, 24.24±4.08, 24.02±4.37 kg/m2 respectively. Most of patients were males 92%, 76%, 100%, 76% in groups I, II, III, and IV respectively.

3.2 Serological and Histopathological Data of the Four Studied Groups Included in the Study

3.2.1 Regarding HbeAg and HbeAb status in the four studied groups

HBeAg was found to be negative in 88%, 92%, 96%, 100% in the four groups respectively. While HBeAb, was positive in 80%, 88%, 84%, 100% in groups I, II, III, and IV respectively. There were 6 (6%) patients with both negative HBeAg and HBeAb (2 patients in group I, 1 patient in group II and 3 patients in group III).

3.2.2 Regarding liver histopathology

Significant difference was detected between the four studied groups regarding liver necroinflammatory activity, significant hepatic pathology (F≥2 and/or A≥2) and hepatic steatosis ($p<0.05$). As regard hepatic necroinflammatory activity according to Metavir scoring system: A0 was 8% in group II, A1 76%, 88%, 72%, 96% in group I, II, III, and IV respectively, A2 4% in group II and IV, 24% and 28% in group I, III respectively. Hepatic fibrosis according to Metavir scoring system : F0 was 4% in group II, F1 was 88% in group I and II, 72% and 100% in group III, IV respectively, F2 was 4%, 8%, 20%, 0% in group I, II, III, and IV respectively, F3 was 8%, 4% in group I, III respectively, F4 was 4% in group III. Significant liver pathology: was detected in 7/25 (28%), 3/25 (12%), 11/25 (44%) and 1/25 (4%) in the four studied groups respectively.

3.2.3 Characteristics of chronic hepatitis B patients with significant hepatic pathology (≥F2 and/or ≥A2)

Twenty two patients (22%) were presented with significant liver pathology, twenty males with only two females, their age ranged from 20 to 48 years (mean=35.54), BMI was 25.6 (19-35.3) kg/m^2, IHA was positive in 2/22 (9%), steatosis >10% of the hepatocyte was 3/22 (13.6%), HBV-DNA level ranged 18-16.000.000 IU/ml (mean = 745.288) and the ALT l level ranged 14 – 116 U/ml (mean = 52.32). Twenty one patients (95.5%) were HBeAg negative and only one (4.5%) was HBeAg positive.

3.2.4 Correlation between ALT level and significant liver fibrosis (≥F2) and activity (≥A2) in chronic hepatitis B patients

Positive correlation was recorded between ALT serum levels and significant liver fibrosis ($r=0.302$, $p < 0.01$) were detected. Also, there was statistically significant positive correlation between ALT and significant liver activity ($r=0.320$, $p < 0.01$).

3.2.5 Correlation between HBV DNA and significant liver fibrosis (≥F2) and activity (≥A2) in chronic hepatitis B patients

A negative Spearman correlation was found between PCR levels and significant fibrosis (F≥2) and Significant activity (A≥2) in chronic hepatitis B patients included in this study ($p> 0.05$).

3.2.6 Candidacy of patients for therapy according to EASL 2009 guidelines compared with liver biopsy with significant pathology

25 / 100 patients fulfilled the typical criteria for treatment (HBV-DNA level ≥ 2,000 IU/ml and ALT level ≥ 1 × ULN), only 7/25 (28%) had significant liver pathology (fibrosis ≥F2 and/or activity ≥A2).50 / 100 patients had either HBV-DNA level ≥ 2,000 IU/ml or ALT level of ≥1× ULN; 14/50 (28%) had significant pathology. 25/100 patients had HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN, significant pathology was found in 1/25 (4%) patients. So, the total number of patients eligible for treatment according to EASL 2009 guidelines in this study was 39/100 (39%) patients while the number of patients eligible for treatment according to liver biopsy at initial presentation (≥ F2 and/or ≥ A2) was only 22/100 (22%) patients (Table 2).

