

Original Article

Seroprevalence of Hepatitis B before Marriage: A Study on Marriage Candidates in the Southeast of Iran; Is It Worthy of Consideration?

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Abstract

Objectives: Hepatitis B virus infection is one of the most common causes of acute and chronic hepatitis worldwide. Furthermore, since most people with hepatitis B virus are asymptomatic, timely diagnosis is important for prevention. This study has evaluated the prevalence of Hepatitis B seromarkers in marriage candidates.

Design: In this Cross-sectional study, 370 marriage volunteers selected by the simple sampling method were investigated in 2011 – 2012.

Results: A total of 185 men and 185 women were investigated. Mean Age of participants was 24.7 years. The prevalence rates of hepatitis B surface antigen (HBsAg), anti hepatitis B surface antibody (anti-HBs) and anti-hepatitis B core antibody (anti-HBc) were 1.1%, 47.6% and 8.9% respectively. Frequency of HBsAg was 0.5% and 1.6% in men and women respectively. The prevalence of anti-HBs was 43.8% in men and 51.45% in women. Anti-HBs was more common in young people and those with higher levels of education and positive history of vaccination. The prevalence of anti-HBc was 8.1% and 9.7% in men and women respectively.

Conclusion: Although Kerman is a low endemic area, due to the high risk of transmission to the spouse and offspring, screening for HBV study before marriage is recommended.

Keywords: Hepatitis B, marriage, seroprevalence

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Introduction

Hepatitis B is the most common cause of acute and chronic hepatitis and a matter of health concern. Chronic infection with hepatitis B virus can cause hepatocellular carcinoma and cirrhosis leading to death. Annually, two billion people around the world have been infected with this virus, 360 million live with chronic hepatitis B and 600 thousands die due to the complications of HBV. It has been estimated that 10% of the general population in some Asian countries such as China and Western pacific areas develop chronic hepatitis B, whilst its prevalence in the US and Northern European countries is under 0.5%.¹ Iran with about 3% of its population as chronic carriers is in a group of countries with moderate endemicity and 35% of Iranian population have evidence of exposure to HBV. Sexual transmission is the most significant mode of HBV spreading in the most developed countries; however the most likely route of transmission in developing countries such as Iran is perinatal transmission.²

Several markers help to diagnose HBV; however one the most useful screening test is hepatitis B surface antigen (HBsAg). This

antigen can be detected 1 – 10 weeks after the exposure; if it stays positive after six months, the patient is considered to develop a permanent and chronic infection. Anti-HBs appears 2 – 16 weeks after HBsAg development, injection of hepatitis B immune globulin (HBIG) or during blood transfusion and indicates the immunological and clinical improvement.³

Hepatitis B core antibody (anti-HBc) which rises early, within 2 – 4 weeks after the exposure, can be detected in serum during acute or chronic phases, but commonly, when it is the only sign of HBV exposure, it indicates an improved state or a remote infection. Negative HBsAg and positive anti-HBc, might be a sign of HBV replication and HBV DNA should be determined in these patients. IgM anti-HBc can be detected in the acute phase of HBV infection and disappears slowly in four months to two years, but IgG anti-HBc may be found in patients who are recovering or in chronic phase of the disease.

While sexual contact is one of the most important ways of virus transmission, especially in young people, Hepatitis B can be prevented by vaccination, which is the most cost-effective way of prevention. Iran is a country with a moderate prevalence of HBV and about 35% of people are exposed to HBV during their life.⁴ On the other hand, in Iran pre-marriage sexual contact is banned because of cultural and religious beliefs and pre-marriage tests are limited to just thalassemia, syphilis or opium addiction and pre-marriage HBV tests are voluntarily performed regardless the age and risk factors of sexual and perinatal transmission in the near future; therefore before marriage is a critical time for evaluation of HBV status in order to implement prevention measures.

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Materials and Methods

This analytical cross-sectional survey was designed to determine the seroprevalence of HBV markers in couples in Kerman city, which is the largest city in the Southeast of Iran, with about 800,000 populations, from September 2011 until August 2012.

All couples who were referred to a marriage consultation center of Kerman city for routine pre-marriage tests, were enrolled in this study by convenient sampling. We did not have any exclusion criteria, so subjects were all couples who were supposed to get married in city. The purpose of the study was explained and volunteers for participation were admitted after obtaining informed written consent. A detailed researcher-made questionnaire was used, including 20 questions covering demographic features (age, sex, educational level, previous marriage, and place of residency) and history of icter, imprisonment, drug consumption, drug injection, tattoo, blood transfusion, and HBV vaccination. All couples filled the questionnaire without any identification data with the help of a trained healthcare worker. Each questionnaire was assigned a code for a follow up purpose. Five milliliters (5 ml) of blood samples was taken after taking blood samples for routine tests from all subjects.

