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Hepatitis B Virus (HBV) and Occult Hepatitis B Virus (OBI) in Hemodialysis Patients: An Epidemiological Observational Study in Kerman, Iran (2022-2023)

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ABSTRACT

Background and Aim: Viral infections are associated with augmented morbidity and mortality risk in Hemodialysis (HD) patients.

Materials and Methods: In this investigation, we have scrutinized the presence of hepatitis B virus (HBV), and occult hepatitis B virus infection (OBI), in HD patients.

In this cross-sectional study, the blood samples and data from 200 hemodialysis patients referred to a dialysis center in Kerman, from January 2022 to March 2023 were collected. Anti-HBV antibodies and HBs Ag were analyzed in the specimens using an enzyme-linked immunosorbent assay (ELISA). All samples were tested for OBI by using a real-time polymerase chain reaction (PCR) assay.

Results: Of the 200 patients considered, 4 (2%) for HBs Ag were positive. Real-time PCR demonstrated OBI in 2 (1%) patients. The majority presence of viral infection was in the age group 50≥ years, self-employment, low education level, and diabetic patients. Furthermore, our study revealed that a higher percentage of HD patients were associated with low educational levels, self-employment, urban residence, and comorbidities such as diabetes and hypertension.

Conclusion: In this study, HBV antigen prevalence was 2%, and OBI prevalence was 1%, indicating a low HBV incidence in HD patients. Our study underscores the importance of lifestyle improvement, increased awareness among lower-educated and lower-skilled individuals, and rigorous disinfection practices in managing risk factors for hemodialysis patients, including HBV and OBI.

Keywords: Hemodialysis, Hepatitis B virus, Risk Factors, Iran

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1. Introduction

HBV infection is one of the leading health problems worldwide. Nearly 2 billion of the world's population have had serological evidence of HBV infection **(1, 2)**, and its overall prevalence is reported to be 2.14% in Iran, with high gastrointestinal-related mortality **(3-5)**. HBV is classified within the family Hepadnaviridae, characterized as a double-stranded DNA virus with a genome size of 3.2 kilobase pairs (kbp), and is one of the most common cause of acute hepatitis in adults **(6-9)**. Although HBV infection is asymptomatic in 50-70% of cases, it is associated with symptoms such as fever, headache, malaise, anorexia, nausea, vomiting, diarrhea, and abdominal pain **(10)**.

HBV can be transmitted through various routes, including blood transfusion and the use of infected blood products, sexual contact, skin-to-skin transfer (such as during tattooing or acupuncture), organ transplantation, and from a mother to her fetus during pregnancy **(11, 12)**. The prevalence of hepatitis B among hemodialysis patients exhibits regional variations, ranging from 3% in Iraq and Tunisia to 14.3% in Vietnam **(13-15)**. However, in Iran, the reported prevalence among hemodialysis patients varies from 0% to 18.2% **(16)**.

In 1970, a novel form of HBV infection known as occult hepatitis B virus infection (OBI) was identified, characterized by the absence of detectable HBsAg in patients, while the presence of the virus genome (HBV DNA) can be detected in peripheral blood mononuclear cells (PBMC) or liver tissue **(17, 18)**. Despite the notable impact of screening tests for hepatitis B surface antigen (HBsAg) in curtailing the transmission of HBV infection among recipients of blood products, these measures have proven inadequate in detecting and preventing the transmission of OBI **(17)**. The risk of acquiring OBI through blood transfusion is significantly higher compared to the risk of acquiring human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections **(19)**. The prevalence of OBI varies among different populations in Iranian cities, ranging from 0% to 63%. Besides, in past studies in different regions of the world, a prevalence of 0 to 36% has been declared among hemodialysis patients **(20, 21)**. Molecular methods such as polymerase chain reaction (PCR) and Real-Time PCR are utilized for the detection of OBI. In particular, Real-Time PCR is a quantitative method with high sensitivity compared to PCR. It can detect even a small number of HBV DNA copies, less than 200 copies, in serum and liver biopsy samples, making it a definitive diagnostic method for OBI **(22)**.

