Seroprevalance of Hepatitis E virus among HIV-1 infected patients in Khartoum

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Abstract

Background: Hepatitis E virus (HEV) is an enterically transmitted pathogen that causes wide scale epidemics of acute hepatitis in highly HEV-endemic areas such as Africa, Asia and the Middle East. HEV can cause chronic infection and cirrhosis in the immunosuppressed, including patients with HIV-1 infection**.** Little is known about HEV and HIV-1 co infection in Sudan.

Objectives: The aim of this study was to detect seroprevalence of HEV antibodies (IgG and IgM) among HIV-1 infected patients in Khartoum, Sudan.

Materials and methods: This was descriptive and cross-sectional study, a total of 92 HIV-1 infected patients were tested for anti-HEV IgM and IgG by, using enzyme linked immunosorbant assay (ELISA), in period from December 2015 to March 2016.

Result: Out of 92 HIV-1 infected patients, anti-HEV IgG and IgM were detected in 21.7% and 8.7% respectively. HIV-1 infected patients complaining of liver cirrhosis and jaundice were 5 (5.43%) and 46 (50%) respectively. The prevalence of anti-HEV IgG and IgM were 100% and 60% within patient with liver cirrhosis, and 32.6% and 10.9% within patients with jaundice.

Conclusions: Regarding IgG, the overall seroprevalence of HEV among study group was high (21.73%), and interestingly it was higher within patients with liver cirrhosis and jaundice.

Introduction:

Hepatitis E is a liver disease caused by the hepatitis E virus, an RNA virus previously known as enterically transmitted non-A, non-B hepatitis, is an infectious viral disease with clinical and morphological features of acute hepatitis, the hepatitis E virus is transmitted mainly through contaminated drinking water. It is usually a self-limiting infection and resolves within 4–6 weeks. Occasionally, a fulminant form of hepatitis develops

(acute liver failure), which can lead to death [1,2]. Although Hepatitis E often causes an acute and self-limiting infection with low mortality rates, it bears a high risk of developing chronic hepatitis in immunocompromised patients with substantial mortality rates. Hepatitis E occasionally develops into an acute, severe liver disease, and is fatal in about 2% of all cases [3]. Chronic HEV can lead to cirrhosis within less than three years, In the setting of immunosuppressed HIV-infected patients with chronic hepatitis E, progression to cirrhosis may be even faster than that observed in HBV (hepatitis B virus) or HCV (hepatitis C virus)/HIV co- infection [4]. Immunosuppression is a recognized risk factor for the persistence of HEV infection, and high rates of HEV/HIV co-infection have been reported in some countries [5]. In 2009, chronic HEV co infection was documented in the United Kingdom (UK) and France in two HIV-infected patients, in association with established cirrhosis. However, little is currently known about the extent or outcomes of HEV and HIV co infection [5, 6]. In study done in Zambia, Hepatitis E virus infection associated with HIV shows 71% prevalence of HEV infection among HIV-positive adults, compared to a prevalence of 24% in HIV-negative adults [7].

Materials and Methods:

This was a descriptive and cross-sectional study conducted in, Khartoum state, Sudan between of December 2015 and March 2016.

A total of 92 blood specimens were collected from HIV-1 infected patients who attending department of HIV medicine at Omdurman teaching Hospital. The investigated patients included 48 (52.17%) male and 44 (47.83%) female. Demographic and clinical data were obtained by direct interviewing questionnaire. Blood specimen was collected from each patient, and serum was separated for anti-HEV immunoglobulin G (IgG) and IgM immunoassays testing. Specimens were tested for anti-HEV IgG and IgM antibodies using enzyme linked immunosorrbent assay (ELISA) (EUROMIUN. UK) .

