Original Article

Chronic hepatitis B infection in a hepatology clinic at a university hospital in Jeddah

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Abstract

Introduction: The outcome of chronic hepatitis B (CHB) infection in a cohort of CHB patients at the hepatology clinics of King Abdul Aziz University Hospital in Jeddah was studied.

Methodology: The results of a complete blood count, prothrombin time, liver function test and hepatitis B virus polymerase chain reaction (HBV-PCR) performed over the previous two to five years of follow-up were reviewed. Results of abdominal ultrasound performed within the last year and the data on the treatment type, duration and resistance were also obtained.

Results: The majority of the 109 patients studied were Hepatitis B e antigen (HBeAg-negative; 87.2%). Male patients had higher serum ALT values compared to females at follow-up. HBeAg-positive patients had higher HBV-PCR levels at diagnosis compared to HBeAg-negative patients. Patients below 40 years of age had higher HBV-PCR compared to those above 40 years. Ultrasound showed liver cirrhosis in 11% of patients. Cirrhotic patients had higher GGT levels compared to non cirrhotic patients. Drug resistance developed in 25% of the 20 Lamivudine-treated patients. The mean duration of treatment was 2.5 ± .47 years.

Conclusion: CHB was predominantly HBeAg negative, with a benign long-term outcome in most patients. Therapy may need to be individualized for patients with high risk of progression.

Key words: Serum ALT; natural history; vaccination; HBV-DNA; PCR; ultrasound; liver cirrhosis

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Introduction

Chronic hepatitis B (CHB) is a worldwide problem, with an estimated 400 million persons infected with the hepatitis B virus (HBV) [1]. CHB is a leading cause of end-stage liver disease and hepatocellular carcinoma [2,3]. Saudi Arabia is similar to other CHB-endemic countries, where CHB is usually an outcome of vertical transmission or transmission early in life from infected family members [2-7].

The natural history of hepatitis B e antigen (HbeAg) positive CHB usually passes through the following phases: the immune-tolerant phase, that can last more than 30 years, in which the hepatitis B virus double stranded Nucleic acid (HBV-DNA) is greater than 20,000 IU/mL and serum ALT is normal; the immune active or immune clearance phase, in which HBV-DNA is greater than 2,000 IU/ML and ALT is elevated; and the inactive or chronic carrier phase, in which HBV-DNA is low or undetectable, ALT is normal, and seroconversion of HBeAg to hepatitis Be antibody (HBeAb) occurs [2,3,8]. HBeAg-negative CHB, which represents the majority of cases in Saudi Arabia and Asia, may have a different natural history, in which elevated serum HBV-DNA greater than 2,000 IU/ml and high serum ALT are recognized as the immune active phase [2].

Previously reported data on CHB from Saudi Arabia have focused on the prevalence of the disease [5-7] and the effect of HBV vaccination on reducing the rate of hepatitis B surface antigen (HBsAg) seropositivity in the country [9,10], but there are no similar data available that focus on the long-term follow-up or treatment outcome. HBV-DNA was found to be the best predictor of the severity of fibrosis, long-term outcome, progression to end-stage liver disease, the risk of hepatocellular carcinoma (HCC) and the response to therapy [2,3,11-14].

Most of the current treatment guidelines for CHB suggest that treatment is indicated in patients with HBV-DNA greater than 20,000 IU/MI for HBeAg-positive CHB and when HBV-DNA is greater than 2,000 IU/ML for HBeAg-negative CHB, together with evidence of active inflammation as reflected by

elevated serum ALT or liver histology in the absence of other causes for liver disease [2,15-18]. Treatment responders among CHB patients will have a reduction in the risk of progression to cirrhosis and HCC [15,16,19,20]. One of the yet unresolved CHB treatment issues is that the current recommendations and guidelines for CHB treatment exclude the groups of patients with lower HBV-DNA levels and patients with high HBV-DNA but normal ALT, who both still have a risk of progression to cirrhosis and HCC [18,21-23]. In addition, most of the orally used nucleotide and nucleoside analogs for treatment of CHB carry variable risks of drug resistance [15,16,24]. Another issue of concern is the implementation of mass vaccination programs for HBV worldwide in children, which has resulted in the emergence of new HBsAg vaccine-escaped mutants of HBV [25]. In this retrospective analysis, we studied the clinical, laboratory, and treatment outcomes of CHB patients in the main university hospital in Jeddah, Saudi Arabia.

Methodology

Study Design

This retrospective, descriptive cohort study followed chronic hepatitis B patients at the hepatology clinic at King Abdul Aziz University Hospital in Jeddah for at least two to three years.

