

# Prevalence of hepatitis C virus infection and response to hepatitis B vaccination among Egyptian school children

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## ABSTRACT

**Background:** A prevalence of hepatitis C virus (HCV) infection is relatively low in children as it was 3% and 9% in the upper and lower Egypt areas, respectively. Most chronic hepatitis C patients are asymptomatic and unaware of their disease before diagnosis. Similarly, hepatitis B virus (HBV) is a major cause of chronic liver disease, particularly cirrhosis and hepatocellular carcinoma, though HBV incidence and infection has been markedly reduced after mass vaccination programs. **Objectives:** To identify the prevalence and risk factors of asymptomatic HCV infection and evaluate response of hepatitis B vaccination among Egyptian children. **Materials and Methods:** Six hundred children (6-17 years) were screened for HCV antibodies (HCV Ab) and hepatitis B surface antibody concentration (HBsAb) was quantitated by enzyme-linked immunosorbent assay. HCV Ab-seropositive was tested for HCV ribonucleic acid by real-time-polymerase chain reaction, complete blood count, and liver function tests. **Results:** 4.7% were diagnosed as HCV Ab-seropositive and 58.5 % as HBsAb-seroprotective (HBsAb  $\geq$ 10 mIU/ml). History of exposure to blood transfusion, frequent intravenous injection, history of prior hospitalization and blood exposure were significantly more likely to be among HCV seropositive. Blood transfusion was considered the most predictable risk factor for HCV infection. There was a significant decrease in HBsAb concentration with increasing age. **Conclusion:** Booster dose of hepatitis B vaccine should be considered to enhance immune protection of the vaccine especially in our endemic area.

**KEY WORDS:** Seropositive; Seroprotective, Hepatitis C Virus; Hepatitis B Surface Antibody Concentration; Immunity

## INTRODUCTION


Infection with hepatitis C virus (HCV) is a major global health care-problem. The World Health Organization estimates that up to 3% of the world's population has been infected with the virus.<sup>[1]</sup> The infection rate ranges from 0.1% in Canada

to 18.1% in Egypt. Indeed, HCV infection is now the leading reason for liver transplantation worldwide.<sup>[2]</sup>

The prevalence of HCV infection is relatively low in children as it was 3% and 9% in the upper and lower Egypt areas, respectively.<sup>[3]</sup>

Most chronic hepatitis C patients are asymptomatic and unaware of their disease before diagnosis. However, they report a consistent and significant reduction in quality of life, even in the absence of severe liver disease.<sup>[4]</sup>

Although blood transfusion, circumcision, vertical transmission, and living in a house with an infected family member are established risk factors for HCV transmission,

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approximately 70% of acquired infections are due to unidentified risk factors.<sup>[5]</sup>

Similarly, hepatitis B virus (HBV) is a major cause of chronic liver disease, particularly cirrhosis and hepatocellular carcinoma, though HBV incidence and infection has been markedly reduced after mass vaccination programs.<sup>[6]</sup>

In 1992, Egypt started a program of universal immunization in infancy. The schedule, which adopted by Egyptian Ministry of Health, was three doses of yeast - recombinant hepatitis B vaccine and administered to all infants at 2, 4, and 6 months of age to coincide with other compulsory vaccines.<sup>[7]</sup>

Seroprotection against HBV infection was defined as having an anti-HBs level of  $\geq 10$  mIU/ml after having received a complete immunization schedules.<sup>[8]</sup>

The term booster dose refers to a vaccination given sometimes after a primary vaccination series and with the aim of providing rapid protective immunity against a significant breakthrough infection.<sup>[9]</sup> Few data are available concerning the long-term immunogenicity of the pediatric doses of hepatitis B vaccines.

### Objectives of the study

1. To estimate the prevalence and some risk factors of HCV infection in school aged Egyptian children.
2. To evaluate the immune response to hepatitis B vaccination in a sample of vaccinated school aged Egyptian children.

