Is it the time to start blood screening for Hepatitis-E?

Dr. Somnath Mukherjee MD, MAMS
Assistant Professor
All India Institute of Medical Sciences
Bhubaneswar
• During last 25 years the safety of blood products in regard to infectious risk (HIV, HBV, HTLV, HCV) has been dramatically improved in developed countries.
• Improvements and awareness also increased in developing countries.
• Still blood is not 100% safe......
• This is because of new and emerging viruses/pathogens and re-emerging old viruses may pose a yet undefined risk to transfusion safety.
Factors Contributing to Emergence of Infectious Disease

- Human demographics and behavior
- Technology and industry
- Economic development and land use
- International travel and commerce
- Microbial adaptation and change
- Breakdown of public health measures
HEP E Virus (Introduction)

• Hepatitis E virus (HEV) is one such virus about which a significant number of studies have been published in the last decade regarding the risk it poses to transfusion safety.

• This virus first recognized retrospectively from samples collected during an epidemic in Delhi in 1955.
**Hep E Virus**

- Later, identified as Hepatitis E during an epidemic in Kashmir in 1978.
- Cause of wide-spread water-borne epidemics and sporadic infections in developing countries.
- Usually causes an acute self-limiting hepatitis, but in some cases fulminant hepatic failure (FHF) with resultant mortality and morbidity.
The main questions arise

• How it is clinically relevant in terms of transfusion transmitted infection of HEP E virus both in developed and developing countries?

• Is it preventable through donor screening? Are there quick, reliable and affordable tests available to screen the virus in blood?
To know the answers

- The characteristics of the virus
- Epidemiology
- Prevalence/Seroprevalence of the virus in the donor population
- Natural history of the disease caused by the virus, chronicity, long-term sequelae and whether it is treatable.
- Duration of asymptomatic viremia, proportion of subclinical disease and whether it is transmissible through blood transfusion.
Characteristics

- Family of herpeviridae, non enveloped, 27-34 nm in diameter.
- Genome 7.2 kb, single stranded RNA
- Three discontinuous partially overlapped ORFs
- Four major genotypes;
  - genotype 1 and 2 restricted to human
  - Genotype 3 and 4 zoonotic, isolated from human and animals
ORF1 encodes a protein of 1693 amino acids containing functional motifs include methyl transferase, protease, RNA helicase, and RNA-dependent RNA polymerase.

ORF2 encodes the viral capsid protein of 660 amino acids responsible for virion assembly, interaction with target cells and immunogenicity.

ORF3, which overlaps ORF2, encodes a small protein of 114 amino acids involved in virion morphogenesis and release.
Epidemiology

- Genotype 1: Asia, Africa
- Genotype 2: Mexico, Africa
- Genotype 3: Worldwide
- Genotype 4: South East Asia
- HEV genotype 1 is more conserved and five subtypes: 1a, 1b, 1c, 1d, and 1e.
- Genotype 2 two subtypes: 2a and 2b.
- Genotypes 3 and 4 are extremely diverse and are divided into 10 and 7 subtypes respectively.
- These genotypes and subtypes helpful in understanding epidemiological characteristics of the virus.
Figure 2: Worldwide distribution of clinical cases of HEV infection
Note, that in several countries, including in South America, there have been occasional reports of HEV3 infection. Countries left blank are those with insufficient data.
Epidemiology

• Developing Countries:
  • HEV 1 and 2 periodic outbreak: water borne and faecal contamination of water.
  • HEV is a major cause of sporadic hepatitis and more than 25% of acute sporadic hepatitis is attributed to hepatitis E in endemic areas.
  • In India, up to 30-70% of acute sporadic hepatitis is attributed to HEV.
HEV 3 and 4

- Responsible for sporadic cases of autochthonous Hepatitis in both developed and developing countries.
- Zoonotic transmission: consumption of raw/undercooked pork/game meat
- Animals such as **boars, deer, cows, sheep, goat, horses and rabbits** susceptible to infection and acting as reservoirs for HEV in nature.
During epidemics, attack rates of 1-15% are seen with a case fatality rate of 0.2% to 4%. The attack rates are higher for males as compared to females and young adults as compared to children. Children present with the anicteric form of the disease more often than adults (21.8% vs. 14.6%). A significantly higher rate of 10-20% of fulminant hepatic failure has been observed in pregnant women especially during the third trimester. Person to person transmission is unusually low. Recently, significant person to person transmission has been reported in Uganda.
Serology and Seroprevalence

Figure 1: Schematic representation of HEV infection, showing virus detection at different sites and serological response.
Seroprevalence

• The presence of anti-HEV IgG antibody has generally been taken as evidence of prior exposure to HEV.
• The duration of persistence of circulating IgG anti-HEV antibodies remains unclear.
• Characteristic regional variations in the age, gender and urban/rural seroprevalence of HEV.
• In western countries, the seroprevalence of HEV IgG antibodies varies from 1% to 20%.
• In most developed countries, the prevalence is in the range of 2-4% in blood donors.
Seroprevalence

HEV IgG seroprevalence range from 13% among donors younger than 30 years to 43% older than 60 years.

