

## Occult Hepatitis B Virus Infection in Nigerian Patients on Haemodialysis

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### ABSTRACT

**Introduction:** Hepatitis B virus (HBV) infection is highly endemic in Sub-Saharan Africa. Occult HBV infection is prevalent among chronic kidney disease (CKD) patients on haemodialysis and frequent blood transfusion.

**Method:** Forty one adult Nigerians with CKD on haemodialysis who were asymptomatic, had no signs of liver disease and tested negative to hepatitis B surface antigen (HBsAg) were recruited. Patients' status with regard to antibody to hepatitis C virus (anti-HCV), human immunodeficiency virus (HIV) I and II were determined by serological test using third generation enzyme-linked immunosorbent assay (ELISA). Quantitative analysis for HBV DNA was estimated by real-time polymerase chain reaction (PCR) using EliGeneHBV UNI reagent according to the manufacturer's protocol.

**Result:** 6 (14.6%) of the patients had occult hepatitis B virus infection with detectable HBV-DNA in their serum. One (16.7%) of the patients with occult Hepatitis B virus infection had co-infection with Hepatitis C virus. Two (33.3%) of the patients with occult Hepatitis B virus infection had co-infection with HIV I & II, compared with 8 (22.9%) of the HBV-DNA negative patients. There was however no statistically significant difference between both groups ( $p = 0.483$  and  $0.629$  respectively).

**Conclusion:** Occult HBV infection is common among Nigerian CKD patients. More studies involving larger population may be necessary to characterize this group of patients

### INTRODUCTION

Hepatitis B virus (HBV) infection is highly endemic in Sub-Saharan Africa with about 14 to 17.1% of the population chronically infected.[1, 2]. Most infections in this region are believed to occur very early in life through various cultural practices such as scarification and some other practices using unsterilized equipments.[1, 2, 3, 4]

Although primarily a hepatotropic virus, HBV infection has also been implicated in the pathogenesis of many glomerular diseases in the kidney, mainly membranous, membranoproliferative and mesangial proliferative glomerulonephritides. [5, 6]

Secondly, hepatitis B infection and transmission in the dialysis population is believed to constitute a major problem in Haemodialysis patients. Due to the parenteral transmission of the virus, haemodialysis patients are at high risk of infection with this virus because they need frequent blood transfusions and undergo other medical and surgical procedures that may be associated with bleeding. The prevalence of HBV infection in these patients has been estimated to be between 5 to 10%.[7, 8]. This

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lower incidence in the dialysis population compared with that of the general population in Nigeria is probably due to selection bias as many dialysis units in the country do not enroll Hepatitis B positive patients for treatment.

General treatment guideline recommends isolation of hepatitis B positive patients from other patients during dialysis. Isolation of HIV positive patients from other non-infected patients in the haemodialysis room is not recommended because of the low risk of transmission.[9]

Occult HBV infection is defined as the detection of HBV de-oxyribonucleic acid (HBV-DNA) in the serum, lymphatic cells or hepatic tissues in the absence of detectable hepatitis B surface antigen (HBsAg) in the serum.[10] Occult infection could be either as a result of HBV infection by low titre of the virus (primary occult infection (POI) or a sequel of acute infection which is resolving (secondary occult infection (SOI).[11]

Antibody to HBV core antigen (anti-HBc) which indicates previous HBV infection and recently, evidence of progressing occult infection is detectable in about 80% of patients with SOI [12]. Naturally acquired antibody to HBsAg (anti-HBs) may be detectable in occult HBV infection [12 –1 5].

Although all dialysis patients are routinely screened before the commencement of dialysis program and also at regular intervals during maintenance dialysis in accordance with the unit's protocol on viral screening, occult HBV infection can be present in the patient. This occult infection is not detected by routinely used viral screening procedures. Occult HBV infection is common in patients with chronic kidney disease (CKD) due to impaired immunity in uraemic states. Prevalence rates of occult hepatitis B infections in the haemodialysis population have been reported to be between 5 to 58% [12,16,17]. Other conditions such as HIV co-infection with HBV and HCV co-infection with HBV are associated with occult HBV infection.<sup>18,19</sup>

The prevalence rate and clinical profile of occult Hepatitis B virus infection in the CKD population in Nigeria is not known. This subgroup of patients may constitute risk of HBV infection to other CKD patients especially when not isolated from other patients during dialysis.

