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Assessment of Hepatitis E Viral Infection and its Effect on Egyptian Pregnant Mothers

A. A- E. Abo-Elkhier[1], K. S. E. Abdel-Wahab[2], M. A. Ali[3], M. A-H. El-Sayed[4], N.A-K. Saleh [5] and A. A. Atef[6]

[1] Biochemistry, El-Sahel Teaching Hospital. [2] Virology, Faculty of Medicine (Girls), El-Azhar University. [3] Virology, National Research Center, [4] Obstetric & Gynecology, El-Sahel Teaching Hospital. [5] Biochemistry, Faculty of Science, Ain Shams University. [6] Biochemistry, Faculty of Science, Ain Shams University, Cairo, Egypt.

The current study was performed to determine the prevalence of HEV in Egyptian pregnant women, and to estimate the risk of prenatal transmission of the virus and its effect in infected pregnant women. A 120 aborted women between 17-36 years old and 120 women aged matched who had normal full term pregnancy in labor were included in the present study. Maternal blood was tested for anti-HEV IgG and IgM antibody and the positive cases were tested for HEV Ag and HEV RNA. The fetal tissue from anti-HEV IgG and IgM positive aborted women and cord serum of infant of anti-HEV IgG and IgM positive full term delivered women were tested for HEV Ag and HEV RNA. Among 120 aborted women, 2.5 % (3/120) 0f them were positive result with anti-HEV IgM, anti-HEV IgG, HEV RNA and HEV Ag, also 11.7 % (14/120) of them gave positive result with anti-HEV IgG, HEV RNA and HEV Ag, and 5.8 % (7/120) of them were positive for anti-HEV IgG only. Also 13 of 120 (10.83 %) full term delivered women were anti-HEV IgG positive only, all results of liver function tests of HEV-RNA positive aborted women appeared to be slightly higher than of HEV RNA negative aborted women, these differences was statistically significant ($P < 0.05$) (Except total protein and alkaline phosphatase). It was found that 3 anti-HEV IgM, anti-HEV IgG, HEV RNA and HEV Ag positive pregnant women transmitted the virus to their all fetus (100 %). While 14 anti-HEV IgG, HEV RNA and HEV Ag positive pregnant women transmitted the virus to 12 (85.7 %) of their fetus and the fetus died and terminated to abortion. However the 7 anti-HEV IgG positive aborted women did not transmitted the infection to their fetus. Also the cord serum of infant of 13 anti-HEV IgG positive full term delivered women gave negative result with HEV-RNA. HEV RNA infected pregnant women transmitted the virus to their fetus and the fetus died and terminated to abortion.

INTRODUCTION

HEV is the etiologic agent of 50% acute enterically transmitted non A, non B hepatitis in developing countries (Bradley, et al, 1987; Emerson and Purcell, 2003). HEV causes, in Egypt sporadic hepatitis (Goldsmith *et al.*, 1992; Hyams *et al.*, 1992; El Zimaity *et al.*, 1993; Kamel *et al.*, 1995; Gomatos *et al.*, 1996; Abdel-Wahab *et al.*, 1996 a and Osman *et al.*, 2002). This disease is a serious medical concern of travelers to tropical areas of the world (Purcell and Ticchurst, 1988). But innate HEV in developed countries is not evident (Emerson and Purcell, 2003). Egypt is a country with endemic HEV of sporadic incidence. Clinically HEV infection may be asymptomatic or

acute or occasionally fulminent, particularly if it infects women in their third trimester of pregnancy and neonates (Lau *et al.*, 1991 and 1995; Song *et al.*, 1991; Hamid *et al.*, 1996). HEV is commonly transmitted from infected mothers to their babies with significant perinatal enfant morbidity and mortality (Khuroo *et al.*, 1995). HEV was found to be associated with abortion (Abdel Wahab *et al.*, 1996 b and Kumar *et al.*, 2001).

