Hepatitis E and Acute Liver Failure in Pregnancy

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Hepatitis E virus is a positive strand RNA virus with three open reading frames which is transmitted predominantly through the fecal contamination of water and food. It is the most common cause of acute liver failure in endemic areas. Pregnant women especially from the Indian subcontinent and Africa are at increased risk of contracting acute HEV infection as well as developing severe complications including ALF. Transmission of HEV occurs from mother to unborn child. Both maternal and fetal complications may occur, including abortion, fetal demise, preterm labor and maternal or neonatal death. The precise reasons for increased susceptibility to HEV infection during pregnancy and associated severe disease are still an enigma. Management is supportive and termination of pregnancy is not recommended as a general rule. Prevention of infection is of vital importance, as availability of clean drinking water can reduce the burden of this disease in the community. There is a need for future research to focus on prevention of ALF in pregnancy and to study the disease pathogenesis, which is not explicitly understood at present. The availability of a vaccine may alter the natural course of the disease in this select population which is at risk. (J Clin Exp Hepatol 2013;3:213–224)

Hepatitis E virus (HEV) is a positive strand RNA virus with 7.6 kb length and has 3 open reading frames (ORF).[1] ORF1 encodes non-structural proteins while ORF2 is responsible for structural region of HEV. Role of ORF3 is yet unclear.[2] It is an important cause of acute liver failure (ALF) in the epidemic and endemic setting.[3–10] While pregnancy does not confer increased susceptibility to hepatitis A, B and C viruses, pregnant women may be more vulnerable to HEV infection. Hepatitis E related ALF is reported predominantly from developing countries, especially the Indian subcontinent and African countries such as Somalia and Sudan but a few case reports of HEV infection in pregnancy are now being reported from developed countries.[11,12] The important issues in HEV related infection in pregnancy include: a) whether acute viral hepatitis (AVH) is a risk factor for mortality in pregnancy b) increased proneness of pregnant females to contract HEV c) severity of liver disease in these patient subgroups as compared to the rest of the population d) the natural course of HEV-ALF among pregnant females and outcome of pregnancy and fetus in such patients. These issues are addressed in the present review.

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Abbreviations: HEV: hepatitis E virus; ORF: open reading frame; AVH: acute viral hepatitis; ALF: acute liver failure; NR: not reported; NANE: non A, non E; OR: odds ratio; CTL: cytotoxic T lymphocytes; P: pregnant; NP: non-pregnant; NK: not known
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HEPATITIS E VIRUS BURDEN

It is widely believed that HEV associated liver disease is confined to developing countries due to lack of safe water supply. However, autochthonous HEV infections have recently been documented from developed countries like France, Japan, United Kingdom and USA.[13–15] While contaminated water and food remains the source of infection in developing world, zoonotic transmission of HEV has been documented in developed industrialized nations.

A recent study by WHO[16] assessed liver disease burden globally and identified that worldwide, approximately 3.7 million people get affected by HEV, with 70,000 mortality in a year. A recent study from Bangladesh analyzed the verbal autopsy data from 4 population based studies to assess the maternal and neonatal mortality rates associated with jaundice during pregnancy. This study reported that upto 19–25% of all maternal deaths and 7–13% of all neonatal deaths were associated with jaundice.[17] The mortality ratio associated with jaundice during pregnancy in Bangladesh was 54–55 per 100, 000 live births, and the neonatal mortality ratio associated with jaundice during pregnancy was 2.2–4.2 per 1000 live births. The study further reported that mortality among pregnant females with HEV-ALF was 58%. These data would indicate that burden of HEV associated liver disease is substantial globally and in HEV infection during pregnancy high maternal and fetal mortality has been documented.

The highest burden of ALF is found in the Indian subcontinent, China and Southeast Asia where HEV is the commonest cause of endemic and epidemic acute hepatitis.[3–10,18] The hepatitis E virus is predominantly transmitted through fecal contamination of water and food.[9,19] The contamination of

Table 1 Studies across the world reporting female predominance and role of pregnancy in ALF.

Country, year	No. of cases (N)	Number of females overall (%)	Pregnancy	Percentage of female patients with pregnancy associated liver failure	Etiology of ALF (HEV)	Overall mortality reported (pregnant females)
USA,[20] 2012	1696	1173 (69%)	16	1.5%	Nil	NR
UK,[21] 2009	422	257 (61%)	? NR	NR	Nil	NR
Germany,[22] 2012	109	69 (63%)	3	NR	Nil	33%
Australia,[23] 2004	80	64 (80%)	? NR	NR	Nil	NR
India,[24] 2008	1015	590 (58%)	249	38.5%	59.4%	54%
India,[25] 2003	180	111 (62%)	49/83	59%	96%	66%
France,[26] 2008	363	–	? NR	2%	Nil	NR
Japan,[27] 2011	856	423 (49%)	? NR	NR	Nil	NR

NR: Not reported.

water supply, in both rural (well water) and urban areas (water pipes) is responsible for outbreaks. The epidemiology of HEV has been discussed in detail in the previous issue of this journal.[18] The epidemiology relevant to HEV-ALF in pregnancy will be discussed in this section.

