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Research Article

Sero-molecular Epidemiology of Hepatitis E Virus in Blood Donors, Gezira State, Sudan: A Cross-sectional Study

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Abstract

Background: Hepatitis E virus (HEV) is a hepatotropic pathogen that causes significant morbidity and mortality in humans. It is an important causative agent of viral hepatitis outbreaks. This study investigates the serological and molecular prevalence of HEV in blood donors attending the Central Blood Bank in Wad Medani City in Gezira State, Sudan.

Methods: The study adopted a cross-sectional descriptive design. A structured questionnaire was used to collect data concerning demographic information and risk factors associated with HEV transmission. All enrolled participants (*N* = 300) were screened for HEV IgG antibodies using commercial ELISA kits, then strong positive samples (*N* = 84) were selected and rescreened for HEV IgM and HEV RNA by RT PCR. SPSS version 24.0 was used for analysis.

Results: Out of 300 male participants, 36.3% (109/300) were positive for HEV IgG. However, only one participant was IgM positive, while the HEV RNA was negative. The highest prevalence rates of the virus were 42 (44.6%) among the age group of 31–40 years, 20 (48.8%) in those who consumed food from outside, 13 (50%) in three to four multiple blood donations, and 5 (62.5%) in those who consumed water from the river source. A significant association of HEV IgG prevalence concerning the occupation of the participants being students or farmers was detected using univariate and multivariate analysis (*P*-value = 0.007).

Conclusion: High prevalence of HEV IgG was demonstrated among the healthy blood donors in this study. Given the possibility of HEV transmission by transfusion from donors to recipients, we recommend that routine screening for HEV should be adopted by blood banks in Sudan.

Keywords: HEV IgG, blood donors, HEV risk factors, HEV IgM, HEV RT PCR, Sudan

1. Introduction

Hepatitis E virus (HEV) initially belonged to the family *Caliciviridae*, but recently after the introduction of molecular techniques, the virus has been reclassified and placed in the *Hepeviridae* family [1]. Although the virus is serotypically homogenous, it has been subdivided into four genotypes: genotypes 1 and 2 are mainly found in humans, whereas genotypes 3 and 4 represent porcine HEVs but can also infect humans [2]. In addition, the family of *Hepeviridae* is phylogenetically divided into two genera, with four species in genus *Orthohepevirus* [3]. HEV prevalence rate in Africa is variable. It was reported to be 45.3% in Egypt [4], 5.4% and 22% in Tunisia [5, 6], and 19.1% in Burkina Faso blood donors [7]. Studies conducted among risk groups revealed the variable distribution of serological markers of HEV [8–10]. Transmission through pooled platelet concentrates was concluded in some studies in Germany [11]. In addition, HEV RNA-positive blood donations from healthy blood donors were observed in Japan [12]. Studies from Japan and Europe showed that a few cases of HEV were transmitted by blood transfusion, and retrospective follow-up of the transfused patients revealed elevated transaminase levels and signs of fulminant hepatitis [13–15]. A retrospective study of transfusion recipients suggested that in HEV-1-endemic areas, HEV could be transmitted by blood transfusion [16]. A similar conclusion was demonstrated in another study, in which some recipients exhibited HEV reactivity [17] after blood transfusion. In the Egyptian population, a study revealed that 80% were HEV IgG positive. However, only 0.26% were found to be HEV RNA positive [18].

A study aiming at molecular detection of HEV RNA revealed negative results among 239 Ghanaian blood donors [19]. Similar results were obtained from American plasma donations [20]. Low rates of HEV RT-RNA were detected in studies conducted among blood donors in Sweden (0.012%), Germany (0.022%) [20], and Egypt [18]. The HEV RNA seems quite difficult to detect and this may require a very large sample size with highly sensitive RT-PCR methods. In a study of blood donations in China, 30 out of 44,816

donations (0.07%) were HEV RNA-positive [21]. In addition, low HEV RNA production rates were concluded by Fischer *et al.*, where they could only detect 7 (0.58%) HEV RNA among 1203 (13.55%) positive HEV IgG [22]. Furthermore, Nassir *et al.* revealed a negative result concerning the HEV-RNA among hemodialysis patients with a positive HEV seroprevalence [23].

On the other hand, the viral distribution concerning the gender and age groups revealed some variation among the studies. For example, a study conducted in Iran showed a difference in HEV IgG by age but not gender [24].

