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ORIGINAL ARTICLE

Prevalence of hepatitis C infection among children with β-thalassaemia major in Mid Delta, Egypt: a single centre study

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Background: Transfusion dependant patients are at a higher risk of acquiring bloodborne infections even under conditions of safe transfusion. This study was designed to determine sero-prevalence of hepatitis C infection and possible associated risk factors in thalassaemic children.

Methods: One hundred and twenty five children with β thalassaemia major (β-TM) were recruited from the Haematology/Oncology Unit, Paediatric Department, Tanta University Hospital, Egypt, between April 2010 and October 2011. Patients underwent history taking, full clinical examination, routine investigations and venous blood sampling. Serum was stored at −20°C till tested for hepatitis C (HCV Ab) and B (HBsAg) by ELISA. HCV Ab positive cases were confirmed by PCR.

Results: All patients were HBsAg negative. HCV Ab ELISA was positive in 76%, negative in 20% and equivocal in 4%. Fifty patients (40%) had positive PCR for HCV. PCR showed low viraemia in 78%, moderate viraemia in 20% and high viraemia in 2%. A positive family history of HCV, history of minor operative intervention and/or dental procedures were significantly associated with higher frequency of HCV infection in thalassaemic children, while amount and frequency of transfused blood, age at transfusion and chelation state were not.

Conclusion: HCV infection is highly prevalent in children with β-TM in Egypt despite strict pre-transfusion blood testing. This should arouse the attention for environmental and community acquired factors. Quality management to insure infection control in minor operative procedures and adding more sensitive tests for blood screening are recommended.

Keywords: HCV, Thalassaemia, PCR, Seroprevalence, Egypt

Introduction

β Thalassaemia major (β-TM) is an autosomal recessive disease which represents a major health problem in the Mediterranean region. It causes high morbidity, early mortality and great deal of psychological and economic burden for the family. Those who are fortunate enough to have regular lifelong blood transfusions and adequate iron chelating therapy can avoid many complications and live a near normal life.[1] However, they can face another kind of clinical challenge if they do not get safe blood (by safe we mean that blood is matched and pre-tested for hepatitis C virus [HCV], hepatitis B virus [HBV] and HIV). Multiple transfused patients are more prone to catch transfusion transmitted viruses, particularly HCV, HBV and HIV.[2] In Egypt, thalassaemia is the most common haemolytic anaemia with carrier rates ranging from 9–16%.[3,4] There are many centres for thalassaemia in Egypt including Tanta Paediatric Thalasaemia Centre of the Haematology/Oncology unit where patients can get safe transfusions and obtain their chelating therapy.

Egypt has the highest prevalence of HCV in the world with a nationwide prevalence of 14.7% for anti HCV Ab in the 15–59 years age group. The prevalence tends to increase with age to reach 46.3% in men aged 50–59 years. Rural areas (such as the Nile Delta) had higher prevalence compared to urban areas (18.3% versus 10.3%, respectively).[5]

HCV infection usually progresses to chronic hepatitis with subsequent cirrhosis, end stage liver disease and hepatocellular carcinoma.[6] The use of sensitive screening tests and donor selection procedures in blood banks has significantly reduced blood transmission of viral infections, however, transfusion dependent patients are still prone to acquire hepatitis B and C infections.[7–16]

The aim of this work was to update the prevalence of HCV sero-positivity by ELISA and RT-PCR and determine the possible

associated risk factors in multiple transfused β-TM children in Tanta University Centre.

Patients and Methods

A prospective, cross-sectional study was conducted between April 2010 and October 2011. Eligible patients aged between 1 and 16 years were recruited from the Haematology/Oncology Unit of the Paediatric Department, Tanta University Hospital, Egypt.

Inclusion criteria for this study was: patients diagnosed with β-TM by clinical picture, CBC and hemoglobin electrophoresis; registered at the Haematology/Oncology Unit, Paediatric Department, Tanta University Hospital; regularly attending the unit for safe packed cell transfusion; received deferoxamine mesylate pump 5 days/week for chelation with variable compliance to therapy. All of our patients were from rural areas and their families were not tested for HCV infection in this study, but some had performed the tests independently.

Patients who had ever received blood transfusion outside our centre or prior to obligatory blood screening for hepatitis viruses and patients who had undergone operative or dental procedures outside equipped hospitals were excluded.

