

Full Length Research Paper

Prevalence of occult hepatitis B virus (HBV) infections in haemodialysis patients in Khartoum State, Sudan from 2012 to 2014

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This study was carried out to detect occult hepatitis B virus (OHB) among haemodialysis patient in Khartoum State, Sudan. Antigen capture enzyme linked immunosorbent assay (ELISA) to detect hepatitis B surface antigen (HBsAg), competitive ELISA to detect Hepatitis B core antibody (HBcAb) antibodies and polymerase chain reaction (PCR) to detect hepatitis B virus (HBV) DNA were used to analyze 100 plasma samples collected from patients in 3 hospitals (El Amel Hospital, Bashair Hospital and Salma Hospital) during the period of 2012 to 2014. Out of the patient sampled, 65 were males and 35 were females (age 18 to 70 years) none of these patients showed signs of clinical hepatitis. The results showed that 9 out of the 100 samples were positive for HBsAg, and were subsequently excluded from the study. Out of the remaining HBsAg negative 91 samples, 38(51.6%) showed positive HBc antibodies and 3 (3.3%) tested positive to HBV DNA using competitive ELISA and PCR, respectively. These results indicated that molecular detection of occult HBV infections in haemodialysis patients in Sudan is of fundamental importance to prevent HBV transmission through contamination of haemodialysis machines.

Key words: Hepatitis B virus (HBV), hemodialysis, 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 of Hepadnaviridae virus (Hunt, 2007). HBV is highly contagious, and is the most commonly transmitted blood

borne virus in the health care setting. Transmission generally occurs from patient to patient or from patients to health care personnel via contaminated instruments or accidental needle-stick or sharps injuries (Mast et al.,

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1993). The virus can be transmitted directly through body fluids to mucous membranes, cutaneous scratches, abrasions, burns or other lesions (Mast et al., 1993). 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 (Mast et al., 1993).

HBV affects all age groups and can lead to liver disease, liver cancer and death in many of those afflicted (www.nfid.org/factheets/hephadult.html). There remain many unanswered questions regarding its pathogenesis and clinical significance, but should be considered a potential risk factor in the development of hepatocellular carcinoma (HCC) whenever it is encountered. The prevalence of occult HBV infection is most common in regions of the world where HBV is endemic, while it is less common in regions with intermediate HBV prevalence rates and least common in areas, where HBV is relatively uncommon (Minuk et al., 2005).

Occult hepatitis B (OHB) is characterized by the presence of HBV DNA in the liver and/or serum in association with negative hepatitis B surface antigen (HBsAg) results (Raimondo et al., 2008; Hollinger et al., 2010). It can be classified further into seroreactive for hepatitis (HB) core antibody with or without seroreactivity to HBsAg. Seronegative subjects are negative for hepatitis B core antibodies (anti-HBc) and hepatitis B surface antibody (anti-HBs) in some cases, serum HBV DNA may be undetectable in patients with occult hepatitis B virus (OHB), although, replicative DNA can be detected in the liver tissue. Occult hepatitis B has been observed in patients with cryptogenic chronic liver disease, in patients with Hepatocellular carcinoma (HCC), in patients with chronic hepatitis C and in patients with fulminate hepatitis (Chemin et al., 2001; Bréchet et al., 2001; Fang et al., 2009). When Occult hepatitis B becomes established, however, the presence of severe liver injury that was due to hepatitis B infection might be preserved obscuring the original cause of injury (Liedó et al., 2011).

Many epidemiological and molecular studies indicate that HBV presence may play a critical role in the development of HCC. Indeed in areas where HBV is present, occult hepatitis B mono infection or co-infection with hepatitis C virus (HCV) was reported to be associated with HCC.

In the world, the incidence of occult HBV was reported to range between 0 to 58%. The incidence of occult HBV in Sudan was reported to range between 0 to 11.5% (Hassanein, 2013; Nafisa et al., 2013). The diagnosis for HBV infection is made following serologic tests for the virus, such as ELISA. ELISA is used to detect HBsAg and HB core antibodies or by molecular biological techniques such as polymerase chain reaction (PCR). On the other hand, diagnosis of occult HBV infection requires sensitive HBV-DNA PCR assay (Ke-Qin, 2002).

The aim of this study is to determine the prevalence of occult HBV infection among haemodialysis patients in Khartoum state hospitals, Sudan.

MATERIALS AND METHODS

Study area and period

This study was conducted on haemodialysis patients in Khartoum State, during the period of 2012 to 2014.

Target population and sample size

A cross-sectional study was carried out from 2012 to 2014 to investigate a population of 100 haemodialysis patients of between 18 to 80 years old, at three hospitals (Bashir Hospital, El Amel Hospital and Dr. Salma Center for Transplantation and Haemodialysis). All participating patients were given written informed consent. Most of patients in this study presented to the clinics were suffering from a variable spectrum of complaints including fever and jaundice. Liver data, liver case status and clinical information were abstracted by reviewing medical records. The collected data included age, gender, date of renal failure, date of sample collection, and patient residence during sample collection. Plasma was separated by centrifugation and stored frozen at -20°C until further analyses.