Identification and treatment of HBV infected patients with CKD could prevent spread of the

infection to other non-infected CKD patients receiving treatment in the same facility and thus contribute to improvement in outcome of CKD treatment.

HbsAg sero-positivity has been reported to be an independent and significant risk factor for death as well as graft failure after transplantation [20].

This study was done to determine the prevalence of occult HBV infection and identify factors associated with occult infection in Nigerian CKD patients on haemodialysis.

## **METHOD**

Forty one consenting adult Nigerians with End Stage Renal Disease (ESRD) on haemodialysis who had no symptoms and signs of liver disease and tested negative to hepatitis B surface antigen (HBsAg) were recruited from the renal units and dialysis centres of the Lagos State University Teaching Hospital, Dialyzer Medical Centre, Oshodi, Lagos.

Five milliliters of whole blood was obtained from each participant into EDTA bottles. The plasma was immediately separated from the whole blood and stored at -80°C till it was analyzed

Patients' status with regard to antibody to hepatitis C virus (anti-HCV), human immunodeficiency virus (HIV) I and II were determined by serological test using third generation enzyme-linked immunosorbent assay (ELIZA). Quantitative analysis for HBV DNA by was estimated by real-time polymerase chain reaction (PCR) using EliGene HBV UNI reagent according to the manufacturer's protocol.

Ethical approval for the study was obtained from LASUTH Health Research and Ethics Committee.

Statistical analysis was done using SPSS version 17.0. Fisher's test was used to compare proportions while comparison of means was done using the student two-tailed t test. P value in either case was significant if it is <0.05.

## **RESULTS**

### ***Biodata of the subjects***

Forty one patients on haemodialysis were recruited into the study. Twenty four (58.5%) were males while 17 (41.5%) were females. The mean age of the

patients was  $42.59 \pm 13.40$  years. Thirty five (85.2%) were in the 21 – 60 years age range. Table 1 shows the age and gender distribution.

**Prevalence of occult hepatitis B virus infection**

Six (14.6%) of the patients had detectable HBV-DNA in their serum. They were 3 (50%) males and 3 (50%) females. Their mean age was  $42.83 \pm 15.47$  years. The gender distribution and mean age were similar to those of the patients who had undetectable

**Table 1:** Age and Gender distribution of the studied subjects

		0-19	20-39	40-59	=60	Total
Patients with occult HBV	M	0	1	3	0	4
	F	0	2	0	0	2
	SUB-TOTAL	0	3	3	0	6
HBV negative patients	M	0	10	10	2	22
	F	0	4	7	2	13
	SUB-TOTAL	0	14	17	4	35

Mean age ( $\pm$ SD) for all studied subjects was  $42.59 \pm 13.40$

Mean age ( $\pm$ SD) for patients with occult HBV was  $42.83 \pm 15.47$

Mean age ( $\pm$ SD) for HBV negative patients was  $42.54 \pm 18.21$

**Table 2:** Comparison of risk factors for hepatitis B virus infection between Patients with occult HBV infection and Patients without HBV infection

	Patients with occult HBV infection	Non HBV infected patients	X <sup>2</sup> /student's values.	t P value
Positive family history of HBV infection (%)	1 (16.7)	1 (2.9)	1.858	0.294
Positive sharing of sharp objects (%)	3 (50)	9 (25.7)	1.345	0.341
Previous scarification (%)	0 (0.0)	4 (11.4)	0.810	1.000
Previous surgery (%)	0 (0.0)	7 (20.0)	1.551	0.568
Multiple sexual partners (%)	1 (16.7)	4 (11.4)	0.094	1.000
Mean number of blood transfusion $\pm$ SD	$3.00 \pm 2.16$	$3.45 \pm 4.00$	-0.218 (CI = -4.644 – 3.747)	0.829
Range of number of dialysis sessions	1 - 8	1 - 36		
Mean number of dialysis $\pm$ SD	$4.50 \pm 2.74$	$6.46 \pm 8.08$	-0.582 (CI = -8.758 – 4.844)	0.564
Range of duration on dialysis (Weeks)	1 - 7	1 - 26		
Mean duration on dialysis $\pm$ SD (weeks)	$2.83 \pm 2.229$	$4.57 \pm 5.215$	-0.797 (CI = -6.148 – 2.672)	0.430

