Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/aaf92496

FREQUENCY OF CHOLESTASIS AMONG THE PATIENTS SUFFERING FROM HEPATITIS E VIRUS

Noman Ul Haq[1*], Bahawal Khan[2], Atif Hussain[3], Muhammad Anwer[4], Zia Uddin[5], Syed Hasnain Abbas Gilani[6], Asmatullah[7]

[1*,4,5]Bolan Medical College Hospital, Quetta, Pakistan
[2,6,7]Pir Abdul Qadir Shah Jillani Institute of Medical Science Khair Pur Mir's Pakistan
[3]Pakistan Kidney and liver Institute Lahore, Pakistan

***Corresponding Author:** Noman Ul Haq
*drnomializai@gmail.com

ABSTRACT

Background: Hepatitis E virus (HEV) continues to be the leading aetiology of acute viral hepatitis in Pakistan, where cholestasis is a common but mostly unrecognised complication, which, among other things, can extend the duration of the disease and impede the recovery.
Objective: To determine the frequency of cholestasis in patients with Hepatitis E virus infection.
Materials and Methods: This descriptive cross-sectional study took place in the Department of Gastroenterology, PGMI/Shaikh Zayed Hospital, Lahore, from 18 March to 18 September 2021. 189 HEV-positive patients who underwent clinical examination and liver function tests for the detection of cholestasis were included in the study.
Results: Cholestasis developed in 90 (47.6%) of 189 patients. The majority of patients were of middle age, and males were more commonly affected. High bilirubin and ALP levels were the principal markers of cholestasis.
Conclusion: Cholestasis is frequently a complication of HEV infection. Therefore, timely identification and observation play an important role in improving prognosis.

Keywords: Hepatitis E, Cholestasis, Liver Function, Jaundice, Pakistan.

INTRODUCTION

Hepatitis E virus (HEV) is a primary etiological agent of acute viral hepatitis in many developing regions like Pakistan, where lack of proper sanitation and polluted water contribute to the spread of the disease. Research in Pakistan indicated an increase in HEV cases, with the main symptoms being jaundice, fever, nausea, and abnormal liver function tests. One of the clinically significant side effects is cholestasis, which denotes the decrease of bile flow, causing the skin to yellow, itch, and the level of bilirubin to rise. A study conducted locally in Pakistan brought it out that cholestasis is frequently accompanied by HEV infection, and its clinical condition may become aggravated further (1). This warrants knowing the rate of occurrence of cholestasis in HEV-positive patients so that provision of prompt diagnosis and treatment can prevent the development of complications. There are different genotypes of HEV, and the severity of the disease is influenced by the immune system and the location of the patient.

Typically, HEV is an acute, self-resolving liver disease, but in a few cases, especially in immunocompromised individuals, the virus may persist and eventually result in chronic hepatitis,

which may lead to other complications. A systematic review indicates that the management of chronic HEV infection is mostly antiviral therapy if necessary, in the case of immunocompromised patients, transplant recipients, or those with prolonged viral shedding (2). Such patients can go through more severe liver damage, which elevates the probability of cholestasis development. Furthermore, the recent research has revealed that HEV infection can even worsen the prognosis in patients with pre-existing chronic liver diseases like hepatitis B and cirrhosis. Liver cirrhosis patients, when infected with HEV, can get their condition deteriorated very fast, can get decompensation, and acute-on-chronic liver failure, which also increase bilirubin levels and might simulate cholestasis (3). This demonstrates that HEV strongly affects bile flow and liver function when it takes place in susceptible patients.

The chronic HEV infection in men has also been reported in the ejaculate, indicating that the virus can be dissociated for a long time even outside the liver. Such persistence may eventually lead to prolonged illness and liver dysfunction, including cholestasis (4). The virus is also able to cause ketoacidosis, a condition in which some of the body's systems are affected, including the nervous system, pancreas, kidneys, and blood cells. The immune-related mechanisms are responsible for these extrahepatic effects and can be a source of complication in the overall disease picture of HEV-infected patients, frequently resulting in the exacerbation of jaundice and cholestatic symptoms (5). HEV is a major global threat that has been underestimated for a long time, and one of the main factors that research has pointed to determining the severity of the disease is the immune response. The sudden flare-up of the immune response leads to the most severe death of liver cells and a decrease in the bile secretion in some patients. The immune reaction to the liver increases the risk of cholestasis in acute HEV infection (6).

