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Seroepidemiology of HBV Infection in Kermanshah- West of Iran; A Population Based Study

Seyed Moayed Alavian [1*], Seyed Vahid Tabatabaei [2], Samad Nourizad [3], Feyzollah Mansouri [3], Nahid Khademi [3], Sedigheh Amini Kafi-abad [4], Ahmad Gharehbaghian [4], Hassan Abolghasemi [4]

[1] Baqiyatallah University of Medical Sciences, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Tehran, IR Iran
[2] University of Muenster, St. Marien Hospital GmbH, Gastroenterology Clinic, Lunen, Germany
[3] Kermanshah University of Medical Sciences, Kurdistan, IR Iran
[4] Iranian Blood Transfusion Organization Research Center, Tehran, IR Iran

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ABSTRACT

Background: hepatitis B virus (HBV) infection is a major public health problem that affects billions of people worldwide. The lack of information on HBV prevalence among the general population is an obstacle to formulate effective policies to reduce the burden of viral hepatitis.
Objectives: This population based serological survey was conducted in Kermanshah province to determine the local prevalence and risk factors of HBV infection.
Patients and Methods: 1979 healthy subjects were selected from all districts of Kermanshah province (in the west of Iran) using random cluster sampling. Subjects between 6 and 65 years of age were included with mean age of 35 ± 13. Serum samples were tested for HBcAb, HBsAg and anti-HDV antibody. To carry out lab tests the third generation of ELISA was used. Various risk factors were recorded and multivariate analysis was performed.
Results: The prevalence of HBsAg and HBcAb in Kermanshah was 0.75% (95% CI 0.44; 1.21) and 8.28% (95% CI 7.13; 9.56), respectively. One case of HDV-Ab was found. Predictors of HBsAg or HBcAb in multivariate analysis were: old age, being male, history of tattooing and history of dental procedure.
Conclusions: approximately 8% and less than 1% of general population in Kermanshah are HBcAb seropositive and active carriers of HBV infection, respectively. Age, sex and history of tattoo and dental procedures are major risk factors of HBV seropositivity in this province.

Implication for health policy/practice/research/medical education:
Lack of adherence to Hygiene protocols by dentists and those who conduct cosmetic invasive procedures are major causes of HBV infection in Kermanshah and should be more closely supervised. We recommend to read this article by health policy makers, gastroenterologists, hepatologists, infectious especialists and Internists.

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* *Corresponding author:* Seyed-Moayed Alavian, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Ground floor of Baqiyatallah Hospital, Mollasadra Ave., Vanak Sq. P.O.Box: 14155-3651, Tehran, IR Iran. Tel/Fax: +98-2188067114, *E-mail:* alavian@Thc.ir

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1. Background

Hepatitis B virus (HBV) infection is a major global health problem and the most serious type of viral hepatitis. WHO has estimated that currently two billion people have been infected by the HBV and more than 350 million people have chronic infection. Annually more than 500,000 deaths occur because of cirrhosis and hepatocellular carcinoma caused mainly by HBV infection (1, 2).

Iran, like other Middle East countries has an intermediate prevalence of hepatitis B chronic infection. The prevalence of chronic carrier state in Iran was reported around 3% in 1980s (3). It is estimated that over 35% of Iranian have been exposed to the HBV and about 3% are chronic carriers (3), ranging from 1.7% in Fars province to over 5% in Sistan-Balouchestan (4). Indeed, the first report regarding HBV infection in Iran was published in 1972 (5). In later studies from 1977 to 1993, the rate of HBV infection was reported between 3.49% and 3.5% in general population and blood donors (3, 5).

In 2008, in a systematic review of literature, available data on seroepidemiology of HBV infection in general population of Iran were gathered and pooled. According to the findings, the HBV infection prevalence in Iran was estimated 2.14% (95% CI: 1.92; 2.35) (6) and the data were available for only 7 out of 30 provinces of Iran. It might be concluded that around 2% of Iranians are HBs Ag positive (7). Kermanshah is one of the provinces with missing data regarding seroprevalence of HBV infection. From the public health view, in addition to seroepidemiology, the distribution of risk factors of HBV infection is of great importance, since with proper intervention, HBV seroepidemiology can be prevented. The major known risk factors for transmission of HBV are from HBsAg positive pregnancy (vertical transmission during delivery) (8, 9),blood transfusion, hospitalization, tattoo, intravenous drug abuse (10, 11) and high risk sexual behaviors (12, 13).

