

PREVALENCE OF HEPATITIS E VIRUS INFECTION AMONG BLOOD DONORS IN A TERTIARY HEALTHCARE FACILITY IN OSUN STATE, NIGERIA

ABSTRACT

Hepatitis E virus (HEV) is currently the most common cause of viral hepatitis in developing countries. While prospective blood donors are routinely screened for HIV, Hepatitis B and C globally in endemic areas, HEV is not included in the protocol, despite the increase in global prevalence of HEV. Till date, only a handful of studies exist on the prevalence of HEV in Nigeria. The aim of this study was to fill this knowledge gap by determining the prevalence of HEV among blood donors at the LAUTECH Teaching Hospital, Osogbo, Osun State, Nigeria. A total of 102 consenting individuals were enrolled into the study between ages 20 and 55. A volume of 5ml blood sample was collected, and the serum harvested, stored at 20°C until ready for analysis. A double antibody sandwich HEV ELISA was performed to analyze the sera in duplicate for HEV antigen, using an ELISA Kit .Of the 102 samples analyzed, 15 (14.7%) tested positive for HEV antigen, confirming the presence of HEV infection among blood donors in the study population.

There was significant association between HEV seropositivity and contact with animals (P=0.010) and alcohol consumption (P=0.000), which means there was a higher chance of donors who consumed alcohol or have had contact with animals to get infected with HEV. However, age, educational status, occupation and gender did not show any statistically significant association with HEV infection. The prevalence of HEV seropositivity was high (14.7%) and a cause for concern as HEV continues to be a major public health issue especially in developing countries.

Keywords: Hepatitis E virus, Prevalence, Blood donors, ELISA, LAUTECH

INTRODUCTION

“Hepatitis E virus (HEV) is a global public health concern, affecting approximately 20 million people, with 3.3 million estimated to have symptomatic infections” [1]. “The WHO estimated that the Hepatitis E virus was responsible for approximately 44,000 deaths in 2015, accounting for 3.3% of the mortality due to viral hepatitis” [1]. “Hepatitis E viral outbreaks have been reported in Asia, the Middle East, Africa and Central America” [2]. “In regions where HEV infection is endemic, it is typically acquired through the faecal-oral route, principally via contaminated water”[3]. “Hepatitis E is diagnosed by detecting viral RNA in the serum or faeces of an infected individual using RT-PCR during the incubation period or the early acute phase of the disease”[4]. “More commonly, hepatitis E is diagnosed by demonstrating the presence of IgM anti-HEV in serum samples or a rising titer of IgG anti-HEV in the serum during the late acute phase or convalescent phase of the illness”[5]. However, even though this serological test is considered specific enough, the sensitivity of these markers has not been determined. Thus, the proportion of hepatitis E cases that may have been missed during diagnosis is uncertain.

“The burden of hepatitis E infection in Nigeria is unclear. This is most likely due to poor or insufficient diagnostic methods available for hepatitis E in Nigeria. However, there have been reports of sporadic cases of hepatitis E infection in Nigeria, with prevalence ranging between 2.7-66.7%” [6-11]. Furthermore, only a handful of studies examines the association of risk factors such as alcohol consumption, blood transfusion etc., with the prevalence of HEV in the country.

“Despite the significance of HEV in public health, especially in resource limited regions of the world, data reporting remained suboptimal, consequent of inadequate surveillance system”[12]. Research done globally on hepatitis E virus (HEV) infection is far fewer

compared with other types of hepatitis virus infections such as HAV, HBV, HCV etc. In Nigeria, there is little information on the prevalence and circulation of HEV infection in the population, particularly among blood donors. The aim of this study was to fill this knowledge gap by determining the prevalence of HEV among blood donors in South west Nigeria.

METHODOLOGY

Study area

The study was conducted between January 2020 to March 2020 at Ladoko Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo, Osun State, Nigeria. LAUTECH is a tertiary healthcare facility that provides health services to the indigenes of Osun State and that of neighbouring States in the south-western geopolitical zone of Nigeria.

