A Study on the Prevalence and the Risk Factors of Hepatitis B Virus Infection in Kurdistan Region, Iraq: A Multicenter Study

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Objectives: The aims of this study were to determine the prevalence of HBV infection and investigate the HBV-related risk factors in the Kurdistan region of Irag.

Methods: Sera samples were collected from 3423 patients visiting three centers and tested for HBV positivity by enzyme-linked immunosorbent assay. A questionnaire was prepared and used by the volunteers who collected data, including age, marital status, and different HBV-related risk factors, through face-to-face interviews.

Results: The mean age of the recruited participants was 28.94 ± 11.17 years. In addition, 873/3423 (25.47%) were males, and 3024/3423(88.34%) were married. Furthermore, HBV infection had a prevalence of 1.37% (47/3243). Multivariate analysis was conducted to identify the predictive factors of HBV infection, which revealed that a history of tattoos and age were predictive factors (P < 0.05). We then stratified our data according to sex. No association was found between the various factors and HBV positivity in males. On the other hand, in females, a significant association was found between the history of tattoos or age and HBV positivity (P < 0.05).

Conclusion: In conclusion, the prevalence of HBV infection was low. Our study showed that a history of tattooing and older age were predictive of HBV infection. Our results could be used as a basis for local elimination programs.

Keywords: Hepatitis B virus, prevalence, risk factors, ELISA, Iraq

Introduction

HBV infection is a global public health issue that is associated with deleterious consequences, including liver cirrhosis, hepatocellular carcinoma, and hepatic failure¹. Approximately 500 million people are infected with HBV, with an estimated 750000 deaths annually. The prevalence of HBV infection varies, ranging from 0.5% in some developed countries to up to 8% in some East Asian countries1. A previous study in Turkey, a neighboring country of Iraq, showed that the prevalence of HBsAg positivity varied markedly from 1% to 14.3%, according to the geographical region of the study and the recruited samples². While the prevalence of HBV infection was below 1% in some regions in Iran, studies from Saudi Arabia showed that approximately 3% of the study populations were infected with the virus^{3,4}. The World Health Organization declared an ambitious plan to eliminate viral hepatitis by 2030. However, it has been previously estimated that only 10% of HBV-infected patients are aware of their infection¹. As such, it is essential to study the risk factors associated with viral transmission to come up with a public health plan for combatting HBV infection. Risk factors for HBV include blood and blood product transfusions, pregnancy, being a healthcare worker, tattoos, drug abuse, and high-risk sexual behaviors^{1,5,6}. To establish new strategies for the elimination of the virus in Iraq, epidemiological data, including risk factors, should be identified.

Since the distribution of HBV risk factors is important for any public health prevention plan, this study investigated the prevalence of HBV and its associated risk factors in multiple centers in the Kurdistan region, Northern Iraq.

Materials and Methods

Blood Samples

Blood samples were collected from volunteers attending Zakho General Hospital, Azadi Teaching Hospital, and Shahid Dr Khalid Teaching Hospital at Koya between January 2019 and February 2021. A 5-cc syringe and needle were used to obtain 5 mL of blood from the volunteers. To separate the sera, blood samples were centrifuged at 1500 rpm for 3 min. Samples were tested immediately for HBsAg or kept frozen at -20°C until the tests were performed.

Questionnaire

A questionnaire was prepared and used by each volunteer. Data were collected through face-to-face interviews, including age, marital status, history of blood transfusion, history of dental procedures, history of surgical 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)

HBsAg, HBc-IgM, and HBc-IgG were analyzed using a commercial ELISA kit (DIA.PRO Diagnostic Bioprobes; Milan Italy), following the manufacturer's instructions. Monoclonal antibodies were fixed to the surface of the micro-wells, and sera samples were added and incubated. Afterward, a secondary monoclonal antibody conjugated with horseradish peroxidase (HRP) was added. Unbound serum proteins and HRP conjugates were washed off. After blocking the

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Percentage

enzymatic reaction, the substrate was added, and the optical density was measured using an ELISA reader.

Ethics

The study was approved by the appropriate ethics committee of the College of Medicine, University of Zakho. Written informed consent was obtained from all patients.

Statistics

Regression analysis was used to assess the risk factors associated with HBsAg. Variables that achieved a P-value of less than 0.2 were included in the multivariate study. Backward elimination was used to determine the HBV-related risk factors. All computations were performed using the Minitab 17 (Minitab; Pennsylvania, United States).

