Review Article

Hepatitis E: A Scorned Virus

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ABSTRACT

Hepatitis E virus (HEV) is one of the most common causes of acute viral hepatitis in developing countries with poor sanitation and hygiene. *HEV* is a non-enveloped RNA virus and its genome is formed by a non-segmented positive sense RNA chain. The virus is classified into four genotypes (1-4) with one serotype. Genotype I is the predominant strain in India. *HEV* infection is an underdiagnosed disease due to wide use of serological assay with low sensitivity. *HEV* infection outbreaks frequently occur due to fecal-oral transmission, usually through contamination of drinking water. Chronic infections are rare, except in organ transplant recipients, patients with hematological malignancy requiring chemotherapy, and individuals with *HIV*. Extrahepatic manifestations include a number of neurological syndromes and renal injury. Treatment is required in chronic infections by reducing immunosuppression in transplant patients and/or the use of antiviral therapy. A subunit vaccine has been shown to be effective in preventing the clinical disease, but it is not yet commercially available.

INTRODUCTION

Hepatitis is a disease characterized by the presence of inflammatory cells in the tissue of the liver. It can be caused by both; infectious (i.e. viral, bacterial, fungal and parasitic organisms) and non infectious (e.g. alcohol, drugs, autoimmune diseases and metabolic diseases) agents. Depending upon the cause, hepatitis can manifest either as an acute or chronic disease. Viral hepatitis represents a major health problem worldwide followed by alcoholic and non alcoholic liver diseases.[1] Viral hepatitis is caused by any of the five hepatotrophic viruses, i.e. *Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV)* and *Hepatitis E virus (HEV)*. These viruses can be distinguished depending on the predominant mode of transmission- water or blood and show significant differences in their epidemiology, presentation, prevention and control. In 2012, about 119,000 cases of viral hepatitis were reported from India. 290,000 cases of acute viral hepatitis were notified to the Integrated Disease Surveillance Programme (IDSP) of the National Centre for Disease Control in 2013.[2] *HEV* is now established as the major etiological agent of the enterically transmitted non A, non B hepatitis. Fecal contamination of drinking water was the reason for the epidemic of 1955-56 in New Delhi which also was the first well documented epidemic of hepatitis E infection in India. A total of 29,000 people were affected in this epidemic and it was originally considered as *HIV* epidemic while later retrospective testing of the stored sera from the affected patients suggested that a novel infectious agent was responsible.[3,4] Later identification and sequencing of its etiological agent, the disease became known as Hepatitis E and its agent as *HEV*.[5] The letter E stands for 'enteric', 'epidemic' or 'endemic' which describe the epidemiology of *HEV*.

There are five genotypes of *HEV* based on whole genome sequencing. *HEV* genotypes 1 to 4 are associated with human infections, while genotype 5 has only been known to infect birds.[6] Human infections with *HEV* have two distinct epidemiological patterns.[7-9] In area with poor sanitation, 1 and 2 genotypes are transmitted between human by the faecal-oral route, usually via contaminated water. This results in frequent sporadic cases and occasional large outbreak. In developed countries, *HEV 4* is transmitted zoonotically from animal reservoirs with sporadic cases that have increasingly been reported in the past few years due to improvements of diagnostic tools and screening strategies. *HEV 3* has been reported from chronic *HEV* infection resulting in chronic hepatitis and cirrhosis in immunocompromised persons.[10]

VIROLOGY

HEV is round non enveloped, positive sense single stranded RNA virus with an icosahedral capsid, 27-34 nm in diameter. It belongs to the family Hepaviridae, genus Orthohepevirus, species orthohepevirus A which includes isolates from human, mammalian and avian *HEV*.[11] The *HEV* genome of 7.2 kb contains three reading frames (ORF) that encode structural and non structural proteins. ORF 1 encodes 1693 amino acids containing functional domains present in the non structural proteins of other positive-strand RNA viruses.[12] These functional domains include methyl transferase, cysteine protease, RNA helicase and RNA dependent polymerase domains. ORF 2, present at 3' end, encodes the viral capsid protein of 660 amino acids that is responsible for virion assembly[13], interaction with target cells[14] and immunogenicity.[15] ORF 3 overlaps the other two ORFs and encodes a small protein of 113 or 114 amino acids that is involved in virion morphogenesis and release.[16-18] A hypervariable region (HVR) having 557 to 641 amino acids is present in ORF 1. The HVR overlaps with the proline-rich sequence that is located between N-terminals of the X-domain and the C-terminal portion of the putative papain-like protease domain. The HVR of different *HEV* strains varies in length as well as in sequence.