The patients who met both inclusion and exclusion criteria were labeled sequentially (268 files) and randomized by simple randomization to include even numbers (134 cases). Among them 125 parents give informed consent to participate in this study. A detailed clinical account was made for each patient with emphasis on age, age at diagnosis, age at start of regular transfusions, inter-transfusion intervals, history of jaundice, operative and dental procedures, family history of hepatitis, parent's awareness about risk of developing parenterally transmitted hepatitis and physical examination findings. All patients included in the study had certified HBV vaccination with three doses at age 2, 4 and 6 months with recombinant Hep Vax vaccine. Complete blood counts with manual differential count, estimation of liver transaminases, serum bilirubin and serum ferritin levels were performed. For virology studies, venous blood sample was obtained, centrifuged at 3000 rpm for at least 20 min at room temperature (18–24°C), and stored at -20°C till tested for HBsAg, and HCV Ab titre by third generation ELISA test (ELISA-3) based on the indirect sandwich principle (Precheck Bio Inc., Anaheim, CA, USA). Positive cases were confirmed by PCR to detect the presence of virus. Quantification of serum HCV RNA levels was performed according to a PCR assay (Amplicor HCV Monitor Test, version 2.0; Roche Diagnostics, Branchburg, NJ, USA).

Interpretation of results was performed according to manufacturer's instructions as follows: for ELISA, patients with a titre <0.9 IU/mL were interpreted as negative, patients with a titre 0.9–1.0 IU/mL were considered equivocal, while patients with a titre >1.0 IU/mL were considered positive. For PCR, a titre <10 IU/mL was considered negative, $10–10^2$ was repeated 2 months later, $10^2–10^5$ was interpreted as low viraemia, $10^5–10^6$ moderate viraemia, while any titre $>10^6$ was interpreted as high viraemia.

All healthy blood donors attending blood bank in the same period (n = 3756) were tested for HBsAg and anti HCV antibody routinely. Their serologic data were used as control.

Statistical analysis

Data were analyzed using SPSS version 13.0 (IBM Corp, Armonk, NY, USA). All values are expressed as mean ± SD and median if not normally distributed. The χ^2 test was used to compare the frequency of clinical parameters, and Mann Whitney U test to compare median difference in clinical and laboratory parameters between HCV-positive and HCV-negative β-TM patients. The clinical and laboratory variables that were previously reported to be associated with higher risk of HCV infection in β-TM patients were correlated to level of viraemia using Pearson correlation test; p-value <0.05 was considered statistically significant for all used tests.

Results

Among 3756 blood donors, 659 (17.5%) had positive HCV antibody and 109 (2.9%) had positive HBsAg by ELISA. Out of the 125 Egyptian thalassaemic children investigated, 74 were males (59.2%) and 51 females (40.8%). Their ages, at the time of data analysis, ranged from 1–16 years with a mean age of 7.6 ± 4.4 years (median 6 years). Among them, 40% had consanguineous parents. History of minor operative procedures (e.g. circumcision, tonsillectomy, sutures) and/or dental procedures was positive in 77 (61.6%) of examined cases. Operative history is significantly more frequent in HCV infected children. Family history of positive HCV antibodies in parents or siblings was detected in 57 out of 104 cases tested apart from this study. Children with HCV had a significantly more frequent positive family history of HCV antibodies when compared with HCV-negative children with β-TM (69% in HCV-positive patients vs 45% in HCV-negative patients, p = 0.03; Table 1). Depending on each patient's individual response, inter transfusion intervals ranged between 2 weeks and 2 months. Twenty five were splenectomized (20%) in equipped hospitals with their wounds healed

Table 1. Frequency of clinical parameters of all patients

	HCV positive n = 50		HCV negative n = 75		χ^2	p
	Frequency	%	Frequency	%		
Consanguinity	21	42	29	39	0.035	NS
Minor operative and dental procedures	38	76	33	44	4.376	0.036
Family history of HCV infection	29/42	69	28/62	45	4.496	0.034
Splenectomy	12	24	13	17	0.469	NS
Anorexia	18	36	29	39	0.013	NS
Jaundice	48	96	72	96	0.217	NS
Abdominal pain	22	44	31	41	0.381	NS
Splenomegaly	39	78	61	81	0.052	NS
Hepatomegaly	40	80	59	79	0.002	NS