ALF is more common in females across the world, except in Japan where its incidence is reported to be equal in males and females. The reasons for female preponderance across different etiologies are not clear. Among the females developing ALF in the Indian subcontinent, significant proportions are pregnant. Studies from the West and Japan have not highlighted this issue, as the number of pregnant patients developing ALF is relatively small. Table 1[20–27] provides reported global data on frequency of female preponderance among ALF patients as well as frequency of pregnancy among females with ALF.

SEROPREVALENCE OF HEPATITIS E VIRUS ANTIBODIES IN PREGNANT FEMALES AND ITS RELEVANCE

Seropositivity of IgM anti-HEV antibody with or without detectable HEV RNA in sera indicates acute HEV infection in endemic regions, whereas serological detection of IgG anti-HEV indicates previous exposure/infection to HEV.

The prevalence of IgG antibodies in various asymptomatic population groups varies from 5% to 90%.[28,29] These antibody levels wane with time and their role in protection against subsequent infection is controversial. In a study from an endemic area in Kashmir, India, the seroprevalence of IgG Ab among a cohort infected with HEV during an epidemic of acute HEV hepatitis decreased to 47% fourteen years after documented acute hepatitis. The seroprevalence in the said area 30 years after the epidemic was 4.5%.[30] Similar decline in IgG anti-HEV titers was also documented in Nepal.[31]

The prevalence of IgG antibodies in asymptomatic pregnant females across the world varies from 3.6[32]–84.3%,[33] the highest being reported in Egypt. None of these patients had any symptoms of liver disease in the past. Seroprevalence of IgG anti-HEV is higher in developing countries than in developed countries as shown in Table 2.[32–38,39] These studies indicate that HEV exposure/infections are frequent in endemic area of HEV and IgG-anti HEV titers decline over time. Further, long term protective role of IgG-anti HEV in such individuals remains unclear.

Table 2 Seroprevalence studies: hepatitis E virus infection in pregnancy.

Author and country	Patients	Pregnancy (trimester)	Pregnancy (IgM HEV/IgG HEV Ab)
Lindemann,[32] Spain.	1040	1st trimester, asymptomatic	IgG 38/1040 (3.6%) IgM: 7/1040 (0.67%)
Stoszek,[33] Egypt	2428	Asymptomatic, 2nd and 3rd trimester	IgG: 2046/2428 (84.3%)
Begum,[34] New Delhi, India	300	16–24 weeks gestation asymptomatic	IgG: 101/300 (33.6%)
Gad,[35] Egypt	116	Asymptomatic	IgG: 68/116 (58.6%)
Mesquita,[36] Portugal	12	Asymptomatic	IgG: 4/12 (25%)
Adjei,[37] Ghana	157	76%: 3rd trimester, 23% 2nd trimester Asymptomatic	IgM: 29/157 (18.4%) IgG: 16/157 (10.2%) Overall, 25% in 2nd and 30% in 3rd trimester +ve
Caron,[38] Gabon, Central Africa	840	Asymptomatic	IgG: 119/840 (14.1%)
Cevrioglu,[39] Turkey.	245	3rd trimester	IgG 31/245: (12.6%)

Table 3 Sporadic studies reporting the AVH/ALF in pregnancy.

Author, year, country	No of pregnant females/total population	No of AVH/ALF	HEV as cause of AVH/ALF	Other causes of ALF in pregnant females	Overall outcome (deaths)	Outcome (maternal deaths) HEV	Relationship to different trimesters
Beniwal,[42] 2003, New Delhi, India	97	AVH: 69 ALF: 28	AVH: 25/69 (36%) ALF: 21/28 (75%)	Non-A, E: 5 HAV: 1 HBV:1	Total : 24/97 AVH: 0, ALF: 24 HEV: 18/24 (75%), Non A, E: 5/24 (21%) HAV: 1/24 (4%)	HEV group: 18/46 (39.1%) expired 18/21 (85.7%) of HEV-ALF died	–
Jaiswal,[43] 2001, Indore, India	273 P: 127 NP: 146 (controls)	P: 83/127 (AVH); 44/127 (ALF) NP: 129/146 (AVH); 17/146 (ALF) 42/44 of ALF (2nd and 3rd trimester)	P (AVH): 40/83 (48%) P (ALF): 33/44 (75%)	HBV: 4.5% HDV: 2.3% Non A, E: 16%	Total: 24/273 P: AVH 3/83 (3.6%); ALF 21/43 (48.8%) HEV: 16/24 (66.6%) Non A, E: 6/24 (25%) HBV: 2/24 (8.4%)	P (AVH) 1/40 (2.5%) P (ALF): 15/33 (45.4%)	Total 15 deaths 1st: 1/2 (50%) 2nd: 4/12 (25%) 3rd: 10/19 (53%)
Rasheeda,[44] 2008, Chennai, India	115 developed jaundice in 1,01,754 antenatal cases		AVH: 86/115 (75%)	–	5/115	3.4%	–
Patra,[45] 2007, New Delhi	220 consecutive P females (2nd and 3rd trimester)	AVH: 129 ALF: 91	AVH: 59/129 (46%) ALF: 73/91 (80%)	–	AVH: 0 ALF: 60/91	HEV group: 54/132 (41%)	Mortality 2nd: 66% 3rd: 78%
Hamid,[46] 1996, Pakistan	5935 antenatal cases 52 had jaundice	AVH: 30/52 ALF: 12/52 Other causes: 10/52	8/12 (75%)	–	2/12 (16.6%)	1/8 (12.5%)	–
Bhatia,[24] 2007, New Delhi, India	1015 ALF P: 249 NP: 341 M: 425		HEV-ALF: 342/1015 (34.4%) P (ALF): 145/249 (59.4%) NP (ALF): 100/341 (30.4%)	HAV: 2 (0.8%) HBV: 7 (2.9%) Dual acute 4 (1.6%) Drugs 6 (2.5%) Chronic markers 12 (4.9%) No etiology 68 (28%)	575/1015 (56.7%) P: 134/249 (53.8%) NP: 195/341 (57.2%) M: 246/425 (57.9%) NS	HEV: P: 74/145 (51%) NP: 46/100 (46%) M: 36/97 (37%) Non-E: P: 52/95 (55%) NP: 132/214 (62%) M: 184/293 (63%) NS	Mortality: 1st: 3/5 (60%) 2nd: 92/171 (54%) 3rd: 39/70 (56%) NS
Khuroo,[47] 2003, Kashmir, India	P: 76 NP: 337	P (76) AVH: 29/76 ALF: 47/76 NP (337) AVH: 303/337 ALF: 34/337	P: 65/76 P (ALF): 45/47 (96%) NP: 140/337 NP (ALF): 14/34 (41.2%)	P (ALF): 2/47 NP (ALF): Non A, E: 14/34 (44.1%) HBV: 5/34 (14.7%)	ALF: 50 P: 25/47 NP: 25/34	ALF: HEV: 30/59 Non-HEV: 20/22	Prevalence of HEV: 1st: 76.9%, 2nd: 88.9%, 3rd: 83.8% (NS) Rate of ALF: 1st: 4/13 (30.8%), 2nd: 12/18 (66.7%), 3rd: 23/37 (62.2%) P: 0.015

