

Hepatitis E Virus IgG in Serum of Pregnant Women

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ABSTRACT

BACKGROUND: The prevalence of anti-HEV in Egypt is among the highest of any country in the world. The outcomes of pregnancy in infected women need to be delineated.

AIM: To screen pregnant females for hepatitis E IgG and to assess the effect of HEV IgG on the pregnancy outcome.

METHODS: 142 consecutive pregnant women were subjected to full history-taking, thorough clinical examination, abdominal ultrasonography and IgG antibody to HEV using ELISA kits. HEV avidity index was estimated for anti-HEV positive cases.

RESULTS: There was a significant elevation of HEV IgG antibody in the serum of pregnant women, especially in the countryside, in

age group more than 30 years and in the third trimester of pregnancy. There was a possible association between HEV IgG antibody and liver fatty infiltration and neonatal jaundice. There was a significant inverse correlation between HEV IgG avidity index and age. No clinical correlation noticed between HEV IgG avidity index and history of previous abortion, the number of pregnancy, complete blood picture (CBC), liver enzymes, INR or serum creatinine. There was no statistically significant association between HEV IgG avidity indices and outcome of gestation.

CONCLUSION: HEV IgG is prevalent in serum of pregnant women but with benign course of both the pregnant lady and her outcome.

Key words: HEV IgG; Pregnant women; Avidity index; Neonatal outcome

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BACKGROUND

Hepatitis E virus (HEV) is endemic in Egypt, but has a relatively benign course with no morbidity, the prevalence of HEV is very high in rural communities, but researchers did not find any cases of acute viral hepatitis or fulminant hepatic failure due to HEV^[1].

The relationship between HEV and pregnancy is attractive due to its high prevalence and severe manifestations in pregnant women in some regions such as North India^[2]. The IgG avidity index was high (> 60%) in patients with previous infection or polyclonal activation but was low (< 40%) in patients with acute infection^[3].

In Egypt, the prevalence of anti-HEV is among the highest of any country in the world approaching 80%; yet, outbreaks have not been reported. A prospective study estimated the HEV incidence at 41.6/1000 person-years^[4].

Stoszek detected no mortality among more than 2000 pregnant women with serological markers for infection^[4].

The aim of this study was to screen pregnant females admitted to Zagazig University Hospitals for anti-HEV IgG and to assess the effect of HEV IgG on the pregnant women and their outcome.

SUBJECTS AND METHODS

Study area and subjects

We conducted this study in the Obstetrics, Gynecology, Medical Biochemistry and internal medicine Departments in Zagazig University Hospitals between June, 2015 and October 2015. Most of the patients are referral cases from various parts of El-Sharqia Governorate.

Inclusion criteria: The cases included were the first 142 consecutive pregnant women admitted to Obstetrics and Gynecology Department during the period of the study.

Exclusion criteria: Subjects with manifestations of chronic liver disease or had positive viral markers for HBV or HCV. Also, patients who refused to give a written consent were excluded from the study.

Patient assessment: All patients were subjected to the following evaluations. (A) A full history-taking. (B) Full clinical examination. C. Abdominal ultrasonography. (D) The samples used for the study were the sera from blood samples drawn from these 142 pregnant women for their routine investigations. Hepatitis B virus surface antigen-core antibody, Hepatitis C virus antibody, CBC, Albumin, Bilirubin, ALT, AST, Creatinine, and Urea. (E) All sera were screened for IgG antibody to HEV using ELISA kits. We score results as positive or negative according to the standard procedures recommended by the manufacturer. Positive and negative controls were included in all the ELISA Microplates assayed. (F) We estimated the neonatal liver functions and birth weight at the time of delivery. (G) HEV avidity index was estimated for anti-HEV positive cases: IgG avidity index was expressed in percentage, as follows: IgG avidity index (%) = (optical density of well with urea/optical density of well without urea) × 100. We considered values below 40% were as low, and those above 60% were considered as high. IgG avidity index values between 40-60% were found to be equivocal.

Ethical considerations

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University, Egypt (ZU-IRB # 1922). All patients gave written informed consent to participate in the study and for performing all appropriate interventions.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for Windows (MedCalc Software bvba, Ostend, Belgium).