Samples were centrifuged (3000 rpm) for separation of serum and were stored in sterile tubes at -20°C until use. Each participant's tube was assigned the same code as his/her questionnaire code. Samples were sent to the target lab regarding the cold chain. All samples were tested for HBsAg (Simense Kit, Germany), anti-HBs (Acon and Diaplus, America), and anti-HBc (Biorad and Delaware, France) using one ELISA Reader according to the manufacture instruction (Human ELISA System, Germany). All positive results were rechecked and some of them were randomly controlled by the Iran Transfusion Center Lab. Data was analyzed through SPSS18 and frequency, relative frequency, and central tendency (mean \pm SD) were used for descriptive analysis and Chi-square or Fisher's exact test for categorical variables, and

Mann-Whitney-U test for continuous variables after evaluation of normality of all continuous data (by Kolmogorov-Smirnov test). P value < 0.05 was considered statistically significant. This study was approved by the Ethics Committee of Kerman University of Medical Sciences with ethical codes of k/91/08.

Results

A total of 185 volunteered couples (370 subjects) participated in this study. Mean age of participants was 24 ± 5.6 years (minimum and maximum of the age for males were 16 and 68 and for females were 12 and 54, respectively). Other demographic data were shown in table 1.

HBsAg, anti-HBs, and anti-HBc tests were positive in 4 (1.1%; CI: 0.1% – 2%), 176 (47.6%; CI: 41% – 50.2%), and 33 (8.9%; CI: 6.9% – 10.9%) subjects respectively. HBsAg was found in three (1.6%; CI: 0 – 3.4%) of female subjects and one (0.5%; CI: 0 – 1.02%) of male subjects. Anti-HBs was positive in 95 (51.4%; CI: 47.7% – 55.1%) of females and 81 (43.8%; CI: 40.2% – 47.4%) of males that 55 (29.7%; CI: 27.4% – 32%) of subjects were the couples who had the same result. Anti-HBc was positive in 18 (9.8%; CI: 7.6% – 12%) of men and 15 (8.1%; CI: 4.2% – 12%) of women, of whom only one man and one woman were supposed to get married. In one of the subjects all three tests were positive. Anti-HBs and anti-HBc tests were positive in 17 (4.6%; CI: 2.5% – 6.7%) cases. HBsAg and anti-HBs tests were positive in two persons (0.5%; CI: 0% – 1.22%).

Among four cases of positive HBsAg, three spouses were anti-HBs negative and at risk for HBV infection acquisition, while it could be prevented easily by pre-marriage vaccination.

Spouses of all four persons with HBsAg positive test were not HBsAg positive and just one of them had positive anti-HBs test. Concordance between couples in seromarkers was shown in table 2.

Of 17 subjects who were anti-HBs and anti-HBc positives, 16 subjects (94.1%; CI: 83% – 105%) had a history of HBV vaccination.

Table 1. Demographic characteristic of people who were enrolled in study.

Age (years)	24 \pm 5.6*
Sex	
Female	185 (50%)
Male	185 (50%)
Education	
Illiterate	2
Primary	10
Middle	40
High	152
University	166
Living status	
Lonely	8
With family	361
With friend	1
Previous marriage	23
Have a child	7
Imprisonment	1
Tattoo	3
Blood transfusion	0
Intravenous drug abuser	0
Icter	0
Cigarette	7
Opium	1
Alcohol	3
Methadone	1
Others	1

*: mean \pm SD

Table 2. concordance between couples in viral seromarkers

		Female					
		HbsAg		Anti-HbsAb		HbcAb	
Male		Positive	Negative	Positive	Negative	Positive	Negative
Positive		0	1	55	26	1	14
Negative		3	181	40	59	17	153

Table 3. Status of HBsAg, anti-HBs, and anti-HBc in 185 couples before marriage in Kerman, 2011 – 2012.

	HBsAg			Anti-HBs			Anti-HBc		
	Positive	Negative	P value	Positive	Negative	P value	Positive	Negative	P value
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Gender			0.54			0.08			0.35
Total	4 (100)	366 (100)		176 (100)	194 (100)		33 (100)	337 (100)	
Male	1 (25)	184 (50.3)		81 (46.6)	104 (53.6)		15 (45.5)	170 (50.4)	
Female	3 (75)	182 (49.7)		95 (53.4)	90 (46.4)		18 (54.5)	167 (49.6)	
Educational level			0.72			0.02			0.09
Illiterate	0 (0)	2 (0.56)		1 (0.6)	1 (0.5)		1 (3)	1 (0.3)	
Primary school	0 (0)	10 (2.8)		5 (2.8)	5 (2.6)		0 (0)	10 (2.97)	
Middle school	0 (0)	40 (10.93)		12 (6.9)	28 (14.4)		3 (9.1)	37 (10.98)	
High school or Diploma	3 (96)	149 (40.7)		65 (36.9)	87 (44.9)		18 (54.6)	134 (39.75)	
University	1 (4)	165 (45.1)		93 (52.8)	73 (37.6)		11 (33.3)	155 (46)	
Living status			0.95			0.53			0.002
Alone	0 (0)	8 (2.2)		3 (1.7)	5 (2.6)		2 (6.1)	6 (1.8)	
With family	4 (100)	357 (97.53)		173 (98.3)	188 (96.9)		30 (90.9)	331 (98.2)	
With friends	0 (0)	1 (0.27)		0 (0)	1 (0.5)		1 (3)	0 (0)	
HBV vaccine status			0.54			0.0001			0.51
Vaccinated	2 (66.6)	190 (52.8)		163 (92.6)	29 (15.5)		16 (51.6)	176 (53)	
unvaccinated	1 (33.4)	170 (47.2)		13 (7.4)	158 (84.5)		15 (48.4)	156 (47)	