P: pregnant; NP: non-pregnant; M: men; AVH: acute viral hepatitis; ALF: acute liver failure; HAV: hepatitis A virus; HBV: hepatitis B virus; Non A, E: non A, non E, NS: not significant.

During epidemics of HEV infection in endemic areas, pregnant females have been documented to be more prone to contract the infection, and more often develop severe liver disease with accompanying increased mortality rate. Even in the sporadic setup in these regions similar observations have been reported. The pregnant female's enhanced susceptibility to HEV is further substantiated by documentation of increased seroprevalence of IgG anti-HEV. However, such increased susceptibility of pregnant females has been predominantly documented in regions hyperendemic for HEV. Recent reports from Portugal[36] and France[40] also suggested proneness of pregnant females to HEV, but such reports are scarce. These differences may be due to higher regional prevalence of HEV with unsafe water source and lack of appropriate sanitation practices facilitating easy HEV exposure to a population where the frequency of pregnancy is higher (e.g. 3% of Indian female population are pregnant at any unit time period).[24]

INCREASED RISK OF MORTALITY IN PREGNANCY DUE TO ACUTE VIRAL HEPATITIS

Maternal mortality in developing countries has been documented to be high due to various reasons. As mentioned earlier, these developing regions are endemic for HEV and frequency of pregnancy is substantial. Proneness of HEV infection among pregnant females is well documented from these regions. Therefore AVH which is usual manifestation of acute HEV infection is likely to increase mortality risk among pregnant females in these geographical areas. Indeed, a retrospective study of pregnant females admitted to intensive care unit evaluated the risk of mortality of various acute illnesses and reported odds ratios (OR) for these to be—acute cardiovascular (OR 5.8), nervous system (OR 4.73), respiratory failure (OR 12.9), disseminated intravascular coagulation (OR 2.4), viral hepatitis (OR 5.8) and intracranial hemorrhage (OR 5.4). Among the major causes of liver failure including viral hepatitis, acute fatty liver of pregnancy and HELLP syndrome, only viral hepatitis was associated with a poor maternal outcome.[41]

PREGNANT FEMALES ARE MORE SUSCEPTIBLE TO CONTRACT HEPATITIS E VIRUS INFECTION

Various reports evaluating etiological spectrum in individuals with acute hepatitis during both epidemic and sporadic settings indicate three distinct observations in India: 1) HEV is the commonest cause of AVH in both sporadic and epidemic settings[3–10] 2) The frequency of HEV as the cause of AVH is significantly higher among pregnant females than in non-pregnant females and males[24,25,42–47] 3) The frequency of ALF is significantly higher in pregnant females than in non-pregnant females and males with HEV infection.[7,24,25,43,45] Tables 3 and 4[7,11,12,42–46,48–50] highlight the sporadic and epidemic studies of HEV induced AVH/ALF reported in pregnant females.

A north Indian study evaluating etiology of AVH in a sporadic setup reported HEV as the etiology of AVH in 82%, 49% and 57% of the pregnant females, non-pregnant females and males respectively.[51] A similar study from Kashmir documented HEV as the etiology in 85.5% and 41.5% of AVH among pregnant and non-pregnant females respectively.[47]

During many epidemics of HEV, increased susceptibility of pregnant females to contract HEV infection has been repeatedly documented. The frequency of ALF is higher (10–22%) among pregnant women with HEV infection than among men and non-pregnant women (1–2%).[52] Further, certain studies have also documented that

Table 4 Attack rates and mortality in HEV infection across different population subgroups in epidemics.