In order to address the diagnostic and treatment needs pertaining to this infection among high-risk patients, as well as the limited research conducted in Iran, our study is designed to assess the prevalence of HBV infection and OBI specifically among hemodialysis patients in the city of Kerman, Iran.

2. Materials and Methods

Study Population and Settings

The cross-sectional study was conducted from January 2022 to March 2023, involving a sample of 200 hemodialysis patients at the Javad Alaemeh Dialysis Centers in Kerman. Ethical approval for this study was obtained from the ethics committee (Code: IR.KMU.AH.REC.1400.201). A questionnaire was specifically developed by the researchers to gather information pertaining to demographic details, patient history, and various tests, which was diligently documented.

Inclusion and Exclusion Criteria

The inclusion criteria encompassed the following: 1. Patients residing within the city of Kerman province, 2. Patients who possessed the mental alertness necessary to fully respond to the questionnaire and expressed complete satisfaction with participating in the study, and 3. Patients diagnosed with chronic kidney disease (CKD) or having a six-month history of dialysis. On the other hand, the exclusion criteria consisted of: 1. Individuals infected with HIV, and 2. Individuals with a previous history of organ transplantation.

Sampling and Preparation of the Specimens

After obtaining consent from the patients and conducting the questionnaire, venous blood samples were taken from 200 patients in the amount of 10 cubic centimeters (cc) using two tubes. The tubes did not contain anticoagulant but included heparin. The collected serum was isolated and stored in a freezer at a temperature of -70 degrees Celsius until nucleic acid extraction or serologic tests. The tube containing the anticoagulant was diluted equally with phosphate-buffered saline (PBS) to facilitate the isolation of PBMCs. The diluted mixture was slowly poured onto a cold ficoll layer, which had the same volume as the blood sample, allowing the separation into two layers. Subsequently, the samples were centrifuged at a speed of 2500 revolutions per minute (rpm) for 30 minutes, resulting in the separation of PBMCs in the formed phase. After two washes with PBS buffer, the PBMCs were mixed with a cell precipitate of 500 microliters (µl) lysis buffer and stored in a -70-degree Celsius freezer until the molecular test was performed.

Serological Test

All serum samples obtained from the patients underwent testing for the presence of HBsAg, HBcAb, and HBsAb using the using enzyme-linked immunosorbent assay (ELISA) Kit (Pishtaz Teb, Iran) in accordance with the guidelines provided by the manufacturer.

DNA Extraction

The isolated sera and PBMCs from patient samples were subjected to total DNA extraction utilizing the SinaPure Viral extraction kit (Sinaclon, Iran), following the manufacturer's instructions. The concentration and purity of the extracted DNAs were assessed using a BioPhotometer (NanoDrop™, N-D 2000, Thermo Scientific, USA). Additionally, the integrity of the extracted DNA was evaluated through electrophoresis on an agarose gel. The extracted DNA samples were stored at a temperature of -70°C until further analysis using a real-time method.

Detection of HBV Genome by Real-time PCR

DNA extracted from each patient's serum and PBMC was tested for HBV genome using a Real-time PCR technique based on SYBR green dye technology. Specific primers from previous studies (Table 1) were used to identify the target sequence in the genome of HBV (23, 24). The mixture of each reaction includes 12.5 µL of RealQ Plus 2x Master Mix, 0.5 ml of each primer, 10.5 µL of PCR-grade H2O, and 1 µL of template in a final volume of 25 µL. The PCR reaction was performed, using the following thermal cycling conditions; 15 minutes at 95°C followed by 45 cycles at 94°C for 15 seconds, 55°C for 1 minute, and incubation at 25°C for 40 seconds. Negative and positive controls were used in each run. For quality control and to ensure the presence of the HBV genome, the real-time reaction product of the positive samples was electrophoresed on an agarose gel, and the results were checked with the gel documentation system. Band 93 bp indicated the presence of the HBV genome.

