

Clinical, laboratory, and virological characteristics of patients with positive hepatitis B surface antigen in Upper Egypt

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Background

Hepatitis B virus (HBV) infection is a major cause of morbidity and mortality, particularly in developing countries.

Aim

The aim of the study was to determine the clinical, laboratory, and virological characteristics of patients with chronic HBV infection in Upper Egypt.

Patients and methods

This descriptive, cross-sectional study included 252 patients with positive hepatitis B surface antigen (HBsAg). It was conducted in the Tropical Medicine and Gastroenterology Department and Outpatient Clinic, Assiut University Hospital (Egypt), from May 2012 to May 2014. All patients underwent clinical evaluation, were administered a questionnaire about risk factors for transmission of HBV, underwent liver function tests, abdominal ultrasonographic examination, and complete blood count, evaluation of serological markers of HBV, and quantitative detection of HBV-DNA.

Results

Of the 252 patients included, 88.5% were male with a mean age of 35.4 years. Arthralgia was the most common complaint (15.5%) and hepatomegaly was the most common finding (8.3%). As regards imaging results (ultrasonographic) the following were found: normal liver in 83.3%, coarse liver in 11.9%, hepatomegaly in 7.5%, splenomegaly in 6.3%, and cirrhosis in 5.9%. As regards laboratory results normal alanine aminotransferase was found in 79.8%, normal aspartate aminotransferase in 85.7%, reduced serum albumin in 4.4%, and low platelet count in 9.9%. The majority of patients (91.7%) were hepatitis B envelope antigen negative; 65.9% of patients were positive for HBV on PCR. No significant differences were found between positive HBV-DNA status (by PCR) and negative HBV-DNA status as regards clinical, imaging, and laboratory characteristics of patients.

Conclusion

Most of the patients had normal liver on ultrasonographic examination and normal liver function tests. No significant difference was found between positive HBV-DNA status (by PCR) and negative HBV-DNA status as regards clinical, imaging, and laboratory characteristics of patients.

Keywords:

egypt, hepatitis B surface antigen, hepatitis B virus infection, PCR, risk factors

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Introduction

Hepatitis B virus (HBV) belongs to Hepadnaviridae, a family of enveloped viruses with an incomplete double-stranded DNA genome of 3.2 kb. HBV can be classified into at least eight genotypes (A–H) based on a divergence in the entire nucleotide sequence greater than 8% [1,2]. Infection with HBV is a major public health problem [3]. Chronic HBV infection is a major underlying etiology in liver cirrhosis and hepatocellular carcinoma (HCC) [4].

In countries with high HBV endemicity (prevalence >8%), the main source of infection is through perinatal transmission from the chronically infected mother or through infection during early childhood [5,6]. Vertical (mother to infant) transmission occurs worldwide and is mainly related to

transplacental transmission of HBV *in utero*, perinatal infection through contact with infectious blood or body fluids from the infected mother at the time of delivery, or postnatal transmission during care or through breast milk [6].

In areas of low HBV endemicity, most infections are acquired by horizontal transmission in early adult life, through intravenous drug use, or through unprotected sexual activities [7]. Other risk factors include working in a healthcare setting, blood transfusions, dialysis, sharing razors or toothbrushes with an infected person, travelling in countries where the infection rate is high, and living in an institution [8,9]. Tattooing and acupuncture led to a significant number of cases in the 1980s; however, this has become less common with improved sterility [9].

Chronic HBV infection in Egypt is an important medical problem. The prevalence of hepatitis B surface antigen (HBsAg) in Egypt is intermediate (2–7%) [10,11].

The infection may be entirely asymptomatic and may go unrecognized [12]. Chronic infection with HBV may be asymptomatic and may progress to liver cirrhosis and HCC [13].

HBsAg is most frequently used to screen for the presence of HBV infection. Most hepatitis B diagnostic panels contain HBsAg and total anti-hepatitis B core (anti-HBc) (both IgM and IgG). Shortly after the appearance of HBsAg, hepatitis B envelope antigen (HBeAg) will appear [14]. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (anti-HBe) will arise immediately after. This conversion is usually associated with a marked decline in viral replication [14].

The aim of the current study was to determine the clinical, laboratory, and virological characteristics of patients with chronic HBV infection in Upper Egypt.

Patients and methods

Type of the study

- (1) Study design: cross-sectional analytic study.
- (2) Study frame: the study was performed in the Tropical Medicine and Gastroenterology Department and Outpatient Clinic, Assiut University Hospital (Egypt), from May 2012 to May 2014.

Inclusion criteria included being positive for HBsAg and negative for hepatitis C virus antibodies.

Exclusion criteria included other identifiable causes of chronic liver diseases, a history of antiviral therapy, and acute hepatitis B infection.