## MATERIALS AND METHODS

### Study type

Cross-sectional analytic study.

### Sample size

600 school aged children ( $N = (Z^2 * P * Q) / (E^2)$ ) [N= Minimal sample size, Z=1.96, P= No of affected population, Q=1-p and E error=.05%](10).

### Sampling method

Multistage random sample was selecting two governorates by simple random then one school from each governorate. After meeting, parents in the parents' day of the school and obtaining consent from those who accepted to participate after their orientation about the objectives of the study (with 90% response rate). Children were selected systematically (every 4<sup>th</sup> child).

### Study duration

2 years (from September 2013) after obtaining approval from Benha ethical committee.

Inclusion criteria were school children aged 6-17 years, received their three doses of HBV vaccine at 2, 4, and 6 months according to the Expanded Program on Immunization in Egypt. The patients with known hepatic diseases or critically ill were excluded from the study.

All children were subjected to full history taking using a predesigned questionnaire for demographic characteristics, socioeconomic state.<sup>[11]</sup> Potential risk factors of HCV infection (transfusions history of blood or blood products, surgical or dental procedures, frequent injections, sharing needles or syringes, sharing razors and tooth brushes with family members, tattooing, ear piercing, circumcision, previous hospitalization, and family history of hepatitis) and factors affecting the immune response to hepatitis B vaccine (obesity, smoking, and any disease or drugs) were recorded.

Thorough clinical examination (weight, height to calculate the body mass index (BMI), proper examinations of the liver size and the skin).

### Laboratory investigations

About 5 ml venous blood samples were collected under complete aseptic conditions; sera were separated into two aliquots and preserved at -20°C until analysis. HCV antibody (HCV Ab) was detected by enzyme-linked immunosorbent assay (ELISA) using INNOTEST HCVsAb intravenous (IV) Kit, distributed by INNOGENETICS GmbH, Hannover, Germany.<sup>[12]</sup> All serum samples of the HCV antibody positive cases were tested for HCV-ribonucleic acid (RNA) by polymerase chain reaction (PCR) using Thermo Fisher scientific real-time PCR system. Hepatitis B surface antibody (HBsAb) was detected by ELISA using ETI-AB-AUK-3, Diasorin kit, for quantitative determination of HBsAb to determine the immune state.<sup>[13]</sup> Seroprotection against HBV infection was defined as having an anti-HBs level of  $\geq 10$  mIU/ml after having received a complete immunization schedules.<sup>[9]</sup> In cases with inadequate immune response HBsAb <10 mIU/ml, serum samples were tested for HBsAg by ELISA using SURASE B-96 (TMB), Medical Technology promedt consulting GmbH, to detect HBV infection.<sup>[13]</sup> For HBsAg positive cases, PCR was done using Thermo Fisher scientific real-time PCR system to confirm the diagnosis. Complete blood count (CBC) was performed by Sysmex XS-800 I cell counter. Liver function tests including total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase, and albumin were done for positive cases by Biosystem A 15 autoanalyzer. ALT was selected to indicate the degree to which liver injury had

occurred, it was considered abnormal if the level was >2 times greater than the upper limit of the normal level, i.e., >90 U/L, where the normal range is 5–45 U/L.<sup>[14]</sup> Prothrombin time was performed by Helena C-H coagulation analyzer.

### Statistical analysis

The collected data were tabulated and analyzed using the computer programs Statistical package for social science version 16. The collected data were summarized in terms of mean±Standard deviation and range for quantitative data and frequency and proportion for qualitative data. Statistical comparisons between the different study groups were carried out using simple univariate tests including the Chi-square test ( $\chi^2$ ) and the Fisher exact test to compare proportions as appropriate and the Student's t-test (*t*) to examine mean differences between two groups. A *P* < 0.05 was considered statistically significant.

