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MORBIDITY AND MORTALITY ASSESSMENT IN ACUTE HEPATITIS-E.

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ORIGINAL ARTICLE

MORBIDITY AND MORTALITY ASSESSMENT IN ACUTE HEPATITIS-E

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Background: Hepatitis-E is an enterically transmitted virus causing acute hepatitis. Mostly it is a self-limiting clinical course, but can be life threatening in certain high risk groups. Pakistan is endemic for Hepatitis-E with limited published literature. The aim of this study is to evaluate the predictors of mortality in patients with acute Hepatitis-E. **Methods:** We analyzed the medical records of 369 adult patients with Hepatitis-E infection admitted at Aga khan University Hospital, from January 1996 to December 2010. Details of their laboratory investigations, clinical course and complications such as FHF and mortality were noted. The outcome was compared, and determinants of mortality were evaluated in important patient subgroups. **Results:** Out of 369 patients with Hepatitis-E, 326 (88.3%) were discharged after full recovery. Out of these 22 (6%) patients had chronic liver disease CLD in this study, of whom 10 (2.7%) expired (p-value <0.001). There were about 67 (18%) pregnant patients, with a mean gestational age of 29.19±7.68 weeks and 5 (1.4%) pregnant patients died (p-value=0.23). A total of 58 (15.7%) patients were co-infected with other hepatotropic virus, and a comparison did not find an increased risk of mortality in this group. **Conclusion:** This study showed that Hepatitis-E is significantly associated with mortality in patients suffering from pre-existing chronic liver disease. Pregnancy was not a determinant of mortality in Hepatitis-E patients in this study, and neither was co-infection with other Hepatotropic viruses.

Keywords: Acute Hepatitis, Hepatitis-E, Hepatitis.

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INTRODUCTION

Hepatitis-E infection is a major form of enterically transmitted non-A, non-B Hepatitis. It is the leading cause of acute Hepatitis in the world, with the highest number of cases reported in the developing regions, i.e., Asia, Africa, Central America, and the Middle East.[1,2] With almost 2.3 billion people affected globally,[3] Hepatitis-E is an emerging pathogen. The virus is responsible for causing several water-borne epidemics of acute Hepatitis at a large scale in developing countries, where it may also cause sporadic cases. About 10–95% of admitted cases of Hepatitis in South Asia have been attributed to Hepatitis-E alone.[4–10] A re-evaluation of the epidemiology of the virus indicates a global distribution that isn't limited merely to the developing world, but with infection occurring due to food borne zoonotic transmission in the developed countries.[11–14]

Hepatitis-E infection is caused by Hepatitis-E virus (HEV), which is a single-stranded positive sense RNA virus belonging to the Hepeviridae family. It is 32–34 nm in diameter, with a genome approximately 7200 bases in length. HEV can be classified into at least four major genotypes (1–4)[15] with genotype 1 being responsible for most cases of epidemic and sporadic Hepatitis-E in the developing world. The virus has an incubation of 15–60 days, and primarily leads an acute and self-limited course. It is transmitted via the feco-oral route, and spread by contaminated water in endemic areas. The clinical presentation can't be distinguished from other forms of acute viral Hepatitis. Asymptomatic infections have also been observed, especially in children, who show a symptomatic to asymptomatic ratio of 1:12, compared to only 1:3 for adults.[8] The highest attack rates are seen in an age range between 15–40 years.[16]

Anti-HEV IgM is used as an acute phase marker for the infection, where as anti-HEV IgG is used to assess exposure to HEV. The latter studied in the healthy population of the developed countries shows a range between 9.3–26%, while that in the general population of India ranges from 4% in the areas of Kashmir and South India to a much higher 29–35% in Delhi.[8,17–19] The number of cases reported in the developed world can usually be traced back to a history of travel.[20] Being an enterically transmitted virus, it is shed in large titers in the human feces[21], which can accumulate in the sewerage that becomes an important source of contamination of drinking water resources.[22] This mode of contamination is a common phenomenon in the developing regions of the world. Infect, studies from India show an HEV prevalence of 41% throughout the year in sewerage water[23], with as high as 57% of its sewerage workers were positive for IgG anti-HEV.[24] Data from Pakistan is no different, with a study done on samples obtained from sewerage water in Rawalpindi and Islamabad discovering 40.7% of the sewerage samples to be contaminated with HEV.[25]

