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Seroprevalence and Risk Factors for Hepatitis B in an Adult Population: The First Report from Birjand, South Khorasan, Iran

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Abstract

Background: Infection with the hepatitis B virus (HBV) is an important global health problem. Knowledge of the geographic distribution pattern of HBV infection can help to control the spread of the disease.
Objectives: To determine the prevalence of HBV infections and risk factors for the disease for the first time in Birjand, Southeastern Iran.
Methods: This was an analytical cross-sectional study conducted in 2013 - 2014 of 5235 HBV cases in Birjand, South Khorasan Iran. Subjects aged 15 - 70 y were selected using the cluster sampling method. Blood samples were taken and tested at a reference laboratory for the hepatitis B core antibody (anti-HBc). Seropositive specimens were tested for the hepatitis B surface antigen (HBsAg).
Results: The mean age (± SD) was 39.07 (± 14.04) y, and 786 (15%) subjects were anti-HBc positive. The prevalence of HBsAg was 1.6% (n = 85). The prevalence of anti-HBc seropositivity was significantly higher in subjects with a lower level of education (P = 0.09), older subjects (P = 0.001), intravenous (IV) drug users (P<0.05), subjects with piercings (P < 0.001), and subjects with a positive history of familial HBV or HCV infection (P < 0.05). It was also significantly higher in those who drank alcohol (P = 0.09) or had a history of blood transfusions (P = 0.001), cupping (P = 0.004), hospital admission (P = 0.03), or endoscopy (P = 0.002). The rate of HBsAg positivity was significantly higher in subjects with a history of cupping (P=0.03), a positive history of familial HBV or HCV infection (P < 0.05), and older subjects (P = 0.015).
Conclusions: Although the frequency of HBsAg seropositivity in the present study was close to that observed in the overall Iranian population, the seroprevalence of anti-HBc was higher, possibly due to the exposure of the elderly to more risk factors. The risk factors were similar. These included a history of blood transfusions, cupping, hospital admission, endoscopy, or familial HBV/HCV infection, in addition to piercings and drug abuse.

Keywords: Hepatitis B, Epidemiology, Risk Factor, Iran, Hepatitis B Surface Antigen

1. Background

Hepatitis B is one of the most common and dangerous infections worldwide. It is more common in developing countries, such as Iran, and other Middle Eastern countries than in developed countries. Its prevalence is associated with the socio-cultural and economic climate. Among Asian countries, the endemicity of hepatitis B is highest in China. In the Middle East, the endemicity is low in Iran, Bahrain, and Kuwait but high in Egypt, Jordan, Oman, Palestine, Yemen, and Saudi Arabia (1).

Preventive vaccination of newborns, increased knowledge of the risk factors for hepatitis B, and vaccination of high-risk groups have led to a marked decrease in hepatitis B virus (HBV) endemicity in recent years in Iran, although it remains high. According to various studies, the prevalence of the hepatitis B surface antigen (HBsAg) varies, but a prevalence of 1.2% - 5% was reported in Iranian provinces (2-4). The prevalence of hepatitis B infection is greater in some eastern provinces, such as Sistan, Baluchestan, and Khorasan, due to their closer ties to Afghanistan, where the prevalence of the disease is higher (5, 6).

The evaluation of risk factors for HBV infection is important for designing strategies to control the disease (7). Depending on the region, many risk factors for hepatitis B infections have been proposed. Changes in the transmission pattern of new cases of hepatitis B in Iran have been reported recently. There have been no comprehensive seroepidemiological studies of the prevalence of hep-

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atitis B in Birjand, the capital of South Khorasan province.

2. Objectives

The present study evaluated the risk factors for hepatitis and the prevalence of the disease in a large group of patients in South Khorasan, Iran.