3.2.7 Candidacy of patients for therapy according to EASL 2009 guidelines compared with liver biopsy with significant pathology with implementation of high ALT levels (42 U/l for men and 38 U/l for women)

15 / 100 patients fulfilled the typical criteria for treatment (HBV-DNA level ≥ 2,000 IU/ml and ALT level ≥ 1 × ULN), only 4/15 (26.7%) had significant liver pathology (fibrosis≥F2 and/or activity ≥A2). 53 / 100 had either HBV-DNA level ≥ 2,000 IU/ml or ALT level ≥1× ULN, 14/53 (26.4%) had significant pathology. 32/100 patients had HBV-DNA level < 2,000 IU/ml and ALT level < 1× ULN, significant pathology was found in 4 / 32 (12.5%) patients.

So, the total number of patients eligible for treatment according to EASL 2009 guidelines with implementation of high ALT levels in this study was 29/100 (29%) patients while the number of patients eligible for treatment according to liver biopsy (≥ F2 and/or ≥ A2) was 22/100 (22%) patients. So, implementation of high ALT levels decreased the number of patient candidates for treatment by 10 % (ten patients) (Table 2).

Table 1. Characteristics of chronic hepatitis B patients with and without significant hepatic pathology

	Patients with significant hepatic pathology	Patients without significant hepatic pathology
Number of patients	22/100 (22%)	78/100 (78%)
Male / female	20/2	66/12
Age	35.54 (20 –48)	31.32 (19 –55)
BMI (kg/m^2)	25.6 (19-35.3)	24.5 (18.4-35)
HBV-DNA level	745.288 (18– 16.000.000)	16.111.317 (17 – 39.000.000)
ALT level U/l mean	52.32 (14 – 116)	30.40 (11 – 84)
HBeAg negative %	21 (95.5%)	73 (93.6%)
HBeAg positive %	1 (4.5%)	5 (6.3%)

3.2.8 Candidacy of HBeAg negative patients for therapy according to ASSLD 2009 guidelines compared with liver biopsy with significant pathology

Only 3/94 patients with HBeAg negative filled into the typical criteria for treatment (HBV-DNA level > 2,000 IU/ml and ALT level > 2 × ULN), but out of the three patients, only one patient (33.3%) had significant liver pathology (fibrosis ≥F2 and/or activity ≥A2). 18/ 94 patients had either HBV-DNA level 2.000-20.000 IU/ml or ALT level of 1-2 ULN, 7/18 (38.9%) had significant pathology. 25/94 patients with HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN, significant pathology was found in 1/25 (4%) patients. 24/94 patients with HBV-DNA level < 2,000 IU/ml and ALT level > 1 × ULN, not involved in ASSALD 2009 guidelines, significant pathology was found in 9 / 24 (37.5%) patients. 23/94 patients with HBV-DNA level > 2,000 IU/ml and ALT level < 1 × ULN, also not involved in ASSALD 2009 guidelines, significant pathology was found in 3 / 23 (13.1%) patients. So, the total number of patients eligible for treatment according to ASSLD 2009 guidelines in this study was 10/94 (10.7%) patients, while the number of patients eligible for treatment according to liver biopsy was 21/94 (22.3%) patients.

3.2.9 Candidacy of HBeAg negative patients for therapy according to ASSLD 2009 guidelines compared with liver biopsy with significant pathology using high ALT levels (42 U/l for men and 38 U/l for women)

Only 1 / 94 patients with HBeAg negative filled into the typical criteria for treatment but there was no significant liver pathology.12 / 94 had either HBV-DNA level 2.000 IU/ml and ALT level 1-2 × ULN, 3/12 (25%) had significant pathology. 32/94 patients with HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN, significant pathology was found in 4 / 32 (12.5%) patients. 17/94 patients with HBV-DNA level < 2,000 IU/ml and ALT level > 1 × ULN, not involved in ASSALD 2009 guidelines, significant pathology was found in 7 / 17 (41.2%) patients. 32/94 patients with HBV-DNA level >2,000 IU/ml and ALT level < 1 × ULN, not involved in ASSLD 2009 guidelines, significant pathology was found in 6 / 32 (18.8%) patients. So, the total number of patients eligible for treatment according to ASSLD 2009 guidelines in this study was 4/94 (4.3%) patients while the number of patients eligible for treatment according to liver biopsy was 21/94 (22.3%) patients. So, implementation of high ALT levels decreased the number of patient candidates for treatment by 6.4% (6 patients).

