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Prevalence of viral hepatitis (A, B, C, and E) infection and co-infection among hospitalized children in Cairo, Egypt

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Abstract

Viral hepatitis is considered a public health issue facing the entire world. The World Health Organization encouraged all countries to work together to eliminate this fatal infection and achieve the 2030 agenda. The present study aimed to investigate the silent infection of viral hepatitis (A, B, C, and E) among hospitalized children in Cairo, Egypt, to control and avoid chronic infection early on. This cross-sectional study included 184 randomly selected hospitalized children from three different hospitals in Cairo, Egypt. They were children aged between a few months to 15 years to determine viral hepatitis infection and co-infection. Antibodies to hepatitis A virus (HAV IgM), hepatitis E virus (HEV IgM), hepatitis C virus (HCV Ab), and hepatitis B virus surface antigen (HBs Ag) were performed by ELISA. If the ELISA results were positive, the viral load was quantified by real-time polymerase chain reaction (RT-PCR). Other laboratory investigations included alanine aminotransferase, aspartate aminotransferase, albumin, and complete blood count. Only five children (2.71%) had HCV Ab positive with no other viral (A, B, and E) co-infections as determined by ELISA. Also, the RT-PCR detected HCV RNA in these ELISA positive children. The remaining children (179/184) were all negative for all hepatitis viruses' markers (HAV IgM, HEV IgM, HBs Ag, and HCV Ab). In conclusion, this study documented that, Cairo hospitals serving Egyptian children had a low prevalence of viral hepatitis (A, B, C, and E). More research with larger sample sizes from hospitals across Egypt is needed.

Keywords: Children, viral hepatitis, Hepatitis C virus, Hepatitis A virus, Hepatitis E virus, Hepatitis B virus.

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Introduction

Comparable to other major infectious illnesses, including tuberculosis, human immunodeficiency virus infection (HIV), and malaria, viral hepatitis poses a significant threat to public health worldwide.[1] When the World Hepatitis Summit 2022 was recently conducted, the World Health Organization (WHO) and the World Hepatitis Alliance encouraged collaborative effort to eradicate viral hepatitis in light of the 700 unexpected and unknown incidents of hepatitis in young children that were reported in 34 countries in the months

prior to the Summit.[2] Rapidly developing symptoms of severe acute hepatitis have caused many children to acquire liver failure, with a few requiring liver transplants.[2] According to the most recent WHO report on hepatitis, it was estimated that 354 million people are currently living with this lethal infection, and at least one person dying every 30 seconds from viral hepatitis. That is more than 1 million deaths each year, more than that due to HIV and malaria merged.[2,3] In addition, the absolute burden of viral hepatitis is growing over time. Between 1990 and 2013, the global death toll due to viral hepatitis climbed from 0.89 million to 1.45 million. In 2013, viral hepatitis was the seventh biggest cause of death worldwide, while in 1990 it was the tenth.[4]

Recent research indicated that liver disease is the leading cause of death in Egypt, where mathematical models estimated a growth in the number of individuals with liver cirrhosis and liver cancer in the coming years.[4,5] Hepatitis C virus (HCV) and Hepatitis B virus (HBV) infections progress through three distinct phases. New infections (mostly asymptomatic but rarely symptomatic in the form of acute hepatitis) may progress into chronic infections (typically asymptomatic), which may develop into consequences such as cirrhosis and hepatocellular carcinoma (HCC) that result in morbidity and mortality.[6] Based on the WHO, one in three people worldwide is infected either with HBV or HCV.[7,8]

A WHO report published in April 2017, estimated that in 2015, 1.34 million people died from viral hepatitis.[9] In addition, it was reported that 275 million people worldwide had chronic HBV infection and 71 million had chronic HCV infection. The report centered on hepatitis B and C, which accounted for 96% of all hepatitis-related deaths.[9] According to a study in 2018, 2 billion people were infected with HBV, 185 million people infected with HCV, and 20 million people infected with hepatitis E virus (HEV).[10] HEV Genotypes 1 and 4 represent a public health problem, as they were accountable for around 56,600 death and acute liver damage in about 3.3 million individuals. And approximately 20% of mortality among HEV-infected pregnant women were observed in the third trimester.[11]