There are cases when the virus gets into the cerebrospinal fluid and causes neurological symptoms, so HEV can go beyond the liver and trigger generalised inflammation that may also affect the liver function and bile flow (7). In irregular cases, HEV infection can be a predisposing factor to chronic liver cancer development. Research studies have revealed that the Hepatitis E virus may become a factor in the development of hepatocellular carcinoma in specific demographics, especially when the infection is persistent. Those with liver cancer are most likely to have jaundice and cholestasis, so it is essential to study the effects of HEV on bile flow (8). According to laboratory research, cell cultures have been created that demonstrate HEV can stay in liver cells for a long time, damaging bile canaliculi and disturbing bile secretion, which leads to cholestasis with a higher probability (9). Even though vaccines are being considered worldwide as a means of controlling HEV infection, especially in high-risk groups, HEV continues to be a significant problem in poorly sanitised areas (10). A few new strains of HEV, such as rat HEV, have also been discovered in humans, indicating that the virus is changing and can be found in populations that were not expected.

In addition, these new strains may behave differently and also influence the frequency of cholestasis (11). It is essential to follow up with HEV-positive patients because some transplant patients or those on immunosuppressive therapy may eventually develop chronic HEV infection. It is essential to keep an eye on HEV RNA levels as this is one of the ways to determine the extent of the disease and also to spot the complications of cholestasis at an early stage (12). Case reports from various regions of the world have demonstrated that HEV can lead to acute-on-chronic liver failure, particularly in people with liver diseases for a long time, resulting in the rapid escalation of bilirubin and cholestatic symptoms (13). Studies have also led to the identification of immune-related proteins like GBP1, which serve as natural defence agents against HEV. When such immune protections are weakened, the virus is able to proliferate extensively, the liver is injured more, and the risk of cholestasis also increases (14)(15). Molecular research on the biology of HEV has unveiled that the virus changes bile secretion routes, liver cells, and immune systems, which overall lead to cholestasis (16).

HEV has been pinpointed as a new leading cause of acute hepatitis in some European regions due to zoonotic transmission. Jaundice is usually prominent in these patients, and their clinical features mostly indicate cholestatic involvement (17). Besides that, chronic HEV infection may lead to CD8+ T-cell exhaustion, the immune system's control over the virus is lost, and the virus eventually

becomes latent, making liver dysfunction more severe, including cholestasis (18). Since cholestasis has the capacity to aggravate symptoms, prolong recovery, and increase patient suffering, knowledge about its occurrence in HEV-infected patients is quite vital. The escalating load of HEV in Pakistan, along with changes in clinical presentation, makes it imperative to state that cholestasis occurs frequently among these patients. This data is of great help to the doctors in screening the patients who have tested positive for HEV, giving room for early treatment and complications prevention.

Objective: To establish the frequency of cholestasis in hepatitis E virus-infected patients and also to assess its clinical importance in facilitating early diagnosis, treatment, and prevention of complications associated with the disease.

MATERIALS AND METHODS

Study Design: Descriptive Cross-sectional study.
Study Setting: Department of Gastroenterology, Postgraduate Medical Institute (PGMI) / Shaikh Zayed Hospital, Lahore.
Duration of the Study: 18th March 2021 to 18th September 2021.
Inclusion Criteria: Patients of any gender who showed the symptoms of acute hepatitis, such as jaundice, fatigue, vomiting, and increased liver enzymes, and were found to be positive for anti-HEV IgM were included in this study. Therefore, individuals above the age of 15 years and diagnosed with Hepatitis E virus infection by serological testing (anti-HEV IgM positive) formed the patient population of this research.
Exclusion Criteria: To avoid confounding factors, patients with known chronic liver disease, obstructive jaundice, hepatitis A, B, C, or D coinfection, those on hepatotoxic drugs, pregnant women, and patients with incomplete data records were excluded.

