ORIGINAL ARTICLE

Immunological Modulation of HAV and HEV Positive Children

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ABSTRACT

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Background: hepatitis E virus (HEV) and hepatitis A virus (HAV) are acute hepatitis that infects mainly children. They are feco-orally transmitted disease. Objectives: detection of the prevalence of HAV and HEV in children with acute hepatitis and their correlation with the immune response. Methodology: 151 hepatic children from Children's Hospital in Assiut were enrolled in our study. Anti-HEV IgM, anti-HAV IgM, liver function tests and the immune response using Flow Cytometry measuring $CD4^+CD25^+Foxp3$ and intracellular IL-10 were measured. Results: in anti HAV-IgM patients, 66.36 % were males and 33.64 % were females, 42.72 % were urban and 57.28 % were rural, while in HEV-IgM positive patients 57.14 % were males and 42.86 % were females. 42.86 % were urban and 57.14 % were rural, the level of $CD4^+CD25^+Foxp3$ was statistically significant (P=0.027), and IL-10 was statistically significant (P=0.007). Conclusion: there was a significant correlation between the presence of the viruses and the immune response.

INTRODUCTION

In our country, communicable diseases are major health problem, and the hepatitis viruses are usually endemic[1] .Hepatitis E is a non enveloped positive-sense ss RNA virus with an icosahedral capsid, classified as a species in the unclassified family Hepeviridae that can infect a wide range of mammalians. Related species of virus, that have not yet been shown to cause human disease, have been found in birds and bat[2].

Hepatitis A (HAV) and hepatitis E (HEV) viruses are self limiting, feco-orally disease transmitted through consuming food or water contaminated by feces as well as closely related to the bad sanitary hygiene; whereas hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) are transmitted through parenteral route and may progress chronic hepatitis [3].

To make diagnosis of hepatitis, several tests should be made to ensure being infected, first of all liver enzymes tests as aspartate transaminase (AST) and alanine transaminase (ALT). Elevated liver enzymes indicate presence of infection, stress, or damage of the liver. Also other blood tests used for diagnosis are ELISA for measuring IgMs for each hepatitis virus and flow cytometry for measuring cytokines produced from the immune response[4].

Treatment may include antiviral medications if it is a virus. In some cases the type of the virus infection needs only healthy diet and rest. In more severe cases admission to hospital is required to treat the disease and prevent the spread of the virus. Providing proper sewage disposal, maintaining good personal hygiene and safe clean drinking water are imperative to control HEV outbreaks[5]

The aim of the present study is to throw light on the prevalence of HAV and HEV in infected children with hepatitis in Assiut governorate, to correlate the immune response with the positivity of the viral infection.

METHODOLOGY

Patients:

Our study was conducted from May 2017 until July 2019. After informed written consent from parents/ guardians. A total number of 151 consecutive children with acute hepatitis between 2-13 years of age attending the Children's Hospital of Assiut University, Assiut, Egypt, were included in the study. Full history was taken and clinical examination was done for each patient at the time of admission to exclude any signs or symptoms of infection present. Basic demographic informations and clinical data were recorded including age, sex and social condition. Approval of the ethics committee, Faculty of Medicine, Assiut University was obtained.

Acute hepatitis in children were diagnosed based on clinical data (Jaundice less than three months of age; right hypochondrial pain, pruritus, anorexia, vomiting,

,tender hepatomegaly with or without ascites, clay stool, , low grade fever and without any stigmata of chronic liver disease); biochemical (elevation of serum bilirubin and ALT).

Data recorded for all study participants included a complete medical history, possible routes of infection and risks of hepatitis exposure in the previous 6 months, liver function tests, clinical examination, and screening for hepatotropic viruses.

Types of samples and Methods of collection:

Venous blood samples (5 cc) were collected aseptically from suspected patients on 3 tubes. A total of 151 blood samples were collected, routine blood screening for alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin and direct bilirubin levels were done. Plasma samples were collected in EDITA tubes for anti HAV-IgM and anti HEV-IgM detection by ELISA kit according to the manufacturer instructions and Heparin tubes for cytokines detection by flow cytometry.

Flow Cytometry analysis of Treg surface and intracellular markers:

It was done using Heparinized tubes to measure the quantities of cytokines as CD4, CD25, Foxp3, Phenotypic analysis of peripheral blood mononuclear cells (PBMCs) in whole blood samples was performed on a fluorescence-activated cell sorter using a set of fluorochrome - labelled monoclonal antibodies against T-reg surface and intracellular markers[11].

