

# Absence of Occult Hepatitis B Virus Infection in Haemodialysis Patients in White Nile State, Sudan

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## Research Article

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## Abstract

This study was carried out to detect occult hepatitis B virus infection in haemodialysis patients in white Nile state, Sudan. Sandwich Enzyme Linked Immunosorbent Assay (ELISA) was used to detect hepatitis B surface antigen (HBsAg), competitive ELISA to detect Hepatitis B virus core antibody (HBcAb) and polymerase chain reaction (PCR) to detect Hepatitis B virus (HBV) DNA were used to analyze 89 serum samples collected from patients in Abass Ibrahim haemodialysis center in white Nile state, Sudan. Of all the patients sampled, 68 were males while 21 were females, none of the patients showed signs of clinical hepatitis. The results showed that 1(1.1%) out of 89 samples was positive for HBsAg and was subsequently excluded from the study. Out of the 88 HBsAg negative samples, 37(42%) (27 males and 10 females) were positive for HBcAb and 0(0%) were positive for HBV DNA with PCR.

**Keywords:** Hepatitis B virus (HBV); Haemodialysis; Polymerase Chain Reaction (PCR); Enzyme Linked Immunosorbent Assay (ELISA); Sudan

## Introduction

Hepatitis B virus (HBV) is a species of the genus Orthohepadnavirus which belongs to the family Hepadnaviridae. HBV is highly contagious, and is the most commonly transmitted blood borne virus in the health care setting [1].

Hepatitis B virus infection is a considerable global health problem and approximately two billion of the

world populations have been infected, of which 250 million live with HBV infection. HBV infection is linked with a wide range of clinical manifestations, including acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carriers, chronic hepatitis, cirrhosis [2,3].

Occult hepatitis B infection (OBI) is defined as the existence of low-level HBV DNA in the serum (<200 IU/mL); cells of the lymphatic (immune) system,

and/or hepatic tissue in patients with serological markers of previous infection (anti-HBc and/or anti-HBs positive) and the absence of serum HBsAg.

Traditionally, HBV is diagnosed by serological techniques to detect antigens or antibodies. The hepatitis B surface antigen (HBsAg) is often used for routine diagnosis since it is considered as the hallmark of infection. During acute infection, antibodies to HBV core antigens (anti-HBc) (initially both IgM and IgG) appear 1–2 weeks after the appearance of HBsAg, while IgG persists during chronic infection. The presence of antibodies to HBsAg (anti-HBs) represents immunity to HBV infection [4].

Several groups are believed to be at risk of OBI. The reactivation of OBI may take place in individuals with a previous history of HBV infection along with immunosuppression or chemotherapy status. Lastly, to prevent the spread of OBI, the screening of HBV DNA should be implemented in blood donors, immunosuppressed patients, organ transplant donors, organ transplant recipients, and individuals with acute rheumatoid arthritis before and after treatment with anti-tumor necrosis factor (TNF)- $\alpha$  [5].

HBV can be transmitted directly through contact of body fluids to mucous membranes, cutaneous scratches, abrasions, burns or other lesions. Indirect transmission can occur from surfaces contaminated with blood or body fluids to mucous membranes. HBV has been shown to survive in dried blood on surfaces at room temperature for at least a week [1].

The aim of this study was to determine the prevalence of occult hepatitis B virus infection in haemodialysis patients without symptoms of liver disease in White Nile State, Sudan.

## Materials and Methods

### Study Design

This is a cross-sectional study.

### Study Area And Period

This study was carried out in Abass Ibraheim center in White Nile state during the period from December 2017 to February 2018.

### Study Population and Sample Size

The study was conducted among haemodialysis patients including 66 males and 23 females who visited

the Abass Ibraheim center in White Nile State. The personal data collected from patients included age, gender, date of renal failure, date of sample collection. Plasma was separated by centrifugation and stored at -20 until further analyses.

### Serology

Commercial ELISA kits (Fortress Diagnostic Automation, INC) were used to detect HBsAg and HBcAb according to the procedure described by the manufacturer.

### HBV DNA Detection

DNA was extracted from patient's material using commercial kit (Analytic jena, INC. Korea) according to manufacturer's instructions. The extracted DNA was stored at -20 until used.