MATERIALS AND METHODS

1-Subjects

The study covered a total of 240 pregnant women between 17- 41 years old who were admitted to Obstetric and Gynecology Department of Sahel Teaching Hospital from January 98 to January 99. 120 of them presented by first trimester abortion, also 120 full term in labor mothers were encountered as control. The data including age of mother, job, number and outcome of previous pregnancy, past history of jaundice or hepatic disorder, surgical operation, blood transfusion were recorded. Obstetric examination done by obstetrician was stated in individual subject's sheet.

2-Samples

Maternal blood samples were taken during abortion or delivery under aseptic condition. Cord blood samples from newborns of control mothers were taken after delivery by disposable syringes from cord vein. After the blood samples clotted they were kept at room temperature for 1 hour for clotting sera were separated, then stored at -50°C. Aborted fetal tissue (1 gm) was added to 4 ml of Hank's BSS with antibiotics and anti-fungus, then stored at -50°C.

3- ELISA

The commercially available ELISA Kits for the detection of anti-HEV IgM and IgG were obtained from Biokit Spain (code 3000 - 1234) and (code 3000 - 1233).

4- HEV Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

HEV RNA was extracted using guanidinum thiocyanate/ phenol/ chloroform technique according to (Chomczynski and Sacchi 1987) method.

The extracted RNA was dissolved in 20µl of DEPC-treated water and mixed with 40 U RNase inhibitor (GIBCO-BRL). The mixture was heated at 95°C for 5 minutes, then chilled on ice. Reverse transcription was done in 20 µl reaction mixture containing 4 µl extracted RNA, 1 µl antisense primer (ET 1.1 R1 5-CAG GGC CCC CAA GTT CTT CT-3), 1 µl of 10 mM dNTPs mixture (Pharmacia Biotechnology), 4 µl of 5 X Reverse transcriptase buffer (Advanced Biotechnology, 500 mM Tris-HCl, pH 8.3, 500 mM KCl, 100 mM MgCl, 40 mM Dithiothreitol), 1 µl (21 U) AMV-Reverse transcriptase and DEPC-treated water to up 20 µl. The reaction mixture was incubated at 40°C For 1 hour (Ray, et al, 1991). Two microliters of the first stranded cDNA was combined with 4 µl of each antisense primer and the sense one (ET 1.1 F1 5 - GCT CAT TAT GGA GAG AGT GTG T- 3), 5 µl 10 X PCR buffer (500 mM Tris-HCL, pH 8.3, 500 mM

KCl, 100 mM $MgCl_2$, 40 mM Dithiothreitol, Advanced Biotechnology), and 1.5 µl of 10 mM dNTPs in a 50 µl reaction volume. Taq DNA Polymerase 0.5 µl (5 U) (GIBCO-BRL) was added after an initial 5 minute incubation at 95 °C. Thirty rounds of amplification were performed under the following conditions; 94°C for 1 minute, 50°C for 2 minute and 72°C for 1.5 minute using Perkin Elmer 9600 thermal cycler PCR products were analyzed by electrophoresis in agarose gel containing ethidium bromide with a lane for DNA molecular weight marker (Ø X 174 DNA /Hinf 1 marker, Promega), then were visualized under UV light. An amplicone of 381 bp molecular weight was considered a positive signal of HEV (cDNA) (Ray, et al, 1991).

5-Biochemical analyses

Total and direct birubin and alkaline phosphatase were determined using Boehringer Mannheim Kits. Total protein and albumin were determined using Biomerieux Kits. AST and ALT were assayed using Randox Kits. Gamma-glutamyl transferase was assayed using Sclavo Kit. The 5'-Nucleotidase was assayed using biomerieux Kit.

RESULTS

Association Between Anti-HEV IgM or IgG Antibody and HEV Viremia

Anti-HEV IgM antibody was positive in 3 cases of abortions [Table 1] (2.5%); was negative in all sera of full term delivered women. This difference was not statistically significant ($P > 0.05$). Also, there was 24 anti-HEV IgG positive among the 120 aborted women (20%) compared to 13 anti-HEV IgG antibody positive (10.83 %) full term delivered women. This difference is statistically significant ($P < 0.05$). Table [1] shows that among the 24 anti-HEV IgG positive serum from aborted women, 17 sera were positive for HEV-RNA by PCR. None of the 13 HEV IgG positive control women, had serum HEV RNA by PCR. This difference was statistically significant ($P < 0.01$) [Table 1].

