Journal of Applied Sciences Research, 8(3): 1517-1523, 2012
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Overview on the Prevalence of Transfusion Transmitted Viral Hepatitis among transfusion-dependant β-thalassemia Egyptian Children

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ABSTRACT

Background: Although the risk of transfusion-transmitted hepatitis has been recently reduced, transfusion-dependent β-thalassemia patients are still at a high risk of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. We aimed to look into the prevalence of HBV and HCV sero-positivity amongst multiply transfused thalassemia patients at our center. **Patients and Methods:** Four hundred and thirty five thalassemic patients (mean age 10.1±6.7 years) were recruited to participate in this study. The records of patients were reviewed and history was taken with emphasis on blood transfusion. Blood samples were withdrawn and tested for hepatitis B surface antigen (HBsAg) using Qualitative chemiluminescent immunoassay (ChLIA), anti-HCV antibodies using third generation ELISA kits in addition to serum ferritin and pre-transfusion hemoglobin. **Results:** Ninety-five/406 (23.4%) patients were infected by HBV and their mean age was significantly higher than HBV sero-negative patients. A significant positive correlation was proved between HBsAg positivity and age (r=0.2, p=0.0001). Among vaccinated cases, the prevalence of HBV infection was significantly lower among those who received the vaccine for less than 10 years (18.9%) when compared to those who received the vaccine for more than 10 years (30.4%) and those who didn't receive the vaccine (30%). Three hundred out of 435 (69%) patients had anti-HCV antibodies; among which 83% were children. Significant positive correlations were found between HCV sero-positivity and both age and serum ferritin of the studied cases (p=0.0001 and 0.007 respectively). **Conclusions:** We still have the highest prevalence of HCV and HBV among thalassemics and therefore, stricter criteria of safe donor selection have to be adopted. Prevalence of HBV infection increased 10-years post vaccination and so, booster dose of HBV vaccine may be mandatory.

Key words: hepatitis; β-Thalassemia; Safe blood transfusion.

Introduction

In Egypt, thalassemias represent the commonest cause of chronic hemolytic anemia. The carrier rate in different studies ranged from 9 to 10.2% (El-Beshlawy, 2004). Early and regular blood transfusion therapy in transfusion-dependant patients of β-thalassemia decreases the complications of severe anemia and prolongs survival (Prati, 2000). Regular blood transfusions for patients of thalssemia carry a definite risk of transmission of certain viruses**,** especially hepatitis C virus (HCV), hepatitis B virus (HBV) and HIV infections (Ansar, Kooloobandi, 2002; Antipa, 1996; Riaz, 2011).

Preventing these infections is one of the most important goals of management of β thalassemia major especially in developing nations where patients receive compromised treatment owing to financial and logistic constraints (Hassanshahi, 2011).

Although the incidence of transfusion transmitted viral hepatitis has been greatly reduced after the introduction of HBV vaccination and application of reliable procedures for blood donors' screening for HCV (Aach, 1991; Donahue, 1992; Schreiber, 1996; Blajchman, Bull, 1995), HCV infection is common in transfusion-dependent thalassemia**.** This is especially true for counties like Egypt where HCV is more prevalent in general population and therefore also among blood donors. Egypt has a very high prevalence of HCV and approximately 20% of Egyptian blood donors are anti-HCV positive (Lavanchy and McMahon, 2000).

The aim of the present work was to assess the sero-prevalence of HCV and HBV in transfusion-dependant β- thalassemia patients at a single Egyptian center.

Materials and Methods

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This was a cross-sectional study included 435 transfusion dependant β-thalassemia patients carried out over 6 mos (from the beginning of March 2009 to the end of August 2009). All patients were diagnosed as β-thalassemia confirmed by hemoglobin electrophoresis and regularly follow up at the Hematology Clinic, New Children's Hospital (tertiary referral teaching hospital), Cairo University.

The study protocol was approved by Cairo University Children's Hospital and was conducted in accordance with the University bylaws for human research. Patients on regular blood transfusion were enrolled. Regular transfusion is defined as (patients on regular-interval transfusion protocols [for a pretransfusion Hb of ≤ 7.0 g/dL]. The study was explained and consent was obtained from all participants or their legal guardians before enrollment.

The records of all the patients were reviewed and all cases were subjected to full history and clinical examination. Data collected included demographics (age and sex), splenectomy status, onset and rate of blood transfusion and chelation therapy. The vaccination data regarding HBV vaccine were obtained from parents and confirmed by revision of their birth certificates.

After explaining the procedure to the patients 4 ml of venous blood was withdrawn under complete aseptic conditions and divided into; 1ml on EDTA to perform hemoglobin level before transfusion. The other 3 ml left to clot and centrifuged to separate the serum to perform the cross matching, estimation of serum ferritin and to detect hepatitis markers.