RESULTS

Most of the cases included in the study were housewives 90.8%, the age of most cases were between 21 and 30 years (mean age of 25.35 ± 5.66). The least size of the sample was in the age group more than 30 years old. We noticed relatively increased the number of rural than urban women (65.5% against 34.5%) (Table 1).

HEV IgG was present in rural individuals (69.9%) compared to (63.3%) in urban. There was no statistically significant relationship between infection and demographic features of the subjects of our study as occupation, age, history of blood transfusion, hypertension or diabetes mellitus. No statistically significant relationship between HEV IgG and history of previous abortion.

HEV IgG was present in 67.6% of the studied group.

Forty-three patients had a high avidity index denoting chronic infection, 26 had a low avidity index indicating recent infection while 27 had intermediate avidity.

Table 1 HEV IgG and its correlation with demographic data.

The studied group	N	Number	Positive anti-HEV IgG Prevalence% (95% CI)	p
Total	142	96	67.6	
Rural women	93	65	69.9	0.648
Urban women	49	31	63.3	
Housewife	129	89	69	0.526
Student	13	7	53.8	
≤ 20 years	42	22	52.4	
21 - 30 years	72	51	70.8	0.235
> 30 years	28	23	82.1	0.128
Blood transfusion	24	15	62.5	0.738
No Blood transfusion	118	81	68.6	
Diabetes mellitus	11	7	63.6	0.867
No diabetes mellitus	131	89	67.9	
1st trimester	47	25	53.2	---
2nd trimester	32	22	68.8	0.378
3rd trimester	63	49	77.8	0.119
Previous abortion	12	7	58.3	0.683
No previous abortion	130	89	68.5	
3-5 pregnancies	66	48	72.7	0.489
1-2 pregnancies	76	48	63.2	

Table 2 Risk factors for HEV infection.

Risk factors		No	Frequency of HEV infection No%		p
Residence	Rural	93	65	69.9	< 0.001
	Urban	49	31	63.3	
Occupation	Housewife	129	89	69	< 0.001
	Student	13	7	53.8	
Age	≤ 20 years	42	22	52.4	
	21 - 30 years	72	51	70.8	0.001
	> 30 years	28	23	82.1	0.002
Blood transfusion	No	118	81	68.6	< 0.001
	Yes	24	15	62.5	
Diabetes mellitus	No	131	89	67.9	< 0.001
	Yes	11	7	63.6	
Trimester	1st	47	25	53.2	
	2nd	32	22	68.8	0.039
	3rd	63	49	77.8	< 0.001
Abortion	No	130	89	68.5	< 0.001
	Yes	12	7	58.3	
Pregnancy	1-2 pregnancies	76	48	63.2	< 0.001
	3-5 pregnancies	66	48	72.7	
Delivery	Vaginal delivery	59	40	67.80%	0.967
	Cesarean Section	83	56	67.50%	

There was a statistically significant high prevalence of HEV IgG in rural subjects, homemakers, and age group > 30 years. Also, there was a substantial increase in infection in the 3rd trimester of pregnancy. We noticed the significant inverse relationship between HEV IgG antibody and history of blood transfusion, diabetes mellitus, and previous abortions.

No statistically significant relationship noticed between HEV IgG and history of chronic diseases like hypertension, diabetes mellitus, congenital heart disease or blood transfusion. There was a statistically significant association between HEV IgG and trimester of pregnancy as it was increased in the 3rd trimester of gestation ($p = 0.024$).

However, there was no statistically significant relationship between prevalence of HEV infection and history of previous abortion, pregnancy number or method of delivery.

There was no statistically significant association between HEV IgG antibody and the cause of hospital admission as vaginal bleeding, placenta previa, pre-eclampsia, hyperemesis gravidarum, premature rupture of membranes, anemia, abdominal colic or cesarean section.

There was no statistically significant relationship with HEV IgG antibody infection and fatty liver or splenomegaly. There was no statistically significant association between HEV IgG antibody and hemoglobin, white blood cells, platelet count, albumin, ALT, AST, total and direct serum bilirubin, INR or serum creatinine level.

