Hepatitis E Virus Infection among People Living With HIV/AIDS in Kano State, Nigeria

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Abstract: Hepatitis E is an emerging viral disease causing acute hepatitis worldwide which may result into a chronic hepatitis especially in immunocompromised individuals. The study determined the prevalence of Hepatitis E Virus (HEV) among people living with Human Immunodeficiency Virus (HIV) infection in Kano State. One hundred and eighty (180) subjects were enrolled for the study and their sera were screened for Hepatitis E Virus Antigen using Enzyme Linked Immunosorbent Assay (ELISA- Wantai Diagnostics, Beijing). Twelve (6.7%) were found to be positive for HEV antigen comprising 7 (58.3%) males and 5 (41.7%) females ($p<0.05$) and was common among subjects aged 35-44 years old (41.7%). Preponderance of HEV antigen was also found among subjects with primary school level of education 5 (41.7%), entrepreneurs 8 (66.7%), those with HIV duration of 3-5 years 8 (66.7%), those on the first line of antiretroviral treatment (ART) 10 (83.3%) or used borehole as a source of water 8 (66.7%). The study provided evidence that HEV is present among people living with HIV in Kano State. There is need to intensify enlightenment campaign among the populace about the disease so as to limit its spread in the community.

Keywords: Hepatitis E virus, HIV/AIDS, Kano

INTRODUCTION

Hepatitis E virus (HEV) is a spherical, non enveloped, single stranded positive sense RNA virus belonging to the genus *Hepevirus* and family *Hepivirideae* (Aggarwal, 2013). HEV has only one serotype and the classification is based on the nucleotide sequence of the genome (Perez-Gracia *et al*., 2015), although there are four genotypes (1-4) (Schlander and Mushahwar, 2001), with 24 subtypes (Teshale and Hu, 2011). Genotypes 1 and 2 exclusively infect humans and can lead to endemic outbreaks in countries with poor sanitation systems and also found in population in developing countries, genotype 3 and 4 are zoonotic and are globally distributed (Lu *et al*., 2006). Study shows that chronic infection with HEV in patients with HIV infection and in patients with non-Hodgkin lymphoma receiving rituximab, suggesting that immunosuppression predisposes patients to chronic infection outside of the transplant setting (Sherman, 2019). The virus is shed in stools of infected persons and enters the body through the intestine (Khuroo and Khuroo, 2015). It is transmitted mainly through contaminated drinking water, other transmission include food borne transmission from ingestion of products derived from infected patients, transfusion of infected blood products and vertical transmission from pregnant mothers to fetus, zoonotic transmission from animals to humans (WHO, 2016). The case fatality rate of HEV-induced hepatitis ranges from 0.5 to 3% in young adults and up to 30% in infected pregnant women (Yuchen and Yan-Jin, 2016). The aim of the study was to determine the burden of HEV infection among people living with HIV in Kano State.

MATERIALS AND METHODS

Study Area

The study was carried out in General hospitals of Rano, Rogo, Wudil, Bichi, Danbatta, Gwarzo Local Government area (LGAs) and Infectious Disease Hospital (IDH), Murtala Muhammad Specialist Hospital (MMSH) and Aminu Kano Teaching Hospital(AKTH) all in Kano metropolis of Kano State.

Kano State is located in Northern Nigeria. Created on May 27, 1967 from part of the Northern Region, it borders Katsina State to the north-west, Jigawa State to the north-east, Bauchi State to the south-east and Kaduna State to the south-west (Khan *et al.,* 2017). It has a population of 9,383,682 (NPC, 2006), located at latitude 11° 30' 00" N and longitude of 8° 30' 00" E, with a total area of 20,131 km². Kano state has a population of 374,000 people living with HIV. The inhabitants of Kano State are mostly Hausa/Fulani who are predominantly traders (Kurawa, 2006) with some percentage being farmers and civil servants. Other tribes such as Igbo and Yoruba with other few minority tribes also inhabit the state (NPC, 2006).