Pre transfusion hemoglobin was determined using Sysmex KX-21N hematology analyzer which utilizes the Non-cyanide Sodium Lauryl Sulfate (SLS) method to determine the hemoglobin level.

Serum was separated within 4 to 5 hours of collection, and each sample was transferred into 4 cryovials and stored at -70°C. All samples were tested for anti-HCV by third-generation enzyme immunoassay for anti-HCV IgG (Abbott AxSYM system version 3, Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. This system is a Microparticle Enzyme Immunoassay (MEIA) for the qualitative detection of these markers in human serum. HCV seropositivity was defined by a ratio of optical density to the cut-off value ≥1. Samples with a ratio between 0.8 and 1.0 (gray zone) were retested in duplicate, and those repeatedly ≥1.0 were considered positive.

Qualitative chemiluminescent immunoassay (ChLIA) was used to confirm the presence of hepatitis B surface antigen (HBsAg) in the serum of 406 patients by means of specific antibody neutralization. Seropositivity for HBs Ag was defined by a ratio of optical density to the cut-off value ≥1. Samples with a ratio between 0.8 and 1.0 (gray zone) were retested in duplicate, and those repeatedly ≥1.0 were considered positive. Ferritin levels were performed by one Abbott Axsym using Microparticle Enzyme Immunoassay (MEIA) according to the manufacture's instructions.

Statistical analysis:

Data management and analysis were performed using SigmaStat program; version 3.5 (Systat Software, Inc., USA). The numerical data were statistically presented in terms of range, mean, standard deviation, median and interquartile range (IQR). Categorical data were summarized as percentages. Comparing categorical variables were done by Chi-square test. Spearman Rank Order Correlation was used. All p-values are two sided and considered significant when P-values less than 0.05.

Results:

In this study, 435 β-thalassemia patients were included; 247 males (56.8%) and 188 females (43.2 %) with a male/female ratio 1.3:1. According to patients' age at enrollment, we have 372/435 (85.5%) children ≤18yrs old and 63/435 (14.5%) adults >18yrs old. Among our cases, blood transfusion was started mainly for persistent worsening anemia with hemoglobin level ≤7g/dl. The average pretransfusional hemoglobin was 5.7± 1.2. Hepatomegaly was evident in all patients, and splenomegaly was noted in 222/435 (51%) of cases. A total of 213/435 (49%) patients were splenectomized and the main indications for splenectomy were increased transfusion demand (76.5%) or symptomatic splenomegaly (23.5%). Table 1 illustrates the basic and demographic data of our patients.

Out of 406 patients for whom HBsAg testing was done, 95 (23.4%) were positive. HBV infection was significantly related to patients' age but not to inter-transfusion period or serum ferritin. The prevalence rate was higher among those who didn't receive the compulsory HBV vaccine (30%) when compared to the vaccinated cases (22%) but this difference was not significant. Among our HBV vaccinated cases, we found that the prevalence rate of HBV infection was significantly lower among those who received the vaccine for less than 10 years (18.9%) when compared to those who received the vaccine for more than 10 years (30.4%) and those who didn't receive the vaccine (30%) (table2).

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Table 1: Baseline data of the studied cases (n=435):

Variable	Studied cases (n=435)
Age (yrs): Mean ±SD (Range)	10.1±6.7 (0.8-31)
Age at 1st transfusion (mos): Mean ±SD (Range)	19.436±22.142 (1-156)
Inter transfusion period (wks): Mean ±SD (Range)	4.39 ± 1.92 (1-12)
Pre-transfusional Hb level (gldl): Mean ±SD (Range)	5.7± 1.2 (2.8-9.3)
Serum ferritin (ng/ml): Mean ±SD (Range)	1930.8±1548.4 (96-9849)
Splenic status (n,%): Splenectomized None splenectomized	 213/435 (49%) 222/435 (51%)
Chelation therapy(n,%): Chelated Not chelated	 200/ 435 (46%) 235/435 (54%)

Table 2: Comparison of data of HBsAg positive and HBsAg negative patients (n=406):

	HBsAg positive (n=95)	HBsAg negative (n=311)	p-value
Age (yrs): Median (IQR)	11.1 (6.4-17.3)	7.6 (4.3-13.0)	<0.001*
Inter transfusion period (wks): Mean ±SD (range)	4.4±2.1	4.5±2.2	0.7
Serum ferritin: Median (IQR)	1558 (997-2501.5)	1438.5 (772-2257)	0.1
HBV compulsory vaccine: Received (n= 346) Not received (n= 60)	 77 (22%) 18 (30%)	 270 (78%) 42 (70%)	 0.2
Age (yrs): ≤10yrs (n=244) 10-18yrs (n=102) >18yrs (n=60)	 46(18.9%) 31(30.4%) 18 (30.0%)	 198 (81.1%) 71(69.6%) 42(70 %)	 0.03*

* Statistically significant p-value

Among our cases a statistically significant positive correlation was proved between HBsAg positive state and age (r=0.2, p=0.0001) table 3.