NS: non significant

Table 2. Comparison between HCV infected and non infected patients

	HCV infected mean ± SD (median) n = 50	Non HCV infected mean ± SD (median) n = 75	Z	p
Age in years	7.2 ± 3.6 (6.5)	7.8 ± 4.9 (6)	0.076	0.942
Age at first transfusion (months)	16.3 ± 13.3 (13.5)	16.1 ± 13.7(12)	0.638	0.523
Inter-transfusion interval (days)	37.2 ± 15.8 (35)	37.2 ± 11.1 (35)	0.086	0.932
Total number of transfused packs	69.4 ± 70.5 (40)	63.9 ± 53.7 (39)	0.344	0.731
Transfused packs (200ml)/year	11.1 ± 4.8 (10)	10.5 ± 4 (10)	0.856	0.327
Ferritin (ng/dL)	2665.0 ± 1514.8 (2500)	2239.9 ± 1128.7 (2400)	0.343	0.734
Range	8000–950	7500–550		
ALT(IU/L)	56.2 ± 20.3	39.2 ± 8.8	7.207	<0.0001
AST(IU/L)	55.3 ± 21.7	38.4 ± 8.7	7.219	<0.0001

Table 3. Correlation between HCV-RNA viral load and other parameters, n = 50

HCV PCR	r	p
Age in years	0.047	0.895
ALT (IU/L)	0.140	0.334
AST (IU/L)	0.143	0.322
Ferritin (ng/dL)	0.222	0.121
Inter-transfusion interval (days)	0.141	0.329
Number of transfused packs	0.118	0.414

by primary intention. Clinically, 96.0% (120) of our cases had jaundice. Hepatomegaly was present in 99 (79.2%), and splenomegaly in 100 (80.0%) (Table 1). None of our cases had features of liver cell failure or extra hepatic manifestations of HCV infection.

Twenty five children (20.0%) were negative for HCV antibodies, five patients (4.0%) had an equivocal titre, and 95 patients (76.0%) were positive for HCV Ab by ELISA. Interestingly, none of the five equivocal cases had PCR positive results in confirmation.

There was no significant difference between HCV positive and negative patients regarding age, number, frequency or total amount of transfused blood or ferritin levels. Transaminases were higher than normal for age (12 IU/L) in most patients. The levels of ALT and AST were doubled or more in 26/50, 27/50 of HCV infected patients, and 23/75, 24/75 in non HCV infected patients ($\chi^2 = 4.804$, $p = 0.028$ and $\chi^2 = 4.458$, $p = 0.035$) respectively. The mean values were significantly higher in HCV positive patients (Table 2).

Infection with HCV was confirmed by PCR testing in 50 out of 100 patients who had positive or equivocal HCV Ab results. Only one patient had high viraemia, 10 had moderate, and 39 had low viraemia. However, the viral load was not correlated to patient's age, transfusion rate, ferritin or transaminase levels (Table 3).

Discussion

Regular safe transfusion and adequate iron chelation therapy are essential to decrease the complications and prolong survival in patients with β-TM.[17] However, if safety of blood transfusion is breached patients are particularly prone to develop transfusion transmitted infections especially HBV, HCV and HIV.[18]

In this work, children with β-TM had an alarmingly high prevalence of HCV antibodies compared to blood donors in the same period (76% vs 17.5%). On the contrary, all children enrolled in our study were negative for HBsAg while blood donors have a prevalence of 2.9%. This is surprising as both HCV and HBV transmission has the same risk factors. However, negative HBsAg cannot rule out occult HBV infection. Also, our results may reflect the protective effect of compulsory HBV vaccine as many blood donors did not receive vaccination while all of our patients had a certified complete vaccination course. Mansour et al.,[15] in a recent study in mid Delta Egypt, reported much higher prevalence (29–44%) of HBV in their thalassaemic patients despite comparable prevalence in blood donors (4%). However, they stated that 'some of their patients had received blood in peripheral centers where viral screening could not be guaranteed". Such practice was an exclusion criterion in our work. Also El-Farmawy et al.[19] reported 12% HBsAg positivity in their repeatedly transfused cases, but only 78% of their patients had completed their 3-dose vaccination course.

Bortolotti et al.[20] studied hepatitis C natural history in Italian and Spanish children, and found that whether infection was post-transfusion or community acquired, children mostly have insidious illness onset, slow rate of progression and lack of clinical signs or symptoms with or without elevated transaminases which hinders the diagnosis and causes underestimation of chronic hepatitis in children. Usually, such silent infection in children is discovered accidentally.[20] Another study by Jara et al.[21] reported anorexia, asthenia and abdominal pain in 14% of HCV children, without jaundice or extrahepatic manifestations. Clinically, none of our patients had features of liver cell failure or extrahepatic manifestations of hepatitis C. The high incidence of hepatomegaly, splenomegaly and jaundice can be related to reticuloendothelial hyperplasia and haemolysis caused by thalassaemia rather than portal hypertension or hepatic affection.

