Original Article

Hepatitis C virus infection in Egyptian children with type 1 diabetes mellitus: A single center study

Hekma Saad Farghaly, Kotb Abbass Metwalley, Heba Ahmed Abd El-Hafeez[1]

Departments of Pediatrics, and [1]Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

ABSTRACT

Background: Only few studies have evaluated the epidemiology and risk factors of hepatitis C virus (HCV) infection in Egyptian children with type 1 diabetes mellitus (T1DM). The present study aimed at measurement of the rates of anti-HCV positivity by Enzyme-Linked Immuno-Sorbent Assay (ELISA) test and of HCV-Ribonucleic acid (RNA) positivity by polymerase chain reaction (PCR) among children with T1DM and to study the possible risk factors of infection. **Settings and Design:** Cross-sectional controlled study. **Materials and Methods:** The study included 150 children with T1DM (Group 1) (mean age 14. 76 ± 6.4 years). Fifty children age and sex-matched were included as control group (Group 2) (mean age 13.62 ± 2.11 years). They were screened for HCV antibodies using third generation ELISA and HCV-RNA positivity by PCR. **Results:** The frequency of anti-HCV positivity by ELISA was significantly higher in children with T1DM (n = 150) in comparison wiith control group (n = 50) (12% vs 6%; P<0.001), while the frequency of HCV-RNA positivity by PCR among the cases testing positive by ELISA was 75% for both diabetic group and control group. There were no significant differences in serum levels of liver biochemical profile in diabetic children with anti-HCV positivity (n = 18) in comparison to those with anti-HCV negativity (n = 132). Residence in rural area, low socioeconomic class and prior hospitalization were significant risk factors for anti-HCV positivity by ELISA. **Conclusions:** The frequency of HCV infection in children with T1DM in Upper Egypt appears to be high and is mainly related to residence in rural area, low socioeconomic class and prior hospitalization. HCV infection in these children is not associated with significant changes in hepatic biochemical parameters. **Recommendations:** Implementation of strict infection control measures are highly recommended to reduce the frequency of HCV infection. Furthermore, the silent evolution of HCV infection in children makes periodic screening of HCV in diabetic children mandatory.

Key words: Alanine aminotransferase, hepatitis C virus, infection, type 1 diabetes mellitus

Introduction

Hepatitis C virus (HCV) infection is a major global healthcare problem, with 170 million carriers worldwide and 3-4 million new cases per year and an overall global prevalence 1-3% with a significant risk of progression to cirrhosis and hepatocelluar carcinoma.[1] The infection rate ranges from as low as 0.1% in Canada to the extremely high rate of 18.1% in Egypt.[2] The prevalence of anti-HCV among Egyptian children in relation to lifestyle is poorly understood. Patients infected with HCV often have minimal clinical evidence of disease. Since HCV treatment has become increasingly effective, it is important to identify silently infected individuals. There are few data on the prevalence of HCV infection in Egyptian children with type 1 diabetes mellitus (T1DM). Up to our best knowledge, this is the first study trying to estimate the prevalence of hepatitis C among children with T1DM and associated risk factors in Upper Egypt.

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.129111

Materials and Methods

Patients

This cross-sectional study included 150 children with T1DM (Group 1). There were 98 boys and 52 girls. In addition, 50 apparently healthy age and sex-matched children, living in the same living conditions, were studied as a control (Group 2). They were 37 boys and 13 girls. Both patients and controls were recruited from Pediatric

Corresponding Author: Dr. Kotb Abbass Metwalley,Assistant Professor of Pediatrics ,Pediatric Endocrinology Unit Department of Pediatrics, Faculty of Medicine, Assiut University, Assiut, Egypt. E-mail: kotb72@yahoo.com

Endocrinology Unit and General Pediatric Outpatients Clinics in Assiut University Children Hospital, Assiut, Egypt. The study protocol was approved by the ethical committees of Assiut University Children Hospital, Assiut, Egypt. Written informed consents were obtained from the parents of both patients and controls.

Inclusion criteria

- Definite diagnosis of T1DM according to the criteria of American Diabetes Association (ADA)[3]
- On insulin replacement therapy
- Age range 3-16 years
- Disease duration >2 years.