Induction and detection of intracellular cytokine (IL-10) by flow cytometry:

three hundreds µl blood sample were cultured in 300 µl RPMI-1640 medium (1:1) in 12×7 mm fluorescence activated cell sorting tube and incubated with 3 µl Phorbol-Myristate Acetate (PMA) and 1 µl Ionomycin as a positive polyclonal non-specific stimulus, for a period of 24 hours at 37 and in 5% CO_2 incubator, 3 µl Brefeldin A was added simultaneously, to block cytokine secretion at the Golgi, allowing for optimal detection of the molecules.

RESULTS

A total of 151 children suffering from acute hepatitis were participated in the research, and 151 blood samples were taken. 110 (72.85 %) of them were diagnosed as HAV-IgM positive (group 1), 14 (9.27 %) were diagnosed as HEV-IgM positive (group 2) and 27

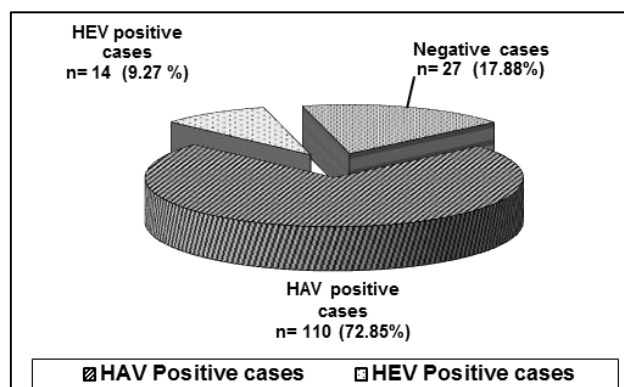(17.88 %) were negative for both HAV and HEV (group 3).

Fig. 1: Presentation of the studied sample into:
Group 1: children with HAV-IgM positive
Group 2: children with HEV-IgM positive
Group 3: children with negative both HAV and HEV IgM

Age in years among group 1 and group 2

In group 1, (mean +SD: 6.21±2.80), while in group 2 (mean + SD: 7.22±2.92), with No significant statistical difference between the two groups.

Sex distribution among group 1 and group 2

In group 1, 66.36 % of children were males and 33.64 % were females, while in group 2, 57.14 % of children were males and 42.86 % were females with no significant statistical difference between the two groups.

Residence distribution of cases

In group 1, 42.72 % of children were urban and 57.28 % were rural, while in group 2, 42.86 % of children were urban and 57.14 % were rural with no significant statistical difference between them.

Liver function test in group 1 and group 2

In group 1, the liver function tests were total bilirubin (mean +SD: 11.308±6.175), ALT (mean +SD: 391.957±207.322) and AST (mean +SD: 552.923±254.839) while in group 2, total bilirubin (mean + SD: 10.870±7.467), ALT (mean +SD: 244.0±122.285) and AST (mean +SD: 504.885±295.074) with No significant statistical difference between the two groups.

In group 1, Direct bilirubin (mean +SD: 5.869±4.175), while in group 2, Direct bilirubin (mean +SD: 6.870±4.352) with significant statistical difference between the two groups. The results are shown in table 1.

Table 1: Liver function tests in group 1 and group 2 patients

		Range	Mean	±SD	T. test	P. value
Group 1	T.Bili	1.20 - 28.26	11.308	6.175	T. bilir (2.299)	0.104
	D.Bili	0.32 - 17.50	5.869	4.175		
	ALT	103.3 - 970.7	391.957	207.322	D. bilir (6.184)	**0.003***
	AST	123.7 - 988.7	552.923	254.839		
Group 2	T.Bili	1.38 - 28.80	10.870	7.467	ALT (2.067)	0.130
	D.Bili	0.43 - 14.5	6.870	4.353		
	ALT	166.5 - 536.9	344.00	122.285	AST (2.799)	0.064
	AST	142.7 - 916.0	504.885	295.074		

CD4$^+$CD25$^+$Foxp3 cells and IL-10 in group 1 and group 2 by flow cytometry

In group 1, CD4$^+$CD25$^+$Foxp3 cells (mean ± SD: 95.215±2.324), while in group 2, (mean ± SD: 97.533±0.776), with significant statistical difference between the two groups.

In group 1, Intracellular IL-10 (mean ±SD: 65.631±24.141), while in group 2, (mean ± SD: 95.333±0.840), with high significant statistical difference between the two groups. (Table 2 and figure 2)