Study population

The following were performed:

- (1) Clinical evaluation (history taking and clinical examination).
- (2) Administration of a questionnaire about risk factors for transmission of viral hepatitis, such as previous blood transfusion, past history of operations, dental manipulation, receiving drugs by intravenous injection, tattooing, family history of HBV, and family history of liver diseases.
- (3) Abdominal ultrasonographic examination.

- (4) Liver function tests serum levels of total and direct bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase].
- (5) Prothrombin time estimation.
- (6) Complete blood count.
- (7) Serological markers of HBV: 5 ml of venous blood was aseptically drawn into plain tubes. The samples were centrifuged within 30 min at 3000 rpm for 10 min; the collected serum samples were divided into aliquots and stored at -70°C . All blood specimens were tested for HBsAg [using Monalisa HBsAg Ultra (Bio-Rad, Paris, France) detection kit], antibody against hepatitis B surface (anti-HBs Ab) [using CTK Biotech Inc. (San Diego, California, USA) detection kit], and total antibody against HBc (anti-HBc Ab) [using Wkea Med Supplies Corp. (New York, USA) detection kit] by means of the enzyme-linked immunosorbent assay technique. Reactive results were confirmed by repeating the test in duplicate. All samples that were confirmed to be HBsAg positive were further tested for HBeAg using microparticles enzyme immunoassay kits (AxSYM; Abbott Laboratories, Wiesbaden, Germany) and HBV-DNA assays.
- (8) Quantitative detection of HBV-DNA: DNA was extracted from 200 ml of serum with a DNA extraction kit (Catalog #57704; Qiagen GmbH, Stockach, Germany), according to the manufacturer's procedure.

HBV-DNA was quantified by real-time PCR using a 7500 fast real-time PCR system (Applied PCR System; Biosystems, Life Technologies, Grand Island, New York, USA), using ready-to-use PCR kits supplied by Artus HBV PCR kit (version 1, Catalog #4506163; Qiagen GmbH) [15].

Ethical considerations

Before enrollment all participants signed a consent certificate after a detailed discussion about the study subject and study aim. Participants were clearly informed that refusal to participate would not prevent them from getting full benefit of the available medical service and treatment. Data were collected by means of personal interviews with participants after ensuring data confidentiality. The study was approved by the Faculty of Medicine Ethical Committee, Assiut University. The procedures followed were in accordance with the ethical standards (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

Statistic analysis

Statistical analysis was performed using statistical package for the social sciences (SPSS, version 17; SPSS Inc., Chicago, Illinois, USA). All data were expressed as mean \pm SD or as frequencies. Continuous variables were compared through Student's *t*-test and proportions were compared with χ^2 -test. Results were presented as mean \pm SD. *P* values less than 0.05 were considered significant.

Results

The study included 252 patients, of whom 88.5% were male. Their median age was 35 years (mean 35.4 ± 10.2 years). All patients were native residents of Egypt. The possible risk factors for HBV infection are summarized in Table 1.

Arthralgia was the most common complaint (15.5%) and hepatomegaly was the most common finding (8.3%), as shown in Table 2.

As regards imaging results (ultrasonographic), normal liver was found in 83.3% of patients, coarse liver in 11.9%, hepatomegaly in 7.5%, splenomegaly in 6.3%, and cirrhosis in 5.9%.

As regards laboratory data elevated ALT more than two-fold was found in 4.8% of patients, elevated AST more than two-fold in 1.2%, reduced serum albumin in 4.4%, and low platelet count in 9.9%, as shown in Table 3. The majority of patients (91.7%) were negative for HBeAg, and 65.9% of patients were positive for HBV on PCR, as shown in Table 4.

No significant difference was seen between positive HBV-DNA status (by PCR) and negative HBV-DNA status in terms of clinical, imaging, and laboratory characteristics of patients, as shown in Table 5.

Discussion

Our study disclosed some important observations. First, dental manipulations, surgical operations, previous blood transfusion, HBV infection in the family, and tattooing were the possible risk factors for HBV infection in our patients. Second, arthralgias, fatigue, and abdominal pain were the main clinical manifestations of chronic HBV infection. Third, most of the patients had normal liver on ultrasonographic examination and normal liver function tests. Fourth, the majority of patients with chronic HBV infection were negative HBeAg and about two-thirds had positive PCR. Fifth, no significant difference was found in clinical, imaging, and laboratory characteristics

between patients with positive HBV-DNA status (by PCR) and negative HBV-DNA status.

