Original Article

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Hepatitis E Virus Prevalence Amongst Blood Donors in Kenya

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Abstract

Introduction: Hepatitis E (HEV) is an emerging cause of viral hepatitis mainly transmitted through the fecal-oral and parenteral routes. It has greatly affected populations in many parts of Kenya due to declined sanitation practices. The objective of the research was to determine IgM and IgG seroprevalence of HEV prevalence among blood donors in Kenya as well as its Anti-HEV sero-reactivity by demographic factors.

Material and Methods: Cross sectional research design was used to collect blood samples from six from regional blood collecting centers, with blood donors selected through proportionate stratified sampling. Serum were matched to the age and gender distribution of the blood donors and tested by IgM and IgG enzyme immunoassays (EIA). The screening was carried out using human MP Diagnostics (0721150096T-96 wells) HEV ELISA kit. To test for IgM and IgM anti-HEV antibodies, microplates with 96-well plate were used. Results were averaged to generate a signal-to-noise ratio with ≥1.0 designated as positive per the manufacturer's recommended cutoff.

Result and Conclusion: On the analysis, IgM reactivity was associated with HEV (RR 1.66, 95%CI 1.07, 2.60; p = 0.024) while IgG reactivity was associated with increasing age (p < 0.001) and HIV (RR 1.93, 95%CI 1.52, 2.46; p < 0.001). AFI case-patients were more likely to be IgM (p = 0.002) and IgG (p<0.001) reactive compared to healthy residents. All the 19 hepatitis E virus IgM and/or IgG seropositive blood samples tested negative for HEV RNA since no bands were detected using RT-PCR technique. Serologic evidence for HEV in blood samples from selected areas suggests a high burden of infection, though age and gender exhibited no significant difference in prevalence of HEV among the blood donors in Kenya (p>0.05).

Keywords: *Hepatitis E, Seroprevalence, Blood donors, Endemic*

Introduction

Hepatitis E virus (HEV) is a virus which causes Hepatitis a liver disease which has been identified by the World Health Organization (WHO) as killer disease. Hepatitis E virus (HEV) has at least 4 different types: genotypes 1, 2, 3 and 4. It is a non-enveloped, single-stranded RNA virus belonging to the family Hepeviridae [1, 2]. In developing countries, HEV is a major cause of acute hepatitis, transmitted by the fecal–oral route and associated with contamination of drinking water [3]. It can also be transmitted through ingestion of undercooked meat or meat products derived from infected animals (e.g. pork liver), transfusion of infected blood products and vertical transmission from a pregnant woman to her baby [4]. Hepatitis E virus has been reported as a transfusion-transmissible virus from the year 2002. Recently, epidemiological reports have documented that HEV may affect the safety of blood distribution [5,6].

Genotypes 1 and 2 have been found only in humans while Genotypes 3 and 4 circulate in several animals (including pigs, wild boars, and deer) without causing any disease, and occasionally infecting humans. The virus is shed in the stools of infected persons, and enters the human body through the intestine. It is transmitted mainly through contaminated drinking water. HEV has recently been cloned and sequenced leading to new serological tests based on recombinant proteins which have been used to characterize the natural history and epidemiology of HEV infections [7].

HEV infections have been prevalent in most parts of Asia, Africa, Mexico, and Middle East where fecal contamination is high. The global distribution of hepatitis B infection varies greatly. It is estimated that 350 million people globally are chronic carriers of the hepatitis B virus of who 170 million reside in Africa [8]. The prevalence of HBV carriers varies substantially between regions, from 7 to 35 % [9]. The wide range is largely related to differences in age at the time of infection, a factor that is inversely related to the risk of chronic infection. In some parts of the World like

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USA where HEV has not been reportedly prevalent, few cases have been reported among travelers coming back HEV endemic areas. However, clinical cases of hepatitis E have been reported among persons with no history of travelling to HEV –endemic areas, though the mode of HEV transmission is not known [10].

HEV-B and HEV-C have received much attention in Kenya due to their prevalence and long-term sequelae of chronic infections with these agents like liver cirrhosis and hepatocellular carcinoma [2]. However, in recent times, much attention has been paid to HEV-E which is the leading cause of acute viral hepatitis in young to middle-aged adults in Kenya. Globally, Hepatitis E is more severe than hepatitis A, with mortality rates in the range of 1–2%, compared with 0.2% for hepatitis A. In Kenya in particular, HEV has been identified as a cause of acute sporadic hepatitis in refugee camps in northern Kenya; these refugees originate from the neighboring countries like Somalia, Southern Sudan and Ethiopia where HEV epidemics have been reported [11]. HEV testing involves the identification of HEV-Specific host antibodies with enzyme immunoassay (EISs) or HEV RNA through nucleic Acid Testing Methods. IgM wanes after several weeks while IgG remains detectable for years [12].