Procedure of IgM: The foil pouch was opened; the number of strips required for the assay was assembled and Sufficient IgG absorbent containing sample diluents were Prepared according to number of samples to be tested. Patient samples were diluted 1:101 with the sample buffer (10µl serum plus 1 ml sample buffer) and incubated for at least 10 m unites at room temperature. Then 100 µl of the calibrator, positive and negative control and the diluted patient sample were dispensed into the wells and incubated for 30 minutes at room temperature while direct sunlight was avoided. The Wash Buffer was diluted by adding 50ml of buffer solution to 450ml distilled water (1:9) and the well contents were aspirated and washed 3 times using an automatic plate washer. After that 100µl of Conjugate was dispensed into each well and incubated for 30 minutes at room temperature and the well contents were

discarded and washed with wash buffer 3 times. Then 100µl of enzyme conjugate (peroxidase-lablled anti-human IgM) was dispensed rapidly into each well and Incubated for 15 minutes, the Substrate reaction was stopped by adding 100µl of Stop Solution to each well and the optical density was read in a microplate reader within 30 minutes. Sera giving an absorbance greater than the cut-off value were considered to be positive.

Procedure of IgG: The foil pouch was opened; the number of strips required for the assay was assembled. Patient samples were diluted 1:101 with the sample buffer (10µl serum plus 1 ml sample buffer) and incubated for at least 10 m unites at room temperature. Then 100 µl of the calibrator, positive and negative control and the diluted patient sample were dispensed into the wells and incubated for 30 minutes at room temperature while direct sunlight was avoided. The Wash Buffer was diluted by adding 50ml of buffer solution to 450ml distilled water (1:9) and the well contents were aspirated and washed 3 times using an automatic plate washer. After that 100µl of Conjugate was dispensed into each well and incubated for 30 minutes at room temperature and the well contents were discarded and washed with wash buffer 3 times. Then 100µl of enzyme conjugate (peroxidase-lablled anti-human IgG) was dispensed rapidly into each well and Incubated for 15 minutes, the Substrate reaction was stopped by adding 100µl of Stop Solution to each well and the optical density was read in a microplate reader within 30 minutes. Sera giving an absorbance greater than the cut-off value were considered to be positive. Obtained data and results were analyzed using the statistical package (SPSS) and the significance of difference was determined using chi square test. Ethical approval was obtained from the Research Ethical Committee Board of AlNeelain University, Sudan, and verbal consent was taken from each patients.

Results:

A total 92 HIV-1 infected patients were tested for anti-HEV IgM and IgG by ELISA, 20 (21.73%) were positive for anti-HEV IgG, while 8 (8.69%) were positive for anti-HEV IgM.

Association between gender - age groups and seroprevalence of anti-HEV- IgM and IgG in HIV infected patients:

The results in table (1) showed that the anti- HEV IgM was detected in 10.42% and 6.82% of both males and females respectively, positivity was slightly high among male than female.

Whereas anti- HEV IgG was detected in 20.83% and 22.73% of both males and females respectively, positivity was slightly high among female than male. Also table (1) demonstrated 8.11% anti-HEV IgM were detected among the age group (20-40 years), 9.62% among the age group (41-60 years), and 0.0% among the age group (61-80 years). While anti- HEV IgG was detected in 13.51% among age group (20-40), 23.08% among age group (41-60), and 100% among age group (61-80), anti HEV IgM was showed high seropositivity among age group 41-60(9.62%), while IgG was high among the age group 61-80 (100%).

Effect of HEV seroprevelance among HIV-1 infected patients with jaundice and liver cirrhosis:

Table (2) showed the contribution of HEV infection to liver cirrhosis and jaundice among HIV-1 infected patients, the prevalence of anti-HEV IgM and IgG was demonstrated in 60% and 100% in patients with liver cirrhosis respectively. Whereas anti-HEV IgM was detected in 10.86% and anti-HEV IgG was detected 32.60% in HIV infected patients with jaundice.

Table (1): Seroprevelance of HEV among HIV-1 infected patients (n=92) regarding their age and gender.

Type of Anti-HEV		Gender		Total No (%)	Age group years No(%)			Total No%	p.value
		Male No (%)	Female No (%)		20-40	41-60	61-80		
IgM	Positive	5 (10.42)	3 (6.82)	8 (8.70)	3 (8.11)	5 (9.62)	0 (0.00)	8 (8.70)	Gender 0.307
	Negative	43 (89.58)	41 (93.18)	84 (91.30)	34 (91.89)	47 (90.38)	3 (100)	84 (91.30)	Age group
Total No%		48 (100)	44 (100)	92 (100)	37 (100)	52 (100)	3 (100)	92 (100)	0.753
IgG	Positive	10 (20.83)	10 (22.73)	20 (21.74)	5 (13.51)	12 (23.08)	3 (100)	20 (21.74)	Gender 0.789
	Negative	38 (79.17)	34 (77.27)	72 (78.26)	32 (86.49)	40 (76.92)	0 (0.00)	72 (78.26)	Age group
Total No%		48 (100)	44 (100)	92 (100)	37 (100)	52 (100)	3 (100)	92 (100)	0.014

Table (2): Distribution of anti-HEV IgM and IgG among HIV-1 infected patients with liver cirrhosis and jaundice.