Methods: A consecutive non-probability sampling method was used to select a total of 189 patients meeting the inclusion criteria. After getting informed consent, each patient went through a complete clinical evaluation, which also included the assessment of symptoms such as jaundice, dark urine, pruritus, abdominal pain, and fatigue. Laboratory investigations were done for all the participants, including serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Anti-HEV IgM serology was used to confirm Hepatitis E virus infection. Cholestasis was identified based on the clinical features supported by the increase in bilirubin and ALP levels, indicating the obstruction of bile flow. All the laboratory tests were done in the hospital diagnostic laboratory by using standardised techniques. Demographic details like age, gender, duration of illness, and history of diabetes were recorded. Data were recorded on a structured proforma and analysed to determine the frequency of cholestasis in HEV-positive patients.

Results

A total of **189 patients** with Hepatitis E virus infection were part of this study. The data reveal the demographic characteristics such as age, gender, and also the duration of disease, presence of diabetes, and frequency of cholestasis among the population under study.

Age Distribution

The age of patients varied between 15 and 70 years, and the **average age was 47.95 ± 14.176 years**. The most significant number of patients was found to be in the age group of **41–50 years**, indicating that middle-aged people were the most prevalent ones. The number of patients in the younger age groups was less.

Table 1: Age-wise Distribution of Patients

Age Group (Years)	Frequency (n)	Percentage (%)
15–20	11	5.8%
21–30	13	6.9%
31–40	45	23.8%
41–50	86	45.5%
51–60	16	8.5%
61–70	18	9.5%

The age distribution is notably biased towards older people, as per the data. This may indicate that the older population has been more exposed to or is more vulnerable to HEV infection.

Gender Distribution

Among 189 patients, **110 were males (58.2%)**, and **79 were females (41.8%)**. The male predominance might throw light on the differences in the exposures, the risk related to the male-dominated occupations, or even the health-seeking behaviour of males.

Table 2: Gender-wise Distribution

Gender	Frequency (n)	Percentage (%)
Male	110	58.2%
Female	79	41.8%

This discovery indicates that males constituted the majority of those affected in the study population, which is in line with the previously known patterns of HEV epidemiology.

Duration of Disease & Diabetes Status

Where patients' sickness periods were different, **103 patients (54.5%)** presented within 3 months, while **86 patients (45.5%)** had symptoms for more than 3 months. Diabetes was similarly checked in the same patients, and the sample showed almost equal distribution, with **94 diabetic patients (49.7%) and 95 non-diabetics (50.3%)**.

Table 3: Duration of Disease and Diabetes Status

Variable	Category	Frequency (n)	Percentage (%)
Duration of Disease	Less than 3 months	103	54.5%
	More than 3 months	86	45.5%
Diabetes Status	Yes	94	49.7%
	No	95	50.3%

These findings show that the majority of patients sought medical care in the early stages, and diabetes, which is a significant comorbidity, was almost equally distributed.

Frequency of Cholestasis

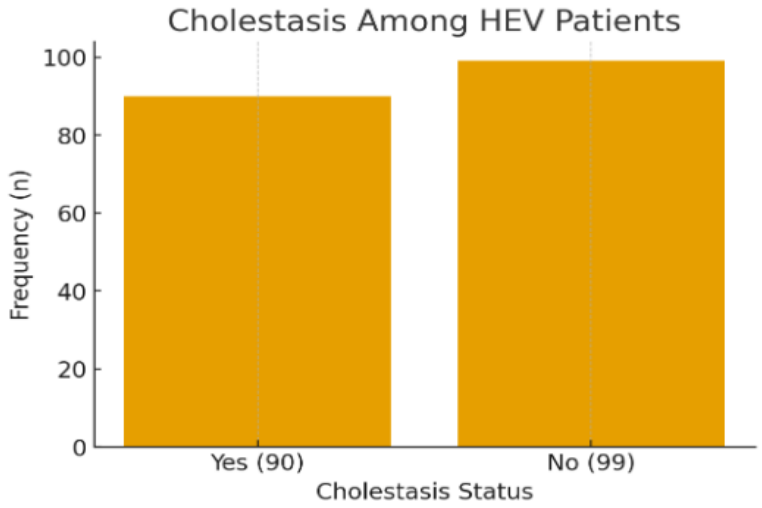
The first goal of the research was to determine how frequently cholestasis occurred in patients with HEV. Among 189 patients:

90 patients (47.6%) had cholestasis

99 patients (52.4%) did not have cholestasis

The data reveal that around **one-half** of the HEV patients experienced cholestasis, which is a significant and frequent complication.