Like most other positive-strand RNA viruses, the *HEV* replication also takes place via a negative-sense RNA intermediate.[18] The *HEV* replication occurs in hepatocytes but also found to replicate in the small intestine, colon and lymph nodes as demonstrated by detection of negative sense RNA intermediates.[19] During replication of *HEV*, a subgenomic RNA produces the ORF2 and ORF3 proteins and the full genomic RNA encodes nonstructural proteins and serves as a template for replication (Figure 1).

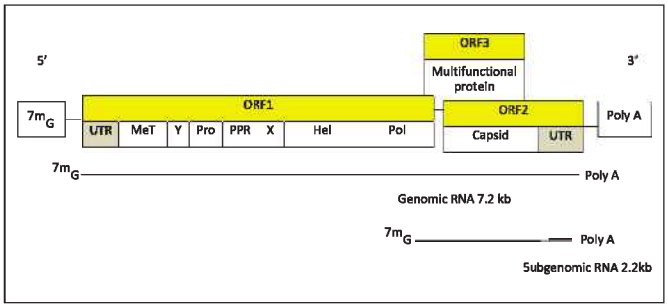

Figure 1: Schematic representation of hepatitis E virus genome

ORF: Open reading frame; UTR: Untranslated region; MeT: Methyltransferase; Y: Domain Y; Pro: A papain like cysteine protease; PPR: Proline-rich (hypervariable region); X: Domain X; Hel: Helicase; Pol: RNA Polymerase.

EPIDEMIOLOGY

The incubation period of *HEV* in human volunteers after oral exposure is normally 4-5 weeks.[20] Four major routes of transmission of *HEV* infection are: (1) Faecal-oral transmission due to contaminated drinking water. (2) Foodborne transmission. (3) Transfusion of infected blood products and (4) Vertical transmission from mother to baby. The outbreaks are usually waterborne and are more common during periods when faecal contamination of water supply is more likely (floods and water scarcity when faecal matter in water bodies becomes concentrated). Consumption of contaminated food (usually meat) from common source is also reported.[21] The attack rates during hepatitis E outbreaks range from 1% to 15% with case fatality rate of 0.2% to 4% and males outnumbering females and young adults.[22] The attack rate of icteric *HEV* infection was found higher in adults (4.9%) than children (1.8%) but anicteric *HEV* infection found to be higher in children (21.8%) than adults (14.6%).[23] A significantly higher rate of 10-20% of fulminant hepatic failure has been observed in pregnant women especially during the third trimester.[24]

Hepatitis E prevalence is highest in the East and the South Asia regions, accounting for about 60% of hepatitis E global incidence and about 65% of global deaths. Despite the high endemnicity of *HEV* in the South Asian region, the sero-prevalence of antibody to *HEV* is only 25% in young adults. Among the Indian population, there is low sero-prevalence until age 15, reaching 40% in young adults. Most acute liver failures diagnosed in India are attributable to *HEV*, and *HEV* is the most common cause of hepatitis during pregnancy. *HEV* Genotype I is the predominant strain in India.[2]

Among females, pregnancy has been reported with higher attack rate. Unlike several other enterically transmitted infections, person to person transmission of *HEV* is uncommon.[25] Epidemics of hepatitis E usually occur in unimodel outbreaks with a highly compressed curve of incidence or, alternatively, prolonged epidemics with multiple peak of incidence.[26]

Hepatitis E is responsible for more than 25% of acute sporadic hepatitis in endemic regions. In India, about 30-70% of acute sporadic hepatitis attributed to *HEV*.[27] Sporadic cases may be attributed to faecal contamination of food and water.[22] Ippagunta et al found *HEV* genomic

