Research Article

Detection of Hepatitis E Virus among HIV Infected Individuals in Ogbomoso, South-western Nigeria

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Abstract | Hepatitis E virus is a foodborne and waterborne virus commonly transmitted faecal-orally and through the transplacenta. Hepatitis E virus is one of the viruses that causes acute and self-limiting infection with a high risk of developing chronic hepatitis in immunocompromised individuals usually leading to liver cirrhosis. Individuals living with Human Immunodeficiency Virus have a greater risk to the virus due to their impaired immunity. The study was carried out to determine the sero-prevalence of Hepatitis E among people living with Human Immunodeficiency Virus in Ogbomoso. The blood sample of consented two hundred and seventy five (275) subjects were obtained and their sera were screened for antibodies to HEV using Enzyme Linked Immunosorbent Assay. Samples were collected over a period of 4 months (September – December, 2014). The mean age and mean $CD4^+$ count of the subjects were 40.14±0.47 years and 387.28±18.91 cells/mm^3 respectively. Out of 275 subjects tested, 15(5.45%; 95% confidence interval) were positive for anti-HEV IgM. Anti-HEV IgM was highest among the age group 11-20 (9.09%), female (7.83%), civil servant (7.37%), > 500 CD4/ mm^3 (7.89%). The multivariate analysis between age and CD4 count shows highest prevalence in the age range 31-40 years with CD4+ count less than 200cells/µl. The seroprevalence shows there is a significant difference between age and $CD4^+$ cell count. The result provides the evidence that Hepatitis E virus is present among HIV individuals in Ogbomoso and they are more susceptible to the virus.

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Background

Hepatitis E virus is a small non-enveloped positive sense single stranded RNA icosahedral virus with a 7.7 kilobase genome that belongs to the Caliciviridae family with divergent clades- an anthropotropic and an enzootic form which subsequently evolved into genotypes 1&2 and 3&4 respectively (Purdy et al., 2010). Among indigenous people, a significant prevalence of HEV specific antibody has been reported in Asia, the Middle East, Africa and several Amerindian Yepka tribes in South America (Teshale et al., 2010a, 2010b).

HEV is excreted in faeces of persons infected with this virus, and is transmitted mainly through the faecal-oral route. Other transmission routes include food-borne transmission from ingestion of products

derived from infected patients, transfusion of infected blood products, and vertical transmission from pregnant women to foetus, zoonotic transmission from animals to humans from exposure to infectious body fluids of infected animals (Zhu et al., 2010; WHO, 2014). Although, humans are considered the natural host for the hepatitis E virus, antibodies to the hepatitis E virus or closely related viruses have been detected in primates and several other animal species (WHO, 2014).

Although HEV often causes acute and self-limiting infection (in that it usually goes away by itself and patient recovers) with low mortality rates in the western world, it bears a high risk of developing chronic hepatitis in immunocompromised individuals with substantial mortality rates (Zhu et al., 2010). HEV occasionally develops into an acute, severe liver disease and is fatal in about 2% of all cases (WHO, 2014).

Chronic hepatitis E virus has been observed in HIV individuals (Jacobs et al., 2014). It can cause rapid liver cirrhosis in HIV individuals with low CD4+ cell counts. Often there are no symptoms and the infection is usually cleared by the body's natural defences, however, immunosuppression is a risk factor for persistence of the infection (Neukam et al., 2014). HEV causes around 20 million infections which results in around three million acute illness and 57,000 death annually (Lozano et al., 2012).

As cases of hepatitis E are not clinically distinguishable from other types of acute viral hepatitis, diagnosis is made by laboratory evaluation of IgM and IgG. Acute hepatitis E is diagnosed when the presence of IgM anti-HEV is detected (Junaid et al., 2014).

The seroprevalence of HEV among HIV individuals is less documented in Ogbomoso and Nigeria, at large, justifying this research. There is therefore an urgent need for research of this nature to provide necessary information for pro-active strategy formulation especially in the study area.

Materials and Methods

Study centre

The study centre was located in Ogbomoso, Oyo State in the tropical belt of South-Western part of Nigeria. Its geographical coordinates are 80 8' 0" North, 40 16' 0" East.

Study population

The study subjects were consented HIV positive individuals living in Ogbomoso, Nigeria.

