

Original Article

Molecular Epidemiology of Hepatitis E Virus Genotypes in Blood Donors in Peshawar, Khyber Pakhtunkhwa

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ABSTRACT

Background: Hepatitis E Virus (HEV) is a significant cause of viral hepatitis worldwide, with a notable prevalence in developing countries. It poses a considerable risk to blood safety, particularly through transfusion-transmitted infections. The epidemiology of HEV, including its genotypes, varies geographically, influencing disease transmission and public health strategies.

Objective: This study aimed to determine the seroprevalence and genotype distribution of HEV among blood donors in Peshawar, Khyber Pakhtunkhwa, and to assess the implications for blood transfusion safety and public health policies.

Methods: A cross-sectional study was conducted with 3,480 volunteer blood donors at the Regional Blood Center, Peshawar. Samples were screened for HEV antibodies (IgM and IgG) using Chemiluminescence Immunoassay (CLIA) and for HEV RNA through Nested RT-PCR. Sociodemographic data were collected and analyzed to evaluate the association with HEV prevalence. The genotypes of HEV RNA-positive samples were determined using specific gene sequencing techniques.

Results: Of the 3,480 donors, 170 (4.9%) were positive for HEV antibodies, with 60 (1.72%) positive for IgM antibodies, indicating recent or ongoing infection. The prevalence of HEV RNA was found to be 0.51%. Genotype analysis revealed the exclusive presence of Genotype 1 among HEV RNA-positive samples. Seroprevalence was higher in males (99.41% of positive cases) and most common in the 18-25 and 25-34 age groups, suggesting age and gender as significant risk factors.

Conclusion: The study indicates a considerable prevalence of HEV among blood donors in Peshawar, with Genotype 1 being predominant. These findings highlight the necessity for routine HEV screening in blood donors, especially in endemic regions, to enhance blood transfusion safety and guide public health interventions.

Keywords: Hepatitis E Virus, HEV Seroprevalence, Blood Donors, Genotype Distribution, Blood Safety, Transfusion-Transmitted Infections, Public Health, Peshawar.

INTRODUCTION

Blood transfusion remains a critical component of contemporary medical practices, providing essential support for patients undergoing acute medical crises that lead to significant blood loss. The paramount importance of ensuring the safety of blood transfusions cannot be overstated, as it serves as a crucial preventive measure against the transmission of infectious agents, both bacterial and viral, from donors to recipients. This safety measure is especially vital in surgical contexts where the likelihood of substantial blood loss is high. Furthermore, blood transfusions play a pivotal role in the treatment of anemia, constituting a fundamental element in the therapeutic strategies for various clinical conditions(1).

Among the viral agents of concern, Hepatitis E Virus (HEV), a small, non-enveloped entity with a positive-sense RNA genome approximately 7.2 kilobases in length and a diameter ranging from 26 to 34 nm, has emerged as a significant global public health challenge. As the foremost cause of acute viral hepatitis worldwide, HEV has been identified as a critical agent of enteric hepatitis(2). Despite the high global incidence of HEV infections, with the World Health Organization estimating around 2.3 billion cases, over 3 million confirmed cases, and 70,000 deaths annually, a large proportion of these infections remain asymptomatic. However, they

have the potential to escalate into severe liver failure. Particularly noteworthy is the association of chronic hepatitis E, primarily with genotype 3, among organ transplant recipients(3, 4).

The Hepeviridae family, to which HEV belongs, has been classified by the International Committee on Taxonomy of Viruses into two genera: Piscihepevirus and Orthohepevirus A. Within the Orthohepevirus A genus, eight genotypes, HEV 1 to HEV 8, have been delineated. The initial two genotypes, HEV 1 and HEV 2, are primarily implicated in human infections, while genotypes HEV 3 and HEV 4 are known to infect both pigs and humans. HEV 5 and HEV 6 have been isolated from wild boars in Japan, whereas HEV 7 and HEV 8 have been identified in dromedaries and Bactrian camels, respectively(5). This diversity in genotypes and host species underlines the complex epidemiology of HEV, which includes zoonotic transmission routes, particularly for HEV 4, through the consumption of undercooked food or direct contact with infected animals(6).

