Molecular Biology

Elfaki et al., Mol Biol 2017, 6:1
DOI: 10.4172/2168-9547.1000184

Research Article OMICS International

Molecular Detection of Hepatitis B Virus DNA in Human Immunodeficiency Virus Infected Patients in Khartoum, Sudan

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Abstract

Background: Since hepatitis B virus (HBV) and human immunodeficiency virus (HIV) have the same modes of transmission, it is usual for them to infect the same host. This study was conducted at Bashair University Hospital during the period from April to July 2013, to detect HBV among HIV-infected individuals using polymerase chain reaction (PCR).

Methodology: A total of 87 subjects (n=87) were included in this observational, descriptive, case-study. The subjects were confirmed as HIV-positive by an ELISA assay, from ages ranged from 16 to 60 years, and from both sexes. From the study participants, serum samples were collected and tested for HBsAg by a capture ELISA assay and for HBV DNA by PCR.

Results: Out of the 87 people who took part in the study, 13 (14.9%) were positive for HBV DNA, while 14 (16.1%) were positive for HBsAg. Six people (6.9%) tested positive for both HBV DNA and HBsAg and 7 people (8.0%) were found to have occult hepatitis B infection (OBI) (i.e., positive for HBV DNA, negative for HBsAg).

Conclusion: From the above findings we concluded that, there is a high percentage of HBV/HIV coinfection in the Sudan. Also, there is an increasing percentage of OBI in HIV patients. Hence, we recommend the screening of HIV-positive subjects for HBV markers and the use of HBV DNA as a marker of OBI in the same population.

Keywords: Coinfection; Hepatitis B; Occult; HIV; PCR; Khartoum

Background

Since Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) share the same epidemiological risk factors and modes of transmission, co-infection between these two viruses is common [1]. HIV co-infection is known to unfavorably influence the course of HBV infection. This is illustrated by the fact that progression to end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC) is more rapid in HIV co-infected subjects than in mono-infected individuals [2].

Furthermore, HBV co-infection is thought to modify the course of HIV infection, particularly in patients receiving Highly Active Anti-retroviral Therapy (HAART), in whom severe hepatotoxicity during treatment is seen. Thus, screening of HBsAg and anti-HBc is recommended before initiation of HAART [3]. Moreover, HIV co-infected patients have lower rates of resolution of HBsAg and high rates of chronic carriage. It is thought that administration of the life-saving antiretroviral therapy (ART) increases the risk for resistant HBV mutants' emergence [4].

Occult Hepatitis B Infection (OBI) is defined as the presence of HBV DNA in the liver and sometimes serum of chronically-infected individuals together with the absence of HBsAgin their blood [5]. It has been shown that OBI represents a risk factor for HCC development [6]. OBI can result in acute and severe overt infection in immunocompromised patients (e.g. HIV-AIDS patients) [7].

Since the prevalence of occult HBV in HIV-positive population is higher than that of non-HIV-infected cohorts [8] and that the prevalence of OBI is variable according to the endemicity of HBV infection in the region [9], one would assume that a high percentage of OBI is present among Sudanese HIV patients, which is not yet known.

Studies conducted to determine the prevalence of HBV and HIV infection in Sudan have focused on mono-infection by both viruses. Given the lack of studies that address the coinfection of HBV/HIV and OBI among HIV-infected individuals in this part of the world, we carried out this study to detect HBV infection in HIV-positive individuals in Khartoum State, Sudan.

Methodology

Study design and participants

This study is classed as an observational, descriptive, case study. HIV-infected patients, both men and women and of different ages, were the source of the samples.

Eighty-seven HIV-positive subjects (n=87), confirmed by indirect enzyme-linked immunosorbent assay (ELISA), were enrolled in the study. They were enrolled on a convenience basis, and blood sample was collected from each of them.

Detection of HBsAg by capture ELISA

Blood samples were collected in plain containers and allowed to clot, then subjected to centrifugation to obtain serum. The obtained serum was stored at -20°C pending processing. Commercial ELISA kits (WKEA Med Supplies Corp, China) were used to detect HBsAg according to the procedure described by the manufacturer.

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Received December 23, 2016; **Accepted** December 27, 2016; **Published** December 31, 2016

Citation: Elfaki Y, Enan KA, Elkhidir IM, Nagi AM (2017) Molecular Detection of Hepatitis B Virus DNA in Human Immunodeficiency Virus Infected Patients in Khartoum, Sudan. Mol Biol 6: 184. doi: 10.4172/2168-9547.1000184

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DNA Extraction and target amplification by Polymerase Chain Reaction (PCR)

DNA was extracted from 150 µL of serum using the Viral Gene-spin® Viral DNA/RNA Extraction Kit (Intron Biotechnology, South Korea).

First, 150 µl of the serum sample was dispensed to an eppendorf tube, followed by a lysis buffer and proteinase K. The DNA was extracted according to the manufacturer's instructions. Finally, the DNA was eluted in 50 µl elution buffer provided with the kit.