Out of 435 patients enrolled during the study period, 300 (69%) patients had anti-HCV antibodies. The frequency distribution of anti-HCV antibodies seropositivity among different subgroups is illustrated in table 4.

A highly significant positive correlation was observed between HCV sero-positive state and both age and serum ferritin (p=0.0001 and 0.007 respectively) table 5.

Table 3: Correlations of HBsAg positive state and patients' variables (n=406):

	r-coefficient	p-value
age	0.174	0.0001*
Age at 1st transfusion	0.0623	0.213
Transfusion interval	-0.0185	0.710
Serum ferritin	0.0746	0.134

* Statistically significant p-value

Table 4: The prevalence of HCV sero-positivity among subgroups:

	anti-HCV positive	anti-HCV negative	P value
	N (%)	N (%)	
Adults (n=63) Children (n=372)	52 (82.5%) 248 (66.7%)	11 (17.5) 124 (33.3%)	0.021*
Males (n=247) Females (n=188)	158 (64%) 142 (75.5%)	89 (36%) 46 (24.5%)	0.013*
Transfused every <8wks (n=382) Transfused every 8-12wks (n=53)	271(70.9%) 29 (54.7%)	111 (29.1%) 24 (45.3%)	0.025*

* Statistically significant p-value

Table 5: Correlation of HCV sero-positive state and patients' variables:

	r-coefficient	p-value
Age	0.311	0.0001*
Age at 1st transfusion	0.066	0.169
Transfusion interval	0.007	0.877
Serum ferritin	0.128	0.007*

* Statistically significant p-value

Discussion:

Fortunately, HBV infection can be, to a great extent, prevented by active immunization. In Egypt, compulsory immunization has been started in 1992 with one of the recombinant HBsAg vaccines and is delivered as a three dose series at 0, 1, and 6 months and these three doses induce a protective response in more than 90% of healthy adults and children (Rivkina, 2002).

This may not be the case among the high risk populations like thalassemic cases, who proved to have some alterations in the immune system as a consequence of iron overload (Froutan-Pishbijari, 2004) as Keating and Nobel (Keating, Noble, 2003) found that the immunogenicity of Hep-B(Eng) was reduced in patients with conditions associated with impaired immune function. However, Froutan-Pishbijari and co-workers (Froutan-Pishbijari, 2004) found that the protective HBsAb level in thalassemic patients was not significantly different from the healthy subjects, but, it was not a long term evaluation as it was a post vaccination evaluation.

Chang (2006) stated that the annual decay rate of hepatitis B surface antibody (anti-HBs) was 10.2% in children who did not receive a booster dose**.** Thus, immunogenicity of hepatitis B vaccine as well as its long-term protection remains controversial (Mokhles, 2009), especially amongst the high risk population.

Among our study population, the prevalence rate of HBV infection was 23.4 %. This is nearly the same rate reported by Abu El-Hassan *et al*. (1993) who found HBsAg in 23.75% of thalassemics and was lower than that reported recently by Mokhles *et al*. (2009) who reported a prevalence rate of 31.3% positive HBV among high risk population including thalassemics but their high rate may be explained by excluding cases who received the vaccine within 10 years before the study. Our results are much higher than a study from Pakistan (Riaz, 2011) demonstrating a rate of 5.1%.

In addition to the low sero-conversion rate and the vanishing protection with age, persistently high prevalence of HBV infection among thalassemics may be explained by the high prevalence of infection among the general population (and therefore also in the blood donors), as Egypt is considered to be a region of intermediate prevalence for HBV infection ranging from 3.2 (El-Gilany, El-Fedawy, 2006) to 4.3% (Frank, 2000).

A recent study carried out on 174 transfusion dependant β-thalassemia patients at our center reported a prevalence rate 0% (Omar, 2011). This marked discrepancy between their results and ours may be explained by the inclusion of HBV vaccinated cases only in addition to the higher percentage of children included in their study.

Our study showed that HBV infection was significantly related to patients' age but not to inter-transfusion period or serum ferritin and positive correlation was found between HBV infection and age ($r=0.2$, $p=0.0001$) which is in line with previous studies which documented decreased protective antibody titer with age (Dentico, 1992; Peces, Laures, 2001); in addition to the added risk of sexual activity and sexual transmission of the virus among adults.