In addition to its role as a major cause of self-limited Hepatitis, the virus possesses certain peculiar characteristics in both its clinical course and outcome that researchers struggle to understand. A crucial association being its aggressive course in pregnant women. Various studies from developing countries showed an increased incidence of mortality in this group, especially in the third trimester.[26–30] Exact cause of increased mortality in pregnant patients is unknown but few studies suggested progression to Fulminant Hepatic Failure (FHF) and obstetric complications as a cause of death,[30,31] with a mortality that ranges as high as 15–20%[32]. Also documented in this hi-risk category of patients is the vertical transmission of HEV, with foetal outcomes that range between intrauterine foetal death to symptomatic and asymptomatic neonatal hepatic infection.[33] Interestingly however, this disproportionately high incidence of infection and mortality that was observed in the pregnant population of developing countries such as India was not shared by the pregnant population group studied in Egypt.[34]

Another equally perplexing trait possessed by the HEV is the severe morbidity it causes in another patient group, i.e., those with pre-existing and advanced chronic liver disease. Studies from India, also highly endemic for HEV, have demonstrated that super-infection with HEV in cirrhotics can be complicated by Acute on Chronic Liver Failure (ACLF) and mortality.[35–38] Other studies done on an entirely different set of patients, i.e., the immuno-suppressed patients who have undergone organ transplant, have suggested that HEV may also progress to a chronic course[39–41], and can cause chronic hepatitis in more than 60% of solid organ recipients[42] thus again controvert its role as a benign self-limiting condition.

The epidemiological profile of Hepatitis-E infection in Pakistan is similar to that witnessed in other countries of South Asia, as being endemic and leading a benign self-limited clinical course in most patients.[43,44] Outbreaks have been documented in Sargodha[46], Abbottabad[46,47] and a major epidemic that was reported in Islamabad in 1993-1994 owing to a malfunctioning water treatment plant that affected about 4,000 people[48]. HEV in Pakistan has also been reported to affect pregnant women mostly in their third trimester, with majority of the women who are young and from the lower economic strata.[49] But despite observations from around the world -and not just South Asia- that clearly depict the role of HEV as a growing and important problem, there still is a significant void in literature from Pakistan on the clinical progression and outcome of HEV that varies amongst the different patient subgroups infected with this disease. In this retrospective cohort study, therefore, we delineate the symptoms, clinical course, biochemistry indices, complications and mortality of HEV in patients treated between 1994 till 2008 at a tertiary care hospital in Karachi, Pakistan. Our aim is to evaluate the predictors of mortality in various patient groups suffering from Hepatitis-E, namely the pregnant population, those with pre-existing chronic liver disease and those patients who were co infected with other hepatotropic viruses.

MATERIAL AND METHODS

This is a cross-sectional review of medical records of 369 patients with Hepatitis-E infection admitted at Aga khan University Hospital, from January 1996 to December 2010. It was approved by hospital ethics committee. We included these patients in our study based on a diagnosis of Hepatitis-E made through patient's history, clinical signs and symptoms and laboratory workup including Enzyme-linked immuno-sorbent assay (ELISA) of Hepatitis-E antibodies (IgM) and liver function tests at the time of admission. Patients <12 years were excluded from the study. Apart from detailed laboratory investigation, we also recorded other co morbid illnesses (i.e., chronic liver disease (CLD), chronic renal failure, diabetes mellitus, ischemic heart disease etc.) The presence of a co-existing Hepatitis infection owing to other hepatotropic viruses (A, B, C or D) was also noted. Length of illness and hospital stay, number of readmissions, and any possible progression to chronicity were also looked for and noted. Pregnancy status of child bearing women was confirmed, with maternal and foetal outcome also being noted.