Indeed, viral hepatitis constitutes a major challenge to public health as it was considered the main reason of death globally. Moreover, the harsh consequences it causes worldwide include liver disease and liver cancer.[12] Several studies agreed that children exhibit silent infection of viral hepatitis, in addition, children who acquired the infection in early stage especially under the age of 5 usually have a great chance to develop chronic infection and its consequences from cirrhosis and HCC.[13-15] Moreover, in areas of high endemicity, more than 90% of children usually get infected with hepatitis A virus (HAV) by the age of 10. However, few develop complications.[16] HBV and HCV infection in children varies from that of adult patients in terms of route of transmission, rate of clearance, advancement of fibrosis, and duration of chronic infection when acquired at birth.[17] Based on previous studies, vertical transmission of HCV from mother to her offspring is the leading cause of children infection worldwide.[10,16] In addition, horizontal transmission, particularly among children, continues to be a major cause of HBV and HCV infection in developing countries including Egypt. Both are blood-borne viruses transmitted through blood transfusions and the use of contaminated syringes and medical tools.[18]Acute HCV signs are uncommon in children, but might appear as fever, fatigue, and muscle pain. And about 20%–25% of acute HCV infections could cure completely on their own, typically by the age of 4 years. In addition, children who do not clear HCV infection on their own typically acquire chronic asymptomatic infection.[16]

Prevention and management techniques for viral hepatitis, such as raising public awareness through education, vaccination, blood donation safety strategies, early recognition of the infection, and efficient medical assistance, could be the key requirements to eradicate viral hepatitis infection.[19] The purpose of this study was to evaluate the silent infection of viral hepatitis (A, B, C, and E) among hospitalized children in Cairo, Egypt, in order to control and avoid chronic infection at an early stage.

Subjects and Methods

Study design and population

This cross-section study, was conducted from March to August 2019, included 184 children, selected using simple random sampling from hospitalized pediatric patients in three different hospitals (El Demerdash hospital, Sayed Galal hospital, and El Hussein University hospital) in Cairo, Egypt. The ages of the study children ranged from a few months (two months) to 15 years. Their blood samples were examined to determine the seroprevalence of viral hepatitis (A, B, C, and E) infection and co-infection.

Samples and data collections

Approximately 2-3 ml of whole blood was collected from each study patient, specimens were left till coagulated at room temperature then centrifuged for 15 minutes at 3000 rpm (Product number: 0068264, Fixed angle rotor, Hettich EBA 20, Germany). The serum was collected and divided into two or three aliquots (0.75- 1 ml/each), then stored at -20 ˚C until used in the study investigations.

Samples preparations and the enzyme linked immunosorbent assay (ELISA) serology testing were performed at the National research Centre. Commercially available ELISA kits provided by Prechek Bio, Inc, USA were used. These included HAV IgM CA. no: HAV IgM (96T), HEV IgM CA. no: HEV IgM (96T), HBs Ag CA. no: HBV911(96T), and HCV Ab CA. no: HCV921 (96T). The assay was performed according to manufacturer's instructions. The final optical density was determined by using an ELISA reader (Serial Number: 4300-2017, CHROMATE, Model: 4300, Awareness Technology, Inc. Palm city, FL 34990, USA).

Biochemical analysis

Patient's medical history and data for complete blood count (CBC) were collected from the patient's medical records. Patient's sera were examined for alanine aminotransferase (ALT), aspartate aminotransferase (AST) by using commercial biochemical kits (ALT/GPT, CODE:11832, AST/GOT CODE:11830, BiosystemS. A. Costa Brava 30,08030 Barcelona Spain), according to manufacturer's instructions. Albumin (ALB) was measured by commercial biochemical kits (Albumin Liquicolor REF:10560, Human Geselischaftfur Biochemica and Diagnostica GmbH, Germany), according to manufacturer's instructions.