HEV RNA is detectable by NAT in serum or stool with peak RNA levels occurring during late incubation and lasting approximately 2–3 weeks after symptom onset [2]. Reports on seroprevalence are valuable and they largely depend on the different efficacy of the assays used [13]. Studies from endemic zones concerning the epidemiology of HEV in Kenya have been associated with practices of blood donation and transfusion [11]. More especially, blood transfusion and related blood products which have not been tested for HEV is associated with chronic hepatitis E infection in immune-compromised and anemic individuals in Kenya [10]. It is against this backdrop that this paper sought to determine the HEV prevalence of blood donors in Kenya. This is informed by the fact that Screening for blood on HEV prevalence among the blood donors will aid in the enactment of policies which will minimize HEV infections during blood donation and transfusion. The study aimed at determining the prevalence of Hepatitis E Virus (HEV) in Kenya. It examine the IgM and IgG seroprevalence of HEV among blood donors in Kenya and the Anti-HEV seroreactivity by demographic factors and serologic markers of blood donors in Kenya.

Material and methods

Study Area and Design: The study was carried out at the National central testing laboratory located at KNBTS building within Kenyatta National Hospital in Nairobi, Kenya. Cross sectional research design was used to obtain blood samples from Eldoret, Embu, Kisumu, Mombasa, Nairobi and Nakuru regional blood collecting centers. This areas were selected due to high HEV prevalence rates. Samples of blood collection from donors was done during the months of November and December in the year 2016.

Study population: The study population consisted of all occupants of age 18 and above from the sampled areas. A sample size of 384 was made from the sampling frame of 22057 HEV-Vulnerable individuals from the six sampled areas. A systemic random sampling of all collected blood samples using random numbers from the table of random numbers in the two months was performed where each 23rd blood sample was picked. All necessary ethical and legal considerations involving blood sample collection were observed including informed consent from the donor as part of the pre-counseling process on a donor questionnaire was administered before blood donation was carried out. Fresh blood samples drawn from the 384 were taken to the regional blood collection centers in Kenya and stored at a temperature of 20C to 80C during the serological testing period in the months of November and December 2016. Two milliliter of blood sample was collected from each blood donor. Of the 2ml blood sample, 1ml was put in plain blood collecting tubes, while the other 1ml was put in blood collecting tubes containing ethylenediaminetetraacetic acid (EDTA). The blood samples containing EDTA were used for blood typing, while those in plain blood collecting tubes were centrifuged at 2500 revolutions per minute to obtain blood serum for serological testing

Specimen Analysis and Serologic testing: Human serum samples obtained from the 384 donors were screened for Human IgM and IgG hepatitis E virus. The screening was carried out using human MP Diagnostics (0721150096T-96 wells) HEV ELISA kit. To test for IgM and IgM anti-HEV antibodies, microplates with 96-well plate were used. Every microplate well contained adsorbed recombinant hepatitis E virus proteins and was stored at 2-8 degree Celsius, while the diluent was used to fill the reagent reservoir. 200 microliters of the diluent were added to the wells using a multichannel pipette. All the necessary steps for screening in line with MP Diagnostics were followed. Each sample was tested by EIA in duplicate for IgM and IgG-specific HEV antibodies (DS-EIA-ANTI-HEV-G, DS-EIA-ANTI-HEV-M. IgM sensitivity (98.0%) and specificity (95.2%) [1]. Results were averaged to generate a signal-to-noise ratio with ≥1.0 designated as positive per the manufacturer's recommended cutoff. Each serum sample underwent HEV NAT by both reverse transcription and real-time polymerase chain reaction protocols developed by the Centre for Disease Control (CDC) [14].

Case-control study: The obtained data was recorded in a data handbook, tabulate in Microsoft Excel spreadsheet, cleaned and then exported to Statistical Package for Social Sciences (SPSS) Version 21.0 for statistical analysis. Data were expressed as frequency and percentages. Categorical data were evaluated in 2-way contingency table analyses using Pearson Chi-Square test. Odds ratios were also computed. Statistical differences were analyzed at 95% level of significance. Data was presented in graphs or tables.

