Associations between HBeAg status, HBV DNA, ALT level and liver histopathology in patients with chronic hepatitis B

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Abstract: Hepatitis B virus (HBV) infection is still a significant healthcare problem all over the world. Between January 2009 and May 2014, a total of 96 patients with chronic hepatitis B (CHB) were enrolled in study. A total of 96 CHB cases were examined. The mean total liver histological activity indices for grade and stage were 6.01±2.46, and 1.6±0.99 and the mean ALT and AST levels were 32.6 ±21.0 IU/L and 25.6 ±11.2 IU/L, respectively. The mean HBV DNA level was 8.9 x10⁶±3.310⁶ IU/mL. Forty (41.7%) patients had HBV DNA <20 IU/Ml (undetectable) and 14 (14.6%) patients had HBV DNA levels between 21 and 2000 IU/mL. Of the total 96 patients, 100% were HBsAg positive, 88 (91.7%) were HBeAg negative and 8 (8.3%) were HBeAg positive. A significant correlation was found between the HBeAg serostatus, HBV DNA level and the histological activity index necroinflammatory total scores (P= 0.034 and 0.000). We found no correlation between the fibrosis score and HBeAg status (P= 0.451). However, a statistically significant difference was found between HBV DNA levels and stage of fibrosis (P= 0.048). A significant relationship was found between the HBeAg status, HBV DNA level and ALT and AST levels (P= 0.000, 0.000, 0.032, 0.024). The HBeAg status of CHB patients should not affect the treatment response or need for long-term follow-up visits with repeat ALT and HBV DNA levels. However, chronic hepatitis patients who are negative for HBeAg may need different short-term follow-up.

Keywords: ALT, HBV DNA Level, HBeAg Status, Liver Histology

1. Introduction

Hepatitis B Virus (HBV) can cause chronic liver infection. This virus is responsible for more than 240 million cases of chronic infection and about 600,000 deaths each year worldwide. It is still a major public health problem in sub-Saharan Africa and East Asia [1]. Infection with HBV does not always lead to chronic hepatitis and <5% of adults progress to chronic HBV infection [2]. Progression to long-term HBV infection occurs in approximately 15-40% of infected patients [3]. Chronic HBV infection may lead to cirrhosis, hepatocellular carcinoma [4], liver failure [3] and/or death [5]. Various factors seems to affect the natural course of hepatitis B infection including age, sex, immune status of the individual, viral load, replication of HBV and other factors [6]. The HBV is usually found in blood, saliva, semen, vaginal secretions, milk and urine [5]. Chronic HBV infection is usually the result of perinatal transmission, early childhood infection, contaminated blood transfusions, unprotected sexual contact or sharing of contaminated needles [7]. Chronic hepatitis is defined using clinicopathological criteria. In chronic hepatitis B infection, a necroinflammatory reaction is seen in the liver, patients have more than six months of clinical findings, and a change is seen in liver biochemical markers. The hallmark of the disease is a persistent rise in the serum aminotransferase levels (alanine aminotransferase, or ALT, is most sensitive) for at least 6 months [8]. The natural history of chronic hepatitis B (CHB) includes a HBeAg positive, immune-tolerant phase that then progresses to a HBeAg-positive, immune-reactive phase, HBeAg-negative, inactive HBV carrier state, HBeAg-negative CHB phase and HBsAg-negative phase (occult infection) [9]. The HBV is a DNA virus and this virus is capable of genome organization, has a replication replication cycle, and affects the host immune response. The HBV can be detected serologically, by isolation of the virus, by detection/identification of HBV DNA polymerase activity and HBV DNA in the serum [10].
Histopathological changes that occur with HBV infection include necroinflammatory activity (inflammation and necrosis) and fibrosis, which are correlated with HBeAg, anti-HBe, ALT, and HBV DNA levels [8, 11]. The guidelines recommend cutoff values of 40 IU/ml for alanine aminotransferase (ALT) levels and 2000 IU/ml for HBV DNA levels [12, 13]. A histologic activity index has been created which uses histological features to determine grade (inflammation) and stage (fibrosis) [8].

2. Methods

2.1. Study Design

This study was approved by the local Institutional Review Board.