†p: positive; ‡n: negative

In whole, 193 subjects had history of HBV vaccine injection (97 ones reported complete course of vaccination) of whom 2 ones were HBsAg positive, 163 ones were anti-HBs and 16 ones were anti-HBc positives. Anti-HBs titers was greater than 10 IU/L in approximately 85% (CI: 80% – 90%) of persons who had received the vaccine ($P = 0.0001$).

Although the number of women who had every three markers (separately) were more than men, but the difference between two sexes was not significant ($P > 0.05$). In addition mean age of participants did not show any statistical significant correlation with HBsAg or anti-HBc positivity ($P > 0.05$), while anti-HBs positivity showed a significant decrease with age increase ($P = 0.001$). Mean age of persons who had received the HBV vaccine was less than unvaccinated persons (23.7 ± 4 years versus 25.8 ± 6.8 years) but this difference was not significant. Thirty out of 193 persons who had received the HBV vaccine did not have anti-HBs positive test (49.7% of vaccinated individuals received incomplete course).

In this study, there was no correlation between positive HBV markers and some well-known risk factors such as history of imprisonment, tattooing, illegal drug consumption, and blood transfusion. Lower prevalence of HBsAg and anti-HBc in subjects with higher educational levels was not significant in our study, but anti-HBs significantly increased with level of education ($p =$

0.02). It could be attributed to more opportunities for vaccination in schools or universities.

Living with family and friends or being lonely was one of the variables that did not affect the HBsAg and anti-HBs. However anti-HBc was seen more in those who were living with friends than in alone subjects ($P = 0.002$), (Table 3).

Discussion

Of 185 couples who had referred for pre-marriage tests, 4 subjects (1.1%) were HBsAg positive, 3 subjects (1.6%) were female and one person (0.5%) was male. In whole, 33 subjects (8.9%) were anti-HBc positive, four of them were positive in the HBsAg test too; and 176 subjects (47.6%) of all subjects were anti-HBs positive. There was not any significant correlation between HBsAg or anti-HBc and sex or age, but a significant difference was obtained between age and anti-HBs.

If we could generalize the obtained results to the whole population, the prevalence of HBsAg that was less than 2%, locates our population in the low epidemic area. This prevalence rate differs with available registered data and some surveys in which the prevalence rates are greater than 2%; for instance, Merat, et al. have reported the prevalence rate of 2.6% for HBsAg and 16.4% for anti-HBc positive in three provinces, including the capital of

Iran, a northern province and a southern one.⁵ According to the mentioned study, Iran is a region with a moderate prevalence of HBV. The obtained results are similar to the results of some other studies including a Turkish study on engaged couples (1.7%),⁶ an Iranian study on engaged men (0.8%),⁷ a seroepidemiologic research in a western province of Iran (0.8% HBsAg), a study on hemophilia patients in our province (1%)⁸ and hemophilia patients in south west of Iran (1.1%).⁹ It should be mentioned that rational higher and various rates of HBV have been found in prisoners (5.8% – 2.1%), thalassemia patients (0% – 13.5%), and in general population of some provinces of Iran.^{10,11} It seems that the lower prevalence of HBV in most recent researches is because of the effectiveness of national vaccination program implemented from 1992 for neonates, adolescents, voluntaries, and high risk groups such as health care workers. In addition, it might be due to the improvement in awareness about prevention and risk factors, as well as easy access to disposable syringes in healthcare centers.

HBsAg positivity did not show correlation with age in our study, which is in contrast to the results of previously mentioned Turkish research and some other Iranian studies, in which HBsAg positivity was seen in older patients more than youth.^{5,6,9} In some other studies on prisoners, patients with thalassemia, or engaged men, HBsAg was not different in various ages;^{5,6,10} a finding that is against the more probability of exposure over time. Perhaps recent sufficient donor blood screening and higher level of knowledge in prisoners, patients, and general population and more access to sterile syringes or private paraphernalia are the causes of this result in the mentioned studies.

