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Risk Factors of Hepatitis B Virus Infection in the Kurdistan Region of Iraq: A Cross-Sectional Study

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To the editor.

Viral hepatitis is a major public health challenge as dictated by the World Health Organization (WHO), with a figure of 254 million people living with hepatitis B infection. It is one of the communicable diseases for which mortality is increasing. In 2022, an estimated 1.2 million new people contracted the infection, and 1.1 million patients died of hepatitis B and related complications, including liver cirrhosis, liver failure, and hepatocellular carcinoma. This datum makes it the second leading infectious cause of death, superseded only by tuberculosis (TB).[1] Hepatitis B infection is caused by hepatitis B virus (HBV), an enveloped DNA virus that is primarily transmitted by exposure to infected body fluids (mainly blood and mucosal secretions). The methods of transmission include unprotected sexual intercourse, intravenous drug injections, transfusion of blood products, dialysis, tattooing, needle-stick injury, and vertical transmission.[2] Despite having both active and passive immunization by an effective vaccine and hepatitis B immunoglobulin, respectively, it has been difficult to control the spread of hepatitis B in developing countries because of the lack of a standardized program to deliver and supervise these immunizations.[3,4] Imperative to any intention of public health authorities fighting against hepatitis B is a set of data on the prevalence and risk factors associated with the disease in order to develop an effective and targeted plan. WHO data clearly show that the worldwide distribution of hepatitis B varies by region, ranging from a prevalence of 0.5% in North and South America to 5.8% in Africa.[1,5] It is also expected that the contribution of each risk factor associated with hepatitis B varies depending on cultural trends, which is extremely important to consider when planning to limit the spread of infections in general and hepatitis B in particular.[6,7] Therefore, we present this study to investigate various risk factors associated with hepatitis B in the Kurdistan region of Iraq.

Materials and methods

Blood samples. Blood samples were taken from 4091 participants attending different hospitals, including Azadi Teaching Hospital, Zakho General Hospital, Duhok Blood Bank, Duhok Obstetrics and Gynecology Hospital, and Zakho Obstetrics and Gynecology Hospital between January 2019 and December 2023. A 5-cc syringe and needle were used to obtain 5 mL of blood from the participants. Then, the blood samples were centrifuged at 1500 rpm for 3 min to separate sera, which were then immediately tested for HBsAg or kept frozen at –20°C until the tests were performed.

Data Collection. A structured questionnaire was prepared and filled out by each study participant. Data were collected through face-to-face interviews, including age, gender, residency, marital status, history of blood transfusion, history of dental procedures, history of general operation, history of tattoos, and history of regular injections. Regular use of injections was defined as the regular use of over-the-counter injections or any other injections.

Enzyme-linked Immunosorbent Assay (ELISA). The presence of HBsAg was detected in serum samples by an ELISA test using a commercial HBsAg ELISA kit (ELISA 480 Test; AVONCHEM, Cheshire, UK) and ELISA 96 microwell plates. First, the anti-HBsAg antibody was applied and fixed to microwells. Subsequently, the sera of the participants were added to the fixed anti-HBsAg antibodies. After incubation, plates were washed to remove any components of the sera that were not bound to the antibodies. Secondary conjugated monoclonal antibodies bound to horseradish peroxidase were then added to the microwells of plates. After incubation, the unbound antibodies and enzymes were washed away. The stop solution and a colored substrate were added to the wells, and the results were recorded through an ELISA reader. The concentration of antigen in a sample is calculated using the optical density (OD). Thus, as per the manufacturer's instructions, the cut-off of HBsAg results are as follows: a. sample (OD) / cut-off value (S/C.O) ≥ 1 = positive; b. sample (OD) / cut-off value (S/C.O) < 1 = negative.

Statistics. Data analysis was computed using the IBM SPSS Statistics Version 25 software. Descriptive analysis was performed to categorize and generate percentages, means ± standard deviation (SD). Then, univariate and multivariate analysis was done by performing binary logistics regression to calculate the crude and adjusted odds ratio (OR), P values, and 95% confidence intervals (CI) for all potential factors associated with HBV infection. Statistical significance was set at p-value < 0.05.

Ethical statement. The study was approved by the College of Medicine Scientific and Ethics Committee at the University of Zakho, Kurdistan Region, Iraq (UoZ18-29). Before enrollment, written informed consent was obtained from all participants.

Results

Demographic Data. The patient characteristics, including potential risk factors for HBV infection, were assessed via a questionnaire; the findings are presented in **Table 1**.

HBV Positivity. Among the 4091 study participants, 150 tested positive for HBsAg (3.67%). The prevalence of HBV positivity among females was 4.77% (136/2851). This was significantly higher than the prevalence of HBV positivity among males, which was 1.13% (14/1240) ($p = 0.001$) (**Table 2**).

Risk factors for HBV infection. Various risk factors for HBV infection were considered, and the following factors were found to be statistically significantly associated with the HBV infection: gender, residence, marital status, receiving an injection, general operation, and history of tattoo (p = 0.001, 0.001, 0.0014, 0.001, 0.001 and 0.001 respectively). Multivariate analysis revealed that the association of surgical operation and history of tattoo to HBV infection is not statistically significant (multivariate p = 0.06325 and 0.0645, respectively) (**Table 2**).

