Prevalence of hepatitis E virus in children with acute hepatitis: one Egyptian center study

Maysaa El Sayed Zaki[1], *Mona Abdel Latif Alsayed*[2], *Hoda Ramadan Ryad Abbas*[3], *Doaa Mabrouk Ahmed*[4,*], *Amany Yusif El Ashry*[5]

Abstract

Introduction **The objective of the present study was to evaluate the prevalence of hepatitis E virus in acute hepatitis in pediatric patients.**

Methods **This was a cross-sectional study including 180 children with acute hepatitis. Blood samples were obtained and subjected to study the serological markers of hepatitis B surface antigen (HBsAg), hepatitis B core IgM (HBc IgM), hepatitis C IgG (HCV IgG) and hepatitis A IgM (HAV IgM), hepatitis E IgM and IgG, cytomegalovirus IgM (CMV IgM) and specific antibodies IgM for Epstein Barr virus by ELISA. Also ELISA attempted the laboratory diagnosis of autoantibodies by performing assay of antinuclear and anti-smooth muscle antibodies. Real time PCR was used for determination of HEV-RNA in samples positive for HEV serological markers.**

Results **From a total of 180 children with acute jaundice 69.4% were males and 39.6% were females with mean age ± standard deviation 5.8±3.5 years. Positive HEV markers were found in 47 patients (26.1%). A comparison between demographic, clinical and laboratory findings in children with positive HEV markers and children negative for HEV markers, revealed significant association with contact of animals (p=0.001), rural residence (p=0.001), presence of positive autoantibodies (p=0.001) and positive HAV IgM (p=0.001). The markers of hepatitis E virus showed significantly higher prevalence in children below age of 6 years (p=0.04).**

Conclusions **HEV infection is more common in preschool age. There is a significant association between contact with animals, rural residence and other hepatitis affection like autoimmune hepatitis and other viral hepatitis viruses such as hepatitis A.**

Keywords **HEV, children, IgM, PCR.**

Introduction

Hepatitis E virus (HEV) is a non-enveloped single strand positive-sense RNA virus that consists of three open-reading frames (ORFs).[1,2] There are eight genotypes of HEV: HEV1 and HEV2 are restricted to humans, HEV3 is found in humans, swine, rabbits, deer and mongooses, HEV4 circulates among humans and swine, HEV5 and HEV6 are found in wild boars, and finally HEV7 and HEV8 were identified in dromedary and Bactrian camels.[3] The mode of transmission differs according to the virus genotype, where genotypes 1 and 2 are transmitted by fecal-oral route and via contaminated water in underdeveloped countries.[4] On the other hand, genotypes 3 and 4 are transmitted through contact with animals or consumption of undercooked meat from affected

Received: 19 February 2020; revised: 27 April 2020; accepted: 01 May 2020.

[1]MD, Clinical Pathology Department, Faculty of Medicine, Mansoura University, El Gomhoria street, Mansoura, Egypt; [2]MD, Pediatric Medicine Department, Faculty of Medicine, Mansoura University, El Gomhoria Street, Mansoura, Egypt; [3]Titles?, Medical Biochemistry Department, Faculty of Medicine, Beni-Suef University, Mohamed Hassan Street, Beni-Suef, Egypt; [4]MD, Medical Microbiology and Immunology Department, Faculty of Medicine, Beni-Suef University, Mohamed Hassan Street, Beni-Suef, 6251, Egypt; [5]MD, Clinical Pathology Department, Faculty of Medicine, Mansoura University, El Gomhoria street, Mansoura, Egypt.