Correlation between HEV Viremia and Antigenemia.

Among 120 aborted women 2.5 % (3/120) were positive for anti-HEV IgM; anti-HEV IgG; HEV-RNA and HEV Ag. While 11.7 % (14/120) were positive for anti-HEV IgG, HEV-RNA and HEV Ag, and 5.8 % (7/120) were positive for anti-HEV IgG only. [Table 2].

Maternal Age and HEV Infection

There was an apparent anti-HEV IgG positivity increase, and HEV-RNA detection decrease with increasing age of aborted women but it proved statistically to be not significant ($P > 0.05$). Also anti-HEV gM was higher in age group 20 - < 30 than the other two groups, but the relation was not statistically significant. [Table 3].

Frequency of Abortion

There was no statistically significant ($P > 0.05$) relationship between maternal age and the history of repeated abortion [Table 3] and between HEV infection of pregnant women and the frequency of abortion (table 4)

Liver Function in HEV Infected and Aborted Mothers

The liver function tests of HEV-RNA positive aborted women were higher than of HEV RNAnegative aborted women and than the normal limits. These differences were statistically significant (P <0.05) except total protein and alkaline phosphatase [Table 5].

Table (1): Comparison of the Incidence of IgM or IgG anti-HEV antibody between aborted and full term delivered women.

	Aborted Women		Full term delivery women		P value
	Frequency	Percent	Frequency	Percent	
Anti-HEV IgM +ve	3	2.5	-	None	P > 0.05
Anti-HEV IgM -ve	117	97.5	120	100	
Anti-HEV IgG +ve	24	20	13	10.83	P < 0.05
Anti-HEV IgG -ve	96	80	107	89.17	
HEV-RNA +ve	17	70.83	None	None	P < 0.01
HEV-RNA -ve	7	29.17	13	100	

Table (2): Hepatitis E viremia and antigenemia among one hundred and twenty aborted women.

Number of Samples	HEV-RNA RT PCR	HEV Ag Dot ELISA	Anti-HEV by ELISA	
			IgM	IgG
3	+ve	+ve	+ ve	+ ve
14	+ve	+ve	- ve	+ ve
7	-ve	-ve	- ve	+ ve
Total	17	17	3	24

Table (3): The incidence of anti-HEV IgM or anti-HEV IgG and HEV RNA among aborted women according to maternal age.

Age group	No of sample	No of anti-HEVIgM Positive Samples		No of anti-HEVIgG Positive Samples		No of HEV-RNA Positive samples	
		Frequency	Percent	Frequency	Percent	Frequency	Percent
< 20	6	0	0	1	16.66	1	16.66
20 - <30	63	2	3.17	12	19.0	9	14.2
30 +	51	1	1.96	11	21.56	7	13.7
Total	120	3	2.5	24	20	17	14.16
P value		> 0.05		> 0.05		> 0.05	

Table (4): The incidence of HEV RNA and anti HEV IgM & IgG positivity in 120 aborted women according to the frequency of abortion.

Frequency of abortion	No of samples	No of anti-IgM positive samples		No of anti-IgG positive samples		No of HEV-RNA positive samples	
		Frequency	Percent	Frequency	Percent	Frequency	Percent
1	84	3	3.57	18	21.43	13	15.48
2	22	-	-	4	19.05	3	14.29
≥ 3	14	-	-	2	14.28	1	7.1
Total	120	3	2.5	24	20	17	14.17
P value		> 0.05 N.S.		> 0.05 N.S.		> 0.05 N.S.	

Table (5): Liver function tests of HEV-RNA positive and negative aborted women.

