Am J Transl Res 2024;16(5):2049-2058
www.ajtr.org /ISSN:1943-8141/AJTR0155835

Original Article

Hepatitis E associated determinants and diagnostic biomarkers during pregnancy and its prenatal consequences in Multan, Punjab tertiary care setting (Pakistan)

Ambreen Aisha[1], Shafqat Abbas[2], Emad M Eed[3], Dildar Ahmed[4], Sabahat Irfan[5], Fariha Ur Rehman[6], Sara Siddique[5], Muhammad Naeem[7]

[1]Department of Biochemistry, Punjab Medical College, Faisalabad Medical University, Faisalabad 38000, Pakistan; [2]Faculty of Allied Health Sciences, Superior University Lahore, Lahore 54000, Pakistan; [3]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia; [4]Department of Biochemistry, Riphah International University Islamabad, Islamabad 44000, Pakistan; [5]Department of Biochemistry, University of Agriculture, Faisalabad 38000, Pakistan; [6]Institute of Microbiology, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan; [7]College of Life Science, Hebei Normal University, Shijiazhuang 050024, Hebei, China

Received February 8, 2024; Accepted May 13, 2024; Epub May 15, 2024; Published May 30, 2024

Abstract: Objective: Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis in the world. Hepatitis E infection is commonly widespread by the fecal oral routes and contaminated water. This study was designed to explore the prevalence and risk factors of hepatitis E infection in pregnant women of the Multan district, Pakistan. Methods: The study comprised of a total of 500 enrolled patients, among which, 105 pregnant females with hepatitis E infection fulfilled the criteria for anti-HEV antibodies. Pregnant women without significant complications and without hepatitis E infection were excluded from this study. Hepatic profile, complete blood count, coagulation markers, and standard protocol were also assessed for fetal maternal hemorrhage. Results: Our results showed that 105 patients (66.66%, CI 95%) had HEV infection with mean age 25±5 years. Serum bilirubin levels were increased in 74 patients (70.47%), aspartate transaminase was elevated > 200 IU/L in 71 patients (67.61%), alanine transaminase was above the 100 IU/L in 65 patients (245 IU/L), and low platelet counts were found in 45 patients (42.85%). Moreover, fetal distress cases were 9 (10.84%) and maternal distress cases were about 11 (13.25%). Fetal mortality cases were 39 (37.14%), and maternal mortality cases were about 22 (20.95%) due to hepatic comma, intravascular coagulation, and hepatic failure. Conclusion: It was concluded that the prevalence of Hepatitis E during pregnancy is associated with high risk factors of unhygienic practices, blood transfusion, and noncompliance with universal infection control techniques. Maternal fatalities and fetal consequences were exacerbated by HEV infection.

Keywords: Viral hepatitis, pregnancy complications, hygiene condition, water borne diseases, fetal outcomes

Introduction

Hepatitis results in inflammation of the liver and contributes to morbidity and mortality, particularly in developing countries [1]. Hepatitis E virus causes about 3 million deaths and 57,000 fatalities throughout the world each year [2]. According to the World Health Organization (WHO), HEV infection was responsible for 44,000 fatalities globally in 2015, accounting for 3.3% of all viral hepatitis deaths [3]. Currently, there is inadequate awareness on the global burden of Hepatitis E infection among pregnant women [4]. Liver cirrhosis is a leading cause of mortality among children and pregnant women especially with pre-existing liver disease [5]. During the third trimester, the mortality risk for pregnant women escalates to 10% to 25%.

Hepatitis E virus (HEV) is a ribonucleic acid (RNA) encapsulated virus spread predominantly

https://doi.org/10.62347/SLWM5449

by fecal oral transmission [6]. As the capsid contains spike glycoproteins and phosphoproteins, the pORF3 gene is thought to play an important role in budding the virion through the Golgi processing and inactivation of the immune system [7]. Majorly, 8 classes of HEV are reported, predominant strains occurring in human infections are (HEV-1) and (HEV-2) genotypes infect only humans by feco-oral transmission due to poor sanitation. Zoonotic sources such as shellfish, rabbits and dear may also serve as infective agent for HEV-3 and HEV-4. Genotype 3 and 4 prevail in industrial countries, with sporadic and clustered cases of hepatitis E infected regions [8, 9].

HEV infection is indistinguishable from other kinds of acute viral hepatitis in terms of its clinical presentation [10, 11]. Acute liver failure is the most common complication of HEV infection, which accounts for 30 to 70% of all cases of acute hepatitis [12-14]. The condition is frequently self-limiting in males and non-pregnant women, with a case-fatality incidence of less than 0.1%. During pregnancy, the mortality rate is much higher because of hormonal and immunological changes. Patients with elevated ALT levels and antibodies IgG and IgM against HEV provide the most significant prognostic information [15, 16]. Hepatitis E infected pregnant women suffer from jaundice and worse obstetrics complications, than the pregnant women bearing other types of hepatitis [17-20]. Immune-compromised patients are prone to develop chronic fibrosis of liver tissues due to hepatitis E [21]. Serological test systems significantly define hepatitis E diagnosis based on seroprevalence of IgG and IgM antibodies against HEV and elevated ALT levels in patients [22].