Molecular analysis

If the ELISA results were positive, the viral load was quantitatively determined by commercial reverse transcription (RT)-polymerase chain reaction (PCR) kits (CA. no. 52904; QIAamp Viral RNA Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions, using a PCR machine (Applied Biosystems 7500 instrument, AB Sciex, Foster City, CA, USA).

Statistical Analysis

The GraphPad Prism version 9.0.2 Software (GraphPad, San Diego, CA) was used for statistical analysis. All numerical results were analyzed for non parametric data chi-square test was used, and for parametric data and normal distribution data unpaired t-test was used. *P*-values <0.05 were considered statistically significant.

Results

Characteristics of studied patients

A total of 184 randomly selected hospitalized children were enrolled in this study from three different hospitals (El Demerdash hospital, Sayed Galal hospital, and El Hussein university hospital) in Cairo, Egypt. The patients aged between few months to 15 years with mean age of 4.8 $\pm$ 2.4. Children were categorized into four age groups (Table 1). There were 104/184 representing (56.52%) males and 80/184 representing (43.48%) females, as presented in Table 1. In addition, the medical history of the patients is summarized in Figure 1.

Table 1. The 184 study children as arranged by age group and gender.

Age Group	Female	Male	Total
	No. (%)	No. (%)	No. (%)
0*-3 Years	41 (42.27)	56 (57.73)	97 (52.72)
4-7 Years	11 (35.48)	20 (64.52)	31 (16.85)
8-11 Years	13 (43.33)	17 (56.67)	30 (16.30)
12-15 Years	15 (57.69)	11 (42.31)	26 (14.13)
Grand Total	80 (43.48)	104 (56.52)	184 (100)

(0* year means from 1 month to 11 months).

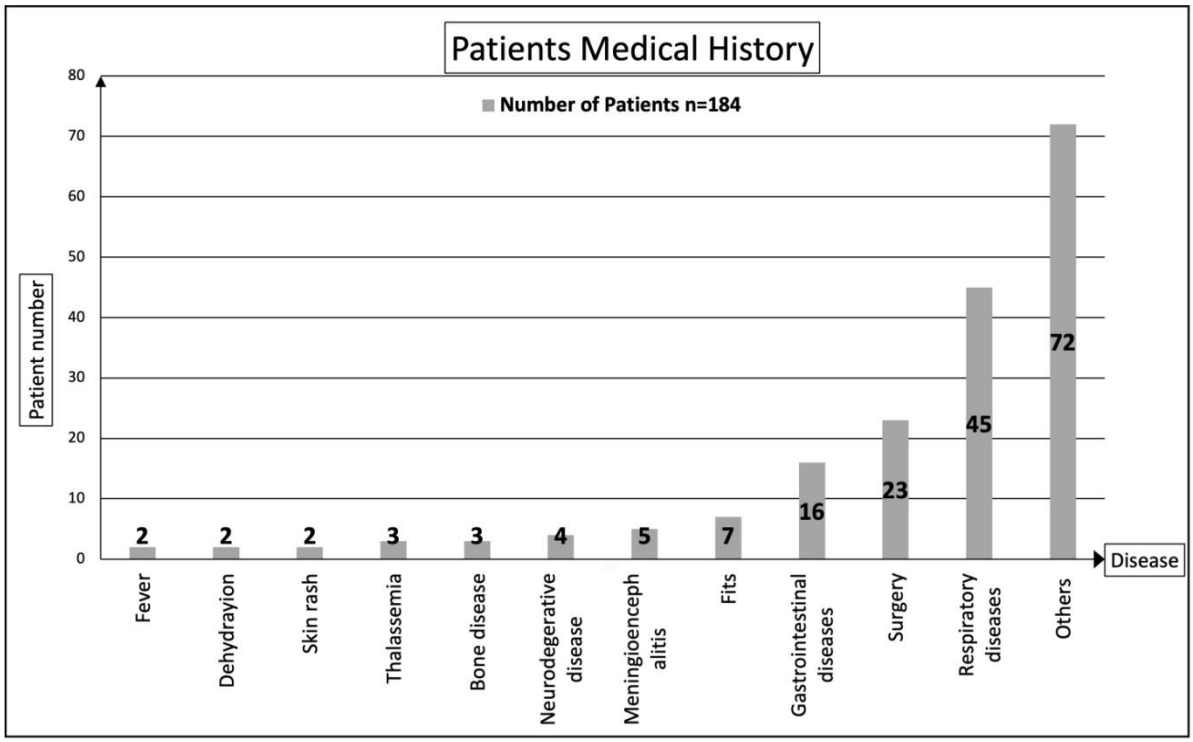

Figure 1. The medical history of the 184 patients showing different categories of diseases.