Results

Volunteers

Sera samples were collected from 3423 patients and tested for HBV positivity. The mean age of the recruited participants was 28.94 ± 11.17 years. In addition, 873/3423 (25.47%) were males, and 3024/3423 (88.34%) were married (Table 1).

HBV Prevalence and Risk Factors

The prevalence of HBV infection in the recruited sample was 1.37% (47/3243). All samples were HBc-IgG-positive, indicating chronic infection. Univariate analysis showed that 14/47 (29.8%) of the HBV-positive patients had a history of blood transfusion, which was significantly higher compared to the HBV-negative patients (602/3376 [17.8%], P = 0.048). In addition, HBsAg positivity was significant associated with a history of tattooing (P = 0.039) and age (P = 0.003) (Table 2). Multivariate analysis was then conducted to identify the predictive factors of HBV infection, which revealed that a history of tattoos and age were predictive factors (Table 2).

We then stratified our data according to sex. No association was found between the various factors and HBV positivity in males (Table 4). On the other hand, in females, HBV positivity was significantly associated with a history of tattoos and age (Table 5). Multivariate analysis confirmed that both history of tattoos and age were predictive factors for HBV positivity (Table 6).

Discussion

HBV is a common public health issue worldwide. Chronic HBV infection is associated with serious complications, such as liver cirrhosis, hepatocellular carcinoma, and hepatic failure. Studies conducted in the Middle East found that the prevalence of HBsAg positivity ranged from less than 3% in Iraq and some regions in Iran to approximately 7% in war-torn Yemen and some districts in the Arab peninsula^{3,4,7}. Specifically, the results of studies conducted in Iraq varied depending on the recruited samples and the geographic regions. In a previous study in Duhok City, Northern Iraq, the prevalence of HBV was 1.14% in blood donors⁸, while it was 0.7% in the middle region of Iraq⁹. In contrast, in a report from Kerbala, Southern Iraq, HBV had a prevalence of 3.5%7. The vast majority of previous studies in Iraq exclusively recruited male blood donors from one center or city. In this study, the prevalence of HBV was 1.37%, which reflected more realistic results in the general population because both sexes were recruited from three different cities.

Variables

Table 1. Characteristics of the study population

Sex	Male	872	25.47
	Female	2551	74.53
Marital status	Married	3024	88.34
	Single	399	11.66
Blood transfusion	Yes	616	17.99
	No	2807	82.01
Surgical operation	Yes	813	23.75
	No	2610	76.25
Dental procedures	Yes	2228	65.09
	No	1195	34.91
Tattoo	Yes	269	7.86
	No	3154	92.14
Injections	Yes	2017	58.92
	No	1406	41.08
Age (Year ± STD)		28.94 ± 11.17	

STD: standard deviation

Table 2. Univariate analysis of the hepatitis B virus-associated risk factors in the recruited population

Factors	HBsAg-Positive (n = 47)	HBsAg-Negative (<i>n</i> = 3376)	Confidence interval	Odds ratio	<i>P</i> -value
Sex (Male)	11	861	0.4522 – 1.7604	0.89	0.7
Marital status (Married)	45	2979	0.7253 – 12.4205	3	0.07
Blood transfusion	14	602	1.0398 - 3.6754	1.95	0.048
Surgical operation	8	805	0.3053 - 1.4094	0.66	0.25
Dental procedure	33	2195	0.6760 - 2.3792	1.27	0.45
Tattoo	8	261	1.1323 – 5.2934	2.44	0.039
Injection	30	1987	0.6777 – 2.2454	1.23	0.5
Age (Year ± STD)	34.42 ± 11.04	28.87 ± 11.15	1.0136 – 1.0579	1.03	0.003

HBV can be transmitted via blood and blood product transfusion, exposure to contaminated blood through needles, tattoos, vertically from mothers to newborns (particularly during delivery), and unprotected sex⁵. It was previously found that the mode of HBV transmission varied in different countries according to age, sex, norms, and societal traditions¹⁰. It is worth mentioning that a blood screening program for blood-borne diseases started in 2007 in the Kurdistan region. In our study, there was a trend of higher HBV positivity in people with a history of blood transfusion. Careful examination of our data showed that all HBV-positive patients were born before 2007, possibly implying that screening programs in these cities have successfully prevented the transmission of the virus. In agreement with a study conducted in China¹¹, we found a significant association between age and HBV positivity. Similarly, a study from Iran showed that HBV positivity was more common in older patients3. Again, this could be explained by the fact that the screening program started in 2007, and older patients had a higher chance of exposure. In addition, HBV vaccination was included in the expanded

Table 3. Multivariate analysis of the hepatitis B virusassociated risk factors in the recruited population

Factors	Coef	SE Coef	T value	<i>P</i> -value
Age	0.00056	0.0002	2.96	0.003
Blood transfusion	0.009	0.005	1.69	0.091
Tattoo	0.017	0.008	2.13	0.033

Coef: coefficient, SE Coef: standard error of the coefficient.

vaccination program in 2000. Therefore, people born before the HBV vaccination program had a higher chance of infection.