There was a statistically significant relationship between HEV IgG avidity index and age as the subjects ≤ 20 years had a high avidity index denoting that increased rate of chronic infection in young subjects ($p = 0.011$). Subjects more than 30 years had the lowest avidity index denoting increased risk of recent infection in this age group.

There was no statistically significant relationship between HEV

IgG avidity index and history of medical disease as diabetes mellitus, hypertension or history of blood transfusion.

There was no significant association between HEV IgG avidity index and trimester of pregnancy, history of previous abortions, the number of current pregnancy, a method of delivery or the cause of hospital admission.

We found a significant relationship between direct serum bilirubin level and avidity index as cases with intermediate avidity index showed increased levels of direct serum bilirubin compared to cases with low or high avidity index. There was no statistically significant relationship between HEV IgG avidity index and CBC, liver enzymes, serum albumin, total bilirubin, INR or serum creatinine levels (Table 4).

There was a statistically significant relationship between low avidity index and fatty liver in cases included in the study denoting possibility of increased risk of liver fatty infiltration with recent HEV infection. However, there was no significant relation between avidity index and splenomegaly.

Table 3 Comparison between pregnant women with negative anti-HEV IgG and those with positive anti-HEV IgG as regarding medical history.

Medical history		All (N = 142)	Anti-HEV IgG						p
			Negative (N = 46)				Positive (N = 96)		
			No.	%	No	%	No.	%	
Diabetes Mellitus	Absent	131	92.3	42	32.1	89	67.9	0.747	
	Present	11	7.7	4	36.4	7	63.6		
Hypertension	Absent	134	93.7	45	33.8	88	66.2	0.271	
	Present	9	6.3	1	11.1	8	88.9		
Congenital heart disease	Absent	134	99.3	45	31.9	96	68.1	0.324	
	Present	1	0.7	1	100	0	0		
Blood transfusion	No	118	83.1	37	31.4	81	68.6	0.558	
	Yes	24	16.9	9	37.5	15	62.5		
Trimester	1st	47	33.1	22	46.8	25	53.2	0.024	
	2nd	32	22.5	10	31.3	22	68.8		
	3rd	63	44.4	14	22.2	49	77.8		
Previous abortion	No	130	91.5	41	31.5	89	68.5	0.331	
	Once	11	7.7	4	36.4	7	63.6		
	Twice	1	0.7	1	100	0	0		
Pregnancy number	1st	42	29.6	15	35.7	27	64.3	0.726	
	2nd	34	23.9	13	38.2	21	61.8		
	3rd	52	36.6	15	28.8	37	71.2		
	< 3	14	9.8	3	51.5	11	78.6		
Delivery	Vaginal delivery	59	41.5	19	32.2	40	67.8	0.967	
	Cesarean section	83	58.5	27	2.5	56	67.5		

§ Chi-square test.

Table 4 The relationship between anti-HEV IgG avidity and laboratory findings.

Laboratory findings	Anti-HEV IgG avidity			p
	Low (N = 26)	Intermediate (N = 27)	High (N = 43)	
Hemoglobin (gm/dL)	0.50 ± 1.20	9.69 ± 1.90	10.42 ± 1.39	0.258
WBC ($\times 10^3/mm^3$)	9.53 ± 1.02	9.71 ± 1.53	9.14 ± 1.34	0.129
Platelet count ($\times 10^3/mm^3$)	220.23 ± 42.96	222.29 ± 61.17	224.04 ± 41.40	0.776
Albumin (gm/dL)	3.61 ± 0.41	3.58 ± 0.32	3.57 ± 0.27	0.815
Total serum bilirubin (mg/dL)	0.79 ± 0.19	0.93 ± 0.27	0.84 ± 0.22	0.131
Direct serum bilirubin (mg/dL)	0.15 ± 0.13	0.23 ± 0.16	0.18 ± 0.14	0.027
AST (U/L)	29.34 ± 13.98	32.81 ± 16.46	30.76 ± 11.26	0.575
ALT (U/L)	24.03 ± 10.93	27.77 ± 15.92	24.74 ± 8.64	0.648
INR	0.97 ± 0.19	1.05 ± 0.29	0.98 ± 0.26	0.569
Creatinine (mg/dL)	0.89 ± 0.26	1.00 ± 0.42	0.88 ± 0.29	0.699

Kruskal-Wallis H test

There was a significant inverse correlation between HEV IgG avidity index and age ($p = 0.004$) as a low avidity index was increased in age group more than 30 years. No clinical correlation noticed between HEV IgG avidity and history of previous abortion, the number of pregnancy, CBC, liver enzymes, INR or serum creatinine.