Detection of viral hepatitis' markers, HAV Ab (IgM), HEV Ab (IgM), and HBsAg, in the study population by using ELISA

None of the 184 studied children was positive for any of the viral hepatitis' markers HAV Ab (IgM), HEV Ab (IgM), and HBsAg.

Detection of (HCV Ab) in the study population by using ELISA

Of the 184 studied children, 5 (2.71%) children were positive for HCV Ab. Among the HCV Ab seropositive children, 2 (40%) were males and 3 (60%) females.

Detection of HCV RNA in the HCV-Ab positive children by using RT-PCR

RT-PCR was used as a more sensitive and confirmatory technique for detection of HCV. HCV RNA was detected in the same five HCV-Ab positive children. However, there was no difference in the positivity between males and females, as presented in Table 2.

Table 2. Viral infection and gender association.

ELISA results	HCV		Total
	Female n (%)	Male n (%)	
Seropositive	3 (60%)	2 (40%)	5
Seronegative	77 (43.02%)	102 (56.98%)	179
p-value	NS		184

Fisher's Exact test was used to determine the association between viral infection and gender.
P > 0.05 is not significant (NS).

Complete blood count

All complete blood count parameters were studied and showed no difference in total leukocyte count (WBCs) between uninfected and infected children (*p*= 0.9991). Platelets counts were slightly higher in the uninfected than the infected children, but this difference did not reach statical significance (*p*=0.5652). However, the difference in the hemoglobin levels between the uninfected and infected children was statically significant (*p*=0.0034), as presented in Table 3.

Liver function tests

Among tested individuals, ALT level was higher in the HCV infected children than uninfected children and showed significant association with viral infection *p*=<0.0001. However, AST showed a slightly higher level in infected children, but the difference did not reach statical significance *p* =0.1176. In contrast, albumin was lower in the infected group than the uninfected group, but the difference did not reach statical significance *p*=0.701, as presented in Table 3.

Table 3. Clinical parameters of the 184 study Children.

Parameter	HCV negative N=179	HCV-positive N=5	*p*-value
CBC			
WBC's (×10^3/mm3)	9.207 ± 1.8	9.998 ± 1.22	NS
Platelets (×10^3/mm3)	322.6 ± 28.9	283.8 ± 53.7	NS
Hemoglobin (g/dL)	10.95 ± 1.7	8.8 ± 1.71	0.0034
Liver Functions			
ALT (U/L)	17.52 ± 2.18	28.2 ± 2.38	<0.0001
AST (U/L)	25.13 ± 1.7	30.2 ± 3.72	NS
Albumin (g/dL)	4.005 ± 0.47	3.84 ± 0.055	NS

Unpaired t- test was used for comparison between means of studied parameters. *P* > 0.05 is not significant (NS).

HCV infection and patient's medical history

Among the five HCV positive individuals, three were thalassemic patients, one child had pneumonia, while the other one suffered from abdominal discomfort. However, none of HCV negative children were thalassemic patients. The relationship between HCV infection and thalassemia is shown in Figure 2, which demonstrated a strong significant relationship between HCV infection and thalassemia (*p*<0.0001). The risk of thalassemia with HCV seropositivity (OR: 65.63) is presented in Table 4. In addition, correlation between HCV infection and the four patient's age groups revealed on, no significant correlation was detected between children's age and HCV seropositivity, as presented in figure (3).