Statistical Analysis

Data obtained from the ELISA and RT-qPCR PCR were analyzed using Statistical Package for the Social Sciences (SPSS), version 22 (SPSS Inc., Chicago, Ill., USA), and Graphpad Prism, version 8.0.2 (Graphpad Software, Inc.). Data are presented as percentages, means, and standard deviation. The P-value<0.05 was considered statistically significant.

Table 1. Oligonucleotide Sequence of primers used in this study

Locus	Oligonucleotide Sequence	Product Size, bp	Ref
HBV-F	5′ GATGTGTCTGCGGCGTTTTAT 3′	93	(23)
HBV-R	5′ GGCAACATACCTTGRTAKTCCAGA 3′		
β-actin-F	5′ ACCGAGCGCGGCTACAG 3′	60	(24)
β-actin-R	5′ CTTAATGTCACGCACGATTTCC 3′		

3. Results

The results revealed that among the 200 patients participating in this study, 59.5% (119 people) of the studied patients were men, while 40.5% (81 people) were women. We found that 80% of the patients were over 50 years old, the average age of all patients was 60.1 ± 13.8, and men had a lower average age than women (58.1±14.9 versus 63.2±11.3, respectively). The prevalence of HBV antigen or antibody in serum and its genome in serum and PBMC (OBI) were 4 and 2 respectively (Figure 1). Although in the studied patients, a percentage of married people (75 %), without university education (84 %), without government jobs (83 %), and living in the city (89 %) account for the largest share, the presence of HBV (serological and molecular) were more observed among people with unemployed and low education (Table 2).

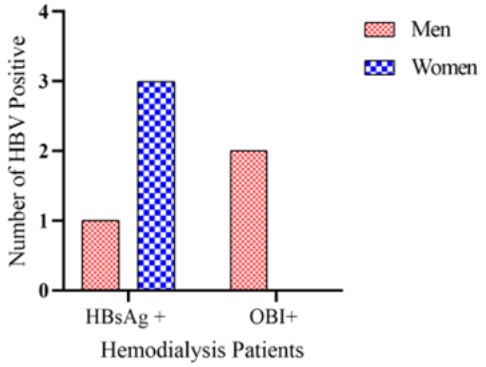

Figure 1. Serological and molecular cases of HBV in hemodialysis patients

Table 2. Demographic parameters in relation to HBV infection status

Parameters		Number (%)	P-value	HBV-(%)	HBV+(%)	P-value
Age	≤50	40(20%)	<0.0001	38(19.6%)	2(33.3%)	0.345
	>50	160(80%)		156(80.4%)	4(66.7%)	
Sex	Male	119(59.5%)	0.007	116(59.8%)	3(50.0%)	0.688
	Female	81(40.5%)		78(40.2%)	3(50.0%)	
Marital status	Married	151(75.5%)	<0.0001	150(77.3%)	1(16.7%)	0.003
	Single	17(8.5%)		15(7.7%)	2(33.3%)	
	Others	32(16%)		29(14.9%)	3(50.0%)	
Education	High school	119(59.5%)	<0.0001	115(59.3%)	4(66.7%)	0.891
	diploma	50(25.0%)		49(25.3%)	1(16.7%)	
	Bachelor and higher	31(15.5%)		30(15.5%)	1(16.7%)	
occupation	Self-employment	166(83.0%)	<0.0001	162(83.5%)	4(66.7%)	0.270
	Government	34(17.0%)		32(16.5%)	2(33.3%)	
Location	Urban	179(89.5%)	<0.0001	174(90%)	5(83.3%)	0.502
	Rural	21(10.5%)		20(10%)	1(16.7%)	

Among the patients, 49% had a history of blood transfusion, 19 individuals had a history of drug abuse, 2 individuals had a record of imprisonment, and 1 individual had a history of thalassemia. However, the presence of HBV (both serological and molecular) did not demonstrate a significant association with these parameters, as indicated in Table 3.