2. Objectives

Since the lack of information on HBV prevalence and distribution of its risk factors among the general population is an obstacle to formulate effective policies to reduce the burden of viral hepatitis, this population based study was designed to determine an accurate estimation of HBV infection epidemiology and associated risk factors in Kermanshah. Kermanshah is a province in the west of Iran with 25,059 km^2 area and the population of 1,879,385 . This province is of great importance since it has common border with Iraq that has an impaired health care system and lacks major health statistics including the HBV infection rate.

3. Patients and Methods

3.1. Study Population

The general population of Kermanshah was studied. Subjects between 6 and 65 years of age were included. The exclusion criteria were: temporal inhabitants of the household, non-Iranian nationalities or those who did not consent to the study or refused to give blood samples. The demographic characteristics of Kermanshah province are shown in *Table 1*.

3.2. Sample Size Calculation

The sample size was calculated by [DEFF*Np (1-p)]/ [(d^2/ $Z^2_{1-\alpha/2}$* (N-1)+p* (1-p)] equation, when N stands for population size (1,440,518), P for Hypothesized % frequency of outcome factor in the population (here 5% ± 2), d for confidence limits as % of 100 (absolute +/- %, here 2%) and DEFF for design effect of cluster surveys which was set to 1. The sample size was calculated 1796 subjects at 99.99% confidence level. This number was rounded up to 2000 subjects.

Table 1. Census Information, 14 Districts of Kermanshah Province

Districts	Area, km^2	Population	Urban, %	Male, %	Literacy, %
Total	25059	1879385	66.8	50.9	82.1
Islamabad	2109	152500	59.8	50.8	79.7
Paveh	811	52783	51.3	49.7	81.3
Salas	1704	38580	22.9	51.3	70.2
Javanrood	772	62797	69.3	50.7	76.9
Dalahoo	1930	42598	30.3	49.4	73.6
Ravansar	1110	45324	36.5	51.4	78.2
Pol e zahab	930	84945	40.8	51.7	78.9
Sanghar	2307	97012	46.3	49.9	77.6
Sahneh	1609	76414	45.8	50.02	78.4
Gasrshirin	2014	24383	65.2	56.8	82.6
Kermanshah	5639	967196	82.7	51.1	85.8
Kangavar	805	80608	60.8	50.6	82.0
Guilan e Gharb	2146	62945	34.8	49.6	74.3
Harsin	1173	91300	58.9	51.03	78.3

3.3. Sampling and Randomization

Clustered random sampling was employed. One hundred clusters of 20 were randomly selected from districts within Kermanshah province. Postal codes or family registry codes were used to select the first household for each cluster randomly. Blood samples were obtained from subjects and questioners were completed by a trained interviewer for each subject. The questionnaires comprised demographic and anthropometric data and also risk factors for HBV infection.

3.4. Laboratory Tests

The blood samples were transferred to the regional laboratory. Serums were separated in local laboratory, then were frozen at -20°C and transferred to the central laboratory of Keyvani institute in Tehran. HBsAg, HBcAb and HDV-Ab were evaluated using Enzygnost HBsAg, 5.0 kit (Dade Behring, Germany) and Hepanostica anti-HBc Uni-Form kit (Biomerieux, France), and DiaSorin ELISA kit (Italy) respectively.

3.5. Ethics

The protocol of study was approved by the Institutional Review Board of the Baqiyatallah University of Medical Sciences, Baqiyatallah Research Center for Gastroenterology and Liver Diseases and Iranian Blood Transfusion Organization. Written informed consent was obtained from participants before data collection.