Multiple logistic regression analysis of being a case of HCV was conducted. Factors that were associated with significant variations in the proportions of cases were included in the preliminary models. Then nonsignificant factors were removed relying on a lowered *P*-value and if the odds ratio of the remaining factors did not change. In the final model, the risk of being a case conditioned on social class, blood transfusion, frequent IV injection, prior hospitalization, and exposure to blood was tested.

## RESULTS

The study included 600 children aged from 6 to 17 years (12.07 ± 3.37). Studied children include 54.3% male, 73.7% lived in rural area, and 46.5% belonged to moderate social class which was higher than that of low or high social classes (37.3% and 16.2%, respectively).

Regarding HCV, 4.7% of children were diagnosed as HCV seropositive by ELISA for anti-HCV while 58.5% were HBV seroprotective (HBsAb ≥10 mIU/ml). A high percent of HCV positive cases tested by ELISA (75%) was positive after PCR test (Table 1).

Table 2 shows significant differences between positive and negative children regarding socioeconomic state. The majority of positive cases belonged to low socioeconomic status followed by middle and high socioeconomic status (60.7%, 25.0%, and 14.3%, respectively; *P* < 0.05).

Children who were exposed to risk factors such as blood transfusion, frequent IV injection, history of prior hospitalization, and blood exposure were significantly more likely to be a case of HCV (60.7%, 92.9%, 82.1%, and 28.6%, respectively) than those who were not exposed (5.8%, 39.3, 39.5, and 8.2%, respectively).

No significant differences were demonstrated regarding history of surgical procedure, nonmedical circumcision, ear piercing, shaving in common barber, using common tools like razors or tooth brush, delivery by aid of physician or birth attendant and history of schistosomal infection.

Positive cases significantly presented with symptoms such as diarrhea, abdominal pain, dark urine, easy fatigue, and poor general health (46.4%, 67.9%, 17.9%, 60.7%, and 50%, respectively). Obviously, school absence was among HCV seropositive children (42.9%) (*P* < 0.001).

Table 3 shows increased risk of HCV infection by blood transfusion as the most predictable risk factor (Exp (b)=10.02, 95% CI = 3.69-27.22).

While there was a significant decrease in HBsAb concentration with increasing age (*P* < 0.05), no significant difference with other factors that affect the immune response to HB vaccine (sex, residence, social classes, weight, and immune state) (Table 4).

HBsAg was done for children with negative immune response to hepatitis B vaccine (HBsAb <10 mIU/ml), 5 cases with positive HBsAg were detected with no hepatitis B viremia when confirmed by PCR. No abnormalities regarding CBC and liver function tests among positive HCV cases were detected.

**Table 1:** Sociodemographic distribution of the studied group

Parameter	Value (600)
Age (year) (mean±SD, range)	12.07±3.37,6-17
Sex	
Male	326 (54.3)
Female	274 (45.7)
Residence	
Urban	158 (26.3)
Rural	442 (73.7)
Social class	
High	97 (16.2)
Middle	279 (46.5)
Low	224 (37.3)
HCVAb by ELISA	
Seronegative	572 (95.3)
Seropositive	28 (4.7)
HbsAb titer	
<10 mIU/ml	249 (41.5)
≥10 mIU/ml	351 (58.5)

HBsAb: Hepatitis B surface antibody concentration, HCV: Hepatitis C virus, ELISA: Enzyme-linked immunosorbent assay, SD: Standard deviation