Patients were later on divided into groups depending upon outcome, such as uneventful recovery, morbidity like Fulminant hepatic failure (FHF) or mortality. A comparison was then made between the patients in these two outcome groups based on their demographic profile, clinical parameters and serum laboratory values. The outcome was compared, and determinants of mortality were evaluated in the following important subgroups that were analyzed in the present study: patients having CLD; patients who were diagnosed with FHF at any time during the course of their hospital stay; pregnant patients, and if they were co-infected with other hepatotropic viruses (A, B, C, D) at the time of admission.

The CLD group was subdivided on the basis of their Child Pugh score into Class A, B, and C. We defined FHF when there is development of encephalopathy and deranged prothrombin time (PT). Diagnosis of CLD was made based on ultrasound and laboratory findings. The management of all FHF

(pregnant or non-pregnant) patients was done in the HDU as per standard guidelines. Ethics approval take from hospital committee and there is no conflict of interest.

Statistical analysis was performed using the SPSS-18.0. A descriptive analysis was done for clinical and the other features, and results are presented as mean±standard deviation for quantitative variables and number (percentage) for qualitative variables. For the analysis, $\chi 2$ test and Fisher's exact test were used for categorical variables, while the independent sample t-test was applied for numerical variables. All *p*-values were two sided and considered as statistically significant if <0.05.

RESULTS

A total of 369 adult patients diagnosed with Hepatitis-E infection were included in this study. 188 (50.9%) patients were males. The mean age of our study group was 32.13±14.27 years; the general characteristics of the study group are presented in table-1. A total of 326 (88.3%) patients were discharged after making a complete recovery, and 43 (11.7%) patients expired during the course of the illness. In the expired group (n=43), there were 5/43 (11.6%) pregnant patients, 10/43 (23.3%) patients suffering from CLD, and 13/43 (30%) patients who were co-infected with other hepatotropic viruses. FHF was the cause of mortality in 27 (63.0%). Laboratory values of discharged and expired patients are given in table-2.

There were 22 (6%) patients suffering from CLD in this study, of whom 12 (3.3%) were discharged, and the remaining 10 (2.7%) patients expired (all patients were having Child Pugh Class C). Superinfection with acute Hepatitis-E in CLD patients was found to be associated with mortality (*p*-value <0.001) as summarized in table-3

There were about 67 (18%) pregnant patients in this study, with a mean gestational age of 29.19±7.68 weeks. FHF was reported in about 14/67 (20.9%) pregnant women. About 5 pregnant patients expired in the study, all due to FHF. About 30/67 (45%) pregnant patients had a full-term delivery, 27/67 (40.3%) patients had a pre-term delivery, 4/67 (6.0%) suffered a miscarriage and 4/67 (6.0%) deliveries resulted in still births. The incidence of an onset of Hepatitis-E in pregnancy was not associated with increased mortality (*p*-value=0.23) in this study. (Table-4)

A total of 58 (15.7%) patients were co-infected with other hepatotropic virus, and a comparison between the discharged vs. the expired group in this study did not find an increased risk of mortality in patients co-infected with other hepatotropic viruses without CLD.

Table-1: General characteristics of study group

Variables	Discharged (n=326)	Expired (n=43)	*p*-value
Age (Years)	31.15±13.5	39.09 ± 17.1	0.005
Gender:			0.72
Male	165 (50.6)	23 (53.5)	
Female	161 (49.4)	20 (46.5)	
Associated Hepatitis:			
Hepatitis A	8 (2.5)	0	0.18
Hepatitis B (HbsAg)	26 (8)	6 (14)	0.23
Hepatitis C	14 (4.3)	4 (9.3)	0.31
Hepatitis B& D	15 (4.6)	3 (7)	0.86
Pregnant:	62/161 (38.8)	5/20 (25)	0.23
Pregnant and FHF	0/161 (0)	5/20 (25)	
Chronic Liver Disease	12 (3.7)	10 (23.3)	<0.001
FHF	37 (11.3)	26 (60.5)	<0.001

