

## Prevalence of overt and occult hepatitis B virus infections among 135 haemodialysis patients attending a haemodialysis centre at Al-Nasiriyah city, Iraq

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### ABSTRACT

**Background and Objectives:** The prevalence of Hepatitis B virus (HBV) infection among haemodialysis (HD) patients has been well documented. In addition to overt infection, occult Hepatitis B infection exists in which a patient who is diagnosed seronegative for Hepatitis B surface antigen (HBsAg) shows positive HBV-DNA on using more accurate molecular methods. This study aims to determine the prevalence of overt and occult HBV infection among the HD patients who had attended Al-Nasiriyah dialysis centre during a two-month period.

**Materials and Methods:** Serological qualitative detection of HBsAg by rapid test (strips), enzyme immunoassay (EIA, HBsAg) and molecular (real-time polymerase chain reaction (real-time PCR)) was conducted for quantitative detection of HBV in HD patients' serum.

**Results:** The prevalence of overt HBV infection among HD patients was 3.7%. The viral load of HBV positive patients was ranging from  $5.85 \times 10^1$  to  $2.16 \times 10^6$  copies/ml of serum with median ( $7.4 \times 10^5$  copies/ml). Occult Hepatitis B was not detected in any of the seronegative HD patients (0%). Overt infection was found more in males (80%) than females (20%) ( $P < 0.05$ ). Similarly, infection was found to be higher among patients who had blood transfusions (80%) than those who had not (20%) with statistical significant  $p < 0.05$ . Although not statistically significant, the mean duration of HD was higher among HBV positive HD patients (17.6) than HBV negative HD patients (14.3). A dual infection of HBV and HCV was not detected in this study.

**Conclusion:** Nosocomial transmissions at HD centres and blood transfusion are important risk factors. Besides serological screening, real-time PCR offers a safeguard against the spread of overt and occult HBV infection and determines the viral load of the positive patients.

**Keywords:** Haemodialysis; Hepatitis B virus infection; Serological; Real-time polymerase chain reaction

### INTRODUCTION

Hepatitis B virus (HBV) infection is a major international health problem. More than 350 million peo-

ple worldwide are estimated to be chronically infected with HBV (1). The clinical impact of HBV infection is represented by hepatic cirrhosis and hepatocellular carcinoma (HCC) (2). In the general population, the prevalence of HBV varies from country to country, ranging from 1% in developed countries to about 8% in developing ones (3). Among many groups susceptible to HBV infection, patients with end-stage renal disease (ESRD) who are on maintenance haemodialysis (HD) are considered to be strong candidates for HBV infection due to prolonged vascular access

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via HD procedure at HD units where the nosocomial transmission of this virus is well documented (4-9). In addition, the impaired immune response of HD patients puts this population under serious risk of Hepatitis B and C virus infections (10, 11). Various global studies have proved that the prevalence of HBV infection is greater among HD patients than in the general population (2, 12-14).

Unlike the appearance of this infection in immune-competent individuals, HBV infection usually tends to be chronic in HD patients due to the immunosuppressive nature of ESRD (4, 5). Chronic HBV infection has three distinct states of chronicity, which can be differentiated serologically. The first one is chronic Hepatitis B characterised by detectable HBsAg in the serum for six months or more. The second is an inactive HBV carrier in which the HBsAg is detectable in the serum with negative HBeAg. The third one is an unusual clinical entity known as occult Hepatitis B infection (OBI) (15).

OBI infection is observed in patients diagnosed as seronegative for HBsAg and have detectable HBV-DNA in the liver tissue and detectable or undetectable HBV-DNA in the serum. When detected in the serum, its concentration is less than 200 IU/ml (16). A majority of OBI patients have Hepatitis B core antibody (HBcAb) and/or Hepatitis B surface antibody (HBsAb) while few patients have no serological marker at all. (5, 15, 17). The clinical impact of OBI infection involves different clinical aspects, including cryptic liver disease, development of HCC, transmission of active HBV infection during blood transfusion and organ transplantation, and its effect on the clinical outcome and treatment response of chronic Hepatitis C (17-19). Reports from various parts of the world reveal that the prevalence rate of OBI in HD patients is heterogeneous and differs from country to country. However, most studies showed that it ranged from 0 to 58% (5, 20). According to previous studies, the prevalence of OBI in HD patients largely depends on the prevalence of HBV infection in the general population of a particular country (18).