3. Methods

A cross-sectional study was conducted in Birjand, the capital of the South Khorasan province of Iran, in 2013 - 2014. Birjand is located in northeastern Iran where the weather is warm and dry. The estimated population of Birjand is about 178,000. The city does not have many industries and is surrounded by deserts. The south Khorasan province borders Afghanistan. The study consisted of 5235 individuals aged 15 - 70 years. The sample size was estimated according to the following formula, in which the overall national prevalence of hepatitis B was considered equal to 3% (Equation 1):

$$n = \frac{Z^2 \mathrm{pq}}{d^2} \quad (1)$$

Where Z^2 is constant, p is the prevalence, q = (1 - p), and d is the estimated error risk. A multistage sampling method (randomized clusters) was used. According to the city's postal areas, 250 clusters were identified. In each cluster, 20 subjects (equal gender distribution ratio) were selected. Equal numbers of males and females were selected from a range of age groups (15 - 24, 25 - 34, 35 - 44, 45 - 54, 54 - 70, and older than 70 years).

After explaining the research project and the importance of hepatitis B, the participants were invited to take part in the study and undergo hepatitis B testing at no cost. The inclusion criteria were aged 15 - 100 years, consented to take part in the study, possessed national identification documents, and had the ability to respond to the interviewer. The exclusion criteria included non-Iranian nationality. After explaining the nature of the research project, each participant completed an informed consent form. The checklist of risk factors and epidemiological characteristics were based on a review of the literature.

Experienced technicians performed the blood sampling, data gathering, and questionnaire administration. Venous blood (8 mL) was collected from each subject. The specimens were placed in sterile-covered storage tubes and stored at -30°C until collection of all the samples. The results were relayed to the subjects.

3.1. Assays

The detection of the hepatitis B core antibody (anti-HBc) (bioELISA anti HBc, Biokit, Barcelona, Spain) in the serum samples was done using a commercially available enzyme-linked immunosorbent assay kit (bioELISA anti HBc, Biokit, Barcelona, Spain). Seropositive specimens were tested for HBsAg (Enzygenost® HBS Ag 5.0, Dade Behring Inc., Newark, U.S.). AST and ALT were analyzed by enzymatic photometry. All measuring laboratory equipment was calibrated. All the subjects underwent a clinical and physical examination by a medical doctor. One individual collected all the data.

3.2. Statistical Analysis

This project was approved by the ethics and scientific committee of Birjand University of Medical Sciences, Iran (approval code: bums.1394.11). The quantitative and qualitative data were analyzed using SPSS, ver. 21 (Chicago, IL, U.S.). For qualitative data, the normal distribution was assessed using the K-S test. For data with a normal distribution, the means ± standard deviation (SD) were computed, and independent T-tests and the Mann-Whitney test were performed. P values were used to evaluate the null hypothesis. Odds ratios (ORs) were calculated to determine the association between one group and another. Qualitative data were described and analyzed using frequencies and prevalence, in addition to the chi-squared test. A P value less than 0.05 was considered statistically significant.

4. Results

The mean age was 39.07 ± 14.04 y, and 2732 (52.2%) participants were females. Most of the subjects were married (n = 4248, 81.1%), and 1567 (29.9%) had a college education. Detailed demographic features are shown in Table 1.

The HBc-positive antibody was detected in 786 subjects, giving an overall prevalence of 15%. The prevalence of HBc seropositivity was significantly higher in males (16.9% in males vs. 13.3% in females; P < 0.001, OR = 1.33, CI = 1.15 - 1.6), those with a lower level of education (26.6% in the illiterate group vs. 8.9% in the college group, P < 0.001), and older subjects (32.1% in those aged 65 - 70 years [oldest group] vs. 3.9% in those aged 15 - 24 years [youngest group]; P = 0.001). Among the known risk factors that were assessed, the prevalence of HBc seropositivity was significantly higher in subjects with a history of blood transfusions vs. those with no history (23.5% vs. 14.7%; P = 0.001, OR = 1.8, CI = 1.3 - 2.5), a history of cupping vs. no history (18.3% vs. 14.4% ; P = 0.004, OR = 1.3 , CI = 1.1 - 1.6), a history of hospitalization vs. no history (16.2% vs. 14.1%; P = 0.03, OR = 1.2 , CI = 1.01 - 1.4), a history of endoscopy vs. no history (19.7% vs.