Hepatitis E, a zoonotic viral disease may end up with dreadful outcomes during pregnancy. This study was designed to explore the prevalence of hepatitis E infection in pregnant women of the district of Multan, Pakistan, and its associated risk factors.

Materials and methods

Study design

A Descriptive cross-sectional study was conducted at Gynae Obstetrics Department, Divisional head quarter Hospital, Multan, for one year from November 2021 to January 2023. All techniques adhered to the STROBE declaration and checklist, and were executed in compliance with the Equator guideline [23]. Among 500 consecutive patients investigated for acute hepatitis E, we identified cases of acute of HEV infection [24, 25]. The case group was compared with a healthy pregnant control group of 20 individuals. The biochemical work-up included liver function and kidney function tests, in order to maintain their identity patients' details were deidentified.

Inclusion criteria

The diagnostic criteria for acute HEV infection were detection of serum anti-HEV immunoglobulins. Patients were screened for HEV virology by IgG quantification. Pregnant patients who reported with clinical diagnosis of jaundice, were enrolled for study, who underwent routine diagnostic investigations and were screened for anti HEV antibodies through ELISA.

Exclusion criteria

Pregnant women without significant complications and without hepatitis E infection were excluded from the study.

Biochemical investigation

Demographic details were collected, all patients signed a consent proforma on assurance of confidentiality to participate in Hepatitis E identification. The participants gave their consent to participate. Venous blood samples were drawn for biochemical and hematological parameters analysis. Hematology included LFTs, CBC and prothrombin time to reveal hepatic profile. Incidence of HEV infection data was collected from the systematic review of the prevalence of HEV disease. Pregnancy was closely followed end point of observation was the natural/artificial termination of pregnancy or death of the woman. Comparison was done between the 'survivor' and 'non survivor' cases regarding the type of viral hepatitis, biochemical and hematological picture to evaluate the factors responsible for maternal mortality [26].

Statistical analysis

The data was statistically analyzed software SPSS version 20. Descriptive statistics were

Table 1. Details of patients under study, gestational period and their percentage

	Observation	No of patients	% of patients
1-	Mean age (years)	25±5	
2-	Gestational age (months)		
	Ist Trimester	30	28.57%
	IInd Trimester	53	50.47%
	III^{3rd} Trimester	22	20.95%
3-	Control involved		
	1st and 2nd trimester	66	
	Late pregnancy	39	
4-	Gravidity		
	One to two	56	53.33%
	Three to 5	49	46.66%

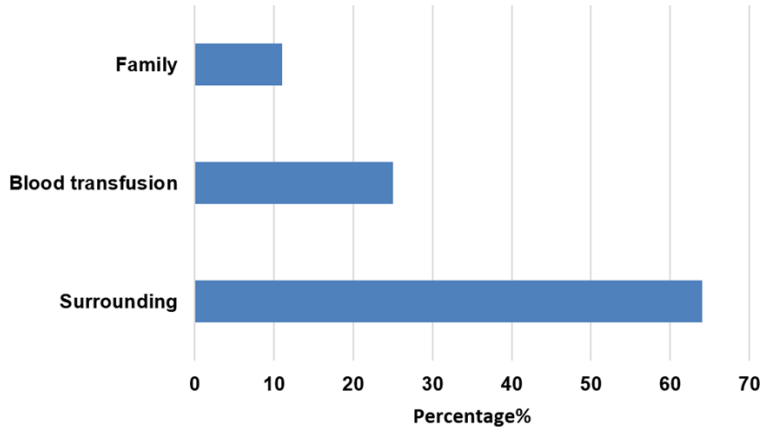

Figure 1. Demographic, clinical disposition of Hepatitis E and possible modes of Hepatitis E virus transmission; Unhygienic practices and contaminated water, *via* food and zoonotic transmission holds 64% of infection. Eleven percent of pregnant patients acquired HEV from the family and direct contact with the nearby inhabitants. Almost 25% HEV transmission occurred by blood transfusion.

applied for clinical, and the other variables and results have been presented as ± standard error of mean for quantitative variables and number (percentage) for qualitative variables. The statistical analysis of ANOVA determined the *P* value. P < 0.05 which was considered as statistically significant [23].

Results

The demographically represented subjects of Hepatitis E had a mean age of 25±5 years (**Table 1**). Mostly, HEV infections are present in persons aged 20 to 30 years in HEV-prevalent areas. **Figure 1** shows the Hepatitis E transmission routes through fecal oral routes and contaminated drinking water from surroundings, blood transfusion and vertical transmission. Blood transfusion is a major factor for HEV transmission cases. The unhygienic surroundings proved transmission of HEV in 64% patients. Some pregnant patients with HEV were distinguished as being infected via a family member (11%). Whereas 25% of women acquired HEV from a blood transfusion within the last 6-8 months.