**Table 2:** Differences between children with seronegative and seropositive HCV

Parameter	Seronegative HCV (572)	Seropositive HCV (28)	Test	P	Risk	95-% CI
Age (year) (mean±SD, range)	12.02±3.33,6-17	13.21±4.17,6-17	#1.84	0.067	-	-
Sex						
Male	309 (54.0)	17 (60.7)	^0.482	0.488	0.76	0.35-1.65
Female	263 (46.0)	11 (39.3)				
Residence						
Urban	153 (26.7)	5 (17.9)	^1.09	0.297	1.68	0.627-4.5
Rural	419 (73.3)	23 (82.1)				
Social class						
High	93 (16.3)	4 (14.3)			-	-
Middle	272 (47.5)	7 (25.0)	^7.01	0.031*		
Low	207 (36.2)	17 (60.7)				
Risk factors						
Blood transfusion	33 (5.8)	17 (60.7)	^98.4	0.001**	25.24	10.94-58.23
Frequent IV injection	225 (39.3)	26 (92.9)	^31.4	0.001**	20.05	4.71-85.3
History of surgical procedure	230 (40.2)	13 (46.4)	^0.428	0.513	1.29	0.6-2.76
Prior hospitalization	226 (39.5)	23 (82.1)	^19.98	0.001**	7.04	2.64-18.79
Circumcision by nonmedical personel	81 (20.5)	6 (28.6)	^0.372	0.542	1.55	0.58-4.12
Ear piercing (females=274)	252 (44.1)	11 (39.3)	^0.247	0.619	0.822	0.378-1.79
+ve family history of HCV	100 (17.5)	5 (17.9)	^0.0	1.0	1.03	0.381-2.76
Shaving in community Barber (males=326)	274 (47.9)	16 (57.1)	^0.913	0.339	1.45	0.674-3.12
Using common razors or tooth brush	86 (15.0)	1 (3.6)	^1.98	0.093	0.209	0.028-1.56
Exposure to blood	47 (8.2)	8 (28.6)	^10.95	0.001**	4.47	1.87-10.69
Delivery						
By doctor	383 (67.0)	17 (60.7)	^0.468	0.494	1.31	0.602-2.86
By birth attendant	189 (33.0)	11 (39.3)				
History of schistosomal infection	2 (0.3)	0 (0.0)	^0.0	1.0	0.953	0.936-0.97
Present history						
Diarrhea	120 (21.0)	13 (46.4)	^10.02	0.002**	3.26	1.51-7.05
Abd. pain	193 (33.7)	19 (67.9)	^13.6	0.001**	4.15	1.84-9.34
Dark urine	15 (2.6)	5 (17.9)	^14.79	0.001**	8.07	2.7-24.12
Easy fatigue	102 (17.8)	17 (60.7)	^30.87	0.001**	7.12	3.24-15.66
Past history						
Jaundice	34 (5.9)	7 (25.0)	^12.38	0.001**	5.28	2.1-13.27
Poor general health	47 (8.2)	14 (50.0)	^46.55	0.001**	11.17	5.03-24.83
School absence because of illness	31 (5.4)	12 (42.9)	^50.75	0.001**	13.09	5.7-30.06

^: FET, ^^: Chi-square test, #: Student *t*-test, \*: Significant ( $P<0.05$ ), \*\*: Highly significant ( $P<0.01$ ) CI: Confidence interval, HCV: Hepatitis C virus, IV: Intravenous

## DISCUSSION

Children with chronic HCV infection are usually free of symptoms, frequently with normal or borderline ALT value.<sup>[14]</sup> Hence, population-based serologic studies are needed to estimate the prevalence of infection and to study risk factors associated with HCV infection.

This study revealed that the prevalence of HCV seropositivity was 4.7% while it was 5.8% in a previous study among healthy

Egyptian children selected from 10 schools in Alexandria with HCV viremia in 75%,<sup>[15]</sup> 8% in children aged 2 months-15 years attending pediatric department, Assiut University,<sup>[16]</sup> 1.9% for children aged <9 years in a community based study in a village in Assiut governorate,<sup>[4]</sup> 2.02% among children aged 1-9 years collected from Cairo University pediatric hospital,<sup>[17]</sup> and 3.6% seroprevalence rate in India.<sup>[18]</sup>

This difference may be attributed not only to the difference in the study design but also to the age of the

study population and partially attributed to sensitivities of screening tests.