Table-2: Laboratory values

	Discharged	Expired	*p*-value
WBC count	14.99±31.9	16.46±11.5	0.76
Platelet count	270.59±549.01	186.28±140.5	0.32
BUN	19.58±27.52	28.80±26.71	0.05
Creatinine	4.89±20.9	2.50±2.7	0.45
Potassium	8.09±52.36	4.21±0.83	0.63
Total Bilirubin			
On admission	9.93±11.1	17.61±15.1	0.002
Peak	15.46±12	21.62±11.9	0.03
Last recorded	6.54±27.8	20.62±12.7	0.01
Direct Bilirubin			
On admission	6.35±7.1	10.15±66.8	0.003
Peak	8.83±.2	10.65±7.10	0.37
Last recorded	4.28±22.1	10.82±8.06	0.27
Indirect Bilirubin			
On admission	4.28±22.1	8.31±7.29	0.59
Peak	8.43±17.2	10.05±6.9	0.71
Last recorded	2.84±10.5	9.04±6.3	0.03
SGPT			
On admission	1257.5±1342.1	860.23±1020.6	0.06
Peak	1314.15±1248.9	1153.57±1072.2	0.74
Last recorded	225.5±446.3	189.33±332.3	0.70
AP			
On admission	238.64±253.7	216.95±168.2	0.59
Peak	309.83±317.5	153.0±51.4	0.14
Last recorded	132.22±84.1	168.53±118.6	0.20
SGOT			
On admission	1171.20±1410.9	1655.53±2427.4	0.31
Peak	1181.91±1074.4	1454.40±1780.9	0.75
Last recorded	176.28±377.3	1028.44±1998.3	0.23
GGT			
On admission	158.76±483.0	101.54±108.9	0.49
Peak	220.02±317.2	67.28±24.4	0.006
Last recorded	98.05±165.4	82.86±106.23	0.72

Table-3: Chronic Liver Disease Patients

Variables	Discharged (n=12)	Expired (n=10)	*p*-value
Age (years)	36.75±14.271	41.70±14.040	0.42
Mean Hospital stay (ds)	*7.08*±4.680	9.30±5.755	0.33
Chronic Liver Disease			
Child Pugh A	1 (0.3)	0	<0.001
Child Pugh B	4 (1.2)	0	
Child Pugh C	7 (2.1)	10 (23.3)	
Spleen			
Normal	179 (88.6)	26 (83.9)	0.45
Enlarged	23 (11.4)	5 (16.1)	
Portal Vein			
Normal	195 (96.5)	22(71)	<0.001
Dilated	7 (3.5)	9 (29)	
Ascites			
None	165 (82.1)	11(35.5)	<0.001
Mild	23 (11.4)	8 (25.8)	
Moderate	10 (5)	10 (32.3)	
Severe	3 (1.5)	2 (6.5)	
Days hospitalized	5.20±5.6	8.91±6.3	<0.001

Table-4: Comparing clinical parameters in pregnant vs. non-pregnant female patients

Variables	Pregnant	Non-pregnant (n=103)	*p*-value
Age (years)	25.51±5.13	34.90±14.38	<0.001
FHF	14 (20.9)	11 (10.7)	0.06
Hospital Stay (Days)	5.72±4.72	5.25±5.10	0.55
Symptoms			
Fever	33 (49.3)	73 (70.9)	0.004
Abdominal pain	31 (46.3)	77 (74.8)	<0.001
Vomiting	37 (55.2)	82 (79.6)	0.001
Associated Hepatitis			
Hepatitis A	2 (3.0)	2 (1.9)	0.77
Hepatitis B (HbsAg)	3 (4.5)	6 (5.8)	0.61
(core B)	4 (6)	2 (1.9)	0.30
Hepatitis C	2 (3)	1 (1)	0.36
Hepatitis B & D	1 (50)	2 (100)	0.24
Diabetes Mellitus	5 (7.5)	20 (19.4)	0.03
Hypertension	2 (3)	16 (15.5)	0.009
Mortality	5 (7.5)	11 (10.7)	0.48

DISCUSSION

Pakistan is highly endemic for the Hepatitis-E virus, Studies report that this benign infection, however, has been associated with poor outcomes in a subset of patients such as those suffering from CLD[35–38], the immuno-compromised solid organ organ-transplant recipients[39–42], and very importantly the pregnant population that shows mortality between 15–20%[26–30,32]. In the present study, we retrospectively reviewed the clinical course of 369 patients admitted with Hepatitis-E infection.