Table 2. Demographic Data of the Study Population

	Islam Abad	Paveh	Salas	Javanrood	Dalahoo
Subjects interviewed, No.	160	40	40	60	60
Samples collected, No.	160	40	40	60	60
Male, %	49	50	50	50	50
Age, Mean ± SD	34 ± 1	36 ± 2	35 ± 2	30 ± 1	36 ± 2
Rural, %	36	100	95	28	33
Currently married, %	72	80	68	60	73
History of blood transfusion, %	0.6	2.5	12.5	3.3	8.3
Addiction, %	0.0	2.5	0.0	1.7	1.7
IV. addiction, %	0.0	0.0	0.0	0.0	0.0
Tattoo, %	20	23	63	32	25
imprisonment, %	0.6	2.5	5	6.7	1.7
	Ravensar	**Pol e Zahab**	**Sanghar**	**Sahneh**	**Ghasr e Shirin**
Subjects interviewed, No.	60	80	100	80	20
Samples collected, No.	60	80	100	80	20
Male, %	50	50	50	46	50
Age, Mean ± SD	34 ± 2	35 ± 2	33 ± 1	35 ± 1	37 ± 3
Rural, %	67	74	39	71	100
Currently married, %	62	70	72	79	70
History of blood transfusion, %	5	15	5	1.3	0.0
Addiction, %	0.0	1.3	8	15.3	0.0
IV. addiction, %	0.0	1.3	6	1.3	0.0
Tattoo, %	25	45	17	31	25
imprisonment, %	0.0	2.5	1	2.5	5
	Kermanshah	**Kangavar**	**Guilan e Gharb**	**Harsin**	**Total**
Subjects interviewed, No.	1060	79	61	100	2000
Samples collected, No.	1039	79	61	100	1979
Male, %	50	51	49	50	50
Age, Mean ± SD	35 ± 0.5	35 ± 2	34 ± 2	37 ± 2	35 ± 0.3
Rural, %	27	48	64	60	40
Currently married, %	67	66	67	75	69
History of blood transfusion, %	2.2	1.3	3.3	3	3.2
Addiction, %	6.5	10.1	3.3	2	5.2
IV. addiction, %	0.2	0.0	0.0	0.0	0.2
Tattoo, %	16	28	18	29	21.3
imprisonment, %	2.3	6.3	3.3	2	2.2

3.6. Statistical Analysis

Statistics of all variables were summarized in *Table 2*. Continuous variables were presented as mean values ± standard deviation (SD), while qualitative and discrete variables were presented as absolute and relative frequencies in the form of percentage. chi –square test was applied to assess associations between categorical variables. The 95% CI for rate of HBcAb or HBsAg positivity was calculated by Mid P-Exact method if NPQ ≤ 5 when N is sample size, *P* is the rate of positive cases and Q is 1 – (rate of positive cases). If NPQ had been ≥ 5, then normal approximation method (Wald) would have been used to make 95% CI. Student's t-test was employed to compare continuous and qualitative variables. Multivariable logistic-regression analysis involving recorded risk factors and patients' characteristics that had significant P value in univariate analysis of HBV serostatus were performed to identify independent predictors of positive HBV seromarkers. A stepwise procedure was deployed at $P = 0.05$ as the threshold level for variables to be entered into and retained in the final model, and $P = 0.1$ as the threshold level for variables to be removed. SPSS version 18 was employed to carry out all computations.

4. Results

A total of 2000 subjects were interviewed in 14 districts of Kermanshah province. 21 subjects who refused to give blood samples or had inadequate samples were excluded. A total of 1979 samples were analyzed. The demographic characteristics of the population under study are given in *Table 2*. Totally 164, 8.29% (95% CI 7.13; 9.56) and 15, 0.75% (95% CI 0.44; 1.21) subjects from 1979 participants were HBcAb and HBsAg positive respectively. Furthermore one case of positive HDV-Ab was found. The rate of positive HBV seromarkers in various districts of Kermanshah is given in *Figure 1*.