The variation in the seroprevalence of HEV antibodies among blood donors in developed countries is notable, with rates ranging widely from 2% to 87%. This variability underscores the potential risk of HEV transmission through blood transfusion from asymptomatic donors, a risk that can be mitigated through the application of Nucleic Acid Testing (NAT) for the detection of HEV RNA(7-9). Despite the acknowledged seroprevalence, there remains a paucity of data regarding the seroprevalence of HEV antibodies and the distribution of its genotypes among blood donors in Pakistan. This study aims to fill that gap by investigating the seroprevalence of the Hepatitis E virus among healthy blood donors at the Regional Blood Center in Peshawar and identifying the predominant HEV genotype circulating among this population.

MATERIAL AND METHODS

The study was designed to investigate the seroprevalence and genotype distribution of the Hepatitis E virus (HEV) among blood donors at the Regional Blood Center in Peshawar, Khyber Pakhtunkhwa, Pakistan. The methodology adhered to the principles outlined in the Declaration of Helsinki for ethical research involving human subjects. The Regional Blood Center Peshawar Ethics Committee and the Medical University Shaheed Zulfiqar Ali Bhutto, Islamabad, had granted approval for the study protocol, ensuring that all procedures were performed in compliance with ethical standards.

For sample collection, approximately 5mL of blood was extracted from each donor bag and immediately transferred into a yellow-capped sterile tube to prevent contamination. The blood samples were then subjected to centrifugation at 14,000 revolutions per minute (rpm) for five minutes. This process enabled the separation of plasma from the whole blood, which was subsequently utilized for serological analyses to detect the presence of HEV antibodies.

The serological examination employed the Chemiluminescence Immunoassay (CLIA) technology, utilizing Wantai's HEV IgM ELISA kit (catalog number WE 71966) and HEV IgG ELISA kit (catalog number WE 7296) (10). This approach facilitated the detection of anti-HEV IgM and IgG antibodies, indicating recent and past infections, respectively. The plasma specimens were stored at -80°C in preparation for further molecular testing.

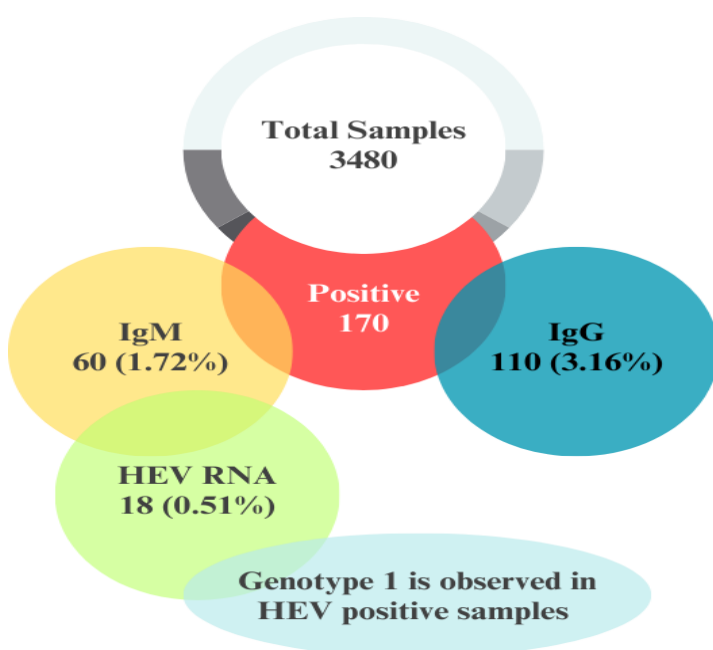


Figure 1 Flowchart of the study characteristics

The molecular analysis involved the extraction of HEV RNA from 200 microliters of serum using the QIAamp Viral RNA Mini Kit, following the manufacturer's instructions(11). This was a critical step for the subsequent Reverse Transcription Real-Time PCR (RT-PCR) analysis. The RT-PCR process commenced with the conversion of RNA into complementary DNA (cDNA) in a 20 µL reaction mixture. Specific gene primers targeting the ORF2 domain of the HEV genome were employed, including a forward primer (GTATGYTYTGCATACATGGCT) and a reverse primer (AGCCGACGAAATYAATTCTGTC). The RT-PCR conditions were meticulously set to ensure the accurate amplification of the HEV genetic material.

