RESEARCH ARTICLE

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Natural History of Chronic Hepatitis B Virus Infection in Ahvaz City, Iran

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Abstract

Objective: A long persistent of Chronic Hepatitis B (CHB) infection may develop liver cirrhosis or hepatocellular carcinoma (HCC) and about one million people die due to HBV -related liver cancer and end-stage liver disease annually worldwide. The natural history of CHB phases comprises four phases: immune tolerant (HBeAg detectable and ALT (Alanine Transaminase) normal, HBeAg-positive immune active (HBeAg detectable, anti-HBe antibodies undetectable and ALT persistently elevated), HBeAg-negative immune active (HBeAg undetectable, anti-HBe antibodies present and ALT persistently elevated), inactive carrier (HBeAg undetectable, anti-HBe antibodies present and ALT normal). The evaluation of chronic hepatitis B phases is a crucial to manage the burden of disease and limit the development of associated complications, such as cirrhosis and hepatocellular carcinoma (HCC). Thus this study conducted to evaluate the natural history of HBV infection in patients with chronic HBV infection in Ahvaz city, Iran. **Methods:** In this study, 71 non-treated CHB individuals were recruited including 44 (62%) males and 27(38%) females. The sera were tested for HBV markers, HBsAg, HBcIgG, HBeAg, and HBeAb. ALT assay and HBV viral load were carried out for each CHB individual. **Results:** Based on the analysis of serological, ALT status and viral load, the results showed: immune tolerance 5(7%), eAg+ Immune Clearance 14(19.7%), eAg- Immune Clearance 29 (40.84%) and Inactive Carrier 23 (32.39%). The HBeAg seroconversion was observed in a male age 18 year. **Conclusion:** The results of the natural history of individuals with chronic hepatitis B phases CHB shows immune tolerance (7%), eAg+ Immune Clearance (19.7%), eAg- Immune Clearance (40.84%) and Inactive Carrier (32.39%). To prevent the consequence of CHB infection, an individual in immune tolerance phase should be tested periodically for ALT level, HBV markers, HBsAg, HBcIgG, HBeAg, HBeAb and HBV viral load. Then decision-making therapy can be applied for CHB patients at early stage of immune clearance.

Keywords: Chronic Hepatitis B (CHB)- HBsAg- HBeAg- Alanine Aminotransferase (ALT)- Real-Time PCR

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Introduction

More than 284 million of the world populations have a persistent hepatitis B virus (HBV) infection (Ott et al., 2012). Even though vaccine and effective antiviral therapies are available, but no cure exists. The prevalence of chronic HBV varies from 1 percent in low-prevalence areas (eg, United States, Canada, Western Europe) to 7% in intermediate-prevalence areas (eg, Mediterranean countries, Japan, Central Asia, Middle East, and parts of South America), to ≥8 % in high-prevalence areas (eg, Western Africa, South Sudan (Schweitzer et al., 2015; Zhang et at., 2016; Bosch et al., 2005). The prevalence of HBV infection was reported about 1.7-5 % in Iran (Makvandi et al., 2015). Some of chronic carriers develop liver cirrhosis or hepatocellular carcinoma (HCC), and about one million people die due to HBV -related liver cancer and end-stage liver disease annually worldwide (Trepo et al., 2014; Wanich et al., 2016, Loho et al., 2016). HBV infection may occurs via infected body fluids prenatally, sexual exposure, blood transfusion and pre-cutaneous inoculation (Jahangirnezhad et al., 2011; Ito et al., 2014). Children acquired chronic HBV infection have a long 'immune tolerant phase' and enters the second 'immune active phase' after 2–3 decades of persistent infection (Kao, 2007; Liaw, 2009; Della et al., 2014). Spontaneous hepatitis B e-antigen (HBeAg) seroconversion is reported to depend on factors such as

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age, ALT level, and HBV genotype. HBV is classified into nine genotypes, A to I, that among them Genotype D of hepatitis B is predominant in Iran (Neisi et al., 2011; Ansari et al., 2015). Differences natural course of HBV infection have been described in different countries and areas that may depend on the genotype of HBV (Croagh et al., 2015).

