Original Article

Occult Hepatitis B Virus in Gezira State Sudan

Gasmelseed N[1*], ElsirA A[1], Elhaj EY[1], Saeed OK[2] and Elbalal M[2]

ABSTRACT

Background: Occult hepatitis B infection (OBI) is simply defined as serologically undetectable hepatitis B surface antigen (HBsAg-ve), despite the presence of circulating HBV DNA.

Objective: The aim of this study was to determine the prevalence of occult HBV among Screened HBsAg subjects in Gezira State, Sudan.

Materials and Methods: A total of 176 subjects including cancer patients, hospital based controls and health care workers were screened for HBsAg by ELISA, and their DNA was extracted by using polymerase chain reaction.

Results: 49/176 (27.8%) were positive for occult HBV as follows: cancer patients had 31/81 (38.2%) cases, while 18.9% were reported among hospital based controls. 11.2%, 2.2% were positive for both HBsAg and DNA respectively. Ten out of the 50 (20%) healthcare workers, who were negative for HBsAg, were found to be positive for HBV DNA, while one out of the 19, who were positive for HBsAg, was found to be positive for HBV DNA. Occult HBV among this study groups showed an approximately equal distribution among males and females 18.2% and 20.5 %, respectively.

Conclusion: This study concluded that the prevalence of OBI among healthcare workers, hospital based controls and cancer patients, is remarkably increasing compared with the prevalence of the disease. More molecular epidemiological studies are needed to delineate a link between OBI and cancer. Proper HBV screening should be carried out to avoid occult hepatitis B infection transmission.

Keywords: Hepatitis B virus, Gezira State, cancer, healthcare workers, Sudan.

Hepatitis B virus(HBV) remains a major public health problem worldwide[1]. Implementation of hepatitis B surface antigen (HBsAg) in routine screening of blood donors in the early 1970s has greatly enhanced transfusion safety[2]. The incidence of transfusion-transmitted hepatitis B has steadily reduced over the last decades[2].

However, it was demonstrated that HBV transmission by blood components negative for HBsAg can still occur[3]. Hence the term occult hepatitis B virus infection (OBI) was introduced.OBI is simply defined as serologically undetectable hepatitis B surface antigen (HBsAg-ve), despite the presence of circulating HBV DNA[4,5]. The residual risk of HBV transfusion transmission is mainly related to blood donations negative for HBsAg that have been collected either during the time between infection and detection of a viral antigen or antibody marker, or during the late stages of infection[1]. Allain reported OBI in several clinical contexts including: (1) recovery from past infection indicated by the presence of hepatitis B surface antibody (anti-HBs); (2) chronic hepatitis with surface gene escape mutants that are not recognized by current assays; (3) chronic carriage without any marker of HBV infection other than HBV DNA (referred to as, sero-negative,); and (4) most commonly in endemic areas, chronic carriage stage with HBsAg too low to be detected and recognized by the presence of anti-HBc as the only serological marker (referred to as anti-HBc alone or isolated anti-HBc) [6].

1.Department of Molecular Biology National Cancer Institute, University of Gezira
2.Department of Internal Medicine, Faculty of Medicine, University of Gezira.
*Correspondence to: Nag_la@yahoo.com

Sudan is classified among countries with a high hepatitis B surface antigen (HBsAg) with endemicity of more than 8%[7]. Exposure to HBV infection ranges from 47% to 78% with HBsAg seroprevalence ranging from as low as 6.8%[8] in central Sudan, to as high as 26% in southern Sudan[9]. Seroprevalence of HBsAg among asymptomatic blood donors ranged from 12.3% in southern Sudan to 17.5% in central Sudan [7].

The aim of this study is to identify the prevalence of occult HBV among screened HBsAg subjects in Gezira State Sudan.

MATERIALS AND METHODS:

This was a prospective, descriptive, hospital based study. HBsAg and HBV DNA were detected in 81 cancer patients, 95 of hospital based controls in addition to 69 Health care workers. All study subjects were recruited from Wadmedani Hospital, Gezira State, Sudan during 2008-2009. Ethical clearance was obtained from National cancer Institute Ethical Review committee.

