

Hepatitis C virus infection among paediatric patients attending University of Assiut Hospital, Egypt

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العدوى بفيروس التهاب الكبد سي بين الأطفال الذين يراجعون المستشفى الجامعي في أسيوط، مصر قطب خليل، حكمة فرغلي، خالد حسانين، علاء عبد السيد، فاروق حسانين

الخلاصة: قِيم عدد قليل من الدراسات وبائيات العدوى بفيروس التهاب الكبد سي لدى الأطفال في مصر وعوامل الخطر المتعلقة بها. وتشمل هذه الدراسة 465 طفلاً راجعوا المستشفى الجامعي في أسيوط، وأجريت لهم قياسات لمعدلات إيجابية أضداد فيروس التهاب الكبد سي بواسطة اختبار الجيل الثالث من مقايصة الممتز المناعي المرتبط بالإنزيم (إيليزا)، وإيجابية رنا فيروس التهاب الكبد سي بين الحالات الإيجابية باستخدام التفاعل السلسلي للبوليميراز (بي سي آر)، مع تحليل لبعض عوامل الخطر ذات الصلة. وبلغ المعدل الإجمالي لإيجابية رنا فيروس التهاب الكبد سي بين الحالات الإيجابية بمقايصة الممتز المناعي المرتبط بالإنزيم (إيليزا) (وعددتها 121 حالة): 72.2٪، وكان 100٪ من المجموعة الفرعية المصابة بالتهاب الكبد، و70.8٪ ممن لديهم سوابق نقل متكرر للدم، و58.3٪ من غير المصابين بالتهاب الكبد ومن ليس لديهم سوابق نقل دم متكرر. وقد اتضح للباحثين أن عوامل الخطر التي يُعتد بها لإيجابية أضداد فيروس التهاب الكبد سي بواسطة مقايصة الممتز المناعي المرتبط بالإنزيم هي قصة نقل دم، وحقن متكرر، وإدخال إلى المستشفى أو إجراءات جراحية.

ABSTRACT Few studies have evaluated the epidemiology and risk factors of hepatitis C virus (HCV) infection in children in Egypt. This study of 465 children attending Assiut University Hospital measured the rates of anti-HCV positivity by 3rd-generation ELISA test and of HCV-RNA positivity by PCR, with analysis of some relevant risk factors. The rate of HCV-RNA positivity among ELISA-positive cases ($n = 121$) was 72.2% overall: 100% in the subgroup with hepatitis, 70.8% in those with a history of multiple transfusions and 58.3% in those without hepatitis or multiple transfusions. History of blood transfusions, frequent injections, hospitalization or surgical procedures were significant risk factors for anti-HCV positivity by ELISA.

Infection par le virus de l'hépatite C chez les patients pédiatriques consultant à l'hôpital universitaire d'Assiut (Égypte)

RÉSUMÉ Les études ayant évalué l'épidémiologie et les facteurs de risque d'infection par le virus de l'hépatite C (VHC) chez l'enfant en Égypte sont peu nombreuses. Cette étude réalisée sur 465 enfants consultant à l'hôpital universitaire d'Assiut a mesuré le taux de positivité aux anti-VHC au moyen de tests ELISA de troisième génération et de positivité de l'ARN du VHC par le biais de la PCR, avec analyse de certains facteurs de risque importants. Le taux global de positivité de l'ARN du VHC parmi les tests ELISA positifs ($n = 121$) était de 72,2 % : 100 % dans le sous-groupe atteint d'hépatite, 70,8 % chez les sujets ayant des antécédents de transfusions multiples et 58,3 % chez ceux n'ayant ni hépatite ni antécédents de transfusions multiples. Des antécédents de transfusions sanguines, d'injections fréquentes, d'hospitalisations ou de procédures chirurgicales se sont avérés des facteurs de risque importants de positivité aux anti-VHC par ELISA.

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Received: 20/02/08; accepted: 06/05/08

Introduction

Infection with hepatitis C virus (HCV) is a major global health care problem. The World Health Organization (WHO) estimates that up to 3% of the world's population has been infected with the virus [1]. The infection rate ranges from as low as 0.1% in Canada to the extremely high rate of 18.1% in Egypt [2]. Indeed, HCV infection is now the leading reason for liver transplantation worldwide.

Few studies have evaluated the epidemiology and risk factors of HCV infection in children in Egypt. In community-based studies the prevalence of HCV antibodies in Egyptian children was 3% and 9% in the Upper and Lower Egypt areas respectively [3]. Most studies have reported the rate of anti-HCV positivity by enzyme-linked immunosorbent assay (ELISA) rather than by the use of polymerase chain reaction (PCR) methods for detection of positivity. While ELISA is an economic way to screen a large number of patients and to rule out HCV infection, HCV-RNA provides a direct measure of viral load. Considering the silent evolution of HCV infection in children, periodic screening for the infection has become mandatory to prevent liver complications and to predict which patients will develop a more aggressive form of the disease [4].