Graph: Distribution of Cholestasis



According to the graph, cholestasis occurred in around 50% of the patients, and there was only a slight difference between the patients with and without cholestasis. The research indicates that Hepatitis E primarily affects middle-aged adult males. Almost half of the total number of HEV patients develop cholestasis, which is a critical clinical condition. It is essential to recognise it at the very beginning as cholestasis may worsen jaundice, increase the itching, prolong the time of recovery, and also require more intensive monitoring. The fact that almost half of the sample had diabetes quite strongly points to the necessity of investigating the involvement of metabolic factors in the progression of HEV.

Discussion

The present study aimed to identify the frequency of cholestasis in patients with Hepatitis E virus infection. The outcome of the study revealed that 47.6% of patients developed cholestasis, which is an outstandingly high figure and shows that cholestasis has become a comorbidity of HEV in our population. The results are in agreement with those of a local Pakistani study, which also found cholestasis as the most common clinical feature in HEV patients, and early diagnosis and monitoring were emphasised by the study (1). The concordance between the local studies and the current research galvanises the argument that cholestasis should not be considered a rare or infrequent manifestation in HEV infection in the region. HEV infection severity depends on the host's immunity, genotype, and comorbidities. A systematic review has indicated that chronic HEV infection is commonly associated with immunocompromised individuals, and such patients have a more severe hepatic dysfunction that may lead to cholestasis (2).

While our research did not specifically examine chronic HEV cases, a significant number of patients in our cohort had extended durations of disease, and those who had symptoms for over three months showed more biochemical abnormalities. This points to the fact that long-term infection may lead to individuals developing cholestasis due to continuous inflammation and obstruction of bile flow. Zhao et al. demonstrated that HEV superinfection in patients with hepatitis B-induced cirrhosis escalates complications, liver decompensation, and mortality, aggravating the condition of the liver (3). The resulting severe hepatic damage leads to an increase in bilirubin levels and the appearance of cholestatic symptoms. To eliminate the risk of confounding, people with chronic liver diseases were not part of our study. However, the well-documented link between HEV and deteriorating liver function is the reason why cholestasis can still occur in healthy individuals when inflammation gets

to be very severe. In fact, a study has revealed that HEV may stay even in such unusual body fluids as semen, implying that viral replication might be occurring in more locations than initially assumed (4). Extensive viral presence in the organism elevates the probability of prolonged inflammatory response, which in turn can cause cholestasis through the injury of hepatocytes and bile canaliculi. The extrahepatic side effects of HEV, comprising neurological, renal, pancreatic, and haematological symptoms, are additional evidence of the virus's capability to induce systemic damage (5). This extensive involvement usually leads to cytokine release and immune activation, both of which can intensify jaundice and biliary stasis. The immune response is the primary factor that determines the HEV severity. Kupke and Werner have pointed out that HEV is a largely unrecognised global problem and that immune-mediated damage is the leading cause of symptoms (6). In case of vigorous immune-mediated hepatocyte damage, the bile secretory apparatus of the liver is severely damaged, resulting in cholestasis.

The different parts where immune damage plays a crucial role have been backed up by experiments, showing that HEV can penetrate the cerebrospinal fluid and cause neurological symptoms, which again demonstrates the inflammatory potential of the virus throughout the body (7). A few studies have also pointed to the viral infection as a source of local and global liver diseases, such as hepatocellular carcinoma (HCC) in specific areas (8). While this research does not directly tackle HCC, patients with liver cancer are the ones who usually manifest cholestatic symptoms, and the connection of HEV with severe liver inflammation is an indication of how bile flow can be disrupted due to virus-induced hepatocellular injury. In vitro studies have demonstrated that HEV is capable of staying in hepatocyte-like cultures for several days, and it is very likely that there is ongoing damage in bile canaliculi even if there is no visible chronic disease (9). This is in line with our observation that almost 50% of the patients were cholestatic, while most of them were in the acute phase of viral infection and not the chronic one.