Exclusion criteria

- Past history of hepatitis before diagnosis of diabetes
- Associated autoimmune diseases
- Diabetic children with abnormal lipid profile.

Methods

- A standardized questionnaire was used to collect socio-demographic data such as age, sex, 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, prior surgical procedure(s) (operations, dental procedures, stitches, abscess drainage), prior hospitalization, hemodialysis, tattooing, ear-piercing (female patients only), circumcision by informal health care provider, and family history of hepatic disease especially HCV positive members
- Socioeconomic status was calculated according to Egyptian score including education of father and mother, crowding index, and sanitation of the house[4]
- Thorough clinical examination including body mass index (BMI) of children which was calculated by using formula body mass index (BMI) = weight (kg)/height (m2). Hepatic assessment included clinical examination and abdominal ultrasound.

Laboratory investigation

- Complete blood count with differential counts
- Liver biochemical profile: Bilirubin (total and direct), albumin, aspartate, and alanine aminotransferases (AST and ALT). Liver biochemistry tests were done by routine methods. ALT (normal up to 40 U/L) and AST (normal up to 40 U/L) were considered elevated, if any elevation above the upper limit was detected
- Mean glycosylated hemoglobin (HbA1c%) of two readings in the year preceding the study. It is measured by Turbidimetric Inhibitory Assay Quantification (TINA-Quant) (Hitachi 911).[5] The American Diabetes Association (ADA) published the target age-specific Hg A1c as follow; <6 years, 7.5-8.5%; from 6 to 12 years, ≤8%; from 13 to18 years, ≤7.5%[6]
- HBsAg, total hepatitis B core antibody (HBcAB), and anti-HCV antibody were detected with commercially available enzyme linked immunosorbent assay (Equipar, 21047 Saronno VA, Italy)
- Children positive for anti-HCV were tested for HCV RNA by reverse transcription-polymerase chain reaction (PCR) by a PCR kit (Cobas Amplicor, Roche Diagnostics).

Statistical methods

Statistical Package for Social Sciences (SPSS) program version 11 was used for analysis of data. Simple statistics such as frequency, arithmetic mean, and standard deviation (SD) were used. Comparison between two groups was done by using Student's *t*-test for quantitative independent variables and Chi square test for qualitative variables. *P* value is considered significant if <0.05. Odds ratio were used to measure the strength of associations.

Results

Table 1 shows demographic and metabolic characteristics of the studied groups.

Table 1: Some demographic and metabolic characteristics of the studied groups

	Diabetic children group 1 (*n*=150)	Control group 2 (*n*=50)	*P* value
Age (years)	14. 76±6.4	13.62±2.11	NS
Sex (male/female)	98/52	37/13	NS
Residence (urban/rural)	18/132	8/42	NS
Socioeconomic class (high/low)	39/111	8/42	NS
Diabetes durations	7.1±1.1	-	-
Insulin dose (U/kg per 24 h)	0.8±0.1	-	-
BMI	16.3±6.3	18.5±7.3	NS
Mean Hb1Ac (%)	11.2	5.1	<0.001

None of the children had hepatomegaly either clinically or by abdominal ultrasound, Data are given as mean±SD unless otherwise specified, BMI: Body mass index, BP: FBG: Fasting blood glucose, HbA1c: Hemoglobin A1c

Table 2: Frequency of seropositivity to hepatitis C virus antibodies among the studied groups by ELISA and HCV–RNA positivity by PCR

	Group 1		Group 2		*P* value
	n	%	*n*	%	
Total tested	150	-	50	-	-
ELISA+ve (% of total)	18	12	3	6	0.01
PCR+ve (% of ELISA+ve)	12	75	2	75	NS

None of the studied children was positive for HBsAg or HBcAb, NS: Not significant, PCR: Polymerase chain reaction, ELISA: Enzyme-Linked Immuno-Sorbent assay, HCV: Hepatitis C virus, RNA: Ribonucleic acid

Table 2 shows frequency of seropositivity to HCV antibodies among the studied groups by Enzyme-Linked Immuno-Sorbent Assay (ELISA) and HCV-Ribonucleic acid (HCV-RNA) positivity by PCR. The frequency of anti-HCV positivity by ELISA was significantly higher in children with T1DM in comparison with control group (12%vs 6%; $P < 0{,}001$) while the frequency of HCV-RNA positivity by PCR among the diabetic cases testing positive by ELISA was 75%, which is same frequency of HCV-RNA positivity by PCR in the control group.

