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Distribution of Different Clinical Forms of Hepatitis B Virus (HBV) Infection among HBV Infected Patients Referred to Al-Zahra Hospital, Isfahan, Iran

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ABSTRACT

Aims: Despite the vast global vaccination programs against the HBV infection, millions of people are chronic HBV carriers worldwide. The present study aimed to evaluate the distribution of different clinical forms of Hepatitis B infection among HBV infected patients to find the frequency of people at risk of developed liver diseases in Isfahan province.

Materials & Methods: This cross-sectional study was performed on 600 HBV infected patients admitted to Al-Zahra hospital in Isfahan from March 2017 to March 2018. Based on the virological markers, HBV infection in participants was categorized into four clinical forms including post-infection immunity, acute hepatitis, asymptomatic carrier state, and chronic active hepatitis. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were used for screening HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe, and viral DNA in serum samples.

Findings: In this study, 308 (51.3%) females and 292 (47.7%) males with HBV infection and the mean age of 39 years were participated, of whom 189 (31.5%), 172 (28.7%), 138 (23%), and 101 (16.8%) participants were found to be in the post-infection immunity, acute hepatitis, asymptomatic carrier state (inactive carrier), and chronic active hepatitis forms of HBV infection, respectively.

Conclusion: The results of this study highlighted the high prevalence of asymptomatic carrier and chronic active hepatitis forms of HBV infection in 20-40 year old patients. Extensive measurements are needed to determine the prevalence of these two mentioned forms of HBV infection in all provinces of Iran in order to control the economic and life burden of disease in people not covered by the infant vaccination programs in Iran.

Keywords: Hepatitis B, Clinical form, Isfahan, Iran

CITATION LINKS

[1] Hepatitis B virus infection. Lancet... [2] Hepatitis B vaccination coverage among health-care workers in Africa: A systematic ... [3] Risks of chronicity following acute Hepatitis B virus... [4] Hepatitis B inactive carriers: An overlooked population? GE Port J Gastroenterol. 2014; 21(6):241-9. [5] Global epidemiology of Hepatitis B virus infection: New estimates of age-specific HBsAg... [6] Medical virology. 4th ed. Academic... [7] Diagnosis of Hepatitis B virus infection through serological and virological markers. Expert Rev Gastroent. 2008; 2(4):553-62. [8] Recommendations for identification and public health management of persons with chronic Hepatitis B virus infection. MMWR Recomm Rep. 2008; 57(RR-8):1-20. [9] Hepatitis B virus infection: Epidemiology and vaccination. Epidemiol Rev. 2006; 28(1):112-25. [10] Hepatitis B virus infection in the general population of Iran: An updated systematic review and meta-analysis. Hepat Mon. 2016; 16(4): e35577 [11] Novel subgenotypes of Hepatitis B virus Genotypes C and D in Papua, Indonesia. J Clin Microbiol. 2008; 46(7):2160-6. [12] Prevalence of Hepatitis B virus infection among Iranian high risk groups: A systematic review and meta-analysis...[13] Risk factors of Hepatitis B virus infection in Turkey: A population-based, case-control study.. [14] Seroprevalence of Hepatitis B surface antigen and Anti-Hepatitis C antibody in zahedan city, Iran.. [15] Distribution and risk factors of Hepatitis B virus infection in... [16] Hepatitis B is a serious health problem in ... [17] Prevalence of national responsiveness to... [18] Prevalence of Hepatitis B virus seromarkers in young adults vaccinated at birth; impact on... [19] Efficacy of Hepatitis B vaccination in under five-year-old children in Iran: A systematic review... [20] Spontaneous relapse in patients with inactive chronic... [21] Hepatitis B in pregnancy... [22] Pathogenesis of Hepatitis B virus infection. Pathol Biol. 2010; 58(4):258-66. [23] Immunobiology of Hepatitis B virus... [24] Carriers of inactive Hepatitis B virus are still at risk for hepatocellular... [25] Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. 2018; 68(3):526–49.