Study population

The study consisted of people living with HIV/AIDS of different age groups, sex, educational and socioeconomic background attending Antiretroviral therapy (ART) clinics of General hospitals of Rano, Rogo, Wudil, Bichi, Danbatta, Gwarzo Infectious disease hospital (IDH), Murtala Muhammad specialist hospital (MMSH) and Aminu Kano teaching hospital(AKTH).

Inclusion criteria

All registered and consenting ART clinic attendees of General hospitals in Rano, Rogo, Wudil, Bichi, Danbatta, Gwarzo Infectious Disease Hospital (IDH), Murtala Muhammad Specialist Hospital (MMSH) and Aminu Kano teaching Hospital (AKTH) were enrolled in the study.

Exclusion Criteria

All new and old clinic attendees and children less than age group 5 years were excluded in the study.

Determination of sample size

The sample size was determined based on the prevalence of HEV of 12.2% in HIV infected patients by Odaibo and Olaleye (2013), using the formula described by Lwanga and Lemeshow, 1991; Sarmukaddam and Grad (2006) below:

$$n = \frac{Z^2 pq}{d^2}$$

Where

n =Minimum sample size

Z = Percentage point of standard normal distribution curve, 95% confidence interval at 1.96

p =Prevalence from previous studies = 12.2%

q =1-p (1-0.122) 0.878

d =Minimum sampling error allowed at 95% confidence limit 0.05

$$n = \frac{(1.96)^2 x 0.0122 x 0.878}{(0.05)^2}$$

$$n = 164$$

The sample size was rounded up to 180 to increase precision and to allow for attrition (10%)

Sampling Selection

The subjects for the study were selected by random sampling technique. The samples were selected based on the population of the three senatorial district; Kano South (Rano, Rogo, Wudil), Kano North (Bichi, Danbatta, Gwarzo) and Kano Central (Infectious disease hospital, Murtala Muhammad specialist hospital, AKTH).

Data Collection

Blood samples were collected from the subject of the study and a questionnaire was used to generate other information such as age, sex, occupation, settlement, educational level, source of water, duration of HIV infection, type of ART regimen and pregnancy for the female subjects.

Blood Collection

Five millilitre of blood was collected aseptically from each of the subjects as described by Cheesebrough (2000). Samples collected were dispensed into a sterile container and was allowed to clot and retract. The serum was separated by centrifugation at 3500rmps for five minutes to avoid hemolysis of red blood cells. The serum separated was transferred aseptically into a plain sterile container and stored at -80^0C until needed for assay (Junaid *et al*., 2014).

Sample Analysis

Blood sample was analyzed for the presence of HEV, using ELISA (Wantai Diagnostics, Beijing).

Principle of the test

The test kit is a two-step incubation, solid-phase antibody "sandwich" ELISA in which polystyrene microwell strips are pre-coated with anti-HEV antibodies directed against the viral antigen. Patient's serum or plasma sample is added into the microwells. In case of presence of HEV Ag in the sample, the pre-coated antibodies will bind to the viral antigen and during the first incubation step; the specific immunocomplex formed is captured on the solid phase. After washing to remove unbound sample proteins, second, anti-HEV antibody conjugated to the enzyme Horseradish Peroxidase (HRP) is added into the wells. During the second incubation step, this antibody will bind to the anti-HEV, HEV Ag complexes immobilized onto the wells during the first incubation step. The unbound HRP conjugate is removed during washing and Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are then added into the wells. In presence of the antibody-antigen-antibody (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HEV Ag remain colorless (Wantai Diagnostics, Beijing).

Test procedures

The test was carried out according to manufacturer's instructions (Hepatitis E virus ELISA Antigen Detection Kit - Wantai Diagnostics, Beijing).