Author, year, country	Total cases	Attack rate	Mortality (case fatality rate)			
			Overall deaths	Men	Non-pregnant	Pregnant
Khuroo,[7] 1981, Kashmir, India	275	AVH: P: 36/208 (17.3%) 1st trimester: 8.8%, 2nd: 19.4%, 3rd: 18.6% NP: 2.1%, M: 2.8% ALF: P: 22.2%, NP: 0, M: 2.8%	10	2.8%	0%	6/8 (75%) All deaths in third trimester
Vishwanathan[48] 1956, Delhi, India	29300	P: 5.3%, NP: 1.5%, M: 2.8%	65	0.2%		5/48 (10.5%)
Tandon,[49] 1982, Azamgarh, India	152	P: 10.3%, NP: 2.3%, M: 2.6%	18	8.4%	13.4%	39%
Bile,[11] 1993, Somalia	11,413	15%	346	2.9%	3.5%	13.8%
Boccia,[12] 2006, Sudan	253	P: 19.3%	45	13.5%		31.1%
Rab,[50] 1997, Islamabad, Pakistan	3827	P: 21.6%, NP: 10.9% 1st trimester: 28.6%, 2nd: 31.4%, 3rd: 40%, (P=0.57) NS	4	0		4/35 (11.4%) All in 3rd trimester

P: Pregnant, NP: Non-pregnant, M: males, NS: not significant.

susceptibility to contract HEV infection during pregnancy increases in a linear fashion with increasing trimester of pregnancy. During epidemics, pregnant women in their second and third trimesters get infected more frequently (12–20%) than men and non-pregnant women (2–4%).[7,12,48,49,52]

However, the above mentioned facts have emanated predominantly from the Indian subcontinent Northern India and Pakistan, Somalia, Sudan and Nepal.[7,11,12,50] The information of HEV and its relation to pregnancy in southern India needs appropriate prospective evaluation.

In the earlier section of the review, a suggestion was made that IgG anti-HEV a neutralizing antibody wanes over time after initial exposure and therefore repeated HEV infection is possible in an individual and cohort. Indeed in cities like Ahmedabad and Kolkata distinct epidemics of HEV infection have been reported at an interval of 8–10 years. Investigation of these epidemics suggests that HEV genotypes (subtypes) during the epidemics were different and further raising possibility of lack of prolonged protective immunity to HEV infection.[53]

PATHOGENESIS

Unfortunately, pathogenetic mechanism of all the 3 aspects of HEV infection: HEV associated ALF, its proneness to infect pregnant females and induction of ALF more frequently in pregnant females is yet unknown.

Rhesus monkeys (*Macacca mulatta*) are established animal models for acute HEV infection.[54,55] Two different studies could not document increased severity of infection in pregnant Rhesus monkeys when infected with HEV.[56,57] The present knowledge about the pathogenesis therefore is inadequate and based on studies with small sample sizes in humans and logical extrapolation of physiological alterations during pregnancy.

Pregnancy is a physiological process. Therefore, the fetus despite being a foreign entity is not rejected by the maternal immune system. Immunological tolerance during pregnancy is responsible for the protection against an alloimmune response directed at the paternal antigens expressed by the fetus. Maternal immune system may tolerate fetal antigens by suppressing cell-mediated immunity while retaining normal humoral immunity.[58] These changes usually occur locally at the fetal–placental interface but may get altered subsequent to systemic immune responses to infection contracted by pregnant females. A brief overview of the mechanisms of immune tolerance is described here.

Normal Pregnancy: Mechanisms of Immune Tolerance

The various mechanisms of maternal immune tolerance include: complement inhibition,[59] phosphocholination,[60] upregulation of programmed death ligand-1 over CD4/CD8 CTLs,[61] alteration of processing and presentation by Major histocompatibility complex, progesterone,[62] shift in Th-1/Th2 cytokine balance, Tregs (regulatory T cells) and placental exosomes. The role of these mechanisms of maternal immune tolerance is still unfolding. Few studies suggest an association with increased risk of infection and severity of HEV; however the exact mechanisms are not clear.

Predisposition to development of ALF in pregnancy: hypothesis

- Maternal factors: malnutrition
- Factors related to pregnancy
  - Switch from Th1 to Th2
  - Hormonal changes: estrogen, progesterone
  - Progesterone receptor (PR) gene mutations (PROGINS)
- Viral factors
  - Viral load
  - Genotype, subtypes, quasispecies
  - Nucleotide substitutions and amino acid substitutions

Malnutrition

The malnutrition theory, which proposed a poor nutritional intake and deficiency of folate leading to a greater risk of infection, was discussed in some initial reports but this has not been corroborated by further studies.[7,43]