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Introduction

HBV has affected more than 2 billion people worldwide, and 257 million people are living with chronic Hepatitis B infection [1]. In 2015, Hepatitis B was estimated to result in 887000 deaths, mostly due to cirrhosis and hepatocellular carcinoma [2]. HBV transmission is mainly mediated by mucosal and percutaneous exposure to the virus present in infected blood or human body fluids [3]. Depending on the immunological response and age of the host at the time of infection, different forms of infection occur following acute HBV infection, including the immune-tolerant, immune-reactive (post-infection immunity), inactive or asymptomatic carrier, and chronic Hepatitis B [4]. Perinatal infections are mostly resulted from the contamination of baby during the parturition, and around 90% of infected infants become chronic carriers. Children between 1 and 5 years old, who acquire HBV infection from siblings or parents secretions are 25-30% likely to be HCV carrier, while chronicity occurs only in 1-5% of adults who are principally infected by sexual intercourse or drug abuse [5-6]. Serology markers are available for the diagnosis of HBV and differentiation between the various clinical forms of infection [6-7]. Markers associated with the infection are Hepatitis B surface antigen (HBsAg), Hepatitis B e antigen (HBeAg), antibody to hepatitis surface antigen (anti-HBs), antibody to HBeAg (anti-HBe), antibody to Hepatitis B core antigen (IgG and IgM) (anti-HBc), and HBV-DNA [6, 8-9]. By applying preventive strategies against the HBV infection, Iran was classified among the HBV low-prevalent areas in 2010 [10]. But population surveillance of different HBV infection clinical forms including chronic hepatitis is currently rare in Iran, and the accurate frequency of individuals who are chronically infected with HBV or asymptomatic carriers is unknown. Continuous regional reports of the prevalence of HBV carriers in Iran help characterize the status of infected people and quantify HBV burden. This may also help stop further transmission of infection and apply appropriate treatment measures.

Objectives: In the light of the mentioned gap, the purpose of this present study was to estimate age- and sex-specific prevalence of different clinical forms of HBV infection, including post-infection immunity, acute hepatitis, asymptomatic carrier state, and chronic active hepatitis in Isfahan province of Iran.

Materials and Methods

HBV patients: In this cross-sectional study, serum samples were collected from 600 HBV infected patients referred to Al-Zahra hospital, Isfahan, Iran from March 2017 to March 2018. Al-Zahra is a public hospital to which HBV-infected patients are referred from all over the Isfahan province for more accurate diagnosis of infection. A checklist containing demographic data such as age, gender, and serological markers was prepared for each participant.

Clinical form determination criteria: Six serum markers along with viral DNA were detected to categorize HBV infection into four clinical forms: a) post-infection immunity; b) acute hepatitis; c) asymptomatic carrier state; and 4) chronic active hepatitis (Table 1) [6-7].

HBV and anti-HBV antigen detection: In this step, 10 mL blood sample was taken from each subject, and sera was screened for the presence of HBsAg, anti-HBs, HBeAg, and anti-HBe using ELISA kit (DIA.PRO, Milano, Italy) according to the manufacturer's instructions. Anti-HBc was evaluated by the kit mentioned above using the competitive enzyme immunoassay.

Viral DNA detection: Sera samples were subjected into viral DNA extraction using commercial DNA extraction kit (GeNet

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Table 1) Serological markers of HBV infection [6-7]

Clinical Conditions	Serological Marker[a] HBsAg	Anti-HBs	total Anti-HBc	IgM Anti-HBc	HBeAg[b]	Anti-HBe	Viral DNA
Post-infection immunity	-	+	+	-	-	+>>-	-
Acute hepatitis	+	-	+	++	+>>-	->>+	+
Asymptomatic carrier state	+[c]	-	+	-[d]	-	+	-[e]
Chronic active hepatitis	+	-	+	+	+	+	+