4. DISCUSSION

Management of CHB in Egypt is convoluted with many amalgamated clinical, economic, social, cultural, and generally poor compliance to both follow-up and treatment strategies. Follow-up strategies recommended by the international guidelines are not feasible in most of Egyptian patients for the following reasons: *First*, Most of Egyptians are not health insured, so they pay the expenses of disease management out of their pockets. *Second*, most of them are not aware of the risk of the disease as they are apparently healthy [2]. *Third*, HBV infected Egyptians usually manifest lately with advanced liver pathology [2]. *Fourth,* HBeAg negative is the most dominant variant in Egypt [12], exemplifying an advanced HBV state with persistent viral replication, and possible rapid progression to liver cirrhosis [13]. *Fifth*, HBV infected Egyptians are mainly genotype D [14], branded with more severe liver disease [15-16]. *Finally*, the uneven measures of serum transaminases and HBV-DNA replicated a puzzling diagnostic bottleneck

[17]. For all these reasons, early liver biopsy and therapy rather than follow-up are mandated.

The limited recommendations for liver biopsy in the international guidelines dealing with chronic HBV infection should be reviewed specifically for Egyptian patients. They should be considered as special entity with their own special guidelines. Treatment decision of chronic HBV is a crucial decision affecting not only the patient socioeconomic and health welfare but also the community progression. In the current study, HBeAg was found to be negative in 88%, 92%, 96%, and 100% in the four studied groups respectively. So, most of the studied patients were HBeAg negative (94%) and only (6%) were HBeAg positive. This is in accordance with El-Zayadi et al who reported that 90-95% of Egyptian patients were HBeAg negative [18], also it is comparable to other studies reported in Middle East and Mediterranean countries [19]. HBeAg disappears early in patients with HBV genotype D, which is the predominant genotype in Egypt, this occurs due to mutations in the pre core and/or basic core promoter regions of the genome that abolish or diminish the production of HBeAg [20].

In this study, there were 6/100 (6%) of the cohorts negative for both HBeAg and HBeAb (two patients in group I, one patient in group II and three patients in group III). This may be due to early disappearance of HBeAg before appearance of HBeAb due to pre core and/or basic core mutations, de novo infection with mutant HBV virus, or hepatitis B virus is producing viruses but not in huge loads so, HBeAb not developed. Regarding; age, body mass index, smoking and diabetes mellitus non-significant differences among all groups was detected (P >0.05). However most of patients were significantly males (92% in group I, 76% in group II, 100% in group III, and 76% in group IV).Histopathological examinations of liver biopsies revealed that significant hepatic pathology (fibrosis stage ≥ 2 and/or necroinflammatory grade ≥ 2) was found in 7/25 (28%), 3/25 (12%), 11/25 (44%), 1/25 (4%) in the four studied groups respectively. So, significant hepatic pathology was only 22/100 (22%) in all the studied patients (15% had significant necroinflamation and 12% had significant fibrosis). El-Zayadi [2] evaluated the histopathological pattern among 40 chronic hepatitis B Egyptian patients exhibit significant activity in 12.5% and significant fibrosis in 27.5%. Shiha et al. [21] reported that out of 44chronic hepatitis B Egyptian patients, 20.4% (9/44) had significant hepatic necroinflamation and 15.9% (7/44) had significant hepatic fibrosis. Nahar et al. [22] who studied 77 CHB patients in Bangladesh, they found that only 9% had significant hepatitis. In contrast, El-Zayadi et al. [19] found that A total of 52 HBeAg-negative CHB patients (46 male and 6 female) aged 20 – 52 years (median = 37.5) were included in the study. (50 %) were presented with moderate-to-severe inflammation (A2 – A3) and 29 (55.8%) had significant fibrosis (≥ F2). Possible explanation come to mind may be, the relatively younger age of our patients compared with the published literature. In most series, the median age of HBeAg negative patients was significantly older at presentation compared to HBeAg positive patients with mean age was 40 years [22], while the mean age in our series was 32.21 years.