		HEV-RNA +ve aborted women	HEV-RNA –ve aborted women	P value
Total bilirubin (N: 0.4-1.0 mg %)	Mean	1.34	0.78	< 0.01
	SD ±	0.31	0.15	
Direct bilirubin (N: 0.2-0.4 mg%)	Mean	0.55	0.25	< 0.01
	SD ±	0.11	0.07	
Total protein (N: 6.5-8.7 g %)	Mean	7.36	7.48	> 0.05
	SD ±	0.57	0.55	
Serum albumin (N:2.5-4.0 g %)	Mean	3.17	3.46	< 0.05
	SD ±	0.37	0.45	
ALT (N:up to 12 U/L)	Mean	20.18	8.24	< 0.01
	SD ±	2.38	1.79	
AST (N:up to 12 U/L)	Mean	17.53	8.18	< 0.01
	SD ±	11.81	1.78	
A L P (N:up to 93 U/L)	Mean	132.47	123.3	> 0.05
	SD ±	22.16	19.36	
γ -GT (N: 7-33 U/L)	Mean	36.59	23.53	< 0.01
	SD ±	4.66	6.46	
5`-NT (N: up to 12 U|L)	Mean	9.65	6.12	< 0.01
	SD ±	2.00	1.49	

Table (6): HEV Vertical Transmission: Correlation of Hepatitis E viremia, antigenemia and anti-HE`V antibody status of aborted fetal tissue and relevant maternal sera.

Code No	Maternal Serum				Fetal Tissue	
	HEV-RNA RT-PCR	HEV Ag Dot ELISA	IgM*	IgG *	HEV-RNA RT-PCR	HEV Ag Dot ELISA
1	+ve	+ve	-	1.901	+ve	+ve
18	+ve	+ve	-	1.200	+ve	+ve
23	+ve	+ve	-	1.258	+ve	+ve
28	+ve	+ve	-	1.211	+ve	+ve
33	+ve	+ve	1.364	2.868	+ve	+ve
38	+ve	+ve	-	2.422	+ve	+ve
42	-ve	-ve	-	1.790	-ve	-ve
43	+ve	+ve	-	1.380	+ve	+ve
44	+ve	+ve	-	1.592	+ve	+ve
46	+ve	+ve	-	2.705	+ve	+ve
51	+ve	+ve	-	1.966	+ve	+ve
54	+ve	+ve	-	2.389	+ve	+ve
64	+ve	+ve	-	2.624	+ve	+ve
68	+ve	+ve	0.519	2.948	+ve	+ve
75	+ve	+ve	-	2.825	+ve	+ve
80	+ve	+ve	-	1.306	-ve	-ve
81	-ve	-ve		0.637	-ve	-ve
85	+ve	+ve	-	2.646	-ve	-ve
88	+ve	+ve	1.586	1.562	+ve	+ve
92	-ve	-ve	-	0.819	-ve	-ve
93	-ve	-ve	-	1.030	-ve	-ve
106	-ve	-ve	-	0.852	-ve	-ve
111	-ve	-ve	-	0.809	-ve	-ve
114	-ve	-ve	-	1.086	-ve	-ve
Total	17	17	3	24	15	15

* An optical density reading (OD) above the cutoff value of IgG at 0.534 λ or of IgM at 0.465 λ is considered positive.

Vertical HEV Transmission

The 3 anti-HEV IgM, anti-HEV IgG, HEV-RNA and HEV Ag positive pregnant women transmitted the virus to their fetus (100 %). While 14 anti-HEV IgG, HEV-RNA and HEV Ag positive pregnant women transmittedthe virus to 12 (85.7 %) of their fetus. But the fetal tissue of the remaining 7 anti-HEV IgG positive aborted women were HEV-RNA negative [Table 6]. In contrast to fetal wasting full term delivered babies were born to 13 anti-HEV IgG positive control women. All their infants cord sera were negative for HEV RNA.

DISCUSSION

While 3 of 120 (2.5 %) aborted women had anti-HEV IgM none of the 120 full term women had it, but this difference was not statistically significant ($P > 0.05$). On the other hand anti-HEV IgG was detected in 24 of 120 (20 %) of the aborted women, compared with 13 of 120 (10.8%) full term delivered women. This difference was statistically significant ($P < 0.05$). HEV RNA and HEV Ag were detected in 17 of 24 (70.8 %) anti-HEV IgG positive aborted women, which indicated active HEV infection, while all the sera from 13/120 (10.83%) anti-HEV IgG positive full term delivered women were HEV RNA negative. This difference in HEV RNA detection in sera was highly significant ($P < 0.01$).