Cell-Mediated Immunity

As mentioned earlier, the pathogenesis of hepatic damage in HEV infection is not known. In a recent report, when post mortem liver biopsies from HEV-ALF patients were evaluated to identify the type of cellular infiltrate, it was seen that activated CD8 CTL (Granzyme +ve, perforin +ve) were markedly higher in liver tissues of such patients than in the liver tissue of healthy control liver (collected intraoperatively during cholecystectomy) and were similar to such infiltrate in HBV-ALF, a known model of Th1 mediated immune injury to liver.[63] Further, CTL epitopes have been documented against both ORF2 and ORF3 of HEV genomes in in-vitro studies.[64] In human studies which include HEV infected pregnant females, a decrease in peripheral CD4 count with increase in CD8 count and altered CD4/CD8 ratio have been documented.[65]

Further, human studies have also documented increase in levels of Th2 associated cytokines (IL4, IL5, IL10 and IL13) and in-vitro obtunded lymphoproliferative response subsequent to nonspecific mitogens like phytohemagglutinin (PHA).[66]

These documentations logically indicate that during pregnancy immune responses get altered, particularly to viral infections and make pregnant females susceptible to such infections. It is well known that predominant immune

response to viral infections in through Th1 mediated response which gets altered during pregnancy. Further, subsequent to viral infection, antigen presenting cells (APCs) present them to naive CD4 cells to initiate immunological cascade to eradicate the infection. In pregnancy CD4 cells have been documented to decrease with enhanced Th2 response which is a well known suppressor of Th1 response.

As a result, due to decreased Th1 response, intracellular hepatocyte HEV replication may be avid, leading to increase in viremia (also documented in one study) with subsequent CD8 CTL mediated damage, specifically affecting the liver. These statements are conjectural and derived from extrapolation of the available data.

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex involved in immune and inflammatory responses, proliferation, cell survival, and apoptosis.[67] In mice lacking p65, there is increased apoptosis leading to degeneration of liver and death by 15–16 days of gestation.[68] The embryonic fibroblasts in these mice have a defect in the tumor necrosis factor (TNF)-mediated induction of messenger RNAs for I kappa B alpha and granulocyte/macrophage colony stimulating factor (GM-CSF). Prusty et al[69] reported a high activity of NF-kB in both the PBMC and postmortem liver biopsy specimens of pregnant ALF patients as compared with those from non-pregnant women and pregnant women with acute viral hepatitis (AVH) without ALF. The expression of p65 was almost absent in the pregnant ALF patients, suggesting a possible role of this pathway in the significant liver damage in these subset of patients.

The regulatory T cells (Tregs) are a subpopulation of T cells which are CD4+ CD25+ and express FoxP3. These cells suppress antigen-specific immune responses and are important for allograft tolerance. An increase in levels of circulating Tregs has been documented during early pregnancy, which peaks during the second trimester and then declines postpartum. Although the role of these cells has not assessed in the pathogenesis of HEV-ALF in pregnant patients, they may be an important player for the severe outcome seen in these patients.

Hormonal Factors

Placental steroid hormones, especially progesterone, are important for maintenance of pregnancy by suppressing the maternal immune response to fetal allograft.[70] Progesterone stimulates the activity of specific enzyme matrix metalloproteinases and adhesion molecules, inhibits antibody production, suppresses T-cell activation and cytotoxicity and modifies the activity of natural killer (NK) cells.[71] The biological effects of progesterone during pregnancy are manifested by a 34 kDa protein called the progesterone induced blocking factor (PIBF), which is released by lymphocytes of pregnant women following binding of progesterone to its receptors. In pregnant women, the PIBF concentration gradually increases until the 37th week of gestation, followed by a slow decrease until term. PIBF signals through the JAK/STAT pathway and has been shown to: alter the cytokine balance resulting in a preferential production of Th2 type cytokines in mice, inhibit NK cell activity through mediation by cytokines, and is responsible for preventing abortion of the fetus. A recent study reported that progesterone receptor (PR) gene mutations (PROGINS) were seen more often in patients with ALF. A reduced expression of progesterone receptor (PR) and progesterone induced blocking factor (PIBF), and a higher IL-12/IL-10 ratio were associated with a poor pregnancy outcome in Hepatitis E virus infection.[72]

Viral Factors

From developed countries, only a few case reports have recently been described of HEV infection in pregnancy.[40,73] Unlike reports of increased severity and mortality in the pregnant females from developing countries, such differences have not been reported from developed countries where HEV is not endemic.[74] This would suggest that factors other than pregnancy- like viral factors—may play a more dominant role.

The HEV viremia is short lived, and lasts for a few days. HEV RNA may not be detected if the patient presents late. A study from North India reported a correlation of viral load with increased severity of infection during pregnancy.[75] In this study, the HEV viral load in ALF pregnant women was $5.87 \times 10^4 \pm 1.5 \times 10^5$ μL/mL as compared to AVH pregnant women 343.29 $\pm$ 216.44 μL/mL. The viral load in ALF and AVH in non-pregnant women was 199.2 $\pm$ 225.5 μL/mL and 13.83 $\pm$ 7.8 μL/mL, respectively.[76] An increased viral replication or release from infected hepatocytes may be responsible for the high viral load seen in these patients. A possibility of pregnancy per se being a risk factor for viral replication has been raised. Though, in this study a high viral load was reported in pregnant females, it may be related to the differences in the clinical presentation of the patients (day of reporting to the hospital). The viremia in HEV is transient. Unless the patients are compared on the same day of presentation, the interpretations of these results may not be appropriate.