In the present study, like previously mentioned Turkish and Iranian studies, no sex difference was seen in HBsAg positives. There was no HBsAg positive subject with a history of blood transfusion, illegal drug consumption, or Imprisonment in our study. It can confirm other transmission routs such as early childhood infections, congenital, or sexual modes.

Anti-HBs was found in 51.4% of women and 43.8% of men and did not show any correlation with gender, but with younger ages ($p = 0.001$) and could be perfectly explained with high efficacious national vaccination from 1992. The evaluation of the impact of vaccination on three markers, we obtained a strong correlation with anti-HBs whilst HBsAg and anti-HBc did not have any correlation with vaccination even after excluding the subjects with immunity due to HBV acquisition, who had concurrent anti-HBc and/or HBsAg.

Anti-HBc was positive in 9.7% of women and 8.1% of men with no significant difference based on sex and age. Interestingly, this prevalence is closer to reports from endemic countries (10% – 20%).

The majorities of HBsAg positive persons had susceptible spouses and were uninformed about their HBV situation. They could be responsible for transmission of HBV to their sexual partners (Table 2). The transmission rate has been different in several studies; while it was about 65% within two years of marriage in a Chinese survey, it has been about 5% in an Israeli survey. According to an Iranian study this value had been 65.3% in husbands of infected wives and 46.3% in wives of infected husbands, many of which could be prevented perfectly (about 89%) with vaccination of couples before marriage.¹²⁻¹⁴ According to another Iranian survey, applying some prevention measures including determining HBV seromarkers seems to be cost effective in comparison to the financial burden of HBV infection outcomes.¹⁵

About 85% of individuals, who had received HBV vaccine, had positive anti-HBs. Although only 50.3% of subjects had completed a course of hepatitis vaccination, we did not know about their last vaccination date and this factor affects the result too. But comparison of this group with unvaccinated cases indicates the efficacy of vaccination. However, there were two individuals with HBsAg and 16 individuals with anti-HBc who had been vaccinated.

According to credible reports, 1–2 months after the latest dose of a triple course, the expected efficacy of HBV vaccine is 94% – 95%. Failure to complete the vaccination course or delayed measurement of anti-HBs can explain the lower prevalence of anti-HBs in vaccinated subjects of our study. The presence of HBsAg or anti-HBc may be a sign of pre-vaccination exposure to HBV (immunity through infection), sensitivity of non-responders to HBV or recent vaccine injection. The relationship between vaccination and anti-HBs was statistically significant even after excluding these cases ($P < 0.005$).

The higher percentage of anti-HBs titers in subjects with higher levels of education suggests more effective prevention programs for these groups of people. Although we could not find any correlation between level of education and HBsAg and anti-HBc positivity, another Iranian study has obtained these two markers of infection more in subjects with lower levels of education.⁵ This discrepancy may be because of different sample sizes or different regions; however the higher level of knowledge of the general population may cause this result (Table 3).

In 17 persons anti-HBc was detectable, of whom 15 ones had a history of vaccination. This could be due to exposure to the virus before vaccination (remote HBV infection), early acute hepatitis because of incomplete vaccination and inadequate immunization, or carrier state (maybe pre-core mutant).

There were 13 (3.5%) subjects with isolated anti-HBc tests. Isolated anti-HBc is seen in the window period, resolving acute infection, several years after acute infection, or rarely remote infection with decreased HBsAg level. Although we did not test subjects for IgM anti-HBc (for diagnosing of acute infection) and HBV-DNA, but according to other surveys, DNA of the virus is detectable between 0% and 12.7% of isolated anti-HBc positives. This difference might be related to HBV infection prevalence in different populations, so that in an area of low endemicity (0.5%) in the center of Iran, none of those with anti-HBc positive test were HBV DNA positive.¹⁶ The clinical significance of positive isolated anti-HBc is unclear. There are a few reports about transmission by blood and organ donation, but not by other routes such as sexual contact.

One of the limitations of this study was its small sample size that included all subjects who could be recruited to the study. Moreover, determination of HBV DNA could confirm the serologic marker results, but we did not do this test in our study because of financial constraints and unavailability of subjects. We did not ask participants about time of receiving vaccines, which is useful for explanation of levels of serum markers.

In addition, we could not find any correlation between recognized risk factors and exposure to HBV. It might be because of reporting bias for the sake of critical premarital situation and sensitive questions about socially unacceptable behaviors; however the number of missing data in all questions about risk factors regardless of their social acceptance was similar, perhaps because of preoccupation with marriage. The discordance between most

couples, as was found in this study, puts them at risk of HBV acquisition. Further well designed studies are required for precise calculation of cost-effectiveness of HBV serologic testing in premarital screening.

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