Subject recruitment procedure

Consenting blood donors were identified, and well-structured questionnaires based on direct and indirect questions to obtain demographic characteristics such as age, sex, marital status, occupation, and education level were administered to those who gave consent to participate. Behavioural characteristics and possible associated risk factors such as the previous history of hepatitis, source of drinking water, and interaction with animals were also recorded. The questionnaires also included other factors such as smoking habits, consumption of alcohol, surgery, and so on. Consenting individuals filled the administered questionnaire before sample collection.

Inclusion criteria

The factors considered for the prospective blood donors to be included in the study before collecting samples were:

1. Willing blood donors,

2. irrespective of sex and who,
3. apparently healthy/fit (with negative HIV, HBV and HCV status), and
4. donors with a moderate or high level of PCV and must not be on hard drugs.

Exclusion criteria

The factors which disqualified some subjects from being considered for participation in the study were:

1. refusal to participate,
2. people with low PCV,
3. individuals with HIV, HBV and HCV positive status, and
4. individuals with a history of drug immunosuppressive therapy or critical illness etc.

Pre-sampling protocol

“Purposive sampling was employed following gradual selection”[13]. “Information was theoretically selected and categorized with relevant criteria such as age, gender, ethnicity, locality, HIV status, history of substance of abuse, history of blood transfusion, source of drinking water, alcohol consumption, contact with animals and sexual activity status. This was to allow maximum variation”[14].

Sample size and sample collection

One hundred nineteen participants were recruited in the study. However, only 102 blood samples were analyzed. A volume of 5ml blood samples was collected into EDTA anticoagulated bottles at the blood donation department in the hospital. Each blood sample was allowed to clot, and the plasma subsequently harvested into a sterile plain container. The blood samples were separated by centrifugation at 3000 rpm for 10 minutes to obtain serum.

The serum samples were then transferred into 2 ml cryovial and stored at -20°C until ready for analysis.

Double antibody sandwich Enzyme Linked Immunosorbent Assay (ELISA)

All of the sera were analyzed in duplicate for HEV antigen (HEV Ag) using an ELISA kit manufactured by Melsin Medical Co., Limited in China with 97% sensitivity and 97% specificity. The assay employs a double-antibody sandwich technique to analyze the presence of HEV Ag in human serum. The double antibody sandwich ELISA was carried out according to the manufacturer's instructions contained in the manual. Briefly, ten microliters of the plasma sample were dispensed into microwell, into which 40µl of sample diluent was added. After that, 100µl of HRP-conjugate reagent was dispensed into each well, and the plate was incubated at 37°C for 60 minutes. The plate was then washed with 400µl of wash solution to remove unattached conjugate. Wash was repeated five times using an automated plate washer. Fifty microliters of Chromogen solution A and B, respectively, were added to each well, followed by incubation at 37°C for 15 minutes. The reaction was stopped by adding 50µl stop solution, and a color change from blue to yellow was observed. The Optical Density (O.D.) was read at 450nm using a microtiter plate reader within 15 minutes.

Statistical analysis and interpretation

The prevalence of HEV Ag was determined from the proportion of the positive individuals in the overall population studied and expressed as percentages. The data entry was done in duplicates using Microsoft Excel 2019 (Microsoft office Inc. for Windows, USA). Statistical analysis was carried out on SPSS software version 25. The Chi-square (χ^2) test was used to determine the relationship between socio-demographic variables and HEV status. Descriptive

statistics significance was set at $p \leq 0.05$. Regression analysis was performed to determine and confirm the association strength between potential risk factors and HEV status.

RESULTS

This study observed an overall seroprevalence of 14.7%, as 15 participants were positive for HEV Ag. The distribution of the positive samples according to demography is presented in Table 1.

Prevalence of HEV status in comparison to socio-demographic characteristics

The distribution of positive samples according to age, sex, educational status, occupation, source of drinking water and direct contact with animals of the study participants are shown in Table 1. Age-specific prevalence of HEV Ag was observed between ages 20 and 50. Out of the 15 positive donors, 6.67% (1/15) were of age 20-25 years, 20.00% (3/15) in the age range 26-30, 20.00% (3/15) in the age group 36-40, 20.00% (3/15) in the age range 41-45 years, 26.67% (4/15) in the age group 31-35 years, and 6.67% (1/15) among donors over 45 years. There was, however, no significant association ($p > 0.05$) between seropositivity and age distribution (Table 2).