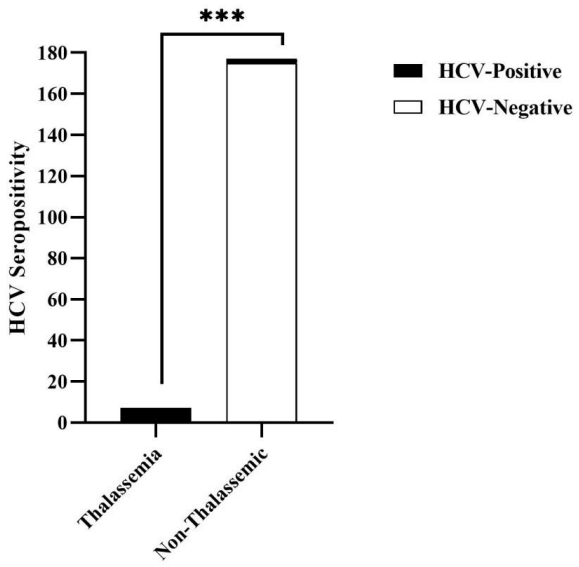

Figure 2. Relationship between anti-HCV antibody positive subjects and thalassemia. There was a significant relation (p <0.0001).

Table 4. Simple logistic regression analysis of HCV seropositivity versus thalassemia.

	HCV Seropositive	Thalassemia	X at 50%
Best-fit values	-3.801	4.207	0.9036
Standard error	0.5056	1.044	0.1964
95% CI (profile likelihood)	-4.978 to -2.943	2.194 to 6.462	0.6019 to 1.6
Odds Ratios	0.02235	67.13	
95% CI (profile likelihood) for odds ratios	0.0069 to 0.05268	8.972 to 640.4	
Significance of Slope			
|Z|	4.031		
p-value	<0.0001		
Likelihood Ratio Test			
Log-likelihood ratio (G2)	14.58		
p-value	0.0001		
Tjur's R2	0.2414		
Cox-Snell's R2	0.0746		
Model deviance, (G2)	45.23		
Fisher's Exact Test			
p-value	0.0003		
Relative Risk	37.93% (95% CI: 8.054 to 162.7)		
Odds ratio	65.63 (95% CI: 9.937 to 400.3)		

$P \leq 0.05$ is significant.

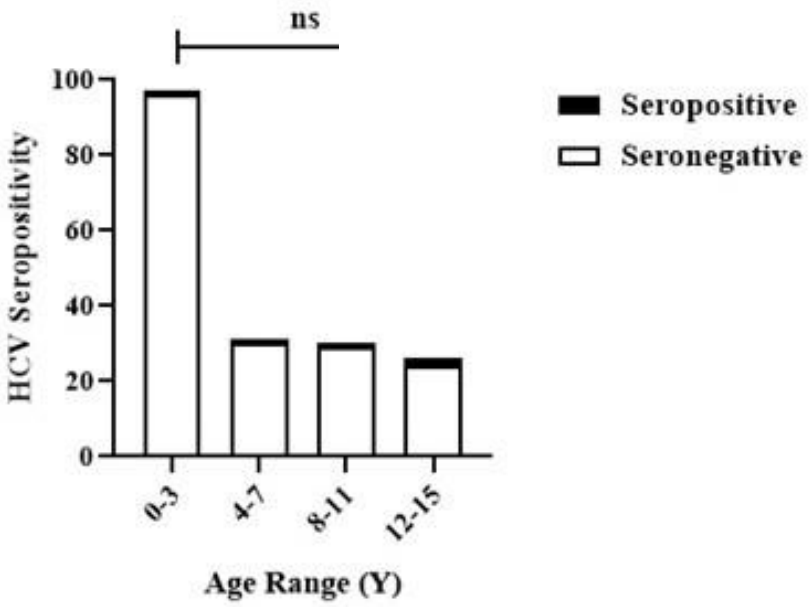

Figure 3. Relation between HCV seropositivity and patient's age groups.
There was no relation between anti-HCV antibodies and the age of study children.