For genotyping, a nested reverse transcription approach was adopted to amplify a core segment of reading frame 2 (nucleotide positions 5261 to 5330), essential for identifying conserved sequences for primer design targeting the HEV genome(13). The amplified products were then subjected to gel electrophoresis to ascertain their size and purified for sequencing. The obtained sequences were analyzed using

Bionumerics Applied Maths and compared against known sequences in the NCBI BLAST database to determine the HEV genotypes present.

Data analysis was performed using SPSS version 22.0, employing a p-Test to determine statistical significance, with a p-value of 0.05 or lower considered indicative of significant findings. This comprehensive methodology ensured the rigorous investigation of HEV seroprevalence and genotype distribution among blood donors, contributing valuable insights to the epidemiological understanding of HEV in the region.

RESULTS

The study meticulously investigated the sociodemographic characteristics and Hepatitis E virus (HEV) prevalence among a substantial cohort of 3,480 blood donors. In this context, a significant emphasis was placed on analyzing gender distribution, revealing a pronounced discrepancy in HEV reactivity between males and females. Specifically, a staggering 99.41% of reactive cases were observed in males, equating to 169 individuals, compared to a mere 0.59% in females, representing only a single individual (Table 2). This gender disparity is underscored by a highly significant p-value of 0.00001, indicating a statistically substantial difference in HEV prevalence between genders.

Age distribution among the donors further delineated the prevalence of HEV, with younger donors aged below 25 years and those within the 25–34 age bracket collectively accounting for a substantial majority (84.71%) of the reactive cases. Notably, the age groups of <25 and 25–34 years exhibited HEV reactivity in 70 (41.18%) and 74 (43.53%) individuals, respectively. This distribution underscores a marked concentration of HEV cases within the younger population segments, a trend that was statistically significant with a p-value of 0.00001, denoting a pronounced variance across different age groups (Table 2).

The educational background of the donors presented an intriguing insight, albeit without a statistically significant association with HEV prevalence (p-value = 0.238). Donors with no formal education and those with primary education constituted the largest proportions of HEV-reactive individuals, at 23.53% and 31.77%, respectively. This observation suggests a nuanced relationship between education level and HEV prevalence, warranting further investigation (Table 2).

Residential patterns offered another layer of analysis, with a slightly higher prevalence of HEV observed in urban dwellers (65.88%) compared to their rural counterparts (34.12%). However, this difference was not statistically significant (p-value = 0.5611), indicating a relatively uniform distribution of HEV prevalence across different residential settings (Table 2).

Regarding the donors' history, a slight majority of the HEV-reactive group were first-time donors (72.35%), in contrast to 27.65% who were repeated and regular donors. This finding, while indicative of the demographic makeup of the reactive cohort, did not present a statistically significant correlation with HEV reactivity (p-value = 0.567) (Table 2).

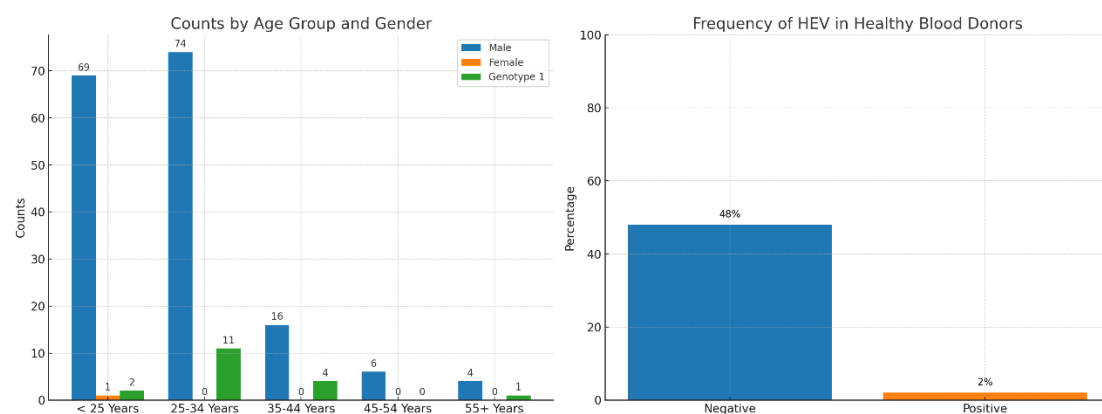


Figure 2 HEV Status, Age and Gender

The prevalence of HEV among blood donors was further contextualized through an international comparison, with studies from India, Algeria, Brazil, Italy, and Poland offering a broad spectrum of prevalence rates. Notably, the study from Poland reported the highest prevalence rate at 43.4% for HEV IgG, contrasting sharply with the lower rates observed in other studies, such as 0.20% in Pune, India, and 0.16% in Setif, Algeria, for HEV IgM. These discrepancies highlight the geographic variability in HEV prevalence and underscore the necessity for localized studies to accurately gauge HEV impact within specific populations (Table 3).

