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Hepatitis E Virus Outbreak in an Internally Displaced Population of Afghanistan: Is it a Risk to Transfusion Safety?

***Akhlaaq Wazeer*[1]**
***Enayatullah Hashemi*[2]**
***Noore Saba*[3]**
***Zahida Qasim*[1,4]**
***Aurooj Fatima*[4]**
***Jahangir Anjum*[4]**
***Raja Tahir Mahmood*[1]**
***Usman Waheed*[2,5]**

[1] Department of Biotechnology, Mirpur University of Science and Technology, Mirpur, Azad Jammu and Kashmir, Pakistan
[2] Afghan National Blood Safety and Transfusion Service, General Directorate of Curative Medicine, Ministry of Public Health, Afghanistan
[3] Peshawar Regional Blood Centre, Provincial Ministry of Health, Khyber Pakhtunkhwa, Pakistan
[4] Mirpur Regional Blood Centre, Department of Pathology and Transfusion Medicine, Divisional Headquarters Teaching Hospital, Mirpur, AJK, Pakistan
[5] Department of Allied Health Sciences, Islamabad Medical and Dental College, Islamabad, Pakistan

Address for Correspondence
Dr. Enayatullah Hashemi
Director, Afghan National Blood Safety and Transfusion Service, General Directorate of Curative Medicine, Ministry of Public Health, Afghanistan.
Email: dr.enayath@gmail.com

Abstract

Objective: The objective of this study was to investigate the Hepatitis E virus (HEV) outbreak in an internally displaced population (IDP) of Afghanistan, specifically in the Jalalabad region near the Torkham border.

Methodology: Blood samples were collected from 544 suspected cases in IDP camps in Jalalabad, Afghanistan, near the Torkham border. The samples were screened for HEV antibodies (IgM and IgG) and alanine aminotransferase (ALT). Subsequently, the samples were transported to the Peshawar Regional Blood Centre in Pakistan for serological and molecular analyses. The data were stored in Microsoft Excel 2013 and analyzed by the IBM Statistical Package for Social Sciences version 24.0 Armonk, NY: IBM Corp. for frequency, percentage, mean, and standard deviation. The Chi-square test was used where appropriate, and a p-value of < 0.05 was considered significant with a 95% confidence level.

Results: Out of the 544 samples serologically screened, 135 (24.81%) tested positive for HEV IgM, while 59 (10.84%) were reactive for HEV IgG. Among these samples, only 41 that were HEV IgM reactive also showed reactivity for HEV IgG. Within the cohort of 135 HEV IgM positive patients, 80 (59.25%) had donated blood for their family or friends at least once in the past three years, and all of them were males. Additionally, among the 135 HEV IgM positive samples, 86 (63.70%) tested positive for HEV RNA. The positivity frequency of HEV RNA was 100% (76/76) for symptomatic cases and 16.94% (10/59) for asymptomatic cases.

Conclusion: Our findings suggest that drinking river water was a potential source of HEV infection in this outbreak. It is prudent to consider hepatitis E as a potential risk to blood safety, especially considering that the majority of the infected cases were males with a history of recent blood donation. Furthermore, it is worth noting that there is currently no screening facility for HEV in any blood establishment.

Keywords: Hepatitis E, outbreak, IDP, Afghanistan, blood safety

Introduction

Hepatitis E virus (HEV) was identified as an epidemic of non-A, non-B hepatitis in the early 1980s.[1, 2] It belongs to the Hepeviridae family of RNA viruses.[3] HEV infection is primarily transmitted enterically and is usually self-limiting, but it poses a potential risk to immunocompromised individuals, leading to significant morbidity and mortality.[4]

The mortality rate can exceed 20% in patients with chronic liver disease, cirrhosis, or pregnancy.[5] According to the World Health Organization (WHO), Hepatitis E virus is responsible for 20 million infections and 44,000 deaths annually, accounting for 3.3% of global deaths due to viral hepatitis.[6]

The high prevalence of HEV among the general population has raised concerns about blood safety, with an increase in cases of transfusion-transmitted HEV.[7, 8] Outbreaks of HEV are frequently reported in countries with limited access to

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clean water, healthcare services, sanitation, and hygiene, among other factors.[9, 10] HEV cases often go undiagnosed or are misdiagnosed as other types of viral hepatitis due to the similar clinical presentation and the lack of specific screening assays for HEV.