### Polymerase Chain Reaction(PCR)

The PCR was performed using primers that are specific for the HBsAg gene of HBV. The primers used consisted of forward primer 5'TCGGAAATACACCTCCTTTCCATGG3' (HBV genome 1353-1377) and reverse primer, 3'GCCTCAAGGTCGGTCGTTGACA-5' (HBV genome 1702-1681). The reaction was performed in 25 $\mu$ l volume using Solis Bio dyne master mix. The volume included: 5 $\mu$ l master mix, 1 $\mu$ l forward primer, 1 $\mu$ l reverse primer, 5 $\mu$ l extracted DNA and 13 $\mu$ l distilled water.

The DNA was amplified in thermocycling conditions using PCR machine. Techno (japan) as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 62°C for 1 min and extension at 72°C for 1 min, with a final extension step at 72°C for 1min. Ten  $\mu$ l of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose gel. The gel was prepared by adding 0.7g of Agarose to 35 ml 5x Tris Borate EDTA buffer, the product was visualized by staining with 0.15% Ethidium bromide using UV gel documentation system INGeNius. The expected size of the surface antigen gene (HBsAg gene) amplicon is 350 Bp.

## Results

### Detection of HBsAg

A total of 89 samples were tested for HBsAg, one sample (1.1%) (Male) was positive for HBsAg by ELISA. 88 samples (98.9%) (67 males and 21 females) tested negative for HBsAg (Table 1).

### Detection of HBcAb

The HBsAg negative samples (88) were tested for HBcAb, thirty seven samples (42%) (27 males and 10 females) were positive for HBcAb while fifty one (58%) samples (40 males and 11 females) tested negative for HBcAb (Table 2).

### Detection of Hepatitis B Virus DNA

A total of 37 samples that were positive for HBcAb and negative for HBsAg were tested for HBV DNA using PCR. No DNA was detected in any of the samples (table 3).

Sex	ELISA HBsAg		Total (%)
	Positive (%)	Negative (%)	
Male	1(1.47)	67(86.5)	68(76.4)
Female	0(0.0)	21(100)	21(23.6)
Total	1(1.1)	88(98.9)	89(100)

Table 1: Frequency of HBsAg among haemodialysis patients.

Sex	ELISA HBcAb		Total (%)
	Positive (%)	Negative (%)	
Male	27(40.3)	40(59.7)	67(76.1)
Female	10(47.6)	11(52.4)	21(23.9)
Total	37(42.0)	51(58.0)	88(100)

Table 2: Frequency of HBcAb among haemodialysis patients negative for HBsAg.

Sex	HBV DNA		Total (%)
	Positive (%)	Negative (%)	
Male	0(0)	27(100)	27(72.9)
Female	0 (0)	10(100)	10(27.0)
Total	0(0)	37(100)	37(100)

Table 3: Frequency of HBV DNA among HBc positive haemodialysis patients.

### Discussion

This study was designed to determine the prevalence of occult hepatitis B virus infection in end stage renal failure patients undergoing haemodialysis in White Nile State. The study subject involved 89 individuals.

The frequency (0%) of occult HBV infection among seroreactive patients to HBcAb in this study is in agreement with the results presented by Kalantari et al in Isfahan, Iran which reported that no patient with occult HBV infection were found among haemodialysis patients

[6]. Another study in Sudan also reported prevalence of occult HBV infection among blood donors was (0%) [7].

However the study findings disagreed with the results obtained by Abdalhafiez AM et al in Sudan where 3 haemodialysis patients (3.3%) were positive for occult HBV infection. In contrast; very high (32%) incidence of OBI was reported by Helaly et al in Alexandria, Egypt in chronic haemodialysis patients [8,9]. On the moderate side; Tomokatsu et al in Japan reported that 6 haemodialysis patients (3.7%) were found to harbor occult HBV, and Andrea et al in northeast Brazil reported that 7 patients (2.3%) were HBV-DNA positive [10,11].

The variation in the reported incidences of occult HBV infection in different studies including this study could be a result of several factors. It could be attributed to the different status of the population investigated, the differences in the sensitivity of the various molecular biology techniques used in detection of HBV DNA, differences in the prevalence of HBV in geographical areas, differences in the storage and age of serum samples used in studies and differences in study sample size [12].

### Conclusion

Despite the 0% level of occult HBV infection reported in this study serological markers of HBV infection should always be backed up with molecular tests to guard against inadvertent transmission of HBV infection especially in haemodialysis settings.

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