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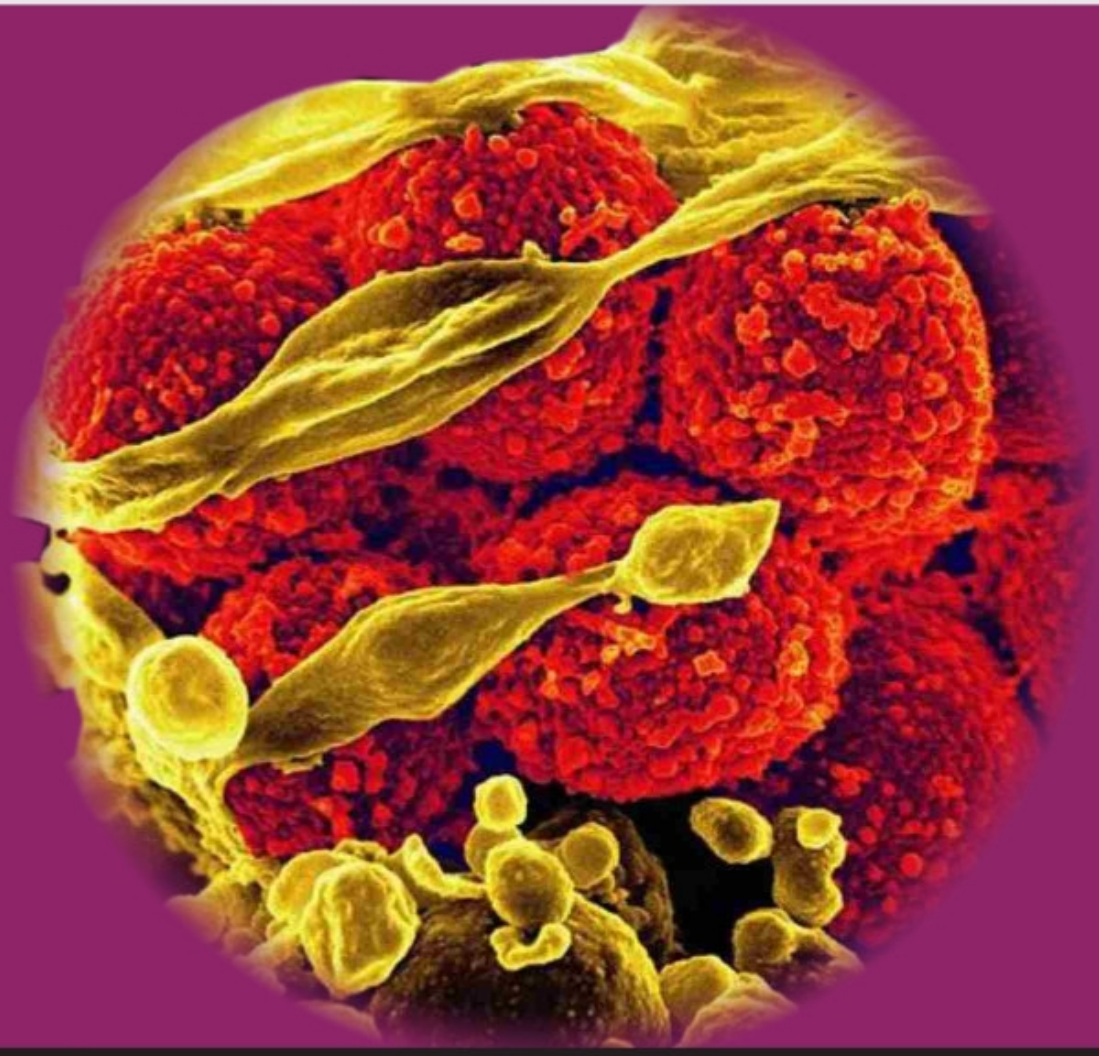

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ORIGINAL RESEARCH ARTICLE

Prevalence of Hepatitis E Virus Infection among Blood Donors seen at the Lagos State University Teaching Hospital, Nigeria

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ABSTRACT

Background:
Hepatitis E virus (HEV), a single stranded RNA virus belonging to the Hepeviridae family usually causes a mild self-limiting infection. Most patients infected with HEV spontaneously clear the virus, whilst in a few cases, chronic hepatitis E infection may develop. Transmission is usually via the faeco-oral route, though emerging evidence indicate that transmission via transfusion of blood and blood products is possible. Presently in Nigeria, the screening of blood for transfusion transmittable infections does not include HEV. Very few studies are available on the prevalence of HEV infection among the Nigerian population and most studies have been conducted amongst specific populations such as, pregnant women.