The results of this study are similar to a previous study by O'Connell *et al.* [16] and to a study by Allwright *et al.* [17]. They reported that HBV was found in all body secretions and excretions, but only blood, body fluids containing visible blood, semen, and vaginal secretions represented a risk of transmission. HBV is transmitted by percutaneous and mucosal exposure to infective blood or body fluids. The main modes of HBV transmission include sexual or close household contact with an infected person, perinatal mother to infant transmission, injecting drug use, and nosocomial exposure. Grogan *et al.* [18] reported that HBV can be transmitted by transfusion of infected blood or blood products, sharing unsterilized injection needles for intravenous drug use, hemodialysis, acupuncture, tattooing,

Table 1 Demographic characteristics and history of patients with chronic hepatitis B virus infection (n = 252)

Characteristics	N (%) (n = 252)
Male sex	223 (88.5)
Age (mean \pm SD) (years)	35.4 ± 10.2
Residence	
Urban	69 (27.4)
Rural	183 (72.6)
Smoking	157 (62.3)
Past history	
Hepatitis	27 (10.7)
Blood transfusion in the past	40 (15.9)
Surgical operations (major or minor)	45 (17.9)
Dental manipulations	51 (20.2)
Parenteral drug abuse	5 (2)
Tattooing	36 (14.3)
Schistosomiasis	10 (4)
Family history	
HBV infection	33 (13.1)
Liver disease	13 (5.2)

HBV, hepatitis B virus.

Table 2 Clinical characteristics of patients with chronic hepatitis B virus infection

Characteristics	N (%) (n = 252)
Symptoms	
Fatigue	33 (13.1)
Abdominal pain	27 (10.7)
Arthralgia	39 (15.5)
Hemorrhage	
Gums	9 (3.6)
Nose	3 (1.2)
Abdominal distension	10 (4)
Signs	
Jaundice	10 (4)
Hepatomegaly	21 (8.3)
Splenomegaly	12 (4.8)
Ascites	10 (4)

Table 3 Imaging and laboratory characteristics of patients with chronic hepatitis B virus infection

Characteristics	N (%) (n = 252)
Imaging data (US)	
Normal liver	210 (83.3)
Coarse liver	30 (11.9)
Hepatomegaly	19 (7.5)
Splenomegaly	16 (6.3)
Cirrhosis	15 (5.9)
Ascites	12 (4.8)
Laboratory data	
ALT	
Normal	201 (79.8)
Raised ≤ 2 folds	39 (15.5)
Raised >2 folds	12 (4.8)
AST	
Normal	216 (85.7)
Raised ≤ 2 folds	33 (13.1)
Raised >2 folds	3 (1.2)
ALP	
Normal	240 (95.2)
Raised	12 (4.8)
Serum total bilirubin	
Normal	235 (93.3)
Raised	17 (6.7)
Serum albumin	
Normal	241 (95.6)
Reduced	11 (4.4)
Prothrombin time	
Normal	232 (92)
Prolonged >3 s than control	20 (8)
Low platelet count	
	25 (9.9)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; US, ultrasonography.

Table 4 Serological markers and virological characteristics of patients with chronic hepatitis B virus infection

Characteristics	N (%) (n = 252)
HBeAg	
Positive	21 (8.3)
Negative	231 (91.7)
Anti-HBc	
Positive	252 (100)
Negative	0 (0)
HBV-DNA PCR	
Positive	166 (65.9)
Negative	86 (34.1)
HBV-DNA value by real-time PCR (IU/ml)	
Mean \pm SD	1536923.26 \pm 2639960.34
Range	50–10342671

anti-HBc, anti-hepatitis B core; HBeAg, hepatitis B envelop antigen; HBV, hepatitis B virus.

and injuries from contaminated sharp instruments sustained by hospital personnel.

Our present study bears some similarities with previous reports by Liaw *et al.* [14], who found that the main clinical manifestations of chronic HBV infection were fatigue, vague abdominal discomfort, nausea and

Table 5 Relationship between hepatitis B virus DNA status (by polymerase chain reaction) and the clinical, imaging and laboratory characteristics of patients with chronic hepatitis B virus infection