*Corresponding author: Doaa Mabrouk Ahmed, doaamicro50@gmail.com

Published June 2020

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

animals and are common in developed countries.[5]

Twenty million cases of HEV are recorded worldwide per year.[6] HEV is endemic in many developing countries, but it is much more common in non-endemic developed countries than previously recognized. [7] Acute viral hepatitis E usually has a mild course and may pass unnoticed in young age, while it is associated with severe complications in pregnant women.[8] The prevalence of anti-HEV in Egypt, especially in rural areas, was very high with up to 84.3% having positive antibodies to HEV in pregnant women,[9] also another two studies showed a relatively high prevalence of anti-HEV IgG in jaundiced patients, 38.1%, and in β-thalassemic children, 24.29%, in Egypt.[10]

The diagnostic markers for hepatitis E include specific antibodies determination by different immunoassay methods and detection of RNA by polymerase chain reaction.[11]

There are few studies about hepatitis E virus prevalence in children with acute hepatitis infection.[12,13]

The objective of the present study was to evaluate hepatitis E virus prevalence in acute hepatitis in pediatric patients. The used diagnostic markers were anti-HEV IgG, anti-HEV IgM and detection of HEV-RNA by polymerase chain reaction (PCR).

Methods

This was a cross-sectional study that was carried out from January 2017 to January 2019, all patients recruited from outpatient's clinic of Mansoura University Children Hospital. Patients were under the age of 18 years old, complaining of acute jaundice for a duration less than one month, without previous history of chronic liver disorders or blood disorders leading to jaundice. Patients with chronic liver disorders, malignancy affecting the liver or drug induced liver injury were excluded from the study. The study was approved by the Mansoura Ethical Committee and the parent of each child participating in the study gave informed consent.

Patients were subjected to full medical history and complete clinical examination. Ten milliliters of blood were collected from each participant and sera was isolated.

Sera was subjected to estimation of liver function tests including albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and direct bilirubin by automated system Dialab48 (Dialab GmbH, Neudorf, Austria).

Viral serological markers were determined by the use of specific enzyme linked immunosorbent assay for each virus. Hepatitis viruses, hepatitis B surface antigen (HBsAg), hepatitis B core IgM (HBc IgM), hepatitis C IgG (HCV IgG) and hepatitis A IgM (HAV IgM) were determined by ELISA (CTK Biotech, San Diego, USA) and specific antibodies for hepatitis E IgM and IgG were determined by ELISA (Adaltis, Guidonia Montecelio, Italy). Specific IgM for cytomegalovirus IgM (CMV IgM) and specific antibodies IgM for Epstein Barr virus were detected by ELISA (My BioSource Inc., San Diego, USA). The laboratory diagnosis of autoantibodies was attempted by performing assay of antinuclear and anti-smooth muscle antibodies by ELISA (My BioSource Inc).

Anti-HEV-IgM by ELISA (Adaltis)

The kit detects specific immunoglobulin M to ORF2 antigen of HEV. The amount of color intensity was measured by micro plate ELISA, which was proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. The wells that appear colorless represent as negative for HEV IgM.

HEV IgG-ELISA (Adaltis)

The principle of the test depends mainly upon the binding of specific IgG in the serum with specific antigen of HEV bound to the microplate. ELISA reader measured the amount of color intensity that was proportional to the amounts of anti-HEV IgG in the samples.

Detection of HEV RNA

HEV RNA extraction

Two hundred microliters of the serum samples were used for extraction of HEV RNA by the use of viral RNA extraction kit from body

fluids (QIA amp viral RNA mini kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Then the extracted RNA was kept at -80°C for further reverse transcription real time polymerase chain reaction (PCR).

Reverse-transcription real time PCR (RT-PCR)

RT-PCR was performed by the use of 10 µL of the extracted RNA according to the manufacturer's instructions of a qualitative HEV RT-PCR kit (TaqMan ampliCube, Mikrogen GmbH, Neuried, Germany) using a RT-PCR instrument (QuantStudio 6 Flex, Applied Biosystems, Carlsbad, CA, USA).

