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Molecular Detection of Occult Hepatitis B Virus among Human Immunodeficiency Virus Patients in Khartoum

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Abstract

Background: Hepatitis B, a potentially life-threatening liver infection caused by the hepatitis B virus (HBV) is a major global health problem. Of the two billion people infected with the virus, more than 296 million are chronic carriers and more than 686,000 die annually from HBV-related complications, including cirrhosis and hepatocellular carcinoma.

Aim: This study was carried out to determine the prevalence of Occult Hepatitis B Virus among Human Immunodeficiency Virus patients in order to establish basic knowledge for future HIV Patient care.

Method: A total number of 88 Serum samples from Human Immunodeficiency Virus patient was collected and tested to determine the HBV exposure rate and the presence of HBsAg and detection of Occult Hepatitis B Virus .

Results: in This study , the mean age of them was 35.98 years, 64.7% (n= 57) were male and 35.3% (n= 31) were female, the exposure rate of HBV was 39.7%,and we found high rate of exposure in male (63%) and We found the high rate of exposure in age group (25-35 years) (45.7%) and The prevalence of HBsAg in study population was 6.82% we found high prevalence rate in female (66.7%) and high prevalence rate in age group (25 – 35years) (66.7%) in the other hand the prevalence of OHB was 2.4% and We found high rate in male 100% and we found similar distribution to infection in just two age group; group (25-35)years(50%) and age group (less than 15 years) (50%) .

Conclusion: This study highlights the urgent need for continued fully screening and education about Occult HBV infection and strategies that ensure future HIV Patient care.

Key words: Occult hepatitis B Virus, HIV, HBV DNA, HBsAg, Anti-HBc.
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Introduction: Hepatitis B, a potentially life-threatening liver infection caused by The hepatitis B virus (HBV) is a major global health problem, Of the two billion people infected with the virus, more than 240 million are chronic carriers (1) , WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year (2), and more than 686,000 die annually from HBV-related complications, including cirrhosis and hepatocellular carcinoma (3).

A growing body of evidence is emerging showing that the prevalence of HBV is significantly higher amongst HIV-positive individuals, presumably because of the shared transmission risks and risk factors (4). HIV generally accelerates the natural course of HBV infection and facilitates faster progression of liver disease to cirrhosis and hepatocellular carcinoma (HCC) (5).

Traditionally, HBV is diagnosed by serological techniques to detect antigens or antibodies. The hepatitis B surface antigen (HBsAg) is often used for routine diagnosis since it is considered as the hallmark of infection. During acute infection, antibodies to HBV core antigens (anti-HBc) (initially both IgM and IgG) appear 1–2 weeks after the appearance of HBsAg, while IgG persists during chronic infection. The presence of antibodies to HBsAg (anti-HBs) represents immunity to HBV infection (6).

Occult hepatitis B (OHB) has been increasingly recognized over the last 2 decades as a public health concern. It is characterized by the presence of Hepatitis B virus (HBV) DNA in plasma ,liver, and/or peripheral blood mononuclear cells (PBMC) of patients with no detectable hepatitis B surface antigen (HBsAg) in serum,Occult hepatitis B infection is common in HIV infected patients (7) .

A study by Coffin et al., for instance, has shown 17.8% and 40% prevalence rates of OHB in serum and PBMC of HIV infected patients respectively (8). Similar study in a cohort of HIV infected people reported 47% prevalence rate of OBH ,In Africa, about 100 million individuals are estimated to be infected with HBV or HCV (9), Also, HBV and HCV infections are highly endemic in Africa and are responsible for 80% of liver cirrhosis and HCC, with HBV being the main cause of end-stage liver disease (10).

HIV-infected people are three to six times more likely to develop chronic or long-term hepatitis B infection because of their suppressed immune systems than individuals without HIV (11).

The global prevalence of HBV/HIV co-infection varies from 1.13% to 59% . In the United States of America [USA], the prevalence of HIV/HBV in children is 2.6% and 4.9% in China (12) .

Reports from Africa have revealed that the prevalence of HBV/HIV co-infection is between 10% and 20% as many countries in sub-Saharan Africa are typically classified as endemic, high, or intermediate countries with HBV infections (13).

In Tanzania, a prevalence of 1.2% was documented in children aged 18 months to17 years while 12.1% was documented by Route et al in Cote d' Ivoire in West Africa (14,15).

Methods: Study design and setting: This was Descriptive Cross-sectional study confined on patient infected with of Human immunodeficiency Virus already Study done in Khartoum state, Sudan at Khartoum North Teaching Hospital and Omdurman Teaching Hospital during April -2021 to August - 2021.