Aim:
To determine seroprevalence rates of anti HEV IgG and IgM amongst apparently healthy blood donors attending the donor clinic of the Lagos State University Teaching Hospital

Materials and Methods:
Sera from 104 blood donors were screened for anti-HEV IgM and IgG using ELISA. Data were expressed as mean +_standard deviation and significant level set at P_<0.05.

Results:
The overall prevalence of anti HEV antibodies was 4.8% with a prevalence rate of 1.9% for anti HEV IgM and 2.9% for anti HEV IgG. All the seropositive participants were male and a higher prevalence was found in participants between 20-40 years. No significant association was seen (P > 0.05) between seropositivity and known risk factors associated with the transmission of HEV.

Conclusion:
Although the prevalence rates of anti-HEV IgG and IgM antibodies are low, there is a risk of HEV infection transmission via blood and blood products.

Keywords: Hepatitis E virus, IgG, IgM, seroprevalence, blood donors.

INTRODUCTION

Hepatitis E virus (HEV) causes acute hepatitis in humans and is usually enterically transmitted. In developing countries, it is a major public health issue and causes large waterborne outbreaks.[1-3]

Infection with HEV may be associated with high mortality rates in certain populations such as pregnant women and the immunosuppressed where infection has been known to result in fulminant hepatitis and associated increase in maternal and foetal morbidity and mortality.[4]

IgM and IgG anti HEV antibodies develop at about the time of onset of symptoms, but usually before the appearance of jaundice; IgM anti HEV antibodies precede IgG anti HEV antibodies by a few days.[4]

The presence of IgM anti-HEV antibodies signifies an acute response to a recent infection and antibody titres decline during convalescence. On the other hand, the presence of IgG anti-HEV antibodies in the serum provides protection against new infections and usually persist for up to 14 years.[5] It is usually impossible to clinically distinguish infection with HEV from other types of acute hepatitis; diagnosis is made by the detection of IgM and IgG anti-HEV antibodies in patients' serum. Acute HEV infection is usually diagnosed with the detection of IgM antibodies to HEV. However, both false positive and negative are common with the assays available. In this regard, additional serologic testing or HEV RNA testing with the use of polymerase chain reaction (PCR) may be required to confirm the diagnosis.

Although historical evidence has suggested that HEV is enterically transmitted with transmission mechanisms similar to those of Hepatitis A virus, the differences in the prevalence of infection of the two viruses and their differential distributions in specific population groups have led to search for additional risk factors associated with HEV infection.[6] HEV has the potential to be transmitted by transfusion of contaminated blood and blood products since it has an asymptomatic blood borne phase and may survive in blood and blood components during processing and storage. Several studies have documented that transfusion with any blood products i.e. red cells, platelets, fresh frozen plasma may transmit HEV and the presence of HEV-RNA has been reported in Europe and North America.[7-10] Currently in Nigeria, the routine screening of donor blood for transfusion transmittable infections does not include HEV. Given the fact that emerging evidence shows that HEV can be transmitted by blood transfusion and infected potential blood donors may never have had the symptoms, HEV has become a transfusion transmittable pathogen of concern and can be a potential threat to transfusion safety, particularly in pregnant women and immunosuppressed clients like those on chemotherapy, transplant recipients and HIV-infected who are given blood transfusion from time to time.

This study therefore aimed to determine the sero-prevalence rates of HEV amongst apparently healthy blood donors attending the donor clinic of the Lagos State University Teaching Hospital, Lagos, Nigeria with a view to ascertaining whether its inclusion in routine screening of blood pre-transfusion is feasible and cost effective.

MATERIALS AND METHODS

Study population

This study was conducted in the Donor Clinic of the Lagos State University Teaching Hospital (LASUTH), Lagos, Nigeria. LASUTH is a 700 bedded tertiary hospital established in 2001.

There are 20 clinical departments in the hospital. The blood donor clinic domiciled in the blood bank is under the purview of the department of Haematology and Blood transfusion. It caters to approximately 40 donors daily

Study Design and sampling technique

This was a cross-sectional study using the non-probability convenience sampling technique.

Study Period

Study was conducted between November 2019 and December 2019.

Inclusion criteria: All donors who met the criteria for blood donation in Lagos State University Teaching Hospital and who gave informed consent were recruited into the study. These criteria include

1. Age: 18 to 60 years

2. Weight and height: Minimum height of 5 feet and minimum of 45kg
3. Haemoglobin level: No less than 12.5g/dl
4. At least 12 weeks interval between donations
5. Blood pressure: not greater than 140/90mmHg; Pulse rate: 50-100 beats/minute and regular
6. Temperature: 36.6-37.2^{0}C.