Paveh was the only district with no detected case of both HBsAg and HBcAb. HBsAg was not detected in the following districts despite HBcAb prevalence of 5 to 10%; Sanghar, Sahneh, Harsin, Guilan- e- Gharb, Ghasre Shirin, Pol e Zahab, Salas and Javanroud. The most prevalent area in respect to HBsAg was Kangavar with rate of 3.79% (95% CI 0.97; 9.98) and in respect to HBcAb was Ghasr e Shirin with rate of 10.00% (95% CI 1.71; 29.29). In univariate analysis, there was no significant difference in the rate of HBsAg ($P = 0.3$) or HBcAb ($P = 0.6$) among various dis-

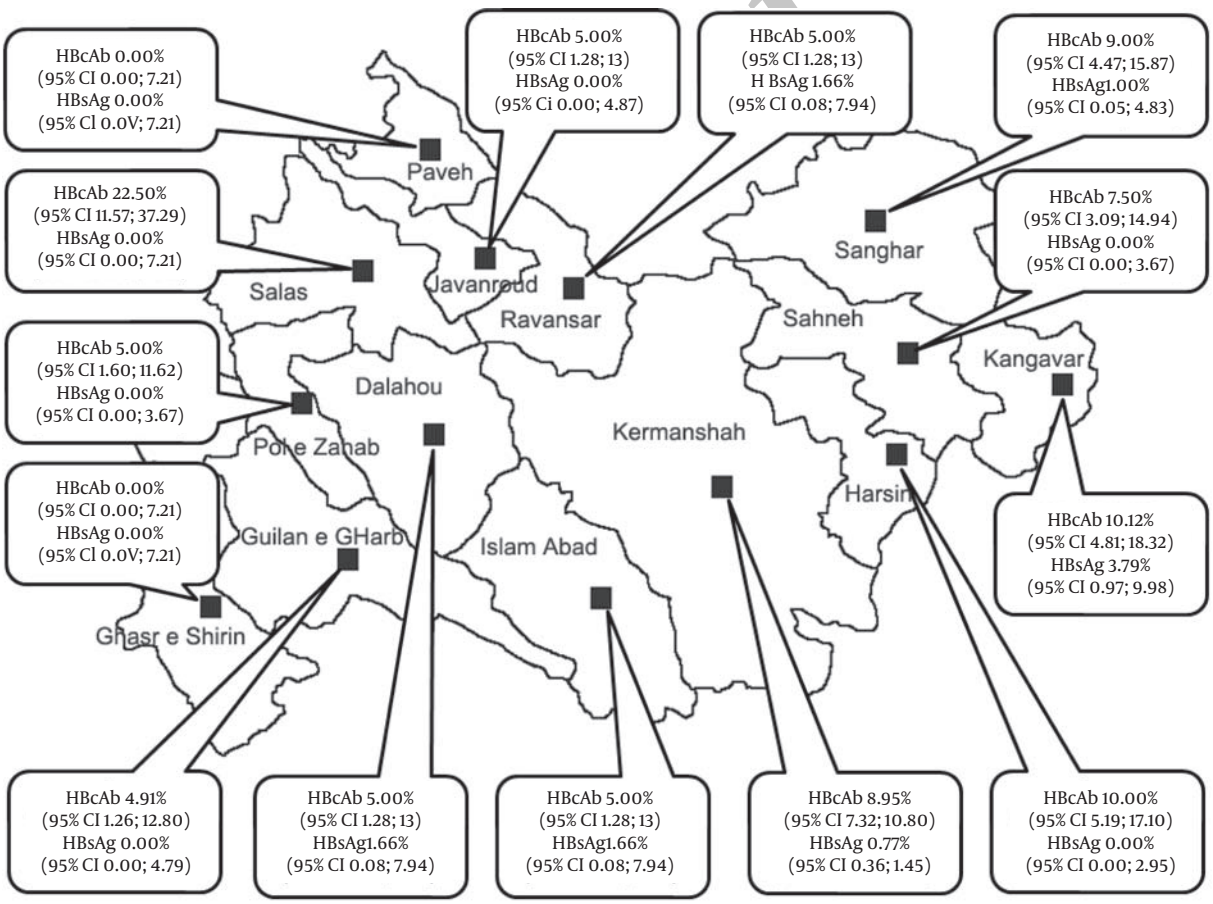

Figure 1. Geographical Distribution of HBcAb and HBsAg in Various Districts of Kermanshah Province