The results of this study found pre-existing chronic liver disease to be significantly associated with mortality in patients suffering from Hepatitis-E, making it the only hi-risk patient group amongst the patients reviewed in this study. Interestingly, pregnancy was not a determinant of mortality in patients infected with Hepatitis-E in this study, and neither was co-infection with other hepatotropic viruses (A, B, C and D). The results of our study in the CLD group to be significantly associated with mortality, is consistent with literature. Kumar *et al*[36] compared 107 cirrhotic patients super infected with HEV to 200 (non-cirrhotic) controls. They found HEV infection in cirrhotics to be associated with rapid hepatic decompensation as well as higher mortality, citing HEV positive status to be an independent risk factor for mortality in cirrhotics, in another study in Nepal also report superinfection with HEV to be a significant predictor of morbidity and mortality in this group, where they attribute hepatorenal syndrome and upper GI bleeding as the most common causes of mortality in these patients. The mortality in CLD patients which we report in our study, however, was due to FHF.

The finding of a higher sero-prevalence and mortality in the pregnant population, who are infected with Hepatitis-E virus, is a crucial association well-documented consistently in the past in various studies through the years. The causes of mortality have been attributed to Fulminant Hepatic Failure and obstetric complications such as haemorrhage.[30,31] Incidence of vertical transmission to the foetus is also well-established. Khuroo *et al*, investigated foetal outcomes in the pregnant group infected with Hepatitis-E, and found that in-utero transmission is possible, and is responsible for foetal outcomes that range from intrauterine foetal death to symptomatic and asymptomatic neonatal liver disease.[31] Studies from Karachi, where Hamid SS *et al*.[50] evaluated the clinical course along with the maternal and foetal outcome in 12 pregnant women who presented with FHF, show maternal and foetal mortality to be at 16.6% and 50% respectively. Interestingly however, the results in the pregnant group in the present study, in which 67 pregnant patients were compared to 103 non-pregnant patients also affected with Hepatitis-E, are not consistent with several previous studies[23–27] that have consistently documented the association between HEV infection during pregnancy and mortality. The onset of complications such as Fulminant Hepatic Failure and the mortality in the pregnant group was not significantly increased than in the non-pregnant group that we studied. This finding does not support literature, with the exception of studies in another highly endemic region, i.e., Egypt, where despite the high sero-prevalence, HEV-related mortality in pregnant women remains negligible. Stoszek *et al*[34] studied the course of HEV in a cohort of 2,428 women living in the Nile Delta in Egypt. They reported an anti-HEV prevalence to be 84.3%, and factors such as older age, large number of siblings, lack of soap usage and contact with cats to be associated with sero-prevalence. None of the pregnant women in this community however, demonstrated clinical hepatitis, FHF or mortality. They attributed lack of clinical hepatitis in that geographical region to early exposure to HEV during childhood, and to a different genotype in the community which was less virulent than the one responsible for the much higher mortality in South Asia. Hepatitis-E genotypying were not done in these patients which might be one reason for mortality difference in pregnant patients. Also difference in the sample size between the pregnant and the non-pregnant patients in the present study could be a reason for this. More prospective studies are required to evaluate the sero-prevalence and mortality in pregnant women with Hepatitis-E are warranted in Pakistan, to ascertain if a less virulent strain is indeed affecting patients in this region.