[a] Arrow means transition in due course from one state to the other
[b] If infected with HBV wild type, not with pre-core mutant
[c] Persisting for more than 6 months.
[d] Low titer
[e] Very low titer

Bio, Korea), and HBsAg gene was detected by PCR technique using specific primers as described previously [11]. The PCR reaction mixture (25 µL) consisted of 2.5 µL of 10xPCR buffer, 20 pmol of forward (5'-CAAGGTATGTTGCCCGTTTG-3') and reverse (5'- AAAGCCCTGCGAACCACTGA-3') primers, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 3U of Taq polymerase (CinnaGen, Iran). Amplification was carried out for 30 cycles (at 95°C for 1 min, at 60°C for 1 min, and at 72°C for 1 min) after an initial denaturation step at 95°C for 7 min. The cycles were followed by a final extension step at 72°C for 5 min. The 258 base pair PCR products were visualized on 2% agarose gel containing ethidium bromide. To determine the sensitivity of the test, different dilutions of DNA from 4 to 4×10^{-6} particles were prepared by serial dilution. In order to determine the specificity of the test, genome of human, *Toxoplasma gondii*, Hepatitis C cDNA, *Mycobacterium tuberculosis*, *Escherichia coli,* and *Saccharomyces cerevisiae* were used as negative controls.

Statistical analysis: Quantitative data were presented by percent. The frequencies of post-infection immunity, acute hepatitis, asymptomatic carrier state, and chronic active hepatitis forms of HBV infection were evaluated in patients according to the patients' age and gender. Statistical analysis was performed by SPSS software Ver. 23 (SPSS Inc., Chicago, IL, USA). Frequency comparison was done using Chi-square test, and $p<.05$ was considered as statistically significant.

Findings

Study population: Gender and age grouping information of 600 patients under study is summarized in Table 2. Males and females showed a relatively equal frequency, and patients were more in the age groups of 20-30 and 30-40 years than in other age groups.

Frequency of HBV infection clinical forms in the total studied population: Of 600 studied subjects, 189 (31.5%) cases were categorized into post-infection immunity group. The frequency of patients with acute hepatitis, asymptomatic carrier state, and chronic active hepatitis forms of HBV infection were 172 (28.7%), 138 (23%), and 101 (16.8%) cases, respectively.

Sex-specific frequency of different HBV infection clinical forms: The statistical analysis results showed that the frequency of patients with post-infection immunity, acute hepatitis, and chronic active hepatitis was equal in two sexes ($p<0.05$); however, there was a significant difference between the male and female patients regarding the

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asymptomatic carrier state frequency (p= .027), and the number of females was higher than males (59.4% versus 40.5%) (Figure 1).

Table 2) Study population grouping results

Parameter	N (%)
Gender	
Male	292(48.7)
Female	308(51.3)
Age group	
<20	37(6.2)
20-30	135(22.5)
31-40	134(22.3)
41-50	85(14.2)
51-60	110(18.3)
>60	99(16.5)
Total	600(100)

Age-specific frequency of different HBV infection clinical forms: Of 189 patients with post-infection immunity state, 43 (22.7%) and 42 (22.2%) patients belonging to the 20-30 and > 60 years age groups, respectively captured the highest frequency, and of 172 patients with acute HBV infection, 37 (21.5%) and 44 (25.5%) patients belonging to the 20-30 and 31-40 years age groups showed the highest frequency. Of 139 asymptomatic HBV carriers, the highest frequency belonged to 34 individuals (24.6%) in the age group of 51-60 years, followed by 32 (23.1%) and 29 (21%) individuals in the age groups of 20-30 and 31-40 years, respectively. The highest frequency of chronic active hepatitis was observed in the age groups of 20-30 and 30-40 years with 23 (22.7%) and 30 (29.7%) patients, respectively (Figure 2). The least frequencies of all HBV infection clinical forms were observed in patients belonging to the age group of <20 years: 9 (4.7%), 17 (9.8%), 7 (5%), and 4 (3.9%) cases for post-infection immunity, acute hepatitis, asymptomatic carrier state, and chronic active hepatitis, respectively (Figure 2).