Type of Anti-HEV		Liver Cirrhosis		Total (%)	Jaundice		Total No (%)	P.value
		Yes (%)	No (%)		Yes (%)	No (%)		
IgM	Positive	3 (60.00)	5 (5.74)	8 (8.69)	5 (10.86)	3 (6.52)	8 (8.74)	Liver Cirrhosis 0.000
	Negative	2 (40.00)	82 (94.25)	84 (91.30)	41 (89.13)	43 (93.47)	84 (91.30)	Jaundice 0.231
Total No (%)		5 (100)	87 (100)	92 (100)	46 (100)	46 (100)	92 (100)	
IgG	Positive	5 (100)	15 (17.24)	20 (21.73)	15 (32.60)	5 (10.86)	20 (21.73)	Liver Cirrhosis 0.000
	Negative	0 (0.00)	72 (82.75)	72 (78.26)	31 (67.39)	41 (89.13)	72 (78.26)	Jaundice 0.010
Total No (%)		5 (100)	87 (100)	92 (100)	46 (100)	46 (100)	92 (100)	

Discussion:

Susceptibility of HIV positive individuals to HEV is a controversial issue. The result of investigations from endemic parts of the world showed higher HEV seroprevalence rates in HIV-infected individuals and also its association with late stage of HIV infection. It is not clear if this is due to an opportunistic infection or common method of transmission [8].

This study was designed to determine the seroprevalence of HEV antibodies in HIV-1 infected patients in Khartoum. The seroprevalence of anti-HEV IgG was (21.73%) and anti-HEV IgM was (8.69%) of the total 92 HIV-1 infected patients. Our above results for anti-HEV IgM are higher than results reported by Scoto *et al,* in 2015 in Foggia and Naples in southern Italy (2.1%)[9] and in Nizhny Novgorod, Russia (1.80%) [10]. While it seems lower

than those reported by Hassan, et al., in Shiraz, Southern Iran (16.4%) [11]. The prevalence of anti-HEV IgG in the present study was found to be higher than those reported in Nizhny Novgorod, Russia (2.80%) [10], and Jardi 2012 Spain (9%) [12], when compared with endemic African countries it was lower than HEV seroprevalence in adult HIV patients from Ghana (45.3%, n=402) and higher than seroprevalence among HIV infected patients in Cameroon (14.2%, n=289) [13]. Several epidemiological factors may explain these differences. In some endemic areas, an association has been observed between higher anti-HEV seroprevalence and lower CD4 cell count [14]. In non endemic areas, the prevalence is uneven, which seems to indicate geographical differences, even between HIV-infected patients in two neighboring regions [15]. The seroprevalence of HEV antibodies among age groups was found to be increase with increasing age which is agreed with Hassan, who reported that the overall seroprevalence of hepatitis E was 26 (16.4%), which had a positive correlation ($P < 0.001$) with age ranging from zero in subjects less than 30 years of age to 47.4% in those aged 50 years or older [11]. Regarding liver cirrhosis and jaundice, interesting results were observed in this study, the seroprevalence of HEV was high in patients with cirrhosis, it was 60% and 100% for anti-IgM and anti- IgG respectively. This observation was in agreement with Jardi *et al*, 2012, who reported that the seroprevalence of HEV was 23% in patients with cirrhosis versus 6% in patients without cirrhosis [12]. An explanation for this finding could be the immune dysfunction was observed in patients with cirrhosis, who present decreased innate immune system activity with a reduction in natural killer cell activity [16].

Conclusions:

Our findings shows high seroprevalence of HEV IgG, IgM among study group, and interestingly it was higher within patients with liver cirrhosis and jaundice.

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