Enrolment of subjects

Consenting HIV positive individuals were enrolled from different HIV/AIDS support groups in Ogbomoso. Enrolment took place between September and December 2014. Thereafter, consenting subjects were enrolled for the study. Demographic and other relevant information were retrieved from the study subjects using a structured questionnaire. Afterwards, blood sample was collected from each of the 275 consenting subjects enrolled for the study. The study protocol was in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All study subjects gave written, informed consent.

Sample collection

Samples were collected in a tube without anticoagulant for serology and with coagulants for the CD4 count analysis. A tourniquet was firmly tied to the upper arm of the subjects while sitting and skin sterilizer with 70% alcohol. The sterile needle was inserted into conspicuous antecubital vein and the plunger of the sterile syringe was withdrawn and pressure applied to the puncture site with a cotton wool to stop bleeding. Blood sample was spun on a bench centrifuge at 3,000rpm for 10 minutes to obtain serum. Serum was separated immediately. Aliquots of serum were made per sample in labelled sterile cryovials which were stored at–20°C until ready for analysis.

CD4 count and serology

CD4 count was determined using standard laboratory procedure with the use of Counter 2 (Version 2.4, Partec Germany). All the 275 samples were subjected to hepatitis E virus specific Enzyme Linked Immunosorbent Assay (ELISA) using the hepatitis E virus IgM detection ELISA kit (WKEA Med Supplies Corp, Changchun, China). The assay was performed according to manufacturer's instructions. The optical density was read using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA) and the result was interpreted according to the manufacturer's instructions.

Statistical analysis

The data obtained from the study were subjected to descriptive statistical analysis. Variance analysis of

serological pattern of hepatitis E virus antibody was analyzed. All interval estimates are 95% confidence intervals. SPSS program for Windows (Version 21.0; SPSS, Inc., Chicago, IL) was used.

Results

Demographic characteristics of the study population

Table 1 shows the age distribution of the study subjects. The highest participant rate was found in the age range of 31-40 years (36.64%) while the lowest was found in 11-20 years (4.00%) and 61-70 years (4.00%) as shown in Table 1. The occupational distribution of the study subjects is shown in Table 1. The CD4+ counts of the study subjects is shown in Table 1, 89(32.36%) were less than 200cells/ µl, 58(21.09%) were between 200-350cells/µl, 38(13.82%) were between 350-500 cells/ µl and 90(32.73%) were greater than 500 cells/µl.

Table 1: *Demographic characteristics of the study population*

Age Range	Frequency	%
11-20	11	4.00
21-30	39	14.18
31-40	100	36.64
41-50	84	30.55
51-60	30	10.91
61-70	11	4.00
Total	275	100
Occupation		
Artisan	85	30.91
Student	8	2.91
Unemployed	17	6.18
Civil Servant	95	34.55
Trading	70	25.45
Total	275	100
Cd4+ Counts Cells/ µl		
<200	89	32.36
200-350	58	21.09
350-500	38	13.82
>500	90	32.73
Total	275	100

Prevalance of HEV IgM among study subjects with age distribution

Figure 1 shows prevalence of HEV IgM in the study subjects. Out of 275 subjects whose serum were tested, the highest prevalence of HEV IgM (4%) was found in persons whose ages were between 11-20 years, and that of others age groups is shown in Figure 1.

Prevalence of HEV IgM of occupational groups among the study subjects

A total of 275 study subjects with different occupational groups were tested for antibody. The prevalence of HEV IgM in traders, artisans, civil servants, students, unemployed and unspecified is shown in Figure 2. Civil servant had the highest seropositive of (7.37%) while student had the least which is (0%).