**Table 3:** Co infection with HIV and HCV

	HBV DNA positive (n=6)	HBV DNA negative (n=35)	Total (N=41)	P value
Anti-HCV positive only (%)	0 (0)	0 (0)	0 (0)	0.483
HIV I & II positive only (%)	1 (16.7)	5 (22.9)	6 (14.6)	0.629
HCV/HIV co infection (%)	1 (16.7)	3 (8.6)	4 (9.8)	P value

HBV DNA in their serum;  $p = 0.679$  and  $0.961$  respectively. The plasma HBV DNA concentration ranged from  $4996.67 \pm 3138.11$  IU/ml.

#### **Risk factors for hepatitis B virus infection**

One (16.7%) of HBV DNA positive patients had a positive family history of HBV exposure, compared with 1 (2.9%) of HBV negative patients ( $X^2 = 1.858$ ,  $df = 1$ ,  $p = 0.294$ ). The HBV DNA positive patients who shared sharp objects with others, had scarifications, multiple sexual partners and previous surgery were 1 (16.7%), ( $X^2 = 1.345$ ,  $df = 1$ ,  $p = 0.341$ ); 3 (50%) ( $X^2 = 0.810$ ,  $df = 1$ ,  $p = 1.000$ ); 0 (0.0%), ( $X^2 = 0.094$ ,  $df = 1$ ,  $p = 1.000$ ) and 0 (0.0%), ( $X^2 = 1.511$ ,  $df = 1$ ,  $p = 0.568$ ) respectively. Table 2 shows these details as well as the range of duration on dialysis, mean duration on dialysis, mode of number of dialysis and range of number of dialysis sessions.

#### **Co-infection with HCV and HIV infections**

One (16.7%) of the patients with positive HBV DNA in the plasma was positive for HCV antibody while 3 (8.6%) patients in plasma HBV DNA negative group was positive for HCV antibody. Two (33.3%) of the HBV-DNA positive patients were positive for HIV I & II, compared with 8 (22.9%) of the HBV-DNA negative patients. There was however no statistically significant difference between both groups ( $p = 0.483$  and  $0.629$  respectively).

### **DISCUSSION**

The observed prevalence of occult HBV infection among Nigerians on haemodialysis in this study was 14.6%. Similar studies in Brazil [21] found a rate of 1%, while 47% of patients in Spanish [22,25] studies,

6% in Iran [23] and 26% in Egypt.[24]. The high prevalence of HBV infection in Nigeria may explain this observation. Other factors may however contribute to this, in view of the fact that a lower rate of occult HBV infection among haemodialysis patients was found in Iran where HBV infection is endemic, with a similar prevalence rate as Nigeria.[25]

Although all patients tested negative for Hepatitis B surface antigen on routine ELISA testing as at the time of screening, however, the high levels of HBV viraemia in those with occult infection in this study is striking. Could this really be peculiar for occult HBV infection among Nigerians on haemodialysis or false negative screening test results? The most plausible explanation is that they are false negative results. Studies in Brazil and Iran found HBV DNA concentration of  $<3.5$  IU/ml [21] and  $<50$  IU/ml [23] respectively in their patients with occult hepatitis B virus infection. The implications of this are enormous. A high level of false negative results from the currently available screening tests in the country would inadvertently place non-infected patients at a high risk of HBV infection as the appropriate infection control mechanisms would not be put in place for the dialysis of the false negative infected patients.