In this retrospective study, all liver specimens were evaluated. The liver specimens were four micrometer thick formalin-fixed and paraffin-embedded sections created from liver biopsies of patients with chronic HBV infection. The specimens were stained with hematoxylin–eosin (H&E), Masson's trichrome, reticulin silver stain and periodic acid-Schiff (PAS) in the State Hospital's pathology laboratory. The liver specimens were four micrometer thick. Histopathological changes that occur with HBV infection were evaluated. The liver specimens were four micrometer thick formalin-fixed and paraffin-embedded sections created from liver biopsies of patients with chronic HBV infection. The specimens were stained with hematoxylin–eosin (H&E), Masson's trichrome, reticulin silver stain and periodic acid-Schiff (PAS) in the State Hospital's pathology laboratory between January 2009 and May 2014. In our study, all cases had a positive hepatitis B surface antigen for at least six months and all patients had not taken interferon or antiviral therapy prior to their liver biopsy.

2.2. Liver Biopsy (Histopathologic Changes)

All cases of chronic hepatitis B were categorized as periportal or perisepal interface hepatitis with piecemeal necrosis (score: 0-4), confluent necrosis (score: 0-6), focal (spotty) lytic necrosis, apoptosis and focal inflammation (score: 0-4), portal inflammation (score: 0-4). The necroinflammatory total scores (grade) were classified into four subcategories: minimal chronic hepatitis (scores 1-3), mild chronic hepatitis (scores 4-8), moderate chronic hepatitis (scores 9-12), and severe chronic hepatitis (scores 13-18). Staging was based on fibrosis, with scores ranging from 0 to 6. The scoring system described by Ishak (modified Knodell) was used. Patients were divided into groups based on fibrosis scores (stage): those with scores ≤2 were classified as mild fibrosis and those with scores 3-6 had advanced fibrosis [14, 15, 16].

2.3. Biochemical Tests; Alanine Aminotransferase (ALT or SGOT) and Aspartate Aminotransferase (AST or SGOT), Albumin, Alpha-Fetoprotein (AFP) Levels

The cutoff value for serum ALT levels was 40 IU/L and was 35 IU/L for AST [12]. The upper limit of normal for serum AFP levels was ≤10 µg/L [17]. The normal range for serum albumin was 3.5-5.5 g/dl. A Cobas 8000 modular analyzer series (the COBAS c502, c701, Roche Diagnostics, Japan) was used to measure ALT, AST, albumin and AFP levels. On the other hand, it has been suggested that the cutoff values for serum ALT and AST should be 30 IU/L for men and 19 IU/L for women [18, 19].

2.4. Serum HBV DNA Levels

The HBV DNA was categorized based on level: ≤20, 20-≤2000, 2000-20,000 and ≥20000 IU/mL (1IU/mL≈5.82 copies/mL). The lower limit of detection was 20 IU/mL and the upper limits of detection was 170000000 IU/mL (the quantitative range of this assay is 1.3-8.2 log IU/mL). The Cobas Amplicor HBV Monitor test (real-time polymerase chain reaction, Roche Diagnostic Systems, COBAS® AmpliPrep® HBV Test, COBAS® TaqMan® 48) was used in this study [12, 20, 21].

2.5. Hepatitis B Viral Markers

The electrochemiluminescence immunoassay (ECLIA) was performed using the Cobas 6000 analyzer series (the Cobas e601 analyzer, Roche Diagnostics, Hitachi, Japan) in order to measure the hepatitis B surface antigen (anti-HBs), hepatitis B surface antigen (HBsAg), Hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe) levels.

2.6. Statistical Analyses

Data were analyzed using Statistical Package for Social Sciences (SPSS) software (version 21.0 for Windows, IBM Corp. Released 2012, IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). The t-test was used to compare averages, a chi-square test was used to determine the dispersion of the two groups (HBeAg positive vs negative), and a Kruskal-Wallis-H test was used to determine the dispersion of HBeAg positive group and a HBeAg negative group. All differences associated with a chance probability of 0.05 or less were considered statistically significant. Continuous variables are presented as mean ± standard deviation (SD).

3. Results and Discussion

Patient characteristics: A total of 96 chronic hepatitis B cases were examined. The mean age of the patients was 42.32±12.6 years (range:16-79 years). There were 55 males and 41 female cases in this study. The clinical, histopathological, virological, biochemical data of the 96 chronic hepatitis B patients were shown in Table 1.

Liver biopsy; necroinflammatory activity and fibrosis: The mean total scores of the liver biopsy based on the Ishak modified Knodell histologic activity index for necroinflammatory activity (grade) and fibrosis (stage) were 6.01±2.46 (range 2 to 13) and 1.6±0.99 (range 0 to 5), respectively. Most patients had mild chronic hepatitis: 14.6% had scores ranging from 1 to 3, 63 patients (65.6%) had scores ranging from 4-8, 18.8% had scores ranging from 9 to 12, and 1% had a score in the 13-18 range. The fibrosis (stage) scores were as follows: 81 patients (84.4%) had mild fibrosis (score 0-2) and 15 patients (15.6%) had advanced fibrosis (scores 3-6).