Classification of Natural CHB infection

The clinical outcome of HBV infection relies on the interaction between virus replication and host immune response (Trépo et al., 2014). HBV-specific T-cell dysfunction has been demonstrated to be associated with HBV persistence (Bertoletti et al., 2012). Based on relationship between viral replication and evolution and the host immune responses, the natural CHB infection has been classified into four phases. Immune Tolerance, HBeAg-positive immune active, HBeAg-negative immune active, inactive carrier. Immune Tolerance, phase is characterized by the existence of HBeAg, HBV-DNA viral load > 10,000-20,000 IU/ml and normal serum ALT level without or mild inflammation in the liver biopsy (McMahon., 2008). After 10-30 years, Immune tolerance phase may enter Immune reactive (also defined as, immune clearance or immune active) phase which characterizes fall in HBV DNA load (>2,000 - <20,000 IU/ml), comprises two phases HBeAg-positive immune active phase (HBeAg detectable, anti-HBe antibodies undetectable, ALT persistently elevated) and HBeAg-negative immune active phase (HBeAg undetectable, anti-HBe antibodies present and ALT persistently elevated) (Tomoko et al., 2017). Several types of mutations have been described in HBV genome in the immune reactive phase which may lead to cirrhosis or hepatocellular carcinoma. The core promoter mutations reduce HBeAg expression but enhance genome replication which may associate with acute liver failure, cirrhosis, and HCC (Liu et al, 2011; Tseng et al, 2015). Cirrhotic and HCC patients also have high prevalence of preS deletions, S region truncations, and spliced HBV RNAs (Abe et al., 2009). The HBx protein is a potent transactivator that activates host genes, including oncogenes (Fisicaro et al, 2009), and mediates in Up/Down regulation of liver specific mirRNAs (Zhang et al., 2017). G145R mutation in the S region might pose a threat to the global vaccination program due to immune escape against anti- HBs and sustained infectivity (Shuping and Peter, 2016).

The immune reactive phase may last several months to several years (McMahon, 2008). The immune reactive phase may enter inactive carrier state that is characterized through anti-HBe positive, low HBV viral load level <2,000 IU/ml with normal /abnormal ALT level (Trépo et al., 2014). A percentage of inactive carriers may face reactivation HBV infection which exhibits the presence of anti-HBe antibody along with elevated HBV viral load level, heightened ALT level and liver inflammatory activity (Lok et al., 2009). Thus, to understand the natural history of chronic hepatitis B phases, effectively reduce the burden of disease and limit the development of associated complications, such as cirrhosis and hepatocellular carcinoma (HCC). Thus our objective was to evaluate the natural history of chronic hepatitis B phases in CHB patients individuals through assaying ALT level, HBsAg, HBeAg/anti-HBe, and HBV DNA viral load.

Materials and Methods

The sera were collected from 71 individuals with chronic hepatitis B who referred to Ahvaz Imam-Khomeni hospital during Feb to Nov 2013. All the patients had persisted HBsAg for more than 6 months. The subjects positive for anti-HCV antibody, HDV antibody, human immunodeficiency virus 1, 2 (HIV -1, 2) antibody, liver cirrhosis, autoimmune disorders or malignant disease were excluded from this study. The risk factors including surgery operation, tattooing, blood transfusion, and presence of HBV infection among family history were recorded from each individual.

Serological Markers

All the patients' sera were tested for HBV markers including HBsAg, HBeAg, anti-HBe, by enzyme-linked immunosorbent assay (ELISA) (DIA. PRO. Kit Diagnostic Bio probes, Milan, Italy).

Biochemical Markers

The ALT assay and total bilirubin assay were measured using Pars Azmoon kit (Tehran, Iran). Upper normal limit (ULN) for ALT was considered <31 U/L for women, and <41 U/L for men. The ULN for total bilirubin was considered 1.2 mg/dl.

HBV DNA Quantification

HBV-DNA was extracted from patient's sera using the High Pure Viral Nucleic Acid kit (Roche, Germany) according to manufacturer instructions. The quantification extracted HBV-DNA was assessed by Real-Time PCR using Artus HBV LC PCR kit (Artus GmbH, Hamburg, Germany) as instructed by the manufacturers and as previously described (Della et al., 2014).

Ethic Status

This project with registration number ETH- 711 has been approved by ethic committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The ethic consent was obtained from each individual participated in this project.