Detection of HBsAg: Five ml of venous blood was collected from each participant into an anti- coagulant EDTA-containing tube after written informed consent. HBV surface antigen (HBsAg) was detected by HBsAg ELISA Kit (Monolisa™ HBs Ag ULTRA, Marnes-la-Coquette-France) according to the manufacture instructions.

Molecular Detection of HBV: Genomic DNA was extracted from the plasma samples using cinnaGenIncDNP™ Kit (DN 8115C) Tehran, Iran. HBV DNA was detected by Polymerase Chain Reaction (PCR), using cinnaGenIncDNP™ PCR Kit as recommended by the manufacturer. HBV Positive samples were identified by presence of a 353 bp PCR product by electrophoresis using 2% agarose gel.

Statistical analysis: Data were expressed as mean and standard deviation (SD) as appropriate. Comparison between HBsAg and HBV DNA were performed using the Chi-square test, and Fisher's exact test as appropriate. Data were collected and analyzed. The Statistical Package for Social Science (SPSS version 16.0) was used in performance of all statistical analyses, and p-value less than 0.05 was considered significant.

RESULTS:

A total number of 245 subjects were selected for this study as follows: 81 were cancer patients, 95 were hospital based controls and 69 were health care workers.

Occult HBV among cancer patients and hospital based controls: 176 subjects, including 81 cancer patients and 95 hospital based controls, were examined for HBsAg and the presence of HBV DNA. The study showed that 49/176 (27.8%) were positive for occult HBV as follows, cancer patients had 31/81 (38.2%) cases, while 18.9% were reported among hospital based controls. 11.2%, 2.2% were positive for both HBsAg and DNA respectively as shown in table (1).

With respect to cancer patients 65.4% (53/81) were males and 34.6% (28/81) were females. Occult HBV was found in 41.5% (22/53) males' cancer patients, while it was seen among 32.1% (9/28) females' cancer patients. Regarding hospital based controls; males

Table (1): Results of HBV in both cancer patients and hospital based controls (N=176).

Type of subject	+ ve HBV DNA -veHBsAg Occult HBV (%)	+ve HBV DNA +veHBsAg (%)	-ve HBV DNA -veHBsAg (%)	Total (%)
Cancer patients	31 (38.2)	9 (11.2)	41 (50.6)	81(100)
Hospital based control	18 (18.9)	2 (02.2)	75 (78.9)	95 (100)
Total	49 (27.8)	11(06.3)	116 (65.9)	176 (100)

Pearson Chi-Square Test *P*= .000 for HBV DNA

were 68.4% (65/95), while females were 31.6% (30/95). Among this group, Occult HBV was detected in 20.0% (6/30) of females and 18.5% (12/65) in males.

Occult HBV among cancer patients washigher in all age groups range except among the group between 21-30 years as shown in table (2).

In contrast to cancer patients, Occult HBV was found to cluster among those less than 20 years and those more than 60 in hospital based controls as shown in table (3).

Occult HBV among Health Care Workers (HCW): Sixty nine of health care workers were screened for HBsAg. 27.5% (19/69) were found to be positive and 72.5% (50/69) of them were negative. The risk of acquisition of occult HBV was founded to be high among young subjects, who were less than 40 yrs, as shown in table (4).The duration of work and experience had a great role in HBV infection among Health Care Workers. Occult HBV was found more among those who worked less than one year and decreased with the increase of duration of work, as shown in table (5).

Table (2): Occult HBV among cancer patients according to age.

Age range	-veHBsAg + ve HBV DNA Occult HBV (%)	+veHBsAg +ve HBV DNA (%)	-veHBsAg -ve HBV DNA (%)	Total (%)
>20	10 (38.5)	01 (03.8)	15 (57.7)	26 (100)
21-30	01 (16.7)	03 (50.0)	02 (33.3)	06 (100)
31-40	05 (50.0)	01 (10.0)	04 (40.0)	10 (100)
41-50	05 (35.7)	01 (07.2)	08 (57.1)	14 (100)
51-60	03 (33.3)	01 (11.1)	05 (55.6)	09 (100)
<61	06 (37.50	02 (12.5)	08 (50.0)	16 (100)
Total	31(38.2)	09 (11.2)	41(50.6)	81

Fisher's Exact Test *P*= .9 for HBsAg *P*= .50 for HBV DNA

Table (3): Occult HBV among Hospital based controls according to age.