Importantly, many countries including Iraq have followed strict rules – such as thorough routine screening for seropositive HBV, vaccination and isolation of HBV positive patients in a separate sector and the use of dedicated dialysis machines to eliminate HBV infection at haemodialysis centres – and these have contributed to significantly minimising HBV contamination (20, 21). However, HD patients

and HD centres are still under serious threat by virtue of the OBI. Since many countries, including Iraq, depend solely on the serological detection of HBsAg to judge whether the patient is free of HBV and to ultimately send them to the haemodialysis sector, which is specialized for seronegative patients, it is not uncommon to find OBI under such circumstances. Thus, the investigation of OBI becomes urgent and requires a highly sensitive method for detecting the virus DNA in the blood, and the best choice for this mission is real-time PCR, considered a golden standard test for detecting OBI (16). Thus, this study aims to investigate the following: the prevalence of HBV infection among the haemodialysis patients at Al-Nasiriyah Haemodialysis Centre and the prevalence of OBI in HD patients, if present.

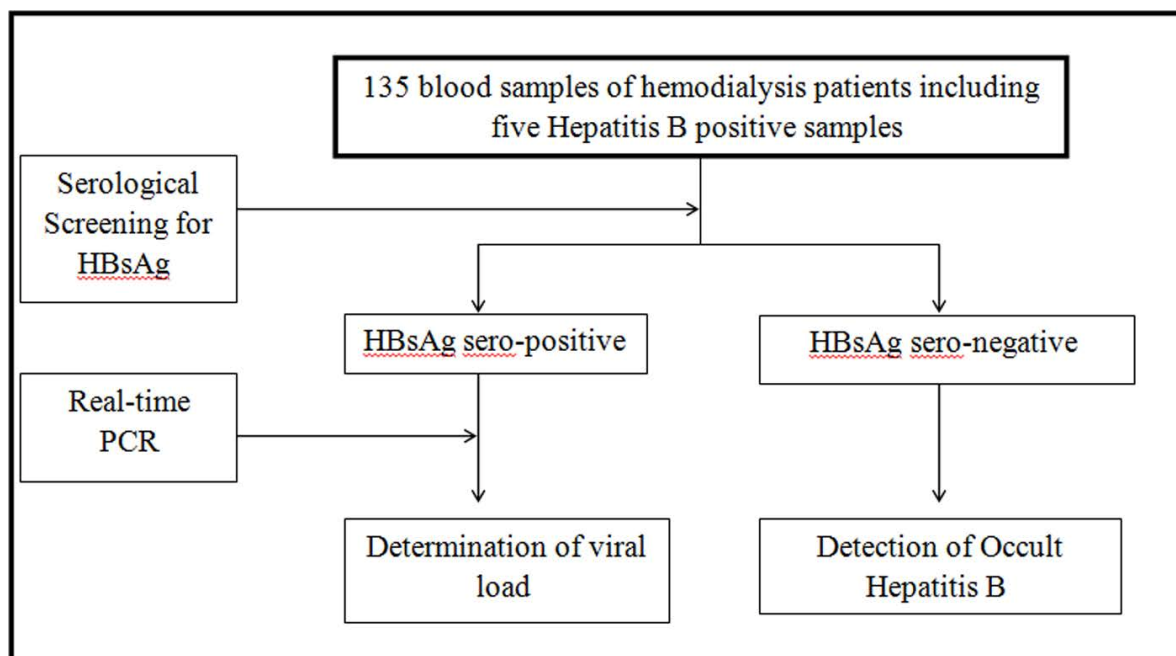
## MATERIALS AND METHODS

135 frozen serum samples were obtained from the laboratory of Nasiriyah Haemodialysis Centre/ Al-Hussein Teaching Hospital. These samples were taken from all the HD patients who had attended the haemodialysis centre at Al-Hussein Teaching Hospital in Al-Nasiriyah city from June to September 2019. All the relevant medical and socio-demographic characteristics – including age, sex, duration of haemodialysis, details on blood transfusion and organ transplantations, Hepatitis B vaccination history – were gathered from the Nasiriyah Haemodialysis Centre's statistical department.