In addition to viral load, mutations in the open reading frame-1 (ORF1) have been associated with acute liver failure.[77] In a study from western India, complete genomes (five each from AVH and ALF patients) were sequenced. HEV genotype-1 sequences from fulminant cases exhibited 150 significantly different nucleotide substitutions when compared to all AVH sequences. At six positions, all ALF sequences showed identical substitutions (1 non-synonymous). Six amino acid changes in ORF1- F179S, A317T, T735I, L1110F, V1120I, and F1439Y—were significantly associated with HEV-type-1 ALF. Four out of the 6 ALF sequences had L1110F and V1120I amino acid

substitutions in helicase domain raising a possibility of these mutations playing a role in determining the outcome of HEV infection.

Effects of Genotype, Geographical Distribution and Pregnancy

Genotypes 1 and 2 of hepatitis E virus are prevalent in hyperendemic regions where the reservoir for HEV seems to be human, and cause outbreaks, sporadic acute hepatitis, acute liver failure, and acute-on chronic liver failure.[18] Genotypes 3 and 4 are more prevalent in USA, Europe, and Japan where the reservoir seems to be represented by pigs, and zoonotic transmission is considered to be the cause of infection of human beings, leading to autochthonous acute HEV. Genotypes 3 and 4 have not been reported to be associated with severe liver disease and the majority of cases appear to represent subclinical infection. Table 5 highlights the difference between various HEV genotypes.

CLINICAL PRESENTATION

HEV infection among general population during epidemics and sporadic situations is usually a self limiting illness. In the clinical setting, during pregnancy, the illness starts with a prodrome, followed by development of jaundice. The acute liver failure in them is recognized by the occurrence of encephalopathy and coagulopathy without history of previous underlying pre-existing liver disease.[78] There is variation in the length of time intervals from the onset of icterus to the development of encephalopathy across different reports. Most large studies report onset of encephalopathy within 4–8 weeks of onset of icterus.[24,25]

In a large single centre study involving 1155 patients of acute liver failure, 249 patients were pregnant. The study reported icterus to encephalopathy interval to be 5.2 ± 4.4 days, pre-encephalopathy interval 7.2 ± 5.8 days, serum bilirubin 14.4 ± 5.8 mg/dl and serum aminotransferase (AST/ALT) levels in thousands. Cerebral edema was present in half of the patients at admission and advanced encephalopathy (Grade 3/4) in approximately 75% of patients. The rapidity of onset of encephalopathy, frequency of cerebral edema, and grades of encephalopathy were similar in pregnant women when compared with non-pregnant women and men with HEV-ALF.[24] Some studies have reported a shorter pre-encephalopathy interval (5.8 ± 5.3 days) and greater incidence of cerebral edema and disseminated intravascular coagulation in pregnant women as compared with non-pregnant women.[25]

Associated complications that may occur during the course of illness include seizures, gastrointestinal bleeding, renal failure and sepsis. Infection and sepsis are the two major causes of mortality in these patients. Whether the pregnancy has an effect on the clinical course of disease is not clear. These patients usually have more complications, as will be discussed later.

Do Pregnant Women with Viral Hepatitis more often Progress to Liver Failure?

There are a few studies in the literature to support this notion, but all of them suffer from selection bias. Most studies describing the course of acute viral hepatitis during pregnancy has included self referred patients. Patients who have more severe illness are more likely to report to the hospital and progress to liver failure. However, there is some evidence that acute hepatitis caused by HEV may be particularly severe among pregnant women and girls, with mortality rates reaching 15–20%. In prospective studies evaluating pregnant patients with acute hepatitis caused by HEV, progression to ALF has been reported in 15–60% of patients.[25,45] In two prospective, comparative studies from Kashmir and Delhi, 55–70% of pregnant women and girls with HEV hepatitis compared with 10%–20% of pregnant women and girls with non-HEV hepatitis progressed to liver failure.[25,45] Hence, increased attack rates of HEV hepatitis and increased progression to liver failure during pregnancy together translate into a higher proportion of ALF during pregnancy in endemic areas.

Does Liver Failure during Pregnancy have a Poor Prognosis?

The disproportionate mortality attributable to ALF among pregnant women and girls in endemic areas is because of their high absolute numbers. HEV leads to a large number

Table 5 Hepatitis E virus: genotypes, geographical prevalence, viral and disease characteristics.

Genotype	Reservoir	Animal to human transmission	Water borne transmission	Geographical distribution	Severity	Chronicity	Relation to pregnancy	Epidemics
1	Human	No	Yes	South Asia, Central Asia, China, sub-Saharan Africa	Yes	No	Cause severe disease	Yes
2	Human	No	Yes	Mexico, Nigeria	Yes	No	NK	Yes
3	Swine	Yes	NK	Industrialized countries, USA, Europe, Japan	No	Yes	NK	No
4	Swine	Yes	Yes	Taiwan, Japan, China	No	NK	NK	No

NK: not known.

of deaths among pregnant ALF patients simply because it is the major cause of ALF in endemic areas. In a study from North India, the severity of liver failure and complications were similar among the pregnant women and girls, age matched non-pregnant women and girls, and men and boys regardless of the cause.[24] Liver failure during pregnancy has a poor prognosis. The influence of pregnancy becomes redundant once liver failure develops.