15.5% of the outcome of cases included in the study suffered from low birth Weight, 17.6% was preterm labor while 15.5% suffered from neonatal jaundice but no cases of stillbirth had been reported.

There was a statistical significance between anti-HEV IgG and newborn jaundice. However, there was no relationship between anti-HEV IgG and preterm labor or low birth weight.

There was no statistically significant association between HEV IgG avidity indices and outcome of pregnancy as regards neonatal jaundice, low birth weight or preterm labor.

DISCUSSION

HEV is an enterically transmitted pathogen and is responsible for a large-scale epidemic of hepatitis around the world, according to the epidemiological data, 3.3 million acute cases and 20 million new cases of hepatitis E are diagnosed each year^[5].

Diagnosis of HEV infection depends on the clinical features and the exclusion of other causes of acute hepatitis; assays of sera make the serological diagnosis for the presence of anti-HEV IgM or IgG by ELISA screening^[6].

HEV infection among pregnant women in Egypt was not associated with a history of jaundice or liver disease^[4]. The reasons for the infrequent clinical hepatitis could be the result of early childhood exposure.

The prevalence of HEV is higher in rural communities, but no cases of acute viral hepatitis due to HEV have been reported^[1]. That study examined a cohort of 2428 pregnant women in the Nile Delta to assess the prevalence of, and risk factors for, anti-HEV and correlated these with a history of liver disease. Anti-HEV prevalence was 84.3%. The subjects included in that study were outpatient pregnant women in rural villages at Nile Delta^[7].

In our study, anti-HEV IgG was present in 67.6% of pregnant women admitted to Zagazig University Hospitals. The cause of this relative difference may be due to small the sample size compared to previous large-scale one and the type of our sample (all cases were inpatients).

Our findings when compared with the international studies, pregnant women showed a higher prevalence of anti-HEV IgG than those in other countries like Spain (2-13%)^[8] and this can be explained by the association between HEV infection and inadequate food and water sanitation

HEV IgG is increased in rural areas. It is in agreement with Medhat *et al*^[11] and Darwish *et al*^[7] in a study at Nile delta. The high prevalence in both studies may be attributed to similar environmental factors as water^[9] and food sanitation also, anti-HEV antibodies have

been detected in swine, rodents, dogs, cattle, sheep, and poultry^[10].

We noticed a high rate of HEV IgG in the third trimester of pregnancy (77.8%) compared to women in their second (68.8%) and first trimester (53.2%). That was in agreement with Matthiesen *et al*^[11] and Singh *et al*^[12].

Anti-HEV IgG positive individuals in our study had normal ALT, AST, bilirubin levels and this is in agreement with Debing and Neyts^[13] and Amer *et al*^[14] but in contrary to a study that was published by Verghese and Robinson^[15] that showed the elevation of liver enzymes and jaundice with HEV infection.

We found no significant laboratory abnormalities in HEV IgG-positive subjects included in the survey as CBC, serum creatinine, prothrombin time, and INR. It is in line with Amer *et al*^[14]. However, in contrary to other studies, there were extrahepatic manifestations due to HEV infection in the form of hemolysis, purpura, glomerulonephritis, and neurological manifestations^[16].

We found that most of the positive cases of HEV antibodies (IgG) showed fatty liver as a significant finding during abdominal examination with ultrasound, which could be a normal variant or due to fat deposition in the liver. There is no clinical significance of liver enlargement or fatty infiltration in relation to HEV IgG positivity. There was a relationship between low avidity index and fatty liver in our cases denoting possibility of increased risk of liver fatty infiltration with recent HEV infection, but, there was no significant relation between avidity index and cases with splenomegaly.