Table 3 shows biochemical liver profile in diabetic children with anti-HCV positivity in comparison to those with anti-HCV negativity. There were no significant differences in serum levels of total and direct bilirubin, albumin, ALT, and AST in diabetic children with anti-HCV positivity in comparison to those with anti-HCV negativity.

Table 4 shows some of the risk factors for anti-HCV positivity by ELISA testing. Significant risk factors were residence in rural area (OR = 4.279; 95% CI: 0.943-19.427), low socioeconomic class (OR = 3.684; 95% CI: 1.017-13.341), and prior hospitalization (OR = 10.036; 95% CI: 1.295-77.762). High HbA1c, uses of either syringes or insulin pen and diabetic durations >5 years were not significant risk factors for anti-HCV positivity by ELISA. The risk effect of some other factors i.e., blood transfusions, surgical operation, circumcision, and ear piercing could not be analyzed, either because the exposure was universal among the studied cases, or because the exposure was nil.

Table 3: Biochemical liver profile in diabetic children with anti-HCV positivity in comparison to those with anti-HCV negativity

	Anti-HCV positivity *n*=18	Anti-HCV negativity *n*=132	*P* value
Total bilirubin (mg/dl)	0.44±0.11	0.39±0.21	NS
Direct bilirubin (mg/dl)	0.12±0.14	0.09±0.11	NS
Albumin (gm/dl)	4.1±1.2	4.4±2.7	NS
ALT (IU/L)	24±8.6	20±9.5	NS
AST (IU/L)	22±7.3	19±6.8	NS

Data are given as mean±SD, NS: Not significant, HCV: Hepatitis C virus, ALT: Alanine aminotransferases, AST: Aspartate, aminotransferases

Discussion

Egypt is a country with a high load of HCV. The prevalence of anti-HCV among Egyptian children ranged between 3% and 9%.[7] The prevalence of T1DM in Egypt is 0.13-0.4%, according to the International Diabetes Federation.[8] In a country like Egypt, with annual births approaching 2 millions, diabetic children are expected to increase between 2,000 and 8,000 cases per year.

In the present study, the rate of anti-HCV positivity among children with T1DM was 12% and prior hospitalization was found to be associated with anti-HCV positivity. El-Karaksy *et al.,*[9] in a recent study, reported a lower rate of anti-HCV of 3.6% among diabetic children attending Cairo Children University Hospital-Egypt. Also, the prevalence in our study was higher than that found in diabetic children and adults in different countries.[10-12] On the other hand, other studies in children revealed that HCV was not associated with diabetes in spite of the high frequency of injections.[13,14] The higher rate of anti-HCV positivity in our study reflect a poor infection control measures. Children with T1DM are usually hospitalized for either monitoring and diabetic control (several weeks) or diet education (a week). Diabetic patients perform self-monitoring of capillary blood glucose by finger puncture several times daily. Particular risk factors for diabetic patients include using shared spring-triggered finger-stick device for glucose self-monitoring or use of multi-dose insulin vials for more than one patient using used syringes in addition to the possibility of patient-to-patient transmission during hospital admission.[15,16] Food and Drug Administration has recommended since 1990 that finger-stick devices be restricted to individual use. Contaminated multi-dose vial (a vial sealed with a stopper intended to be accessed with a needle and syringe on more than one occasion) was likely the source of transmitting HCV in nosocomial outbreaks. Some of the practitioners misused multi-dose vial by reentering the vial with a needle that had already been injected into the patients.[15]

