Epidemiology of Hepatitis E Virus infection in a Cohort of 3,480 Blood Donors in Mirpur, Azad Jammu & Kashmir, Pakistan

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Abstract

Introduction: Hepatitis E is caused by hepatitis E virus (HEV), with varying clinical appearances ranging from asymptomatic cases to acute liver failure, particularly in immunocompromised individuals and pregnant women. In Pakistan, despite evidence of transfusion-transmitted HEV infections, routine HEV RNA blood donor screening is not practiced. This study investigates the prevalence of HEV IgM, IgG antibodies, and RNA among blood donors in Mirpur, Azad Jammu & Kashmir (AJK), to assess their potential role as HEV carriers.

Methodology: This cross-sectional study, conducted at the Mirpur Regional Blood Centre, evaluated 3,480 blood donors from October to December 2023. Blood samples were screened for anti-HEV IgG and IgM immunoglobulins using Wantai ELISA, with reactive IgM samples subjected to HEV RNA detection via nested RT-qPCR. Donor demographics and medical histories were collected. Statistical analyses were performed using SPSS, with a significance threshold of $p \leq 0.05$.

Results: Anti-HEV IgG and IgM antibodies were detected in 9.25% and 1.03% of donors, respectively, with an overall HEV seroprevalence of 10.28%. HEV RNA was found in 0.11% of samples. Past infection rates increased significantly with age but showed no gender differences. All HEV RNA-positive donors were asymptomatic males, predominantly aged >30 years.

Conclusion: The study highlights the presence of recent HEV infections (1.03%) and potential transfusion-transmission risk. While no direct evidence of transfusion-transmitted HEV was observed, implementing HEV RNA screening in blood banks is recommended to enhance transfusion safety, especially for immunocompromised patients.

Key words: Hepatitis E Virus, Transfusion-transmitted, RNA,

Introduction

Hepatitis E is an intestinal diseases caused by hepatitis E virus (HEV). Only a small portion of patient infected with HEV showed sign and symptoms likewise hepatitis A virus.[1] The first

report of HEV infection was from India in 1978 during the hepatitis epidemic after a faecal contamination of drinking water.[2,3] The epidemic was first identified as Hepatitis V virus occurrence; though, later it was credited to HEV contagion on retrospective study.[4]

Both epidemic and sporadic hepatitis can be caused by HEV. In most cases, the infection is asymptomatic or self-limiting. However, serious clinical consequences, such as fulminant hepatic failure with a high mortality rate (20–30%), can result from acute infection in pregnant women.[5]

HEV is a single-stranded RNA virus without envelop that is a member of the Orthohepevirus A species, Orthohepevirus genus, and Hepeviridae family. Five of the eight different Orthohepevirus A genotypes (HEV-1, -4, and -7) are capable of infecting humans.[6] Only humans are infected by HEV-1 and -2. Other species like pigs, deer, wild boars, rabbits (HEV-3 and -4) and camelids (HEV-7) are also infected by the other three genotypes.[7] While HEV-4 is more common in Asia but is also found in Europe, HEV-3 is found all over the world. A person who consumed camel milk and flesh was the first to be found infected with HEV-7.[8] The majority of HEV-3 or HEV-4 infections are asymptomatic, and those over 40 years of age are primarily affected by the clinical disease. These infections (mostly HEV-3, but occasionally HEV-4 and HEV-7) can become chronic in immunocompromised persons and even cause liver cirrhosis.[9, 10]

According to the World Health Organization, there are about 20 million individuals infested by the HEV infection each year (July 2023), greater than 3.3 million acute HEV characteristic cases of, affecting 70,000 deaths, are reported per annum.[11]

Immunoglobulin (IgM) is first antibody at a week 4, followed by immunoglobulin G (IgG) at week 5 after the infection.. The typical duration of IgM positivity is between 8 to 12 weeks. Viremia seems in period of acute infection in second week and normally lasts for 3 weeks to month but can preceding for 2 months.

Geographical location, dietary and hygiene practices, and environmental factors all influence the prevalence of HEV contact, which varies among and within developing and industrialized countries. HEV is typically spread by consuming food and water tainted with HEV infectors.[12] Animals are now the main source of HEV infection in humans due to improvements in sanitary infrastructure and water supply in developed countries. However, the primary cause of illnesses in developing low-income countries is fecal-oral, typically through contaminated water. Furthermore, within the past 20 years, there has been a documented rise in transfusion transmitted HEV contagions from the blood donors having no symptoms, which result in severe diseases for immunocompromised patients.