Specific primers and a double-marked probe for the amplification and detection of HEV RNA were present in the kit. The forward primer was (JVHEVF; 5′-GGTGGTTTCT-GGGGTGAC-3′), the reverse primer was (JVHEVR; 5′-AGGG-GTTGGTTGGATGAA-3′), while the probe was (JVHEVP; 5′-TGATTCT-CAGCCCTTCGC-3′).[14] The internal control was transcribed to cDNA, amplified and detected in the same RT-PCR preparation. False negative results that occur as a result of inhibition of the RT-PCR reaction can be excluded. Probes for the detection of the internal control were marked with yellow dye. It was possible to detect both target sequences in a single reaction.

Statistical analysis

The data was analyzed by the use of Statistical Package for Social Science (SPPS v24, IBM Corp., Armonk, NY, USA). The qualitative data was expressed as numbers and percentages and compared by the use of Chi-Square test and p was considered significant if <0.05. The quantitative data was analyzed as mean and standard deviation (SD). The risk factor assessment was measured by binary logistic regression and p was considered significant if it was <0.05.

Results

The study included 180 children with acute jaundice. They were 125 (69.4%) males and 55 (30.6%) females with mean age±SD 5.8±3.5 years. The residence was rural for 50.5% of the children and urban for 49.5% of them. The most prevalent virological markers were HEV-IgG in 25.6%, followed by HAV-IgM in 15.6% and CMV-IgM in 11.7%. Lower prevalence rates were determined for HCV-IgG 8.9%, EBV-IgM 7.8% and HBsAg and/or HBc IgM 6.7%. Autoantibodies were present in 5.0% of the patients as detected by positive antinuclear and anti-smooth muscle antibodies. HEV-IgG was positive in 46 patients (25.6%), HEV-IgM was positive in 10 patients (5.6%) and HEV-RNA was found in 6 patients (3.3%) of the patients – Table 1.

Table 1. Demographic, clinical and laboratory findings of the patients

Parameter	Patient characteristics
Sex **Male** **Female**	 125 (69.4%) 55 (30.6%)
Age (mean±SD), years	5.8±3.5
Residence **Rural** **Urban**	 91 (50.5%) 89 (49.5%)
Contact with animals	42 (23.3%)
Fever	53 (29.4%)
Abdominal pain	40 (22.2%)
Arthralgia	34 (18.9%)
Albumin (mean±SD), g/dL	4.2±0.4
Total bilirubin (mean±SD), mg/dL	4.3±1.8
Direct bilirubin (mean±SD), mg/dL	2.1±1.5
ALT (mean±SD), IU/L	43.5±20.7
AST (mean±SD), IU/L	54.7±25.5
HAV-IgM	28 (15.6%)
Autoantibodies	9 (5.0%)
HCV IgG	16 (8.9%)
HBsAg and/or HBc IgM	12 (6.7%)
HEV IgG	46 (25.6%)
HEV IgM	10 (5.6%)
HEV-RNA	6 (3.3%)
CMV IgM	21 (11.7%)
EBV IgM	14 (7.8%)

Table 2. Comparison between patients positive for HEV markers and patients negative for HEV markers