The prevalence of Hepatitis E was determined while Hepatitis A, B, C and patients with liver disease were excluded. Of the 500 subjects, 105 cases (21%) were serologically positive for IgG antibodies. Among these 30 (28.57%) had IgG antibodies in the 1st trimester, while 53 cases (50.47%) were in 2nd trimester, 22 cases (20.95%) had reported with symptoms of nausea and jaundice in late pregnancy (**Table 1**). Patients presented during primary, or 2nd gravida were 56 (53.33%), while 49 (46.66%) had 3rd to 5th gravida, the trimester of pregnancy was not a significant factor for prediction of mortality.

Among the LFTs, serum bilirubin was raised in 74 patients (70.47%) (**Table 1**). Liver enzyme ALT level was > 200 IU/L (SEM 482.54 IU/L±513.77) in 71 pregnant women (67.61%) while AST was above the 100 IU/L (SEM 362.61±430.2371). Creatinine level determined was > 1.5 mg/dL with SEM 1.32±1.44 (**Table 2**). The average serum bilirubin, serum protein, prothrombin time, blood urea, and creatinine levels were not significantly different between cases and healthy controls. While liver enzymes (**Table 3**) ALT & AST were significantly high in HEV pregnant with *p* value 0.042 and 0.054 respectively. The bilirubin levels and TLC were markedly increased (12.45±7.33 and 35867±5631) in infected patients, indicating

Table 2. Hepatic profile and biochemical parameters of HEV+ pregnant patients (n = 105)

Biochemical Markers	Clinical parameters	No of cases and % age on different levels of parameter		
LFTs	Serum Bilirubin	> 1.5 mg/dL 74 (70.47%)	< 1.0 mg/dL 31 (29.52%)	
	SGPT	< 50 IU/L 34 (32.38%)	> 50 IU/L 71 (67.61%)	
CBC	Hb	Normal > 10 mmHg 13 (12.38%)	Anemia < 7 mmHg 49 (46.66%)	Severe anemia < 5 mmHg 43 (40.95%)
	Platelet count	> 1,50000 26 (24.76%)	< 1,50000 79 (75.23%)	
Coagulation profile	Prothrombin time	> 12.5 sec 56 (54.28%)	11-12.5 sec 48 (45.71%)	

Table 3. Comparison of parameters among the control group and HEV positive group

Hematological parameters	G1 = HEV infected pregnant cases	G2 = Healthy controls	*P* value
Hemoglobin mg/dL	7.2±2.12	8.34±.243	0.54
TLC	35867±5631	12849±3031	0.023*
Bilirubin	12.45±7.33	6.31±4.55	0.165
AST (IU)	362.61±430.23	267±324.95	0.054*
ALT (IU)	482.54±513.77	329±356.03	0.042*
Prothrombin time (sec)	26.0±23.44	18.02±16.33	0.802
INR	3.14±3.01	2.402±1.46	0.593
Urea mg/Dl	36±44.92	30.71±31.82	0.203
Creatinine	1.32±1.44	0.89±1.23	0.834

*Statistically significant

Table 4. Fetal and maternal consequences

Maternal Outcomes	Number	% age
Survived	83	79.04%
Dead	22	20.95%
Fetal outcomes	Number	% age
Survived	66	62.85%
Dead	39	37.14%

liver tissue damage with 0.0165 and 0.023 *p* values (**Table 3**).

The complete blood picture included hemoglobin level and platelet cell count, patients showed moderate anemia to severe anemia due to Hepatitis E. The majority of pregnancies were facing severely anemic condition, 43 cases (46.66%) had Hb < 5 mm Hg Hb level during Hepatitis E as compared to the cases showed normal Hb ≥ 10 mm Hg Hb level, that is in 13 cases (12.38%) (**Table 2**). The Hb level in the infected was SEM 7.2±2.12 (**Table 2**). Very low platelet count < 1,50000 was recorded in 79 (75.23%) patients, while 26 (24.76%) patients had platelet count > 1,50000. Another 48 (45.71%) had PT (11-12.5 sec) with less coagulopathies. Several women lost their lives 22 (20.95%) during the battle against Hep E (**Table 4**; **Figure 2**). Presence of encephalopathy at the time of admission correlated very closely with maternal mortality along with neonatal expiry and IUD 39 (37.14%), fetal distress 9 (10.42%), 11 (13.25%) maternal distress, and 8 (9.63%) experienced preterm delivery. Further cases were divided into subgroups of surviving patients and non-surviving. When IUD, still birth and expiry facing patients were compared with those of surviving ones, and the most important feature was that TLC and bilirubin were significantly noticed with *p* value 0.023 and 0.0085 respectively (**Table 5**).