In our study, the frequency of HCV positivity by PCR among ELISA positive cases was 75% in agreement with other studies.<sup>[15,16]</sup>

The difference in the frequency of PCR positivity among ELISA positive cases may be attributed to the clearance of HCV-RNA while the subjects remains anti-HCV positive<sup>[19]</sup> or false positive ELISA results.<sup>[20]</sup>

Our results observed increased percentage of HCV infection among males (60.7%), rural areas (82.1%), and low socioeconomic class (60.7%) which is in concordance with Eassa et al.,<sup>[21]</sup> who mentioned that males had about 5 times significant higher risk of acquired HCV infection than females.

**Table 3:** Logistic regression detecting risk factors of getting HCV infection

Risk factors	Exp (b)	P	95% CI
Social class			
High	1.0 (reff)	0.49	
Moderate	0.986	0.983	0.275-3.53
Low	0.557	0.256	0.203-1.53
Blood transfusion	10.02	0.001**	3.69-27.22
Frequent IV injection	8.41	0.011*	1.63-43.27
Prior hospitalization	1.08	0.905	0.319-3.63
Exposure to blood	1.46	0.477	0.516-4.12

\*: Significant ( $P < 0.05$ ), \*\*: Highly significant ( $P < 0.01$ ), IV: Intravenous, HCV: Hepatitis C virus

Low socioeconomic class and rural area residence are significant risk factors for HCV infection as illiterates in low social class may not acquire the scientific knowledge to change their attitude and improve their behavior.<sup>[15,22]</sup>

In this study, children who were exposed to risk factors such as blood transfusion, frequent intravenous injection, history of prior hospitalization, and exposure to blood were significantly borne to be a case of HCV while no significant differences were demonstrated regarding surgical procedure, nonmedical circumcision, ear piercing, shaving in common barber, using common tools such as razors and tooth brush, delivery by aid of physician or birth attendant and history of schistosomal infection. This is in agreement with Medhat et al.<sup>[3]</sup> who revealed a significant association of HCV infection with history of blood transfusion, surgery and suture but nonsignificant association with delivery, endoscopy or dental extraction. Among those 20 years old or younger, no risk factors were clearly associated with anti-HCV positivity; however, circumcision by informal health-care providers was marginally associated with anti-HCV antibodies.<sup>[5]</sup>

Regarding blood transfusion, in Egypt, blood transfusion remains an important risk factor for HCV transmission. (the high anti-HCV positivity in blood donors;<sup>[23]</sup> technical and financial factors that limit anti-HCV screening;<sup>[3]</sup> inappropriate storage of ELISA kits; use of rapid anti-HCV antibody detection kits which have low sensitivity and specificity and use of ELISA rather than PCR in screening of blood before transfusion, which misses the window period in which anti-HCV positivity cannot be detected.<sup>[24]</sup> Furthermore, unsafe and unnecessary frequent

**Table 4:** Differences between children with negative HBsAb titer and positive HBsAb titer

Parameter	Negative HBsAb titer (<10=249)	Positive HBsAb titer (≥10=351)	Z test	P	Risk	95% CI
Age (year) (mean±SD, range)	12.47±3.37,6-17	11.79±3.36,6-17	#2.42	0.016*	-	-
Sex						
Male	140 (56.2)	186 (53.0)	^^0.614	0.433	0.878	0.633-1.22
Female	109 (43.8)	165 (47.0)				
Residence						
Urban	70 (28.1)	88 (25.1)	^^0.695	0.405	0.856	0.593-1.24
Rural	179 (71.9)	263 (74.9)				
Social class						
High	38 (15.3)	59 (16.8)			-	-
Middle	114 (45.8)	165 (47.0)	^^0.563	0.755		
Low	97 (39.0)	127 (36.2)				
Other factors affecting the immune response to HBV vaccine						
Obesity	17 (6.8)	23 (6.6)	£0.96	0.169	1.05	0.546-2.0
Decreased immunity	17 (6.9)	16 (4.6)	£0.174	0.43	1.54	0.763-3.11