Inclusion criteria

Patients were included if they had regular follow-up at the hepatology clinic at least three times per year. The patients' test results (hepatitis B virus serology, serial LFT, and hepatitis HBV polymerase chain reaction HBV-PCR done at three to six month intervals, as well as the results of abdominal ultrasound performed at six-month intervals and the results of the fasting serum lipids done within the last year) also had to be available in the patients' records or in the hospital information system.

The patients were excluded if they had another chronic liver disease such as hepatitis C or HIV, or if the patient's data were incomplete.

Data collection

Age, sex, duration of infection, and nationality were noted for each patient.

The data on the clinical evidence of progression to de-compensated cirrhosis (development of ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and variceal bleeding) were obtained from the patients' records.

We also reviewed the lab results, including the liver function test at diagnosis and then at six to twleve months, at two years, and at three to four years (if available). These results were assessed by the Dimension Clinical Chemistry System (Dade Behring Inc, Newark, USA) Using the Siemens Health Care Diagnostic, which is one of the main diagnostic chemistry methods approved by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). This method uses the Flex reagent cartridge (Dade Behring Inc, Newark, USA); after calibration, the plasma sample and the reagent are delivered to the system that automatically processes the sample and delivers the results. The laboratory reference normal values at our center are as follows: serum alanine aminotransferase (ALT) normal 30-65 U/L; aspartate amino transferase (AST) normal 15-37 U/L; alkaline phosphatase (ALP) normal 50-136 U/L; gamma-glutamyl transferase (GGT) normal 5-85 U/L; total protein (TP) normal 64-82 g/L; albumin (Alb) normal 35-50 g/L; total and direct bilirubin normal 0-17 and 0-5 umol/L, respectively.

The results of a complete blood count (CBC) at the time of diagnosis and at the follow-up were also obtained: white blood cells (WBC) normal 3-11 K/ul; hemoglobin (Hg) normal 12-17 g/dl; platelets count (plate) normal 100-400 K/u l.

The results of serial prothrombin time (PT; normal 10-14 seconds) were reviewed to identify patients who had prolonged PT at baseline or at follow-up.

Hepatitis serology was performed using ELISA (enzyme linked immunosorbent assay) for hepatitis B virus (hepatitis B surface antigen HBSAg, hepatitis B e antigen HBeAg, hepatitis B e antibodies HBeAb, hepatitis B core antibodies HBcAb) and hepatitis C virus (HCVAb) in all patients. In two patients who developed exacerbation, the results of antibody to hepatitis A virus (IgM) – anti-HAV IgM were obtained at the time of the acute exacerbation.

The results of polymerase chain reaction (PCR) testing with Roche diagnostic were reviewed. The Cobas AmpliPrep/Cobas TaqMan HBV Test, v2.0 (Roche Diagnostic, Basel, Switzerland) which is an *in vitro* nucleic acid amplification test for the quantitation of HBV-DNA in human plasma and serum, using the Cobas AmpliPrep Instrument for automated specimen processing and the Cobas TaqMan analyzer, (both from Roche Diagnostic, Basel, Switzerland) was used to measure HBV DNA levels at baseline and during the follow-up. It is a

fully automated real-time PCR technology using the total nucleic acid sequence that detects all HBV genotypes A-H including pre-core mutants. The results for HBV-DNA were obtained at diagnosis, six months and at one, two and three to five years.

The results of abdominal ultrasound and/or computed tomography done within the last year before inclusion were reviewed for the presence of fatty liver changes, ascites, radiological features of portal hypertension, and hepatocellular carcinoma.

We collected the data of patients who received treatment, including the medication name, dose, duration of treatment, treatment response, and development of resistance.

Using the statistical package for social science (SPSS15) system (SPSS Int, Chicago, USA), we obtained means, standard deviations and the frequencies. The independent T test was used to compare means between serum ALT and HBV-PCR levels; between HBeAg-positive and HBeAg-negative patients; between males and females; and between ages either above or below 40 years. The independent T test was also used to compare the serum ALT and GGT between cirrhotic and non cirrhotic patients. Correlation analysis was used to compare the ALT levels at different intervals.