Duration of Viremia in Pregnancy

Small sample size studies have reported that the HEV viremia in the general population is usually short lived, for 4–6 weeks after the acute infection with HEV.[79–81] Maximum reported duration of viremia in serum is upto 112 days.[82] A recent study[34] reported a significantly higher proportion of viremia in pregnant as compared with non-pregnant women (88.3% vs 27.6%) at day 15. The effect of the duration on the severity of the disease and the outcome of pregnancy is not clear.

Maternal Complications

Severe complications may occur in patients with acute liver failure in pregnancy. The complications are reported to be more in the second and the third trimester. The maternal complications include preterm labor, spontaneous abortions and death due to acute liver failure.[45] A study from North India, involving 132 pregnant women with HEV infection, reported obstetric complications in the form of antepartum hemorrhage (23%), postpartum hemorrhage (14%) and premature rupture of membranes (9%). Coagulopathy is responsible for the increased risk of bleeding seen in these patients.

Maternal to Fetal Transmission

Transmission of the hepatitis E virus occurs commonly from mother to fetus. The transmission rates have been reported to be high varying from 33 to 100%.[82,84] Intrauterine HEV infection has been postulated as one the causes for increased complications in both mother and child.[85] One of the factors associated with transmission include maternal HEV RNA positivity.[83] Kumar et al in a study from UAE reported a mother to infant transmission rate of 100% in pregnant patients who were HEV RNA positive.[84] Table 6[45,83–86] highlights various studies reporting the maternal and fetal complications and maternal–fetal transmission of HEV.

Fetal Complications and Outcomes

The clinical presentation in the neonate may be as hypothermia, jaundice, anicteric hepatitis, acute liver failure,

Hepatitis E

Table 6 Pregnancy and HEV: Vertical transmission.

Author, year and country	No of HEV +ve pregnant women	No of fetus HEV +ve	Maternal complications and deaths	Fetal complications	Fetal follow up
Kumar,[84] 2004, UAE	28 2 died undelivered 22 AVH 4 ALF	26/26 (100%) HEV RNA +ve	2 died undelivered 1 ALF died	10 babies: 8 term, 2 premature between 34 and 36 weeks 5 neonates had elevated ALT Deaths: 2 1 each had hypothermia and hypoglycemia	All surviving infants were negative by 9 months of age. Mother to infant transmission 100%
Khuroo,[85] 2003, Kashmir, India	26 5 died undelivered 11 AVH 15 ALF	15/19 (79%)	5 died undelivered 9/15 (60%) ALF died Total delivery (21) Abortions 2 Premature 4 Full term 15	Icteric hepatitis: 7 Anicteric hepatitis: 5 Elevated bilirubin, normal AST/ALT: 3 Deaths: 7/19 (37%) Prematurity: 1 Icteric HEV: 3 Anicteric HEV: 2 Hyperbilirubinemia: 1	All surviving babies HEV RNA negative by 32 weeks
Singh,[83] 2003, New Delhi, India	22 8 AVH 14 ALF	3/6 (50%)	14/22 (63.6%) died All ALF died	–	–
Kumar,[86] 2004, New Delhi, India	28 19 AVH 9 ALF	6/18 (33%)	7/26 died (All ALF) 5/26 died undelivered	Prematurity: 66%	–
Patra,[45] 2007, New Delhi, India	132 59 AVH 73 ALF	–	54/132 (41%)	Preterm delivery (90%) Abortions (8%) Still birth (54%)	

AVH: acute viral hepatitis, ALF: acute liver failure.

recurrent diarrhea, fever or stillbirth.[85] Hypoglycaemia may occur, the liver function test abnormalities include elevated bilirubin alone, elevated aminotransferases or a combination of both. The diagnosis is made by the presence of IgM HEV antibody and/or HEV RNA positivity.

In a sporadic setting, among all pregnant women infected with HEV, still births have been reported in 54% and neonatal deaths in 17%[45] whereas in an epidemic, fetal deaths including intrauterine and neonatal deaths were reported to be 12.4% in HEV related AVH and 75% in HEV-ALF patients.[85] Among the survivors, the disease is self limiting and the viremia is short lasting, lasting for a maximum of 32 weeks.[85] Recurrent HEV infection and long term sequelae have not been reported till date among survivors. In comparison to ALF due to non-E causes, fetal complications are reported more in HEV.[45] HEV-ALF is associated with increased mortality and hence, the intrauterine fetal deaths are expected to be high. It is still not clear from the available data whether the increased fetal complications occur due to maternal ALF or vertical transmission of HEV. We need more prospective studies to answer this question.

MANAGEMENT

HEV infection is usually self limiting and, in the absence of complications, does not require therapeutic intervention. In cases of uncomplicated viral hepatitis symptomatic treatment leads to improvement. The management in HEV induced ALF in pregnancy is no different from other patient groups. There is increased risk of complications as discussed above, the occurrence of each of these needs to be managed on case to case basis. The principles of management of abortions, preterm labor, premature rupture of membranes and still birth are same as for a normal pregnancy. There is an increased risk of bleeding due to associated coagulopathy.