The prevalence rate of anti-HEV IgG among the aborted women in our study is nearly similar to a published report from Egypt, (Abdel-Wahab et al,. 1996 b). Anti-HEV IgG in pregnant women from north Africa was 14.4 % (Rogez *et al.*, 1993); from Pakistan, in 21.6 % of 162 of pregnant women (Rab *et al.*, 1997); 15 % of pregnant women in Kyrghyzstan, Russia (Fedorova *et al.*, 1996). In 17.7 % (38 of 214) in women in Campinas (Goncales et al. 2000). And in other report in 1 % (3/304) of pregnant women from Brazil. In Switzerland anti-HEV IgG was found in 2.1 % of pregnant women (Lavanchy *et al.*, 1994) which reflect the lower rate of HEV endemicity in industrialized versus developing countries.

In the present study, the three (100 %) anti-HEV IgM positive aborted women transmitted HEV RNA and infection to their fetuses. This relation between anti-HEV IgM positivity of mother and HEV RNA positivity of their aborted fetuses was statistically highly significant ($P < 0.01$). While among 24 anti-HEV IgG positive aborted women in the first trimester of pregnancy 15 (62.5 %) of their aborted fetal tissue were HEV RNA positive. Also 15 among 17 (88.23%) HEV RNA and HEV Ag positive aborted women, transmitted the virus to their fetuses. This relation was also statistically highly significant ($P < 0.01$). Thus HEV infection during first trimester of pregnancy may be the possible cause of spontaneous abortion in (15/120) i.e. 12.5 % pregnancies in our study.

The present study, showed vertical HEV transmission from HEV infected pregnant women to their fetus. When 3 anti-HEV IgM, anti-HEV IgG, HEV RNA and HEV Ag positive pregnant women transmitted the virus to all their aborted fetuses (100 %), While anti-HEV IgG, HEV RNA and HEV Ag positive pregnant women transmitted the virus to 14 (85.7 %) of their aborted fetuses. These results indicated that in a community where HEV is endemic exposure of pregnant women to HEV infection in first trimester led to vertical transmission and spontaneous abortion.

Khuroo et al. (1996) reported vertical transmission of HEV in first half of pregnancy. Abdel-Wahab *et al.* (1996 b) also reported that IgM anti HEV positive anicteric mildly febrile viremic pregnant women, terminated by spontaneous abortion.

In our study, it was observed that the prevalence of anti-HEV IgG increased by age from 16.66 % (1/6) in < 20 years old to 19 % (12/63) in the 20 - < 30 years old, then reached 21.56 % (11/51) in women aged 30 and over. The relation between age of aborted women and the anti-HEV IgG positivity was not statistically significant ($P > 0.05$). While anti-HEV IgM was higher in age group 20 - < 30 than the other two group. The relation between age of aborted women and the anti-HEV IgM positivity was not statistically significant ($P > 0.05$). There was negative relationship between the age and HEV RNA positivity of aborted women, since it decreased from 16.66 % in < 20 years old to 14.2 % in the 20 - < 30 years old, then reached 13.7 % in women aged 30 and over. However this relation was not statistically significant ($p > 0.05$). Thus it can be speculated that elder pregnant women were experiencing a re-infection with HEV that stimulated an IgG boaster antibody response with short viremia, while the youngest had primary infection with longer HEV viremia.

The present study indicated that past history of surgical operation, or blood transfusion, or repeated abortion was not statistically significant ($p > 0.05$). risk factor in HEV infected pregnant Egyptian mothers.

In the present results, the mean value of liver function tests (except alkaline phosphatase and protein) of HEV RNA positive aborted women appeared to be slightly higher than the mean value of HEV RNA negative aborted women. These differences were statistically significant ($P < 0.05$). However alkaline phosphatase showed apparent increase above the normal range. Similar results were observed in HEV infected aborting Ethiopian women (Tsega *et al.*, 1992).

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