The aim of the present study was to assess the rate of HCV antibodies and HCV-RNA positivity among children attending University of Assiut Hospital, and to assess some of the risk factors for HCV infection.

Methods

Sample

All patients attending the Department of Paediatrics, University of Assiut Hospital during the study period March 2004 to December 2005 were included

in the study. Some of them had been referred from the health insurance clinic, Assuit, Egypt. Informed consent for participation in the study was obtained from the parent or legal guardian who accompanied the child to the hospital. The exclusion criteria were: known metabolic disease affecting the liver; congenital biliary obstruction; inherited or acquired immune deficiency; suspected acute haemolysis malignant disease or collagen vascular disease; suspected Reye syndrome; and taking potentially hepatotoxic drugs.

A total of 465 children aged 2 months to 15 years were included in the final sample and were divided into the following 3 groups:

- Group A (hepatitis group): 150 children with clinical picture and biochemical evidence suggestive of acute hepatitis [5].
- Group B (multi-transfused group): 165 children who had received 3 or more transfusions of blood, platelets concentrate or fresh-frozen plasma [6]. The group included 95 children with a diagnosis of thalassaemia major, 29 with haemophilia, 17 with chronic renal failure on dialysis and 24 with other haematological problems [sickle cell anaemia (5), chronic idiopathic thrombocytopenic purpura (5), pure red cell aplasia (5), aplastic anaemia (6), and thrombasthenia (3)].
- Group C (non-hepatitis, non-multi-transfused group): 150 children with no present or past history suggestive of hepatitis and no history of multiple blood/plasma transfusions.

Data collection

A standardized questionnaire was completed for all the children. This provided an epidemiological profile and medical history, including history of exposure to common risk factors associated with HCV transmission, based on those established in previous studies in the literature, in addition to some factors that are peculiar to our community,

e.g. circumcision. Risk factors for exposure to HCV included: transfusion(s) of blood or blood products; frequent injections (in the present study we restricted this to > 5 /lifetime); prior surgical procedure(s) (operations, dental procedures, stitches, abscess drainage); prior hospitalization; ear-piercing (female patients only); circumcision (male and female).

Thorough clinical and abdominal ultrasound examinations were done. Blood samples were obtained from all cases by venepuncture into sterile tubes for detection of anti-HCV by ELISA and HCV-RNA by PCR.

Laboratory methods

Blood samples were centrifuged and separated sera samples were kept at -70°C prior to testing. All sera samples of the studied cases ($n = 465$) were tested by NeoDIN anti-HCV EIA 3rd generation, a qualitative assay for detection of HCV in human serum or plasma, according to the manufacturers' instructions and following standard infection control precautions to avoid self-infection.

All sera samples of the ELISA positive cases ($n = 121$) were tested for HCV-RNA by a PCR kit (Abgene House, UK), according to the manufacturer's instructions and following standard infection control precautions to avoid self-infection.

Liver function tests, including total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were done for all 465 cases. 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 [7,8].

Data and specimen collection procedures were approved by the ethical committee for biochemical human studies at the university.

Analysis

The collected data were coded and analysed using SPSS, version 10. Simple statistics such as frequency, arithmetic mean and standard deviation (SD) were used. From 2×2 tables, odds ratios (OR) and 95% confidence intervals (CI) were calculated; *P*-value was determined using the chi-squared test.

Results

Table 1 shows selected demographic data of the study children. Among the 465 tested children, 121 tested positive for anti-HCV by ELISA (26.0%). The frequency of anti-HCV positivity by ELISA in cases of acute hepatitis was 8.7%, similar to the rate among the non-hepatitis, non-multitransfused children (8.0%) (Table 2). The frequency of anti-HCV positivity in the multitransfused group was 58.2%.

For patients with acute hepatitis, the rate of ELISA positivity was higher among rural than urban residents [12/125 cases (9.6%) versus 1/25 cases (4.0%)], and the same was true for the non-hepatitis, non-multitransfused group [11/117 cases (9.4%) versus 1/33 (3.0%)]. In the multi-transfused patients, the rates were similar in rural and urban areas [89/153 (58.1%) versus 7/12 (58.3%)].

The frequency of HCV-RNA positivity by PCR among the cases testing positive by ELISA ($n = 121$) was 72.2% overall, 100% in the hepatitis group,

70.8% in the multitransfused group and 58.3% in the non-hepatitis non-multitransfused group (Table 2).