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Table 1. Demographic Characteristics of the Participants

Item/Status	No. (%)
Gender	
Male	2503 (47.8)
Female	2732 (52.2)
Marital status	
Single	873 (16.7)
Married	4248 (81.1)
Divorced	20 (0.4)
Widow	94 (1.8)
Educational status	
Illiterate	575 (11)
Primary	960 (18.3)
Pre-high school	584 (11.2)
High school	1549 (29.6)
College	1567 (29.9)
Age group, y	
15 - 24	942 (18)
25 - 34	1253 (23.9)
35 - 44	1051 (20.1)
45 - 54	1007 (19.2)
55 - 64	714 (13.6)
65 and older	268 (5.1)

14.5%, P = 0.002, OR = 1.5 , CI = 1.14 - 1.9), a history of oral drug addiction vs. no history (26.6% vs. 14.7%, P < 0.001, OR = 2.1, CI = 1.4 - 3.1), and a history of intravenous (IV) drug abuse vs. no history (44.4% vs. 15%, P = 0.03, OR = 4.5, CI = 1.2 - 16.9). In addition, the prevalence of HBc-seropositivity was significantly higher in subjects who had at least one positive case of hepatitis B in the family (37.3% vs. 14.5%, P < 0.001, OR = 3.5, CI = 2.4 - 5.1) or at least one positive case of hepatitis C in the family (39.1% vs. 14.9%, P < 0.001, OR = 3.7, CI = 1.6 - 8.5).

In contrast, the numbers of HBc-seropositive cases were lower in those with a history of piercing compared to those with no such history (12.2% vs. 16.4%; P < 0.001, OR = 0.71, CI = 0.84 - 0.59). A history of hepatitis B vaccination (13.1% vs. 15.7%, P = 0.03, OR = 1.23, CI = 1.03 - 1.5) and use of dental services (13.5% vs. 15.7%, P = 0.03, OR = 0.84, CI = 0.99 - 2.43) were also less common among HBc-positive antibody cases in comparison with HBc-negative antibody cases (Table 2).

In the study, 85 (1.6%) of the subjects were HBsAg positive, 2.1% of whom were males and 1.2% of whom were females. (P = 0.01, OR = 1.74, CI = 1.12 - 2.7). HBsAg seropositivity was detected in 0.5% of subjects in the youngest age group and 2.2% of subjects in the oldest age group (P = 0.015). HBsAg seropositivity was present in 2.5% of cases with a history of cupping vs. 1.5% in those with no history (P = 0.03, OR = 1.7, CI = 1.04 - 2.9), 16.1% of cases with at least one positive case of hepatitis B in their family vs. 1.3% in those with no history (P < 0.001, OR = 14.7, CI = 8.5 - 25.6), and 17.4% of subjects with at least one positive case of hepatitis C in their family vs. 1.6% in those with no history (P < 0.001, OR = 13.3, CI = 4.4 - 40) (Table 2).

The mean ± SD of ALT among the anti-HBc-positive cases was 23.9 ± 16.1 mg/dL compared to 23.6 ± 16.3 mg/dl in the negative cases (P = 0.004), whereas the AST was not significantly different between these two groups (P = 0.07). The mean ± SD of ALT and AST in those with a positive HBsAg test was 29.8 ± 31.3 and 25.2 ± 16.3 mg/dL, respectively, compared to 32.5 ± 15.9 and 21.02 ± 11.6 mg/dL, respectively, in those with no positive test (P = 0.022 and P = 0.002, respectively, Table 3).

5. Discussion

The prevalence of HBc antibody and HBsAg seropositivity in the present study was 15% and 1.6%, respectively. These values are similar to those reported in other regional and national research. The prevalence of the HBc antibody was significantly higher in men, older subjects, and those with a low level of education.

In the next the two decades, the prevalence of HBsAg and HBc antibodies in Iran will decrease due to a national hepatitis B vaccination program in the neonatal and infancy periods. The higher incidence of HBV infection in the older groups in the present study was expected, as the possibility of contact with HBV increases with older age (7).