Discussion

In underprivileged nations, HEV causes waterborne acute hepatitis. Furthermore, the infection is more prevalent in areas with poor sanitation and hygiene conditions. Patients with low socioeconomic status have no access to clean drinking water, and their residential conditions include inadequate drainage and incorrect

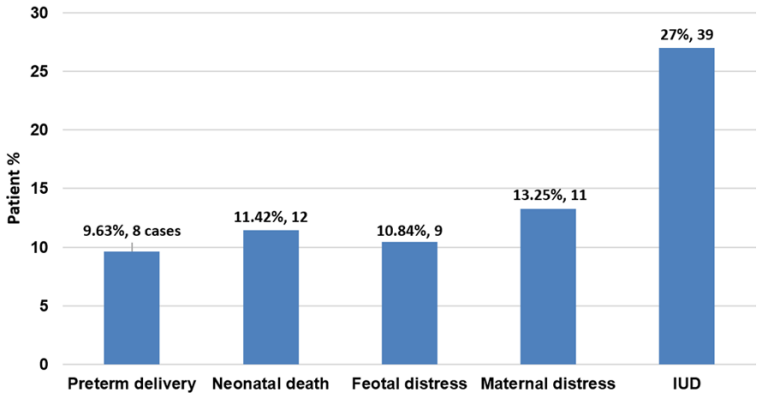

Figure 2. Maternal and foetal outcomes of pregnant patients with Hepatitis E. Intrauterine deaths and maternal distress were the worst-case situations (39 and 11 cases respectively). Most fatalities went through premature labour ending to delivery (8 cases), which either occurred naturally or was induced for medical reasons. Neonatal deaths reported in 12 cases, while 9 fetus experienced distress.

sewage treatment [27]. Women and children face severe morbidity due to unawareness of hygiene practices. As basic health units are not well equipped with diagnostic laboratories, so the samples were drawn from the patients and transferred to tertiary health care units. HEV has an incubation period of 2 to 10 weeks. Hepatitis E virus (HEV) infection symptoms include anorexia, fever, myalgia, backaches, rashes, arthralgia, and vomiting (**Table 6**).

Most major instances of HEV severe infection are observed in pregnant women, who are more vulnerable and may exhibit clinical development linked with poor results (3000 stillbirths are annually recorded due to HEV3). One hundred and five out of 500 pregnant women were screened to be HEV positive serologically after testing positive for IgG. The seroprevalence of anti-HEV (IgG) was found to be high, the patient's percentile in early and last trimesters are 28.57% and 20.95%, respectively (**Table 1**), which are significantly lower compared to the patients experiencing it during the 2nd trimester of pregnancy 50.47% ($P \leq 0.05$), which corresponds to the findings by Sultana and Alvi studies [28, 29]. Among pregnant women, the case fatality rate is 20%, and this rate increases during the second and third trimesters, as narrated by Patra, similar to the current study 20.95%, expiry was recorded in (**Table 4**). HEV infection during pregnancy, especially in the third trimester may lead to acute liver failure (**Table 1**).

Hepatitis E transmission takes place majorly through fecal oral course and contaminated drinking water from surroundings, yet blood transfusion and vertical transmission are also associated [28, 29]. Blood transfusion is now considered as a new risk factor for HEV transmission. Cases of Hep E are reported after blood transfusion from HEV positive blood donors. Despite greater knowledge about HEV, the etiology of the virus is still unclear. HEV infection can be fatal if left untreated and can range from a hardly perceptible infection to a fulminant liver. HEV infection is possible in people with chronic liver disease (CLD) and a kidney or liver transplant [30]. Hepatitis E can spread through contaminated needles, medical waste, and healthcare waste, posing numerous hazards if not handled properly [31].

Anemic condition of mothers and neonates are prevalent features in infected pregnant women [32, 33]. Anemia is also reported in glucose-6-phospate dehydrogenase deficiency. Hemolytic anemia has been documented following HEV infection in up to 23% of patients, and may elevate by 70% in individuals with G-6PD deficiency [34, 35]. A low hemoglobin level 7.2±2.12 mg/dL rendered 43 (40.95%) patients from moderate to severe anemia. Seth et al reported that thrombocytopenia in HEV pregnant women, which can be correlated with anemia, was found in 49 (46.66%) patients with Hb 7 mmHg [36]. Thrombocytopenia can be caused by hypersplenism, decreased hepatic thrombopoietin production, bone marrow suppression by hepatotropic virus, and therapy of anti-platelet autoantibodies and immune complexes [37, 38]. In the current study, 79 (75.23%) patients showed very low platelet < 1,50,000 counts while prothrombin time was raised (**Table 2**). There was prolonged prothrombin time > 12.5 sec in 56 individuals (54.28%). HEV accelerates liver fibrosis and leads to decompensated cirrhosis [39, 40]. Cirrhosis can cause fulminant hepatitis failure (FHF), especially in pregnant women, with a mortality rate of up to 30%. Liver function tests

Table 5. Hematological parameters compared for surviving patients with outcomes with that of non-survival

Hematological parameters	HEV infected pregnant cases who delivered (n = 78)	Cases of IUD, still birth or expiry (n = 27)	*P* value
Hemoglobin	7.72±2.34	7.39±2.23	0.54
TLC	3586±4021	15490±3031	0.023*
Bilirubin	12.05±5.82	16.31±6.91	0.0085*
AST	312.51±509.62	267±409.09	0.548
ALT	467.29±568.12	319±439.11	0.314
Prothrombin time	14.59±14.57	26.68±32.47	0.052*
INR	1.26±1.04	2.02±2.01	0.0041**
Urea	38±28.05	31±28.65	0.563
Creatinine	0.82±	0.96±	0.561

*Statistically significant, **Statistically highly significant.