Statistical Analysis

The data were analyzed by SPSS software version 16 by Independent T-Test, One-way ANOVA, Kruskal-Wallis and Mann-Whitney U. Differences were considered significant when a P value was < 0.05.

Results

Of the 71 patients, 44 (62%) were male individual and 27 (38%) female individuals, with a male/female ratio of 1.62. The median age of the CHB patients was 32 years old (range: 18–64 years old). The natural history of chronic hepatitis B phases of all the patients with CHB were further categorized into four groups according to

Table 1. CHB Patients in Different Phases

Age groups	Immune Tolerance n=5 (7.%)	eAg+ Immune Clearance n=14 (19.7%)	eAb+ Immune Clearance n=29 (40.84%)	Inactive Carrier n=23 (32.39%)	Total (n=71)
< 20	1	1	1	-	3 (4.2%)
20-29	2	3	4	6	15 (21.1%)
30-39	1	6	8	8	23 (32.4%)
40-49	1	2	7	5	14 (19.7%)
50-59	-	-	7	2	10 (14.1%)
>60	-	2	2	2	6 (8.5%)

Above table shows the number of CHB cases in deferent stages in deferent age group, Chiq- squre test was perform to evaluate association, X^2, 14.26; df, 15; p-value = 0.5; indicated no relation was founded, means, the number of cases in various stage had the same pattern in different age group.

Table 2. Displays CHB Phase in Males and Female

Gender	Immune Tolerance (n=5)	eAg+ Immune Clearance (n=14)	eAb+ Immune Clearance (n=29) p	Inactive Carrier (n=23)	Total (n=71)
Male	3 (6.8%)	7 (15.9%)	22 (50%)	12 (27.3%)	44 (62%)
Female	2 (7.4%)	7 (25.9%)	7 (25.9%)	11 (40.7%)	27 (38%)
Total	5 (7%)	14 (19.7%)	29 (40.8%)	23 (32.4%)	71

Table 2, shows the number of cases of female and male in deferent stag. Again Chi-squre test showed there was no relation between age and stage, or the number of cases in various gender had same pattern in different stage; X^2, 4.17; df, 3; p-value= 0.244.

the HBeAg status, levels of aminotransferase (ALT) and HBV DNA, as described previously (Trépo et al., 2014; Bertoletti et al., 2012; Tomoko et al., 2017). 5/71 (7%) of the patients were in immune tolerance (IT) phase, included 3(4.25%) males and 2 (2.81%) women. The IT was characterized by HBeAg positively, high levels of serum HBV DNA, normal or low levels of ALT. 43/71 (60.56%) of the patients were in immune active phase, among them 29/71 (40.84%) males and 14/71 (19.71%) females. In this phase 7 males and 7 females were positive for HBeAg immune active phase and 22 males and 7 females were positive for HBeAb immune active phase. The Immune clearance was characterized by relatively lower levels of serum HBV DNA than IT phase, increased or fluctuating levels of ALT. 23 patients were in inactive carrier (IC) phase included 12 males and 11 females. The IC patients were characterized by very low or undetectable serum HBV DNA levels and normal serum ALT. Table 1 shows CHB patients classified in different phases. Table 2, display CHB phase in males and female. The most frequent risk factor among the participants were related to presence of HBV infection among the parent or the siblings of CHB patients (34%) and the lowest were related to tattooing (5.6%) among the CHB as display in Table 3.

Table 3. Risk Factors among the CHB Individuals

Category	number
Surgery Operation	23 (32.5%)
Tattooing	4 (5.6%)
Blood Transfusion	5 (7%)
Parent, Siblings	24 (34%)
Spouse	5 (7%)
Ther Risk Factors	39(54.92%)