Acute liver failure patients should be ideally managed in an intensive care unit, with continuous, noninvasive cardiac, oxygen saturation and blood pressure monitoring. Elective ventilation should be done for patients with grade IV encephalopathy and for those with grade III encephalopathy and evidence of cerebral edema. All patients should be started on prophylactic antibiotics for prevention of infection. The preferable mode of delivery is vaginal. Fresh frozen plasma is used in cases of active bleeding. Successful liver transplantation has been reported in pregnant females with acute liver failure.[87,88] It can be considered in selected cases.

Acute fatty liver of pregnancy (AFLP) has a strong genetic component and geographical variation in its prevalence. Termination of pregnancy is recommended for improving prognosis in AFLP. On the other hand, in case of HEV associated ALF in pregnancy, there is no rationale for actively terminating pregnancy in HEV endemic regions with the hope of improving the outcome of the patient. Unless the mother is critically ill, there is no evidence to support stopping breast feeding in order to prevent transmission. The anti-HEV antibody and HEV RNA are present in the colostrum of HEV infected mothers in significantly lower levels than in the maternal serum. Breast feeding appears to be safe for these infants.[89]

PROGNOSIS/OUTCOME

Prognostic Models

The ability to predict which patients with ALF will recover with medical management alone and who will succumb without liver transplantation is of paramount importance. Static variables that correlate with survival are age and etiology. Multiple prognostic models have been proposed for ALF. In a large Indian study,[8] the following variables present at admission have been identified as independent risk factors for patient outcomes: (i) age $\geq$ 40 years (ii) bilirubin $\geq$ 15 mg/dL; (iii) prothrombin time prolongation $\geq$ 25 s (iv) clinical features of cerebral edema. With an increasing number of risk factors, mortality increases; with three or more factors it is 93%. In another study from India, clinical prognostic indicators (CPI) reported include age $\geq$ 50 years, Jaundice encephalopathy interval (JEI) >7 days, grade 3 or 4 encephalopathy, presence of cerebral edema, prothrombin time $\geq$ 35 s, and creatinine $\geq$ 1.5 mg/dL. Presence of any 3 of 6 CPI was superior to MELD or KCH in identifying survivors and non-survivors.[90] Pregnancy per se or its duration of gestation does not affect the prognosis[24] and HEV etiology is associated with a better prognosis than other causes of ALF.[25]

ALF is a dynamic process in which variables determining prognosis at admission change over time, and thus the clinical course varies accordingly. A new prognostic model from India, ALF early dynamic (ALFED) model[91] is based on four variables: arterial ammonia, serum bilirubin, international normalized ratio and hepatic encephalopathy > grade II. This model takes into account the values of these variables over 3 days. The performance of the ALFED model has been reported to be superior to the King's College Hospital criteria and the Model for End stage Liver Disease score.

PREVENTION

HEV infection occurs as a result of transmission via enteric route through contaminated water and food. An improvement in sanitary conditions, availability of clean drinking water, proper sewage disposal, public education and promotion of awareness regarding hygienic defecation habits are the mainstay for the prevention of hepatitis E. Simple awareness that of the fact that these viruses can be eradicated by boiling water at 100 °C or by appropriate chlorination can be very effective.[52] Immunoglobulins are not effective in the prevention of

HEV infection in the sporadic setting[92] and also in pregnant women during epidemics.[93]

VACCINATION

Two candidate vaccines against HEV have been tested in endemic regions, providing short term protection in about 95–98 per cent of the people. The safety of baculovirus-expressed 56 kDa vaccine has been tested in the Nepalese[94] population; however the study population was predominantly males. The HEV 239 vaccine has undergone trials in the Chinese population.[95] During the trial, 37 women in the vaccine group and 31 women in the control group who were pregnant inadvertently received the vaccines. Adverse reactions were similar in both the pregnant and non-pregnant women. No fetal complications were seen. The weights, lengths, and gestational ages of the babies born to the mothers in the vaccine group and the placebo group were comparable. The preliminary data suggests that these vaccines should be safe in pregnancy. HEV vaccination has been discussed in a separate article in this issue of the journal. If made readily available, they are likely to benefit and protect the population at risk, especially in endemic areas.

FUTURE RESEARCH

There is a wide variation in frequency of ALF, both during epidemics and in the sporadic setting. The animal study and human data are inconsistent. In the absence of animal models, there is a need for prospectively designed multicenter studies with large sample sizes and clearly defined questions. The focus should be on understanding the immunology of HEV, to understand host–virus interactions occurring in various age groups and in the state of pregnancy.

CONCLUSIONS

Pregnant women are more prone to develop HEV infection. Its pathogenesis is still not completely understood. The occurrence of Hepatitis E virus infection during pregnancy is more often complicated by ALF, and there is increased risk of mortality. However, once HEV-ALF occurs, its natural course remains similar to ALF due to other etiologies. There are increased maternal and fetal complications. Premature deliveries are more frequent. However, data on abortion, still births, intrauterine deaths and teratogenicity is unclear.

CONFLICTS OF INTEREST

All authors have none to declare.

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