Characteristics	PCR+ (n = 166)	PCR- (n = 86)	P-value
Clinical			
Fatigue	21 (12.6)	12 (13.9)	0.146
Abdominal pain	17 (12.2)	10 (11.6)	0.251
Arthralgia	27 (16.3)	12 (13.9)	0.276
Hemorrhage			
Gums	6 (3.6)	3 (3.5)	0.193
Nose	2 (1.2)	1 (1.2)	0.552
Abdominal distension	7 (4.2)	3 (3.5)	0.386
Jaundice	7 (4.2)	3 (3.5)	0.187
Hepatomegaly	15 (9)	6 (7)	0.210
Splenomegaly	8 (4.8)	4 (4.6)	0.136
Ascites	7 (4.2)	3 (3.5)	0.298
Imaging data (US)			
Normal liver	145 (87.3)	65 (75.6)	0.195
Coarse liver	19 (11.4)	11 (12.8)	0.322
Hepatomegaly	13 (7.8)	6 (7)	0.187
Splenomegaly	10 (6)	6 (7)	0.297
Cirrhosis	10 (6)	5 (5.8)	0.484
Ascites	8 (4.8)	4 (4.6)	0.463
Laboratory data			
ALT			
Normal	131 (81.3)	70 (81.4)	0.537
Raised ≤ 2 folds	27 (16.3)	12 (13.9)	0.310
Raised >2 folds	8 (4.8)	4 (4.6)	0.463
AST			
Normal	141 (84.9)	75 (87.2)	0.295
Raised ≤ 2 folds	23 (13.8)	10 (11.6)	0.189
Raised >2 folds	2 (1.2)	1 (1.2)	0.549
ALP			
Normal	158 (95.2)	82 (95.3)	0.589
Raised	8 (4.8)	4 (4.6)	0.403
Serum total bilirubin			
Normal	155 (93.4)	80 (93)	0.429
Raised	11 (6.6)	6 (7)	0.316
Serum albumin			
Normal	159 (95.8)	82 (95.3)	0.496
Reduced	7 (4.2)	4 (4.6)	0.230
Prothrombin time			
Normal	152 (91.6)	80 (93)	0.309
Prolonged >3 s than control	14 (8.4)	6 (7)	0.353
Low platelet count			
	17 (10.2)	8 (9.3)	0.257

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; US, ultrasonography.

vomiting, anorexia, sometimes arthralgia and rash, followed by bleeding gums. Studies by Tosti *et al.* [19] also reported the same findings.

In the present study, our results also showed that the majority of patients had normal liver on ultrasonographic examination (83.3%) and normal liver function tests: normal ALT levels (79.8%), normal AST levels (85.7%), normal total bilirubin (93.3%), normal

serum albumin (95.6%), and normal Prothrombin time (92%). Our study has some similarities with previous reports by Lok *et al.* [20], who reported that most of the patients with chronic HBV infection had normal liver and normal liver function tests.

The replicative phase of chronic HBV infection is characterized by the presence of HBeAg and HBV-DNA. The nonreplicative phase is characterized by the absence of HBeAg and HBV-DNA. Several HBeAg-negative patients have persistent high HBV replication associated with liver inflammation and ongoing fibrosis [3]. HBeAg-negative chronic HBV infection is mostly associated with mutations in the precore and basal core promoter regions that result in prevention or reduction of HBeAg synthesis without affecting the replicative ability of the virus [3,21]. Several studies have shown a strong relationship between HBV genotypes and mutations in the precore and core promoter regions that abolish or diminish the production of HBeAg [21–23].

In the present study, we demonstrated that most of the patients (91.7%) were HBeAg negative (presumably indicating precore or core promoter mutation). Studies by Sitnik *et al.* [23] reported similar findings. Zaky *et al.* [24] mentioned that in Upper Egypt 94% of hepatitis B-infected persons were HBeAg negative and 41% had positive HBV-DNA on PCR. However, in the study by Zahran *et al.* [25], only 72% of hepatitis B-infected patients were HBeAg negative.

Most chronic HBV infections are characterized by high HBV-DNA levels (>20 000 IU/ml). Lower HBV-DNA values (2000–20 000 IU/ml) are common in HBeAg-negative chronic infection, which is associated with a low likelihood of spontaneous disease remission [26]. Detection of HBV-DNA level is a criterion for determining the state of infection, the risk of progression toward cirrhosis and HCC, identification of patients who need antiviral therapy, determining response to therapy, and identifying the emergence of drug resistance [27]. Chu *et al.* [27] and Ledesma *et al.* [28] confirmed the relationship between viral load level and liver damage in patients negative for HBeAg, whereas Alam *et al.* [29] reported that low-level viral load is not always an indication of improved conditions and in some patients it indicates advanced disease. In an Iranian study by Taghavi *et al.* [30], it was reported that there was no statistically significant difference between patients with positive and those with negative HBV-DNA in terms of age, sex, clinical manifestations, and liver enzyme levels.

Our results are in agreement with these studies as it showed that there was no significant difference

in clinical, imaging, and laboratory characteristics between patients with positive HBV-DNA status and those with negative HBV-DNA status.

Conclusion

The main clinical manifestations of chronic HBV infection were arthralgias, fatigue, and abdominal pain, followed by bleeding gums. Most of the patients had normal liver on ultrasonographic examination and normal liver function tests. The majority of patients with chronic HBV infection were HBeAg negative. There was no significant difference in clinical, imaging, and laboratory characteristics between patients with positive HBV-DNA status (by PCR) and those with negative HBV-DNA status.

Acknowledgements

Conflicts of interest

None declared.

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