Sample collection and Data: 88 Serum sample that we were collected it from lab that already have samples of patients who follow up in at Khartoum North Teaching Hospital lab and Omdurman Teaching Hospital lab and kept frozen at -18^{o}C and Personal data (Gender /Age) obtained from the investigation request or files of HIV patient using datasheet.

Statistical analysis: Statistical analysis of database was prepared and processed by using Statistical Package for Social Science (SPSS.) program version 20.X2 and T test was used when appropriate p value of less than 0.05 was considered statistically significant.

Serological testing: Initially, all samples were screened for HBsAg using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from RecombiLISA™ by (CTK , USA ,lot ; E0 531S2f00) and samples that were HBsAg negative were further screened for anti-HBc Ab using ELISA commercial kit for the qualitative detection of antibodies to hepatitis B core antigen (anti-HBc) in human serum, using kits for (Fortress Diagnostics Limited,ref; bxe0761a,LOT;HBc-2202-1).

DNA extraction: DNA from HBsAg negative samples was extracted from 200 µl from serum using the (PowerPrep™ Viral DNA / RNA Extraction Kit **(**CatNo.E 0 0 0 7 / Kogenebiotec /Seoul, Korea 08507**))**was used for the purification of viral DNA in serum,The Kit includes all reagents necessary for purification of viral nucleic acids from serum according to the manufacturer's instructions The extracted DNA was amplified immedia- tely after extraction or stored at −20 °C awaiting subsequent amplification.

Polymerase chain reaction(PCR) for HBV: The PCR was performed by processing the extracted DNA from serum with primers were detected the specific portion of surface gene, the primers used consisted of**:-**

Forwardprimer**:**5'ATCCTGCTGCTATGCCTCATCT -3'

Reverseprimer:3GAACCACTGAACAAATGGCACT-5' (15).

The reaction was performed in 20 µl volume using Maxime PCR blue mix master mix (Intron Co, Korea).The volume included:,(total 20 µl): 1 µl forward primer, 1 µl reverse primer, 4 µl extracted DNA and 14µl distilled water as show in table The DNA was amplified in thermocycling conditions using PCR machine (Techne co, Japan) as follow: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60.5°C for 30 sec and extension at 72°C for 1 min, with a final extension 72°C for 7 min as show in table (2-2),5 µ l of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose.the PCR products will be electrophoresed in 2% agarose gel prepare in1 X TBE buffer Boiled for 30 sec in microwave oven and cool to50°C), adding ethidium bromide (3 µl) stain and evaluate under ultraviolet light, The specific DNA product for HBV was 294 bp of each sample will determine by identifying the bp amplified DNA bands in comparison with the100-bp DNA ladder will be used as DNA size marker .

Results: Description of the Study Population: A total of eighty eight Human Immunodeficiency Virus were enrolled in this study. Four samples (4.54%) of the total subjects were aged less than 15 years, 8 (9.09%) samples were found to be between (15-24 years) while 34(38.6%) samples were found to be between (25-35years).on the other hand we found 27(30.6%) samples in age group (36-45 years) also we found 15(17.04) samples in age group more than 45 years the distribution of the gender among study population showed 57(64.7%) was male and 31(35.3%) was female.

Prevalence of Anti-HBc among HIV patient: among the 88 samples there was 35(39.7%)

samples tested positive for Anti-HBc as showed in Table (1)

Table (1) prevalence of anti-HBc among HIV patient:

Variables	Patients (n=88)	Percent(%)
positive	35	39.7%
negative	53	60.3%

Distribution of Anti-HBc among the Gender And Age Group: Among 35 Anti–HBc positive samples, we found two samples among Age group (Less than 15 years) and three samples among age group (15-24years)**,**we also found 16 samples among age group(25-35years) and 9 sample among age group (36-45years),also five samples among age group (more than 45years) there is no significant association between Anti-HBc positive samples and Age group (P.value:0.799).

Variables	Subjects	Percentage (%)
Age : (years)		
Less than 15	2	5.8%
15 – 24	3	8.6%
25 – 35	16	45.7%
36 – 45	9	25.7%
More than 45	5	14.2%
Gender :		
Male	22	63%
Female	13	37%

Among 35 Anti–HBc positive samples, **22** (63%) were male and **13**(37%) were female, there is no significant association between Anti-HBc positive samples and gender (P.value:0.239), as shows in table (2).

Table (2):Description of the Anti-HBc patients Population:

Prevalence of "Overt" HBV infection among HIV patient: Among the 88 samples there was 6 samples tested positive for HBsAg as shows in table (3) .

Table (3) prevalence of "Overt" HBV infection among HIV patient:

Variables	Patients (n=88)	Percent (%)
HBsAg :		
positive	6	6.82%
negative	82	93.18%

Distribution of "Overt" HBV infection among the Age Group And Gender: Among six HBs Ag positive samples, we found four samples among Age group (25-35years) and two samples among age group (36-45 years)**,** there is no significant association between overt HBV infection and Age group (P.value:0.509).Also among HBs Ag positive samples, **2** (33.3%) were male and **4**(66.7%) were female, there is no significant association between overt HBV infection and gender (P.value:0.095),as shows in table (4).