As mentioned, nearly everywhere in the globe, the possibility of transfusion-transmitted HEV has increased. These findings led to the implementation of universal or selective HEV RNA screening of blood donors in many European countries and a few others since 2012.[13] However, regular or targeted HEV blood donor screening has not yet been implemented in Pakistan. This experimental study aimed to assess the prevalence of IgM, IgG immunoglobulin and HEV RNA in the blood donor's sera as representatives of the broad inhabitants.

Methodology

The Divisional Headquarters (DHQ) Teaching Hospital, Mirpur's Ethical Review Board granted approval for this study by letter number DHQ/ERB-10/2234. The Mirpur Regional Blood Centre, located within the DHQ Teaching Hospital in Mirpur, Azad Jammu & Kashmir (AJK), Pakistan, collected 3,480 blood donor samples at random between October and December 2023. Every blood donor met the national requirements for blood donations and gave written authorisation for the use of their blood samples for research purposes.[14] During routine donor screening, all donor samples were examined for syphilis, malaria, anti-HIV-1/2, anti-HCV, and HBsAg. The donors filled out a questionnaire regarding their medical history and demographics such as age and sex.

The Wantai HEV-IgG and HEV-IgM ELISA (Wantai, Biologic Pharmacy Enterprise, Beijing, China) was used to examine all blood specimens for anti-HEV IgG and anti-HEV IgM immunoglobulin. The tests were accomplished in compliance with the standard guidelines. The statistical determination of the assay's cut-off values was based on the mean OD value of the -ive controls at wavelength of 450 nm plus 0.12. The ORF2-region-expressed recombinant antigens are the focus of both the IgG and IgM anti-HEV testing. Absorbance value of the test sample/absorbance value of the negative control (P/N) was used to express absorbance values. Positive samples were those with a P/N $\geq$ 2.5, the cut-off value. Specimens were subjected to PCR (polymerase chain reaction) testing when P/N values were near to the cut-off value.

HEV RNA was only examined in specimens that were anti-HEV IgM reactive. In accordance with the standards guidelines, 200 µl of each sample was used to extract HEV RNA. Using the commercial kits QIAGEN OneStep RT-qPCR Kit and HotStar Taq Master Mix (Qiagen, Hilden, Germany), nested RT-qPCR was used to identify HEV RNA in anti-HEV IgM positive plasma samples using previously described primer sets.[15] Following the use of a viral DNA/RNA extraction kit to extract viral RNA from 200.00 µl of serum, PCR was performed as follows; reverse transcription at 50°C for 5 minutes, denaturation at 95°C, 40 cycles of denaturation at 95°C for 10s, and annealing and extension at 60°C for 30s. Five genome equivalent (GE) copies of HEV plasmid DNA were found using the TaqMan® assay. This analytical method has a 95% LOD (limit of detection) of 7.9 IU/ml and can detect all four genotypes of HEV with a 95% LOD in samples with a HEV RNA viral load between 7.9 and 17.7 IU/ml.

The prevalences of anti-HEV IgG, anti-HEV IgM, HEV RNA, and their 95% confidence intervals (CI) were calculated. Statistical analysis was carried out using SPSS version 25.0 for Windows (SPSS Inc., Chicago, USA). A *p* value of 0.05 or less was considered to indicate significant differences.

Results

A total of 3,480 blood donors (89.66% males, 10.34% females; median age: 27.4 yrs; while age range: 18-51 yrs) were enrolled in the investigation and assessed for HEV infection markers. Among the 3,480 blood specimens received from donors in the Mirpur Regional Blood Centre,anti-HEV IgG, and IgM positivity rate among donors were 9.25% (322/3,480; 95% CI 8.28% - 10.21%) and 1.03% (36/3,480; 95% CI 0.69% - 1.36%), respectively. HEV seropositive were those patients who have anti-HEV IgG or IgM positive (reactive) results.

The HEV seroprevalence was 10.28% (358/3,480; 95% CI 9.27% - 11.28%). No substantial variances in relation to anti-HEV IgG seropositivity were established between males and females (9.55% and 6.67% were positive, respectively). Past infection prevalence (Anti-HEV IgG positivity) increased significantly with age, ranging from 7.62% amongst donors aged 18-30 years to 12.63% amongst donors 30 years and older (Table 1).

Following their enrolment in the study, 3,480 blood donors (89.66% males, 10.34% females; median age: 27.4 years; age range: 18-51 years) had their HEV infection markers tested. Anti-HEV IgG and IgM positive rates amongst donors were 9.25% (322/3,480; 95% CI 8.28% - 10.21%) and 1.03% (36/3,480; 95% CI 0.69% - 1.36%), respectively, out of the 3,480 blood samples obtained from donors. Donors were considered HEV seropositive if they showed any reactive anti-HEV IgG or IgM findings. Hence, 10.28% (358/3,480; 95% CI 9.27% - 11.28%) was the HEV seroprevalence. Males and females did not vary knowingly in their anti-HEV IgG seropositivity (9.55% and 6.67% were positive, respectively). The prevalence of past HEV infection rose significantly by oldness, from 7.62% among donors aged 18-30 to 12.63% among donors aged 30 and above (Table 2).