Results

A total of 124 patients with more than two years of follow-up were tested for HBV-PCR within the study period, but only 109 patients who met the inclusion criteria, *i.e.,* who were both HBeAg-positive and HBeAg-negative, were studied. In the end, 72 males and 37 females were included. The majority (79 patients) were Saudi (72.5%), and 30 (27.5%) were non-Saudi. HBeAg-negative CHB was more common, with 95 such patients (87.2%). Fourteen (12.8%) patients were HBeAg-positive (Table 1). The mean age was 39.06 years (SD14.824). Patients with HBeAg-positive CHB were more likely to be younger, with a mean age of 27.6 years, compared to 40.99 years in HBeAg-negative CHB patients; although this difference did not reach statistical significance (P value = 0.093; Table 1). The mean duration of follow-up was 3.96 SD2.818. The shortest follow-up period was two years and the longest was 15 years. The mean serum ALT at diagnosis was 70.741 U/L (95% CI 38.985-102.49). The mea serum ALT level was not different from base line at one year and at three years of follow-up for both HBeAg-positive and HBeAg-negative patients. There was no difference in the mean serum ALT between the HBeAg-positive and negative patients at diagnosis and after three to four years of follow-up.

Male patients with both HBeAg-positive and HBeAg-negative CHB were more likely to have higher serum ALT compared to female patients; this was more evident at two to three years of follow-up and thereafter (Table2).

Only 19.3% and 18.85% of the 109 patients had serum ALT levels less than 30 U/L at baseline and at three to four years of follow-up, respectively. The majority of the patients with serum ALT more than 30 U/L were male (72% and 70% at baseline and at three to four years of follow-up respectively). All female patients who had serum ALT higher than 30U/L, had changes of fatty liver on abdominal ultrasound or hyperlipidemia or both, but only one had normal abdominal ultrasound.

Patients with HBeAg-positive CHB had higher HBV-PCR at diagnosis with a mean of 7.1513x107IU/ml SD1.7549x108 compared to HBeAg-negative patients with a mean of 2.5566x106IU/ml SD1.8102x107 ($P < 0.001$). This difference was maintained throughout the follow-up. Patients younger than 40 years had higher HBV-PCR compared to patients older than 40 years (2.1804x107IU/ml SD 9.54503x107 compared to.5437x106IU/ml SD 8.30634x106IU/ml respectively; $P = 0.003$).

Female patients were more likely to have higher HBV-DNA levels at diagnosis with a mean of 2.1173x107IU/ml SD 1.09443x108 compared to males with a mean of 6.50578x106IU/ml SD1.2.74484x107 ($P = 0.035$).

The highest HBV–PCR level was above the detectable range of 1.1x108 IU/ml after six dilutions and it was observed in two HBeAg-positive patients. The lowest level of PCR was undetectable. Lower HBV- DNA was associated with lower serum ALT on the linear regression analysis (Standardized coefficient Beta -.013; $P = < 0.001$).

Table 1. Demographic parameters in chronic hepatitis B patients at King Abdul Aziz University Hospital, Jeddah.

HBe antigen status	Mean Age years	Duration since Diagnosis Years	Sex		Nationality		Total
			Male	Female	Saudi	Non Saudi	
Positive	40.74 ±2,98	3.97 ±.59	8	6	9	5	14 12.8%
Negative	27.74 ±6.26	3.93 ±1.37	64	31	70	25	95 87.2%
Total	39 ±2.82	3.96 ±.53	72 66.1%	37 33.9%	79 72.5%	30 27.5%	109 100%

Table 2. Serum Alt levels for male and females at 2-3 years of follow-up

e Ag status	Mean and SDS serum ALT for males	Mean And SD serum ALT for females	P value
All patients	56.534 U/L SD 32.935	35.96U/L SD 8.599	<0.001
Negative	54.87U/L SD 32.163	34.143U/L SD 8.5	0.005
Positive	66.833U/L SD 38.933	43.6U/L SD 3.286	0.002

For both HBeAg-negative and HBeAg-positive CHB patients, HBV-DNA levels at diagnosis, one year, and two to four years were not different; however, twenty-six (27.37%) HBeAg-negative patients had HBV-PCR above 2000 IU/mL at base line and all through the follow-up. They also had higher mean serum ALT levels at diagnosis (109.824 IU/L SD285) compared to patients with HBV-PCR lower than 2,000 (46.52 IU/L SD25.256 P =.005). Ten patients with HBeAg-negative CHB had undetectable HBV-PCR levels throughout the follow-up and had elevated serum ALT levels above the upper limit of normal.

The mean platelet count for all the patients was 247 k/ul (range 64-521). The mean WBC count for 108 patients was 6.69 k/ul (range 3.1-18). One patient who developed chronic myeloid leukemia with a WBC of 59.9 k/ul during the follow–up was excluded from the WBC analysis.