Out of those 22/100 patients with significant liver fibrosis and necroinflamation, there was 21/94(22.3%) HBeAg negative patients and 1/6(16.7%) HBeAg positive patients. This comes in agreement with Mahtab and Rahman [23], who studied 80 CHB patients, they found that 23% patients with HBeAg positive CHB had moderate to severe chronic hepatitis, in contrast to 36% patients with HBeAg-negative. Also, Rahman et al. [24] studied 155 patients (102 HBeAg-positive and 55 HBeAg-negative patients) and found that 20.8% of patients with HBeAg-negative opposite 18.6% of patients with HBeAg-positive CHB had moderate to severe chronic hepatitis and 28.3% of patients had significant hepatic fibrosis as opposed to 19.6% of patients with HBeAg-positive CHB. These findings were consistent with the concept that HBeAg-negative patients have more advanced disease than HBeAg-positive CHB patients [25]. For example, in a large series from the Mediterranean area, 29-38% of patients had cirrhosis at the time of their first presentation [26]. Also a Turkish study done in 2003, included 179 patients with CHB and revealed a significantly more necroinflamation and fibrosis in HBeAg negative CHB [27]. The Egyptians report similar observation of less severe histologic liver disease in HBeAg-positive CHB compared to HBeAg-negative patients in their series of 670 patients [28]. Significant liver pathology (F≥2 and/or A≥2) was 4/50 (8%) in patients who had normal ALT levels at initial presentation (two patients had F2 and one patients had A2 in group II while one patients had A2 in group IV).

Table 2. Candidacy of patients for therapy according to EASL 2009 guidelines compared with liver biopsy with significant pathology

	Decision according to EASL guidelines	Stratification of patients based on the decision of EASL	According to EASL guidelines	Liver biopsy with significant pathology (≥F2 and /or ≥A2)
HBV-DNA level: ≥ 2,000 IU/ml and ALT level: ≥ 1 × ULN	Treat		25	7
HBV-DNA level ≥ 2,000 IU/ml or ALT level of ≥1× ULN	Consider liver biopsy and treat if moderate to severe histological disease present	25	14	14
HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN	Follow up	50	None	1

Table 3. Candidacy of patients for therapy according to EASL 2009 guidelines compared with liver biopsy with significant pathology after implementation of high ALT levels

	Decision according to EASL guidelines	Stratification of patients based on the decision of EASL	Number of patients eligible for treatment	
			According to EASL guidelines	Liver biopsy with significant pathology (≥F2 and /or ≥A2)
HBV-DNA level: ≥ 2,000 IU/ml and ALT level: ≥ 1 × ULN	Treat	15	15	4
HBV-DNA level≥ 2,000 IU/ml or ALT level of ≥1× ULN	Consider liver biopsy and treat if moderate to severe histological disease present	53	14	14
HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN	Follow up	32	None	4

Table 4. Candidacy of HBeAg negative patients for therapy according to ASSLD 2009 guidelines compared with liver biopsy with significant pathology

	Decision according to ASSLD guidelines	Stratification of patients based on the decision of ASSLD	Number of patients eligible for treatment	
			According to ASSLD guidelines	Liver biopsy with significant pathology
HBV-DNA level > 2,000 IU/ml and ALT level > 2 × ULN	Treat	3	3	1
HBV-DNA level: 2,000-20.000 IU/ml and ALT level: 1-2 ULN	Liver biopsy	18	7	7
HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN	Follow-up	25	None	1
HBV-DNA level < 2,000 IU/ml and ALT level >1 × ULN	Nothing	24	None	9
HBV-DNA level > 2,000 IU/ml and ALT level <1 × ULN	Nothing	23	None	3

Table 5. Candidacy of HBeAg negative patients for therapy according to ASSLD 2009 guidelines compared with liver biopsy with significant pathology using high ALT levels

	Decision according to ASSLD guidelines	Stratification of patients based on the decision of ASSLD	Number of patients eligible for treatment	
			According to ASSLD guidelines	Liver biopsy with significant pathology
HBV-DNA level > 2,000 IU/ml and ALT level > 2 × ULN	Treat	1	1	1
HBV-DNA level: >2,000-20.000 IU/ml and ALT level: 1-2 ULN	Liver biopsy	12	3	3
HBV-DNA level < 2,000 IU/ml and ALT level < 1 × ULN	Follow-up	32	None	4
HBV-DNA level < 2,000 IU/ml and ALT level >1 × ULN	Nothing	17	None	7
HBV-DNA level > 2,000 IU/ml and ALT level <1 × ULN	Nothing	32	None	6