Concerning acute HEV infection, the presence of HEV RNA in blood and stool is short-lived, and becomes undetectable in serum at 3-4 weeks and in the stool at six weeks after the onset of clinical symptoms in asymptomatic subjects, viremia can last for 4-6 weeks^[6]. Given the limited performance of serological markers, IgG avidity index is a useful tool that is simple to use for the diagnosis of HEV infection. Given the problems encountered for the detection of viral RNA or anti-HEV IgM, combinations of markers are required for

Table 5 The correlation between Avidity Index and the study parameters.

Variables	Avidity Index (%)	<i>p</i>
Age (years)	-0.239	0.004
Previous abortions	-0.054	0.604
Number of pregnancy	0.032	0.755
Hemoglobin (gm/dL)	-0.048	0.639
WBC ($\times 10^3/\text{mm}^3$)	-0.149	0.148
Platelet count ($\times 10^3/\text{mm}^3$)	0.041	0.694
Albumin (gm/dL)	-0.094	0.36
Total serum bilirubin (mg/dL)	0.126	0.221
Direct serum bilirubin (mg/dL)	0.1	0.333
AST (U/L)	0.044	0.667
ALT (U/L)	0.014	0.892
INR	-0.047	0.648
Creatinine (mg/dL)	-0.072	0.488

r Spearman's correlation coefficient

Table 6 Neonatal outcome in relation to infection.

		Low birth weight		Preterm labor		Neonatal jaundice	
		Absent (120)	Present (22)	Absent (117)	Present (25)	Absent (120)	Present (22)
HEV IgG	negative	42	4	36	10	43	3
	positive	78	18	81	15	77	19
P		0.121		0.371		0.041	
HEV avidity	Low (26)	23	3	21	5	20	6
	Intermediate (27)	20	7	25	2	21	6
	High (43)	35	8	35	8	36	7
P		0.406		0.381		0.737	

§ Chi-square test.

reliable diagnosis. IgG avidity index may, therefore, help to improve the diagnosis of acute hepatitis E infection. So, we use HEV avidity index to check for recent infection.

We noticed increased prevalence of HEV IgG in age group more than thirty years, and this was in agreement with Amer *et al*^[14].

In our study, no cases of fulminant hepatitis had been reported, and mortality rate of our subjects was zero, and this was in line with other studies in Egypt^[17-18]. It was in contrary to a study in North India^[2] and a study in Darfur in Sudan^[19].

Concerning delivery method, we found no statistically significant relationship between HEV IgG and delivery method. Also, We found that the neonatal jaundice is increased in cases with positive HEV IgG as 19.8% of total cases with neonatal jaundice were delivered by HEV IgG positive mothers while HEV IgG negative mothers delivered only 6.5%. It was in agreement with Khuroo *et al*^[20] denoting a possible relation between HEV infection in the mother and the possibility of occurrence of neonatal jaundice.

Also, there is no significant relationship between HEV IgG and both low birth weight and preterm labor, and that was in agreement with Navaneethan *et al*^[21]. In contrast, another study upon HEV in pregnant women in Iran denoted that infected babies suffered from prematurity, mild anicteric neonatal hepatitis or jaundice, stillbirth, preterm labor, perinatal death or neonatal death soon after birth^[22] and^[23]. It is explained by the difference in virus virulence in both studies.

In our study, follow up six months, there was no mortality, and this was in agreement with El Sayed Zaki *et al*^[23]. In a study published upon HEV infection in pregnant women in South Asia, HEV infection is typically severe during the third trimester of pregnancy, mortality rates among pregnant women in the third-trimester range from 10%-25%. It may be due to the differences in major histocompatibility complex (MHC) phenotypes in this country as compared to the other endemic areas^[24].

However, the findings and conclusions of this study are limited by the small sample size. So, a further larger-scale prospective survey of HEV infection among pregnant women should be conducted to validate our findings, to analyze in more detail the clinical and the epidemiological features of this disease and to evaluate the cost-effectiveness of antenatal HEV screening in Egypt. Also, we had no available data about HEV state of our subjects before the study.

CONCLUSIONS

HEV IgG is prevalent in the serum of pregnant women but with a benign course of both the pregnant lady and her outcome.

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