A higher HEV IgG seroprevalence rate of 26.7% concluded by Ahmed *et al.* among Sudanese blood donors that are significantly associated with a young age group might raise the potential risk for HEV transmission in recurrent blood transfusion by those donors.

2. Materials and Methods

2.1. Study design and samples collection

The study was a cross-sectional, hospital-based descriptive study conducted among blood donors attending the central blood bank in Wad Medani city, the capital of Gezira state, Sudan. This blood bank provides blood donation services to all localities of Gezira State. Therefore, donors in this study could be representatives of the population in Gezira State.

After testing negative during the routine screening of HBV, HCV, syphilis, and HIV for the blood transfusion, 300 blood donors were recruited for this study. Before the sample collection, informed consent was taken from all participants and the demographic data, clinical data, and risk factors associated with HEV transmission were documented using a structured questionnaire. About 5 ml blood was collected from each participant in an EDTA container using standard techniques. The plasma was separated into two aliquots, then stored at –40°C until used for HEV serological screening by ELISA and HEV RNA by RT-PCR.

2.2. Serological screening for HEV

The screening was performed using ELISA for HEV IgG and HEV IgM. For the interpretation of the negative results for HEV IgG or HEV IgM, the positive results, and the equivocal results, the cut-off value was set as <1, >1, and 0.9–1.1, respectively,

according to the manufacturer's instructions. The equivocal reading was later retested for confirmation.

2.3. Molecular detection for HEV-RNA

The Primer design^{TM} genesis® (UK manufacture) kits were used for the extraction and real-time PCR of HEV-RNA detection. The kits are specifically designed for in vitro highly purified RNA extraction and quantification of HEV genomes. The primers and probe sequences in the kit had 100% homology with a broad range of HEV sequences based on comprehensive bioinformatics analysis. Both RNA extraction and real-time PCR were carried out at the National Public Health Laboratory, Khartoum, Sudan, using an RT-PCR Thermo cycler (Rotor gene 6000, Germany). The DNA/RNA Extraction Kit solution (EXP date: 17/10/ 2017, Batch No, 66606, G25493, 66601, 66605, P15079S, 66628, 66622, SHB68127V, and 66596) and the HEV-RNA-specific primer and probe mix (Serial number JN 160305-49345, Batch number PD2879, Expiry date 17.10.2017) were used according to the manufacturer's protocol.

2.4. Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS), version 24.0. Descriptive statistics for HEV prevalence and different variables are illustrated in Tables 1 and 2. Table 1 demonstrates the frequencies among the localities and Table 2 presents the frequencies regarding the demographic and risk factors for virus transmission. The Chi-square test for categorical variables was calculated. Further, for the logistic regression of univariate and multivariate analysis, an odds ratio (OR) with a 95% confidence interval (CI 95%) was calculated, and the statistical significance was defined as $P < 0.05$.

3. Results

3.1. Epidemiological and demographical analysis of the study groups

All blood donors recruited for the current study were male, aged between 18 and 50 years and belonging from different localities of Gezira State, Sudan (Tables 1 & 2). Out of the 300 blood donors, 109 (36.3%) were HEV IgG-positive and 191 (63.7%) were negative (Figure **1**). The highest HEV seroprevalence was found among participants in the age

group of 31–40 years, with a percentage of 44.6%, and the lowest rate was predicted among the age group of 18–30 years with a prevalence of 32.5% (Table 2).

3.2. HEV IgG seroprevalence and the associated risk factors localities

The distribution of seroprevalence of HEV IgG among the localities were as follows: 34 (31.5%) in Wad Medani locality, 30 (34.1%) in South Gezira locality, 16 (59.3%) in Managil, 15 (37.5%) in East Gezira, 10 (41.7%) in Um Algura, and 4 (30.7%) in Hasahesa localities ($P > 0.5$).

3.2.1. Occupations

Higher prevalence of the HEV IgG was calculated among eight (47.1%) farmers, while no prevalence of the virus was found among health workers and policemen (Table 2). A significant association was calculated among students and farmers regarding the HEV IgG distribution and the factors of the disease in univariate and multivariate analysis ($P = 0.007$), Table 2.

3.2.2. Animal contact

The HEV IgG seroprevalence regarding the risk of contact with the animals out of 300 blood donors resulted in 26 (46.3%) for blood donors with animal contact and 83 (34%) with no animal contact ($P= 0.083$), Table 2.