Table 4: Some of the risk factors for anti-HCV positivity by ELISA testing

Risk factor	Anti-HCV positivity *n*=18 (%)	Anti-HCV negativity *n*=132 (%)	*P* value	Odds ratio	95% CI (upper-lower)
Residence in rural area	16/18 (88.9)	86/132 (65.2)	0.003	4.279	0.943-19.427
Low socioeconomic class	15/18 (83.3)	76/132 (57.60)	0.029	3.684	1.017-13.341
Prior hospitalization	17/18 (94.44)	83/132 (62.9)	0.005	10.036	1.295-77.762
Duration of diabetes>5 years	10/18 (55.5)	78/132 (59)	0.775	1.16	0.38-3.44
Use of syringe	17/18 (94.4)	120/132 (90.9)	0.82	0.31	0.21-2.13
Use of insulin pen	1/18 (5.56)	12/132 (9.09)	1.0	0.59	0.03-4.88
High Hb1Ac	12/18 (66.6)	92/132 (69.7)	0.79	0.87	0.28-2-82

HCV: Hepatitis C virus, Enzyme-Linked Immuno-Sorbent assay

In the present study, diabetic children from rural area or from low socioeconomic class are 4 and 3 times are at increased risk to have anti-HCV positivity respectively, these children did not have their own glucometer, they used to check their blood sugar by a common use glucometer in pharmacies which can lead to cross HCV infection. The seroprevalence of anti-HCV antibodies in the control group of our study was much lower than the observed prevalence of an earlier Egyptian study in children (11.8%).[17] On the other hand, present results were higher than that reported by EL-Raziky *et al*., (2007),[18] which was 2.02% in Egyptian children. However, these present results lie within the recorded range of anti-HCV prevalence (3-9%) in other studies.[7,19] This variation in seroprevalence can be attributed to the smaller number of healthy children of group in the current study; or may be attributed to the different laboratory methods used for detection of anti-HCV.[20]

In the present study, none of the studied children was positive for HBsAg or HBcAb. A marked drop is observed in HBV infection in children (<15 yr), who received compulsory vaccination against hepatitis B.[21] In Egypt, compulsory vaccination against HBV has been adopted since 1993.

In the present study, the frequency of HCV-RNA positivity by PCR among ELISA-positive diabetic cases and control group was 75%. Other studies have reported rates of HCV-RNA positivity by PCR among children with anti-HCV positivity by ELISA as 40%.[9] 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;[22] HCV being present in very small amounts in the blood, requiring very sophisticated techniques to pick it up;[23] use of a unsuitable primer set due to the existence of various genotypes in different geographic locations;[24] or false-positive ELISA results.[25] 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.[26] 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.[22,25] 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, there was no significant difference of ALT and AST levels between anti-HCV seropositive and seronegative children with diabetes. In general, there are conflicting data about the role of AST and ALT in supporting the diagnosis and follow up of HCV infection. Some investigators found that all affected children had normal levels of transaminases.[27]Others reported that most chronically infected children with HCV have mildly elevations in ALT levels[28] and also, it was stated that chronic hepatitis C may even occur in adult patients with persistently normal ALT levels with slow or absent progression to cirrhosis after 10 years of follow-up.[29,30] In contrast, persistently elevated ALT levels was recorded in several Egyptian pediatric and adult studies and consequently, HCV infection is not always benign in Egyptian children, hence the authors suggested those tests to be useful and dependable markers in the non-invasive diagnosis of HCV.[31,32]

Limitations of the study

- Small sample size
- Some of the data were obtained by self or caretaker report, which is vulnerable to reporting bias
- The relatively small number of hepatitis C positive cases by ELISA did not allow a very sound study of the effect of the risk factors in acquiring HCV infection.

Conclusions

The frequency of HCV infection in children with T1DM in Upper Egypt appears to be high and is mainly related to residence in rural area, low socioeconomic class and prior hospitalization. HCV infection in these children is not associated with significant changes in hepatic biochemical parameters.

Recommendations

Implementation of strict infection control measures are highly recommended to reduce the frequency of HCV infection. Furthermore, the silent evolution of HCV infection in children makes periodic screening of HCV in diabetic children mandatory.

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Cite this article as: Farghaly HS, Metwalley KA, El-Hafeez HA. Hepatitis C virus infection in Egyptian children with type 1 diabetes mellitus: A single center study. Indian J Endocr Metab 2014;18:197-201.

Source of Support: This study was a thesis presented for the degree of Medical Doctor (MD) which supported by Tehran University of Medical Sciences, Tehran, Iran, **Conflict of Interest:** No.