Original article

Occult hepatitis B in Egyptian thalassemic children

Olfat Shaker[1], Amal Ahmed[2], Inas abdel Satar[3], Hamza El Ahl[4], Wafaa Shousha[5], Wahid Doss[2]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt*
[2]*Department of Medical Biochemistry, National Hepatology and Tropical Medicine Institute, Cairo, Egypt*
[3]*Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt*
[4]*Tabarak Children's Hospital, Cairo, Egypt*
[5]*Department of Biochemistry, Faculty of Science, Helwan University, Helwan, Egypt*

Abstract

Introduction: Thalassemia is hereditary anemia which requires lifelong transfusion as treatment, and hepatitis viral infection is one of the risks of repeated transfusions. Hepatitis B outbreaks in health-care settings are still a serious public health concern worldwide. Blood samples negative for HBsAg but positive for HBV-DNA, with or without the presence of HBV antibodies, are classified as "occult" HBV infection (OBI). This study investigated the prevalence of occult HBV infection in Egyptian thalassemic children.

Methodology: Eighty patients admitted to the Faculty of Medicine, Cairo University Hospital, were involved in this prospective study. Strict inclusion criteria were set to nullify the effect of confounding variables and further minimize selection bias. The following laboratory investigations were performed: complete blood count (CBC); serum AST and ALT; albumin; bilirubin; HBsAg; HBeAg; HBcAb; HCV-RNA; and HBV-DNA.

Results: All our patients had no clinical manifestation suggestive of hepatitis. Molecular biology studies revealed positivity for HCV and HBV at 25% and 32.5% respectively.

Conclusion: The estimated risk of acquiring hepatitis B and C infection in children receiving multiple blood transfusions is surprisingly high. Moreover, occult hepatitis B infection is a considerably risk.

Key words: occult HBV; hepatitis C virus; DNA; thalassemia; pediatrics

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Introduction

Occult hepatitis B (OHB), which is defined as the presence of hepatitis B virus DNA (HBV-DNA) without HBV surface antigenemia (HBsAg), has been a matter of debate for years, but its existence and clinical relevance are supported by Chemina and Trépo [1]. Thalassemias are among the most common genetic disorders in the world [2]. Patients with thalassemia have disturbances in hemoglobin chain production which leads to anemia requiring long-term and multiple transfusions, increasing the risk for transfusion-related viruses, including hepatitis B and C viruses [3]. Though regular blood transfusion improves the overall survival of patients with beta-thalassemia, it carries a definite risk of infection with blood-borne viruses [4]. HBV is resistant to breakdown, can survive outside the body, and is easily transmitted through contact with infected body fluids. HBV is second only to tobacco as a known human carcinogen [5]. Hepatitis B virus (HBV) presents a higher residual risk of transmission by transfusion than hepatitis C virus (HCV) or human immunodeficiency virus (HIV). While most infectious blood units are removed by screening for hepatitis B surface antigen (HBsAg), there is clear evidence that transmission by HBsAg negative components occurs during the serologically negative window period and the late stages of infection. HBV-DNA without HBsAg is a concern for transmission from transfusion or transplantation. Patients from countries highly endemic for HBV are more likely to develop occult HBV infections [1]. OHB may follow recovery from infection, displaying antibody to hepatitis B surface antigen (anti-HBs) and persistent low-level viraemia, escape mutants undetected by the HBsAg assays, or healthy carriage with antibodies to hepatitis Be antigen (anti-HBe) and to hepatitis B core antigen (anti-HBc). Occult HBV may impact in several different clinical contexts, including the transmission of the infection by blood transfusion or

organ transplantation and its acute reactivation when an immunosuppressive status occurs. Moreover, much evidence suggests that it can favour the progression of liver fibrosis and above all the development of hepatocellular carcinoma (6)

The present study was undertaken to investigate the prevalence of occult HBV infection, identified by the presence of HBV-DNA in multiple transfused children with thalassemia and negative HBV surface antigen. We also aimed to correlate the results with hepatitis C infection.