Table 1: Sociodemographic Characteristics of Blood Donors (n=3,480)

Variable	Reactive	Non-Reactive	Total	P-value
Gender				0.00001
Male	169 (99.41%)	2,758 (83.32%)	2,927	
Female	1 (0.59%)	552 (16.68%)	553	
Age Group				0.00001
< 25	70 (41.18%)	980 (29.61%)	1,050	
25–34	74 (43.53%)	1,009 (30.48%)	1,083	
35–44	16 (9.41%)	502 (15.17%)	518	
45–54	6 (3.53%)	478 (14.44%)	484	
Above 55	4 (2.35%)	341 (10.30%)	345	
Education				0.238
No formal education	40 (23.53%)	849 (25.65%)	889	
Primary school	54 (31.77%)	929 (28.07%)	983	
Secondary school	44 (25.88%)	727 (21.96%)	771	
Grad & postgrad	32 (18.82%)	805 (24.32%)	837	
Residence				0.5611
Urban area	112 (65.88%)	2,108 (63.68%)	2,220	
Rural area	58 (34.12%)	1,202 (36.32%)	1,260	
Donor History				0.567
First time	123 (72.35%)	2,460 (74.32%)	2,583	
Repeated/Regular	47 (27.65%)	850 (25.68%)	897	

Table 2: Prevalence of HEV Among Blood Donors in Various Studies

Author	Location	Year	Screening Mode	Sample Size	Positive	Prevalence
Tripathy et al.	Pune, India	2019	IgG/IgM	2,447	433/5	17.70%/0.20%
Gajjar et al.	Ahmedabad, Gujarat	2014	IgM	460	22	4.78%
Boukhrissa et al.	Setif, Algeria	2022	IgM/IgG	434	7/74	0.16%/17.05%
Silva et al.	Brazil	2019	IgG	281	281	7.1%
Marcantanio et al.	Rome, Italy	2019	IgM/IgG	198	2/7	1.01%/3.5%
Grabarczyk et al.	Poland	2018	IgM/IgG	12,664	39/1340	1.27%/43.4%

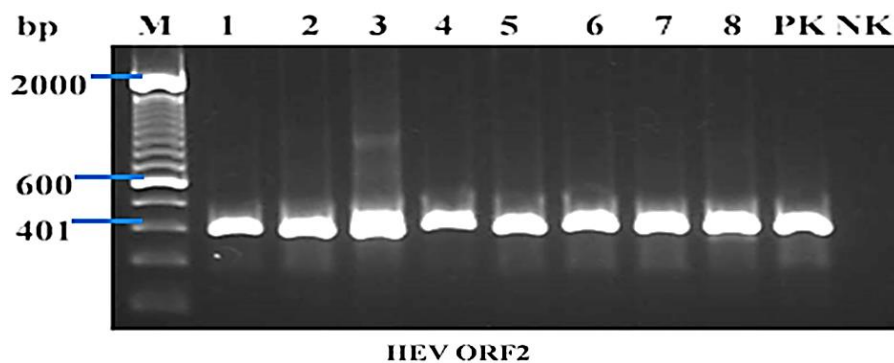


Figure 3 Gel image displaying 401 bp HEV ORF2 fragments from healthy donor samples using Nested RT-PCR. Lanes 1-8 are HEV-positive, "PK" is the positive control, "Nk" the negative control, and "M" the 100 bp DNA ladder

The graphical analysis presented on the single canvas further embellishes these findings, showcasing not only the stark gender disparity in HEV reactivity but also the distribution across different age groups. This visual representation, complemented by data labels for immediate clarity, emphasizes the higher incidence of HEV among younger male donors, a pattern that aligns with the detailed numerical analysis provided in the tables. Furthermore, the graph depicting HEV frequency among healthy blood donors, with

48% negative and 2% positive, offers a concise visual summary of the overall HEV landscape within the studied cohort, encapsulating the core findings of the research in a comprehensible and accessible manner.