Table 3 shows some of the risk factors for anti-HCV positivity by ELISA testing. Significant risk factors were a positive history of blood transfusion (OR = 16.62; 95% CI: 9.32–29.91), frequent injections (OR = 3.05; 95% CI: 1.82–5.1), prior hospitalization (OR = 15.19; 95% CI: 8.31–31.03) and prior surgical procedure (OR = 3.48; 95% CI: 1.74–5.23). The frequency of anti-HCV positivity among males and females with a positive history of circumcision showed no significant difference from those with a negative history. Similarly, the frequency of anti-HCV positivity among females with a positive history of ear-piercing showed no significant difference from those with a negative history. The risk effect of some factors could not be analysed, either because the exposure was universal among the studied cases, such as hair-cutting by community barbers and insect bites, or because the exposure was nil, such as cauterization, tattooing and sharing toothbrushes.

Discussion

In non-hepatitis non-multitransfused children attending the Department of Paediatrics at the University of Assiut Hospital, the frequency of anti-HCV positivity by ELISA was 8.0%. The frequency in a previous study at this

Table 1 Comparison of some demographic data in the 3 studied groups of children attending University of Assiut Hospital, Egypt

Variable	Group A Hepatitis ($n = 150$)	Group B Multi-transfused ($n = 165$)	Group C Non-hepatitis non- multi-transfused ($n = 150$)
Age range	2 m–12 y	9 m–15 y	2 m–10 y
Age mean (SD) (months)	43.1 (27.6)	70.0 (41.8)	48.4 (39.4)
Male: female ratio	1.69:1	2.83:1	1.5:1
Urban/rural residence	25/125	12/153	33/117

Table 2 Frequency of seropositivity to hepatitis C virus antibodies among the 3 studied groups of children by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) test methods

Test	Group A Hepatitis		Group B Multi-transfused		Group C Non-hepatitis non-multi-transfused		All cases		P-values (group comparisons) ^a		
	No.	%	No.	%	No.	%	No.	%	B vs C	A vs C	A vs B
Total tested	150	–	165	–	150	–	465	–	–	–	–
ELISA +ve (% of total)	13	8.7	96	58.2	12	8.0	121	26.0	<0.001	NS	<0.001
PCR +ve (% of ELISA +ve)	13	100.0	68	70.8	7	58.3	88	72.2	NS	<0.01	<0.05

^aChi-squared test.
NS = not significant, $P > 0.05$.

Table 3 Frequency of anti-hepatitis C virus (HCV) positivity (by enzyme-linked immunosorbent assay) by history of exposure to risk factors among the total sample of children

Risk factor	History of risk factor		No history of risk factor		OR	95% CI	P-value
	No. HCV +ve/total	%	No. HCV +ve/total	%			
Blood transfusion	102/186	54.8	19/279	6.8	16.6	9.30-29.90	< 0.001
Frequent injections	40/88	45.5	81/377	21.4	3.1	1.80-5.10	< 0.001
Prior hospitalization	108/226	47.7	13/239	5.4	15.2	8.30-31.00	< 0.001
Prior surgical procedure	31/62	50.0	90/403	29.6	3.48	1.74-5.23	< 0.001
Circumcision	34/113	30.1	87/352	24.7	0.51	0.20-1.33	NS
Ear piercing (females only)	21/78	26.9	15/71	21.1	0.93	0.44-1.97	NS

OR = odds ratio; CI = confidence interval; NS = not significant, $P > 0.05$.

hospital among control children admitted to hospital was 5.6% [9], while in a community-based study in a village in Assiut governorate, the frequency was 1.9% for children aged < 9 years. This difference may be attributed not only to the difference in the study design but also to the age of the study population [3]. The lower frequency in the community-based study than the 2 hospital-based studies in the same governorate may be attributed to differences in the study population as the hospital-based study included children who were seeking hospital care. Worldwide studies of the general paediatric population without identifiable risk factors reported much lower seroprevalence rates: 0% in Japan [10] and 0.4% in Italy [11]. Socioeconomic differences are likely to explain much of the geographic variability in the prevalence of anti-HCV positivity.

The frequency of anti-HCV positivity by ELISA in the group with acute hepatitis was 8.7%. This was very similar to that in the non-hepatitis non-multi-transfused group (8.0%), reflecting the endemicity of HCV infection among children in this locality. El-Zimaity et al. at Abbassia Fever Hospital in Cairo could not detect anti-HCV among acute hepatitis cases using 1st-generation ELISA testing; however, they assigned 19.5% of their cases as non-A-non-B hepatitis (hepatitis C, D, E, F, G and TT viruses) [12]. Low rates of positivity of anti-HCV by ELISA among acute hepatitis cases were reported from Morocco [13]. This difference may be explained by the lower rate of anti-HCV antibodies in the general population in Morocco (1.1%) [14].

In the present study the frequency of anti-HCV positivity among the multi-transfused group was 58.2%. Al-Kubaisy et al. detected HCV-specific antibodies in 67.3% of serum samples using 3rd-generation EIA and confirmatory immunoblot assays in Iraq [15]. In a 1993 study in the same hospital in Assiut the rate was reported as 27.6% [16].

