Original Article

Nosocomial Transmission of Hepatitis B Surface Antigen and Anti-Hepatitis C Virus among Hemodialysis Patients

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Abstract

Hepatitis B and C virus (HBV), (HBC) infection is a major public health problem worldwide, that endangers patients' lives, resulting in serious health consequences due to clinical comorbidities such as liver cirrhosis and hepatocellular carcinoma. Even though accessible resources for vaccination, testing, and therapeutic interventions, the incidence of HBV in hemodialysis remains crucial. The study aimed to detect HBsAg and anti-HCV in HD patients and the control group. In a retrospective case-control, the hospital-based study was carried out on 110 participants 60 HD patients and 50 control, ELISA method was used to detect HBsAg and anti-HCV in HD patients and control sera. The prevalence rate of HBV infection was 26.3%, among HD patients, and the control group was 45 %, and 4% respectively. While the overall prevalence of HCV was 1,8 % and it was 3.3% among the HD patients, there were no cases of HBC infection among the healthy group. A statistically significant correlation between HBV infection and HD was revealed (P-value 0.019). The prevalence of HBV was extremely high among HD patients. The findings highlight the importance of performing a constructive intervention for rapid identification, and diagnosis using PCR technique, also therapeutic interventions of infected patients, and vaccination of those with non-protective anti-HBs antibodies, in an attempt to reduce morbidity and mortality in HD patients.

Keywords: Hepatitis B virus, Hepatitis C virus, Hemodialysis, Serodiagnosis

Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide, that endangers patients' lives, resulting in serious health consequences due to clinical comorbidities such as liver cirrhosis and hepatocellular carcinoma. Even though accessible resources for vaccination, testing, and therapeutic interventions, the incidence of HBV in hemodialysis remains crucial [1, 2] HBV and HCV cause the majority of chronic liver diseases worldwide and can be spread via intravenous fluids, sexual, or vertical routes. Vertical transmission (VT) is the most common route of hepatitis virus transmission among children, accounting for 1–28 percent of HBV cases and 3–15 percent of HCV cases. The timing of infection and infectious routes during pregnancy, delivery, and parturition can help determine the perinatal transmission of hepatitis viruses [3, 4].

According to its effects on the liver; HBV was considered a hepatotoxic virus, that is; can establish a permanent state of chronicity in the infected individual due to immune depletion leading to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Currently, 3.5 percent of the world population is reported as chronically infected with HBV, which accounts for about 240 million people globally. The genomic -structure of HBV consists of a double-stranded DNA, and eight genotypes were identified labeled from A to H and four subgenotypes have been described. The main serological markers of HBV are HB surface antigen (HBsAg), anti-HBs, HBe Ag and anti-HBe, and anti-HBc IgM and IgG [5, 6].

HCV is also another hepatotropic RNA virus that causes induced liver damage which increased susceptibility to cirrhosis and hepatocellular carcinoma. Around 64 and 103 million people across the world are chronically infected. The comparative significance of the two most frequent HCV

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transmission risks are correlated with, blood transfusion and intravenous drug use (IVDU). HCV has a high genetic diversity and is characterized by seven genotypes (GTs), each with a distinct geographical predominance [7-9]. In hemodialysis, blood is forced to remove from the patient's circulation and pumped past the dialysis membrane using needles and plastic tubing. Harmful substances and contaminants transmit through the dialysis membrane into the dialysis solution, which is then disposed of, and the blood is returned to the patient; both are aided by the typical immunological impairment that emerges in renal dysfunction and interrupts the patient's capability to reduce such viruses. The higher likelihood of nosocomial HBV or HCV infection in HD patients has been associated with diminished cellular immunity and blood transfusions [10, 11].

The risk of acquiring hepatitis C (HCV) infection as there is no vaccine available for clinical use right now and a range of variables have been involved in modifying the efficacy of DNA vaccines, including host, target antigenic region, primary strategies, adjuvant existence or total lack, dosing frequency, and, which further generates a vaccination program that can be used to enhance vaccines performance. [12]. Recombinant DNA technology has been used to develop the Hepatitis B (Hep B) vaccine. A plasmid incorporating the HBsAg gene is introduced into popular baker's yeast HBsAg, two single-antigen vaccines, Engerix-B® and Recombivax HB® are covalently linked with aluminum The vaccine is typically administered to children in three 6-dose sequences at the ages of 0 months, 1 to 2 months, and 18 months [13]. So current study aimed to detect HBsAg and anti-HCV among Sudanese patients who underwent hemodialysis to detect the possibility of nosocomial transmission.