3.2.3. Food source

The HEV IgG prevalence concerned the sites of food sources out of the 300 blood donors resulted in 74 (32.6%) at home, 20 (48.8%) outside the home, and 15 (46.9%) at home and outside the home, Table 2.

3.2.4. Water source

Out of the 300 blood donors, 100 (36.2%) HEV IgG-positive used wells as water sources, 5 (62.5%) used rivers, and only 4 (25%) used Hafeer (a hole in the ground used to collect water during the rainy season for using it later, Table 2.

3.2.5. The number for blood donation

The HEV IgG prevalence concerning the numbers for the respondents who donated blood resulted in 89 (35.6%) donating blood once or twice, 13 (50%) donating three to four times, and 7 (29.2%) donating more than four times, Table 2.

3.2.6. HEV IgM and HEV RNA analysis

Out of the 84 blood donors with HEV IgG strong positive samples, only 1.2% (1/84) were positive for HEV IgM, while no HEV RNA was predicted amongst any of them by using an RT-PCR assay.

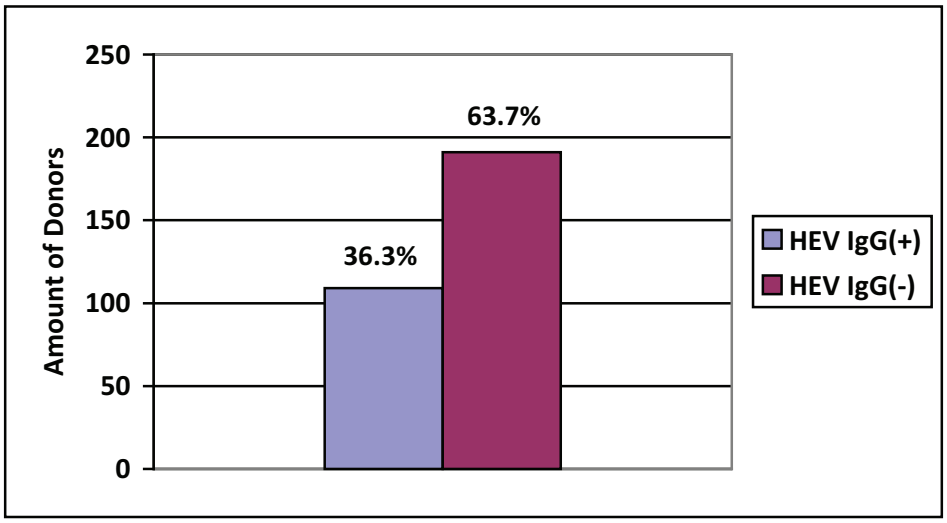

Figure 1: The distribution of HEV IgG among blood donors (*N* = 300).

TABLE 1: The HEV IgG distribution among blood donors according to Gezira State localities (*N* = 300).

Variables	HEV IgG			Univariate			Multivariate		
	Negative	Positive (%)N = 109	Total	OR	CI 95%	P-value	OR	CI 95%	P-value
Wad Medani	74	34 (31.5)	108	0.967	0.278–3.362	0.958	0.854	0.240–3.030	0.806
Um Alqura	14	10 (41.7)	24	0.622	0.149–2.601	0.516	0.587	0.139–2.475	0.468
South Algezira	58	30 (34.1)	88	0.859	0.244–3.022	0.813	0.805	0.227–2.857	0.737
East Algezira	25	15 (37.5)	40	0.741	0.194–2.830	0.661	0.664	0.171–2.58	0.555
Almanagil	11	16 (59.3)	27	0.306	0.075–1.246	0.098	0.305	0.074–1.252	0.099
AlHasahisa	9	4 (30.7)	13	0.306	0.075–1.246	0.098	0.305	0.074–1.252	0.099
Total	191	109 (36.3)	300						

OR, odds ratio; C, 95% confidence interval; HEV, hepatitis E virus.

Table 2: The HEV IgG distribution among blood donors according to demographic and risk factors.