Table 6. Prevalent symptoms of Hepatitis E among pregnant patients

Common symptoms	Less common symptoms
Jaundice	Myalgia
Anorexia	Headache
Lethargy	Pruritis
Vomiting	Arthralgia
Back ache	Rashes
Abdominal pain	Neurological disorders

unveiled 74 patients (70.47%) showed raised serum bilirubin > 1.5 mg/dL (SEM 12.45±7.33) (**Tables 2**, **3**), as also mentioned by Kamar, profoundly elevated serum ALT, AST and bilirubin levels are reported in prodromal and icteric phases of the viral course, respectively [39, 40]. Liver disorder was ascertained by a high level of Alanine transaminase (ALT) in the majority of pregnant patients (71 (67.61%)% having more than 50 IU/L) (**Tables 2**, **3**). High maternal mortality rate along with fetal complications were greater than in the current study (n = 22, 20.95%) (**Table 4**) [28]. It was reported the highest mortality rate (56%), among the HEV-infected pregnancies was in the third trimester. The HEV seroprevalence was 10% with an incidence rate of 46 infections per 1,000 person-years between 2008 and 2010 in a cohort study held in Bangladesh.

Figure 2 shows the maternal and foetal outcomes of pregnant patients with Hepatitis E. Intrauterine deaths and maternal distress were the worst-case situations (39 and 11cases respectively). Most fatalities went through premature labour ending in delivery (8 cases), which either occurred naturally or was induced for medical reasons [41]. Neonatal deaths were reported in 12 cases, while 9 fetus experienced distress. HEV accounted for about 60% of instances of acute hepatitis among pregnant women, 41% of them had ALF with high maternal mortality, antepartum hemorrhage, congenital abnormalities, and IUD in HEV-infected mothers versus non-HEV-infected individuals. Premature birth, stillbirth, and miscarriage have been linked to HEV infection during pregnancy, in addition to the conventional risks, which correlate with our findings [42].

Figure 3 Shows the molecular mechanism leading to liver cirrhosis in hepatitis E during pregnancy. Pregnancy is associated with the secretion of estrogen, progesterone and Human chrionic gonadotrophin (HCG). Hormonal surge and cell mediated immune response suppression by flooding cytokines IL-4, IL-10, TNF, INF-γ and tumor growth factor (TGF) production influenced hepatitis E infection in pregnancy. Whereas, decreased nutritional status and NF-kB promoted viral attack. Further viral replication is enhanced by viral proteins [43]. Decreased CD4/CD8 cell proportion and elevated levels of hormonal steroids, decreased expression of receptors for progesterone, interleukin flooding cytokines IL-4, IL-10, TNF, INF-γ and TGF production influenced hepatitis E infection during pregnancy and viral burden, all of which are risk factors for fulminant hepatitis during pregnancy. As a result, pregnant women with HEV, particularly those in their second and third trimesters are at high risk of poor maternal fetal outcomes, according to systematic

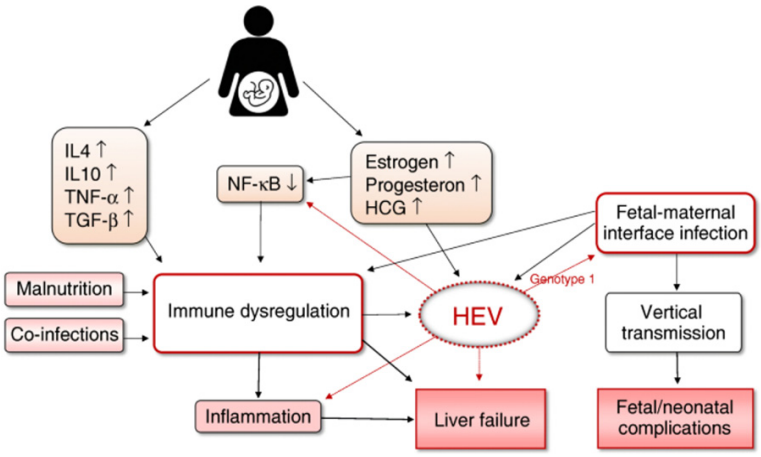

Figure 3. Flow sheet depicting causal mechanisms leading to liver cirrhosis in hepatitis E during pregnancy. Pregnancy brings surge of estrogen, progesterone and Human chrionic gonadotrophin (HCG). Hormonal surge and cell mediated Immune response suppression by flooding cytokines IL-4, IL-10, TNF, INF-γ and tumor growth factor (TGF) production influenced hepatitis E infection in pregnancy. Whereas, decreased nutritional status and NF-kB encourages viral attack. Further viral replication is enhanced by viral proteins.

analysis in various observational research [43]. Altered hormones status like beta HCG, progesterone and estrogen levels can increase viral infection and liver damage. Whereas the immune responsive mechanism (TGF, IL6, INF-γ and cytokines raised levels) also increase susceptibility of pregnant patients towards any viral invasion (**Figure 3**). To facilitate the transmission of HEV, the placenta generates enzymes and cytokines that suppress cell-mediated immunity at the point of maternal-fetal contact.