Calculation of results

Each microplate was considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results were calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. The results were read using a dual filter reader plate (The result was not subtracted from the print report values of the specimen) (Wantai Diagnostics, Beijing).

Interpretation of results

Negative Results (A / C.O. < 1): Specimens giving A value less than the Cut-off value were negative for this assay, which indicates that no HEV antigen have been detected with this kit, therefore there are no serological indications for infection with HEV.

Positive Results (A / C.O. ≥ 1): Specimens giving A value equal to or greater than the Cut-off value were considered initially reactive, which indicates that HEV antigen have probably been detected with this kit.

Border line (A / CO. = 0.9-1.1): Specimens with A value to Cut-off ratio between 0.9 and 1.1 were considered borderline. (Wantai Diagnostics, Beijing).

Data analysis

The data generated was analyzed using OpenEpi 2.3 initiative. Chi square (X^2) and Fishers Exert were used to determine the relationship between HEV and the associated risk factors. A probability value (0.05) was considered significant.

RESULTS

Out of the 180 known HIV seropositive subjects were screened for HEV, 12 (6.7%) were found to be seropositive. Participant in the age group of 35 - 44 years had the highest prevalence with 41.7% followed by 25 - 34 years (33.3%) and 45 - 54 years (25%) respectively. The least prevalence was obtained from age <24 years and >54 years with 0.0%, P = 0.907 (Table 1)

Table 1: Distribution of Hepatitis E virus with respect to age group

Age group (Years)	No of sample Screened	No of sample Positive for HEV	Prevalence of HEV (%)
5 – 14	9	0	0.0
15 – 24	8	0	0.0
25 – 34	59	4	33.3
35 – 44	63	5	41.7
45 – 54	34	3	25
55 – 64	5	0	0.0
65 – 74	2	0	0.0
Total	**180**	**12**	**6.7**

P = 0.907

The relationship between HEV positivity and gender distribution in this study shows that male participants had the highest prevalence of 58.3% while the female recorded 41.7% prevalence. P = 0.043 (Table 2).

Table 2: Prevalence of Hepatitis E virus with respect to gender

Gender	No of samples Screened	No positive for HEV	Prevalence (%)
Female	127	5	41.7
Male	53	7	58.3
Total	**180**	**12**	**100**

Fisher Exert Test P = 0.043

Table 3a revealed that there was no significant difference in the prevalence of HEV infection among the studied subjects based on their settlements (P = 0.768) with both in the rural and urban areas recording a prevalence of 50%.

It also shows that subjects with primary school education had a prevalence of (41.7%), while those with tertiary education qualification and Islamic education recorded the lowest prevalence of (16.7%), P=0.762.

Based on occupational statues of the subjects, the prevalence of HEV was found to be highest among subjects that were Entrepreneurs (66.7%), while the lowest prevalence was found among House wives (16.7%) and public servants (8.3%) (P=0.659) (Table 3a).

The prevalence of HEV was found to be associated with the duration of HIV infection among the studied subjects as those whose HIV status was established between 3-5 years recorded the highest prevalence of 66.7% while those whose status was established within the age of 9-11 years recorded the lowest prevalence of 8.3% (P=0.0310) (Table 3a).

The study also revealed that the prevalence of HEV among the subjects varies with the stage or category of Antiretroviral (ART) drugs taken by the subjects and shows that HEV infection was insignificantly most prevalent among those subjects on first line ART (83.3%) and low among subjects on second line ART (8.3%) (P=0.7650) (Table 3b).

The prevalence of Hepatitis E virus with respect to source of water used by the study subjects was found to be higher among subjects that used borehole water (66.7%) as their water source and lowest among those that use Tap and well water with a prevalence of 16.7% each, P=0.963 (Table 3b).

Finally, the study revealed that there was a significant relationship between status/stage of pregnancy and HEV prevalence among the female subjects of the study (P = 0.0025) where those whose pregnancy was in the first and second trimester had a prevalence of 8.3% each, while those that were not pregnant had a prevalence of 25% (Table 3b).