Materials and Methods

Study Design

A retrospective observational case-control study was carried out.from August- ٢٠٢١ to January -٢٠٢٢ hospital-based study was carried out in a hemodialysis center at Parents Charitable Hospital located in Omdurman city, the center contains 15 hemodialysis machines, and receives a capacity of 20 - 30 patients daily; coming from inside and outside Khartoum state.

Sample Size and Sample Technique

The overall sample size was 110 participants, specified as, 60 hemodialysis patients (case group) and 50 control individuals, using non-probability, convenience sampling technique was used in this study to select the case and control groups.

Inclusion Criteria

HD patients who were seronegative for HBV and HCV infections by immunochromatographic assay were enrolled in the case group, for the control group, any non HD -patient, co-patient, or hospital staff were enrolled in the control group. Critically ill patients at the time of obtaining blood samples and participants whose sera were not sufficient to perform the ELISA test were excluded from the study. Following verbal consent, a constructed questionnaire was designed to obtain baseline and clinical data from study subjects.

Methodology

Blood sample: From each participant; 5 ml of blood specimen was withdrawn in a plain container, after clotting of the blood all specimens were centrifuged for 5 minutes and the serum was divided in another a plain container and then labeled with serial number for case and control and stored at -20°C until further analyses. Before performing the ELISA test all samples were transferred to plain Eppendorf cups and labeled with specific labeled numbers as case or control.

Laboratory Method

Method of HBs Ag Detection

Each serum sample was analyzed by enzyme-linked immuno-sorbent assay (ELISA), based on a 'Sandwich' principle of Negative controls (e. gB1, C1, D1), two Positive controls (e.g., El, F1), and one Blank (e.g.,>E1, F1), and one Blank (e.g., AL, neither samples nor HRP Conjugate should be added into the blank well) are encountered, 20µl of Sample Diluent was then added to each well except the Blank. And 100µl of control positive, control negative, and specimen into their respective wells and incubate for 60 minutes at 37°C. Add 50µl HRP Conjugate to each well except the Blank and incubate for extra 30 minutes at 37°C. At the end of the incubation, wash each well 5 times with diluted wash buffer. Each time, allow the microwells to soak for 30-60 seconds. A total of 50µl of Chromogen A and 50µl Chromogen B solution were added into each well including the Blank and mixed by tapping the plate gently. Incubate the plate at 37°C for 15minutes avoiding light. Using a multichannel pipette or manually, add 50µl Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HBsAg positive sample wells. Calibrate the plate reader with the blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630mm. Calculate the Cut-off value.

Method of Anti HCV Detection

Principle of the Assay

The indirect ELISA technique was used to identify HCV antibodies in a two-step incubation process. Pre-coated polystyrene microwell strips encompass recombinant, strongly immunoreactive antigens correlating to the core and non-structural zones of HCV (Fourth generation HCV ELISA). Anti-HCV-specific antibodies, if revealed, will connect to the solid phase pre-coated HCV antigens throughout the first incubation step. After rinsing the wells to eliminate unbound serum proteins, rabbit anti-human IgG antibodies conjugated to horseradish peroxidase (HRP-Conjugate) are added. During the number of plates produced

simultaneously. The calculations are done by linking the optical density (OD) amount of every sample to the plate's cut-off value (C.O.). If the cut-off reading is based on a single filter plate reader, the outcomes should be calculated by deducting. For result interpretation; samples with absorbance value less than or exactly equivalent to the cut-off value are considered negative, while samples with absorption spectra greater than or equal to the cut-off value are considered positive.

Statistical Analysis Methods

SPSS version 23 was used to evaluate quantitative data, and the distinction between categorical variables was investigated by Pearson Chi-square analysis. When the P-value was less than 0.05, it was considered significant. The Independent Samples T-Test was used to determine the correlation between case and control and the study variables.