Parameter	Positive hepatitis E markers (n=47)	Negative hepatitis E markers (n=133)	Chi-square value	df value	95% confidence interval		P value
					Lower bound	Upper bound	
Gender			0.364	1			
Male	31 (65.9%)	94 (70.7%)			0.395	1.634	p=0.334
Female	46 (34.1%)	39 (29.3%)					
Age (mean±SD), years	4.9±2.9	6.1±3.6					p=0.061
Fever	12 (25.%)	41 (30.8%)	0.469	1	0.363	1.632	p=0.313
Contact with animals	42 (89.4%)	0 (0%)	155.023	1	11.673	65.256	p=0.001
Abdominal pain	12 (25%)	28 (21.1%)	0.403	1	0.591	2.796	p=0.328
Arthralgia	12 (25%)	22 (16.5%)	1.832	1	0.778	3.848	p=0.212
Albumin (mean±SD), g/dL	4.2±2.9	4.2±0.4					p=0.712
Total bilirubin (mean±SD), mg/dL	4.2±1.9	4.3±1.7					p=0.911
Direct bilirubin (mean±SD), mg/dL	1.8±0.5	2.1±1.5					p=0.213
ALT (mean±SD), IU/L	42.2±2.7	43.9±20.4					p=0.623
AST (mean±SD), IU/L	57.8±26.3	53.5±25.6					p=0.331
Residence							
Rural	35 (74.5%)	56 (42.1%)	14.551	1	1.912	8.410	p=0.001
Urban	12 (25.5%)	77 (57.9%)					
Autoantibodies	7 (14.9%)	2 (1.5%)	13.108	1	2.289	57.394	p=0.001
EBV IgM	1 (2.1%)	18 (13.5%)	2.831	1	0.026	1.578	p=0.107
CMV IgM	6 (12.8%)	15 (11.3%)	0.075	1	0.419	3.165	p=0.583
HCV IgG	5 (10.6%)	11 (8.3%)	0.240	1	0.433	4.022	p=0.409
HBsAg	4 (8.4%)	8 (6.01%)	0.348	1	0.417	5.069	p=0.485
Hepatitis A IgM	16 (34.04%)	12 (9.02%)	16.550	1	2.233	12.129	p=0.001

A comparison between demographic, clinical and laboratory findings in children with positive HEV markers and children negative for HEV markers, revealed a significant association with contact of animals (p=0.001), rural residence (p=0.001), presence of autoantibodies (p=0.001) and positive HAV IgM (p=0.001) – Table 2.

The total patients with positive HEV markers were 47 (26.1%). There were combined serological markers, positive IgG, IgM and RNA for HEV in 4 patients, positive HEV-RNA and HEV IgM in 2 patients and isolated positive HEV-IgG and/or positive HEV IgM in 42 patients – Table 3.

Table 3. Distribution of positive markers for HEV

	Number	Percentage from total positive HEV markers* (47.1%)
Isolated positive HEV-IgG and/or positive HEV IgM	42	89.4%
Positive HEV-RNA and HEV IgM	2	4.2%
Positive IgG, IgM and RNA for HEV	4	8.5%

*Total positive markers for HEV are 47

Hepatitis E virus markers demonstrated a significantly higher prevalence in children younger than 6 years of age (p=0.004), their percentage in children less than 6 years was 57.4% while in children above 6 years it was 42.6%.

Discussion

Hepatitis E virus is an endemic virus in Egypt and is responsible for acute self-limited hepatitis especially in young age children.[15]

In the present study the serological and molecular markers for HEV were positive in 26.1% of 180 children with acute jaundice. The rate of HEV in patients with acute hepatitis range from 4% up to 42% in previous studies.[16,17]

In a study from Upper Egypt, Assuit, the prevalence of HEV was similar to the present study.[13] Discrepancies in the prevalence of acute HEV infection between studies may be attributed to the variation in the demographic and economic pattern of the patients studied, discrepancies in sanitary conditions and sewage disposal and early childhood HEV exposures, producing long-lasting immunity or modifying subsequent responses to exposure to the virus. Moreover, the used diagnostic methods influence the prevalence determination of HEV.[18]

In the present study, three markers were used for studying the presence of HEV: HEV IgG, HEV IgM and HEV-RNA. There were combined serological results among the patients. The presence of IgM can indicate presence of recent infection with HEV in the past few months and the presence of HEV IgG can be detected both in recent and remote exposure to HEV. Most studies indicate that HEV IgG titers peak around 4 weeks after infection and rapidly decline.[19,20] On the other hand, real viremia is suggested by the existence of HEV RNA. The available kits for detection of antibodies for HEV are useful in endemic areas and can be used as an active surveillance method for detection of seroprevalence to HEV.[21] The acute viral hepatitis is usually associated with mild symptoms and patients usually are not diagnosed due to absence of active surveillance for acute viral hepatitis in Egypt. However, the active monitoring of acute hepatitis in children and the leading etiology of such clinical issues can aid in the epidemiological understanding of the condition.[17]