sequences in 40% of the sewage specimen in a city.[28] Hazam et al detected *HEV* RNA in 4.25% of sewage samples and 1.42% of drinking water samples. Serology and/or RT-PCR were used and 29.08% cases of viral hepatitis in the community reported due to *HEV*.[29] Many cases of transfusion transmitted *HEV* infection has been reported around the globe.[30-33] *HEV* has been detected in blood of some blood and plasma donors.[34-38] An anti-HEV IgG prevalence rate of 7.8% to 45% has been reported in volunteer blood donors in endemic areas, in contrast to 1-4% in industrialized countries.[34] In developed countries, a number of cases have been due to zoonotic transmission. Pigs to human transmission of *HEV* have been shown. It is also seen that animals like boar, deer, cows, sheep, horses and rabbits are susceptible to infection and act as natural reservoirs for *HEV*.[39] Uncooked or undercooked infected pork or game (wild boar, deer or rabbit) meat can be the reason for zoonotic transmission.[40-42] It has been found that *HEV* remains viable after heating to 56°C for 1 hour and cooking temperature of 71°C for 20 minutes are required to fully inactivate the virus.[43] Direct contact with *HEV* infected animals is another possible route of transmission.[44-46] Seroprevalence studies show that veterinarians and swine handlers are more likely than the general population to be anti-HEV IgG positive.[47] Hepatitis E has been reported in homosexual men, which suggests a sexual mode of transmission.[48] Data from endemic areas is scarce; however, Bali et al reported the spread of hepatitis E in an active group of male homosexuals in a village in North India.[49]

CLINICAL COURSE

Acute Hepatitis: In most patients, HEV cause a self-limiting illness which lasts for a few weeks. Symptoms develop with fever and nausea followed by abdominal pain, vomiting, anorexia, malaise and hepatomegaly. In developed countries patients are usually middle-aged/elderly males while in developing countries patients are usually children and young adults.[50] Jaundice follows after the prodromal symptoms subside, although at times there may be anicteric hepatitis.

Chronic Hepatitis: Genotype 3 has been exclusively reported in almost all the cases of chronic hepatitis.[7] The source of infection in immunocompromised patients is often unknown but thought to be ingestion of pork or deer in most cases. The alanine aminotransferase (ALT) level is usually 1000-3000 IU/litre, but the range is wide. Rarely, in some patients ALT level is normal in blood samples taken at the time of viremia.[51] In majority of patients, the disease is self limiting, with symptomatic and biochemical recovery within 4-6 weeks. In two groups of patients who have underlying chronic liver disease have poor prognosis and individuals who are immunosuppressed often develop chronic infection.[52] Fulminant hepatic failure due to massive liver necrosis is seen in a small subset of patients who present with encephalopathy and coagulation disorders.

Extrahepatic Manifestations: *HEV* is also responsible for wide range of extrahepatic disorders ranging from neurological syndromes to renal injury, pancreatitis and haematological problems.

1. Neurological disorders: These are the most frequent extra-hepatic manifestations. In *HEV* patients, these are Gullain-Barre Syndrome,[53,54] Bell's palsy,[55] neurologic amyotrophy,[56] acute transverse myelitis[57] and acute meningoencephalitis.[58-65] *HEV* infections can cause glomerular disease with two different histological patterns: membranoproliferative and membranous glomerulonephritis.[67] These injuries were observed in immunocompetant patients,[68,69] kidney and liver transplant patients.[67] Cryoglobulinemia have been observed in chronic patients.[61]
2. Pancreatitis: Acute pancreatitis has been associated with *HEV* infections.[62-64]
3. Rheumatologic Manifestations: These are arthralgia, myalgia, skin rashes and cryoglobulinemia have been reported in chronic *HEV* infection.[65]
4. Haematological disorder: Thrombocytopenia and aplastic anemia have been reported for acute *HEV* infection.[66-67]

   A few cases of chronic hepatitis E have also been reported in immunocompetent patients.[68,69]

Pregnancy: Many studies have shown excess mortality in pregnant females with *HEV* infection. 20-25% mortality occurs usually in third trimester.[70] Obstetric problems such as haemorrhage or eclampsia, or development of fulminant hepatic failure are the reason for mortality.

Patient with pre-existing Liver disease: *HEV* patients with underlying chronic liver disease frequently develop acute or subacute liver failure. In a study by Acharya et al on patients with decompensated chronic liver disease, patients who had decompensation because of *HEV*

infection had a significantly worse prognosis than patients who had decompensation due to other causes.[71]

Possible Zoonotic Agent: It is considered that humans are natural host for *HEV*. Few studies have suggested *HEV's* role as zoonotic virus. MH Kuniholm et al in their study in USA found a significant association of *HEV* seropositivity and animal contact among people who have pets in the home.[72] Another study by Y Yu et al carried out in China found the overall prevalence of anti-HEV IgG in the general population ranging from 32% among individuals who had frequent contact with swine to 21% among individuals whose contact was rare. The prevalence rate was found 81.6% where the contact with swine was more than three months.[73] The higher incidence of *HEV* infection observed among the meat-eating donors in comparison with vegetarian donors indicates that consumption of meat is an important risk factor for *HEV* infection.[74]