Exclusion criteria: All donors who failed to meet the inclusion criteria

Sample Size Determination

Sample size was calculated using Daniel formula, $n = Z^2 pq/d^2$.[11] Where n is the sample size and p the prevalence of anti HEV antibodies 4.9% (0.049).[12] A minimum sample size of 71 obtained, but a total of 104 subjects were recruited.

Sample Collection

Five millilitres of blood was collected from each subject from the antecubital vein under aseptic techniques. The blood was dispensed into plain sterile tubes and allowed to clot and retract. This was centrifuged at 3500 rpm for five minutes and the serum samples transferred into 2mls cryovials and stored at -80℃ till analysis. The interval between sample collection and analysis was approximately 2 weeks.

IgG and IgM antibodies were detected using enzyme linked immunosorbent assay (ELISA) kits for the detection of IgM and IgG anti HEV antibodies manufactured by Melsin Medical Co., Ltd, Jilin Province, China and testing was done according to the manufacturer's instruction. For the IgM kit, the specificity ranges from 95.3% to 100% while the sensitivity is between 97.62% and 100%. The specificity of the IgG kit was 99.96% to 99.08% and the sensitivity 99.99% to 100%.

Ethical Consideration and Clearance

Ethical approval was obtained from the Health Research and Ethics Committee of LASUTH REF NO: LREC/06/10/1275. All participants that were found to be seropositive were recalled and referred to the gastroenterology clinic for further evaluation and management.

Participants' Informed Consent

The participants were informed about the study as well as their rights (of refusing to participate in this study) and benefits (recall and disclosure of any positive results and consequent referral to the gastroenterology clinic in LASUTH for further evaluation and management). A written informed consent was obtained by means of voluntarily signed consent forms. They were not coerced in any way to participate in this study. The study was at no cost to the participants.

Statistical Analysis

Data was analysed using SPSS version 20.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The continuous variables were given as means ± standard deviation (SD). The Pearson chi squared test was used to test for association between discrete variables. P value was considered to be statistically significant when ≤ 0.05.

RESULTS

Sociodemographic characteristics of study participants

One hundred and four (104) blood donors were recruited into the study and comprised of 98 (94.2%) males and 6 (5.8%) females. The mean age of the study participants was 31.96 ± 7.90 years with a range of 18-56 years. (Table 1). The participants were classified into 3 age groups: <20 years, 20-40 years and 41-60 years and majority (89%) were between 20 and 40 years. The other socio-demographic

characteristics of the study participants are shown in Table 1.

Table 1: Sociodemographic Characteristics of Study Participants

Demographic Variable	Frequency n = 104 (100%)
Sex Distribution	
Male	98 (94.2)
Female	6 (5.8)
Age Group (years)	
<20	1 (0.9)
20-40	89 (85.6)
41-60	14 (13.5)
Level of Education	
No Formal	4 (3.8)
Primary	10 (9.6)
Secondary	40 (38.5)
Tertiary	41 (39.4)
Postgraduate	7 (6.7)
Missing	2 (1.9)
Religion	
Christian	69 (66.3)
Islam	35 (33.6)

Distribution of anti HEV antibodies in study participants

Among the 104 participants, the overall prevalence of anti HEV antibodies was 4.8% with a prevalence rate of 1.9% for anti HEV IgM and 2.9% for anti HEV IgG. All the seropositive participants were male and fell within the age group 20-40 years (Table 2).

Table 2: Seroprevalence rate of anti HEV antibodies among study participants

Anti HEV antibodies	IgM n (%)	IgG n (%)
Positive	2(1.9)	3(2.9)
Negative	102 (98.1)	101 (97.1)
Total	104 (100)	104 (100)

Correlation between IgM and IgG seropositivity and sociodemographic characteristics

Using ANOVA, there was no significant association between anti HEV seropositivity and age, sex distribution, level of education, type of residence, type of toilet facilities, source of water and history of blood transfusion (Tables 3 and 4).

DISCUSSION

Although Hepatitis E virus infection is one of the commonest causes of acute viral hepatitis, it remains the least diagnosed of all the viral hepatitis.[13] According to the World Health Organization (WHO), HEV causes 20 million infections annually with more than three million cases of acute hepatitis and approximately 60,000 deaths from the infection.[14] Hepatitis E virus commonly leads to a relatively harmless self-limiting acute infection; however, in some specific populations such as, during pregnancy and immunocompromised patients, in whom acute liver failure may occur.