Anorexia and abdominal pain were frequently met in our thalassaemic patients, but we cannot relate them to HCV infection as they are subjective nonspecific symptoms and their frequencies were not significantly different among patients with or without HCV infection.

Regarding transaminases, most of our thalassaemia patients had elevated enzymes (>12 IU/L). It is worth mentioning that all of our patients are chelated by deferoxamine mesylate which does not affect liver enzymes; lack of adherence to chelation therapy with subsequent high ferritin levels reported in some of our patients can explain this enzymopathy. Elevated transaminases noticed even in HCV negative patients may reflect the hemosidrotic hepatic burden of thalassaemia due to the effect of iron overload as previously reported.[1]

HCV infected patients had significantly higher enzymes than non infected. Similar results were reported in recent Egyptian studies,[14,15] which may represent the augmented burden of thalassaemia and HCV infection. But this significant elevation in transaminases was not correlated to viral load. The independently fluctuating patterns both in transaminases and HCV viraemia with discrepancy between viral load and transaminase level is part of the natural disease history in HCV infection.[22]

Over the last decade, seroprevalence of HCV Ab among β-TM in different countries including Egypt ranged from 12.5–100%.[8–15] This wide variation may reflect different geographic localities with variable HCV prevalence in the donor populations, different sensitivity and specificity of tests used, small sample size or variable patient selection criteria.

In our cohort, the frequency of HCV Ab seropositivity was 76%; this is nearly 7-fold the prevalence in controls. It was also much higher than HCV prevalence reported in non-transfused Egyptian children both in upper Egypt (3%[23]) and Nile Delta (9%[24]). This reflects the definite risk of HCV infection in repeatedly transfused β-TM children even if the blood was pretested for hepatitis with ELISA. Thalassaemic patients may catch infection due to infected blood collected from a donor in window phase during which the blood tested negative with ELISA. To avoid window phase the donated blood should be tested with a sensitive test dating earlier than antibody formation. PCR examination effectively covers this period, but it is expensive and only a few Egyptian centres provide it for blood screening; unfortunately, our centre is not one of them.

Comparing our results with other studies done in Egypt among thalassaemic children, we recorded 76% HCV seropositivty and 40% HCV RNA positivity. These results were comparable to most reports but lower than those of Ragab et al.[14] who found all thalassaemic patients in their sample to be sero positive for HCV Ab with HCV viraemia confirmed in 64% of them. This variation may be related to the different sensitivity and specificity of ELISA kits used which may cause contrasting prevalence rates. The higher PCR positivity detected in their patients may be related to unmentioned risk factors. Minor surgical and dental procedures were significantly higher in HCV infected patients in our study. Such history was not mentioned in their study. Acquisition of HCV infection was not related to age, age at starting transfusion, frequency, amount and duration of blood transfusion in our patients. Similar results were reported by Ragab et al.[14] On the contrary, Mansour et al.[15] report age, frequency and amount of transfused blood as risk factors associated with higher frequency of HCV infection in the thalassaemic children in their study. However, their patients had history of transfusion in peripheral centres where pre-testing of blood is not ensured. The more frequently a child gets a transfusion the higher chance to get one unsafe transfusion in those peripheral centres. So the risk may be related to unsafe blood rather than the frequency itself.

Conclusion

Transfusion dependent Egyptian thalassaemic children in Tanta Paediatric thalassaemia centre have a high prevalence of HCV infection despite pretesting of blood. Adding PCR to blood pretesting can reduce the risk of the window phase. Absence of hepatitis B surface antigenaemia in this cohort does not rule out occult HBV infection, however, it puts much stress on the protective effect of vaccination. Strict donor selection to those who have received vaccination may be advised.

The association of positive family history and history of minor operative and dental procedure in absence of obvious transfusion-related risk factors in our patients arouses attention towards environmental factors. Considering safety measures to prevent community-acquired hepatitis C, and quality control measures to insure strict infection control both during testing and administration of blood are highly recommended.

Authors' contributions: MRS and HHS raised the study question, carried out the clinical assessment and collected data and samples; MRS, IAK and HHS designed the study protocol; HMN and SHA carried out the ELISA and PCR determination, and analysis and interpretation of these data. HHS drafted the manuscript; MRS, HHS and HMN critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. MRS and HHS are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: The study protocol was approved by the medicine ethical committee of Tanta faculty. Informed consent was obtained from each child's parent or guardian before participation in the study.

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