Additionally, those subjects whose stage of pregnancy was not established as at the time of the study had a HEV prevalence 58.3% (Table 3b).

Table 3a: Distribution of Hepatitis E virus in relation to risk factor among studied subjects

Risk factors	No screened	No positive foe HEV	Prevalence (%)	P value
Settlement				
Rural	80	6	50	
Urban	100	6	50	0.768
Total	**180**	**12**	**6.7**	
Educational Level				
Adult Education	1	0	0	
Islam Education	64	2	16.7	
None	1	0	0	
Nursery	2	0	0	0.659
Primary	52	5	41.7	
Secondary	33	3	25	
Tertiary	27	2	16.7	
Total	**180**	**12**	**6.7**	
Occupation				
Civil Servant	6	0	0	
Entrepreneur	93	8	66.7	
Farmer	10	2	16.7	
House wife	27	1	8.3	
Public Servant	24	1	8.3	0.659
Pupil	7	0	0	
Retiree	1	0	0	
Student	4	0	0	
Unemployed	8	0	0	
Total	**180**	**12**	**6.7**	
Duration (Years)				
0 – 2	74	3	33.3	
3 – 5	46	8	66.7	
6 – 8	27	0	0	
9 – 11	23	1	8.3	0.0310
12 – 14	7	0	0	
15 – 17	3	0	0	
Total	**180**	**12**	**6.7**	

Table 3b: Distribution of Hepatitis E virus in relation to risk factor among studied subjects

Risk factors	No screened	No positive foe HEV	Prevalence (%)	P value
ART regimen				
First line	162	10	58.3	0.7650
Second line	9	1	8.3	
Nil	9	1	8.3	
Total	**180**	**12**	**6.7**	
Source of water				
Borehole	110	8	66.7	
Pond	6	0	0	
River	1	0	0	
Tap	29	2	16.7	0.963
Well	34	2	16.7	
Total	**180**	**12**	**6.7**	
Stage of pregnancy				
First trimester	1	1	8.3	
Second trimester	4	1	8.3	
Non applicable	53	7	58.3	0.002508
Not pregnant	122	3	25	
Total	**180**	**12**	**6.7**	

Key: ART = Anti retroviral therapy

DISCUSSION

The findings of this study revealed that 6.7% of the studied subjects who are Immunosupressed with HIV had HEV infection. This is not surprising as earlier studies by Kaba *et al*. (2011) revealed that Hepatitis E infection is an emerging infection in Immunosupressed patients across the globe most especially in developing countries. Odaibo and Olaleye (2013) also reiterated that Hepatitis E virus (HEV) among other related viruses has been found to be responsible for acute and chronic hepatitis in immunocompromised patients such as HIV/AIDS.

Compared to the findings of the present study, Fainboim *et al*. (1999) also reported a similar prevalence of 6.7% in Argentina. This similarity may be due to similar cultural activities of the studied subjects. However, the results of this study were slightly higher than those reported by Kaba *et al*. (2011) in France. This could be as a result of higher standard of living, better medical and socioeconomic facilities and lower risk of exposure to infection by HEV and HIV because of high level of awareness and literacy in the area. Kolawole *et al*. (2015) also reported a higher prevalence of 5.45% from Ogbomosho (South Western Nigeria). Other studies revealed higher prevalence rate of HEV compared to the results of this study, such as those in Ghana (45.3%) and Cameroon (14.2%) (Torsten *et al.,* 2013). A prevalence of 12.2% was reported by Odaibo and Olaleye (2013) from Ibadan while Junaid *et al.* (2014) reported 31.1% from Plateau State. The variations in the reports of HEV prevalence in other studies compared to the present study could be explained based on the fact most studies of HEV were targeted on the antibodies which may indicate a past or recent infection, however, this study targeted the viral antigen for the presence of an active infection which may lead to the low prevalence of HEV infection in the targeted subjects.