Secondly, the false negative patients would have benefited from anti-viral therapy to prevent liver damage in line with the European Association for the Study of the Liver guideline.[26]

Frequent haemodialysis and blood transfusion are risk factors for HBV infection for patients on haemodialysis[7]. In this study, the mean number of haemodialysis sessions among those with occult HBV infection was similar to the non HBV infected patients ( $p = 0.564$ ), likewise the mean number of blood

transfusion ( $p = 0.824$ ). The practice of using a dedicated machine for patients with detectable HBsAg in haemodialysis unit reduces the spread of HBV infection. However, the risk of contracting the infection from those with occult HBV infection would persist as the appropriate infection control mechanisms would not have been put in place.

Acquiring HBV infection from blood obtained from donors with occult HBV infection is another possibility. Occult HBV infection has been reported in 17.2% of 302 HBsAg-negative blood donors in Egypt [27] and 1.26% of 302 blood donors from Cambodia [28]. The risk of acquiring this infection is would be greatly reduced if blood transfusion rates are reduced with the use of erythropoietin stimulating agents.

Other known risks for HBV infection in this study were also not significantly higher among those with occult hepatitis B infection. These are positive family history of HBV infection, scarification marks, sharing of sharp objects and previous surgery ( $p = 0.294, 0.996, 0.341$  and  $0.568$  respectively). The small sample size may account for this.

Co-infection with HCV status in this study was not significantly associated with having occult HBV infection ( $p = 0.483$ ). In a study in Egypt for instance, 72% of 25 haemodialysis patients with occult HBV infection in Alexandria, had HCV co-infection.[24] an Italian study also found a similar high rate co-infection with 66% of patients with HCV virus infection testing positive for occult HBV infection [29]. This may have been conveniently attributed to the high prevalence of HCV infection in these countries where prevalence rates of 14.7% in Egypt [30] and 26% in Southern Italy [31] have been reported. Another study in Egypt however, observed that the prevalence of occult HBV infection were similar among haemodialysis patients who were anti-HCV positive and those who were negative (6.3% versus 3.8%,  $p = 1.0$ ). [18]

Positive HIV status has been associated with occult HBV infection. Occult HBV infection prevalence rates of 10.7 and 19.8% have been reported in HIV positive patients [32, 33]. Both HBV and HIV share the same route of transmission. 11.9% of HIV infected persons in Nigeria were HBsAg

positive [3] and this is about the same prevalence rate as in HIV negative persons in Nigeria.[1, 2]

The prevalence of HBV infection among CKD patients would be expectedly higher than 5% reported earlier taking the observed prevalence of occult HBV infection among haemodialysis patients in this study into consideration.[8] As noted above, occult HBV infection poses a potential risk for HBV infection to non-HBV infected patients on haemodialysis. Current guidelines however recommend screening of patients for HBV prior to commencement of haemodialysis, frequent screening and immunization of high risk patients while on maintenance dialysis to prevent HBV infection in this group of patients [9]. Anti-viral therapy is recommended for all HBV infected patients prior to renal transplantation to prevent a flare of acute HBV infection and subsequent liver disease due to immunosuppression therapy in the post-transplantation period.

No definite mechanism has been identified so far as being responsible for occult HBV infection. Some of the reasons given for high prevalence of occult HBV infection among CKD include higher number of blood transfusions, HIV co-infection, HCV co-infection, positive antibody to hepatitis B core antigen (anti-HBc), [12] while some studies have shown an association with some of these factors, others have not demonstrated this. None of these factors was statistically significant in this study. This may be due to the small sample size. A study involving more subjects to identify the risk factors for occult HBV infection and further characterize these subjects is suggested.

## **CONCLUSION**

Occult HBV infection is common among Nigerian patients on haemodialysis. Identification and appropriate management of occult HBV infection in haemodialysis patients is desirable, as this will reduce inadvertent transmission of this infection. No risk factor for occult HBV infection in haemodialysis patients in this study was identified. More studies involving larger sample sizes are however necessary to further evaluate the clinical spectrum of this infection in this group of patients.

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