Biochemical tests: The mean levels of ALT, AST, albumin
and AFP were 32.6 ±21.0 IU/L (range 8 to 113 IU/L), 25.6 ±11.2 IU/L (range 12 to 84 IU/L), 4.6 ±0.3 (g/dL) (range 3.69 to 5.4 g/dL), and 3.4 ±2.8 µg/L (range 22 to 0.6 µg/L), respectively. Low AST (<35 IU/L) and ALT (<40 IU/L) levels were detected in 86.5% and 76% of the cases, respectively. In all cases, the albumin level was in the normal range. Serum AFP levels were elevated (>7 µg/L) in only 8 patients (8.3%).

**Serum HBV DNA levels:** The mean level of HBV DNA was 8952091.6±33829772.4 IU/mL (range 21 to 170 million IU/mL). In our study, 40 cases (41.7%) had undetectable (<20 IU/mL) levels of HBV DNA, 14 (14.6%) had levels ranging from 21 to 2000 IU/mL, 22 (22.9%) had levels ranging from 2001 to 20000 IU/mL, and 20 (20.8%) had levels >20001 IU/mL.

**HBeAg and anti-HBeAg status:** All patients were HBsAg positive in this study. However, 88 cases (91.7%) were HBeAg negative and 8 cases (8.3%) were HBeAg positive. In our study, 84 cases (87.5%) were negative for anti-HBe and 12 cases (12.5%) were positive for anti-HBe. The clinical, liver histology, biochemical, and virological data HBeAg Serostatus patients was shown in Table 2.

<table>
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<tr>
<th>Table 2. HBeAg positive or negative distribution of patients with demographic, liver biochemical test and virological data</th>
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<td><strong>HBV DNA &lt;20 IU/mL</strong></td>
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**Correlation of liver histology with gender, ALT, AST and AFP levels:** There was not a correlation between liver necroinflammatory total scores (grade) and gender, ALT, AST and AFP levels (P=0.601, 0.344, 0.368, respectively). We found no significant difference in liver fibrosis with regards to gender, ALT, AST and AFP levels (P=0.430, 0.360, 0.431, 0.451, respectively).

**Relationship of liver histology with HBeAg status and serum HBV DNA levels:** A significant relationship was found between the HBeAg serostatus and the histological activity
index necroinflammatory total scores (P= 0.034). The majority of patients who were HBeAg negative had a score of 4 to 8. There was also a significant correlation between liver necroinflammatory total scores and the HBV DNA level (P= 0.000). Our study showed that all patients with low or undetectable serum HBV DNA levels (<20 and 21-2000 IU/mL) had necroinflammatory total scores below eight. We found no correlation between the fibrosis score and the HBeAg status (P= 0.451). However, a statistically significant relationship was seen between HBV DNA levels and fibrosis stage (P= 0.048). In our study, 52 patients (54.1%) had mild fibrosis (stage 0-2) and these patients also had a HBV DNA level of ≤2000 IU/mL (Figure 1A-B-C).

**Correlation between HBeAg status, HBV DNA level and biochemical tests:** No statistically significant relationship was found between HBeAg serostatus and HBV DNA level (P= 0.508). A significant relationship was found between HBeAg status and ALT and AST levels (P= 0.000, 0.000). The HBeAg status was not correlated with the AFP level (P= 0.076). The serum HBV DNA level was significantly correlated with ALT and AST levels (P= 0.032, 0.024, respectively). The HBV DNA level was not associated with the AFP level (P= 0.202). About 80.6% of HBeAg negative patients had an ALT level of ≤40 IU/L. Of the 88 HBeAg negative patients, 80 had an ALT level ≤35 IU/L. Approximately 60.2% of patients with an ALT level of ≤40 IU/L also had a HBV DNA level ≤2000 IU/mL.

**Inactive chronic HBV carriers:** In our study, there were 88 patients with HBeAg-negative chronic hepatitis B. Of these cases, HBeAg-negative had serum HBV DNA levels ≤2000 IU/mL and 43 (40.3%) had normal serum ALT levels. Of these cases, 28 (31.8%) had inflammatory activity total scores ≤8 and 34 (37%) had fibrosis scores ≤2.