Ethical Approval

This study was conducted after the approval was taken from the hemodialysis centers, Research Committed and Program/Alfajr collage, and Medical Laboratory Science Program.

Results and Discussion

Demographic Data of the Participants

The overall participants in this study were 110, 60 of them were HD patients who were considered a case group, and 50 were considered a control group, **Figure 1**. 54.5 % of all participants were males (60/110), while the females were 45.5 % (50/110). **Figure 2** The majority of the participants the aged more than 60 years 46.4% (51/110) **Table 1**. age mean was 52.1years. 85.5% (94/110) of the participants have their residence in Omdurman around the HD center, this is good to facilitate the movement of the participants towards and from the HD center **Table 1**. 47.3 % (52/110) of the studded population had a history of hypertension this highlights the high prevalence of this disease in our setting, while D. M occurrence was 8.2% (9/110), and 9.1% (10/110) of the studded population had both Hypertension and D.M. **Table 1**.

Since the age the majority occurred at the range of more than 60 years, the HBV vaccine was limited to 84.5% (93/110) of all participants who were non vaccinated against HBV **(Table 1)**. In our study, 46.4% (51/110) of the HD patients had a history of HD with a duration between 1-10 years **(Table 1)**.

Again, the age of the participants gives a reflection on the occupation, that is, 59.1% (65/110) of them stayed at home without work. **Table 1**, and 37.3% (41/110) were educated at the university level.

Table 1. Demographic data of the participants

Type	Variable	Frequency n=50	Percent %
Gender	Male	60	54.5
	Female	50	45.5
	Total	110	100
Age	20-39	27	24.5
	40-59	32	29.1
	More than 60	51	46.4
	Total	110	100
Residence	Omdurman	94	85.5
	Khartoum	7	6.4
	Out Khartoum state	9	8.2
	Total	110	100
Chronic disease	Hypertension	52	47.3
	D.M	9	8.2
	Hypertension and D.M	10	9.1
	No	39	35.5
	Total	110	100
HBV vaccine	Yes	17	15.5
	No	93	84.5
	Total	110	100
Duration of HD	Less than one year	9	8.2
	1 - 10 years	51	46.4
	More than 10 years	1	0.9
	No	49	44.5
	Total	110	100
Occupation	Worker	37	33.6
	Employee	8	7.3
	No work	65	59.1
	Total	110	100
Education Level	Un literate	35	31.8
	Primary	34	30.9
	University	41	37.3
	Total	110	100

The Prevalence of HBV Infection

The overall prevalence of HBV infection among case and control groups was 26.3% (29/110), and among the case (HD patients) the prevalence was 45% (27/60), and the control group 4% (2/50). 68.9 %(20/29) of these infected individuals were males and 62% had a history of hypertension (18/29). Also, 72.4% (21/29) of them had not taken the HBV vaccine and 27.5% (8/29) of vaccinated individuals had positive results for HBV showing that the immune response toward the HBV vaccine remains unsatisfactory.

None of the infected participants had a history of jaundice or needle injury (0/29). **Table 2**, According to these results;

there was a significant association between HBV infection and history of hypertension (P-value = 0.049), not taking HBV vaccine (P-value = 0.019), and duration of HD more than one year (P-value = 0.000), and absence of needle injury (P-value = 0.012). In **Table 2**, on the other hand, we did not find any association between HBV infection and gender (P-value = 0.057), age (P-value = 0.222), or history of jaundice (P-value = 0.222) **(Table 2)**.

Table 2. Association between HBV infection and study-variables

Type	Variable	HBV infection		Total	P-value
		Negative	Positive		
Gender	Male	40	20	60	0.057
	Female	41	9	50	
	Total	81	29	110	
Age	20-39	20	7	27	0.222
	40-59	21	11	32	
	More than 60	40	11	51	
	Total	81	29	110	
Chronic disease	Hypertension	34	18	52	0.049
	D.M	8	1	9	
	Hypertension and D.M	6	4	10	
	No	33	6	39	
	Total	81	29	110	
HBV vaccine	Yes	9	8	17	0.019
	No	72	21	93	
	Total	81	29	110	
Duration of dialysis	Less than one year	6	3	9	0.000
	1- 10 year	28	23	51	
	More than 10	0	1	1	
	No	47	2	49	
	Total	81	29	110	
Jaundice	No	79	27	106	0.228
	Yes	2	2	4	
	Total	81	29	110	
Needle injury	No	81	27	108	0.012
	yes	0	2	2	
	Total	81	29	110	