Discussion

The current study was performed on 600 HBV infected patients to find the gender- and age-related frequency of HBV infection different clinical forms.

In this study, 292 (48.7%) males and 308 (51.3%) females were found to be infected with Hepatitis B virus (Figure 1). Unlike other studies in which the number of HBV infected males was higher than females [10], the total number of HBV infected male and female patients in this study was relatively equal. HBV infection risk factors are more common in men, but women should also be evaluated in terms of HBV infection risk factors in the country, especially because the number of women with high-risk activities is increasing in Iran [12].

The age grouping results (Figure 2) showed that most HBV infected patients belonged to the age groups of 21-30 (22.5%) and 31-40 (22.3%) years. The results were in accordance with the results of other seroepidemiological studies in which a higher number of HBV infected patients in these age groups was reported [13-15]. This sounds to be reasonable since these age groups besides of occupational exposures are more likely to participate in social activities, substance abuse, and sexual intercourse [16]. On the other hand, the lowest rate of HBV infection incidence was related to the age group below 20 years. The likely reason for this result is the 93.5% responsiveness of HBV vaccination program in Isfahan [17]. The results of this study were also supported by the results of Safar et al. (2014) and Najafi et al. (2018), indicating that neonatal HBV immunization induced effective protection

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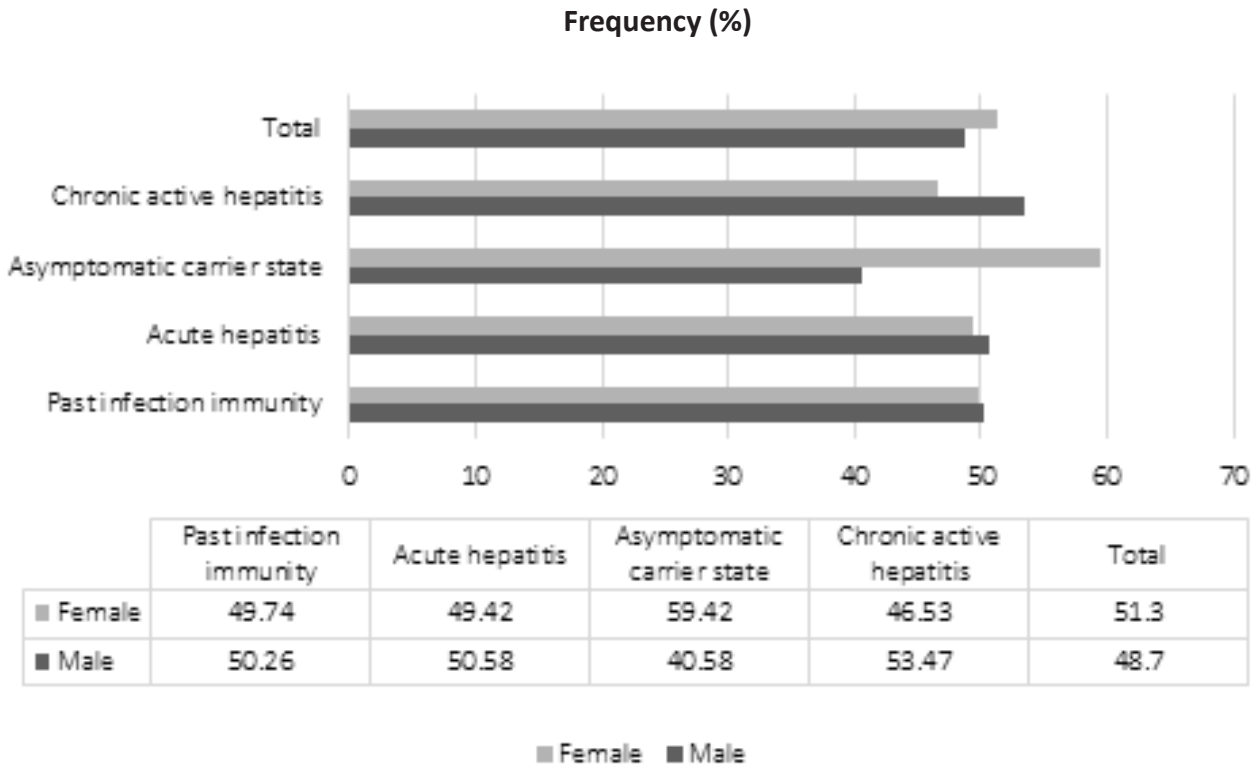
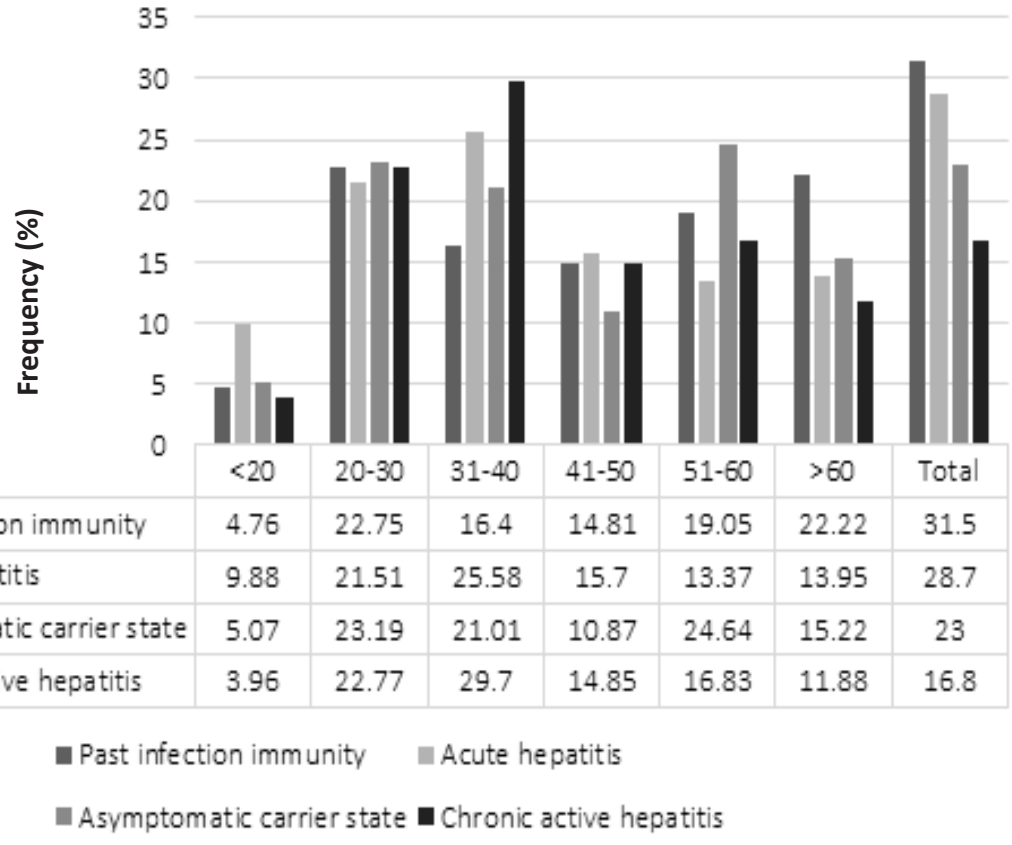


	Past infection immunity	Acute hepatitis	Asymptomatic carrier state	Chronic active hepatitis	Total
Female	49.74	49.42	59.42	46.53	51.3
Male	50.26	50.58	40.58	53.47	48.7