Table 1: Serological results of HEV biomarkers

HEV Biomarkers	Reactive (%)	Confidence Interval (95%)
Anti-HEV IgG	322 (9.25%)	8.28% - 10.21%
Anti-HEV IgM	36 (1.03%)	0.69% - 1.36%
HEV Seroprevalence	358 (10.28%)	9.27% - 11.28%

Table 2: Blood donors' data and HEV serology results on the basis of gender & age

Blood donors (n=3,480)		Total	Anti-HEV IgG (Pos)	Anti-HEV IgG (Neg)	Confidence Interval (95%)	p-value
Gender	Male	3,120/3,480 (89.66%)	298/3,120 (9.55%)	2,822/3,120 (90.45%)	0.08%-0.10%	0.5572
	Female	360/3,480 (10.37%)	24/360 (6.67%)	336/360 (93.33%)	0.04%-0.09%	0.1008
Age	18-30	2,348/3,480 (67.47%)	179/2,348 (7.62%)	2,169/2,348 (92.38%)	0.06%-0.08%	0.0060
	31-51	1,132/3,480 (32.53%)	143/1,132 (12.63%)	989/1,132 (87.37%)	0.10%-0.14%	0.0001

Four of the 36 anti-HEV IgM reactive samples tested positive for HEV RNA, according to the results of the RT-qPCR test. HEV RNA samples had a median viral load of 1,554 IU/ml, with a range of 54 to 2,500 IU/ml. Therefore, HEV RNA's overall positive rate was 0.11% (4/3,480). With mean age of 37.4 yrs, the demographic information of RT-qPCR reactive donors showed that they were mostly male (75%; 3/4).

Discussion:

Given the lack of routine testing for HEV infection in Pakistan and the paucity of information on the epidemiology of HEV in blood donors, the purpose of this investigation was to assess the seroprevalence of HEV exposure amongst blood donors from the northern Pakistani state of Azad Jammu and Kashmir.

Hepatitis E is an emerging infection that transfusion medicine is concerned about, as seen by the mounting trend of the HEV contagion amongst the donors and recipients of transfusion worldwide.[16] In a recent review by our team, it was found that since 2004, there have been 86 documented cases (from 20 studies) of HEV infection resulting from blood transfusions.[17] These cases were confirmed by testing the pre-transfusion & post-transfusion HEV infection status of the blood transfusion recipient and linking the genotype resemblance with that of the intended blood donors, which is characterized as 'trace back' in transfusion medicine.[18] Globally, as of 2024, 26 studies have reported TT-HEV infections, while 101 research articles have testified the prevalence of the HEV amongst the donors.[19]

The prevalence of anti-HEV IgM shows variation amongst diverse nations, with a higher reported prevalence of anti-HEV IgM in endemic regions and developing countries. When we compared our findings of 1.03%, some countries showed a lower percentage of prevalence including China 0.71%,[20] Scotland 0%,[21] India 0.2%,[22] Qatar 0.58%,[23] Italy 0.4%,[24] and the USA 0.58%.[25] On the other hand, some countries have shown a comparatively higher prevalence namely Nepal 2.98%,[26] Burkina Faso 1.9%,[27] and Croatia 4.44%.[28] One of ours previously study from Peshawar, Pakistan exhibited anti-HEV IgM prevalence of 2.04% in 2020.[29]

At the molecular level, our findings of 0.11% for HEV RNA prevalence is slightly higher than those reported from Japan (0.005%),[30] China (0.06%),[31] Germany (0.08%),[32] Australia (0.001%),[33] and Sweden (0.01%).[34]

These dissimilarities may be attributed to the sensitivity and specificity of the tests used and the variation in the epidemiology of HEV in diverse areas of the biosphere.

Conclusions:

Although this study did not find any direct evidence of HEV transmission through transfusion, 1.03% of blood donors tested positive for anti-HEV IgM, indicating a recent infection that could increase the risk of the transfusion transmitted HEV infections. For immunocompromised individuals, transfusion-transmitted HEV infections pose a concern and may result in serious or even fatal consequences.[35]

All anti-HEV IgM reactive donors were non-symptomatic, none of them showed the development of the chronic HEV infections, and no HEV re-infection was also observed. Our findings lend credence to the idea that HEV RNA screening of blood donors makes the blood safe for recipients, particularly for patients with immunosuppression.

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