Table 3. Patient histories in relation to HBV infection status

Parameters		Number (%)	P-value	HBV- (%)	HBV+ (%)	P-value
Blood transfusion history	No	102(51.0%)	0.777	99(51.0%)	3(50.0%)	1.000
	1≤	98(49.0%)		95(49.0%)	3(50.0%)	
thalassemia	Yes	1(0.5%)	<0.0001	1(0.5%)	0(0.0%)	Incalculable
	No	199(99.5%)		193(99.5%)	6(100%)	
History of drug use	Yes	19(9.5%)	<0.0001	18(9.3%)	1(16.7%)	0.455
	No	181(90.5%)		176(90.7%)	5(83.3%)	
Prison history	Yes	2(1.0%)	<0.0001	2(1.0%)	0(0.0%)	Incalculable
	No	198(99.0%)		192(99.0%)	6(100.0%)	
Cause of dialysis	Diabetes	65(32.5%)	<0.0001	62(32.0%)	3(50.0%)	Incalculable
	Hypertension	28(14.0%)		27(13.9%)	1(16.7%)	
	Diabetes and Hypertension	54(27.0%)		54(27.8%)	0(0.0%)	
	Polycystic	17(8.5%)		16(8.2%)	1(16.7%)	
	Kidney atrophy	5(2.5%)		4(2.1%)	1(16.7%)	
	Others	31(15.5%)		31(16.0%)	0(0.0%)	

Patients undergoing dialysis treatment presented with various underlying causes, with the majority of cases attributed to diabetes mellitus and/or high blood pressure, accounting for 73% of the cases (as shown in Table 3). Interestingly, all cases of HBV infection were observed exclusively in diabetic patients. However, it is worth noting that the presence of HBV antigen, antibodies, and genome did not exhibit a significant correlation with any of the causes associated with CKD, as demonstrated in Table 3.

The average levels of three laboratory tests, namely alanine transaminase (ALT), aspartate aminotransferase (AST), and triglycerides (TG), among the patients were within the normal range. Additionally, the levels of these three laboratory markers among patients who tested positive for viral markers of HBV (both serology and molecular HBV) were also within the normal range, as depicted in Figure 2.

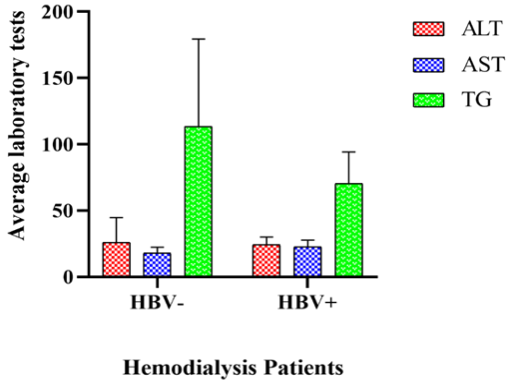

Figure 2. Patient laboratory tests in relation to HBV infection status

4. Discussion

The significant prevalence of blood-borne viral infections, including HBV, among recipient groups such as thalassemia and hemodialysis patients, is a major concern and health issue in the country, particularly in endemic regions. On the other hand, OBI, which is a special type of HBV infection and cannot be detected routinely with serological methods such as ELISA kits, has doubled these concerns. This type of infection can increase the risk of HBV transmission among high-risk groups such as hemodialysis patients and lead to kidney complications and irreversible complications such as various cancers related to this virus, especially hepatocellular carcinoma. Therefore, the present study aimed to investigate the prevalence of HBV and OBI in hemodialysis patients who are considered a high-risk group. The findings of this study provide insights into the overall prevalence of HBV and OBI in the southeast region of Iran, reflecting the broader prevalence within the society.