The combined data from tables and graphical representations paint a comprehensive picture of HEV prevalence and sociodemographic characteristics among blood donors, highlighting significant gender and age-related disparities, alongside an

exploration of educational and residential influences. These insights are crucial for tailoring effective screening strategies and public health interventions to mitigate the risk of HEV transmission through blood transfusion.

DISCUSSION

In the exploration of viral hepatitis, a condition that poses a significant public health challenge, especially in developing regions like the Middle East and Africa, this study has shed light on the prevalence and molecular epidemiology of the Hepatitis E virus (HEV) among blood donors in Peshawar, Khyber Pakhtunkhwa. HEV, often associated with outbreaks in countries with lower socioeconomic conditions such as India, Pakistan, Bangladesh, and Nigeria, has seen sporadic cases in economically advanced regions of Europe and South America, marking it as an entity of growing concern, particularly in the realm of transfusion services(20, 21). The seroprevalence of HEV among volunteer blood donors in this study was found to be 4.9%, indicating a higher prevalence when compared to figures reported in developed countries like Brazil (6.4%) (22) and England (2.8%)(23), yet lower than the prevalence observed in Chennai, India (11.2%)(27). This variation in prevalence rates underscores the influence of regional healthcare management and access to clean water on the incidence of HEV(28).

Of the 3,480 donors screened, 170 tested positive for HEV antibodies, with 60 (1.72%) being positive for IgM antibodies, suggesting an ongoing or recent infection. This rate aligns closely with findings from other studies across various countries, illustrating a consistent pattern of Anti-HEV IgM prevalence(7, 18, 29, 30). The age distribution among the study participants highlighted an increased risk of HEV infection with advancing age, especially prominent in the younger age groups, a trend also observed in other international studies(31, 32). Educational attainment emerged as a notable demographic factor, with a significant portion of those testing positive for HEV having only primary education(33), pointing towards education as a potential risk factor.

The prevalence of HEV viral RNA in this study was 0.51%, comparable to findings in India (0.53%)(34) but higher than rates reported in European countries, indicating geographical disparities in viral RNA prevalence among blood donors(35, 36). The detection of Genotype 1 exclusively among HEV RNA-positive samples underscores the predominance of this strain in the region, mirroring findings from similar studies in Iran and India(34, 38). This contrasts with the global distribution of HEV genotypes, where Genotypes 1 and 2 are commonly found in Asia and Africa through waterborne transmission, while Genotypes 3 and 4, associated with zoonotic transmission, are more prevalent in industrialized nations(39, 40).

This investigation not only contributes to the understanding of HEV epidemiology but also highlights the importance of integrating HEV screening into the standard protocols for blood transfusion services, especially in areas with high HEV prevalence. The exclusive presence of Genotype 1 in this study's HEV RNA-positive samples suggests a localized circulation of this strain, necessitating targeted public health strategies to mitigate the risk of transfusion-transmitted HEV.

The study, while comprehensive, acknowledges certain limitations, including its cross-sectional nature and the focus on a single geographic region, which may not fully represent the diversity of HEV genotypes. Furthermore, the reliance on self-reported data for some sociodemographic variables could introduce bias. Future research should aim to encompass a broader geographic area and include longitudinal studies to track HEV incidence and genotype variations over time. Implementing routine screening for HEV in blood banks, especially in endemic regions, alongside public health campaigns to raise awareness about HEV transmission, could significantly reduce the risk of transfusion-associated HEV infections. This study's findings emphasize the need for continued surveillance and research into HEV, forming a foundation for future efforts to combat this public health challenge.

CONCLUSION

This study highlights the significant prevalence of Hepatitis E Virus (HEV) among blood donors in Peshawar, Khyber Pakhtunkhwa, with a particular emphasis on the exclusive presence of Genotype 1. The findings, revealing a 4.9% seroprevalence of HEV antibodies and a 0.51% prevalence of HEV viral RNA, underscore the critical need for integrating HEV screening into the standard protocols for blood transfusion services in endemic regions. These insights carry profound implications for human healthcare, suggesting that targeted public health strategies, including routine screening and educational campaigns, are essential to mitigate the risk of transfusion-transmitted HEV infections. Such measures will not only enhance blood safety but also contribute to the broader efforts in controlling the spread of HEV in populations at risk.

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