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High Incidence of Occult Hepatitis B Infection (OBI) among Febrile Patients in Atbara City, Northern Sudan

Razan A Bashir[1], Tarteel Hassan[2], Abdel Rahim M El Hussein[3], Khalid A Enan[3*] and Isam M Elkhidir[4]

[1]*Department of Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Sudan*

[2]*Department of Parasitology and Medical Entomology, University of El Imam El Mahdi, Sudan*

[*3]*Department of Virology, Central Laboratory, Ministry of High Education and Scientific Research, Khartoum, Sudan*

[4]*Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan.*

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ABSTRACT

Background: Hepatitis B virus (HBV) remains a major public health problem worldwide that accounts for significant morbidity and mortality. About one third of the world population have serological evidence of past or present hepatitis B virus infection and more than 350 million people may be affected by chronic HBV infection. The aim of this study was to detect the prevalence of hepatitis B virus among febrile malaria and typhoid negative patients in Atbara city, River Nile State, northern Sudan.

Material and methods: A total of 89 blood samples were collected from febrile malaria and typhoid negative patients including 44 females and 45 males. Sandwich Enzyme Linked Immunosorbent Assay (ELISA) was used to detect Hepatitis B Surface Antigen (HBsAg) and competitive ELISA to detect Hepatitis B Core Antibody (HBcAb) antibodies. Detection of HBV-DNA was carried out by Real time-PCR and Conventional-PCR.

Results: Out of 88 samples, 44 (50%) samples were positive for HBcAb and all samples were negative for HBsAg. HBV DNA was detected in 16 (18.2%) and 1 (1.1%) of the samples using real time-PCR and conventional-PCR, respectively.

Conclusion: This study had showed high prevalence of Occult Hepatitis B infection (OBI) among febrile patients in Atbara town northern State where hepatitis B infection seems to be endemic.

Keywords: Hepatitis B virus, Occult hepatitis B, Febrile patients, Atbara, Sudan

INTRODUCTION

HBV is a double stranded DNA virus classified in the virus family Hepadnaviridae. HBV infection may result in subclinical or asymptomatic infection, acute self-limited hepatitis, fulminant hepatitis or chronic hepatitis which can lead to cirrhosis or hepatocellular carcinoma [1,2]. Signs and symptoms of acute hepatitis include nausea, abdominal pain, vomiting, fever, jaundice and dark urine, changes in stool color and hepatomegaly or splenomegaly [3].

Hepatitis B viral infection is endemic in the developing countries of Africa [4]; the highest endemicity (>8%) is seen in some sub-Saharan countries such as Nigeria, Namibia, Gabon, Cameroon, Burkina Faso. Other countries like Kenya, Zambia, The Ivory Coast, Liberia, Sierra Leone and Senegal are considered areas of intermediate endemicity (2%-8%), while low endemicity level (<2%) was shown in Egypt, Tunisia, Algeria and Morocco, located in the north of the continent. Sudan is classified among the African countries with high HBV endemicity [5]. According to the most recent World Health Organization estimate, two billion people worldwide have serologic evidence of past or present HBV infection 360 million are chronically infected and at risk for HBV-related liver disease. And approximately 600,000 die each year [3].

HBV is transmitted by percutaneous or mucosal exposure to infected blood or other body fluids, it has been observed

Corresponding author: Dr. Khalid A Enan, Assistant Professor, Head Department of Virology, Central Laboratory-The Ministry of Higher Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan, Tel: +249912651103; Fax: +2499155183855; E-mail: khalid.enan@gmail.com

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with numerous forms of human contact including perinatal/mother-to-child; household (nonsexual); sexual; needle-sharing; and occupational/health-care-related [3]. Various reports have shown different prevalence rates of HBV among different Sudanese populations using different techniques. Prevalence rates of 6.25% in blood donors [6], 78% in soldiers [4], 18.7% in a survey among healthy subject [7] and 5.6% in pregnant woman [8] were reported.

The aim of this study was to detect the prevalence of hepatitis B virus among febrile malaria and typhoid negative patients in Atbara city, Sudan.

MATERIAL AND METHODS

Study area

This study was conducted in different health centers in Atbara city, northern Sudan during the period of July to December 2018.

Study population and sample size

A total of 88 blood samples were collected from febrile malaria and typhoid negative patients including 44 females and 45 males. The age of the patients ranged between 15-60 years.

Most of the patients complained of fever and other symptoms like headache and general body pain. All relevant information including personal data such as name, gender were collected from each patient after obtaining full consent.

5 ml blood samples were collected in EDTA tubes from each patient and centrifuged at 3000 rpm for 5 min. Obtained plasma was then stored at -20°C until used.

Serological testing

Commercial ELISA kits; Diagnostic Bioprobe (Italy) and Diagnostic Automation/Cortez Diagnostics (USA) were used for detection of HBsAg and HBcAb, respectively according to the manufacturers' instructions. All positive samples for HBcAb were then tested for HBV DNA using real time-PCR and conventional-PCR.