Risk Factors	Index group	Reference group	OR (95% CI)	P value
Male vs. Female	98 (981)	66 (994)	1,56 (1,13; 2,16)	0,01
Urban vs. Rural	93 (1167)	70 (781)	0,88 (0,64; 1,22)	0,44
Age 46 65vs. 6-2g	107 (504)	21 (783)	9,78 (6,03; 15,86)	0
Age 3Œ45vs. 6-29	36 (682)	21 (783)	2,02 (1,17; 3,5)	0,01
Smoking	47 (307)	117 (1668)	2,4 (1,67; 3,45)	0
Married vs. notmarried	148 (1429)	16 (546)	3,83 (2,26; 6,47)	0
Transfusion yes vs. no	6 (64)	157 (1896)	1,15 (0,49; 2,7)	0,76
Addiction yes vs. no	17 (112)	147 (1863)	2,09 (1,21; 3,6)	0,01
Tattooing yes vs. no	60 (421)	104 (1550)	2,31 (1,65; 3,24)	0
Traditional cupping yesvs. no	15 (110)	149 (1861)	1,81 (1,03; 3,21)	0,04
Dental procedure yes vs. no	153 (1562)	11 (413)	3,97 (2,13; 7,39)	0
HospitalLzation yes vs. no	84 (905)	80 (1068)	1,26 (0,92; 1,74)	0,15
Imprisonmentyes vs. no	7 (42)	154 (1872)	2,23 (0,97; 5,11)	0,06

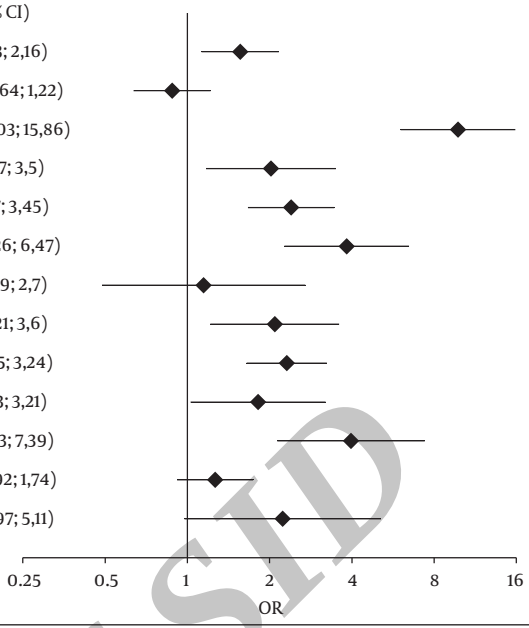

Figure 2. Risk Factors Associated With HBcAb or HBsAg in Univariate Analysis

Risk Factors	OR (95% CI)	P value
Male vs. Female	1,62 (1,1; 2,38)	0,01
Age 4&65 vs. 6-29	6,02 (3,29; 11,01)	0
Age 30-45 vs. 6-29	1,33 (0,7; 2,52)	0,38
Smoking	1,3 (0,82; 2,06)	0,26
Married vs. nomarried	1,3 (0,66; 2,55)	0,45
Addiction yes vs. no	1,09 (0,58; 2,04)	0,79
Tattooing yes vs. no	1,75 (1,21; 2,53)	0
Traditional cupping yes vs. no	1,01 (0,55; 1,85)	0,97
Dental procedure yes vs. no	2,18 (1,14; 4,17)	0,02