Table (4): Description of the Overt HBV infection Population:

Variables	Subjects	Percentage (%)
Age :(years)		
Less than 15	0	0%
15 – 24	0	0%
25 – 35	4	66.7%
36 – 45	2	33.3%
More than45	0	0%
Gender :		
Male	2	33.3%
Female	4	66.7%

Prevalence of Occult HBV infection among HIV patient: Table (5) shows that among the 82 samples there was already tested negative for HBsAg we found 2(2.4%) sample positive for HBV DNA .

Table (5) Prevalence of Occult HBV infection among HIV patient:

Variables	Patients (n=82)	Percent (%)
HBVDNA		
positive	2	2.4%
negative	80	97.6%

Distribution of the OBI among Gender And Age : Among the two HBV-DNA positive samples , we found One samples among age group (less than 15 years), one sample among Age group (25-35years) and there is no significant association between OBI infection and Age group (P.value:0.087).Among the two HBV-DNA positive samples, we found two male (100%) there is no significant association between OBI infection and gender (P.value:0.125). as shows in table (6).

Table (6): Description of the Occult HBV infection Population:

Variables	Subjects	Percentage (%)
Age :(years)		
Less than 15	1	50%
15 – 24	0	0%
25 – 35	1	50%
36 – 45	0	0%
More than45	0	0%
Gender :		
Male	2	100%
Female	0	0%

Discussion: Laboratory detection of Hepatitis B virus infection is crucial for global control and prevention of HBV disease. Among HIV infected individuals under HAART, the increased longevity may facilitate emergence of chronic liver disease which is often a cause of increased morbidity and mortality, a significant proportion of this burden may be attributed to occult hepatitis B virus infection since it has been shown to have hepatopathogenic potential (17).

In this study, HBc total Ab or the exposure rate was 39.7%, which is lower than The exposure rate reported in Sudan and South Florianópolis (62.8%, 71.2%) respectively (18, 19).) Also we found the exposure rate was similar to studies done in Ethiopia, São Paulo and Southeast (Campinas) (39.5%, 38.6% and 44.0%) respectively (19, 21).Also we found the exposure was higher than studies done in Southern Brazil(27.3%) (21).

In this study, we found high rate of exposure in male (63%) our findings was agree with study done by hatim mudawi in sudan (61.8%)(17), also We found the high rate of exposure in age group (25-35) years(45.7%) .

The prevalence of HBsAg in study population was 6.82%, which is lower than study done by Hatim mudawi in sudan and study done by Rui in South Florianópolis in regions of Brazil (11.7%, 24.3%) respectively (18,19) and we found Slightly similar to study done in Ethiopia (5.5%) (21).

Also we found the our prevalence was higher than studies done in Southern Brazil (2.3%) (20). we found high prevalence rate in female (66.7%) our findings was disagree with study done by hatim mudawi in sudan (57.1%)was male.(18),we also found high prevalence rate in age group (25 – 35)years (66.7%) .

In this study, the prevalence of OHB was 2.4%, OBI the observed prevalence it lower than study done by Yousif in in Khartoum and study done by

hatim mudawi in sudan (9.8%,26.8%) respectively (22,18), and slightly similar to study done by George in Cameroon and study done in India by Saha and study done by Salpini in Yaoundé, Cameroon (5.9%,6.3,6.9%) respectively (22,24,25). Also we found the prevalence was higher than studies done by Cohen in Netherlands (0%) (26),we found high rate in male (100%)it is disagree with study done by George in Cameroon (85.%) was female and agree with study done by hatim mudawi in sudan (61.5%) was male (18),we found similar distribution to infection in just two age group; group (25-35) years (50%) and age group (less than 15) years (50%)was disagree with study done by George in Cameroon rate in age group (25-35)years(30%) and age group (36-45) years (23).

It should be noted however that the prevalence of OBI is dependent on the Sensitivity of the DNA assay used, demography and the population studied (27),Thus, in the Sudan study that used a more sensitive real time PCR method compared to the conventional PCR method used in this study. We propose that the difference in prevalence with our study and the Yaoundé study may be due to demographics and sensitivity of method.among HIV patients, several studies conducted worldwide have reported prevalence of OBI ranging from 0% to more than 90% (26, 28).

Conclusion: Our current study was done to detect the prevalence of occult hepatitis B infection among HIV patient. The exposure rate to HBV infection was 39.7% and the prevalence of HBsAg was 6.82%, and the OBI was (2.4%). In the present study there is no significant association between our findings and demographic data.

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