There have also been instances of HEV replication inside the placenta [36]. Another cohort study of HEV epidemic in Sudan revealed 220 jaundiced women among 1133 pregnant women recorded over 3 months (attack rate, 19.4%) [44]. The outbreaks in Pakistan 1994 and 2002 in Abbottabad showed hyperendemic infection of HEV in small study designs which are good indicators of general population prevalence, morbidity and considerable number of mortalities in pregnant women [45].

Liver function tests unveiled that 42% patients had raised serum bilirubin more than 50 mg/dm^3. Profoundly elevated serum ALT, AST and bilirubin levels are reported in prodromal and icteric phases of viral course, respectively. Liver disorder was ascertained by high level of serum glutamate pyruvate transaminase (SGPT) in the majority of pregnant patients (66% having more than 100 U/dm^3). Raised SGPT is a conspicuous marker of tissue damage of liver, which provided drastic maternal and fetal consequences in acute hepatitis E. During pregnancy fulminant liver failure chances are increased. Considering fetal outcomes, data was gathered on preterm fetal distress, IUD, premature deliveries (**Figure 3**). Survey from Bangladesh reported 9.8% of post-partum complications, premature delivery, stillbirth, abortion and IUD are associated with Hep E [32]. The HEV seroprevalence was 10% with an incidence rate of 46 infections per 1,000 person-years in year 2008 to 2010 in a cohort study held in Bangladesh. In another cohort study of HEV epidemic in Sudan revealed 220 jaundiced women among the 1133 pregnant women recorded over 3 months with attack rate of 19.4%. A high incidence of fetal and neonatal mortality due to abortion, preterm delivery, miscarriage, and stillbirth among both deceased and alive pregnant women was reported by Khuroo [15].

Scarcity of knowledge in the way to health goals requires collaboration of community and health care systems. The situation needs to acquaint lay persons with information to convey the issue importance and prioritize hygiene in their lives [6, 42, 46]. The vertical transmission linked to prenatal and intra-partum testing could not be demonstrated due to limited resource settings and noncompliance of patients. Indorsing vaccination and sanitation services, in locations of dense population along with effective collaboration is needed to achieve health objectives. This study was not designed to explore the incidence of hepatitis E through vertical transmission which can be a future prospect.

Conclusion

Hepatitis E is a zoonotic viral disease that may end up with dreadful outcomes during pregnan-

cy. The major causes of infection were unhygienic practices and contaminated water supply. The findings suggest HEV infection induces an elevation in hepatic profile including AST, bilirubin, and prothrombin time. Severe anemia, TLC and low platelet counts are cursors for hepatic damage in this study which predisposes HEV infected pregnancy to increased morbidity and mortality rate. IUD, premature delivery, and postpartum complications were major outcomes of HEV infection during pregnancy.

Acknowledgements

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-138).

Disclosure of conflict of interest

None.

Abbreviations

HEV, Hepatitis E virus; HepE, Hepatitis E; LFTs, liver function tests; AST, Aspartate transaminase; ALT, Alanine transaminase; ELIZA, Enzyme linked immunosorbent assay; CBC, complete blood count; SGPT, Serum glutamate pyruvate transaminase; G-6-PD, Glucose 6 Phosphate Dehydrogenase.

Address correspondence to: Ambreen Aisha, Department of Biochemistry, Punjab Medical College, Faisalabad Medical University, Faisalabad 380-00, Pakistan. E-mail: aishafmu@gmail.com; Sara Siddique, Department of Biochemistry, University of Agriculture, Faisalabad 38000, Pakistan. E-mail: ranasara99@gmail.com

References

[1] Lavanchy D. Chronic viral hepatitis as a public health issue in the world. Best Pract Res Clin Gastroenterol 2008; 22: 991-1008.

[2] Ahmad T, Nasir S, Musa TH, AlRyalat SAS, Khan M and Hui J. Epidemiology, diagnosis, vaccines, and bibliometric analysis of the 100 top-cited studies on Hepatitis E virus. Hum Vaccin Immunother 2021; 17: 857-71.

[3] Al-Shimari FH, Rencken CA, Kirkwood CD, Kumar R, Vannice KS and Stewart BT. Systematic review of global hepatitis E outbreaks to inform response and coordination initiatives. BMC Public Health 2023; 23: 1120.