In recent times, HEV outbreaks have been reported in regions experiencing humanitarian crises and emergencies, such as war-torn areas and internally displaced population (IDP) camps.[11-13] In Afghanistan, where an ongoing emergency has led to the displacement of individuals from remote areas to Jalalabad and the eastern parts of the country near the Torkham border with Pakistan, refugee camps have emerged, straining the available sanitation facilities. In October 2021, a high number of cases with HEV-like symptoms were reported from these camps. Given that the country's transfusion system heavily relies on family replacement donors, the national blood service (ANBSTS) collaborated with the Regional Blood Centre in Peshawar to investigate the outbreak. Therefore, the specific goal of this study was to investigate the HEV outbreak to assist in preventing future incidents.

Methodology

Blood samples of 544 suspected cases were collected from IDP camps in Jalalabad, Afghanistan leading to the Torkham border. Samples were screened for HEV antibodies (IgM and IgG) and alanine aminotransferase (ALT). Samples were transported to the Peshawar Regional Blood Centre in Pakistan (45 kilometres from the Afghan border) where the serological and molecular analyses were performed. HEV antibodies testing was accomplished by the chemiluminescence immunoassay using the Architect i2000SR system (Abbott Diagnostics, Pvt Ltd, USA).

For qualitative RNA detection, HEV RNA was extracted from an IgM-positive sample using the extraction kit from Qiagen. For RNA detection, an RT-PCR assay was employed. The primers were used following a study by Yin *et al.,*[14] including forward primer (JVHEVF: 5′-GGTGGTTTCTGGGGTGAC-3′), reverse primer (JVHEVR: 5′-AGGGGTTGGTTGGATGAA -3′) and probe (JVHEVPmod: 5′-FAM-TGATTCTCAGCCCTTCGC- MGB-3′). RT-PCR conditions were as follows: 30 minutes at 42 °C, 10 seconds at 95 °C, 40 cycles at 95 °C for 5 seconds, and 34 seconds at 60 °C. A cycle threshold value < 37 was perceived as confirmatory for HEV RNA. A questionnaire assessing possible risk factors, demographic data, previous history of blood donation, and clinical symptoms was filled out by the study subjects.

Ethical approval of the study was granted by the Ethical Board of the Peshawar Regional Blood Centre, Pakistan. The privacy and confidentiality of participants were ensured at all levels during the research comprising of non-disclosure of respondent's identity and the use of a distinctive identification number. The study was implemented in agreement with the Helsinki Declaration (World Medical Association).[15] The clinical, as well as epidemiological data, were retained only for therapeutic and research purposes. All study participants provided informed consent before the collection of the blood samples.

The data were stored in Microsoft Excel 2013 and analyzed by the IBM Statistical Package for Social Sciences version 24.0 Armonk, NY: IBM Corp. for frequency, percentage, mean, and standard deviation. The Chi-square test was used where appropriate, and a p-value of < 0.05 was considered significant with a 95% confidence level.

Results

Of the 544 samples serologically screened, 135 (24.81%) tested positive for HEV IgM, while 59 (10.84%) were reactive for HEV IgG. Only 41 of the HEV IgM reactive samples were also HEV IgG reactive. Of the 135 HEV IgM reactive participants, 102 (75.55%) were males (age range 12 - 63 years; mean 29.6), while 33 (24.55%) were females (age range 16 - 49 years; mean 24.7). None of the females was pregnant at the time of testing. Among this 135 cohort of patients, 80 (59.25%) had donated blood at least once for their family or friends in the past three years and they were all males.