Serology

Commercial ELISA kits (Biorex, United Kingdom) were used to detect HBsAg and HB core antibodies (precheck Bio, Inc, USA) according to the procedure described by the manufacturer.

HBV DNA detection

DNA extraction

DNA was extracted from patient's materials using commercial Kit (Vivantis, Malaysia) according to manufacture instructions. The extracted DNA was stored at -20°C till used.

Polymerase chain reaction (PCR)

The PCR was performed by processing the extracted DNA from plasma with primers that are specific for the HBsAg gene of HBV. The primers used consisted of forward primer 5'-TCGGAAATACACCTCCTTTCCATGG3' (HBVgenome1353-1377) and reverse primer, 3'GCCTCAAGGTCGGTCGTTGACA-5' (HBV genome1 702-1681). The reaction was performed in 20 µl volume using Solis Bio dyne master mix. The volume included: 4 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 9 µl distilled water. The DNA was amplified in thermo-cycling conditions using PCR machine Techno (Japan) as follow: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min, with a final extension 72°C for 7 min. 10 µl of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose, the gel was prepared by adding 0.7 g 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 surface

Table 1. Frequency of HBsAg in haemodialysis patients at Khartoum hospitals, Sudan.

Gender	ELISA HBsAg		Total (%)
	Negative (%)	Positive (%)	
Male count	58 (58)	7 (7)	65 (65)
Female count	33 (33)	2 (2)	35 (35)
Total	91 (91)	9 (9)	100 (100)

Table 2. Frequency of HBcAb in haemodialysis patients at Khartoum hospitals, Sudan.

Gender	ELISA HBcAb		Total (%)
	Negative (%)	Positive (%)	
Male count	31 (34)	27 (29.6)	58 (63.7)
Female count	22 (24)	11 (12.1)	33 (36.2)
Total	53 (58.2)	38 (41.7)	91 (100)

Table 3. Frequency of HBV DNA in haemodialysis patients at Khartoum hospitals, Sudan.

Gender	PCR HBsAg gene		Total (%)
	Negative (%)	Positive (%)	
Male count	57 (62.6)	1 (1.1)	58 (63.7)
Female count	31 (34)	2 (2.2)	33 (36.2)
Total	88 (96.7)	3 (3.3)	91 (100)

antigen gene (sAg gene) amplicon was 350 bp.

RESULTS

Detection of HBsAg

A sum of 100 samples were tested for HBsAg, Ninety one samples (91%) (58 males and 33 females) were negative for HBsAg by ELISA. On the other hand, nine samples (9%) (7 males and 2 females) samples tested positive for HBsAg (Table 1).

Detection of HBcAbs

A total of 91 samples that were HBs negative were tested for HBcAb using ELISA. Out of these fifty three samples, (58.2%) (31 males and 22 females) were negative for HBcAbs while thirty eight samples (41.7%) (27males and 11females) samples tested positive for HBcAbs (Table 2).

Detection of Hepatitis B DNA

A total of 91 that were negative for HBsAg were tested for

HBV DNA using PCR. HBV DNA was detected in 3 (3.3%) samples that were also positive for HBcAbs, of which 2 (2.2%) were females and 1 (1.1%) was a male. The rest of the samples were negative (Table 3, Figure1).

DISCUSSION

The present study was aimed to assess and determine the presence of occult hepatitis B among haemodialysis patients in Khartoum, Sudan. The frequency (3.3%) of occult hepatitis B infection among haemodilysis patients in the present study corroborates the result in the literature, among haemodilysis patients that ranges between 0 to 58% in countries such as Canada, Turkey, Italy, Spain, Iran, Brazil and Egypt (Lai et al., 2003; Hass et al., 2005; Sagnelli et al., 2008; Ana Cecilia et al.,2012).

In this study, 26 out of 91 (23.6%) samples were found seroreactive to anti core antigen and 3 (11.5%) out of 26 were found to harbor OHB DNA, representing 3.3% of the total patient investigated. This incidence was similar to the results found in Egypt and some other countries (Edey et al., 2010; Albuquerque et al., 2012; Abu et al., 2012; Minuk et al., 2004; Yakaryilmaz et al., 2006). Conflicting results have been reported on the frequency of OHB in Sudan. In one study, 3 samples (11.5%) HBV-