Discussion

Assessment of the prevalence of hepatitis A, B, C, and E in children in Egypt can provide important data for research of the epidemiology, risk factors, prevention of transmission, and early detection of these infections. This can help in developing more effective prevention, early detection, and treatment strategies. The present study aimed to investigate the silent infection and co-infection of viral hepatitis (A, B, C, and E) among some of hospitalized children in Cairo, Egypt. Such data would help in controlling and avoiding chronic infection and its consequences in the early stage.

Based on two studies conducted by Hasan et al., 2016, and Zaki et al., 2020, the incidence of HEV among Egyptian children was slightly high, about 26% were HEV IgM positive in both of the two studies, which disagreed with our results.[20,21] As in our study, none of the 184 study children was positive for HEV IgM indicating very low incidence of HEV infection among Egyptian children. While according to Abdelmawla et al., 2019, only 4 out of 140 Egyptian thalassemic children's patients were positive for HEV IgM, which matched the present study's findings, and indicated very low incidence of HEV infection among Egyptian children.[22]

In addition, Rasheed et al., 2022, found that 75 % (150/200) children were HAV-IgM positive and 25% (n=50) were HEV-IgM positive. The children mean age was 7.3± 3.0 years, and the majority belonged to the 6–10 years age group, which disagreed with our results and indicating high incidence of HAV among children.[23]

Globally, data of many studies agreed with our study results, which reported that HBV prevalence among children become very low due to vaccination. A study in the Philippines reported that only 15 (0.8%) children out of more than 2000 children were HBsAg positive.[24] In addition, a Chinese study reported that prevalence of HBV was <0.5 % for children born after 2011.[25] Also, a study in Ukraine reported that 8 (0.2%) children out of more than 4000 tested children were positive for HBV.[26] Moreover, according to a study by Jing et al., 2020, the worldwide prevalence of HBV among children declined to about 0.1%.[27] In our study, there was no single HBsAg positive child out of

the 184 studied children, agreed with other studies results and indicated very low incidence of HBV infection among children.[27]

Our results agreed with those of Hasan et al., 2016, reported that only one out of 123 children was HCV positive, and there was no association between infection with viral hepatitis and age or sex.[20] Moreover, a study conducted in Zambia by Phiri, 2015, evaluated the prevalence of viral hepatitis B and C among HIV positive children and found that only one child (0.5%) out of 187 studied children was positive for HCVAb.[28] In addition, according to a study by Seerat et al., 2020, conducted on 3500 children only 66 (1.88%) were positive for HCV.[18]

Findings of a study by Rasheed et al., 2022, agreed with our results and noted that only ALT was significantly higher in viral hepatitis positive patients (p = 0.04), while other biochemical parameters did not exhibit any significant difference (p>0.05).[23] In addition, findings of two studies by Ahmadi et al., 2018 and Mohamed et al., 2019 agreed with our study data and reported that elevated serum ALT and AST levels, and decreased PLT counts were all linked with anti-HCV seropositivity.[29,30]

Moreover, findings of a study conducted in Russia, included 301 children, totally agreed with our study results, observed high ALT and AST levels, but low levels of albumin and platelets counts among HCV infected children.[31] In contrast to our findings, Abdullah, 2018, reported that the hemoglobin levels were not different between seropositive and seronegative subjects.[32] In addition, a study in Pakistan reported that 26 out of 72 thalassemic children aged between 3 and 15 years were HCV positive, and concluded that blood transfusion, ear piercing, and dental procedures were the major reasons for acquiring HCV infection.[33]

In conclusion, our study findings showed that there was a very low incidence of HCV with no A, B, or E viral hepatitis in three hospitals serving children in Cairo Egypt. However, additional research studies with larger study sample sizes from more hospitals in various parts of Egypt are necessary.

Author Contributions

AT, SS; designed the research plan, organized the study and participated in the main role of editing and revising the manuscript. RE and EM; carried out all laboratory tests and coordinated the data analysis, collected and supervised all clinical issues of patients. EM and RE; participated in writing of the manuscript. RE had a major contribution role in following-up all steps of the study. All authors read and approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Medical Research Ethics Committee, National Research Centre (approval #: 314 dated 2019).

Informed consent

A signed consent was taken from the children's guardians before being enrolled in the study.

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