

Original Article

Seroprevalence of Hepatitis E Virus Infection Amongst Pediatric Acute Leukemias at the National Cancer Institute in Egypt

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ABSTRACT

Background: Hepatitis E virus (HEV) is one of the important causes of hepatitis epidemics in the developing world. This study aimed to investigate the prevalence of HEV antibodies in pediatric acute leukemics.

Setting: The pediatric service of the National Cancer Institute, Cairo University, Egypt.

Subjects: One hundred and seventy five children, 88 newly diagnosed acute leukemic children and 87 healthy siblings of 42 of the patients as controls.

Methods: ELISA testing for anti-HEV IgM and anti-HEV IgG was done on the sera of the patients and controls.

Results: A higher exposure rate to HEV (IgG antibodies) was noticed in acute leukemia patients, 26/88 in comparison to 15/87, in the sibling control group. Acute HEV infection diagnosed by anti-HEV IgM Ab was higher in normal siblings (6%) compared to (2%) in

leukemic children. Analysis showed that HEV seropositivity was seen mostly in leukemic children at preschool age (< 5 Y) and the exposure to HEV infection was higher among young adults (> 10 Y). HEV seropositivity was higher among females leukemic children 15/26 (58%) compared to males 11/26 (42%). Leukemic children living in rural area are exposed to a higher risk of hepatitis E infection (58%) compared to (42%) among urban population. Leukemic children with a previous history of blood transfusion showed significant increase in the seropositivity (69%) compared to (45%) in the non-transfused children.

Conclusion: The data indicate a higher susceptibility of the children with acute leukemia for HEV infection. Ensuring a clean drinking water supply remains the best preventive strategy. Recombinant vaccines may be particularly useful for these patients.

KEYWORDS: acute leukemia, children, Egypt, hepatitis E virus

INTRODUCTION

Hepatitis E, previously known as enterically transmitted, enteric, or epidemic hepatitis, is a worldwide public health problem^[1]. Hepatitis E virus (HEV) is a common cause of acute sporadic hepatitis with a frequently prolonged viremia in children living in Egypt^[2]. In developing countries where hepatitis A and E infections are endemic, severe complications can arise in case of mixed infections. These may contribute to most of the mortality caused by acute liver failure during childhood^[3]. Although the mortality rate due to HEV infection is usually low (0.07-0.6%), the illness may be particularly severe among immunocompromised individuals. In addition, the rate of anti-HEV sero-positivity was found to increase with the progression of HIV infection^[4]. Acute liver disease in HEV infection may occur in immunocompromised leukemic children as well, particularly when coupled with other risk factors such as living in rural areas rather than urban

populations^[5]. High mortality rates reported in Asia following blood transfusion were due to HEV^[6]. Our aim was to study the seroprevalence of HEV in pediatric acute leukemics and their siblings, as well as the different factors associated with the infection.

PATIENTS AND METHODS

Patients

The present study was conducted on 88 newly diagnosed pediatric patients with acute leukemia who presented to pediatric oncology service of NCI, Cairo University from March 1995 till August 1998. Eighty-seven siblings of 42 out of these patients were also included as normal controls. All patients were initially subjected to a full history, clinical examination in addition to a battery of investigations done for diagnostic purposes.

Collection of blood samples

5 ml of blood sample was collected from each individual, followed by serum separation. Sera

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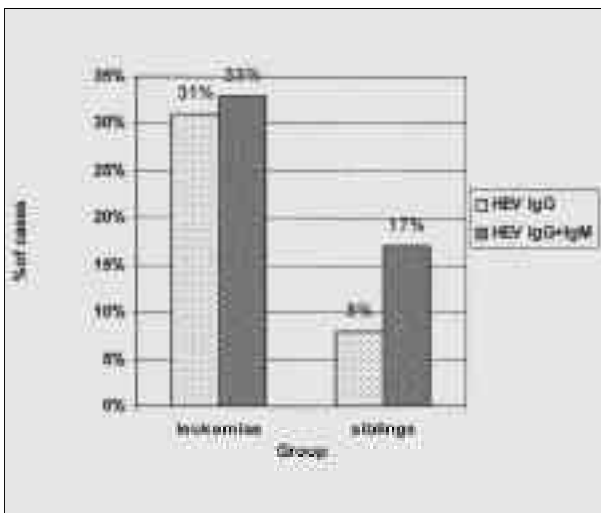