Apart from known conventional risk factors, such as IV drug abuse, sexual contacts, piercings, familial infections, and tattoos, we found that some local, traditional unsafe behaviors, including traditional cupping and oral drug abuse, appeared to be risk factors for positive HBc antibody and HBsAg tests. Traditional cupping (bloodletting) is often performed in nonstandard health care units, and the practice is not regulated by the government. The risk of transmission in exposed cases is increased (5). In a study in Mashhad in northeastern Iran, the nearest geographical and culturally similar city to Birjand, Fathimoghaddam et al. reported that traditional cupping, in addition to drug abuse, was the major risk factor for hepatitis B infections in their study population (5). Rafatpanah et al. demonstrated that HTLV-1 infection was higher in patients with a history of traditional cupping in Mashhad (8). To prevent infections transmitted via blood, governmental regulation

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Table 3. Liver Function Test Results and Viral Test Findings[a]

Item	HBc Antibody Positive	HBc Antibody Negative	P Value	HBsAg Positive	HBsAg Negative	P Value
ALT	23.9 ± 16.1	23.6 ± 16.3	0.004	29.8 ± 31.3	23.5 ± 15.9	0.022
AST	21.4 ± 9.6	21.04 ± 12.1	0.07	25.2 ± 16.3	21.02 ± 11.6	0.002

[a]Based on the Mann-Whitney test.

of traditional cupping and piercing practices in our local area seems to be warranted.

In this study, the most important risk factor for hepatitis B infection was familial infections. For HBsAg, the risk of a positive test was 13 and 14 times greater in subjects with a family history of hepatitis B and C infections, respectively, vs. those with no such history, and while this ratio for the HBc antibody was estimated more than 3.5 times in these cases. This finding was previously confirmed in nucleotide studies (9). Thus, families should be informed about how viral hepatitis is transmitted.

The risk factors for hepatitis B can differ, depending on cultural, educational, socio-economic, and geographical variables. Population-based cross-sectional studies and seroepidemiological surveys can shed light on the status of hepatitis B in the target population (2, 10).

In Iran, many studies have tried to determine the prevalence of HBV infections and risk factors for the disease in different regions in the country. A brief review on some of these is provided in Table 4.

In the present study, it is worth nothing that the prevalence of HBc-positive and HBsAg-positive cases was higher in men. Thus, in Birjand, men seem to have more contact with the HBV. This may be due to occupational exposure or addiction behaviors.

The subjects with a positive HBc antibody test had a lower history of using dental services than those with a negative test. Thus, they may be less likely to take care of their health (25). A previous study demonstrated that public health was associated with the prevalence of hepatitis B infection in this geographical region (26).

Birjand is a city of high immigrant diversity (mostly Afghan). Previous studies reported that the prevalence of hepatitis B infections was higher among Afghan immigrants and that the higher rate of hepatitis B infections in eastern Iran could be associated with the number of Afghan immigrants (27, 28). Another study suggested that all immigrants, regardless of the time since they had left their home countries, should undergo screening for major transmissible infections (29). Moreover, Behzadi et al. recommended that HBV screening of immigrants should be take place at entrances to country borders (27). The same study strongly recommended free vaccination and treatment programs and improving the level of HBV knowledge among Afghan immigrants in Iran.

As shown in Table 4, the mean prevalence of HBsAg seropositivity in Iran was 0.8% - 2.5%, although a prevalence of 6.3% was reported in Golestan. The observed prevalence in the present study was in the middle of this range. The geographical pattern in the prevalence of HBsAg changed according to various risk factors in the area. More studies are needed to determine the role of different risk factors in the increased frequency in various cities, such as Zahedan, Tehran, and Golestan. The higher rate of drug abuse, increased use of risky interventions (e.g., cupping), and a lower level of knowledge about hepatitis may be keys to the different pattern of risk factors in Birjand compared to other cities and provinces.