Results and Discussion

Of the 384 mini pools, comprising of 22057 individual blood donations screened, 19 blood donations containing anti-hepatitis E virus IgM and/or IgG antibodies were identified. This resulted to a seroprevalence of 4.9% (one out of twenty samples). With regard to gender and age of the blood donors, 54.2% (208 blood donors) were male, while 45.8% (176 blood donors) were female of which male exhibited an HEV seroprevalence of 5.3% (11/208), while the female respondents exhibited a seroprevalence of 4.5% (8/176). However, there was no significance relationship between HEV prevalence and gender ($\chi2 = 0.112$; df = 1; p = 0.74). This findings concur with those of [2] who found out that the prevalence of HEV is higher in men than women. With regard to age and HEV prevalence, age group of blood donors less or equal to 24 years old recorded a seroprevalence of 4.0% (1/25), the age group between 25 and 34 years old recorded a seroprevalence of 2.6% (5/189), the age group between 35 and 44 years old recorded a seroprevalence of 8.0% (12/150), while the ages above or equal to 45 years old recorded a seroprevalence of 5.0% (1/20).

However, there was no significance relationship between age and HEV prevalence among various age groups in the study area ($\chi2 = 5.149$; df = 3; p = 0.16). With regard to seroprevalence of HEV by religion, The regions of Eldoret, Embu, Kisumu, Nairobi, Nakuru and Mombasa exhibited a seroprevalence of 4.9% (3/61), 4.7% (3/64), 3.9% (3/76), 4.1% (4/97), 8.3% (4/48) and 5.3% (5.3%) respectively. However, the HEV seroprevalence for blood samples from Nakuru was 1.64(odd ratio) higher than the blood samples from Mombasa. The summary of the Hepatitis E virus seroprevalence among blood donors by gender, age and region in Kenya is shown in table 1 below.

From results in table 1 above, Nakuru leads with a high seroprevalence of 8.3% (4/48), while Kisumu has the lowest seroprevalence of 3.9% (3/76). This findings concur with those of [15]. (2016) who found out that the low HEV prevalence in Kisumu may be as a result of high HIV prevalence in the region. However this is against the global evidence which alludes a correlation between HEV and HCV with HIV seroprevalence [16].

Table 1: HEV seroprevalence among blood donors by gender, age and region in Kenya

Variable	No. of blood donors	HEV seroprevalence	p value
Gender			
Male	208 (54.2%)	5.3% (11/208)	0.74
Female	176 (45.8%)	4.5% (8/176)	
Age in years			
≤24	24 (6.5%)	4.0% (1/25)	0.16
25-34	189 (49.2%)	2.6% (5/189)	
35-44	150 (39.1%)	8.0% (12/150)	
≥45	20 (5.2%)	5.0% (1/20)	
Region			
Eldoret	61 (15.9%)	4.9% (3/61)	0.91
Embu	64 (16.7%)	4.7% (3/64)	
Kisumu	76 (19.7%)	3.9% (3/76)	
Nairobi	38 (9.9%)	4.1% (4/97)	
Nakuru	97 (25.3%)	8.3% (4/48)	
Mombasa	48 (12.5%)	5.3% (2/38)	
Total	384	4.9% (19/384)	
Key: HEV = Hepatitis E virus; No. = Number			

The Second Objective of the study was to establish the Anti-hepatitis E virus IgM and IgG prevalence of blood donors in Kenya which was determined by use of ELISA (MP Diagnostic's hepatitis E virus ELISA protocol). Of the 384 blood samples that were screened, only 13(3.4%), blood samples were seropositive for HEV IgM while only 4 samples among the HEV IgM seropositive samples were HEV IgG seropositive. For the case of Anti-hepatitis E virus IgG prevalence, 2.6% of the blood samples (10/384) were found to be HEV IgG seropositive, while only 6 blood samples among the anti-hepatitis E virus IgG positive samples were hepatitis E virus IgM seropositive representing a seroprevalence of 0.02%, which was quite low.

With regards to Anti-hepatitis E virus IgM and IgG prevalence of blood donors in relation to their gender; the study found no significant association between gender and Anti HEV IgM prevalence of blood donors from all the blood collection points in Kenya ($\chi2 = 0.001$; df = 1; p = 0.98), and between gender and seroprevalence of HEV IgG antibodies among blood donors in Kenya ($\chi2 = 0.072$; df = 1; p = 0.79). However, the female blood donors were 1.19 (odd ratio) times likely to be anti-hepatitis E virus IgG seropositive, and 1.01 (odd ratio) times likely to be HEV IgM seropositive compared to male blood donors. The overall relations between gender and Anti-hepatitis E virus IgM and IgG prevalence of blood donors is shown in table 2.