DIAGNOSIS

Laboratory abnormalities in liver enzymes and liver function tests such as elevated serum levels of alanine animotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin and gamma-glutamyl transferase are the keys for diagnosis of *HEV* infection. Histopathological changes, such as focal necrosis and modest inflammation, in liver are seen during the acute infection. Cholestatic hepatitis is common and a 'pseudoglandular' alteration of hepatocyte plates has been noted. The cases of Hepatitis E are difficult to distinguish clinically from other types of acute viral hepatitis. The diagnosis is made either indirectly by detection of serum anti-HEV antibodies or directly by detecting *HEV* genome in blood or other bodily fluids. *HEV* infection elicits both IgM and IgG antibodies. Commercially available enzyme immunoassays and rapid immunochromatographic kits can detect the presence of IgM or IgG antibodies induced by the four major genotypes of *HEV*, representing a single serotype.[75] Cross reactivity of *HEV* antigen with no other pathogen has been reported. There is considerable variability in sensitivities and specificities of these tests which makes comparison of the diagnosis of *HEV* infection using different tests difficult.

Anti-HEV IgM is a marker of acute infection. Enzyme immunoassay and immunochromatographic assays with varying sensitivity and specificity are commercially available. The presence of anti-HEV IgG without anti-HEV IgM works as a marker for previous infection. Determination of the concentration of anti-HEV IgG in the sera can be helpful in finding the anti-HEV IgG level which prevents the infection after natural exposure or vaccine administration during clinical trials.[76,77] *HEV* RNA can be detected in blood and stool at the peak of the acute serological response. *HEV* RNA becomes undetectable in blood about three weeks after the onset of symptoms but can be detected in faeces for another two weeks. Reverse transcriptase polymerase chain reaction (RT-PCR) can be used to detect *HEV* RNA, but unfortunately such tests are not widely available. In such conditions, epidemiological evidence may help in establishing the diagnosis. Another nucleic acid amplification technique, the loop mediated isothermal amplification (LAMP) assay, has been developed for the detection of *HEV* RNA.[78] The LAMP assay is quicker than RT-PCR and does not need special equipment, making it suitable in resource constricted area. The World Health Organization (WHO) has established a number of international standards which is an important step in both standardization of *HEV* RNA detection and accurate quantification.[79] This also provides control material for comparing the analytical sensitivities of nucleic acid based methods.

TREATMENT AND PREVENTION

Keynotes for prevention of transmission of *HEV* infection are proper treatment and safe disposal of human excreta, safe drinking water supply and improved personal hygiene. Administration of normal immune globulin manufactured from plasma obtained in the areas where *HEV* is endemic was unsuccessful in preventing *HEV* infection.[80] Successful treatment of chronic hepatitis E in *HIV* positive patients with pegylated interferon (IFN)-α and Ribavirin has been reported.[81,82] Two vaccines have been developed for the prevention of *HEV* infection. In phase trail, vaccines were given to 2,000 healthy volunteers from Nepalese Army who lacked detectable anti-HEV antibodies. There was 95.5% efficacy after administration of three doses during a median follow up of around two years.[83] However, this vaccine never progressed beyond Phase II.

Zhu et al published the results of a randomized, double blind Phase III trial of a recombinant *HEV* vaccine among a much larger group of healthy adults where three doses (30 mcg/dose) were given at zero, one and six months and 100% efficacy was observed during twelve months follow up. At 4.5 years of follow up, the vaccine was found to

have 86.8% of efficacy. This vaccine has been licensed for use in the People's Republic of China, but it is not certain if and when this vaccine will be licensed for human use in other part of the world.[84] The exact role of *HEV* vaccines remains unclear and data regarding its safety and efficacy in persons with chronic liver diseases and other vulnerable population are needed prior to making recommendations for its widespread use

CONCLUSION

HEV is leading cause of non A, non B enterically transmitted Acute Viral Hepatitis in India. Prevention strategies cannot be recommended at this time as the associated risk factors to the transmission of sporadic *HEV* is unknown. *HEV* infection remains underdiagnosed. Currently there is wide variation in diagnostic test for *HEV* and there is a need for standardization of assays. Further research studies are needed to know more about the prevalence and incidence of *HEV* infection as well as to boost the knowledge of its natural events and treatment.

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