Age range	-veHBsAg + ve HBV DNA Occult HBV (%)	+veHBsAg +ve HBV DNA (%)	-veHBsAg -ve HBV DNA (%)	Total (%)
>20	07 (26.9)	2 (07.7)	17 (65.4)	26 (100)
21-30	03 (14.3)	00 (00.0)	18 (85.7)	21 (100)
31-40	03 (21.4)	00 (00.0)	11 (78.6)	14 (100)
41-50	02 (16.7)	00 (00.0)	10 (83.3)	12 (100)
51-60	00 (00.0)	00 (00.0)	11 (100)	11 (100)
<61	03 (27.3)	00 (00.0)	08(72.7)	11 (100)
Total	18 (18.9)	2 (11.2)	75 (78.9)	95

DISCUSSION:

Occult HBV infection OBI diagnosis is based upon detection of HBV DNA when HBsAg is absent. It is very important to define the optimal methodology to test this marker to prevent false positive results, depending on HBsAg assay sensitivity. The gold standard for OBI diagnosis is the study of extracted DNA (from liver or blood) [10]. A total of 245 subjects, including 176 cancer patients, and hospital based controls, and 69 health care workers, were examined for HBsAg and the presence of HBV DNA in this study. The results showed that occult infection was higher among cancer patients compared with the hospital based control (38.2%, and 18.9%, respectively). Occult HBV among Health care workers was 20%. Wong et al found that 73% of patients with apparently unidentifiable

Table (4): Distribution of occult HBV with age among health care workers.

Age range	+ ve HBV DNA -veHBsAg Occult HBV (%)	-ve HBV DNA -veHBsAg (%)	Total (%)
>20	01 (50.0)	01 (50.0)	02 (100)
21-30	06 (26.1)	17 (73.9)	23 (100)
31-40	02 (16.7)	10 (83.3)	12 (100)
41-50	01 (12.5)	07 (87.5)	08 (100)
51-60	00	04 (100)	04 (100)
<61	00	01 (100)	01 (100)
Total	10 (20.0)	40 (80.0)	50

Table (5): Distribution of occult HBV among HCW by duration of the work.

Duration of the work in years	+ ve HBV DNA -veHBsAg Occult HBV (%)	-ve HBV DNA -veHBsAg (%)	Total (%)
1<	03 (42.9)	04 (57.1)	07 (100)
2-5	04 (28.6)	10 (71.4)	14 (100)
6-9	01 (14.3)	06 (85.7)	07 (100)
10-13	00 (00.0)	07 (100)	07 (100)
14-17	00 (00.0)	04 (100)	04 (100)
18-21	02 (25.0)	06 (75.0)	08 (100)
22-25	00 (00.0)	03 (100)	03 (100)
Total	10 (20.0)	40 (80.0)	50

causes for HCC were HBV-OBI related[11]. Cancer patients and health care worker are more susceptible for HBV infection than the hospital based control because they are at high risk of infection.

Occult hepatitis B infection has a major impact on transfusion safety[12]. The standard test for detection of occult infection is the amplification of HBV DNA. However, the serological assay for the long lasting antibody response to the highly immunogenic HBV core antigen represents a qualified candidate as a surrogate for DNA amplification. The high prevalence of OBI among both hospital based control and health care worker was (18%), (20%) respectively, who make a good pool for blood donation. Hence the implementation of core antibody testing becomes important step towards continuous improvement in blood safety[10].

Many epidemiological and molecular studies had indicated that OBI is a potential risk factor for development of HCC and Non -Hodgkin's lymphoma (NHL)[13]. In this Study, cancer patients who were less than 20 years old had a high rate (38.9%) of Occult hepatitis B infection as compared to hospital based controls. This may suggest that that cancer patients may had maternal infection of HBV.A high percentage of OBI was found among health care workers who worked less than one year. This may suggest that the experience may play a role in protection against OBI.

CONCLUSION:

This study concluded that the prevalence of OBI among both healthcare workers and hospital based controls cancer patients is remarkable increasing compared with the prevalence of the disease. It recommended that a proper screening program for HBV has to be implemented so as to avoid the issue of OBI transmission. However further molecular genetics and prospective molecular epidemiological studies are needed to display the role of OBI in cancer diseases.

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