These results agreed with the study done by Borg et al. [29] who reported that considerable liver pathology can be found in 10–20% of HBsAg and HBeAg negative individuals with the presence of normal aminotransferase concentrations. Martinot-Peignoux et al. [30] founded that minimal activity was in hepatitis B patients with normal enzymes. Also, Yuen et al. [31] reported that patients with persistently normal ALT levels had a low degree of necroinflamation and almost absent fibrosis. Other studies suggesting that patients with persistent normal serum ALT levels have no or minimal disease progression [32]. Hu et al. [33] reported that among HBeAg negative patients with normal ALT, 15% had significant liver pathology. As, the serum ALT is a marker of hepatic necrosis and inflammation, and patients with normal ALT levels are generally considered to have minimal liver injury with negligible risk of liver-related mortality [34]. In contrast, El Zayadi et al. reported that 45.5% of HBeAg-negative patients with normal ALT level exhibited significant liver pathology [24]. In Bangladesh, Alam et al. [35] found that 23.1% of HBeAg-negative chronic hepatitis B subjects had significant necroinflamation and 10.8% had significant fibrosis despite normal ALT level. This contrast may be due to the lower Keeffe ALT levels we used in our study (30 IU/l for males and 19 IU/l for females) and the relatively younger age of our patients < 40 years compared with the published studies (the mean age was 32.21 years). Significant liver pathology was 28/50 (56%) in patients with elevated ALT (> ULN) at initial presentation (seven patients in group I and eleven patients in group III).

Lindh et al. [36], evaluated histopathological features of 124 hepatitis B e antigen-negative and found that 42% of patients with ALT >ULN had significant necroinflamation, *while* Chan et al. [37] found that 69% cases with ALT >ULN ad significant activity in 55 HBeAg-negative chronic HBV-infected patients. Lesmana et al. [38], reported that in a total of 145 patients, mean age was 41.50 ± 10.74 years, there were 59.3% patients with necroinflammatory grades A2–A3 and 62.1% patients with fibrosis F2–F4. Roushan et al. [39], found that there was a trend for higher serum ALT levels to associate with more histological necroinflamation as reported by other researchers. This cleared by the fact that ALT is normally intracellular enzyme (mainly hepatic cells), and low levels found in the plasma represent the release of cellular contents during normal cells turnover, so elevation of plasma ALT level indicates damage to cells rich in these enzymes, such as viral hepatitis [40,41]. Also, significant liver pathology was 12/50 (24%) in patients with HBV DNA level < 2000 IU/mL (eleven patients in group III and one patient in group IV). El-Zayadi et al. [19] results had shown that 5 of 19 patients (26.3%) with serum HBV-DNA level below 2,000 IU/ml had significant fibrosis. Also, Chotiyaputta et al. [42], reported that 7 of the 25 (28%) patients with HBV DNA <2000 IU/mL at the time of biopsy had significant liver pathology. Nahar et al. [22], who studied 77 CHB patients in Bangladish, they found that 28.6% patients with serum HBV-DNA level below 2,000 IU/ml had significant hepatic fibrosis. The low HBV-DNA level among Egyptian patients may be attributed to the high prevalence of HBeAg-negative variants, different genotype (genotype D), longer duration of infection (owing to the very early age of infection with hepatitis B virus, this state is reached after a very prolonged immune tolerant and immune reactive phase, during which considerable liver damage may have occurred) as well as ethnic and geographic differences [43].