[4] Murrison LB and Sherman KE. The enigma of hepatitis E virus. Gastroenterol Hepatol (N Y) 2017; 13: 484-491.

[5] Perrin HB, Cintas P, Abravanel F, Gerolami R, d'Alteroche L, Raynal JN, Alric L, Dupuis E, Prudhomme L, Vaucher E, Couzigou P, Liversain JM, Bureau C, Vinel JP, Kamar N, Izopet J and Peron JM. Neurologic disorders in immunocompetent patients with autochthonous acute hepatitis E. Emerg Infect Dis 2015; 21: 1928-34.

[6] Supply WUJW and Programme SM. Progress on sanitation and drinking water: 2015 update and MDG assessment. Vacc Immun 2021; 17: 857-71.

[7] Ikram A, Hakim MS, Zhou JH, Wang W, Peppelenbosch MP and Pan Q. Genotype-specific acquisition, evolution and adaptation of characteristic mutations in hepatitis E virus. Virulence 2018; 9: 121-32.

[8] Nimgaonkar I, Ding Q, Schwartz RE and Ploss A. Hepatitis E virus: advances and challenges. Nat Rev Gastroenterol Hepatol 2018; 15: 96-110.

[9] Wu C, Wu X and Xia J. Hepatitis E virus infection during pregnancy. Virol J 2020; 17: 73.

[10] Abhinandan HS, Dewan V, Choudhary M, Singh A, Baghel S and Dhankhar B. Hepatitis E: A rare cause of immune thrombocytopenic purpura. Ind J Case Rep 2020; 12: 310-12.

[11] Shalimar, Sonika U, Kedia S, Mahapatra SJ, Nayak B, Yadav DP, Gunjan D, Thakur B, Kaur H and Acharya SK. Comparison of dynamic changes among various prognostic scores in viral hepatitis-related acute liver failure. Ann Hepatol 2018; 17: 403-12.

[12] Roman S, Panduro A, Aguilar-Gutierrez Y, Maldonado M, Vazquez-Vandyck M, Martinez-Lopez E, Ruiz-Madrigal B and Hernandez-Nazara Z. A low steady HBsAg seroprevalence is associated with a low incidence of HBV-related liver cirrhosis and hepatocellular carcinoma in Mexico: a systematic review. Hepatol Int 2009; 3: 343-355.

[13] Sclair SN and Schiff ER. An update on the hepatitis E virus. Curr Gastroenterol Rep 2013; 15: 304.

[14] Khuroo MS, Kamili S and 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: 519-523.

[15] Khuroo MS. Hepatitis E and pregnancy: an unholy alliance unmasked from Kashmir, India. Viruses 2021; 13: 1329.

[16] Wedemeyer H. Is hepatitis E really a problem? Clinical Hepatology Practice in 2016. Hepat Inter 2016; 11: 12.