The main route of transmission is via the faeco-oral route; however, transmission via transfusion of blood and blood products can occur; vertical transmission via an infected mother to her unborn child has also been documented.[7-10,12,15]

The overall seroprevalence rate of anti-HEV antibodies in our study was 4.8%. This is similar to that reported by Kaufmann and colleagues, who in their study done in Switzerland obtained a prevalence rate of 4.9%.[12] John-Olabode and colleagues (2017) in their study also done among blood donors in Lagos State documented a seroprevalence rate of 6.6%.[16]

Other studies have documented varying prevalence rates ranging from 3.8%, to as high as 49.7%.[17,18] Various epidemiological studies in different regions of the world have shown that there is a wide variation in seroprevalence rates; the rates tend to be higher in developing countries and lowest in developed countries.[19,20] These differences seen in prevalent rates may be as a result of variation in regional seroprevalence rates; and rates may differ even within the same country.

Table 3: Anti HEV IgM antibodies and the sociodemographic characteristics of participants

Demographic Variable	IgM positive n (%)	IgM negative n (%)	Total n (%)	F-value	P-value
Sex Distribution					
Male	2 (2.1)	96 (97.9)	98 (100)		
Female	0 (0)	6 (100)	6 (100)	**0.123**	**0.727**
Age group					
<20	0 (0)	1 (100)	1 (100)		
20-40	2 (2.2)	87 (97.8)	89 (100)		
>40	0 (0)	14 (100)	14 (100)	**1.833**	**0.179**
Education					
No formal	0 (0)	5 (100)	5 (100)		
Primary	0 (0)	11 (100)	11 (100)		
Secondary	1 (2.5)	39 (97.5)	40 (100)		
Tertiary	0 (0)	41 (100)	41 (100)		
Postgraduate	1 (14.3)	6 (85.7)	7 (100)	**1.026**	**0.314**
Water source					
Well	0 (0)	3 (100)	3 (100)		
Tap	2 (2.0)	99 (98.0)	101 (100)	**0.059**	**0.808**
Toilet facilities					
Water closet	2 (2.0)	96 (98.0)	98 (100)		
Pit toilet	0 (0)	5 (100)	5 (100)		
Bush	0 (0)	1 (100)	1 (100)	**0.111**	**0.740**
Residence type					
Rural	0 (0)	1 (100)	1 (100)		
Semi-urban	0 (0)	4 (100)	4 (100)		
Urban	2 (2.0)	97 (98.0)	99 (100)	**0.091**	**0.764**
Previous transfusion	0 (0)	1 (100)	1 (100)		
Yes	2 (2.0)	101 (98.0)	103 (100)	**0.020**	**0.889**
No					
Smoking					
No	2(2)	98 (98)	100(100)		
Yes	0(0)	4(100)	4 (100)	**0.080**	**0.778**
Alcohol					
No	2 (2.5)	77 (97.5)	79 (100)		
Yes	0 (0)	25 (100)	25 (100)	**0.637**	**0.427**

Table 4: Anti HEV IgG antibodies and sociodemographic characteristics of participants

Demographic variable	IgG positive n (%)	IgG negative n (%)	Total n (%)	F-value	P-value
Sex Distribution					
Male	3 (3.0)	95 (97.0)	98 (100)		
Female	0 (0)	6 (100)	6 (100)	**0.186**	**0.667**
Age group					
<20	0 (0)	1 (100)	1 (100)		
20-40	3 (3.4)	86 (96.6)	89 (100)		
>40	0 (0)	14 (100)	14 (100)	**0.092**	**0.762**
Education					
No formal	0 (0)	5 (100)	5 (100)		
Primary	0 (0)	11 (100)	11 (100)		
Secondary	3 (7.5)	37 (92.5)	40 (100)		
Tertiary	0 (0)	41 (100)	41 (100)		
Postgraduate	0 (0)	7 (100)	7 (100)	**0.501**	**0.481**
Water source					
Well	0 (0)	3 (100)	3 (100)		
Tap	3 (3.0)	98 (97.0)	101 (100)	**0.090**	**0.765**
Toilet facilities					
Water closet	3	95	98		
Pit toilet	0 (0)	5 (100)	5 (100)		
Bush	0 (0)	1 (100)	1 (100)	**0.168**	**0.683**
Residence type					
Rural	0 (0)	1 (100)	1 (100)		
Semi-urban	0 (0)	4 (100)	4 (100)		
Urban	3 (3.0)	96 (97.0)	99	**0.137**	**0.712**
Previous transfusion				**0.030**	**0.863**
Yes	0 (0)	1 (100)	1(100)		
No	3 (3.0)	100 (97.0)	103 (100)		
Smoking					
No	3 (3)	97 (97)	100		
Yes	0 (0)	4 (100)	4 (100)	**0.121**	**0.728**
Alcohol					
No	2 (2.5)	77 (97.5)	79 (100)		
Yes	1 (4)	24 (96)	25 (100)	**0.144**	**0.706**