Table 2: CD4$^+$CD25$^+$Foxp3 cells and IL-10 between group 1 and group 2

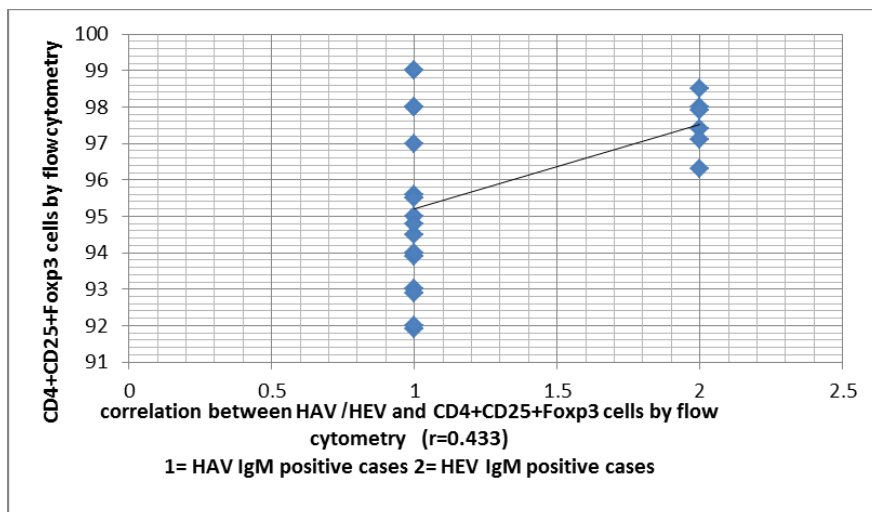|		Range	Mean	±SD	T. test	P. value
Group 1	**CD4**+**CD25**+**Foxp3** cells	91.9-99.0	95.215	2.324	CD25 Positive cells (5.619)	**0.027***
	Intracellular IL-10	30.0-94.7	65.631	24.141		
Group 2	**CD4**+**CD25**+**Foxp3** cells	96.3-98.5	97.533	0.776	IL-10 (8.816)	**0.007***
	Intracellular IL-10	94.3-96.4	95.333	0.840		

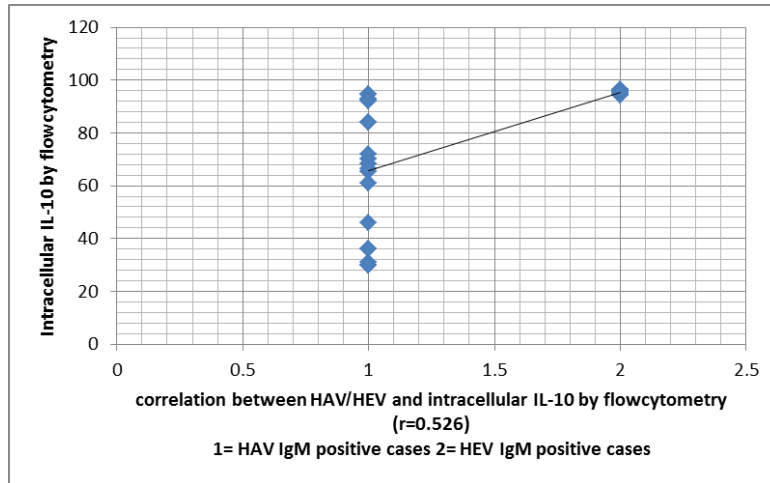**Fig. 2:** Positive correlation between HAV/HEV and CD4$^+$CD25$^+$Foxp3 cells

Fig. 3: Positive correlation between HAV/HEV and intracellular IL-10
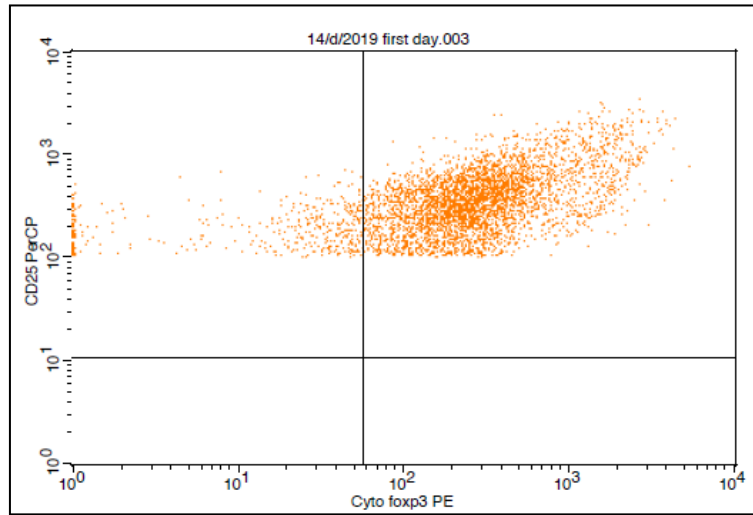

