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Research Article

Prevalence of Occult Hepatitis B Virus Infection in Febrile Patients in West Kurdofan State, Sudan

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Abstract

The current Study was carried out to detect occult hepatitis B virus infection in febrile patients in West Kurdofan 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 in 100 plasma samples collected from patients in 5 health centers in West Kurdofan state, Sudan. Of all the patients sampled, 71 were females while 29 were males with the mean age of ± 38.80 and a standard deviation of ± 17.055, none of the patients showed signs of clinical hepatitis. The results showed that 1 (1%) out of 100 samples was positive for HBsAg and was subsequently excluded from the study. Out of the 99 HBsAg negative samples, 40 (40.4%) (8 males and 32 females) were positive for HBcAb and 7 (7.8%) were positive for HBV DNA with ELISA and PCR, respectively. These results suggest that, with just the use of HBsAg detection marker for HBV, this virus will remain a potential threat to the community and to health workers and this publication highlights the importance of the establishment of PCR as a routine method for the diagnosis of hepatitis B virus infections.

Keywords: *Hepatitis B Virus (HBV); Febrile Patients; Enzyme Linked Immunosorbent Assay (ELISA); Polymerase Chain Reaction (PCR)*

Introduction

Hepatitis B virus (HBV) affects all age groups and can lead to liver disease, liver cancer and death in many of those afflicted [1]. The presence of hepatitis B virus (HBV) nucleic acid in the blood or liver of hepatitis B virus surface antigen (HBsAg) negative patient is called occult hepatitis B virus infection (OBI) [2]. Occult HBV infection are more frequently detected in individuals with antibodies to hepatitis B core antigen (anti-HBc) [3], as a unique marker of HBV infection [4,5]. However recent studies suggest that up to 20% of individuals with occult HBV could be negative even for anti-HBc antibodies or any other serological indicator of exposure to HBV [6]. This infection may persist in some individuals for years without emerging symptoms of overt HBV infection and is characterized by very low viral load (usually below 104 copies/mL) [7].

The availability of HBcAb and HBV DNA with undetectable HBsAg, either indicates resolved infection (most common), or resolving acute infection, or low level chronic infection [8]. Co-infection, immunosuppression, or drug abuse can enhance HBV DNA levels without an increase of HBsAg [9-11].

Hepatitis B virus infection remains a major health problem leading to considerable morbidity and mortality worldwide although vaccines and antiviral treatments are available [12].

HBV infect the liver, causing both acute and chronic diseases. According to the World Health Organization (WHO), approximately 2 billion people worldwide have been infected with the hepatitis B virus (HBV), 350 million people are chronic carriers of the virus and 600 000 die each year as a result of either acute or chronic infections with the virus [12,13]. Approximately 10% of adults and 90% of children infected with HBV become chronically infected [14].

The prevalence of OBI is quite variable depending on the level of endemicity of the disease in different parts of the world, the different assays utilized in the studies, and the different populations studied [15]. Occult HBV 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 [16]. However, the trend of the prevalence is not yet documented in Sudan since only few studies have been conducted [17,18].

The virus 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 [19].

OBI has been observed in blood donors [20], trans-plant recipients [21], in HIV-infected individuals [22], mentally ill patients [23,24] and the general population [25]. Data on HBV occurrence among these patients are limited, and data in the literature have primarily focused on the prevalence of HBV infection.

The aim of this study was to determine the prevalence of occult hepatitis B virus infection in febrile patients without symptoms of liver disease (General population) in West Kurdofan State, Sudan.

Materials and Methods

Study area

This study was carried out in different health centers of Alkhuwey, Al murkab, Al mugaisim, Omlobana and Al dudiyalocated in West Kurdofan State, Sudan during the period of January to July 2016.

Study population and sample size

A total of 100 blood samples were collected from febrile patients including 71 females and 29 males. The mean age of the patients was ± 38.80 with a standard deviation of ± 17.055.