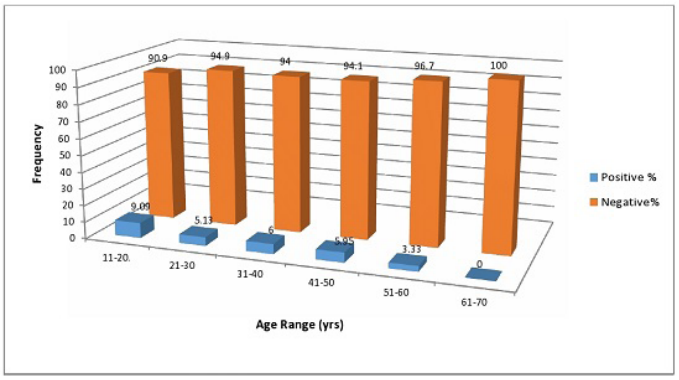

Figure 1: *Age distribution of HEV IgM among the study subjects*

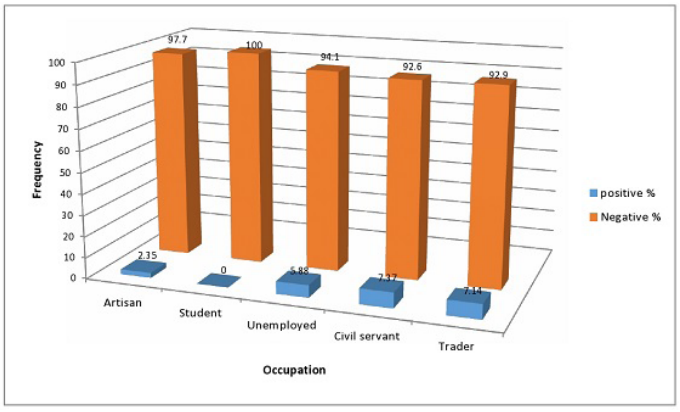

Figure 2: *Occupational distribution of HEV IgM among the study subjects*

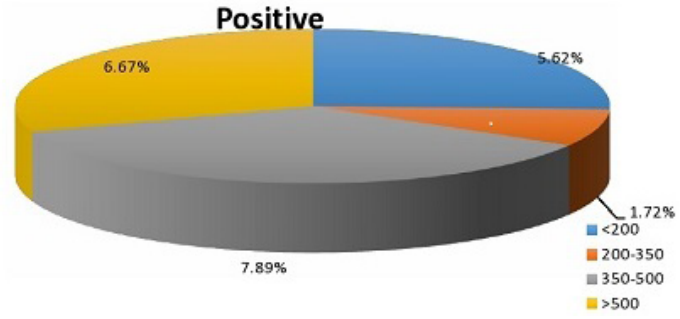

Figure 3: *Prevalence of HEV IgM in relation to CD4+ count among the study subjects*

Prevalance of HEV IgM among study subjects with CD4+ count

The prevalence of HEV among the study subjects with respect to their CD4+ counts is shown in Figure 3. Subjects with CD4+ count between 350-500cells/

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µl had the highest seropositivity of 7.89% while those with CD4+ count between 200-350cells/µl had the least seropositivity of 1.72%.

Analysis using logistic regression of seroprevalence of HEV IgM using age and CD4 count of the subjects

As shown in Table 2, a significant difference was observed in the mean seropositive group in the analysis of logistic regression for age and CD4+ among subjects with a mean of 40.14 for age, 387.28 mean of CD4+cells/µl.

Table 2: *Seroprevalence logistic regression of HEV comparing age and CD4+ count*

Group	No of subjects	Mean
Age	275	40.14 ± 0.47
CD4+ Count	275	387.28±18.91

Values = means± standard error

Multivariate seroprevalence analysis of HEV IgM using age and CD4+ count

Figure 4 shows the multivariate analysis between age and CD4 count. The highest prevalence is seen in the age range 31-40 years with CD4+ count less than 200cells/µl. However, there is significant difference between the mean age and CD4.

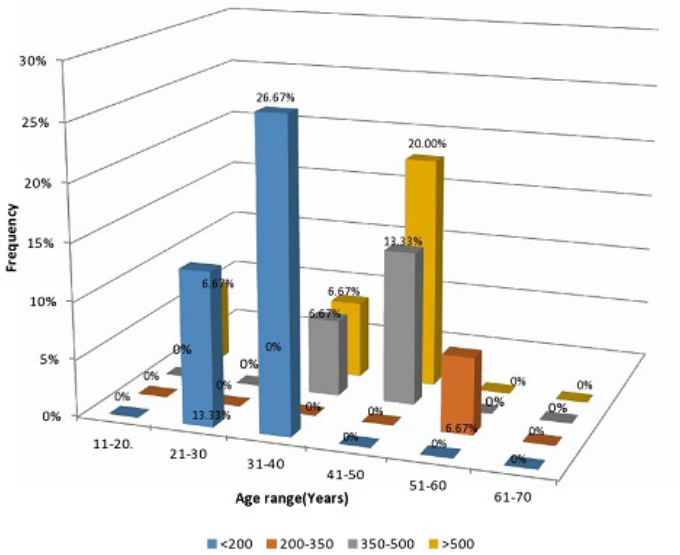

Figure 4: *Prevalence multivariate analysis of HEV IgM in respect to age and CD4+ count*

Discussion

Hepatitis E virus infection has been described as an emerging infection among immunosuppressed patients across the globe, most especially resource-limited countries (Kaba et al., 2011). Hepatitis E have been shown to be a causative agent of acute and chronic hepatitis in severely immunocompromised patients such as organ transplant recipients and person with HIV infection (Odaibo and Olaleye, 2013).