Tattoos are also a risk factor for HBV infection. In a meta-analysis investigating the relationship between HBV and tattooing, tattooing was found to be a risk factor for acquiring the infection^{12,13}. Similarly, we found a significant association between HBV positivity and tattooing. This is a challenge for controlling HBV in the Kurdistan region as tattooing is performed in illegitimate shops or as traditions by untrained personnel.

In a study conducted in Turkey, sexual contact with HBV-positive patients was the main risk factor associated with HBV infection¹⁴. Illegitimate sexual contact could not be included in the study because the ethics committee prohibited the inclusion of questions probing this in the questionnaire since it is a sensitive issue in our conservative society, particularly among females. We believe that even if questions related to this topic were included, the patients would not have provided honest answers.

Previously, the vast majority of studies from Iraq recruited blood donors^{7,9,15,16}. Careful examination showed that all donors were male. Therefore, we believe that the majority of studies in this country had not investigated the HBV-related risk factors in females. Therefore, we stratified our data according to sex. We found no association between HBVrelated risk factors and HBV positivity in males. On the other hand, both univariate and multivariate analyses showed that tattoos and age were associated with HBV positivity in females. These results were difficult to explain, necessitating further studies to explore this.

Table 4. Univariate analysis of the hepatitis B virus-associated risk factors in male

Table 1. Oil value analysis of the nepatitis b vitas associated risk factors in males						
Factors	HBsAg-Positive (n = 11)	HBsAg-Negative (n = 861)	Confidence interval	Odds ratio	<i>P</i> -value	
Marital status	11	598	0 - 0	23	0.9	
Blood transfusion	3	98	0.7619 – 11.1889	2.9	0.153	
Surgical operation	2	361	0.0661 - 1.4330	0.3	0.094	
Dental procedure	8	539	0.4196 - 6.0479	1.59	0.5	
Tattoo	1	95	0.1021 - 6.3685	0.8	0.8	
Age (Year ± STD)	45.16 ± 14.4	31.5 ± 19	0.9950 - 1.0752	1.03	0.09	
Injection	5	334	0.3981 - 4.3425	1.31	0.65	

Table 5. Univariate analysis of the hepatitis B virus-associated risk factors in females

Factors	HBsAg-Positive (n = 36)	HBsAg-Negative (n = 2515)	Confidence interval	Odds ratio	<i>P</i> -value
Marital status	34	2378	0.2277 – 4.0297	0.95	0.9
Blood transfusion	11	504	0.8581 – 3.5918	1.76	0.13
Surgical operation	6	444	0.3868 - 2.2598	0.94	0.9
Dental procedure	25	1656	0.5773 – 2.4073	1.18	0.6
Tattoo	7	166	1.4742 – 7.9139	3.4	0.011
Age (Year ± STD)	32.63 ± 9.45	28.41 ± 8.88	1.0135 - 1.0754	1.04	0.008
Injection	25	1653	0.5804 – 2.4201	1.19	0.6

Table 6. Multivariate analysis of the hepatitis B virusassociated risk factors in females

Factors	Coef	SE Coef	T value	<i>P</i> -value
Age	0.00067	0.0002	2.54	0.011
Blood transfusion	0.01	0.005	1.65	0.1
Tattoo	0.01	0.009	2.79	0.005

Coef: coefficient, SF Coef: standard error of the coefficient.

The strength of our study was the relatively large and diverse sample, as the majority of previous studies recruited specific populations, such as blood donors or patients on renal dialysis^{8,17}. As for its limitation, we only recruited patients who were visiting the said hospitals, rather than random patients in the community. Therefore, a populationbased study is needed to investigate the prevalence and risk factors of HBV infection. This project is of exceptional importance for public health providers in the region because, to the best of our knowledge, this was the first study to recruit samples from more than one city with a relatively large sample size.

Conclusion

In conclusion, the prevalence of HBV infection was 1.37%. Our project showed that a history of tattooing and older age were predictive of HBV infection. Tattooing centers in the region should be legalized, trained, and monitored to prevent the further spread of the infection. Screening for HBV infection should focus on people born before 2000.

Conflict of Interest

None.

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