Comparison between demographic, clinical and laboratory findings in children with positive HEV markers and children negative for HEV markers, revealed significant association with contact of animals, rural residence, presence of autoimmune hepatitis and positive HAV IgM. These findings were on contrary to a previous study from Egypt.[13] However, the findings of HEV markers association with rural residence and contact with animals were reported in previous studies.[22] Wu et al. reported that acute HEV infection may be related to the presence of autoantibodies.[23]

The combined infection with both hepatitis A virus and hepatitis E virus was reported in a previous study;[24] this may be attributed to the same route of transmission of both viruses i.e., fecal-oral route. Moreover, a previous study demonstrated a high prevalence of hepatitis E virus in autoimmune hepatitis.[25]

There is a need for larger cohort studies to find out if this is association is due to immune response triggering by HEV leading to the expression of autoimmune hepatitis or it is just a coincidence of viral etiology affecting children with autoimmune hepatitis.

The prevalence of HEV markers was significantly higher at <6 years age. A similar finding was reported by a previous study from Pakistan.[26] Perhaps, HEV infections occur during early childhood.

Conclusions

The present study highlights that hepatitis E frequently occurs among children with acute hepatitis. The infection is more common in preschool age. There is a significant association between other hepatitis causes such as autoimmune hepatitis and other viral hepatitis viruses such as hepatitis A virus and hepatitis E virus.

Authors' contributions statement: MESZ designed the research plan, organized the study and participated in the main role of editing and revising the manuscript. HRRA and AEA carried out all laboratory tests and coordinated

the data analysis. MALA collected and supervised all clinical issues of patients. The corresponding author is DMA, who had a major contribution in writing of the manuscript and had a role in following-up all steps of the study. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

Acknowledgements: The authors would like to thank all participants and other healthcare workers who helped all through the process of the research.