According to the information obtained from the laboratory of the haemodialysis centre, five samples were previously diagnosed as Hepatitis B seropositive and three samples were hepatitis C seropositive by ELISA. The frozen serum was directly transported via cooled containers to the molecular biology research unit at Technical Institute/Southern Technical University for serological and molecular screening. Fig. 1 summarises the methodology.

Firstly, all the samples were subjected to serological screening for HBsAg using two serological techniques – rapid test (HBsAg strip, CTK, BIOTECH, U.S.A) and Enzyme Immune assay (EIA) kit (Foresight® USA) – for the qualitative detection of HBsAg in the serum.

Secondly, seropositive and seronegative serum samples were subjected to DNA extraction using commercial available viral nucleic acid extraction



**Fig. 1.** Methodology for detection of overt HBV infection and Occult hepatitis B virus Infection (OBI) serological and molecular methods

kit (Gnaid® Viral Nucleic Acid Extraction Kit, Taiwan). The purity and concentration of extracted DNA was checked by Nanodrop (NAS-99, Taiwan). Real-time PCR was used to determine the viral load of seropositive samples and to investigate the OBI in seronegative samples. A quantitative HBV real-time PCR kit (AccuPower® HBV Quantitative PCR kit, Bioneer, Korea) was used, which targeted the X region of the viral genome for detection and quantitation of the HBV in the serum. The reaction mixture and thermo-cycling conditions were prepared following the manufacturing company's instructions (Bioneer, Korea). The absolute quantitation of HBV-DNA in the serum samples was determined using the ready to use five HBV-DNA standards provided with the kit, the concentration of standards HBV-DNA was  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$  copies/ml. Internal process control (IPC) provided with kit was used to evaluate the reaction efficiency and to determine the presence of reaction inhibitors if present. Negative control (DEPC distal water) was used as control negative along with each sample. The amplification reactions and result analysis (copy number/ml, threshold, baseline, slope, reaction efficiency) were done automatically with a real-time PCR thermo-cycler (Exicycler™ 96, Bioneer®, Korea).

**Statistical analysis.** The chi-square test ( $\chi^2$ ) was used to make comparisons between groups of Hepatitis B positive and negative patients. Data were presented as percentages, median, and differences were considered as statistically significant only if  $P < 0.05$ .

**Ethical approval.** This study was approved by scientific committee of Al-Nasiriyah Technical Institute/ Southern Technical University. Serum samples were provided by the laboratory of Haemodialysis Centre/ Al-Hussein Teaching Hospital according to written approval agreement obtained from Health Directory in Thi-Qar Province. A written approved consents were obtained from all participants after informing them about the project.

## RESULTS

The demographical characteristics of the patients are presented in Table 1. The mean age was  $56.044 \pm 14.77$  (22-85 years). The number of males were higher (90, 66.7%) than females (45, 33.3%) with statistically significant differences ( $P < 0.05$ ). The duration of HD of these patients at Al-Nasiriyah Haemodialysis Centre ranged from 1 to 36 months with a mean of  $12.77 \pm 6.95$ .

According to their medical history, the majority of patients (100/135, 74.07%) had not undergone a blood transfusion previously, while 35 (25.93%) had undergone blood transfusions for past surgical operations before attending the haemodialysis centre. None of the patients had organ transplantations, and only three (2.2%) patients had been diagnosed as seropositive for Hepatitis C virus by the haemodialysis centre laboratory. Eighty five (63%) patients had a history of HBV vaccination and 50 (37%) had no history of hepatitis B vaccination.