Vaccination is now part of the global discussion strategies for HEV prevention. The European Society has highlighted the necessity of HEV vaccination, especially in the predisposed groups (10). If HEV infection were limited through vaccination schemes, then the number of different types of complications co-occurring with HEV, such as cholestasis, would also be lower. Firstly, there is a growing body of evidence that shows that unusual HEV strains, such as rat HEV, may eventually be able to infect humans and create problems for us in terms of difficulties in diagnosis (11). These strains may act differently, and in the future, they may cause different rates of cholestasis. Monitoring of chronic HEV infection in transplant patients, in particular, has revealed changes in HEV RNA levels which reflect episodes of deteriorating liver function (12). As a result, when HEV replication is high, cholestasis may occur due to the aggravation of the inflammation. Reports of cases also confirm this sequence of events, as it is presented in a report of a patient with alcohol-related chronic liver disease who, observing HEV infection, developed acute-on-chronic liver failure with severe jaundice and cholestatic features (13).

Cellular immune system components and mechanisms, for instance, also affect liver injury associated with HEV. Glitscher et al. located GBP1 as a significant limitation factor against HEV when this protein is low or is out of function, HEV replication goes up, causing the release of more hepatocellular inflammatory reaction and cholestasis (14, 15). Furthermore, Lin and Zhang stated that HEV impairs the secretion of bile, damages the organelles and immune pathways, and all these changes are involved in the development of cholestasis (16). In Europe, HEV genotype C has been cited as the source of the new wave of acute hepatitis, and patients have been reported to present mainly with bright yellow discolouration of the skin and mucous membranes, indicating that cholestasis is the major factor in HEV infection (17). Another research illustrated that chronic HEV infection may probably result in CD8+ T-cell exhaustion to the extent that the virus is not controlled, and liver damage continues, leading to an increase in cholestatic syndrome (18). Our study's results are in agreement with the worldwide literature, which suggests that cholestasis is one of the most clinically significant features of HEV infection and that it should be kept in mind at all

times. The proportion of patients with cholestasis was almost 50%, so the occurrence of this complication in areas with HEV has become quite normal, as emphasised in Pakistan.

Conclusion

The current investigation revealed that cholestasis is not only prevalent but also a serious condition in hepatitis E virus-infected patients. Almost 50% of the study population was affected by this condition. Such a high occurrence necessitates that medical practitioners should always consider the possibility of cholestasis in patients with HEV, especially those who come with a history of jaundice of long duration or an increase in bilirubin. The results are consistent with worldwide and regional data, which indicate that HEV is capable of causing such inflammatory changes in the liver that finally lead to the immune system attacking the liver and the patient suffers from obstructive cholestasis. A patient with cholestasis identified at an early stage was given time treatment, better supervision, and safeguarded from other sequelae. The investigation singles out as well the significance of the biochemical routine, which must be inclusive of bilirubin, ALP, and liver enzymes, in every case of HEV. Nevertheless, given the disease burden in Pakistan, these outcomes point out the necessity for a restored public health approach in terms of better hygiene and awareness to lower HEV infection and its sequelae. Another study can be helpful to determine the long-term effects and risk factors of cholestasis secondary to Hepatitis E Virus.