*Pearson Chi-Square test was used
*P-value < .05 is significant

The Prevalence of HCV

In this study, the prevalence of HCV was 1.8% (2/110) in the case and control group, and it was 3.3% (2/60) among the HD patient and 0% (0/50) in the control group. The two infected individuals were males, their age more than 60 years, had a history of hypertension, none of them was vaccinated against HBV, and both had a duration of HD of more than one year, also they had no history of jaundice or needle injury. Besides the low prevalence of HCV infection compared with HBV infection in our setting, there was no significant association between HCV infection and gender, age, duration of HD, hypertension, jaundice, and needle injury shown in **Table 3**. In this study, no co-infection with HBV and HCV was detected.

Table 3. Association between HCV infection and study-variables

Type	Variable	HBV infection		Total	P-value
		Negative	Positive		
Gender	Male	58	2	60	0.193
	Female	50	0	50	
	Total	108	2	110	
Age	20-39	27	0	27	0.308
	40-59	32	0	32	
	More than 60	49	2	51	
	Total	108	2	110	
Chronic disease	Hypertension	50	2	52	0.518
	D.M	9	0	9	
	Hypertension and D.M	10	0	10	
	No	39	0	39	
	Total	108	2	110	
HBV vaccine	Yes	17	0	17	0.520
	No	91	2	93	
	Total	108	2	110	
Duration of dialysis	Less than one year	9	0	9	0.502
	1- 10 year	49	2	51	
	More than 10	1	0	1	
	No	49	0	49	
	Total	108	2	110	
Jaundice	No	104	2	106	0.846
	Yes	4	0	4	
	Total	108	2	110	
Needle injury	No	106	2	108	0.782
	Yes	2	0	2	
	Total	108	2	110	

*Pearson Chi-Square test was used *P-value < .05 is significant

Association between Case, Control Groups, and HBV&HCV Infections

In this study, there was a significant difference between the prevalence of HBV infection in case (HD patients) (45%) and the prevalence among the control group (4%) (P-value = 0.000) shown in **Table 4**. Also, there was a significant difference in HBV infection among non-vaccinated individuals in HD patients, and the control group (P-value = 0.000) shown in **Table 4**. But there was no significant difference between case & control in the gender, age, and

HCV infection (P. value 0.212, 0.626, 0,196) respectively shown in **Table 4**.

Table 4. Correlation between case and control and the study-variables

Variables	Type	Mean	Std. Deviation	P value
Gender	Case	1.4	0.5	0.212
	Control	1.5	0.5	
Age	Case	2.2	0.8	0.626
	Control	2.3	0.9	
HBV vaccine	Case	1.7	0.5	0.000
	Control	2.0	0.0	
HBV infection	Case	1.5	0.5	0.000
	Control	1.0	0.0	
HCV infection	Case	1.0	0.2	0.196
	Control	1.0	0.0	