It is also suggested that some health care settings are reservoirs for the transmission of infections especially where running water and materials for hand hygiene are lacking, thus studies conducted in such settings may present higher prevalence rates.

The prevalence of Antigen positivity of HEV in this study was found to be more prevalent among subjects aged 25-54 years while it was absent among those aged <25 years and above 54 years, as such HEV prevalence decreases with advancing age. This observation clearly indicates that HEV infection may be associated with exposure as subjects in this age bracket are likely more engaged with various life activities which may increase their risk of acquiring the infection. The reports of this study agree with those of Adesina *et al*. (2009) and WHO (2014). However, it contracts the report of Junaid *et al.* (2014) which indicates that HEV positivity increases with age.

This study recorded a higher prevalence of HEV positivity in males than females (P= 0.043); this is consistent with previous studies (Vivek *et al*., 2010; Goumba *et al*., 2011; Junaid *et al*., 2014) and contrary to a study by Kolawole *et al.* (2015). Higher prevalence in men could be attributed to the fact that men are more exposed to risk factors associated with acquisition of HEV such as farming, using contaminated river water, disposal of human and animal waste, involvement in environmentally related works.

Educational level of the subjects shows that subjects with the lowest level of education (primary) had the highest prevalence of HEV. This observation is not surprising, as low educational level is associated with low socioeconomic status which usually leads to lack of knowledge on how to avoid possible risk factors associated with HEV hence high risk of exposure to HEV. This agrees with the study carried out in Egypt (Amer *et al*., 1996), Spain (Buti *et al*., 2006) and Plateau State (Junaid *et al*., 2014).

Prevalence of HEV infection had been observed in this study to be associated with the duration of HIV infection. This might be due to the high replication of the virus, low immunity status of the subjects and the type of ART used by subjects which was not the highly active ART (HAART) as observed by the study.

The use of borehole as a source of water in this study shows that subjects using borehole water had higher risk of HEV infection which is contrary to the reports of other studies (Galiana *et al*., 2008; Eker *et al*., 2009; Junaid *et al*., 2014). It is likely that all sources of water could serve as a possible means of transmission, but borehole as a source of water was perhaps implicated due to low treatment of the water before consumption especially sub-standard borehole, lack of proper transportation from the source of water to the house hold and in some cases there is contact of borehole water with sewage.

Finally, the findings of this study revealed that the prevalence of HEV was significantly associated with pregnancy (P= 0.0025). This study shows that pregnant women in both the first and second trimester had a prevalence of 8.3%, and agrees with the findings of Sing *et al.* (2003), Beniwal *et al.* (2003) in Ludhiana, Kumar *et al.* (2004) in New Delhi, and Junaid *et al.* (2014) in Plateau State. This might be due to similarity in socioeconomic status, suppression of the immune system as well as cultural and hygiene practices. The WHO (2016) revealed that a unique feature of ~~with~~ HEV infection in pregnancy is the high mortality rate in infected pregnant women which can reach up to 25%. Other common complications of HEV infection in pregnancy include death of mother and fetus, abortion, premature delivery or still (Smith, 2001). The reported prevalence of HEV of 8.3% among pregnant women in this study likely underscores the importance of HEV in pregnancy in Nigeria which according to Hogan *et al.* (2010) has been is listed among the six countries that account for 50% of global estimate of maternal deaths.

CONCLUSION

The result of this study shows that 6.7% of people living with HIV in Kano State are co-infected with HEV. The prevalence of HEV infection among the subjects was shown to be insignificantly higher in males; those aged 25 to 54 years, those with primary education, those on first line ART and those that use borehole water as their water source. The study recognizes that HEV remains a public health problem in Nigeria and therefore identifies the need for early diagnosis and proper management of HEV infection in addition to provision of safe drinking water by the relevant authorities.

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