The amplification and detection of HBV DNA was carried out by a conventional PCR method in a thermal cycler. For amplification, 5 µL of HBV DNA was added to a 15 µL reaction mixture. HBV primers (HBV-F: 5'-TCGGAAATACACCTCCTTTCCATGG-3', HBV-R: 5'-GCCTCAAGGTCGGTCGTTGACA-3') were used for the PCR reaction. The PCR reaction mixture contained in addition to 5 µl DNA, 0.5 µl of each primer and 10 µl GoTaq ready-to-use master mix (Promega, USA), in a 20 µL total reaction volume. Samples were first denatured at 94°C for 5 min, then thermocycled for 35 cycles (1 min at 94°C, 1 min at 62°C, 1.5 min at 72°C) and final extension was done at 72°C for 7 min. PCR products were separated in a 1.5% agarose gel, then stained with ethidium bromide and viewed under UV light. A result was considered positive when a band of the appropriate size was visible in the gel (350 bp) (Figure 1). Standard procedures for reducing contamination were strictly followed.

Data analysis and presentation

The data obtained were analyzed and presented using Statistical Package for Social Science (SPSS) computer software version 11.5 for Windows. Significance of differences was determined using Chi-square test. Statistical significance was set at $P<0.05$.

Results

Detection of HBsAg and HBV DNA among HIV-infected patients

14 out of 87 HIV-positive patients were found HBsAg positive (16.1%) by using ELISA.

However, when the same HIV-positive patients were examined by conventional PCR, HBV DNA was detected among 13 of them (15.0%) as displayed in Table 1.

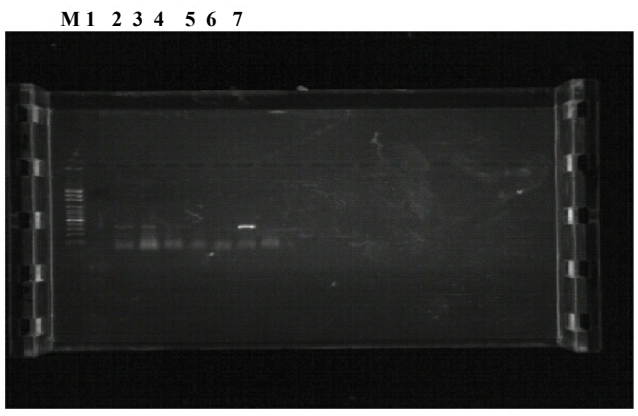

Figure 1: HBV DNA result (350bp) on 1.5% agarose gel. Lane 1 shows negative control, lanes 2-6 show results in five patients, lane 7 shows positive control, M: 100 bp DNA marker.

		HBV DNA		Total
		Positive	Negative	
HBsAg	Positive	6	8	14
	Negative	7	66	73
Total		13	74	87

Table 1: Percentages of both HBV DNA and HBsAg in HIV-positive patients.

		Sex		Total
		Males	Females	
HBV DNA	Count	7/50	6/37	13
	Percent	14.0%	16.0%	15.0%

(P=0.774)

Table 2: The effect of gender of HIV-positive patients on detection of HBV DNA.

		Age Group					Total
		15-24	25-34	35-44	45-54	55-65	
HBV DNA	Count	0	6	5	2	0	13
	Percent	0%	6.9%	5.7%	2.3%	0%	14.9%

(P=0.818)

Table 3: The effect of age of HIV-positive patients on detection of HBV DNA.

Detection of both HBsAg and HBV DNA in HIV-positive patients

6 of the 87 HIV-positive patients (6.9%) were shown positive for both HBsAg and HBV DNA, 8 HIV-positive patients were found positive (9.2%) for HBsAg alone and 7 subjects were detected positive for HBV DNA (8.0%) alone.

The effect of gender of HIV-positive patients on detection of HBV DNA

The results reveals that 7 out of 50 males (7/50) were found HBV DNA-positive (8.0%) and 6 out of 37 females (6/37) were shown positive (6.9%) for HBV DNA (Table 2).

The effect of age of HIV-positive patients on detection of HBV DNA

No single HIV-positive patient was found HBV DNA-positive in the age groups 15-24 and 55-65 years. However, 6 (6.9%), 5 (5.7%) and 2 (2.3%) HIV-positive patients were found HBV DNA-positive among the age groups 25-34, 35-44, and 45-54 years, respectively, with no significant difference (P=0.818) between them (Table 3).

Discussion

Eighty-seven HIV-positive patients (n=87) were randomly selected for the present study, 50 of them were males (57.5%) and 37were females (42.5%), with mean age of 35. The results obtained in the study were similar to those obtained by Ladep et al. in Nigeria, where the rate of HBV/HIV coinfection was 17.8% using HBsAg as a marker [10]. Also, Utsumi et al. [11] in Indonesia and Geretti et al. [12] in China reported results consistent with our findings (15.3%, and 16.7%, respectively), using ELISA to detect HBsAg.