*Independent Samples T-Test was used
*P-value < .05 is significant

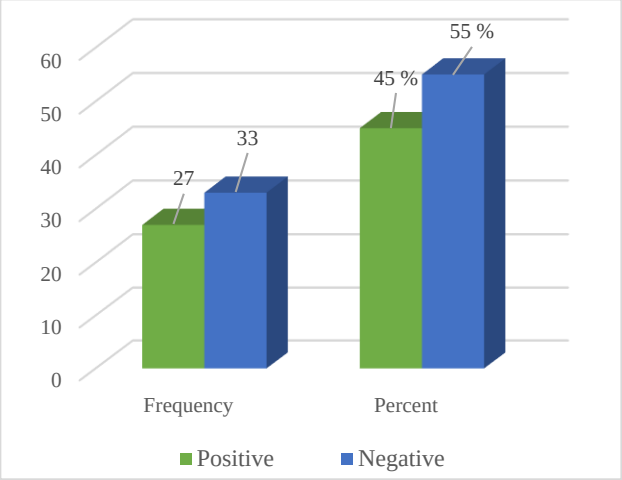

Figure 1. HBV infection among HD patients

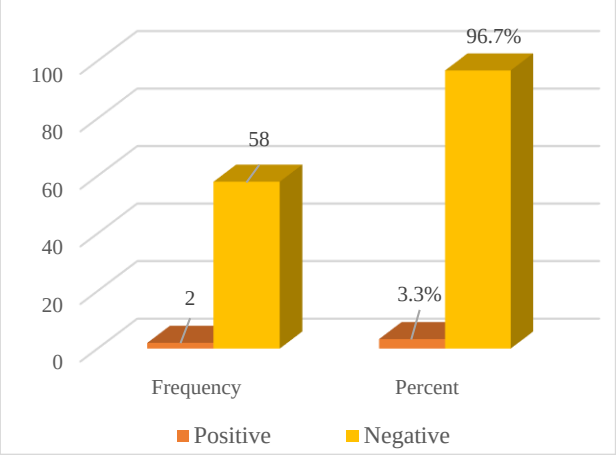

Figure 2. HCV infection among HD patients

The predominance of hepatitis viral infection and genetic markers dissemination among hemodialysis patients in Sudan is undisclosed in recent years. On the other hand, patients undergoing hemodialysis are at a greater likelihood of hepatitis B and C virus acquisition [13]. To take a glance into these concerns and the likelihood of nosocomial transmission. So current study aimed to detect HBsAg and anti-HCV among Sudanese patients who underwent hemodialysis.

According to the present results, 26.3 percent of the population was infected with HBV. This result was less than the rates reported in different studies in Sudan, in which the rate of exposure to the HB virus ranged from 47% to 78% and varies from 6.8% in central Sudan to 26% in Southern Sudan [14] and in many countries of Northern Africa, Asia, and South America, up to 70% [15, 16].

The overall frequency of anti-HCV was (1.8%) less than (40%), which has been documented in Syria, Iran, Tunis, and Senegal [16, 17] and less than (17.3%) in Sudan [17, 18].

In our study, there was a high prevalence of HBV infection among HD (45%) which was high compared with another study carried out in Sudan, in which the prevalence of HBV among HD (5%) and for HCV was 6% C [18, 19], and the incidence of HBV was higher in males (68.9%) than in females (31.1%). In another study, the prevalence of hepatitis B surface antigen was slightly increased among the males' group (54.6%) [19, 20].

Eventually, the present study revealed that the prevalence of HBV increased among advanced age, as it gradually increased after the age of 60 years (37.9%), in contrast to the prevalence in age groups less than 30 years. In another study in India [20, 21], the majority of patients were found to be 41-60 years of age (41.3%) accompanied by 21-40 years (31.5%) and then at 61-80 years (23.9%). In our study 27.5% (8/29) of vaccinated individuals had a positive result for HBV, this result differed from the result found by [19] and none of the vaccinated patients were considered HBsAg positive [18].

Strict preventive measures such as rigorously enforced overall aseptic techniques and guidelines, extremely cautious disinfection and devices sterilization, as well as adequate clinical testing of patients' blood, and regular monitoring of hepatic enzymes should be implemented as a common procedure in Sudanese dialysis centers. Even if the immune system to the hepatitis B vaccine persists inadequate, vaccination is an essential method of preventing HBV spread among many patients on long-term dialysis. Isolation of HBsAg patients diagnosed by dialysis rooms, personnel, and machines remains a major factor in avoiding HBV infection in dialysis units.

Limitation of the Study

The study population was selected from one hospital, so the need for studying more population from different hospitals

are recommended. Molecular screening of HBV and HCV infections for the HD patients using highly sensitive methods such as real-time PCR was not included.

Conclusion

Throughout this study, the predominance of HBV among HD patients was extremely high (45%), implying that HD was a significant risk for HBV infection. The findings highlight the importance of performing a constructive intervention for rapid identification, and diagnosis using a highly sensitive protocol such as PCR technique, therapeutic interventions of infected patients, and vaccination of those with non-protective anti-HBs antibodies, in an attempt to reduce morbidity and mortality in HD patients.

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