Heat treatment of plasma samples

Substrate for HBV real-time and conventional PCR was prepared by heat treatment of plasma without DNA extraction [9]. In brief, 25 µL of specimens was diluted 2-fold with nuclease-free water. The mixture was briefly vortexed and heated at 95°C for 5 min, then at 100°C for approximately 5 min. The mixture was then centrifuged at 12 000 g for 3 min. The supernatant was reserved and 5 µL were used in RT-PCR and conventional PCR for detection of HBV DNA.

Real-time PCR

Detection of HBV DNA was performed using real-time PCR on the Rotor 5 plex real-time PCR machine (Germany) according to the protocol developed by Garson et al. [10]. Commercial kit (innuMIX Q PCR MasterMi`x probe_analytic jena_Germany) was used according to manufactures protocol. Amplification and detection was carried out under the following cycling conditions: 1 cycle of 95°C for 15 min and 45 cycles of 95°C for 15 s and 60°C for 60 s.

Conventional 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 µl volume using (iNtRON, Korea) master mix. The volume included: 5 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 13 µl distilled water.

The DNA was amplified in thermo cycling conditions using PCR machine Heal force-classic (China) 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.

5 µl of the amplified product was then subjected to direct analysis by gel electrophoresis in 2% Agarose gel, and visualized by staining with 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.

RESULTS

Out of 88 samples, 44 (50%) samples were positive for HBcAb. HBV DNA was detected in 16 (18.2%) and 1 (1.1%) of the samples using real time-PCR and conventional-PCR, respectively **(Table 1)**.

Table 1. Frequency of HBV marker among febrile patients.

Tests / Results	Positive results	Negative results	Total
HBsAg ELISA	0 (0%)	88 (100%)	88 (100%)
HBcAb ELISA	44 (50%)	44 (50%)	88 (100%)
HBV real-time PCR	16 (36.3%)*; (18.2%)**	28 (63.7%)*; (31.5)**	44 (100%)
HBV conventional PCR	1(2.3%)*; (1.1%)**	43(97.7%)*; (49.4%)**	44 (100%)

**calculated from total HBcAb positive samples (n=44)*
*** calculated from the total samples tested (n=88)*

DISCUSSION

Occult hepatitis B virus in Sudan had been detected in different populations; it recorded 15.1% in HIV positive patients [11-15] and among hemodialysis patients it was 0% in white Nile state and 3.3% in Khartoum state [16,17], but much higher prevalence were observed in blood donors, cancer patients, renal transplant patients and patients with hematological disorders, recording 38%, 38.2%, 51.4% and 53.3% prevalence rates, respectively [18-21] which is similar to our current finding (36.3%) when using RT-PCR. In Africa similar studies were done regarding OBI revealed that a rate of 7.4%, 8.7% and 7.3% in Libya, Nigeria and Burkina Faso, respectively [22-24]. The variation of OBI rates according to global studies may be affected by populations of the study, demography and sensitivity of used assay [23,25].

The high prevalence of OBI recorded in the present study indicates that HBV infection is endemic in Atbara town, northern Sudan. In Africa more than 50% of the adult population may have serologic evidence of past hepatitis B infection [7]. In this study, the prevalence rate of HBsAg was 0% and HBcAb was 50%. Similar study in Tanzania found prevalence rates of 4.3%, and 29.3% for HBsAg and HBcAb, respectively [11].

Among the 44 (50%) HBcAb positive samples in our study, HBV-DNA was detected in 16 (36.3%) of them. All HBV DNA positive samples were found to be negative for HBsAg; this is consistent with the definition of occult hepatitis B (OBI). Possible explanations for occult HBV are: (1) mutations in 'α' epitope of the S gene could alter the antigenicity of HBsAg, causing the failure of anti-HBs to neutralize HBsAg; (2) or due to decline in HBV genome replication and expression; (3) also altering HBx and/or overlapping core promoter function can reduce HBV replication due to mutation in HBx ORF region [12].

The frequency of OBI (18.2%) in febrile patients without any signs or symptoms of clinical hepatitis in our study is higher than that found (12.5%) in a previous study conducted in West Kurdofan State, Sudan [13]. This rate is also much higher in comparison with previous data (3%) from immigrants with fever from sub-Saharan African countries to Australia [14].

The variation in our results regarding HBV DNA detection by using real time PCR (36.3%) and conventional PCR (2.3%) may be due the fact that we used heat treatment plasma without DNA extraction [9]. This may indicate that heat treatment method is more suitable to use with RT-PCR than conventional PCR but this also may be due to the higher sensitivity of RT-PCR.

CONCLUSION

It is concluded from our study that there is a need for HBV testing in individuals presenting with febrile illnesses in areas where HBV are endemic using serological and molecular methods. Finally and to the best of our knowledge the present study represents the first report on HBV infection in River Nile State.

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