Discussion

Examine patients in immune tolerance phase and in the initial entrance immune clearance by performance of HBsAg and ALT assay periodically, effectively reduce the burden of disease and limit the development of associated complications, such as chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC) (Jean-Pierre., 2016). In this study, we explored the characteristics of adult with HBV infection. Three patients were below twenty years among them one male individual with immune tolerance aged 15 years, two, one male HBeAg-positive immune active phase and the other was female HBeAb-positive immune active phase aged 18 years. It was reported children who are infected prenatally remain in the immune tolerant phase and can be lasted in adolescence or early adulthood (Livingston et al., 2007). In this survey, 5 (7%) of CHB individuals were in immune tolerance phase. One aged below twenty and four were observed in age group 20 to 49 years. They might have acquired HBV infection before national vaccination program which has been launched since 1993 for all neonates in Iran (Alavian, 2007). Wang G et al., have investigated 166 patients with CHB and found 43 (25.9%) cases in immune tolerant phase, 71 (42.77%) in immune active phase and 52 (31.32%) in inactive carrier phase (Wang et al., 2017). In the present study, 14/71 (19.71%) individuals with positive HBeAg immune active phase, included 10 (14.08%) male and 4 (5.6%) female. The positive HBeAg immune active phase has been observed immune clearance phase in all of age groups and varied from <18 years to > 60 except in group age of 50-59 years. In our study 29/71 (40.85%) of CHB individuals included 22 (30.98%) male individuals

and 7 (9.85%) female individuals were categorized in negative HBeAg immune clearance phase. The frequency of CHB individuals in negative HBeAg immune active phase have been observed in the all age groups. Assis et al., from Brazil, have determined the stages of the natural history of chronic hepatitis B in 110 patients with mean age of 42.95±12.52 and reported 6 (3.4%) cases presented with immune tolerance; 16 (9.1%) HBeAg-positive immune clearance phase including, 50 (28.6%) HBeAg-negative immune clearance, 38 (21.7%) were inactive carriers phase (Assis et al., 2015). Several mutations have been described in HBV genome during negative HBeAg immune clearance phase, Precore and core promoter mutations which suppress HBeAg expression and result in HBeAg seroconversion to HBeAb (Shuping et al., 2016; Poustchi et al., 2008; Tseng et al., 2015). In the present study 23 CHB individuals including 12 (16.9%) males and 11 (15.49%) females were recognized in inactive carrier phase. The frequency of individuals in negative HBeAg immune active phase has been found in the all age group, except age group <20 years old. Chan et al., from Hong Kong have reported 22/117 (18.8%) of chronic hepatitis B with longitudinal follow-up for 99±16 months were in inactive carriers phase (Chan et al., 2010). With regards to Chiq- squre test the evaluation of association, X^2=14.26, df=15, in all cases in different stage of CHB phases showed the same pattern in different age group (p=0.5). The results of Chi-squre test showed there was no relation between age and stage, or the number of cases in various gender, had same pattern in different CHB phases , X^2=4.17, df=3, p-value=0.244. It has been investigated that patients with genotypes C and D infection carry a higher risk of cirrhosis and HCC development than those with genotypes A and B (Ni et al., 2004). In Iran HBV genotype D is the most prominent among the patients with HBV infection (Makvandi et al., 2015; Neisi et al., 2011; Ansari et al., 2015; Poustchi et al., 2008). The association of HBV genotype D has been reported among the patients with cirrhosis and hepatocellular carcinoma in Iran (Ansari et al., 2015; Abdolmohammadi R et al., 2016).

There are several risk factors involved in HBV transmission, blood transfusion, history of surgery, tattooing, and history of intra-familial hepatitis (Jahangirnezhad et al., 2011; Ito et al., 2014; Gheorghe et al., 2013; Croagh et al., 2014). In our survey the risk factors in CHB individuals were surgery operation (32.5%), tattooing (5.6%), blood transfusion (7%), infection of parent and siblings (34%), and spouse (7%). In the currently available guidelines, the recommendation is that HBV-DNA, ALT, and HBeAg be analyzed together and with great care for the indication of the biopsy and therapy decision making (European Association, 2012). To improve treatment and management of CHB patients, it is suggested, ALT assay, HBeAg/anti-HBe, and HBVDNA viral load by real time PCR should be implemented for CHB individual in immune tolerance phase.

In conclusion, the results of natural chronic hepatitis B infection among the 71 CHB revealed that immune tolerance (7%), eAg+ Immune Clearance (19.7%), eAg- Immune Clearance (40.84%) and Inactive Carrier (32.39%). To manage CHB patients, it is recommended, ALT level, HBeAg/anti-HBe, and HBVDNA viral load by real-time PCR should be carried out for CHB cases in immune tolerance phase periodically. CHB patients entering at early stage of immune clearance phase should be treated with current medication to stop further mutation in HBV genome, liver fibrosis, cirrhosis and HCC.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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