The seroprevalence according to sex showed positivity of HEV Ag of 73.33% (11/15) among the male blood donors and 26.67% (4/15) in the female participants (Table 1). There was no significant association ($p > 0.05$) between HEV positivity and the sex of blood donors (Table 2).

Considering the source of drinking water, the highest prevalence of HEV Ag; 86.67% (13/15) was observed in participants who drank from taps/well water and 13.33% (2/15) among those with bottled/sachet water as their source of drinking water. In comparison, those who drank from other sources than those listed had a prevalence of 0.00% (0/15). There was no

significant association ($p>0.05$) between HEV positivity and the source of drinking water (Table 2).

Occupation-wise, 20.0% (3/15) was observed in participants who were traders, 33.33% (5/15) in participants who were artisans, 33.33% (5/15) among civil servants, 0.00% (0/15) in both healthcare workers, and unemployed donors, and 13.33% (2/15) in participants who had occupations other than those listed (Table 1). There was no significant association ($p>0.05$) between HEV positivity and participants' occupation (Table 2).

Concerning educational status, an HEV Ag prevalence of 46.67% (7/15) was recorded in participants who were graduates of tertiary educational institutions, 13.33% (2/15) was recorded among undergraduates, 33.33% (5/15) in participants who had only secondary school education, and 6.67% (1/15) in individuals who had other forms of education other than those listed (Table 1).

The prevalence of contact with animals showed positivity of HEV Ag of 46.67% (7/15) among the donors that had direct contact and 53.33% (8/15) among those who did not. A significant association ($p<0.05$) between HEV positivity and contact with animals was recorded (Table 2).

Association of possible risk factors with HEV status

The distribution of positive samples according to age, sex, marital status, educational status, occupation, alcohol, source of drinking water and contact with animals of the study participants are shown in Table 1. Statistical analysis showed no significant association ($P>0.05$) between HEV positivity and marital status, although most of the blood donors were married. Similarly, most participants (70.59%) were males, but there was no statistical association between sex and HEV positivity. The study revealed that HEV Ag was present in 31.8% of the blood donors who had contact with animals which also showed a strong

statistical correlation ($\chi^2=6.548$, $df=1$, $P=0.010$) with the infection (Table 2). Furthermore, alcohol consumption also showed a statistical association with HEV positivity ($\chi^2=13.722$, $df=1$, $P=0.000$), which means there is a higher chance of donors who consume alcohol getting infected. At the same time, there is a 4-fold risk of getting infected with HEV among those who had contact with animals (95% C.I. = 1.321-13.356, OR = 4.2) (Table 3). Age, however, did not show any statistical association ($\chi^2=3.167$, $df=6$, $P=0.788$) (Table 3). In multivariate logistic regression analysis, the possible determinant factors that showed statistical association with HEV positivity in the individuals enrolled in the study were occupation, alcohol, source of water and contact with animals ($P < 0.05$). Linear regression analysis showed a statistically significant association between the parameters evaluated and the presence of HEV Ag in the study population ($P < 0.05$) with possible risk factors such as alcohol, occupation, contact with animals, source of water and educational status. In all these statistical analyses, alcohol consumption and contact with animals strongly correlated with HEV positivity.

Table 1: Socio-demographic characteristics of the study participants including the distribution of HEV positive cases.

Parameter	Level	Frequency	Percentage (%)	Distribution & Percentage of positive samples (n=15)
Age	20-25	14	13.7	1 (6.67%)
	26-30	24	23.6	3 (20.00%)
	31-35	23	22.5	4 (26.67%)
	36-40	18	17.6	3 (20.00%)
	41-45	11	10.8	3 (20.00%)
	46-50	7	6.9	1 (6.67%)
	51-55	5	4.9	-
Sex	Male	72	70.59	11 (73.33%)
	Female	30	29.41	4 (26.67%)
Marital status	Single	36	35.29	4 (26.67%)
	Married	66	64.71	11 (73.33%)
	Divorce	0	0	-
Educational status	No formal	0	0	-