Fig. 1: Seroprevalence of HEV Ab among leukemic children and their siblings

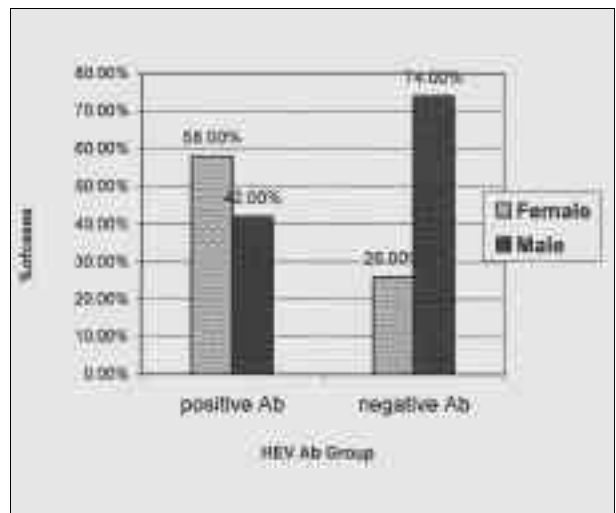


Fig. 2: Seroprevalence of anti-HEV Ab and sex in leukemic children

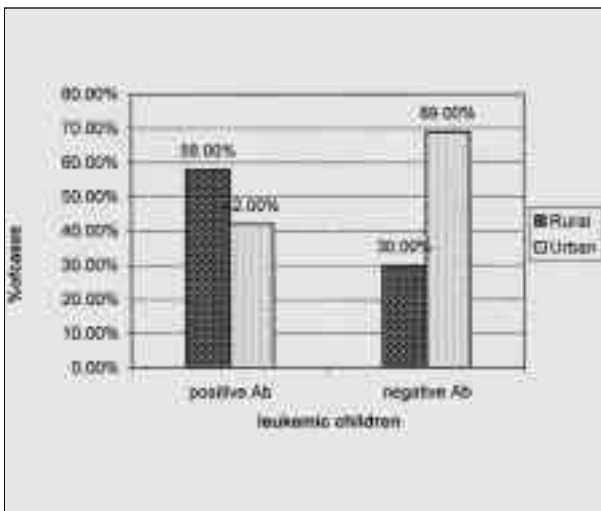


Fig. 3: Seroprevalence of anti-HEV Ab and residence in leukemic children

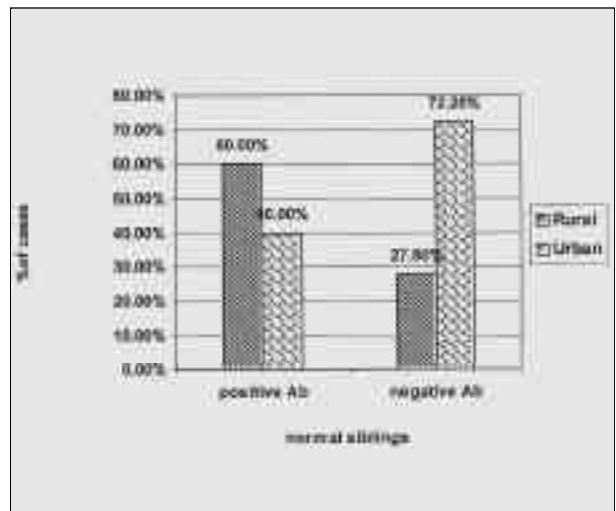


Fig. 4: Seroprevalence of anti-HEV Ab and residence in normal siblings

were then aliquoted and kept at -20° C until used. The serum level of ALT was determined colorimetrically using kits from Biomerieux (France). An abnormal ALT value was defined as 45 Reitman Frankel units/ml or greater.

Serological markers of hepatic viral infection

Detection of anti-HEV IgG Ab and anti-HEV IgM Ab were done by enzyme-linked immunosorbent assay (ELISA) using equipar ELISA kits (Sarono, Italy) employing synthetic peptides from two of the three open reading frames (ORF2 & ORF3) of the HEV RNA molecule encoding conservative immunodominant epitopes of HEV. The tests were done according to the manufacturer’s instructions.

Statistical analysis

SPSS (Statistical package for social sciences) was used for analyzing the data. Chi-square test was used for comparison of proportions. P value is significant at 0.05 level^[7].

RESULTS

Age and sex distribution of the 175 individuals included in the study is shown in Table 1. The median age of the 88 pediatric acute leukemics was six years, and that of the 87 apparently healthy siblings was 6.5 years. Preponderance of males was observed among both acute leukemia patients and normal controls with M/F ratio: 1.8:1 and 1.5:1 respectively (Table 1).