Among our cases, the overall prevalence rate was higher among those who didn't receive the compulsory HBV vaccine (30%) than those who received the vaccine (22%) but this difference was not significant. This was in line with Singh *et al* (2003) who found that the frequency of HBV infection in β-thalassemic patients was similar in vaccine responders and nonresponders, where they found a number of mutations in the S gene, which could have implications for viral replication as well as virus-host cell interaction. However, we found that the lowest prevalence rate of HBV infection was among those who received the vaccine for less than 10 years (18.9%).

This was in agreement with *Mokhles and associates* (Mokhles, 2009) who tested the protective level of HBsAb and presence HBsAg among high risk population including thalassemics who received the vaccine for more than 10years and found a lower protective titer and a higher infection rate when compared to healthy controls but the difference was not significant which may be attributed to the much smaller sample size of the studied group.

HBV infection among our patients was not related to inter-transfusion period –as a representative of the total of transfusions- or serum ferritin**.** This contradicts with the results of Singh *et al* (2003), who reported that the prevalence of serological markers increased with the number of blood transfusions.

On the other side, no vaccine is so far available against hepatitis C, and the only effective protective measure against this virus is provision of HCV negative blood for transfusion and it's well known that HCV hepatitis is more threatening than HBV hepatitis due to a greater risk of chronic liver disease (William, 1992).

In Transfusion dependant β-thalassemia patients, the prevalence of HCV seropositivity has been observed to vary from 11.1 to 43%, 44.7% and 63.8% in different studies (William, 1992; Rehman and Lodhi, 2004).

Egypt has possibly the highest HCV prevalence in the world as 10%–20% of the general population are infected in the country (Arthur, 1997; Nafeh, 2000; El-Zayadi, 2001; Habib, 2001; Hassan, 2001), with children constituting about 17.5% of HCV-infected Egyptians (El-Zanaty, Fatma and Ann Way, 2009). Studies from upper and lower Egypt revealed that the prevalence of HCV in children is 3 and 9%, respectively (Medhat, 2002; El-Zanaty, Fatma and Ann Way, 2009).

The Egyptian Demographic Health Survey [EDHS] tested a representative sample of the entire country for HCV antibody. The overall prevalence (percentage of people) positive for antibody to HCV was 14.7%. EDHS reported that 9.8% continue to have HCV RNA. That means almost 10% of the total population are infected and are infectious to other people (El-Zanaty, Fatma and Ann Way, 2009).

Among our study population, the prevalence of hepatitis C infection was 69%. This high prevalence rate could be related to the relatively high prevalence of HCV in the general population. Our results are in concordance with previous studies reporting HCV antibody in 74.22% and 73% (Zaki, E.S., 2003) while recent studies showed lower rates of 64% and 51.7% (Ragab, 2010).

In the current study, patients consisted of 372/435 (85.5%) children and 63/435 (14.5%) adults with mean age 10.1±6.7 years. Anti-HCV sero-positivity was 52/ 63 (82.5%) in adults and 248/372 (66.7%) in children (p=0.021) and significant positive correlation was proved between HCV sero-positive state and age (r=0.3, p=0.0001). This may be explained by the added risk of sexual activity and therefore sexual transmission in adults as well as the total number of blood transfusions.

A significant positive correlation was found between HCV sero-positive state and serum ferritin (P=0.007) in agreement line with previous studies (Garrido Serrano, 2001).

To our knowledge, no previous studies related the HCV infection rate to either sex but our results shows that the prevalence of HCV seropositivity was 75.5% among females and it was significantly higher than in males (64%) (P=0.013).

The majority of our patients were transfused every 4 weeks (mean 4.39 ± 1.92 weeks) range 1-12weeks and no correlation was found between HCV infection and the inter-transfusion period. However, we found that the prevalence of HCV infection was significantly higher in patients who received blood at intervals less than 8 weeks than those who received blood at longer intervals. This was in concordance with studies reporting higher risk of HCV viremia with a shorter inter-transfusion interval (Ragab, 2010; Laosombat, 1997).

We concluded that HBV infection is still prevailing among thalassemics especially 10-years post vaccination and booster dose of HBV vaccine may be mandatory and should go in hand with reevaluation of its immunogenicity and long term protection. Also, HCV virus infection is still highly prevailing among those patients and therefore, stricter criteria of safe donor selection have to be adopted to minimize the risk of transfusion transmitted HCV.

Acknowledgment

We thank all β-thalassemia patients who were included in our study. We would like to express all our appreciations to our colleagues, nurses and technicians in the clinical pathology unit and hematology unit who facilitated this work.

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