The results of the serological and molecular methods for the detection of HBV infection are presented in Table 2. The serological detection of HBsAg by both rapid test (HBsAg strips) and EIA test revealed that 5 out of 135 (3.7%) patients were diagnosed as seropositive and 130 (96.3%) were seronegative. Molecular detection by real-time PCR revealed that all the seropositive samples showed positive HBV-DNA

with median viral load ( $7.4 \times 10^5$ ) and a copy number ranging from  $5.85 \times 10^1$  to  $2.16 \times 10^6$  per ml of serum (Fig. 2). On the other hand, none of the 130 seronegative patients showed positive HBV-DNA, indicating that the prevalence of OHB infection was 0%.

According to the table above, the prevalence of HBV infection among HD patients was determined accurately with the serological method (EIA) and molecular method (real-time PCR), and it was 5/135 (3.7%).

Distribution of HBV infections among the HD patients, based on their demographical characteristics, is presented in Table 3. The results of this study showed that the mean age of the Hepatitis B positive patients was  $47.8 \pm 20.2$  years, which was slightly lower than the Hepatitis B negative patients ( $56.12 \pm 15.1$ ) with no statistical differences ( $P > 0.05$ ). The prevalence of Hepatitis B infection was found to be higher in males (4/5, 80%) than females (1/5, 20%).

Although not statistically significant ( $P > 0.05$ ), the mean duration of haemodialysis was higher for Hepatitis B positive patients ( $17.6 \pm 1.14$ ) than those free of Hepatitis B infection ( $14.3 \pm 6.7$ ). Regarding blood transfusions, 4 out of 5 (80%) of the HBV positive patients had received blood previously before beginning the haemodialysis. With regard to a dual infection of HBV and HCV, this study found that the three seropositive HCV (previously confirmed by Haemodialysis Centre Laboratory) were seronegative for HBV, the prevalence of dual infection was 0%. This study found that all hepatitis B positive patients had not vaccinated at beginning of haemodialysis and they were hepatitis B positive at haemodialysis admission.

**DISCUSSION**

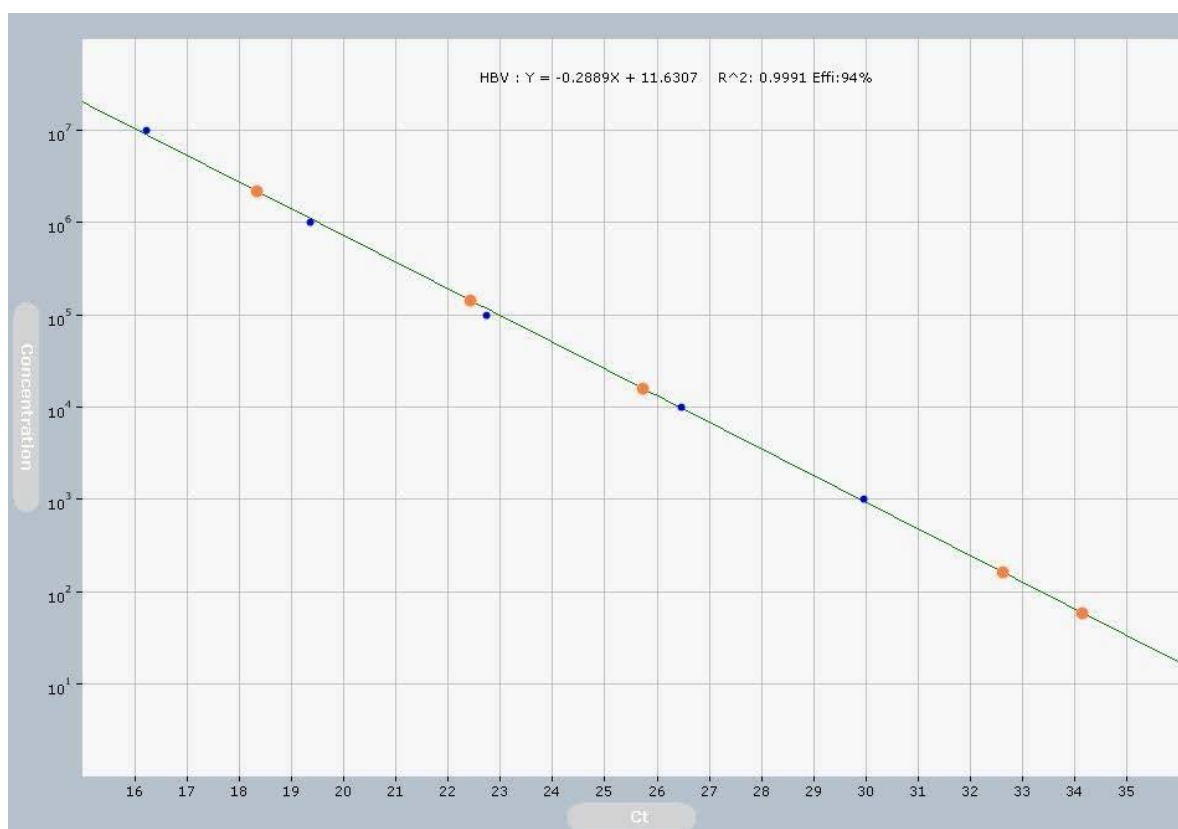
The prevalence of Hepatitis B in Iraq has previously been studied (22, 23). Nevertheless, most of these