In this study a low rate (5.5%) of HEV among HIV positive individuals was found. Although this rate is low as compared to the previously reported among similar populations in industrialized countries (Kaba et al., 2011; Crum-Cianflore et al., 2012; Kenfak-Foguena et al., 2011; Clemente-Casares et al., 2003), it is also higher than that reported among some population groups HEV endemic areas of Africa (Adjei et al., 2010), and Asia (Khuroo et al., 1994; Labrique et al., 2010). It is much lower than the rates of HEV infection recorded in Nigeria (44%) (Kaba et al., 2011), (12.5%) (Odaibo and Olaleye, 2013), Ghana (45.3%), Cameroon (14.2%) (Torsten et al., 2013) and Plateau (31.1%) (Junaid et al., 2014), among the same subjects. However, the rate is higher than the result obtained from the study done in Switzerland (2.6%) (Kovari et al., 2010).

The antibody positivity rates appeared to decrease with age, and increase was marked among the age group 11-20 years with the highest prevalence. The lowest prevalence was among subjects of 61-70 years. This agreed with results from a similar study by WHO (2014), where the prevalence of anti-HEV IgM was highest in ages 15-40 years. This finding from the current study explains that the use of antiretroviral drugs does not completely eradicate the virus even though the level of CD4 count is boosted.

Furthermore, the current study significantly associated civil servants (7.37%) with HEV IgM prevalence as compared to the traders (7.14%). This may be due to the fact that the civil servants are more engaged in the behaviour such as eating of uncooked food at work, hand washing etc can put them at more risk to the infection which is tantamount to poor awareness and knowledge about Hepatitis E virus infection.

The current study recorded higher prevalence among females than males. This is consistent with what had been recorded by Odaibo and Olaleye (2013) and Kovari et al. (2010). A logical reason could be that in the current study area, generally, most women are more exposed to HEV infection risk factors such as behavioural or cultural practices. However, in contrast, Junaid et al. (2014) in Plateau reported a higher

prevalence among males than females. The reason for this disparity may be linked to various interplays of differences in cultural practice among other factors.

The prevalence of anti-HEV IgM by CD4 count among the study subject range from <200cells/mm^3, 200-350cells/mm^3, 350-500cells/mm^3 and >500cells/mm^3, with the highest prevalence marked within 350-500cells/ mm^3. The least was within 200-350cells/mm^3. The mean CD4 count from this study is higher than that of Odaibo and Olaleye (2013), who reported a mean count of 351cells/ µl from a study conducted.

The multivariate analysis comparing age with CD4+ count showed a high prevalence in the age group 31-40 years with CD4+ count <200cells/mm^3 which is consistent with what was recorded by Banas et al. (2013). This result suggests that patients whose CD4 count range between 100-350 cells/ mm^3 are at risk of HEV infection. The statistical analysis comparing the age with CD4 count shows a significant relationship between the mean age and CD4 count of HIV individuals that are positive to anti-HEV IgM since the mean age and CD4 count are significantly different (p-value ≤ α level 0.05).

Findings from this study suggests that socioeconomic status and education is generally the biggest factor behind higher rates of HEV infection irrespective of age, sex, location, occupation, and other factors.

Proper management and screening for hepatitis E virus and HIV should be reinforced in order to identify those at risk of transmission to whom preventive measure can be instituted, through the most effective approach. Government's intervention is solicited for provision of molecular laboratory for proper diagnosis of HEV and other infection in order to enhance safety for immunocompromised patient. PCR testing for HEV RNA should be conducted for HIV-infected persons (especially those with low CD4 cell counts). HEV remains an under-recognized and significant public health problem in Ogbomoso, and this calls for further investigation.