Discussion. The prevalence of hepatitis B is not uniform and ranges from 0.5% in North and South America to 5.8% documented in Africa.[1,5] While this study is not a prevalence study because the sample collection was not random, HBV positivity was shown to be 3.67%, a rate that is higher than that of previous studies in the same region, which ranged from 0.54% to 1.37% during 2016 to 2022.[4,6,8,9] Our results are similar to what was found recently in Iran (3.4%) and Turkey (3%).[10,11] Although the reported prevalence of HBV positivity is high in our region, it is lower than the prevalence reported before the implementation of the HBV vaccine and its inclusion in the Expanded Program on Immunization in Iraq in 2000. Such a reduction was observed in other countries where vaccination programs have significantly reduced HBV prevalence over time.[12] In many high-income countries, the evaluation of

Table 1. Patient Characteristics.

Variables	Category	Number (n)	Percentage (%)
Gender	Female	2851	**69.69%**
	Male	1240	**30.31%**
Residence	Rural	706	**17.26%**
	City	3385	**82.74%**
Marital Status	Married	3628	**88.68%**
	Single	463	**11.32%**
Received Blood	Yes	693	**16.94%**
	No	3398	**83.06%**
Received Injection	Yes	2069	**50.574%**
	No	2022	**49.43%**
Dental Procedure	Yes	2615	**63.92%**
	No	1476	**36.08%**
General Operation	Yes	1154	**28.21%**
	No	2937	**71.79%**
History of Tattoo	Yes	393	**9.61%**
	No	3698	**90.39%**

Participants' age ranged from 1 to 85 years, with a mean age of 29.33 ± 10.74 standard deviation.

Table 2. Statistical analysis.

Potential factors		N. HBV Positive (%)	N. HBV Negative (%)	Univariate analysis		Multivariate analysis	
				P-value	Crude OR	P-value	Adjusted OR
Gender	Female	136 (4.77%)	2715 (95.23%)	0.001	4.387	0.001	5.995
	Male	14 (1.13%)	1226 (98.87%)				
Residence	Rural	63 (8.92%)	643 (91.08%)	0.001	3.714	0.001	3.44
	City	87 (2.57%)	3298 (97.43%)				
Marital Status	Married	147 (4.05%)	3481 (95.05%)	0.0014	6.475	0.00764	4.91
	Single	3 (0.65%)	460 (99.35%)				
Received Blood	No	125 (3.68%)	3273 (96.32%)	0.928	1.0205	0.944	1.0164
	Yes	25 (3.60%)	668 (96.4%)				
Received Injection	No	120 (5.93%)	1902 (94.07%)	0.001	4.288	0.001	4.671
	Yes	30 (1.45%)	2039 (98.55%)				
Dental Procedure	No	62 (4.20%)	1414 (95.80%)	0.173	1.2591	0.997	1.00
	Yes	88 (3.37%)	2527 (96.63%)				
General Operation	Yes	65 (5.63%)	1089 (94.37%)	0.001	2.003	0.06325	1.419
	No	85 (2.90%)	2852 (97.1%)				
History of Tattoo	Yes	30 (7.63%)	363 (92.37%)	0.001	2.464	0.0645	1.542
	No	120 (3.25%)	3578 (96.75%)				

HBsAg is performed using molecular techniques, which offer higher sensitivity and specificity. However, in our study, we used ELISA, and the differences in diagnostic approaches should be considered when comparing HBV prevalence across different regions.[13,14]

The rate of HBsAg positivity among females was 4.77%, which was higher than the previously reported HBsAg positivity among exclusively pregnant women in Zakho (1.13%).[9] This finding is very important considering that women also have the possibility of vertically transmitting HBV to their offspring, who have a high risk of becoming chronically infected, especially when prenatal planning and treatment are not received.

Living in a rural area was significantly associated with HBsAg positivity as 8.97% of rural participants were infected compared to 2.57% of city residents; this aligns with a previous study result from Ethiopia.[15] However, no such difference was reported in another study conducted in our region.[9] The higher prevalence might be associated with the lack of knowledge regarding HBV, limited healthcare access, or preventive programs in rural areas. Furthermore, being married was also identified as predictive of HBsAg positivity, which might be due to the sexual transmission of the disease. Further studies are needed to investigate this.

A history of receiving blood was not associated with HBsAg positivity, similar to previous studies conducted in the region,[4,9] conceivably due to the viral screening practices required before donating blood or receiving a transfusion. No significant association between HBV infection and a history of prior dental procedures was identified.

In our region, therapeutic injections are readily delivered by untrained professionals in unsupervised settings. However, our study revealed that the rate of HBsAg positivity among those who have not received injections is significantly higher than those who received such injections (5.93% compared to 1.45%). This might be due to selection bias or different vaccination rates among such groups.

A history of surgical operation was found to be significantly associated with HBsAg positivity, but after a multivariate analysis, the association was not significant. Two other studies in the same region were also not able to identify an association,[4,9] but other studies in the same region[16] and from China[17] did find a significant correlation between the history of surgery and HBV positivity. Finally, a history of tattooing was similarly found to be significantly associated with HBsAg positivity, but after multivariate analysis, the association was not significant. While previous studies also could not find such associations,[4,16] an association was found between the history of tattooing and HBV infection in a study from the same region.[9] It is important to consider that the use and reuse of contaminated instruments in tattooing have the potential of transmitting the disease. The loss of association between a history of general operation or tattooing and HBV infection after multivariate analysis might indicate a confounding effect from other factors.

Our study's strengths include a large sample size, inclusion of both genders, all ages, and several centers. However, it also has limitations. First, it was a cross-sectional study design with questionnaires that were liable for recall bias and limited geographical coverage.

To conclude, 3.67% of the participants in this study were HBsAg positive; the predictive factors of infection were female gender, rural residency, and being married. Further population-based studies with larger sample sizes are needed to unify the results and be used to build a strong healthcare infrastructure to screen for hepatitis B.

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Competing interests: The authors declare no conflict of Interest.

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