Also, Attia et al. in Cote d'Ivoire [13], Mohammadi et al. in Iran [14] and Saha et al. in India [15] reported similar results to those revealed by our study (13.4%, 14.5%, and 15.19%, respectively), while higher rates of coinfection were reported by Franzeck et al. who detected HBsAg in 28% of HIV-positive patients in Tanzania [16]. This rate of coinfection could reach 90% according to Sud et al. depending on the prevailing risk factors in a given population [17].

The rate of coinfection revealed by our study was higher than those reported by Maitaimi et al. in Urumqi, China [18], Tremeau-Bravard et al. in Nigeria [19] and Saravanan et al. [20] in South India, who reported rates of 6.1%, 7.9% and 9%, respectively. This variation of results could be attributed to ethnic differences, as shown by a study done by Barth et al. in South Africa, which reported a rate of coinfection of 0.4% among South Africans, 4.4% among Caucasians and 8.9% among African immigrants [21].

Alter suggested that the prevalence of HBV/HIV coinfection in Western Europe and the US could range between 6 and 14%, citing prevalence according to geographic regions, and efficiency of certain exposures in transmitting the infection as the reasons for this range [22].

Presence of HBV DNA in the absence of HBsAg, with or without the presence of anti-HBc antibodies, is defined as occult hepatitis B infection (OBI) [1]. Concerning OBI, results of our study were similar to those reported by Panigrahi et al. in India, who found the prevalence of OBI to be 10.7% in HIV-positive patients [23]. However, these results differed from those obtained by Ma et al. in China [24], Utsumi et al. in Indonesia [11] and Attia et al. in Cote d'Ivoire [13], who found the frequencies of OBI to be as high as 30.5%, 27.1% and 21.3%, respectively. Barth et al. [21] in South Africa reported OBI percentage of 10% among South African HIV patients, 3.2% among HIV-positive Caucasians and 1.4% among African immigrants having HIV infection, suggesting ethnic differences as a cause of this difference in frequency of OBI.

Maimaiti et al. in Urumqi, China [18] reported lower rate of OBI than those revealed by our study (3.8%).This difference could be due to the sensitivity of assays that detect HBsAg and the contrasting prevalence of HBV infection [19].

Several mechanisms have been suggested for the occurrence of OBI, these include integration of HBV genome into the host cell genome, strong suppression of HBV replication, escape mutants of HBV that cannot be detected by HBsAg assays, window period, and the persistence of immune complexes that mask HBsAg, rendering it undetectable [20]. According to Raimondo and Pollicino, HBV can be transmitted from OBI patients during blood transfusion and organ transplantation. Also, patients of OBI are at risk of developing liver fibrosis, and hepatocellular carcinoma (HCC) [25].

Strarner in the US screened 3.7 million HBsAg-positive donated blood samples for HBV DNA and showed that 9 of them were positive for HBV DNA, suggesting the use of nucleic acid testing (NAT) to detect potentially infectious HBV during the window period before seroconversion [26].

Furthermore, Bhatti et al. recommended that Pakistani donors who are HBV DNA negative should only be selected as regular donors of blood, not depending only on the detection of HBsAg [27]. Moreover, similar recommendation came forth from Mahtab et al. in Bangladesh, who highlighted the importance of using other means of screening HBV patients in Bangladesh [28].

Concerning treatment of HBV in HBV/HIV co-infected patients, several guidelines have recommended the use of combination therapy with tenofovir/lamivudine or since those drugs are regarded as first-line anti-HIV agent [6]. There is a high rate of lamivudine resistance occurring in HBV/HIV-coinfected patients receiving lamivudine as monotherapy against hepatitis B in their ART regimen. Thus, screening HIV patients for HBV markers and using more than one medication active against HBV in combination therapy for HIV infection in HIV/HBV coinfected patients is recommended by WHO and the US Department of Health and Human Services [8].

Conclusion

The findings of the present study disclosed a high percentage of HBV infection among HIV-positive patients. Also, OBI is an increasingly significant problem in HIV-infected patients. Furthermore, some variation was seen between the results of ELISA and PCR.

Authors' Contributions

YEOM carried out sample collection, immunoassays and molecular analyses and drafted the manuscript. AMN contributed to the drafting of the manuscript and acquisition of funding. KAE participated in data acquisition and critically revised the manuscript for intellectual content. IME conceived of the study.

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This article was originally published in a special issue, **Molecules involved in cell signaling pathways** handled by Editor(s). Dr. Masood Alam Khan, Al-Qassim University, Saudi Arabia

Citation: Elfaki Y, Enan KA, Elkhidir IM, Nagi AM (2017) Molecular Detection of Hepatitis B Virus DNA in Human Immunodeficiency Virus Infected Patients in Khartoum, Sudan. Mol Biol 6: 184. doi: 10.4172/2168-9547.1000184

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