Serology

The total exposure of anti-HEV IgG and IgM antibodies was higher in acute leukemia cases 26/88 (29.5%) compared to their normal siblings 15/87 (17%) but it was not statistically significant (P = 0.11). However, when comparing the same total exposure to HEV in the 42 acute leukemic (in whom their siblings were available) with their siblings, a significant difference was noticed between the two groups (P = 0.001), Table 2, Fig. 1. In contrast to

Table 1
Distribution of age and sex in all subject groups

Group	No. of cases	Age (years)		Sex		
		Range	Median	Male	Female	M:F Ratio
Acute leukemias (with sibling as controls)	42	3-17	6	31	11	2.8:1
Acute leukemias (no sibling as controls)	46	2-16	6	26	20	1.3:1
Acute leukemias (Total)	88	2-17	6	57	31	1.8:1
Siblings	87	7/12-27	6.5	53	34	1.55:1
Total	175	7/12-27	6	110	65	2:1

Table 2
Prevalence of HEV antibody among leukemia children and their siblings

Group	No. of cases	HEV seropositive				Total	(%)
		IgG No. (%)	IgM No. (%)				
Acute leukemias (with sibling as controls)	42	13 (31)	1 (2)		14	(33)	
Acute leukemias (no sibling as controls)	46	7 (15)	1 (2)		12	(26)	
Acute leukemias (Total)	88	20 (22.7)	2 (2)		26	(29.5)	
Siblings	87	7 (8)	5 (6)		15	(17)	
P value		0.001			0.08		

seroprevalence of IgG, seroprevalence of anti-HEV IgM was found higher in the normal siblings (6%) compared to their leukemic children (2%), (Table 2).

Factors associated with anti-HEV Ab seroprevalence

Leukemic children at the preschool age (< 5 Y) had a higher percentage of anti-HEV Ab (54%), though not significant, when compared to those with negative anti-HEV Ab (37%), ($P = 0.16$), and also when compared to leukemic children more than 10 years (15%), (Table 3). The same results were also found among normal siblings, (Table 4).

A higher prevalence of anti-HEV Ab was found among females (58%) compared to males (42%) in acute leukemia patients (Table 3, Fig. 2). On the contrary, the prevalence of anti-HEV Ab was found more among males (67%) compared to females (33%) in the normal sibling group (Table 4).

Acute leukemic children living in rural areas had significantly higher anti-HEV Ab (58%) compared to urban population (42%) of the same group. (Table 3, Fig. 3). Similarly a higher percentage of anti-HEV Ab was found among normal siblings living in rural areas (60%)

Table 3
Risk factors related to HEV in 88 leukemic children

Factor	Seroprevalence of HEV N=88			
	Positive Ab N = 26		Negative Ab N = 62	
	No.	(%)	No.	(%)
Age				
5Y	14	(54)	23	(37)
>5Y- 10Y	8	(31)	22	(35.5)
>10Y	4	(15)	17	(27)
Sex				
Male	11	(42)	46	(74)
Female	15	(58)	16	(26)
Residence				
Rural	15	(58)	19	(30)
Urban	11	(42)	43	(69)
Blood transfusion	18	(69)	28	(45)
Abnormal ALT	4	(15)	20	(32)

Table 4
Risk factors related to HEV in 87 sibling controls

Factor	Seroprevalence of HEV N=87			
	Positive Ab N = 15		Negative Ab N = 72	
	No.	(%)	No.	(%)
Age				
5Y	7	(46.6)	34	(47)
> 5Y- 10Y	3	(20)	13	(18)
> 10Y	5	(33)	25	(34.7)
Sex				
Male	10	(67)	43	(60)
Female	5	(33)	29	(40)
Residence				
Rural	9	(60)	20	(28)
Urban	6	(40)	52	(72)

compared to the urban population (40%), ($P = 0.005$), (Table 4, Fig. 4). The seroprevalence of anti-HEV Ab among leukemic children was significantly associated with a previous history of blood transfusion (69% versus 45%) among those with negative anti-HEV Ab of the same group ($P = 0.009$), (Table 3).

The percentage of abnormal levels of ALT value among acute leukemic children with negative anti-HEV Ab was higher (32%), though not significant than that found in those with positive anti-HEV Ab (15%) present in the same group.

DISCUSSION

Hepatitis E virus (HEV) is believed to be the primary agent responsible for enterically transmitted non A, non B hepatitis (ET-NANBH). Hepatitis E is recognized as an important health concern in Asia, Africa, and Central America^[8]. Hepatitis virus infections are hyperendemic in Egypt. Hepatitis C virus (HCV) and hepatitis E virus (HEV) infections are highly prevalent, and in

fact they may be responsible for more hepatic morbidity than schistosomiasis and HBV^[9]. Under immunocompromised conditions, especially impairment of cell mediated immunity like pregnancy or leukemia, hepatitis E can lead to a severe infection causing a high mortality rate^[6].