With regard to the relationship between age of the blood donors and Anti-hepatitis E virus IgM and IgG prevalence, the study found no significant association between seroprevalence of HEV IgM and age ($\chi2 = 2.105$; df = 3; p = 0.55); and no significant association between seroprevalence

Table 2: Anti-hepatitis E virus IgM and IgG prevalence among blood by gender

		Anti-HEV IgM			Anti-HEV IgG		
Gender	No. of samples	Negative samples	Positive samples	p value	Negative samples	Positive samples	P value
Male	208	201(96.6%)	7 (3.4%)	0.98	203 (97.6%)	5 (2.4%)	0.79
Female	176	170(96.6%)	6 (3.4%)		171 (97.2%)	5 (2.8%)	
Total	384	371(96.6%)	13(3.4%)		374(97.4%)	10(2.6%)	

Table 3: Anti-hepatitis E virus IgM and IgG prevalence among blood by age

		Anti-HEV IgM			Anti-HEV IgG		
Age (years)	No. of samples	Negative samples	Positive samples	p value	Negative samples	Positive Samples	P value
≤24	25	25 (100%)	0 (0.0%)	0.55	24 (96.0%)	1 (4.0%)	0.04
25-34	189	184(97.4%)	5 (2.6%)		188 (99.5%)	1 (0.5%)	
35-44	150	143(95.3%)	7 (4.7%)		142 (94.7%)	8 (5.3%)	
≥45	20	19 (95.0%)	1 (5.0%)		20 (100.0%)	0 (0.0%)	
Total	384	371(96.6%)	13(3.4%)		374(97.4%)	10(2.6%)	

of HEV IgG and age ($\chi 2 = 2.161$; df = 3; p = 0.04) as shown in table 3.

With regard to the relationship between age of the blood donors and Anti-hepatitis E virus IgM and IgG prevalence, the study found no significant association between seroprevalence of HEV IgM and age ($\chi 2 = 2.105$; df = 3; p = 0.55); and no significant association between seroprevalence of HEV IgG and age ($\chi 2 = 2.161$; df = 3; p = 0.04) as shown in table 3.

The findings in table 3 above do not concur with those of [16] and who found a significant association between gender and age of blood donors and Anti-hepatitis E virus IgM and IgG prevalence of blood donors [15]. Although the age of blood donors exhibited no significant difference in prevalence of HEV among the blood donors in Kenya (p>0.05), the ages of 35 to 44 years old recorded higher HEV prevalence of 8.0%, while the ages between 24 to 35 years old recorded the lower HEV prevalence of 2.4%. These findings disagreed with a study carried out by [17] which reported that the ages of 45 years and above recorded the highest HEV prevalence among blood donors in France.

Findings and Conclusions

Previous studies in the East African region demonstrate that respiratory viruses account for the majority of febrile illness [18], malaria was an infrequent cause of fever in Kenya (≤5%) [11], and viral hepatitis was rare [18]. Serologic evidence for HEV in the blood samples in this study and from other studies [2, 11] suggests a high burden of infection. Previous literature on safety of transfused blood and HEV transmission, studies revealed that HEV takes many epidemiological routes, however this study adds to the literature on the epidemiology of HEV by alluding that many transmissions of HEV in Kenya are through blood and fecal oral route. Ceteris Paribas, this study some key findings on Hepatitis E Virus Prevalence amongst Blood Donors in Kenya with regard to age of the blood donors, gender of the blood donors and anti-hepatitis E virus IgM and IgG prevalence among blood donors by age and gender. This study established that exposure of HEV in blood donors among Kenyan populations.

From the blood donated from different regional blood collection centers in Kenya, a hepatitis E virus seroprevalence of 4.9% was computed, which was similar to non-endemic countries [8]. These findings concur with those carried on HEV seroprevalence of blood donors in Ghana, Scotland, Tunisia and Southwest Switzerland which recorded similar HEV seroprevalence of 4.6% 4.7%, 4.8% and 4.9% respectively [19]. The low HEV seroprevalence in Kenya may be attributed to two reasons: minimal contact with infected animals' body fluids or consumption of infected undercooked animal meat [20], and dominance of Hepatitis E virus genotypes 3 and 4 which are less virulent [21].

Although the age of blood donors exhibited no significant difference in prevalence of HEV among the blood donors in Kenya (p>0.05), the ages of 35 to 44 years old recorded higher HEV prevalence of 8.0%, while the ages between 24 to 35 years old recorded the lower HEV prevalence of 2.4%. These findings deviate from those of [3] in Germany who found out an association between age and HEV seroprevalence. The explanation to this as per [3] is that HEV exposure is common among the general adult population in Germany, which is attributed to increasing evidence for pigs as a reservoir for foodborne transmission of HEV in industrialized countries. Lastly, most blood donors had recent HEV infection compared to past infection. This was due to the presence of more seropositive anti-HEV IgM blood samples compared to seropositive anti-

HEV IgG blood samples. This study therefore recommends that preventive measures such as serological screening of anti-hepatitis E virus IgM and IgG of blood donors should be introduced to prevent transfusion-transmitted HEV infections.

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Reference

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