Chronic hepatitis B (CHB), found in about 2% of the population, is usually diagnosed after the hepatitis B surface antigen (HBsAg) has been positive for at least 6 months [22]. Liver biopsies are required to diagnose chronic hepatitis B. Histologic changes (indicative of the degree of damage) have high prognostic value (predictive of progression to cirrhosis). When administering anti-viral medication, it is useful to obtain pre- and post-treatment biopsies to track response to treatment [14]. The immune reactive HBeAg-positive phase is characterized by high levels of aminotransferases, HBeAg positivity, lower serum HBV DNA levels and moderate/severe histologic activity [13]. Patients who are inactive HBV carriers are HBeAg negative, anti-HBe positive, have normal ALT levels, minimal/no histological changes and fibrosis and undetectable/low levels of HBV DNA (serum HBV DNA <2,000 IU/mL or ≤10 000 copies/mL) [6, 20, 23]. The purpose of treatment of chronic hepatitis B is to prevent progression to end-stage liver disease, cirrhosis and hepatocellular carcinoma [20]. In order to be candidates for treatment, CHB patients usually have to be HBeAg positive and HBeAg negative [13].

Patients with CHB have elevated ALT levels, necroinflammatory activity on liver biopsy and active HBV replication [7]. One should be consider liver biopsy in chronic hepatitis B patients who are in one of the following groups: 1) HBeAg positive, HBV DNA ≥20,000 IU/mL, minimally and persistently/intermittently elevated ALT, age >40 years, HBeAg seroconversion that does not occur spontaneously (3-6 months); 2) HBeAg negative, HBV DNA ≥2000 IU/mL, minimally elevated ALT, age >40 years; 3) HBeAg negative, HBV DNA ≥2000 IU/mL, minimally elevated ALT, age >40 years, ALT persistently/intermittently elevated [24].

The Gastroenterology Society of Australia recommends that CHB patients with HBV DNA levels > 2000 IU/mL receive treatment regardless of age due to the high risk for HBV-related HCC [25]. Chen CJ et al. reported that serum HBV DNA level ≥10,000 copies/mL is a major risk factor for hepatocellular carcinoma irrespective of HBeAg status, ALT level and the presence of cirrhosis [26]. The investigations on this subject are numerous, still perfect results have not been obtained. Nevertheless, the mortality rate is low today, owing to the contributions of the investigators. Studies differ with regards to the significance of histological parameters, biochemical tests, viral markers and viral load in CHB infection. In a previous study, Shao et al. reported that HBV DNA levels had no significant statistical association with liver histology regardless of HBeAg status [27]. In our study, we found that the necroinflammatory total scores were correlated with HBeAg status. However, a previous study by Alam et al. found that there were no associations between HBV DNA levels, necroinflammatory score and HBeAg status [28]. In our study, we found no correlation between the fibrosis score and HBeAg status. However, we did find a correlation between the HBV DNA levels and fibrosis stage. Madan et al. showed that HBV DNA levels correlated with liver histology (stage and grade) and levels of ALT [29]. Similarly, we found that patients with low or undetectable serum HBV DNA levels usually had a liver histological activity index score of less than 8. This opinion has been supported by some authors. In another study by Peng et al., in HBeAg negative patients, high levels of HBV DNA had were positively correlated with the histological activity index (inflammatory and the fibrosis scores). In the same study, elevated HBV DNA levels were correlated with high ALT and ALT levels and with increased liver necroinflammatory processes regardless of patients HBeAg status [30]. Similarly, we found that the serum HBV DNA level was correlated with ALT and AST levels. Xiao et al. found a significant relationship between HBeAg positivity and elevated HBV DNA, but no associations were noted between liver histology (stage and grade), ALT levels and HBeAg status. In the same study, HBeAg status was not correlated with HBV DNA levels or degree of necroinflammation/fibrosis. Furthermore, the HBV DNA level was positively correlated with the degree of fibrosis in patients with normal ALT levels [31]. Similarly, we found no association between the fibrosis score and HBeAg status. Importantly, another study showed a correlation between the mean HBV DNA level and liver necroinflammatory score (grade). There was no correlation between HBV DNA level...
and liver fibrosis (grade), sex, age or HBeAg status. In addition, patients with serum HBV DNA ≥104 copies/ml who needed a liver biopsy and were considering treatment were more likely to have higher a histologic activity index [32]. Zacharakis et al. reported that it is beneficial to follow HBV DNA levels in CHB patients who are HBeAg negative, as the HBV DNA levels correlate with the progression of hepatic damage [33].