Our study however, did not indicate increased mortality in patients who had serological

evidence of mixed Hepatotropic viral infection. Our results are consistent with the results reported by Kumar *et al*.[51] Arora NK *et al*[52] however, found dual infection with Hepatitis-E and Hepatitis A to be the largest etiological subgroup of acute hepatic failure in their study. In the present sample that we evaluated, Hepatitis A and E, both of which are transmitted feco-orally, did not show significant mortality.

CONCLUSION

The study reiterates the role of Hepatitis-E as a significant predictor of mortality in patients with chronic liver disease. South Asian countries like Pakistan and India still remain highly endemic for this virus that has caused major epidemics with high mortality in cirrhotics and pregnant population. Our results did not find an association between HEV infection during pregnancy and mortality, and follow-up prospective studies are required with a larger sample size and Hepatitis-E genotyping to evaluate the clinical profile in this population. Being a region that has remained highly endemic for HEV, we will benefit substantially if the safe and effective vaccine is incorporated as soon as possible especially, chronic liver disease patients and the pregnant patients would undoubtedly be most eligible candidates for the vaccine.

REFERENCES

1. Gupta DN, Smetana HF. The histopathology of viral hepatitis as seen in the Delhi epidemic (1955-56) Indian J Med Res 1957;45:101–13.
2. Emerson SU, Purcell RH. Running like water-the omnipresence of Hepatitis-E. N Engl J Med 2004;351(23):2367–8.
3. World Health Organization. Viral hepatitis. October 28, 2010, date last accessed. Available from: http://apps.who.int/gb/ebwha/pdf_files/A62/A62_22-en.pdf.
4. Sheikh A, Sugitani M, Kinukawa N, Moriyama M, Arakawa Y, Komiyama K, *et al.* Hepatitis-E virus infection in fulminant hepatitis patients and an apparently healthy population in Bangladesh. Am J Trop Med Hyg 2002;66(6):721–4.
5. Bansal J, He J, Yarbough PO, Sen S, Constantine NT, Sen D. Hepatitis-E virus infection in eastern India. Am J Trop Med Hyg 1998;59(2):258–60.
6. John R, Abraham P, Kurien G, Chandy G, Sridharan G. Sporadic Hepatitis-E in southern India. Trans R Soc Trop Med Hyg 1997;91(4):392.
7. Clayson ET, Myint KS, Snitbhan R, Vaughn DW, Innis BL, Chan L, *et al*. Viremia, fecal shedding, and IgM and IgG responses in patients with Hepatitis-E. J Infect Dis 1995;172(4):927–33.
8. Khuroo MS, Rustgi VK, Dawson GJ, Mushahwar IK, Yattoo GN, Kamili S, *et al*. Spectrum of Hepatitis-E virus infection in India. J Med Virol 1994;43(3):281–6.
9. Acharya SK, Panda SK, Saxena A, Gupta SD. Acute hepatic failure in India: a perspective from the East. J Gastroenterol Hepatol 2000;15(5):473–9.
10. Shrestha SM. Enteric Non-A, Non-B Hepatitis in Nepal: Clinical and Epidemiological Observations. Amsterdam, the Netherlands: Elsevier Science Publishers BV; 1991. pp. 265–75.
11. Khuroo MS, Khuroo MS. Hepatitis-E virus. Curr Opin Infect Dis. 2008;21:539–43.
12. Pavio N, Meng XJ, Renou C. Zoonotic Hepatitis-E: animal reservoirs and emerging risks. Vet Res 2010;41(6):46.
13. Dalton HR, Bendall R, Ijaz S *et al.* Hepatitis-E: an emerging infection in developed countries. Lancet Infect Dis 2008;8(11):698–709.