Most of the patients complained of fever and other symptoms like headache and general body pain. Patient's positive for Malaria and Typhoid Fever were excluded from the study. All relevant information were collected from each patient after obtaining full consent including personal data such as name, age, sex and occupation, as well as health data such as clinical symptoms and previous liver disease.

Samples of blood (five milliliters) were withdrawn from each patient and centrifuged at 3000rpm for 5 minutes to obtain plasma which was then stored at -20˚C until further analysis.

Serological testing

Commercial ELISA kits (Diagnostic Automation/Cortez Diagnostics inc. USA) were used for HBsAg and HBcAb detection according to the manufacturers' instructions.

DNA extraction

DNA was extracted from patients' plasma using commercial DNA extraction kit (Intron biotechnology, Inc. Korea) according to the manufacturers' instructions. The extracted DNA was stored at -20˚C until used.

Polymerase chain reaction

The PCR was performed using primers that are specific for the HBsAg gene of HBV. The primers used consisted of forward primer 5'TC GGAAATACACCTCCTTTCCATGG3'(HBV genome 1353-1377) and reverseprimer, 3'GCCTCAAGGTCGGTCGTTGACA-5' (HBV genome 1702-1681). 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 follows: 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 step at 72°C for 7 min.

Ten µl 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 and the product was visualized by staining with 0.15% Ethidium bromide using UV gel documentation system INGeNius (synoptics limited, England). The expected size of the surface antigen gene (HBsAg gene) amplicon was 350 bp.

Statistical analysis

The data for the continuous variables and the clinical data are provided as simple frequencies. HBsAg, HBcAb were analyzed by comparing with the patients sex while OBV was analyzed by comparing with sex and clinical symptoms.

Results

Detection of HBsAg

A total of 100 samples were tested for HBsAgand one sample (1%) (Male) was positive for HBsAg by ELISA. The rest of the samples (99%) (28 males and 71 females) tested negative for HBsAg (Table 1).

Sex	ELISA HBsAg		Total (%)
	Positive (%)	Negative (%)	
Male	1 (1.0)	28 (28)	29 (29)
Female	0 (0.0)	71 (71)	71 (71)
Total	1 (1.0)	99 (99)	100 (100)

Table 1: *Frequency of HBsAg in Febrile Patients.*

Detection of HBcAb

Of the 99 samples tested for HBcAb, forty samples (40.4%) (10 males and 30 females) were positive for HBcAb, while fifty-nine samples (59.6%) (41 females and 18 males) were negative for HBcAb (Table 2).

Sex	ELISA HBcAb		Total (%)
	Positive (%)	Negative (%)	
Male	10 (10.1)	18 (18.2)	28 (28.3)
Female	30 (30.3)	41 (41.4)	71 (71.7)
Total	40 (40.4)	59 (59.6)	99 (100)

Table 2: *Frequency of HBcAb in HBsAg negative Febrile Patients.*

Detection of Hepatitis B virus DNA

A total of 40 samples that were positive for HBcAb and negative for HBsAg were tested for HBV DNA using PCR. HBV DNA was detected

in 7 samples (17.5%) (3 males and 4 females) while 33samples (82.5%) (4 males and 29 females) were negative for HBV DNA (Table 3).

Sex	HVB DNA		Total (%)
	Positive (%)	Negative (%)	
Male	3 (7.5)	7 (17.5)	10 (25)
Female	4 (10)	26 (65)	30 (75)
Total	7 (17.5)	33 (82.5)	40 (100)

Table 3: *Frequency of HBV DNA in BHcAb positive in Febrile Patients.*

Cross tabulation of the OBV DNA and clinical symptoms showed that occult hepatitis B virus was detected in patients with body pain, abdominal pain and Urinary Tract Infection (UTI) but no occult hepatitis B virus was detected in patients manifesting fever and headache (table 4). There was no significant relationship between sex and OBV infection (p = 0.240).