	Secondary	40	39.21	5 (33.33%)
	Undergraduate	21	20.60	2 (13.33%)
	Graduate	37	36.27	7 (46.67%)
	Others	4	3.92	1 (6.67%)
Alcohol	Yes	6	5.89	4 (26.67%)
	No	96	94.11	11 (73.33%)
Source of drinking water	Tap/well water	78	76.47	13 (86.67%)
	Bottled/sachet water	13	12.75	2 (13.33%)
	Others	11	10.78	-
Direct contact with animals	Yes	22	21.57	7 (46.67%)
	No	80	78.43	8 (53.33%)
Occupation	Trader	8	7.84	3 (20.00%)
	Artisans	39	38.24	5 (33.33%)
	Civil servant	22	21.57	5 (33.33%)
	Healthcare worker	7	6.86	-
	Unemployed	6	5.88	-
	Others	20	19.61	2 (13.33%)

Table 2: HEV seroprevalence infection in comparison to socio-demographic, clinical and environmental factors.

Parameter	Participants' variables	Positive (%)	Negative (%)	Df	Chi-square	P-value
Age	20-25	1(7.1%)	13(92.9%)	6	3.167	0.788
	26-30	3(12.5%)	21(87.5%)			
	31-35	4(17.4%)	20(82.6%)			
	36-40	3(16.7%)	15(83.3%)			
	41-45	3(27.3%)	8(72.7%)			
	46-50	1(14.3%)	6(85.7%)			
	51-55	0(0.0%)	5(100%)			
Sex	Male	11(15.3%)	61(84.7%)	1	0.064	0.801
	Female	4(13.3%)	26(86.7%)			
Alcohol	Yes	4(11.1%)	32(88.9%)	1	13.722	0.000
	No	11(16.7%)	55(83.3%)			
Educational	No formal	0(0.0%)	0(0.0%)			

status	Secondary	5(12.5%)	35(87.5%)	4	2.623	0.453
	Undergraduate	2(9.5%)	19(90.5%)			
	Graduate	7(18.9%)	30(81.1%)			
	Others	1(25.0%)	3(75.0%)			
Occupation	Trader	3(37.5%)	5(62.5%)	5	7.020	0.219
	Artisans	5(12.8%)	34(87.2%)			
	Civil servant	5(22.7%)	17(77.3%)			
	Healthcare worker	0(0.0%)	7(100.0%)			
	Unemployed	0(0.0%)	6(0.0%)			
Source of drinking water	Others	2(10%)	18(90%)	2	2.140	0.343
	Tap/well-water	13(16.7%)	65(83.3%)			
	Bottled/sachet water	2(15.4%)	11(84.6%)			
Contact with animals	Others	0(0.0%)	11(100.0%)	1	6.548	0.010
	Yes	7(31.8%)	15(68.2%)			
	No	8(10.0%)	72(90.0%)			

Key:df: Degrees of freedom; P-value: Probability of obtaining results as extreme; where $P < 0.05$ is considered significant

Table 3: Possible risk factors correlation and HEV

Variable	Participants' variables	Positive	Negative	OR	95% C.I.	P-value
Alcohol	Yes	4(11.1%)	32(88.9%)	15.45	2.530-94.419	0.000
	No	11(16.7%)	55(83.3%)			
Contact with animals	Yes	7(31.8%)	15(68.2%)	4.2	1.321-13.356	0.010
	No	8(10.0%)	72(90.0%)			

Key:OR: Odds ratio; C.I.: Confidence interval

DISCUSSION

Hepatitis E is a liver disease caused by the hepatitis E virus (HEV) which is one of the five known human viral hepatitis (A, B, C, D and E). HEV has a global distribution but is more common in developing countries and areas with poor or compromised sanitary and hygienic conditions particularly in parts of Africa and Asia. According to WHO, about 20 million HEV infections occur worldwide yearly and in 2015, about 44,000 deaths were recorded [1]. HEV infection with varying prevalences have been documented in different parts of Nigeria [8-10,15-17] but only one study has shown the prevalence among blood donors [11].