Figure 1) The frequency of different clinical forms of HBV infection in males and females



	<20	20-30	31-40	41-50	51-60	>60	Total
Past infection immunity	4.76	22.75	16.4	14.81	19.05	22.22	31.5
Acute hepatitis	9.88	21.51	25.58	15.7	13.37	13.95	28.7
Asymptomatic carrier state	5.07	23.19	21.01	10.87	24.64	15.22	23
Chronic active hepatitis	3.96	22.77	29.7	14.85	16.83	11.88	16.8

Figure 2) The frequency of HBV infection clinical forms in different age groups

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against the infection and reduced chronic infection frequency in vaccinated young adults in Iran [18-19].

Based on the serological and virological markers, Hepatitis B infection has been divided into different clinical forms [6-7]. In the present study, 189 (31.5%), 172 (28.7%), 138 (23%), and 101 (16.8%) patients were categorized into post-infection immunity, acute hepatitis, asymptomatic carrier state (inactive carrier), and chronic active hepatitis groups, respectively.

In this research, the frequency of acute and chronic active hepatitis was relatively equal in two sexes, but the number of women with inactive HBV carrier state was significantly higher than men (p=.027) (Figure 1). Studies showed that male gender is consistently associated with the risk of relapse in asymptomatic carriers [4]. A higher rate of reactivation in male patients was also reported in another study in Iran [20]. Aside from the higher risk of relapse in male carriers and transmission of disease by asymptomatic carriers, female carriers are considered as serious risk factors for neonatal hepatitis occurrence since vertical transmission is the main cause of baby infection and chronic hepatitis occurrence. Considering the high number of female asymptomatic carriers reported in this study, prevention strategies like the prescription of Hepatitis B immunoglobulin (HBIG) and vaccination of newborns should be implemented to reduce the rate of vertical transmission [21].

According to the results, 189 (31.5%) patients were identified with acute hepatitis-related markers. These people are potentially infectious and considered as the major reservoirs of virus transmission to others [9]. Also, in some cases depending on patient's immune response and age, the disease may persist or progress to chronic hepatitis [22-23]. The results of the present study showed that 17 (9.88%) patients with acute hepatitis belonged to the age group below 20 years. This could be related to the non-immunization of these people or low efficacy of HBV vaccination (because of certain diseases or disorders in these individuals)[12].

In this study, a noticeable proportion of patients with chronic active (101 cases) and inactive (139 cases) carrier forms were categorized in the age groups of 20-30 and 31-40 years, respectively (Figure 2). The reason for this observation is rooted in the fact that patients in these age groups (more precisely 26-<60) were born before the start of the neonatal HBV vaccination program in Iran in 1993 [17]. Active and inactive carriers with the age ranges from 20-40 years show more work, sexual, and fertility activities, and are more at risk of cirrhosis or hepatocellular carcinoma [24-25]. According to the results reported in this study, for these types of HBV infection, preventive strategies including assessing HCC clinical risk scores [25], improving public awareness, and implementing diagnosis/treatment programs (up to virus clearance) from before the disease progression to liver-related death should be seriously followed by health care systems.

To the best of our knowledge, this was the first study in which the frequency of various clinical forms of HVB infection was determined in one population. In addition to patients with acute hepatitis, who are infectious carriers, a noticeable number of individuals were found to be in chronic active and asymptomatic carrier states with no clinical manifestations. These people increase the possibility of vertical and horizontal HBV infection transmission, and there is a risk of disease development to severe liver disease over time.

Conclusion

Thus, continuous epidemiological reports

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from HBV infection clinical forms in different provinces of Iran is needed to control the burden of HBV infection in people not covered by the infant vaccination program in Iran.

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Ethical Permissions: The study protocol was conducted following the confirmed institutional guidelines of the university of medical sciences.

Conflict of interests: None declared by Authors.

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