14. Meng XJ. From barnyard to food table: The omnipresence of Hepatitis-E virus and risk for zoonotic infection and food safety. Virus Res 2011;161(1):23–30.
15. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global Hepatitis-E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol 2006;16(1):5–36.
16. Gust ID, Purcell RH. Report of a workshop: waterborne non-A, non-B hepatitis. J Infect Dis 1987;156(4):630–5.
17. Mathur P, Arora NK, Panda SK, Kapoor SK, Jaikhani BL, Irshad M. Sero-epidemiology of Hepatitis-E virus (HEV) in urban and rural children of north India. Indian Pediatr 2001;38(5):461–75.
18. Das K, Agarwal A, Andrew R, Frosner GG, Kar P. Role of Hepatitis-E and other hepatotropic virus in aetiology of sporadic acute viral hepatitis: A hospital based study from urban Delhi. Eur J Epidemiol 2000;16(10):937–40.
19. Daniel HD, Warier A, Abraham P, Sridharan G. Age-wise exposure rates to Hepatitis-E virus in a southern Indian patient population without liver disease. Am J Trop Med Hyg 2004;71(5): 675–8.
20. Centers for Disease Control and Prevention (CDC) Hepatitis-E among U.S. travelers, 1989-1992. MMWR Morb Mortal Wkly Rep 1993;42(1):1–4
21. Rao VC and Melnick JL, Environmental Virology. Wokingham: Van Nostran Reinhold (UK) Co Ltd. 1986; pp. 10–17.
22. Pusch D, Oh DY, Wolf S, Dumke R, Schröter-Bobsinm U, Höhne M, *et al*. Detection of enteric viruses and bacterial indicators in German environmental waters. Arch Virol 2005;150(5):929–47.
23. Ippagunta SK, Naik S, Sharma B, Aggarwal R. Presence of Hepatitis-E Virus in Sewage in Northern India: Frequency and Seasonal Pattern. J Med Virol 2007;79(12):1827–31.
24. Vaidya SR, Tilekar BN, Walimbe AM, Arankalle VA. Increased risk of HEV in sewage workers from India. J Occup Environ Med 2003;45(11)1167–70.
25. Ahmad T, Waheed Y, Tahir S, Safi SZ, Fatima K, Afzal MS, *et al.* Frequency of HEV contamination in sewerage waters in Pakistan. J Infect Dev Ctries. 2010;4(12):842–5.
26. Kumar A, Beniwal M, Kar P, Sharma JB, Murthy NS. Hepatitis-E in pregnancy. Int J Gynaecol Obstet 2004;85:240–4.
27. Khuroo MS, Kamili S. Aetiology, clinical course and outcome of sporadic acute viral hepatitis in pregnancy. J Viral Hepat 2003;10:61–9.
28. Beniwal M, Kumar A, Kar P, Jilani N, Sharma JB. Prevalence and severity of acute viral hepatitis and fulminant hepatitis during pregnancy: a prospective study from north India. Indian J Med Microbiol 2003;21(3):184–5.
29. Medhat A, el-Sharkawy MM, Shaaban MM, Makhlouf MM, Ghaneima SE. Acute viral hepatitis in pregnancy. Int J Gynaecol Obstet 1993;40(1):25–31.
30. Tsega E, Krawczynski K, Hansson BG, Nordenfelt E. Hepatitis-E virus infection in pregnancy in Ethiopia. Ethiop Med J 1993;31(3):173–81.
31. Hussaini SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. Severe Hepatitis-E infection during pregnancy. J Viral Hepat 1997;4(1):51–4.
32. Khuroo MS, Teli MR, Skidmore S, Sofia MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. Am J Med 1981;70(2):252–5.
33. Khuroo MS, Kamili S, Khuroo MS. Clinical course and duration of viremia in vertically transmitted Hepatitis-E virus (HEV) infection in babies born to HEV-infected mothers. J Viral Hepat 2009;16(7):519–23.
34. Stoszek SK, Engle RE, Abdel-Hamid M, Mikhail N, Abdel-Aziz F, Medhat A, *et al*. Hepatitis-E antibody seroconversion without