£: Z test, ^^: Chi-square test, #: Student t-test, \*: Significant ( $P < 0.05$ ), CI: Confidence interval, HBsAb: Hepatitis B surface antibody concentration, HBV: Hepatitis B virus, SD: Standard deviation

injections which were prescribed and administrated by untrained medical providers and reuse of syringes may occur, which is likely to contribute to bloodborne pathogen transmission.<sup>[16]</sup>

Nosocomial transmission of HCV through patient in a hematological ward, during colonoscopy in a gastrointestinal diseases unit and in a hemodialysis unit.<sup>[25]</sup>

Our results showed that symptoms such as diarrhea, abdominal pain, history of fatigue, poor general health, and school absence because of illness were significantly more common among HCV positive cases (no hepatomegaly or splenomegaly). This is in agreement with El Raziky *et al.*,<sup>[17]</sup> but Bortolotti *et al.*,<sup>[26]</sup> showed that HCV in children is usually asymptomatic.

Although our results revealed no abnormalities in CBC and liver function tests, some investigators reported that most chronically infected children with HCV have mild elevations in ALT levels.<sup>[27]</sup> HCV infected adults are more likely to have low neutrophil and platelet counts but no association was detected between anti-HCV status and anemia or peripheral blood cell components.<sup>[28]</sup>

HBV infection is preventable with safe and effective vaccine. In 1992, Egypt started a program of universal immunization in infancy. The duration of protection after HB vaccination of infants is unknown as anti-HBs antibodies disappeared by 5 years of age in most children who were vaccinated with HB vaccine from birth. Although most children showed immunologic memory, one-third failed to demonstrate an anamnestic response to a booster dose.<sup>[29]</sup>

About half (58.5%) of the studied children were seroprotective (HBsAb  $\geq 10$  mIU/ml with a significant decrease in HBsAb concentration with increasing age.

Studies that follow children administrated with HB vaccines during infancy and early childhood in countries with a high endemicity of chronic HBV infection have shown that 47.9% of Iranian children had protective level of HBsAb  $\geq 10$  mIU/ml at 10 years after primary vaccination<sup>[30]</sup> and more than 50% of participants had measurable anti-HBs levels of at least 10 m IU/ml.<sup>[31]</sup> HBsAb concentration dropped rapidly among all participants, they reported that 5 years after vaccination, 6% only of all children had HBsAb  $\geq 10$  mIU/ml and 3% of them at 10 years retained the protective titer.<sup>[32,33]</sup>

Our study revealed that sex, weight, and immune state had no significant effect on the immune response to HB vaccine which disagreed Tsega *et al.*,<sup>[34]</sup> who found that many host and immunization factors affect the immune response and duration of immunity; host factors include age, weight, immune competence of the host, smoking habits, BMI, genetics, and socioeconomic state. Males retain HBsAb titers

of higher values than females<sup>[32]</sup> but Fang *et al.*,<sup>[35]</sup> found the reverse. El-Sawy and Mohamed<sup>[36]</sup> showed that there was no statistical significance between boys and girls who had similar mean Anti-HBs levels in all age groups.

Antibody level becomes less in persons undergoing more stress<sup>[37]</sup> and smokers.<sup>[38]</sup> In contrast with our results Keating and Nobile,<sup>[39]</sup> reported that greater BMI was associated with reduced immune response. However, we did not find such differences, may be due to the same level of stress in both male and female school aged children and also none of our participants was smoker and small percent of obese individuals were in our study group.<sup>[40]</sup>

## CONCLUSIONS

Asymptomatic HCV seropositivity is detectable in 4.7% among Egyptian children attending Benha University hospital. History of blood transfusion, frequent intravenous injections, prior hospitalization, and blood exposure were important risk factors for HCV infection in Egypt. Seroprotection rate against hepatitis B in school aged children previously vaccinated at birth was 58.5%, booster dose of hepatitis B vaccine should be considered to enhance immune protection of the vaccine especially in our endemic area. Avoidance of unnecessary blood transfusion and intravenous injections, and implementation of strict infection control measures are highly recommended to reduce the frequency of HCV infection.

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