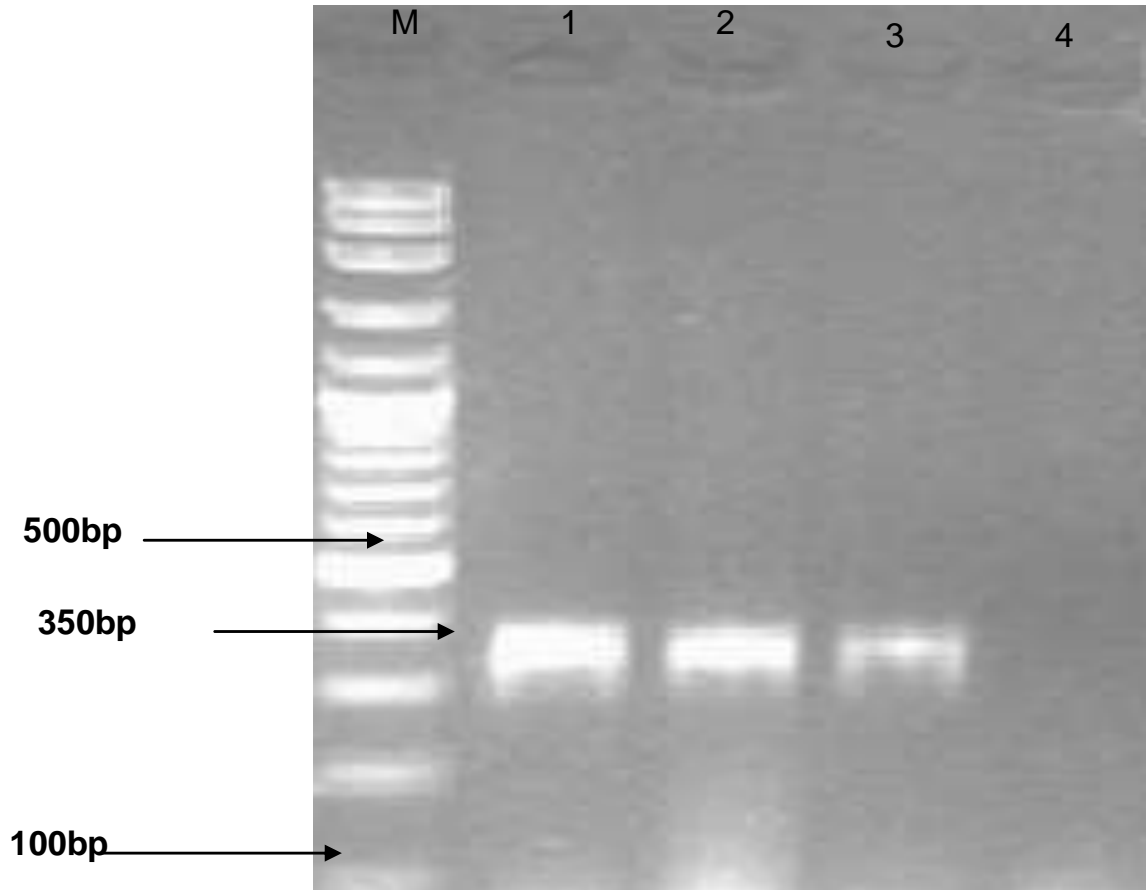


Figure 1. HBVDNA result (350bp) on 2% agarose gel. lane1 shows positive control, lanes 2 and 3 show results in two patients, lane 4 show negative control, M: 100bp DNA Marker.

DNA-positive out of 26 sample positive HBcAb has a strong evidence to support the presence of occult hepatitis B infections in hemodialysis patients (Hassanein, 2013). While in another study, no patients with occult HBV were detected (Nafisa et al., 2013). Although, the results of the present study support the existence of OHB in hemodialysis patients, it is clear that more studies are needed to fully elucidate the incidence of OHB in Sudan.

The discrepancy in the reported incidences of occult HBV infection between several studies, including that of this study, could be due to several factors. It could be attributed to difference in the sensitivity of the various molecular biology techniques used in detection of HBV DNA, differences in the prevalence of HBV in geographical area, and differences in the storage and age of serum samples used in studies (Hisham et al., 2010).

Despite the fact that none of the patients in this study showed prior HBV infection, yet of cases OHB were detectable albeit at low incidence. However, it is possible that patients undergoing chance haemodialysis therapy may have either lower response rate to previous hepatitis

B vaccination compared or have transient responses to vaccination as result of profound immune suppression (Hisham et al., 2010). Finally, the PCR method as used in this study, proved to be highly sensitive and specific method for detection of occult HBV. This is supported by the fact that this study was able to detect HBV using ELISA technique.

Conclusion

The low level of occult HBV reported this study clearly showed that serological markers of HBV infection should always be backed up with molecular tests to investigate possible occult HBV infection. It also indicate that virus genotypes identification in patients with occult HBV infection should be expanded through further studies, in order to have better understanding of the clinical laboratory, and epidemiological characteristics of such infection. Currently, molecular biology test such as PCR are not implemented in routine clinical haemodialysis in Sudan. Hence, PCR method as described in the study should be used as routine test in hemodialysis centers' to

prevent virus transmission in haemodialysis units.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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