



Natural History of Chronic Hepatitis B Virus Infection Based on Laboratory Testing

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Abstract

Background: Understanding of the natural history of chronic HBV infection is useful for presenting the optimal management of chronic HBV infection. The aim of this study was to evaluate the natural history of chronic hepatitis B infection.

Methods: In this cross sectional study, 219 untreated chronic hepatitis B patients from Jan. 2010 to Aug. 2012 were included. The subjects were classified in four groups based on serological, biochemical and molecular testing. Serological tests for HBeAg and HBeAb were performed by ELISA method. HBV DNA viral loads were detected by using Light Cycler instrument and ALT/AST levels were assessed by automatic analyzer.

Results: The majority of subjects (94.1%) were HBeAg negative. Of 13 HBeAg positive patients, 5 (2.3%) and 8(3.7%) were considered as immunetolerant and immune reactive, respectively. Of 206 HBeAg negative patients, there were 142 (64.8%) patients in inactive or low replicative phase and 64(29.2%) were in HBeAg negative chronic hepatitis B phase.

Discussion: The lowest rate of subjects were in immunetolerant phase and most of them had above 20 years old that confirmed successful neonatal vaccines in our country. The highest rate of chronic HBV infected patients were in low replicative phase of chronic hepatitis B infection. Although, it is not recommended to treat these patients, but liver function and also liver biopsy should be considered in patients above 40 years of age.

Keywords: Natural history, Chronic Hepatitis B, HBeAg, HBV viral load, Real time PCR

Introduction

In spite of introduction of vaccination programs, hepatitis B (HBV) remains as an important cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. Approximately one third of world's population has serological evidence of past or present HBV infection, and as a results, 400 million people are chronically infected with variety of clinical outcomes ranging from inactive or low replicative to progressive chronic hepatitis B (CHB) (1). It is estimated that 35% of Iranian people have been exposed to HBV and two million Iranian adults have chronic HBV infection (2).

The HBV prevalence and the main route of HBV transmission differ geographically. In developing regions with high prevalence of HBsAg such as south East Asia, China, Sub Saharan Africa and Amazon Basin most commonly infection acquired in infancy or in early childhood. In part of Eastern and Southern Europe, Middle East, Japan and part of South America, the prevalence of HBV infection is intermediate and infection is acquired in infants or childhood. So mixed pattern of transmission including infant, early childhood and adult transmission exist in these areas. In developed countries such as North America, Northern and

Western Europe and Australia, HBV prevalence is low and most infection is acquired in adulthood through sexual contact or injecting drug use (3). The natural history of chronic HBV infection is variable and affected by viral factors as well as host factors and classified in four phases. The initial immune response to chronic HBV infection depends on the age at which the patient acquired it (4).

In perinatal transmission of infection, the natural history of CHB initiated with immune tolerant. In this phase, virus is tolerated by host immune system, so liver histology and ALT levels are normal. HBeAg and high levels of HBV DNA are detectable. After 10 to 40 years, many of these patients experience the second phase that called immune active phase. This phase is characterized by variable elevation of ALT level, the presence of HBeAg and lower level of HBV DNA compared with the immune tolerant phase. The immune response is very active, resulting in liver damage. In most patients, seroconversion of HBeAg to HBeAb occurs and viral replication suppressed by immune response, resulting in low level of HBV DNA or undetectable levels. These patients have normal ALT level with very low risk of liver inflammation and referred to low replicative phase. On the other hand, some patients with negative HBeAg have higher HBV DNA level compared with low replicative patients and have more severe liver damages. This patients who are in HBeAg-negative CHB phase have active liver disease demonstrated by elevated ALT level. The virus is unable to produce HBeAg due to precore/core mutation in HBeAg-negative CHB phase (5, 6).

Understanding the natural history of chronic HBV infection is useful for optimal management of patients to predict the prognosis, to classify the risk of hepatocellular carcinoma and to choose appropriate candidates for antiviral therapy. The aim of this study was to investigate the natural history of chronic HBV infection in chronic HBV patients, based on laboratory examination including HBeAg, HBeAb, HBV viral load and ALT level testing.

Materials and Methods

In this cross sectional study, 219 CHB patients (based on positivity in HBsAg over 6 months) who referred to IBTO research center, Molecular Diagnostic Lab from Jan. 2010 to Aug. 2012 were considered. All patients gave their written consent to include in this study and the questionnaire forms were filled for all subjects. All plasma samples were collected and stored at -70 °C before performing the HBV DNA quantitative PCR assay.