disease in highly endemic rural Egyptian communities. Trans R Soc Trop Med Hyg 2006;100(2):89–94.
35. Monga R, Garg S, Tyagi P, Kumar N. Superimposed acute Hepatitis-E infection in patients with chronic liver disease. Indian J Gastroenterol 2004;23(2):50–2.
36. Kc S, Sharma D, Basnet BK, Mishra AK. Effect of acute Hepatitis-E infection in patients with liver cirrhosis. J Nepal Med Assoc 2009;48(175):226–9.
37. Kumar Acharya S, Kumar Sharma P, Singh R. Hepatitis-E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death. J Hepatol 2007;46(3):387–94.
38. Mahtab MA, Rahman S, Khan M, Karim MF. Hepatitis-E virus is a leading cause of acute-on-chronic liver disease: experience from a tertiary centre in Bangladesh. Hepatobiliary Pancreat Dis Int 2009;8(1):50–2.
39. Kamar N, Selves J, Mansuy JM, Ouezzani L, Péron JM, Guitard J, *et al.* Hepatitis-E Virus and Chronic Hepatitis in Organ-Transplant Recipients. N Engl J Med 2008;358(8):811–7.
40. Kamar N, Mansuy JM, Cointault O, Selves J, Abravanel F, Danjoux M, *et al.* Hepatitis-E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. Am J Transplant 2008;8(8):1744–8.
41. Haagsma EB, Niesters HG, van den Berg AP, Riezebos-Brilman A, Porte RJ, Vennema H, *et al.* Prevalence of Hepatitis-E virus infection in liver transplant recipients. Liver Transpl 2009;15(10):1225–8.
42. Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C. Factors associated with chronic hepatitis in patients with Hepatitis-E virus infection who have received solid organ transplants. Gastroenterology 2011;140(5):1481–9.
43. Qureshi H, Shahid A, Mujeeb SA. Exposer rate of Hepatitis-E (IgG) in a selected population of children and adults in Karachi. J Pak Med Assoc 2000;50(10):352–4.
44. Saeedi MI, Mahmood K, Amanullah, Ziauddin M, Ilyas N, Zarif M. Frequency and clinical course of Hepatitis-E in tertiary care hospitals. J Coll Physicians Surg Pak 2004;14(9):527–9.
45. Iqbal M, Ahmed A, Qamar A, Dixon K, Duncan JF, Islam NU, *et al.* An outbreak of entericallv transmitted non-A non-B hepatitis in Pakistan, Am. J Trop Med Hyg 1989:40(4):438–43.
46. van Cuyck-Gandré H, Zhang HY, Tsarev SA, Warren RL, Caudill JD, Snellings NJ, *et al*. Short report: Phylogenitically distinct Hepatitis-E viruses in Pakistan. Am J Trop Med Hyg 2000;62(2):187–9.
47. Bryan JP, Iqbal M, Tsarev S, Malik IA, Duncan JF, Ahmed A, *et al.* Epidemic of Hepatitis-E in a military unit in Abbottabad, Pakistan. Am J Trop Med Hyg 2002;67(6):662–8.
48. Rab MA, Bile MK, Mubarik MM, Asghar H, Sami Z, Siddiqi S, *et al*. Water-brone Hepatitis-E virus epidemic in Islamabad, Pakistan: A common source of outbreak traced to the malfunction of a modern water treatment plant. Am J Trop Med Etyg 1997;57(2):151–7.
49. Shams R, Khero RB, Ahmed T, Hafiz A. Prevalence of Hepatitis-E virus (HEV) antibody in pregnant women of Karachi. J Ayub Med Coll Abbottabad 2001;13(3):31–5.
50. Hamid SS, Jafri SM, Khan H, Shah H, Abbas Z, Fields H. Fulminant hepatic failure in pregnant women: acute fatty liver or acute viral hepatitis? J Hepatol 1996;25(1):20–7.
51. Kumar A, Yachha SK, Poddar U, Singh U, Aggarwal R. Does co-infection with multiple viruses adversely influence the course and outcome of sporadic acute viral hepatitis in children? J Gastroenterol Hepatol 2006;21(10):1533–7.
52. Arora NK, Nanda SK, Gulati S, Ansari IH, Chawla MK, Gupta SD, *et al.* Acute viral hepatitis types E, A, and B singly and in combination in acute liver failure in children in north India. J Med Virol 1996;48(3):215–21.

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