Competing Interests

The authors have no competing interests to declare

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References

- Adjei AA, Tettey Y, Aviyase JT, et al. (2010). Unexpected elevated alanine aminotransferase, aspartate aminotransferase levels and hepatitis E virus infection among persons who work with pigs in Accra, Ghana. *Virology Journal*. 7(7):336. http://www.virologyj.com/content/7/1/336
- Banas B, Tausch U, Hofstädter F, et al. (2006). Infection with hepatitis E virus: first report of a chronic case and molecular characterization of the virus. *J Clin Virol*. 36: S162. http://dx.doi.org/10.1016/S1386-6532(06)80503-2
- Clemente-Casares P, Pina S, Buti M, et al. (2003). Hepatitis E virus epidemiology in industrialized countries. *Emerging Infectious Diseases*. 9(4):448-454.
- Crum-Cianflone NF, Curry J, Drobeniuc A, et al. (2012). Hepatitis E virus infection in HIV-infected persons. *Emerging Infectious Diseases*. 18(3):502-506.
- Jacobs C, Chiluba C, Phiri C, et al. (2014). Seroepidemiology of hepatitis E virus infection in an urban population in Zambia: strong association with HIV and environmental enteropathy. *J. Infect. Dis*. 209(5):652-7.
- Junaid SA, Agina SE, Abubakar KA (2014). Epidemiology and associated risk factors of hepatitis E virus infection in Plateau State, Nigeria. *Virology: Research and Treatment*. 5:15-26. http://dx.doi.org/10.4137/VRT.S15422
- Kaba M, Richet H, Ravaux I, et al. (2011). Hepatitis E virus infection in patients infected with human immunodeficiency virus. *Journal of Medical Virology*. 83(10):1704-1716. http://dx.doi.org/10.1002/jmv.22177
- Kenfak-Foguena A, Schoni-Affolter F, Burgisser P, et al. (2011). Hepatitis E virus seroprevalence and chronic infections in patients with HIV, Switzerland. *Emerging Infectious Diseases*. 17(6):10744-1078. http://dx.doi.org/10.3201/eid1706.101067
- Khuroo MS, Rustgi VK, Dawson GJ, et al. (1994). Spectrum of hepatitis E virus infection in India. *Journal of medicine Virology*. 43(3):281-286. http://dx.doi.org/10.1002/jmv.1890430316

- Kovari H, Ledergerber B, Battegay M, et al. (2010). Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis B or C virus co-infection. *Clin Infect Dis*. 50:502–11. http://dx.doi.org/10.1086/649922
- Labrique AB, Zaman K, Hossain Z, et al. (2010). Epidemiology and risk factors of incident hepatitis E virus infections in rural Bangladesh. *American Journal Epidemiology*. 172(8):952-961. http://dx.doi.org/10.1093/aje/kwq225
- Lozano R, Naghavi M, Foreman KL, et al. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380 (9859): 2095– 128.
- Neukam K, Barreiro P, Macias J, et al. (2013). Chronic hepatitis E in HIV patients: rapid progression to cirrhosis and response to oral ribavirin. *Clin. Infect. Dis*. 57:465–468. http://dx.doi.org/10.1093/cid/cit224
- Odaibo G.N, Olaleye DO (2013). Hepatitis E virus infection in HIV positive ART naïve and experienced individuals in Nigeria. *World Journal of AIDS*. 3:216-220.
- Purdy MA, Khudyakov YE, Khudyakov (2010). Evolutionary history and population dynamics of hepatitis E virus. *PLoS ONE 5 (12): e14376*. http://dx.doi.org/10.1371/journal.pone
- Teshale EH, Grytdal SP, Howard C, et al. (2010a). Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clin Infect Dis*. 50(7):1006-10.
- Teshale EH, Howard CM, Grytdal SP, et al. (2010b). Hepatitis E epidemic, Uganda. *Emerg Infect Dis*. 16(1):126-9.
- Torsten F, Sarfo FS, et al. (2013). Hepatitis E virus infections in HIV-infected patients in Ghana and Cameroon. *Journal of Clinical Virology*. 58(1):18-23.
- WHO (2014). The global prevalence of hepatitis E virus and susceptibility: a systematic review. WHO/IVB/10.14. www.who.int/vaccines-documents/
- Zhu FC, Zhang J, Zhang XF, et al. (2010). Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet*. 376(9744):895-902.