The prevalence of HBV antigen by ELISA assay among hemodialysis patients in Kerman was found to be 2%, while the prevalence of OBI by Real-time PCR technique was 1% in this study. These results indicate a relatively low prevalence of HBV in the studied population. In comparison, the serology and molecular findings regarding HBV presence among the patients in this study were lower than the average prevalence reported in a meta-analysis conducted by Mohammadi, and Keshtkar **(25)** in Iran. The meta-analysis reported a range of 0.87% to 8.86% prevalence among different Iranian cities, with an overall average of 3% **(25)**. Furthermore, compared to Zahedi's study which was conducted in Kerman in 2012 and reported a 7% prevalence for HBV, the results of this study indicate a 5% decrease in the prevalence of HBV among hemodialysis patients. Several factors can account for this decrease. Firstly, the availability and increased utilization of the HBV vaccine compared to the past have played a significant role. The vaccination efforts have likely contributed to reducing the incidence of HBV infection among the studied population. Secondly, there has been an improvement in the screening protocols for infectious agents in the dialysis ward, ensuring that patients are thoroughly evaluated for the presence of antigens or antibodies of infectious agents. This enhanced screening process aids in identifying infected individuals and implementing appropriate measures to prevent transmission. Moreover, the use of separate dialysis machines for patients infected with infectious agents has further contributed to reducing the risk of cross-contamination. Additionally, adherence to standard sterilization and disinfection protocols for devices and departments, as well as the implementation of general training and proficiency standards, have played a vital role in curbing the transmission and spread of infectious agents among patients **(26)**.

Previous studies have identified the male gender as a risk factor for HBV infection, and this current study also found a higher prevalence of HBV-positive patients among men. Several reasons contribute to this gender disparity. Risky behaviors, such as drug abuse, unprotected sexual relations (including sex with men or MSM), and alcohol consumption, are known to increase the risk of HBV infection. Men may be more engaged in these behaviors, thereby exposing themselves to a higher risk of HBV transmission. Additionally, occupational factors and the nature of certain types of work may also contribute to a higher prevalence of HBV among men, as they may be more exposed to infection in certain work environments **(27, 28)**.

In this particular study, no significant relationship between age and HBV infection was identified. However, it is worth noting that in a study conducted by Mohaghegh Shelmani, and Karayiannis **(29)** in Iran in 2017, the age group between 25 and 59 years was reported as the most high-risk group for hepatitis B infection. Several factors contribute to this increased risk within this age range. Firstly, individuals within this age group may include a proportion of non-vaccinated individuals who have not received the hepatitis B vaccine. Lack of vaccination can increase susceptibility to infection. Secondly, an increase in risky behaviors, including unprotected sexual relations, has been reported as a significant factor contributing to the higher incidence of HBV infection within this age group **(29)**.

This study identified low levels of education and self-employment as additional risk factors for HBV infection. Consistent with previous research, low levels of education and self-employment can contribute to a lack of awareness regarding the prevention of viral infections, particularly HBV infection. Individuals with limited education may have less access to information about preventive measures and may not fully understand the importance of safe handling practices. Moreover, individuals who are self-employed may not have the same level of occupational safety protocols and training compared to those in formal employment settings. This can increase the risk of exposure to HBV and other infectious agents **(30)**.

This study highlighted diabetes mellitus, high blood pressure, and polycystic kidney disease as the most common causes of patients requiring hemodialysis. These findings are consistent with numerous previous studies conducted in different regions. For instance, a study conducted in Persian Gulf countries reported a prevalence of diabetes mellitus ranging from 45% to 74% among hemodialysis patients. This emphasizes the significant impact of diabetes mellitus as a leading cause of chronic kidney disease requiring dialysis treatment. Additionally, high blood pressure and polycystic kidney disease also contribute substantially to the prevalence of hemodialysis patients **(31)**. Also, the prevalence of high blood pressure in various studies has been reported as 50-60% and even up to 85% among hemodialysis patients **(31)**. On the other hand, diabetic patients, because of frequent percutaneous blood exposure, are considered at risk for HBV infection **(32)**.

5. Conclusion

In this study, HBV antigen prevalence was 2% using ELISA, and OBI prevalence was 1% via Real-time PCR, indicating a low HBV incidence in HD patients. This infection was most prevalent in individuals aged 50 and above, self-employment patients, and those with lower education levels. Additionally, positive HBV cases were more identified in diabetic patients. Our study highlights the significance of enhancing lifestyle, raising awareness among individuals with lower socioeconomic status and educational attainment, and implementing rigorous disinfection protocols for both dialysis facilities and equipment. These measures are pivotal in effectively managing and mitigating risk factors associated with hemodialysis patients, including HBV and OBI.

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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