Abdominal ultrasound showed evidence of liver cirrhosis in ten males and two females (11% of all patients), 11 of whom were HBeAg-negative and one who had HBeAg-positive CHB. Three of the cirrhotic patients were below the age of 40 years: a 12-year-old and a 32-year-old both had quantitative HBV-PCR < 400 at every measurement), and a 25-year-old was HBeAg-negative with HBV-PCR > 20000 u/l on all occasions. Of 27 patients (24.8%) who had fatty liver on abdominal ultrasound examination, 42.8% had hyperlipidemia. The remaining 70 patients (64.2%) had normal abdominal ultrasounds. Nine (18.7%) of 48 patients above 40 years of age had liver cirrhosis on abdominal ultrasound examination. The serum ALT levels were higher in patients with ultrasound-diagnosed cirrhosis and fatty liver compared to those with normal ultrasounds (Table 3. Similarly, the serum GGT level was higher in patients with fatty liver and cirrhosis compared to patients with normal abdominal ultrasounds (Table 3).

Table 3. Serum ALT and GGT for Patients with normal abdominal ultrasound compared to the patients who had cirrhosis or fatty liver on abdominal ultrasound examination

	Mean and SD for patients with normal US	Mean And SD for cirrhotic patients P Value		Mean And SD for patients with fatty liver P value	
ALT U/L	42.04 SD17.313	250.58 SD 474.66	< .001	59.814 SD 28.44	.001
GGTU/L	23.550 SD20.74	115.8 SD186.42	< .001	38.52 SD 42.3	.002

Two HBeAg-negative CHB patients had reactivation and acute hepatitis with the reappearance of e antigen and HBeAg. The first one cleared the HBV-DNA after the acute exacerbation and

developed a high level of HBsAb; the other progressed to de-compensated cirrhosis. One 69-year-old patient progressed to clinically evident cirrhosis at the last year of follow-up, and he developed ascites.

Four patients had liver biopsy, two of whom were HBeAg-positive CHB with very high levels of HBV-DNA; one had elevated ALT and the other had normal ALT. In both, the liver biopsy showed active disease grade 3/stage 2-3. The other two had HBeAg-negative CHB; one 26-year-old male patient had HBV–DNA less than 2,000 IU/mL each time and normal ALT, while his liver biopsy showed grade 2 and stage 2 disease. The second patient was treated with Lamivudine for three years and his HBV-DNA was undetectable for more than two years before the biopsy; however, he had elevated serum ALT and the liver biopsy was consistent with non-alcoholic steatohepatitis (NASH).

Twenty-eight patients received treatment during the follow-up, six of whom were HBeAg-positive and 22 of whom had HBeAg-negative CHB. Twenty patients received Lamivudine; five of them developed Lamivudine resistance. Adefovir at 10 mg was added to Lamivudine in two patients, and the other three were shifted to Entecavir at 1 mg. Four of the Lamivudine-treated patients still maintained remission on Lamivudine after four to five years of follow-up. One patient was treated only with Adefovir 10mg daily. Seven patients received Entecavir alone at 0.5 mg. The mean duration of treatment was 2.5 ± 0.47 years. All the treated patients are still maintained on treatment with very low or undetectable HBV-DNA.

Discussion

Our data show that the majority of our studied patients were HBeAg-negative (87.15%) and they were mostly males; this may not be an outcome of selection bias because previous reports from CHB-high endemic areas showed that the majority of patients had HBeAg-negative CHB [4,26]. Baig found a significant gender difference in CHB infection, with male predominance occurring during all stages of CHB infection; this finding was attributed to the possible protective effect of estrogen on hepatocytes in females, preventing the development of chronic liver disease [27].

Most of our patients were Saudis, and this finding is consistent with the national data showing high prevalence of CHB among Saudis compared to non Saudis in the country [8,10]. Patients with HBeAg negative CHB were more likely to be older than HBeAg positive patients, which may be related to the natural history of vertically transmitted CHB and HBeAg sero-conversion over the years as the disease progresses from the immune–active phase to the active hepatitis B carrier stage [2-4,28]. In our cohort, the duration of follow-up is unlikely to represent the duration of the infection, as most if not all our patients had acquired the infection either through vertical transmission or early in life during childhood. This discrepancy can be explained by late diagnosis, as most of these patients were asymptomatic and were only discovered during screening or at the time of blood donation. The serum ALT was maintained within the normal reference range in 83.5% and 87% of patients at one year and three to four years of follow-up, respectively; previous data for CHB patients maintaining normal ALT during follow-up showed a benign course for the disease [2-4,29]. Ayed *et al.* showed that HBeAg-positive patients had higher serum ALT levels compared to HBeAg -negative patients [30]; however, in our cohort, the ALT levels were not different between the two groups. This difference in the results of the two studies may be explained by the fact that not all HBeAg-negative patients are chronic carriers; instead, some of them have active HBeAg-negative CHB [2,3] . We found that low levels of HBV-DNA were associated with lower serum ALT levels. Shanmugam *et al.* and Shao *et al.* demonstrated a positive correlation between HBV-DNA and serum ALT levels [31,32].