Of the 135 HEV IgM positives, 86 (63.70%) were positive for HEV RNA. For 76 cases with symptoms, the positivity percentage of HEV RNA was 100% (76/76), while it was 16.94% (10/59) for asymptomatic cases.

About 76/135 (56.29%) participants had raised ALT levels (> 45 IU/L) with symptoms consistent with hepatitis. These cases were designated as cases of symptomatic acute HEV. Among these 76 acute cases with symptoms, 56 (73.68%) had ALT levels > 10,000 IU/L whereas 20 (26.32%) had > 1,000 U/L. Among these 76 cases, 49

(64.47%) communicated weakness, 39 (51.31%) had dark urine, 36 (47.36%) had no appetite, 21 (27.63%) had a fever, and 49 (64.47%) had jaundice or pain in the abdomen.

Possible risk factors ad demographic features were assessed through a questionnaire. The results indicated there was a statistically significant association ($p < 0.05$) between drinking river water and soap hand-washing with IgM seropositivity. There wasn't a statistical association ($p > 0.05$) concerning IgM seropositivity and gender, age group, or occupation.

Table I: Demographics and laboratory findings of study participants

Variables	Total patients (n=544)
Mean age (range)	29.6 (12 - 63 years)
Sex M/F	395/149 (72.61%/27.39%)
HEV IgM Pos	135 (24.81%)
HEV IgG Pos	59 (10.84%)
HEV IgG/IgM Pos	41 (7.53%)
HEV RNA Pos	86 (15.80%)
HEV IgM /ALT (> 45 IU/L)	76/135 (56.29%)

Discussion

HEV is a well-known causative agent of acute viral hepatitis; however, complications are generally self-limiting. HEV also has the potential to cause life-threatening complications during pregnancy.[16]

The findings of our study revealed that 135 (14.5%) individuals had recent acute infections, with 76 subjects (56.29%) reporting symptoms consistent with acute hepatitis and 59 (43.70%) being asymptomatic. This asymptomatic rate was higher compared to previous studies.[17, 18] However, a study from England showed lower results compared to our findings.[19]

Our results demonstrated that all 76 symptomatic cases had elevated levels of alanine aminotransferase (ALT) (>45 IU/L), with 73.68% of them having very high ALT levels (>10,000 IU/L). Most of the asymptomatic cases had normal liver function. Therefore, it can be concluded that HEV infection can result in varying impacts on liver functionality, possibly due to host-virus interactions.

Previous studies have provided evidence of higher HEV IgG positivity in multiply transfused patients compared to healthy individuals, suggesting the potential transmission of HEV through blood transfusions.[20-23] Our study revealed that 59.25% of individuals positive for HEV IgM were blood donors. Considering the higher proportion of asymptomatic patients, this poses a potential threat to blood safety, especially as the blood donors are predominantly from specific family networks where there may be familial pressure to donate blood.

The main symptoms of symptomatic HEV infection included weakness, jaundice or abdominal pain, and dark urine, among others. It is important to note that these symptoms are non-specific to viral hepatitis, and therefore the diagnosis of hepatitis E should be based on antibody screening and nucleic acid detection.

No significant associations were observed between age, gender, and acute HEV infection. Regarding drinking water, we investigated the water source, and most participants consumed river water near the IDP camp.

Conclusion

Our findings, therefore, indicated that drinking river water was a likely source of HEV infection in the current outbreak. It is prudent to consider hepatitis E as a potential risk to blood safety, especially considering that the majority of the infected cases were males with a history of recent blood donation. Furthermore, it is worth noting that there is currently no screening facility for HEV in any blood establishment. Genotyping should be performed to determine the specific HEV type responsible for the outbreak. The results of this study will also contribute to the preparation of a future response to similar outbreaks.

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