“Many studies have shown that blood products such as red blood cells, platelets and fresh-frozen plasma can transmit HEV. In Nigeria, there have been a few studies on the prevalence of HEV in the general population but there is little data on the potential risk of HEV transmission in contaminated blood from HEV infected asymptomatic blood donors. This study therefore aimed to generate basic information on the prevalence of HEV infection among blood donors in a tertiary health facility in Osogbo, Osun State, Nigeria. The study confirmed the presence of HEV infection among blood donors with a seroprevalence of 14.7%. This is similar to the prevalence of 13.4% reported in Ekiti State, Nigeria” [18]. However, the prevalence recorded in this study is higher than that (6.6%) reported in Lagos State, Nigeria [11]. A possible reason for the disparity in prevalence may be as a result of variations in the sanitary conditions in these two States. For instance, the **blood donors in Lagos State had access to better sources of drinking water and sanitary conditions** [11] in comparison to those considered in this study, as 76.5% of the participants had well water as their source of drinking water. It is a well-known fact that hepatitis E virus is transmitted via contaminated water [3], therefore having a large proportion of participants obtaining their drinking water from unhygienic sources is a major risk factor.

In comparison to other African countries, the HEV prevalence observed in this study is higher than the prevalence of 5.4% (Tunisia) and 4.6% (Ghana) reported among blood donors, respectively [19,20]. However, the HEV seroprevalence in this study is lower than the seroprevalence of 42% reported in Zambia among apparently healthy individuals [21]. Variations in availability of safe drinking water, basic sanitation practices and other risk factors, between these countries may account for the observed difference in seroprevalences.

In accordance with previous studies [8,22,23], this study showed that males accounted for a higher rate of HEV antigen seropositivity than females. However, this is not always the case as there have been reports of higher rates of HEV antigen seropositivity in women than in men [8, 24,25]. A possible reason for why we have more HEV seropositivity in males than females could be due to the fact that majority of blood donors in Nigeria are men [26]. The reason for this gender bias still remains unclear. This study, however, did not show any statistical association between gender and HEV seropositivity.

“In developing countries, the age-specific seroprevalence profiles reveal that HEV infection is usually limited to adult population between ages 15 and 35 years” [26]. The present study shows a distribution of prevalence of HEV between the ages of 20-50, with the highest prevalence occurring between ages 31-35 years. This finding is somewhat consistent with [18] who observed the prevalence of anti-HEV antibodies to be highest in ages 20–40 years. However, just as in gender, there was no significant statistical association between age and HEV seropositivity.

“Regarding marital status, a higher prevalence (16.7%) was recorded among married donors compared to single donors (11.1%). This agrees with previous reports” [8,27]. “This is possibly attributed to the increased sexual activity and person-to-person contact among the married participants as compared to singles in the study” [8]. There was, however, no statistically significant association between marital status and HEV seropositivity.

The major route of HEV transmission is the fecal-oral route[28]. In this study, 76.47% of the participants obtained their drinking water from unhygienic sources. Therefore, it was unsurprising that we observed a statistically significant association between HEV seropositivity and unhygienic water sources ($p < 0.05$). This result is consistent with another report that established contaminated water as the main source of HEV infection[29].

There has been established link between alcohol consumption and clinical manifestation of HEV infection [8,30]. Thus, it was not surprising that in our study, there was a statistically significant association between alcohol consumption and HEV seropositivity ($P < 0.05$). Similar association has been reported [31]. However, it is important to state that the exact role alcohol plays in the manifestation of HEV infection remains unclear.

Furthermore, the educational status of blood donors considered in this study seemed not to play a role in HEV seropositivity. This is in contrast with previous studies[19,32], where participants with low level of education had increased risk for HEV positivity. Also, considering contact with animals, this study showed a statistically significant association ($P < 0.05$) with HEV seropositivity. This is in accordance with other findings that established contact with animals as a means of HEV transmission[8,17].

CONCLUSION

In summary, a high HEV seroprevalence of 14.7% was observed in this study, which is statistically significant among blood donors. This demonstrates the risk of HEV transmission by blood transfusion and merits further nationwide investigation. Furthermore, it drives home the need for awareness among healthcare workers, necessitating the screening of potential blood donors for HEV.

Ethical Approval and Consent

Ethical approval to carry out this study was obtained from the Research Ethics Committee of Ladoko Akintola University of Technology (LAUTECH) teaching hospital, Osogbo, Osun State, Nigeria.

Informed written consent was obtained from individuals enrolled in the study. Consenting individuals filled the administered questionnaire before sample collection.

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