Methodology

Inclusion criteria

This prospective study was conducted in the Department of Medical Biochemistry, Faculty of Medicine, Kasr El Aini, Cairo University, in the period from August 2008 to February 2010. The study protocol was approved by the institutional committee for the protection of human subjects and conformed to the guidelines of the 1975 Declaration of Helsinki. Strict inclusion criteria were set to nullify the effect of confounding variables and further minimize selection bias. The cases were selected according to the following criteria: naïve male or female children (2 to 12 years old) with thalassemia major on chronic blood transfusion therapy. The sample size of this study was 80 thalassemic children.

Patients with clinical signs of acute hepatitis and patients positive for hepatitis B surface antigen were excluded from this study.

The study patients were classified into two groups according to residence: urban and rural regions. Every patient fulfilling the inclusion criteria was allocated to one of two intervention groups. Group 1 included 40 naïve patients from urban regions, while Group II included 40 naive patients from rural regions.

Besides the necessary investigations needed to fulfill the selection criteria, all individuals included in this study were subjected to the following:

Medical history and physical examination

Full history with special reference to risk factors of liver diseases such as previous HCV exposure was taken. Complete medical examination stressing upon the manifestations of hepatitis as jaundice, hepatomegaly, and tenderness in the right hypochondria was performed.

Laboratory investigations

Serum AST, ALT levels, albumin, total, and direct bilirubins were measured by automated spectrophotometric apparatus.

Serum markers of HBsAb, HBeAg, HBcAb, HBs Ab and AFP were done by using a DiaSorin kit, (DiaSorin S.p.A.,Vercelli, Italy) according to the manufacturer instructions.

Serum ANA was measured by using an immuofloursence Diasorin Kit according to the manufacturer's instructions.

Molecular biology tests

Quantitations of HBV-DNA and HCV-RNA in serum were done by using Real Time PCR (Stratagene, La Jolla, USA).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 9.0 (IBM SPSS, Armonk, NY, USA) was used for analysis of data. Data was summarized as mean and SD. Chi Square was used for analysis of qualitative data. Sensitivity, specificity, positive predictive value and negative predictive value of HbcAb, HbeAg and HbsAb were evaluated.

Results

The current study included 80 patients with thalassemia major on multi-transfusion therapy; half of them (40 patients) live in rural areas whereas the other 40 patients resided in urban areas. There were 54 males constituting 67.5% of the study patients. The patients' ages ranged from two to 12 years with a mean of 7.4 ± 3.2. Twenty-eight patients representing 35% of the study group were younger than six years of age and 52 patients representing 65% were older than six years of age.

All our patients had no clinical manifestation suggestive of hepatitis infection. Table 1 shows the laboratory results of our patients with no indicator of hepatitis infection with normal enzymes, direct bilirubin and serum albumin levels. The relatively higher total bilirubin level is attributed to the hemolytic anemia.

All our patients had negative hepatitis B surface antigen, whereas 26 (32.5 %) patients were actually infected as they were positive for HBV- DNA as detected by PCR. From the 26 HBV-DNA positive patients, 24 patients (representing 30% of the whole study group) were positive for hepatitis B core antibodies, 16 (20%) were positive for hepatitis B e antigen, and two patients were positive for surface

Table 1. Demographic and laboratory data of thalassemic patients included in the study

Variables	Minimum	Maximum	Mean ± SD
Age (yrs)	2	12	7.4 ± 3.2
ALT (U/L)	12.0	49.0	24.1 ± 9.6
Total bilirubin	0.5	4.2	1.8 ± 1.1
Direct bilirubin	0.0	0.9	0.4 ± 0.3
Albumin	3.5	4.5	4.1 ± 0.3
AFP	3.3	10.0	6.9 ± 1.8

AST: Aspartate transaminase, ALT: Alanine transaminase, AFP: Alfa feto-protein

antibodies, indicating an overlap of more than one seromarker in some patients. Twenty patients out of the whole study group (representing 25%) were positive for HCV-RNA as detected by RT-PCR. All patients who were HCV positive were also found to have occult HBV infection (HBV-DNA positive) indicating that the source of infection might be the same.