**Table 1.** Demographic characteristics of HD patients attending Al-Nasiriyah haemodialysis centre during the period from June to September 2019.

Characters	Values	P-Value
Age (Range)	56.0448 ± 14.775 (22-85 years)	
Gender (%)		
Male	90 (66.7%)	< 0.05
Female	45 (33.3%)	
Haemodialysis duration	12.7776 ± 6.952 (1-36)	
Blood transfusion (%)		
Yes	35 (25.93%)	< 0.05
No	100 (74.07%)	
Hepatitis C (%) infection		
Positive	3 (2.2%)	< 0.05
Negative	132 (97.8%)	
Hepatitis B vaccination history		
Yes	85 (63%)	<0.05
No	50 (37%)	

**Table 2.** Serological and Molecular detection of Hepatitis B infection among HD patients

	Serological Marker		Molecular Marker	
	Strip	HBsAg ELISA N (median of OD)	HBV-DNA Positive (Median of copy number/ml serum)	Negative
Seropositive	5	5 (2.45)	5 ( $7.4 \times 10^5$ )	0
Seronegative	130	130 (0.031)	0	130
Prevalence		3.7%	3.7%	



**Fig. 2.** A viral load of HBV positive patients that underwent maintenance HD at Al-Nasiriyah Hemodialysis Center. The orange dots represent HBV positive patients, the blue dots represent standard DNA, the X axis represents the number of Ct and the Y axis represents the Log of Viral load.  $Y = 0.2889x + 11.6307$   $R^2 = 0.9991$ , Efficiency = 94%

**Table 3.** The distribution of HBV infection among HD patients according to their demographic Characteristics

Patient charters	HBV positive patients	HBV negative patients	P-value
Age	47.8 ± 20.2	56.12 ± 15.1	P > 0.05
Gender Male			
Female	4 (80%)	86 (66.15%)	P < 0.05
	1 (20%)	44 (33.85%)	
Duration of haemodialysis	17.6 ± 1.14	14.3 ± 6.7	P > 0.05
Blood transfusion			
Yes	4 (80%)	31 (23.84%)	P < 0.05
No	1 (20%)	99 (76.16%)	
Hepatitis C infection			
Positive	0	3	
Negative	5	127	
HBV vaccination			
Yes	0	85	P < 0.05
No	5	45	

studies have focused on the general population and had employed serological markers such as HBsAg, HBsAb and HBcAb to determine its prevalence. Determining the prevalence using only serological techniques subjected the results to variations since various serological markers were used in different studies and also due to the possibility that Hepatitis B may have gone undetected in HBV positive patients by virtue of OHB. Thus, this study has used the most accurate molecular method to detect and determine the viral load of HBV inpatient serum with real-time PCR besides screening for HBsAg with EIA technique.