Fig. 4: Percentage of CD4$^+$CD25$^+$Foxp3 in cells in first day
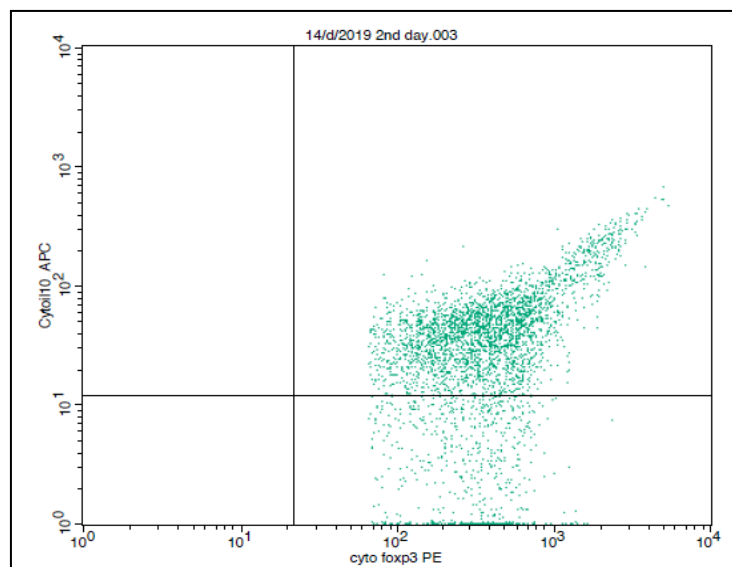

Fig. 5: Percentage of IL-10 in infected cells in the second day

DISCUSSION

In the current study, age classification of studied children with acute HAV showed that infants and young children represented the major proportion of patients 72.85 % which was similar to the report of Fouad *et al* (72.3%)[6].

The percentage of males in our study who had HAV were 66.36% which is higher than that obtained in another study (56.5%)[6], and the percentage of females who had HAV were 43.46% which is higher than that obtained in our study 33.64%.

The seroprevalence of HEV in the general population ranges from 2.3%-37.5% and is higher in males than in females. Khalil study[7] showed that the prevalence of HEV was 9.27 % and is higher in males than females (57.14%).

In some developing countries, (HEV) infection is endemic, and outbreaks occurring in poor water sanitation areas. It is rare in developed countries, although increasing numbers of autochthonous cases have been reported in recent years. many studies of the prevalence of seropositive HEV infection have been performed in developing countries in Mexico and Asia , with widely various results, ranging from a very low prevalence of 0.7 to 2.6% in children and young people aged 0 to 20 years to more than 67.7% in rural areas in Egypt[8]. In our study the prevalence in children suffering from HEV was 57.14% and 57.28 % for children with HAV in rural.

Alanine amino-transferase (ALT) level was significantly higher in viral hepatitis patients. The level being 258.2 ± 91.73 as compared to normal control (11 ± 3.42). Aspartate amino-transferase (AST) levels were significantly raised in viral hepatitis patients. The levels being 157.80 ± 67.8 as compared to normal control (13 ± 3.54). The aminotransferases (trans-aminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases such as hepatitis. The pattern of elevation of the amino-transferase can help in diagnosis. In many acute hepatocellular disorders, the ALT level is $\geq$ the AST level, while the AST\ ALT ratios = 1 for normal and was 0.65(<1) for viral hepatitis [9]

In our study the levels of ALT for HAV infected cases were 391.957 ± 207.322 which indicate liver hepatitis, and the level of AST was 552.923 ± 254.839 indicating hepatitis as compared to the normal levels of them. Also the levels in HEV were 344.00 ± 122.285 and 504.885 ± 295.074, respectively.

Data found in our study had a significant correlation between circulating Treg cells with Anti-HEV IgM and Anti-HAV IgM patients, suggesting that the up regulation in Treg cells might be associated with an increase in HEV IgM. CD4+CD25+Foxp3 cells 95.215 ± 2.324 and IL-10 65.631 ± 24.141 with HAV infection while in HEV infection 97.533 ± 0.776 and 95.333 ± 0.840, respectively.

Cytokines are known to affect the T_{regs} cell number and activity negatively/positively in pathological conditions. Several studies showed that, there is increased $CD4^+CD25^+FoxP3^+$ T cells which inhibit IFN-γ expression. It is known that microbial infections causing activation of $CD4^+CD25^+$ T cells, leading to an overall increase of anti-inflammatory cytokine IL-10. [10]

CONCLUSION

Hepatitis A virus is the major cause of acute viral hepatitis being responsible for more than half of acute hepatitis cases. In our study the percentage of HAV infected children was more than those with HEV. Also males were more than females in both viruses, and infection in rural areas shows higher percentage than infection in urban areas. HEV is an emerging pathogen, causes significant disease in endemic countries and is the leading cause of enterically transmitted viral hepatitis illness globally. The percentage of cytokines as $CD4^+CD25^+Foxp3$ and IL-10 were high in hepatitis E infection than hepatitis A infection and also it was significant.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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