Martinot-Peignoux and colleagues examined the inactive HBsAg carrier state and found that the average serum HBV DNA level was 1300 copies/ml and that all patients had a necroinflammatory total score less than seven (consistent with mild chronic hepatitis) [34]. In our work, all patients with low serum HBV DNA levels (<2000 IU/mL) had necroinflammatory total scores less than 8. All patients were 82 (85.4%) cases with a necroinflammatory total scores, grade ≥4, and had 15 cases (15.6%) with a fibrosis, stage≥3. In our study, of the patients with HBeAg negative CHB infection, 10 (11.4%) had HBV DNA >105 copies/Ml (mean: 2809015 IU/Ml, =1.6×107 copies/mL), 7 (7.8%) had a staging score ≥4 and 9 (10.2%) had a grade score ≤2. Inactive chronic HBV carriers have a good prognosis [23]. In our study, 40.3% (43/88) of HBeAg negative CHB patients had HBV DNA levels ≤ 2000 IU/mL and normal serum ALT levels. These results are consistent with a previous study in which 31.8% of HBeAg negative CHB patients had inflammatory activity total scores ≤8. Mild hepatic fibrosis was present in 38.6% (34 of 88) patients in our study. HBeAg negative CHB patients were more likely to have lower HBV DNA levels and less necroinflammatory changes, but there was no relationship between fibrosis and ALT levels (P=0.025, 0.000 and 0.714, respectively). Papatheodori dis et al. reported that the usefulness of treatment in patients with minimal necroinflammatory changes is uncertain [35]. However, the study by Kim et al. recommended anti-viral therapy in HbeAg negative CHB because spontaneous regression is unusual and the course of the disease is worse without therapy [11]. Saikea et al. suggested that treatment should be initiated in patients with higher ALT levels, high HBV DNA levels and active necroinflammation on liver biopsy regardless of HbeAg status. Furthermore, it should be noted that no correlation has been seen between histological activity and normal ALT levels [36].

Some authors have reported that HBV DNA levels fluctuate over time in CHB patients [27, 37, 38]. Inactive chronic HBV carriers (HBeAg-negative) are particularly only do not assume the serum in monitoring the course of the patient cutoff serum HBV DNA levels. The serum levels of HBV DNA have been not used as an indicator of changes in HBeAg levels [37]. In some HBeAg-negative cases, there is increase in serum HBV DNA and normal ALT levels [38]. In addition, spontaneous seroconversion from HBeAg to anti-HBe does not occur in all cases [39]. Whereas, there are unfortunately many exceptions. The rate of spontaneous HBeAg seroconversion is low in untreated patients and occurs in 8-15% of patients in Central Europe and USA and <2% of pediatric patients in Asia [40]. This is an extremely important point.

Figure 1. Chronic hepatitis, HBeAg negative. A-portal/peribiliary inflammation (Hematoxylin-Eosin, original magnification ×40 objective). B-Masson trichrome stain, dense staining of collagen fibrous septa (advanced fibrosis, original magnification ×40 objective). C-Reticulin stain, reticulin network and nodular architecture (original magnification ×200 objective). Patient: 45-year-old-female, HBeAg positive, HBV DNA, 8.2 × 10⁷ IU/mL, ALT, 100 IU/L, SGOT, 84 IU/L.
4. Conclusion

In conclusion, Chronic hepatitis B has specific clinical and histological behavior and increases the risk of end-stage liver disease/cirrhosis and HCC. The course of HBeAg-negative chronic hepatitis is not available to markers only cutoff HBV DNA. Treatment decisions, including whether to perform a liver biopsy, are important. HBV DNA levels were correlated with the liver necroinflammatory activity index, fibrosis stage and serum ALT, AST levels in this study. However the HBV DNA level was not correlated with the HBeAg serostatus. A significant relationship was found between the HBeAg status and liver histological inflammatory scores. The HBeAg status of CHB patients should not affect the treatment response or need for long-term follow-up visits with repeat ALT and HBV DNA levels. However, chronic hepatitis patients who are negative for HBeAg may need different short-term follow-up. In order to more effectively manage the disease and decide on treatment, HBeAg negative chronic hepatitis B patients should be referred for a liver biopsy.

List of Abbreviations Used

HBV, Hepatitis B Virus; HCC, Hepatocellular carcinoma; ALT, Alanine aminotransferase, AST, Aspartate aminotransferase; Alb, Albumin; AFP, Alpha-fetoprotein; CHB, Chronic hepatitis B; HBsAg, Hepatitis B surface antigen; anti-HBs, hepatitis B surface antigen; HBeAg, Hepatitis B e antigen; anti-HBe, hepatitis B e antibody

Acknowledgements

Written informed consent was obtained from the patient for the publication of this report and any accompanying images.

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