Also high seroprevalence among blood donors in south west France and South West England.

In India, the seroprevalence of HEV IgG in young adults is around 20-40% rises with age.

In contrast, in Egypt, 65% of the children below 10 years are HEV seropositive suggesting a widespread exposure early in life.

The reasons for variation in patterns of seroprevalence with respect to region, gender and age are still not clear.
Course and treatability

- Varied manifestation acute self limiting to fulminant hepatitis.
- Acute: malaise, fever, jaundice after prodromal phase
- Lab Profile: ↑ unconjugated Bilirubin, ↑ ALT,
- Deranged coagulation
- Small group: develop prolonged illness and cholestasis
Fulminant Hepatitis

• In high endemic areas
• A small subset especially pregnancy in third trimester at higher risk of developing massive liver necrosis, encephalopathy, coagulopathy.
• Mortality very high (20%)
• Recent investigations in India showed that pregnant women infected with HEV-1 had an increased risk of developing fulminant hepatitis in comparison to non-pregnant women.
• **Approximately two thirds of the diseased women had a premature delivery, and about half of them died from acute liver failure.**

Susceptible group and Chronic hepatitits

- higher morbidity and mortality: Pre-existing liver disease and responsible for rapid decompensation and death in patients with chronic liver disease of diverse aetiology such as chronic hepatitis B and C, Wilson's disease and autoimmune and cryptogenic liver disease
Chronic HEV

- Mainly caused by HEV3, no report from HEV 1 and 2
- Persisting HEV RNA in serum or stool more than 6 months
- Most cases with solid organ transplant and Hematopoietic Stem Cell Transplant recipient.
- Individuals with HIV
- Pt. with Hematological malignancy and under chemotherapy
<table>
<thead>
<tr>
<th></th>
<th>Immunocompetent</th>
<th>Immunosuppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Presentation</strong></td>
<td>Often symptomatic</td>
<td>Rarely symptomatic</td>
</tr>
<tr>
<td><strong>ALT at diagnosis</strong></td>
<td>$\geq 1000-3000$ IU/L</td>
<td>$\geq 300$ IU/L</td>
</tr>
<tr>
<td><strong>HEV genotype</strong></td>
<td>Genotype 1, 2, 3, or 4</td>
<td>Only genotype 3 HEV infection has been reported in this population</td>
</tr>
<tr>
<td><strong>HEV diagnostics</strong></td>
<td>Increase in IgG and IgM PCR is positive in 75%</td>
<td>Serological testing is unreliable, and seroconversion might never occur The diagnosis should be established by PCR</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Resolving hepatitis</td>
<td>Chronic infection occurs in 60% of patients, and 10% develop cirrhosis</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Ribavirin has been used in very few patients presenting with severe acute hepatitis</td>
<td>Interferon-α and ribavirin are effective treatments for treating chronic HEV infection in this population; a 3-month course of ribavirin therapy is recommended</td>
</tr>
</tbody>
</table>

ALT=alanine transaminase. HEV=hepatitis E virus.

Table 1: Hepatitis E virus infection in immunocompetent and immunosuppressed patients

Is HEV is Transfusion transmissible?

• The high rate of asymptomatic/subclinical HEV infection worldwide

• In recent years, studies have shown asymptomatic viremia in blood donors which is suggestive of ongoing subclinical infection.

• In a study by Gotanda et al. 6700 Japanese blood donors with elevated ALT, 479(7.1%) were HEV seropositive of which 6 had detectable HEV RNA in blood. Three donors, seronegative for HEV, also had detectable HEV RNA.

• In another study, Sakata et al. have shown the presence of HEV RNA in blood donors with elevated ALT levels.

• Arankalle and Choube from Pune, India, have shown 1.5% (3/200) of blood donors to be positive for HEV RNA and suggested the possibility of transmission by transfusion.
Hepatitis E virus in blood components: a prevalence and transmission study in southeast England

Findings
79 donors were viraemic with genotype 3 HEV, giving an RNA prevalence of one in 2848. Most viraemic donors were seronegative at the time of donation. The 79 donations had been used to prepare 129 blood components, 62 of which had been transfused before identification of the infected donation. Follow-up of 43 recipients showed 18 (42%) had evidence of infection. Absence of detectable antibody and high viral load in the donation rendered infection more likely. Recipient immunosuppression delayed or prevented seroconversion and extended the duration of viraemia. Three recipients cleared longstanding infection after intervention with ribavirin or alteration in

Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012
Clinical relevance

• Retrospective study in transfusion recipient: not very consistent
• However, studies from endemic areas have suggested the possibility of transmission through blood transfusion
• No data on effects of transmission of HEV through blood have been described in the subset of patients at high risk of fulminant hepatic failure such as pregnant females, patients with pre-existing liver disease and solid organ transplant recipients.
Detection and Diagnosis

- **Cell culture:** human cell lines (A549, No. RCB0098, RIKEN BRC Cell Bank, Tsukuba, Japan, and 196 PLC/PRF5, hepatocarcinoma cell line, ATCC No. CRL-8024)

- **Serology:** 95% of acute HEV infections are detected as being reactive for IgM class antibodies in 1-4 weeks after onset but disappear after 3 months.
Serology and diagnosis

• IgG class antibodies too are detected very strongly during the acute phase of infection and are known to persist for several months/years.
• Serological samples have to be confirmed by rise of anti-IgG titre in second sample collected 8-10 days later.
• The serologic determination of an HEV infection is usually performed by ELISA tests and confirmed by immunoblot.
Serology and diagnosis

• Genetically engineered peptides derived from ORF2 and ORF3 of genotypes 1 or 2 serve as target antigens.