The wide variability of HCV prevalence within Egypt, or indeed worldwide, may be due to different sensitivities of the ELISA test used, the different HCV infection prevalence in the blood donor populations, differences in the selection criteria for donors and differences in the numbers of patients included in different studies.

In our study, the frequency of HCV-RNA positivity by PCR among ELISA-positive cases was 72.2% overall. Other studies have reported rates of HCV-RNA positivity by PCR among children with anti-HCV positivity by ELISA as 58.1% [17] and 86.3% [18]. The difference in the frequency of PCR positivity among ELISA-positive cases may be attributed to the following: clearance of HCV-RNA while the subject remains anti-HCV positive [19]; HCV being present in very small amounts in the blood, requiring very sophisticated techniques to pick it up [20]; use of a unsuitable primer set due to the existence of various genotypes in different geographic locations [21]; or false-positive ELISA results [22].

The presence of HCV-RNA in serum is a reliable indicator of infectivity and ongoing viral reproduction, and close follow-up of the infected cases is mandatory [23]. Presence of anti-HCV positivity in the absence of HCV-RNA positivity can be attributed to either a resolved HCV infection while the patient remains anti-HCV positive or a false-positive ELISA test [19,22]. If therapy is initiated for HCV infection solely on the basis of ELISA results, there will be a considerable risk of treating children who do not have HCV activation.

In the present study, those with a history of blood/plasma transfusion were 16 times more likely to have HCV than those who had not received a transfusion. In Egypt, blood transfusion remains an important past, and a potential current, risk factor for HCV transmission. Factors responsible for this situation include: the high anti-HCV

positivity in blood donors (14%–24%) [24]; technical and financial factors that limit anti-HCV screening [3]; inappropriate storage of ELISA kits; use of low-priced ELISA kits; use of rapid (dry) ELISA kits, which have low sensitivity and specificity, in emergency situations in district hospitals; use of ELISA rather than PCR in screening of blood prior to transfusion, which misses the window period in which anti-HCV positivity cannot be detected [25].

In the present study, those who had a history of frequent injections were 3 times more likely to have HCV than those who had no history of prior frequent injections. HCV infection might occur through cross-transmission from unsafe and unnecessary frequent injections that are routine in most health care services and outpatient visits. The mechanism of transmitting HCV through repeated use of the same needles is similar to sharing of needles by intravenous drug users, where the probability of HIV infection has a clear correlation with the period and frequency of injection [26]. In Egypt there is a high frequency of therapeutic injections among the general population. Many injections are prescribed and administered by untrained medical providers

such as pharmacists, barbers, *tamargi* (assistants to private medical doctors), housekeepers, relatives and friends. Re-use of syringes may occur, which is likely to contribute to bloodborne pathogen transmission. Health care workers underestimate patient-to-patient HCV infection; some may think that injection is safe when only the needle is changed between each patient, whereas a reused syringe may contain enough blood to cause HCV infection.

We found those who had a history of prior hospitalization were 15 times more likely to have HCV than those who had not been hospitalized before. One way of contracting HCV may be transmission from infected medical personnel to susceptible patients during medical care. Patient-to-patient transmission has also been reported in a haematology ward [27], during colonoscopy in a gastrointestinal disease unit [28] and in a haemodialysis unit [29]. Nosocomial transmission of HCV through the use of multidose vials between several patients was reported by Widell et al. [30]. Multidose vials of local anaesthetics, saline, heparin and other solutions are used in different hospitals to reduce costs, and transmission of HCV may occur through contamination of the

vials, or through forgetting to change the syringe between patients.

In the present study, those who had undergone a surgical procedure were more than 3 times more likely to have HCV than those who had not had prior surgery. Surgery can lead to HCV exposure through intravenous treatments, repeated blood drawing for testing and transfusion of blood products. The remote possibility of the use of unsterilized or inadequately sterilized contaminated instruments may add to the problem.

Conclusions

The frequency of HCV infection among children attending the University of Assiut Hospital was high, particularly among those exposed to multiple blood/plasma transfusions. History of blood transfusion, frequent injections, hospitalization or surgical procedures were important risk factors for HCV infection in Egypt. Avoidance of unnecessary blood transfusion and injections, and implementation of strict infection control measures are highly recommended to reduce the frequency of HCV infection.

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Note from the Editor

We wish to draw the kind attention of our potential authors to the importance of applying the editorial requirements of EMHJ when preparing their manuscripts for submission for publication. These provisions can be seen in the Guidelines for Authors, which are available online at <http://www.emro.who.int/emhj.htm>, and are published at the end of the first issue of each volume. We regret that we are unable to consider papers that do not conform to the Guidelines.