HBsAg, HBeAg and HBeAb were tested by ELISA method (DIA.PRO, Milan, Italy) and ALT/AST levels assessed by automatic analyzer (Pars Azmoon Kit). HBV DNA was extracted from 0.2ml plasma by using the QIAamp DNA Blood Mini kit (Qiagen, Mainz, Germany) according to manufacturer's protocol. Extracted DNA was eluted from the QIAamp spin column with 200µl elution buffer, as the template for the real time PCR (q RT-PCR assay). HBV-DNA loads were measured by using Artus HBV LC PCR kit (Hilden, Germany) based on manufacturer's instruction on the Light Cycler instrument (Roche Diagnostics-version 2). Briefly: 5µl of purified DNA was added to 12 µl of master mix and 3 µl of Mg²⁺ in each capillary. A standard curve was drawn automatically with the Light Cycler software in each run using five quantification standards concentration of HBV DNA to analyze HBV DNA viral load. Quantitative results were determined in IU/ml unit on the basis of Artus HBV LC PCR kit handbook. In each run, we used plasma negative sample and internal control to prevent false positive results and false negative results, respectively.

According to European Association for the study of the liver disease (EASL 2012), subjects were classified in four phases based on serological, biochemical and molecular criteria (7).

Statistical analysis

Clinical and laboratory data were described as mean, median, standard deviation and range. To evaluate any correlation between qualitative variables and quantitative, X² and Mann-Whitney tests

were used. *P* value less than 0.05 was considered as significant. Statistical analysis was done using SPSS version 16.

Results

Of 219 CHB patients, there were 105(45.3%) male and 127(54.7%) female with mean age 40.08 ± 12.65 years, and age range 17-73 years. Twenty hundred and six out of 219 CHB patients (90.5%) were HBeAg negative. No significant differences were on sex and age between HBeAg negative patients and HBeAg positive patients ($P < 0.05$, data not shown).

There were 5(2.3%), 8(3.7%), 142(64.8%) and 64(29.2%) of patients in immunetolerant, immune reactive, inactive or low replicative and HBeAg negative chronic hepatitis B phases, respectively.

Discussion

Our knowledge about the natural history of chronic HBV infection is improving during last decades. According to European Association for the study of the liver disease (EASL 2012), the natural history of chronic hepatitis B was described to be in four phases based on serological, biochemical and molecular criteria, including: 1. Immune tolerant phase with HBeAg Positive, HBeAb negative, Viral DNA > 20000 IU/ml and normal ALT level. 2. HBeAg-positive CHB (Immune reactive) phase with HBeAg Positive, HBeAb negative, Viral DNA > 2000 IU/ml and elevated ALT level. 3. Low replicative phase with HBeAg negative, HBeAb positive, Viral DNA < 2000 IU/ml and normal ALT level. 4. HBeAg-negative CHB phase with HBeAg negative, HBeAb positive, viral DNA > 2000 IU/ml and elevated ALT level (7, 8).

The HBV prevalence and the main rout of HBV transmission are related each other. In the last decade, Iran was classified as an intermediate prevalence of HBV infection and perinatally acquired HBV infection was the main route of transmission of HBV. Recent studies have shown the changing epidemiology of hepatitis B virus infection in Iran (9, 10). In this study, the minority

(2.3%) of subjects was in immune tolerant phase and most of immune tolerant patients had over 20 years old that is the first phase in patient acquired perinatally. It is very likely that they acquired HBV infection before the implementation of vaccination program for neonates. It could be as a result of successful vaccination program for neonates.

These patients usually remain in this phase for years. This long course causes the increasing in risk of liver complication. Immune tolerant patients should be considered for monitoring of HBeAg and liver function every 3-6 months, to detect a rise in alanine aminotransferase (11-13).

In this reported study, the highest rate of the subjects was in low replicative phase (64.8%). This phase may last lifetime without reactivation of HBV infection or HBsAg seroconversion. Although on the basis of recent hepatitis B treatment guidelines, it is not recommended to treat these patients, but liver function and also liver biopsy should be considered in patients above 40 years of age (11-13).

In the present study, the rate of patients in HBeAg negative CHB was higher than those patients in HBeAg positive CHB phase (29.2% versus 3.7%) that reported in other studies. Recent studies in Europe, Asia, and the United States show an increasing in prevalence of HBeAg-negative CHB patients and a decreasing in prevalence of HBeAg-positive chronic hepatitis B. This shift can be due to decrease in new HBV infection cases and has strongly affecting treatment strategies (14, 15).

It is reported that 7-30% of CHB patients are infected with HBV variants that presented little or no HBeAg due to precore/core mutation (16). The prevalence of HBeAg negative CHB was reported to be 33% in Mediterranean, 15% in Asia Pacific and 14% in the USA and Northern Europe. In our study the prevalence of HBeAg negative CHB was 29.2%. Some reasons for the differences in prevalence of HBeAg negative CHB in different regions of the world are related to differences in study design or HBV genotypes variability (16).

Conclusion

According to our study on natural history of CHB in Iranian patients, we showed that the high rate of patients were in low replicative phase. Approximately half of the subjects in low replicative phase had above 40 years old, who should be followed for establishing cirrhosis and treatment is recommended for patients with confirmed cirrhosis and detectable viral DNA. It is possible that these patients would be missed within the public healthcare system in which HBV DNA testing is expensive or not available.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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