Clinical Symptoms	HVB DNA		Total (%)
	Positive (%)	Negative (%)	
Fever & body pain	3 (20)	12 (80)	15 (37.5)
Fever & headache	0 (0)	2 (100)	2 (5)
Fever & abdominal pain	1 (16.6)	5 (83)	6 (15)
Fever & UTI	3 (17.6)	14 (20)	17 (42.5)
Total	7 (12.5)	33 (82.5)	40 (100)

Table 4: *Clinical symptoms and HBV DNA Cross tabulation.*

Result

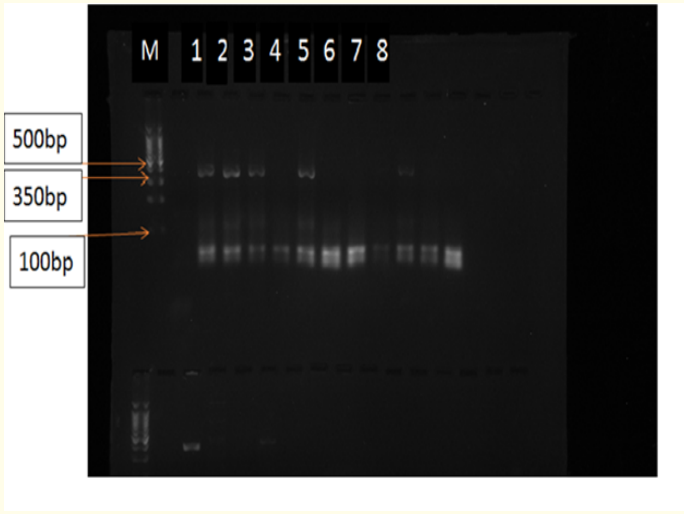

Figure 1: *HBVDNA PCR result (350bp) on 2% agarose gel. Lane 1 show positive control, lanes 2, 3 and 5 show positive results in three patients, lane 4 show negative control, lane 6, 7 and 8 show negative results in three patients, M: 100bp DNA Marker.*

Discussion

Conflicting results have been reported on the frequency of occult hepatitis B virus infection in Sudan. In one study, no patients with occult Hepatitis B virus infection were found in blood donors [26], while in another study 3 samples (3.3%) were found to harbor occult hepatitis B virus in Hemodialysis patients [27]. In another study 96 samples (26.8%) tested positive for occult HBV infection in HIV patients [28].

The current study was carried out to assess and determine the presence of occult HBV infection among febrile patients in West Kurdofan State, Sudan. None of the patients showed signs or symptoms of clinical hepatitis.

The frequency (17.5%) of occult HBV infection among seroreactive patients to HBcAb, which represent 7% of the total number of patients investigated, authenticates the range of occult HBV infection among patients with predisposing factors, that lies between 0 to 58% in countries like Egypt, Nigeria, Colombia, Australia, Korea and South Africa [29-32].

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 differences in the populations investigated, the 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 samples used in studies [31,32].

Despite the fact that none of the patients in this study showed clinical symptoms of HBV infection, OHB was detectable at a moderate incidence.

Finally, the PCR method, as used in this study, proved to be highly sensitive and a specific method for detection of occult HBV infections. This is supported by the fact that this study was able to detect HBcAb using ELISA technique.

Conclusion

This study highlights the fact that HBsAg may not be an effective tool for diagnosis of HBV infections in our study population. The level of occult HBV infection reported in 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 indicates the need for the identification of the virus genotypes of patients with occult HBV infection for better understanding of the clinical laboratory and the epidemiological characteristics of the infection. Currently, a molecular test such as PCR, is not in use as a routine laboratory investigation for HBV in most of the health centers in Sudan. Hence, PCR method as described in this study should be used as routine test for HBV infections in the hospitals in the country.

Finally, despite the current study supporting the presence of occult hepatitis B virus infection in febrile patients, more studies need to be conducted to fully clarify the incidence of occult HBV infection in the general population in Sudan.

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