In addition, significant liver pathology was detected in 10/50 (20%) of patients with HBV DNA level > 2000 IU/mL (seven patients in group I and three patients in group II). A finding negated the role of HBV DNA level as an accurate pointer to liver damage in Egyptian chronic hepatitis B patients. In contrast, El-Zayadi et al. [19], had shown that 24 of 33 patients (72.7%) with HBV DNA level >2000 IU/ml had significant fibrosis. Also, Nahar et al. [22], had studied 77 CHB patients in Bangladesh with HBV DNA level >2000 IU/ml, and found that 71.4% patients had significant hepatic fibrosis. This discrepancy in results may be due to the relatively younger age of our cohorts compared to other studies. Significant pathology was detected in 1/25 (4%) of patients in group IV (patients with both PCR level < 2000IU/mL and normal ALT <1 × ULN). This came in agreement with Fateen et al. [44], had studied 30 inactive HBsAg carrier Egyptians, without evident significant hepatic fibrosis or necroinflamation (0%) using the modified ALT level (30 IU/ml in male and 19 IU/ml in female). Another study among Egyptian patients who had PCR level < 2000IU/mL and normal ALT levels, 16% had significant hepatic fibrosis (Metavir score ≥2) [19]. Kumar et al. [45], in india reported that inactive HBV patients had 21% with significant necroinflammation and 13.8% had significant fibrosis. In Bangladish, Al-Mahatab et al. [46]

reported that 26% of inactive HBV carriers had significant necroinflammation and 11% had significant fibrosis. This difference may be due to different definitions of normal ALT level between the studies. A non-significant correlation was found between serum HBV-DNA levels and significant liver activity and fibrosis ($p>0.05$). This in agreement with Shao et al. who found that Serum HBV DNA level is not correlated to histological grade or stage of liver disease [47]. Another negation to this correlation was assured by Alam et al. [35] either in positive or -negative HBeAg patients. However the interplay between HBV DNA level and extent of liver histology in HBeAg-negative CHB patients was mentioned in many reports [48,49,50]. This unresolved debate may be referred to the dissimilar approaches along with the well documented denial of a direct cytopathic role of HBV in liver disease progression with more concerns directed to lessened immune responses [49]. The strong positive correlation between ALT level and significant inflammation ($p=0.01$) and significant fibrosis ($p=0.01$), came in agreement with Shiha et al. and Alam et al. [29,35]. A notion denoting that high transaminase levels might be mirroring an active histological disease in chronic HBV patients [36]. Otherwise, El-Zayadi et al. [19] found a weak positive correlation between the ALT level and the grade of inflammation, with no correlation between the ALT level and the stage of fibrosis. Also, Zaky et al. [51], reported that there was a positive correlation between the mean ALT level and the grade but no correlation with the stage of hepatitis.

In the present study, number of patients who eligible for treatment according to EASL 2009 guidelines in this study were 39/100 (39%) and decreased to 29/100 (29%) With implementation of high ALT levels versus 22/100 (22%) patients when decision to treat is based on liver biopsy. While Total number of HBeAg-negative patients eligible for treatment according to ASSLD 2009 guidelines in this study were 10/94 (10.7%) patients and decreased to 4/94 (4.3%) with implementation of high ALT levels versus 21/94 (22.3%) when decision to treat is based on liver biopsy.

IN El-Zyadi [19], 2009's study; on AASLD guidelines only 16/52 (30.8%) of chronic HBV patients were capitulated for treatment, when liver biopsy was the mainstay; they were heightened to 29/52 (55.8%). On implementation of decreased ALT level recommended by AASLD (30 U/l for male and 19 U/l for female patients) had yielded an increase in treatment eligible patient numbers by 4% (two patients), with no reported change relying on liver biopsy.

5. CONCLUSION

The negated role of liver biopsy in chronic HBV Egyptian patient's treatment decision even with implementation of the recommended lower ALT levels should be revised. The striking discrepancy between treatment indications according to serum markers and liver histology using either HBV treatment EASL 2009 or ASSLD 2009 guidelines would be considered. Liver biopsy should be recommended in all chronic HBV patients with elevated ALT activity regardless of viremia levels and treatment should be given in cases with appropriate histological findings. Patients with ALT < ULN and HBV DNA < 2000 IU/mL can be considered as true inactive HBV carriers, who require neither liver biopsy nor immediate therapy but continued follow-up. Using low ALT level (Normal values: male up to 30U/Land female up to 19U/L) during decision for management of chronic HBV Egyptian patients especially for inactive carriers also should be substantiated.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or compatible ethical standards.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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