Well-known risk factors for hepatitis B infection are a history of imprisonment, tattooing, illegal drug consumption, blood transfusions, and lower education levels. Living with family and friends or being lonely was reported to be risk factor for hepatitis B infections, although not all studies confirmed these findings.

In conclusion, the prevalence of HBsAg seropositivity seems to be lower in the central provinces of Iran than in the eastern and northern provinces. Patients with abnormal levels of ALT or AST and those exposed to the aforementioned risk factors should undergo hepatitis B screening. The prevalence of hepatitis B infection is decreasing in Iran and worldwide because of vaccination programs (30). However, information is needed on the characteristics of the spread of hepatitis B and associated risk factors in subprovinces to design a target plan to control hepatitis B-related mortality and morbidity in the elderly and prevent transmission in the at-risk younger population. A map of the spread of hepatitis B in different areas would help to anticipate risk factors for the disease.

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Table 4. A Review of the Literature on Previous Similar Studies in Iran

Province or City	Author	Year	Sample Size	HBsAg Positive, %	HBc Antibody Positive, %	Main Risk Factors
Birjand	Ziaee et al. (3)	2015	5235	1.6	15	Male, age, drug abuse, family history
Tehran	(4, 11-13) 4 studies	2001 - 2007	7870	2.2	N/A	Male
East Azerbaijan	3 studies (4, 14, 15)	2001 - 2005	5320	1.3	N/A	Male
Golestan	3 studies	2003 - 2007	4931	6.3	N/A	Male
Isfahan	Ataie	2006	816	1.3	N/A	
Hormozgan	Merat	2006	1988	2.4	N/A	Male
Zahedan	Ansari-Moghaddam (6)	2008 - 2009	2578	2.5	N/A	Male, married, older age
Kermanshah	Kazerani	1999 - 2003	6820	1.3%	N/A	N/A
Nahavand	Alizadeh et al. (16)	2006	1824	2.3	7.8	History of surgery or imprisonment
Kurdistan	Alavian et al. (17)	2012	1613	0.8	5.02	Older age, marriage, intrafamilial HBV transmission
Mashhad	Fathimoghaddam et al. (5)	2011	1652	1.39	N/A	Older age, married, Afghani, and traditional cupping
Amol	Keyvani et al. (18)	2014	6146	0.9	10.5	A history of hepatitis among first-degree family members, a history of tattooing, a history of previous hepatitis, male sex, and age
Kohgiloyeh and Boyerahmad	Khosravani et al. (19)	2009 - 2010	2009	1.2	N/A	Male, drug abuse, level of education, and place of residence
Kerman	Hayatbakhsh et al. (20)	2011 - 2012	370	1.1	8.9	Living status
Yazd	Javadzadeh Shahshahani et al. (21)	2013	254760	0.25	N/A	Recipient of allogenic whole blood donation
Mazandaran)	Mousavi et al. (22)	2011 - 2013	2650	0.08	N/A	N/A
Chaharmahal and Bakhtiari	Moezzi et al. (23)	2012 - 2013	3000	1.3	N/A	Male, aged over 55 y, farmer, and nonpublic occupation
Sistan Baluchestan	Salehi et al. (24)	2012	3989	3.38	23.5	Marital status and addiction

Abbreviation: N/A, Not available

Footnotes

Authors' Contribution: All the authors had a role in the research design and data collection and in writing the final draft. Amin Saburi and Gholamreza Sharifzadeh analyzed the data. Masood Ziaee acted as chief coordinator.