References

1. Shahid M, Ali A, Mahmood H. Frequency of Cholestasis Among the Patients Suffering from Hepatitis E Virus. Pakistan Journal of Medical & Health Sciences. 2022 Dec 14;16(10):705-.
2. Gorris M, van Der Lecq BM, van Erpecum KJ, de Bruijne J. Treatment for chronic hepatitis E virus infection: a systematic review and meta-analysis. Journal of Viral Hepatitis. 2021 Mar;28(3):454-63.
3. Zhao H, Ye W, Yu X, Hu J, Zhang X, Yang M, Sheng J, Shi Y. Hepatitis E virus superinfection impairs long-term outcome in hospitalized patients with hepatitis B virus-related decompensated liver cirrhosis. Annals of Hepatology. 2023 Mar 1;28(2):100878.
4. Horvatits T, Wißmann JE, Johne R, Groschup MH, Gadicherla AK, Zur Wiesch JS, Eiden M, Todt D, Reimer R, Dähnert L, Schöbel A. Hepatitis E virus persists in the ejaculate of chronically infected men. Journal of Hepatology. 2021 Jul 1;75(1):55-63.
5. Wu J, Xiang Z, Zhu C, Yao Y, Bortolanza M, Cao H, Li L. Extrahepatic manifestations related to hepatitis E virus infection and their triggering mechanisms. Journal of Infection. 2021 Sep 1;83(3):298-305.
6. Kupke P, Werner JM. Hepatitis E virus infection—immune responses to an underestimated global threat. Cells. 2021 Sep 2;10(9):2281.
7. Abravanel F, Nicot F, Lhomme S, Cazabat M, Drumel T, Velay A, Latour J, Belliere J, Cintas P, Kamar N, Izopet J. Hepatitis E virus quasispecies in cerebrospinal fluid with neurological manifestations. Vaccines. 2021 Oct 19;9(10):1205.
8. Klöhn M, Schrader JA, Brüggemann Y, Todt D, Steinmann E. Beyond the usual suspects: Hepatitis E virus and its implications in hepatocellular carcinoma. Cancers. 2021 Nov 22;13(22):5867.
9. Pellerin M, Hirchaud E, Blanchard Y, Pavio N, Doceul V. Characterization of a cell culture system of persistent hepatitis E virus infection in the human HepaRG hepatic cell line. Viruses. 2021 Mar 4;13(3):406.
10. Dudman S, Zerja A, Hasanoğlu İ, Ruta S, van Welzen B, Nicolini LA, Yonga P, Øverbø J, Rawat S, Habibovic S, Kim TB. Global vaccination against hepatitis E virus: position paper from the European Society of Clinical Microbiology and Infectious Diseases Viral Hepatitis Study Group. Clinical Microbiology and Infection. 2025 Feb 1;31(2):201-10.
11. Parraud D, Lhomme S, Péron JM, Da Silva I, Tavitian S, Kamar N, Izopet J, Abravanel F. Rat hepatitis E virus: presence in humans in South-Western France?. Frontiers in Medicine. 2021 Sep 3;8:726363.

12. Affeldt P, Di Cristanziano V, Grundmann F, Wirtz M, Kaiser R, Benzing T, Stippel D, Kann M, Kurschat C. Monitoring of hepatitis E virus RNA during treatment for chronic hepatitis E virus infection after renal transplantation. Immunity, Inflammation and Disease. 2021 Jun;9(2):513-20.
13. Fantilli A, López Villa SD, Zerega A, Di Cola G, López L, Wassaf Martínez M, Pisano MB, Ré VE. Hepatitis E virus infection in a patient with alcohol-related chronic liver disease: a case report of acute-on-chronic liver failure. Virology Journal. 2021 Dec 9;18(1):245.
14. Glitscher M, Himmelsbach K, Woytinek K, Schollmeier A, Johne R, Praefcke GJ, Hildt E. Identification of the interferon-inducible GTPase GBP1 as a major restriction factor for hepatitis E virus. Journal of Virology. 2021 Mar 10;95(7):10-128.
15. Lin S, Zhang YJ. Advances in hepatitis E virus biology and pathogenesis. Viruses. 2021 Feb 9;13(2):267.
16. Rivero-Juarez A, Frias M, Perez AB, Pineda JA, Reina G, Fuentes-Lopez A, Freyre-Carrillo C, Ramirez-Arellano E, Alados JC, Rivero A. Orthohepevirus C infection as an emerging cause of acute hepatitis in Spain: First report in Europe. Journal of Hepatology. 2022 Aug 1;77(2):326-31.
17. Kemming J, Gundlach S, Panning M, Huzly D, Huang J, Lütgehetmann M, Pischke S, Zur Wiesch JS, Emmerich F, Llewellyn-Lacey S, Price DA. Mechanisms of CD8+ T-cell failure in chronic hepatitis E virus infection. Journal of Hepatology. 2022 Oct 1;77(4):978-90.