Although this present study failed to show any significant correlation between seroprevalence rates and various environmental conditions like source of drinking water, type of sanitary facilities etc, it could be postulated that the availability of these facilities may be responsible for these regional differences. Perhaps another study done with a larger sample sized may confirm or refute this observation. Another reason for the wide variation in prevalence rates of studies conducted even in the same region may be the differences in the ELISA kits used for the detection of anti-HEV antibodies. Previous studies have revealed that anti-HEV antibody kits vary widely in both their sensitivity and specificity.[21-23] In this study the specificity of the IgM kit was between 95.3% and100% while the sensitivity was between 97.62% and 100%. The specificity of the IgG kit was between 99.96% and 99.08% and the sensitivity between 99.99% and 100%.

In addition, HEV causes an acute viral infection; therefore, it is obvious that a study conducted among apparently healthy subjects may be unlikely to capture acutely infected individuals

who would obviously not have met the inclusion criteria for this study.

Anti HEV IgM and IgG antibodies usually develop at the time of symptomatic illness, with IgM preceding by a few days. Anti HEV IgM is secreted in acute response to a recent infection with HEV and levels rapidly reduce during the recovery phase. The presence of IgM signifies immunity and provides protection against subsequent infections.

Most cases of HEV infection may not be distinguished clinically from other types of viral hepatic infections, diagnosis is usually confirmed by the presence of anti HEV IgG and IgM and acute hepatitis E is diagnosed when anti HEV IgM is detected.[18] However, both false positive and negative are common with the assays available. In this regard, additional serologic testing or HEV RNA testing with the use of Polymerase Chain reaction (PCR) which remains the gold standard for diagnosis may be required to confirm the diagnosis of an acute infection.

This present study documents an IgM anti-HEV prevalence rate of 1.9% and this rate is comparable to the prevalence of 1.3% among blood donors in another centre in Lagos recorded by John-Olabode and colleagues (2017).[16] This value is however lower than the 4.8% prevalence rate obtained by Gajjar *et al* (2014) in India.[24] Again, this difference may be as a result of the factors earlier discussed.

All the participants who tested positive for anti-HEV antibodies were males, it is in agreement with previous researches.[12,16,18,25-27] The reason for this sex difference may be due to the fact that males make up the vast majority of blood donors in Nigeria and recruitment of participants into this study was unintentionally skewed in favour of the male sex.

The seroprevalence of anti-HEV antibodies in developing countries is higher between the ages of 15-35 years.[28] In this present study, the prevalence of anti-HEV antibodies was highest in the age group 20-40 years, a finding that is consistent with that of Adesina and colleagues (2009) who made the same observation.[29] It is worthy of note that the prevalence of anti-HEV antibodies is highest in this age group who make up the majority of blood donors in our environment. This is of particular concern because of the possibility of HEV transmission via blood and blood products and the fact that most patients with acute HEV are usually asymptomatic or mildly symptomatic.

Contrary to other reports, this study found no association between anti-HEV seropositivity with alcohol consumption and smoking.[16, 30] Interestingly, an association between a history of blood transfusion and anti-HEV seropositivity was also not observed. This is in contrast to other studies that found a strong association between the history of blood transfusion and anti-HEV seropositivity.[16,18,31]. No significant association was found between anti-HEV seropositivity and other variables including level of education, source of water and toilet facilities ($P>0.05$).

CONCLUSION

Although this study reports a low seroprevalence rate of anti-HEV IgM and IgG antibodies among apparently healthy blood donors in Lagos, Nigeria, it shows nonetheless that there is a potential risk of transmitting HEV via the transfusion of blood and blood products. This becomes especially worrisome in immunocompromised patients and pregnant women who are at a higher risk of developing acute fulminant hepatitis and liver failure. A larger scale community- based study to determine the prevalence of HEV infection in Lagos, Nigeria would be useful.

Study Limitations: The study used the ELISA method to determine the sero-prevalence of HEV in the general population instead of the HEV PCR which is the gold standard. The small sample size of 104 used to determine prevalence of the HEV antibodies in the general population could have impacted on the statistical significance due to a type 2 sampling

error; however, funding was a major challenge.

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Conflict of Interest: The authors declare no conflict of interest.

Author's Contributions

Study conceptualization and design: UEI, OF. Data acquisition: ET, IA. Data analysis and interpretation UEI, AA. Manuscript drafting and review: all the authors. Final manuscript approval: all the authors.

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