Higher serum ALT in the studied male patients compared to females on longer follow-up could be explained by the existence of a normal sex difference in serum ALT levels between males and females, with higher ALT in males. These differences have been demonstrated by several studies [33,34]. On the other hand, the failure to demonstrate this difference of serum ALT between both sexes at baseline or at shorter times of follow-up may be due to that some of the patients from both sexes had elevated serum ALT from acute hepatitis or were still in the immune-active stage before they progressed to the immune-tolerant stage, with normalization of serum ALT over time.

HBeAg positivity was associated with high HBV-DNA; similar findings have been reported by Feld *et al.* and Hussain *et al.* [35,36]. This relationship can be explained by the gradual reduction in serum HBV-DNA level as the disease progresses to the chronic carrier stage and HBeAg

seroconversion [2,3] . This may also explain why patients younger than 40 years had higher HBV-PCR compared to older patients.

In our cohort, female patients had HBV-DNA levels higher than those of the males; this observation differs from the findings of Baig *et al.*, who determined that male patients had higher HBV-PCR. In our cohort, 10 out of 12 cirrhotic patients were male, while Baig *et al.* and Pan *et al.* showed that the risk of developing complications including HCC was also higher in male patients than in females [27,28] . The elevated serum ALT in 10 patients with persistent low viral load may be due to NASH.

Higher HBV-PCR was associated with a higher rate of ultrasound-diagnosed cirrhosis, although the relationship did not reach statistical significance at P = 0.08; multiple previous studies on larger numbers of patients with longer follow-up showed that HBV-DNA is the primary independent predictive factor of the progression of fibrosis and the development of HCC [12,13,37-39].

Patients with ultrasound-diagnosed cirrhosis or NAFLD had higher serum ALT and GGT values as compared to those with normal abdominal ultrasounds, which supports the previous data on the value of the use of ALT and GGT as non-invasive biochemical markers in the assessment of the progression of liver disease [40-43].

Reactivation of CHB can result in either the clearance of the HBV or the progression of liver disease [3,44]; in our two patients with reactivation, the first cleared the HBsAg and then developed HBsAb, and the second progressed to de-compensated cirrhosis.

Two patients with persistently normal ALT had histologically active disease on liver biopsy, one of whom showed low HBV-DNA levels at every time point. Similar findings of histological progression in the presence of normal serum ALT levels and low levels of viremia have been previously reported [38,45].

Only 25.69% of our cohort received treatment because most of the internationally accepted treatment guidelines require both elevated serum ALT and high HBV-DNA greater than 20,000 IU/mL in HBeAg-positive and greater than 2,000 IU/mL in HBeAg-negative patients [15,16,17]. However, the most recent EASL guidelines recommend treatment for both HBeAg-positive and HBeAg-negative patients when HBV-DNA is greater than 2,000 IU/ml, and also advise that all patients with any elevation in serum ALT above the upper normal range should be considered for treatment [18]. One quarter of our Lamivudine treated patients developed drug resistance, while previous reports on the long-term treatment with Lamivudine had shown higher chances of resistance with prolonged Lamivudine treatment [15-17,46]. In view of the current treatment guidelines that are mostly applicable to HBeAg positive CHB, a considerable number of HBeAg negative CHB patients are not treated and are still at risk of progression to liver cirrhosis [47]. The modification of these guidelines in the future may widen the criteria of treatment so that a larger number of patients will be offered therapy. On the other hand, prolonged treatment with oral antiviral agents is expected to result in drug resistance and more viral escape.

Conclusions

We have demonstrated that the outcome of the follow-up of CHB in our cohort from Saudi Arabia is similar to that in other countries endemic for CHB infection. High HBV-DNA level is a positive indicator of disease progression. The absence of symptoms and presence of normal serum ALT at the current reference levels does not rule out the progression of liver disease; furthermore, the utilization of non-invasive diagnostic markers may help to identify disease progression in those patients. In view of the recent definitions of normal serum ALT levels, treatment may need individualization in high-risk patients. More local data about the disease and the treatment will provide more accurate figure about CHB in Saudi Arabia.

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