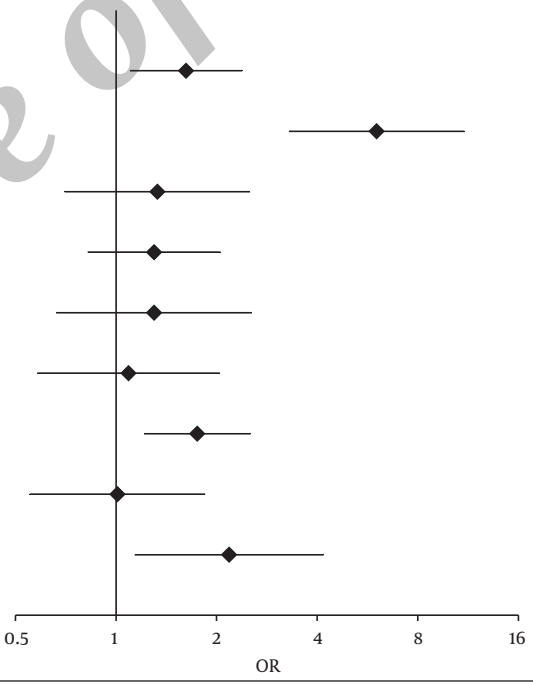

Figure 3. Risk Factors Associated With HBcAb or HBsAg in Multivariate Analysis

Table 3. Seroprevalence of HBcAb in Male and Female Subjects in Different Age Groups

	Male, %	Female, %	Total, %
6–29 Years	3	2.2	2.7
30–45 Years	6.8	4.1	5.3
46–65 Years	25.2	17	21.2

tricts. The age-specific seroprevalence of HBcAb for each sex is indicated in *Table 3*. It can be noticed that HBcAb seroprevalence and the difference between males and females increase with age group.

In univariate analyses, sex, age, smoking, marital status, addiction, tattoo, history of dental procedures and traditional cupping were significantly associated with HBcAb or HBsAg seropositivity (*Figure 2*). In multivariate analysis sex,

age, tattoo and history of dental procedure were independent predictors of HBcAb or HBsAg seropositivity (*Figure 3*).

5. Discussion

The HBV infection has affected a large number of world population and is a major public health problem in developing countries such as Iran. In this country, the mass vaccination program which started in 1993 and reached 94% coverage in 2005 has led to decrease of HBV prevalence from about 3.5% in 1990s to 2.14% in 2000s (1, 5). Unfortunately there were no prior data available from Kermanshah province so we could not monitor changing epidemiology of HBV seroepidemiology after vaccination. The current survey indicated that around 8% of general population in this province had previous exposure to HBV and less than 1% were active carriers. Based on the finding it can be estimated that approximately 18000 active carriers are living in this province that act as a reservoir for transmission of HBV to those with household contact and non-immunized individuals. Furthermore these patients are at increased risk of cirrhosis and hepatocellular carcinoma that impose heavy burden on Iranian public health system.

In comparison with the published articles from different parts of Iran, Kermanshah is low endemicity for HBV infection which can be an impact of HBV vaccination during the recent 20 years in Iran (14).

In multivariate analysis, sex, age, tattoo and history of dental procedures were independent risk factors for HBV seropositivity. Males, elder subjects, and individuals with history of tattoo and history of dental procedures had higher probability of HBV seropositivity. Age is a common risk factor that is reported almost in all of seroepidemiologic studies of HBV infection. The reason is that the risk and cumulative frequency of high risk behaviors increase with age and consequently increase the rate of HBV infection. Furthermore in the religious society of Iran and particularly Kermanshah, many of high risk behaviors such as tattoo, sexual activity outside the family and illegal activities such as addiction and consequently imprisonment are severely considered as taboo for women. Since these behaviors are mostly confined to male population, women have lower rate of HBV seropositivity. Another reason for this claim is that higher age leads to higher difference in seropositivity of males and females (*Table 3*). In addition to age and sex, the current survey indicated that invasive interventions such as tattoo and dental procedures play important roles in transmission of HBV infection in Kermanshah. 10 to 14% of subjects with history of dental procedures and tattoo had HBV seropositivity. It is postulated that dentists and dental staff are infected and transmit the virus to their patients more than any other occupation (15). This implies that with adherence to safety precautions we can significantly reduce HBV infection in general population.

Approximately 8% and less than 1% of general population in Kermanshah are HBcAb seropositive and active carriers of HBV infection, respectively. Age, sex and history of tattoo and dental procedures are major risk factors of HBV seropositivity in this province.

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The authors declare that they have no conflicts of interest relevant to the manuscript.

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