According to the results of the current study, the prevalence of Hepatitis B infection among HD patients was 3.7%. This finding is in agreement with the results of a study conducted in the north of Iraq which found the prevalence of HBV infection among HD patients to be 3.2% (24). Our finding was higher than that obtained by a study conducted in 2015

at Karbala city in central Iraq, where the prevalence is 1/165 (0.6%). On the other hand, our finding was lower than that recorded in Basra city which is 200 km south of Thi-Qar Province, where 31.14% of HD patients are HBsAg positive and 31% are positive for HBV-DNA by real-time PCR (25). This variation could be attributed to the heterogeneity of Hepatitis B prevalence among HD patients in different provinces and in various haemodialysis centres throughout Iraq. Variations in the prevalence of Hepatitis B infection in different haemodialysis centres of the same country has been recorded previously (4, 26, 27).

The results of the current study supports the suggestion that the prevalence of HBV infection among HD patients may be greater than its prevalence in the general population as the endemicity of Hepatitis B in a particular country shapes the prevalence of HBV infection in HD patients (2, 28). Iraq was considered to have intermediate endemicity of Hepatitis B (3%) according to previous studies (23, 29). Thus, locally, the result of this study seems to be reasonable and to fit within the normal range in Iraq.

At the global level, beginning from the neighbouring countries of Iraq, the results of this study are in close association with those reported in Iran (2.7%, 1.2% and 4.6%) (13, 30, 31) and in Libya (2.6%) (27). On the other hand, our findings are lower than Jordan (5.9%) (32) Turkey (13.3%) (33) and Saudi Arabia (10%) (31). The prevalence of Hepatitis B infection among HD patients differs from country to country (4). Globally, based on a large scale study on 8618 HD patients from 305 dialysis centres in western Europe and USA, the prevalence of HBV ranges from 0 to 6.6 (34).

According to this study, the prevalence of OHB infection is 0%. Similar results have been recorded in many global studies, (6, 35-37) where it has been estimated that the prevalence of OHB infection ranges from 0 to 58%. However, the detection of OHB where it depends on serum samples alone may underestimate the true prevalence of OHB. Additionally, the best sample to be studied is a liver biopsy, but obtaining a liver biopsy from HD patients is very difficult. Thus, serum samples are the most common approach to identify OHB (16).

Regarding the age and sex of the HD patients, the mean age of the positive patients was 47.8%, and no relation was found in terms of age. However, this

study recorded a higher rate of infection in males than females. Similar findings had been recorded in previous studies (4, 8, 27, 38, 39). Although not statistically significant ( $P > 0.05$ ), the mean HD duration of positive patients ( $17.6 \pm 1.14$ ) was higher than negative patients ( $14.3 \pm 6.7$ ). Such an observation can be explained based on the aspect of nosocomial transmission of HBV in HD units as longer duration represents longer exposure to infection (27). In fact, similar results have been recorded by many authors (25, 30, 39). Blood transfusion has been noted as an important risk factor for the prevalence of HBV infection among HD patients (12). This study recorded a strong association between blood transfusion and HBV infection, which is in agreement with previous studies (27, 40). Although screening of transfused blood for HBV has been applied in many countries, blood transfusion is still a source of infection in HD patients (12).

## CONCLUSION

Although the haemodialysis centre at Al-Nasiriyah follows the rules of HD infection control and practice, HVB is still recorded among HD patients. Even though it has low prevalence, nosocomial transmission has contributed to some extent. However, blood transfusion is highly suggestive of the prevalence of HBV among HD patients. Using more accurate molecular method real-time PCR for screening HD patients for HBV besides the use of serological method will eliminate the possibility of spreading HBV infection in haemodialysis centres, especially those hidden infections that cannot be detected via serological methods. Though this study found that the prevalence of OHB infection at Al-Nasiriyah is 0%, more studies must be conducted on this topic at the other provinces in Iraq.

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