Variables		HEV IgG			Univariate			Multivariate		
		Negative	Positive (%)N = 109	Total	OR	CI 95%	P-value	OR	CI 95%	P-value
Age group (yr)	18-30	135	65 (32.5%)	200	1.038	0.185–5.817	0.966	0.876	0.152–5.028	0.882
	31-40	52	42 (44.6%)	94	0.619	0.108–3.546	0.59	0.544	0.093–3.178	0.499
	>40	4	2 (33.3%)	6	0.619	0.108–3.546	0.59	0.544	0.093–3.178	0.499
Occupation	Casual Workers	117	87 (42.6%)	204	1.195	0.443–3.223	0.724	1.086	0.397–2.97	0.873
	Drivers	16	6 (27.27%)	22	0.579	0.369–5.946	0.579	1.345	0.331–5.471	0.678
	Policemen	8	0	8	2164	0.000–0.000	0.997	2033	0.000–0.000	0.997
	Engineers	7	1 (12.5%)	8	6.222	0.623–62.159	0.12	6.222	0.618–62.6	0.121
	Teachers	3	2 (40%)	5	1.333	0.176–10.120	0.781	1.112	0.144–8.60	0.919
	Health workers	2	0	2	21643	2164–216437	–	1805	1805–1805	–

OR, odds ratio; C, 95% confidence interval; HEV, hepatitis E virus.

4. Discussion

Studies assessing the risk of HEV transmission through blood transfusion are relatively fewer than others conducted for HCV and HBV. Researchers from Japan and Europe indicated that a few cases of HEV were transmitted by blood transfusion and later developed elevation of transaminase levels and fulminant hepatitis [13–15]. Another study showed that some recipients exhibited HEV reactivity after a blood transfusion from an HEV-infected donor [17].

According to our study, HEV IgG seroprevalence among healthy blood donors was 36.3%. This result was higher than that revealed by Ahmed *et al.* [25] among Sudanese blood donors (26.7%) and lower than that reported among Egyptian blood donors (45.3%) [4], but higher than what was reported by other studies from other African countries [6, 7, 19]. Based on the results of this study, the highest prevalence of HEV IgG seroprevalence was among those aged 31–40 years, 42 (44.6%). This finding could reflect a higher probability of virus exposure among this age group, which might be due to occupational hazards (e.g., farmers and animal contact as approved in the study) or other factors, for instance, this age group might be more likely to consume food outside the home which is a known risk factor for foodborne infections.

However, in other studies, such as that conducted by Adjei *et al.*, HEV seroprevalence was more among the elderly than in younger persons. It should be noted that statistically there was no significant association between HEV prevalence and age group among blood donors in our study and this is consistent with a study conducted in the Netherlands [9].

It was obvious, in our study, that those people who drank the river water were at a higher risk of getting infected (5; 62.5%), compared to those drinking from well sources (100; 36.2%) and Hafeer (4; 25%). Some evidence of significant association regarding the virus prevalence concerning the water sources revealed the highest risk of river sources compared to the well source and the lowest risk of Hafeer sources, OR = 5.000, *P* = 0.084 and OR = 5.145, *P* = 0.081; *P* = 0.081, OR = 5.000, CI 95% = 0.806–31.002, respectively, Table 2. This might be due to a higher likelihood of contamination of the rivers by animal and human products than the wells.

Furthermore, our study identified those with a previous history of multiple blood donations have a higher HEV IgG seroprevalence 13 (50%). This is consistent with the results from other studies [13–15, 17] and as the majority of them are young, this leads to the possibility of later donations in the future which could be an additional risk for transmission.

Although, the HEV IgM seropositive in this study was the only donor; therefore, it was a limitation for our study to carry out statistical HEV IgM interpretation. However, the result still increases the probability to transfuse HEV-contaminated blood to those patients with compromised immunity [16]. HEV RNA concluded in a negative result for all blood donors, and that might be for our sample size limitation to carry out the HEV RT-PCR, some authors obtained a similar result for a negative HEV-RNA [9, 20, 24]. This study is novel in Gezira State, Sudan that showed relations and association of risk factors for HEV and blood transfusion. The limitation of the study was the small sample size and the eclipsing period of HEV, and the collection time of the viruses and ages; therefore, we could not reliably assess the true prevalence of IgM- and RNA-positive cases.

5. Conclusion

The results of this study could suggest a high probability of HEV transmission through blood transfusion as reflected by the high HEV seroprevalence among healthy donors. The risk factors that could be identified in this study include the occupation (mainly farmers), animal contact, and river sources for drinking.

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Ethical Considerations

Not applicable.

Competing Interests

None declared.

Availability of Data and Material

The dataset generated during this study is available from the corresponding author on reasonable request.

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