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Table 2. Demographic Characteristics of the Participants and Their Test Results

Item/Status	HBc Antibody Positive	P Value (OR and CI)	HBsAg Positive	P Value (OR and CI)
Gender		< 0.001 (1.33 and 1.15 - 1.6)		0.01 (1.74 and 1.12 - 2.7)
Male	424 (16.9)		52 (2.1)	
Female	362 (13.3)		33 (1.2)	
Education		< 0.001		0.09
Illiterate	153 (26.6)		12 (2.1)	
Primary	206 (21.5)		17 (1.8)	
Pre-high school	97 (16.6)		10 (1.7)	
High school	190 (12.3)		32 (2.1)	
College	140 (8.9)		14 (0.9)	
Age group				
15 - 24	37 (3.9)	< 0.001	5 (0.5)	0.015
25 - 34	99 (7.9)		15 (1.2)	
35 - 44	158 (15)		21 (2)	
45 - 54	218 (21.6)		24 (2.4)	
55 - 64	188 (26.3)		14 (2)	
65 - 70	86 (32.1)		6 (2.2)	
Alcohol drinking		0.09		-
Yes (38)	2 (5.3)		0	
No (51970)	784 (15.1)		85 (1.6)	
Smoker		0.09		0.66
Yes (367)	66 (18)		7 (1.9)	
No (4868)	720 (14.8)		78 (1.6)	
Blood transfusion		0.001 (1.8 and 1.3 - 2.5)		0.34
Yes (196)	46 (23.5)		1 (0.5)	
No (5039)	740 (14.7)		84 (1.7)	
Cupping		0.004 (1.3 and 1.1 - 1.6)		0.03 (1.7 and 1.04 - 2.9)
Yes (802)	147 (18.3)		20 (2.5)	
No (4433)	639 (14.4)		65 (1.5)	
Major surgery		0.98		0.6
Yes (1235)	217 (17.6)		18 (1.5)	
No (4000)	569 (14.2)		67 (1.7)	
Minor surgery		0.03		0.24
Yes (1470)	221 (15)		19 (1.3)	
No (3765)	565 (15)		66 (1.8)	
Hospitalization		0.03 (1.2 and 1.01 - 1.4)		0.93
Yes (2304)	374 (16.2)		37 (1.6)	
No (2931)	412 (14.1)		48 (1.6)	
Dental service		0.035 (0.48 and 0.99 - 2.43)		0.68
Yes (1709)	231 (13.5)		26 (1.5)	

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No (3526)	555 (15.7)		59 (1.7)	
Endoscopy		0.002 (1.5 and 1.14 - 1.9)		0.08
Yes (507)	100 (19.7)		13 (2.6)	
No (4728)	686 (14.5)		72 (1.5)	
Non-IV drug abuse		≥ 0.001 (2.1 and 1.4 - 3.1)		0.07
Yes (139)	37 (26.6)		5 (3.6)	
No (5069)	749 (14.7)		80 (1.6)	
IV drug abuse		0.03 (4.5 and 1.2 - 16.9)		0.7
Yes (9)	4 (44.4)		0	
No (5226)	782 (15)		85 (1.6)	
Tattoo		0.72		1
Yes (81)	11 (13.6)		1 (1.2)	
No (5154)	775 (15)		84 (1.6)	
Illegal sexual contact		0.5		0.52
Yes (44)	5 (11.4)		1 (2.3)	
No (5191)	781 (15)		84 (1.6)	
Needle stick		0.85		1
Yes (36)	5 (13.9)		0	
No (5199)	781 (15)		85 (1.6)	
Hepatitis B vaccination		0.03 (1.23 and 1.3 - 1.5)		0.76
Yes (1340)	176 (13.1)		23 (1.7)	
No (3895)	610 (15.7)		62 (1.6)	
Family member with hepatitis B		≥ 0.001 (3.5 and 2.4 - 5.1)		≥ 0.001 (14.7 and 8.5 - 25.6)
Yes (118)	44 (37.3)		19 (16.1)	
No (5117)	742 (14.5)		66 (1.3)	
Family member with hepatitis C		0.004 (3.7 and 1.6 - 8.5)		≥ 0.001 (13.3 and 4.4 - 40)
Yes (23)	9 (39.1)		4 (17.4)	
No (5212)	777 (14.9)		81 (1.6)	
Piercings		≥ 0.001 (0.71 and 0.84 - 0.59		0.08
Yes (1686)	205 (12.2)		20 (1.2)	
No (3549)	581 (16.4)		65 (1.8)	

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