References

1. Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology.1991;185:120-31. https://doi.org/10.1016/0042-6822(91)90760-9
2. Ruggeri FM, Di Bartolo I, Ponterio E, Angeloni G, Trevisani M, Ostanello F. Zoonotic transmission of hepatitis E virus in industrialized countries. New Microbiol 2013;36:331-44. https://doi.org/10.1007/978-1-4614-7522-4
3. Webb GW, Dalton HR. Hepatitis E: an underestimated emerging threat. Ther Adv Infect Dis 2019;6: 2049936119837162. https://doi.org/10.1177/2049936119837162
4. Pérez-Gracia MT, Suay B, Mateos-Lindemann ML. Hepatitis E: an emerging disease. Infect Genet Evol 2014;22:40-59. https://doi.org/10.1016/j.meegid.2014.01.002
5. Kamar N, Izopet J, Pavio N, et al. Hepatitis E virus infection. Nat Rev Dis Primers 2017;3:17086. https://doi.org/10.1038/nrdp.2017.86
6. World Health Organization. 2019. Hepatitis E: fact sheet. Accessed on: 30 October 2019. Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-e
7. Murrison LB, Sherman KE. The enigma of hepatitis E virus. Gastroenterol Hepatol (N Y) 2017;13:484-91.
8. Xu C, Wang RY, Schechterly CA, Ge S, Shih JW, Xia NS, et al. An assessment of hepatitis E virus (HEV) in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence for HEV transmission to 362 prospectively followed recipients. Transfusion 2013;53:2505-11. https://doi.org/10.1111/trf.12326
9. Stoszek SK, Abdel-Hamid M, Saleh DA, et al. High prevalence of hepatitis E antibodies in pregnant Egyptian women. Trans R Soc Trop Med Hyg 2006;100:95-101. https://doi.org/10.1016/j.trstmh.2004.12.005
10. Abdelmawla D, Moemen D, Darwish A, Mowafy W. Hepatitis E virus prevalence in Egyptian children with transfusion-dependent thalassemia. Braz J Infect Dis 2019;23:40-4. https://doi.org/10.1016/j.bjid.2019.01.007
11. Ahmed A, Ali IA, Ghazal H, Fazili J, Nusrat S. Mystery of hepatitis E virus: recent advances in its diagnosis and management. Int J Hepatol 2015;2015:872431. https://doi.org/10.1155/2015/872431
12. Aboulata AA, Ahmad MS, Shaban MM, Zayd KM, Abd El-Moktader AM. Prevalence of hepatitis E virus in Egyptian children presented with minor hepatic disorders. Egypt J Immunol 2005;12:71-6.
13. Hasan G, Assiri A, Marzuuk N et al. Incidence and characteristics of hepatitis E virus infection in children in Assiut, Upper Egypt. J Int Med Res 2016;44:1115-22. https://doi.org/10.1177/0300060516659575
14. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods 2006;131:65-71. https://doi.org/10.1016/j.jviromet.2005.07.004
15. Romanò L, Paladini S, Tagliacarne C, Canuti M, Bianchi S, Zanetti AR. Hepatitis E in Italy: a long-term prospective study. J Hepatol 2011;54:34-40. https://doi.org/10.1016/j.jhep.2010.06.017
16. Bayhan Gİ, Demiören K, Güdücüoğlu H. Epidemiology of hepatitis E virus in children in the province of Van, Turkey. Turk Pediatri Ars 2016;51:148-51. https://doi.org/10.5152/TurkPediatriArs.2016.4119
17. Zakaria S, Fouad R, Shaker O, et al. Changing patterns of acute viral hepatitis at a major urban referral center in Egypt. Clin Infect Dis 2007;44:e30-6. https://doi.org/10.1086/511074
18. Blackard JT, Rouster SD, Nady S, et al. Genotypic characteristics of symptomatic hepatitis E virus (HEV) infections in Egypt. J Clin Virol 2009;46:140-4. https://doi.org/10.1016/j.jcv.2009.07.007
19. Labrique AB, Thomas DL, Stoszek SK, Nelson KE. Hepatitis E: an emerging infectious disease. Epidemiol Rev 1999;21:162-79. https://doi.org/10.1093/oxfordjournals.epirev.a017994
20. Bryan JP, Tsarev SA, Iqbal M, et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. J Infect Dis 1994;170:517-21. https://doi.org/10.1093/infdis/170.3.517
21. Aggarwal R. Diagnosis of hepatitis E. Nat Rev Gastroenterol Hepatol 2013;10:24-33. https://doi.org/10.1038/nrgastro.2012.187
22. Li P, Liu J, Li Y, et al. The global epidemiology of hepatitis E virus infection: a systematic review and meta-analysis. Liver Int 2020. https://doi.org/10.1111/liv.14468
23. Wu J, Guo N, Zhu L, et al. Seroprevalence of AIH-related autoantibodies in patients with acute hepatitis E viral infection: a prospective case-control study in China. Emerg Microbes Infect 2020;9:332-40. https://doi.org/10.1080/22221751.2020.1722759
24. Wang L, Li M. Study on hepatitis A virus and hepatitis E virus co-infection. Int J Inf Dis 2018;73:221. https://doi.org/10.1016/j.ijid.2018.04.3916

25. van Gerven NM, van der Eijk AA, Pas SD, et al. Seroprevalence of hepatitis E virus in autoimmune hepatitis patients in the Netherlands. J Gastrointestin Liver Dis 2016;25:9-13. https://doi.org/10.15403/jgld.2014.1121.251.hpe

26. Cevahir N, Demir M, Bozkurt AI, Ergin A, Kaleli I. Seroprevalence of hepatitis E virus among primary school children. Pak J Med Sci 2013;29:629-32. https://doi.org/10.12669/pjms.292.2821

Please cite this article as:
Zaki MES, Alsayed MAL, Ryad Abbas HR, Ahmed DM, El Ashry AY. Prevalence of hepatitis E virus in children with acute hepatitis: one Egyptian center study. GERMS 2020;10(2):88-94.
doi: 10.18683/germs.2020.1189