In the present study, the 88 cases of Egyptian childhood acute leukemics with a median age of six years was similar to that reported from the USA^[10]. Male preponderance also coincided with other reports^[11]. The prevalence of HEV IgG in the 42 acute leukemic (31%) was significantly higher than their normal siblings (8%) ($P = 0.001$), and there was a lower prevalence of anti-HEV IgM in the 42 leukemic (2%). A possible explanation may be that the acute leukemics, who are immunocompromised patients, are more susceptible to infection either subclinically or from blood transfusion than their normal siblings. In addition, the higher prevalence of anti-HEV IgM Ab among healthy siblings, indicates substantial hepatitis E outbreak in the endemic areas^[12].

Nine percent of healthy siblings had acute infection and became immune to HEV, as diagnosed by anti-HEV IgM Ab, compared to 2% in their leukemic relatives. This indicates that leukemic children might be exposed to HEV infection subclinically from their siblings or from blood transfusion. Also further analysis showed that seven out of 87 (8%) of the healthy siblings had anti-HEV IgM Ab, whereas the leukemic relatives showed seronegative result (28/42) to anti-HEV Ab. The latter are at a higher risk of developing severe hepatitis E infection. Therefore, leukemic children should be protected from contracting HEV by isolating them from their siblings till a recombinant vaccine is available. Most HEV infections in children are minimally symptomatic and immunity is usually long lasting whereas severe disease tends to occur in nonimmune adults^[13]. Another study showed that HEV replicates in hepatocyte cytoplasm. Liver cell injury is caused by host immune response and possibly a direct cytopathic effect. HEV infection is common in young adults aged 15-40 years, this stands in contrast to the pattern of childhood acquisition of hepatitis A virus infection in developing countries^[14]. A possible reason for this is waning of the immunity acquired by exposure to HEV in early childhood, allowing later re-infection^[12]. This is matched with the results of the present study where higher HEV seropositivity (54%) was found among leukemic children at the preschool age (< 5 Y) compared to only (15%) among leukemic children more than 10 years.

The disease designated as non-A non-B hepatitis, was most common in males and was

usually mild except in pregnancy women when a high mortality rate due to depression in cell mediated, immunity, was noted^[15]. In contrast, the results of the present study showed significantly higher seropositivity to anti-HEV Ab among females of leukemic children compared to males. Hepatitis E virus, unlike HBV, is transmitted by fecal-oral route, and has caused spectacular water-borne outbreaks of jaundice, primarily in crowded places and in places with inadequate environmental sanitation^[15]. A study by Trinta *et al*^[16] showed that individuals living in rural areas had higher percentage of seropositivity to HEV Ab compared to urban population. The same findings were also reported in Egypt^[15]. Similarly in the present study, leukemic children living in rural areas had significantly higher prevalence of HEV Ab compared to urban population. Some studies describe two situations that favor epidemics: firstly, flooding, which causes raw sewage to contaminate drinking water and secondly, dry conditions, where the concentration of hepatitis E virus in water remains undiluted. Boiling of water prevented transmission, and this strategy has to be the mainstay of prevention until water supplies and sewage processing systems can be improved in these countries^[17].

Anti-HEV seropositivity was accumulated in groups of patients with various liver and non-liver pathologies and certain groups at risk for blood-borne infections^[18]. In the present study leukemic children with a previous history of blood transfusion had significantly higher serum rates of anti-HEV Ab compared to those without history of blood transfusion, indicating that blood transfusion is a risk factor for HEV transmission. Liver injury and abnormal liver function test can be observed among leukemic children due to various reasons like, leukemic infiltration, viral hepatitis, other than HEV, and cytotoxic drugs. The present study showed a higher percentage of abnormal ALT level among leukemic children with negative anti-HEV Ab compared to those with positive anti-HEV Ab. A study by Clayson *et al*^[18] showed that the proportion of HEV RNA positive sera increased from days 0-3 to days 8-11 in the presence of decreased ALT activity suggests an uncoupling of viral replication and liver injury. One possible explanation is that HEV virions may be released from hepatocytes by a mechanism other than cell lysis. Another possibility is that the virus detected in serum after the decrease in ALT activity, was released after replication in tissues other than liver^[18].

CONCLUSION

The data obtained in our study indicated a high susceptibility of children with acute leukemia for

HEV infection. HEV viremia should be tested in such individuals. Ensuring clean drinking water supply remains the best preventive strategy. Recombinant vaccines are being developed that may be particularly useful for these patients. This is the first study done in Egypt seeking hepatitis E virus infection amongst leukemic children and calling for further studies about the viral effect and immunologic response in other immunocompromised patients.

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