[17] Sultana R and Humayun S. Fetomaternal outcome in acute hepatitis e. J Coll Physicians Surg Pak 2014; 24: 127-30.
[18] Paternoster DM, Fabris F, Palù G, Santarossa C, Bracciante R, Snijders D and Floreani A. Intra-hepatic cholestasis of pregnancy in hepatitis C virus infection. Acta Obstet Gynecol Scand 2002; 81: 99-103.
[19] Rein DB, Stevens GA, Theaker J, Wittenborn JS and Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 2012: 55: 988-997.
[20] Ahmad T, Anjum S, Sadaf Zaidi NU, Ali A, Waqas M, Afzal MS and Arshad N. Frequency of hepatitis E and Hepatitis A virus in water sample collected from Faisalabad, Pakistan. Ann Agric Environ Med 2015; 22: 661-4.
[21] Murad EA, Babiker SM, Gasim GI, Rayis DA and Adam I. Epidemiology of hepatitis B and hepatitis C virus infections in pregnant women in Sana'a, Yemen. BMC Pregnancy Childbirth 2013; 13: 127.
[22] Mansoor M, Raza H and Tariq R. Feto-maternal outcome in HEV infection. Ann King Ed Med Uni 2011; 17: 86.
[23] Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC and Vandenbroucke JP; STROBE Initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg 2014; 12: 1495-9.
[24] Goodyear MD, Krleza-Jeric K and Lemmens T. The declaration of Helsinki 2007. BMJ 2007; 335: 624-625.
[25] Kotrlik J and Higgins C. Organizational research: determining appropriate sample size in survey research appropriate sample size in survey research. Info Techn Lear 2009; 19: 43.
[26] Sahai S, Mishra V, Ganga D and Jatav OP. Viral hepatitis in pregnancy--a study of its effect on maternal and Foetal outcome. J Assoc Physicians India 2015; 63: 28-33.
[27] Vandenbroucke JP, Elm EV, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ and Egger M; Strobe Initiative. Strengthening the reporting of observational studies in epidemiology (STROBE). Ann Int Med 2007; 147: W-163.
[28] Patra S, Kumar A, Trivedi SS, Puri M and Sarin SK. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. Ann Intern Med 2007; 147: 28-33.
[29] Kamar N, Bendall RP, Peron JM, Cintas P, Prudhomme L, Mansuy JM, Rostaing L, Keane F, Ijaz S, Izopet J and Dalton HR. Hepatitis E virus and neurologic disorders. Emerg Infect Dis 2011; 17: 173-9.
[30] Guerra JAAA, Kampa KC, Morsoletto DGB, Junior AP and Ivantes CAP. Hepatitis E: a literature review. J Clin Transl Hepatol 2017; 5: 376-383.
[31] Tahira K, Junaid K, Ali H, Afzal S, Ayub RM, Khan A, Khan AA and Dawood M. Occupational health hazards and needle stick injuries among medical laboratory workers. Ann King Edw Med Uni 2020; 26: 391-6.
[32] Kmush BL, Labrique A, Li W, Klein SL, Schulze K, Shaikh S, Ali H, Engle RE, Wu L, Purcell RH, Mehra S, Christian P, West K Jr and Nelson K. The association of cytokines and micronutrients with hepatitis E virus infection during pregnancy and the postpartum period in rural Bangladesh. Am J Trop Med Hyg 2016; 94: 203-11.
[33] Boisson S, Engels D, Gordon BA, Medlicott KO, Neira MP, Montresor A, Solomon AW and Velleman Y. Water, sanitation and hygiene for accelerating and sustaining progress on neglected tropical diseases: a new Global Strategy 2015-20. Int Health 2016; 8 Suppl 1: i19-21.
[34] Bazerbachi F, Leise MD, Watt KD, Murad MH, Prokop LJ and Haffar S. Systematic review of mixed cryoglobulinemia associated with hepatitis E virus infection: association or causat. Gastroenterol Rep (Oxf) 2017; 5: 178-84.
[35] Fousekis FS, Mitselos IV and Christodoulou DK. Extrahepatic manifestations of hepatitis E virus: an overview. Clin Mol Hepatol 2020; 26: 16-23.
[36] Seth A and Sherman KE. Hepatitis E: what we think we know. Clin Liver Dis (Hoboken) 2020; 15 Suppl 1: S37-S44.
[37] Riveiro-Barciela M, Bes M, Quer J, Valcarcel D, Piriz S, Gregori J, Llorens M, Salcedo MT, Piron M, Esteban R, Buti M and Sauleda S. Thrombotic thrombocytopenic purpura relapse induced by acute hepatitis E transmitted by cryosupernatant plasma and successfully controlled with ribavirin. Transfusion 2018; 58: 2501-5.
[38] Kamar N, Lhomme S, Abravanel F, Marion O, Peron JM, Alric L and Izopet J. Treatment of HEV infection in patients with a solid-organ transplant and chronic hepatitis. Viruses 2016; 8: 222.
[39] Rayis DA, Jumaa AM, Gasim GI, Karsany MS and Adam I. An outbreak of hepatitis E and high maternal mortality at Port Sudan, Eastern Sudan. Pathog Glob Health 2013; 107: 66-8.
[40] Boccia D, Guthmann JP, Klovstad H, Hamid N, Tatay M, Ciglenecki I, Nizou JY, Nicand E and Guerin PJ. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. Clin Infect Dis 2006; 42: 1679-84.
[41] Papatheodoridis GV, Papakonstantinou E, Andrioti E, Cholongitas E, Petraki K, Kontopoulou I and Hadziyannis SJ. Thrombotic risk factors

and extent of liver fibrosis in chronic viral hepatitis. Gut 2003; 52: 404-409.
[42] Kumar A, Beniwal M, Kar P, Sharma JB and Murthy NS. Hepatitis E in pregnancy. Int J Gynaecol Obstet 2004; 85: 240-244.
[43] Bigna JJ, Modiyinji AF, Nansseu JR, Amougou MA, Nola M, Kenmoe S, Temfack E and Njouom R. Burden of hepatitis E virus infection in pregnancy and maternofoetal outcomes: a systematic review and meta-analysis. BMC Pregnancy Childbirth 2020; 20: 426.
[44] Van Cuyck-Gandre H, Zhang HY, Tsarev SA, Warren RL, Caudill JD, Snellings NJ, Begot L, Innis BL and Longer CF. Short report: phylogenetically distinct hepatitis E viruses in Pakistan. Am J Trop Med Hyg 2000; 62: 187-189.
[45] Bryan JP, Iqbal M, Tsarev S, Malik IA, Duncan JF, Ahmed A, Khan A, Khan A, Rafiqui AR, Purcell RH and Legters LJ. Epidemic of hepatitis E in a military unit in Abbotrabad, Pakistan. Am J Trop Med Hyg 2002; 67: 662-668.
[46] Butt AS and Sharif F. Viral hepatitis in pakistan: past, present, and future. Euroasian J Hepatogastroenterol 2016; 6: 70-81.