• Recent available antibody assays (Abbott and Genelabs) have adequate sensitivity and specificity.

• Antigen detection systems are also used for diagnosis of early/acute and persistent infections. (Wantai HEV-Ag enzyme-linked immunosorbent assay (ELISA))
Molecular Diagnosis

Evaluated the analytical sensitivity and performance of three HEV RT-PCR assays (RealStar HEV reverse transcription-PCR [RT-PCR], hepatitis@ceeramTools, and ampliCube HEV RT-PCR) for screening of individuals for HEV infections (ID-nucleic acid amplification technology [ID-NAT]) and for blood donor pool screening (minipool-NAT [MP-NAT]).
Result and conclusion:

The applicability of HEV antigen (HEV-Ag) screening was compared to that of RT-PCR screening and detection of HEV-IgM antibodies using seroconversion panels of 10 HEV genotype 3-infected individuals.

Four individuals revealed a positive HEV-Ag detection result, with corresponding viremias ranging from 1.92E03 to 2.19E05 IU/ml. The other six individuals showed no presence of HEV-Ag although the corresponding viremias were also in the range of >1.0E03.

Anti-HEV-IgM antibodies were detectable in seven donors; one donor presented parallel positivities of HEV-Ag and anti-HEV IgM.

The evaluated NAT methods present powerful tools providing sensitive HEV detection with high reproducibility for both ID-NAT and MP-NAT. Application of HEV-Ag or anti-HEV IgM screening is currently inferior for the early detection of HEV infection due to the decreased sensitivity compared to NAT methods.
Any other methods of donor/blood product screening

- Donor Screening: Since HEV infections are usually asymptomatic, specific questions regarding an HEV infection do not appear to be meaningful.

- Other screening methods: non-enveloped virus, SD inactivation procedure of plasma or treatment with amotosalen is considered as ineffective.

Hepatitis E transmission by transfusion of Intercept blood system–treated plasma

2 cases of HEV transmission by 2 units of Intercept treated plasma originating from the same donor. BLOOD, JANUARY 2014 VOLUME 123, NUMBER 5
What should be testing Strategy for HEV in India

• **Background:**
  - Asymptomatic/subclinical infection
  - Very high seroprevalence with increasing age in endemic region.
  - Major concern on blood safety issue in specific group of recipients/patients:
    - Pre-existing liver disease, organ transplant, immunocompromised, pregnancy.
What should be testing Strategy for HEV in India

Q1: Should we go for universal Molecular/NAT testing for HEV?

Should we screen blood products for hepatitis E virus RNA?
In view of the prevalence of hepatitis E infection in the general population (and therefore in potential blood donors) and the severe consequences of hepatitis E infection in immuno compromised patients, systematic screening of blood products for markers of hepatitis E infection should be implemented in countries where hepatitis E is endemic, including Germany, Sweden, and France. Because serological testing is poorly sensitive, hepatitis E nucleic acid testing should be considered. www.thelancet.com Vol 383 January 18, 2014

- Pros: Powerful and superior method, recommended in developed countries.

- Not FDA approved NAT test as yet.

- Cons: Expensive, labour intensive, special training for working in molecular Lab.
What should be testing Strategy for HEV in India

• Q2: Should we go for alternative testing?

In developing countries, a simple and non laborious test such as HEV-Ag, enabling early detection of viral infection prior to the occurrence of IgM antibodies, would enhance the management of HEV infection. [Detection of HEV antigen as a novel marker for the diagnosis of hepatitis E. J. Med. Virol. 2006; 78:1441–1448.]

The combination of anti-HEV IgM and HEV-Ag compensates for the delay in the detection of anti-HEV IgM (Wantai HEV-Ag assay. Journal of Clinical Microbiology June 2014 Volume 52 Number 6
What should be testing Strategy for HEV in India

• Q3: Should we go for selective HEV screening of blood products for high risk patients in endemic areas?
  
• As HEV does not have significant impact on immunocompetent recipient.
  
• Selective screening could be a rational approach;
  
• These patients often require repeated transfusions, exposed to greater risk of transmission of HEV, prolonged viremia and long term sequelae.
Conclusion

• Still debatable
• No evidence of significant morbidity in most recipients.
• However, serious consequences in certain groups of pt.
• No definitive modalities of treatment.
• Licensed vaccines still not available
• **Consideration should be given to selective screening for patients at high risk**
Thank you