Thirty-four patients (representing 42.5% of the study group) were positive for antinuclear antibodies. By analyzing the relationship between hepatitis B and C infection (proved by PCR test) and the autoantigenicity represented by presence of antinuclear antibody, we found a highly positive association with a P value of 0.001 for each, as shown in Table 2.

As presented in Table 3, both HBV-DNA and HCV-RNA were significantly higher in patients older than six years of age with P values of 0.009 and 0.005 respectively, and this significant association was also consequently elicited with respect to antinuclear antibody (P value 0.05).

By correlating hepatitis infection to patient gender, we found that males had a significantly higher rate of both hepatitis B and C infections and also a higher rate of antinuclear antibody (Table 4).

Hepatitis B core antibody had the highest rate of association with occult infection (92.3% sensitivity and positive and negative predictive values of 100% and 96.4% respectively) while the least related antibody was the surface antibody.

By comparing the results according to residency, we found that HCV infection was equally distributed but occult HBV infection was higher in rural areas; however, the rate was not statistically significant. Furthermore, no significant difference could be elicited between patients residing in urban areas and those having rural residency regarding different markers of hepatitis infection except with respect to infectivity represented by hepatitis B e antigen, which was significantly higher in patients from rural areas with a P value 0.02 (Table 5).

Comparing laboratory results of patients from rural and urban areas, we found significantly higher ALT and AFP in patients residing in rural areas, whereas other clinical parameters such as total bilirubin, direct bilirubin, AST, and albumin levels showed no significant differences.

Discussion

Thalassemia is hereditary anemia with lifelong transfusion as treatment, and although regular blood transfusion improves the overall survival rate of patients with beta-thalassemia, it carries a definite risk of infection with blood-borne viruses, especially hepatitis viral infection [4,7].

Because the prognosis for thalassemia has much improved, with better survival rates, it is mandatory to reduce its morbidity and mortality by recognizing and presumptively treating infections in these patients as quickly as possible [8]. The true burden of HBV infection in the thalassemic population, using the more sensitive molecular detection methods, has not been estimated [9].

Occult HBV may be observed in several different clinical contexts, including the transmission of the infection by blood transfusion or organ transplantation and its acute reactivation when an immunosuppressive status occurs. Moreover, much evidence suggests that it can favor the progression of liver fibrosis and the development of hepatocellular carcinoma [10]. Altindiş *et al*. (11) recommended screening of blood units by sensitive PCR-based methods for occult HBV infections, even if they were negative for HBsAg, to prevent or at least to decrease the transmission risk of HBV infection, which is still an important health problem.

In this study, occult hepatitis B infection detected by HBV-DNA testing constituted 32.4% of thalassemic children, which is similar to results from India [9] where Singh *et al*. reported HBV DNA detection in 23 of 70 (32.8%) thalassemic children with only one positive for HBsAg. The frequency of HBV infection in thalassemia was similar in vaccine

Table 2. Comparison between HCV-RNA and HBV-DNA of thalassemic patients included in the study in relation to ANA

Variables	Negative ANA (46) N(%)	Positive ANA (34) N (%)	P value
HCV-RNA :			
Negative (60)	44 (95.7)	16 (47.1)	0.0001*
Positive (20)	2 (4.3)	18 (52.9)	
HBV- DNA:			
Negative (54)	46 (100)	8 (23.5)	0.0001*
Positive (26)	0 (0)	26 (76.5)	

P-value is significant if < 0.05*

Table 3. Comparison between HCV-RNA, HBV-DNA and ANA of thalassemic patients included in the study in relation to age groups

Variables	< 6 yrs (28) N (%)	> 6 yrs (52) N (%)	P value
HCV- RNA :			
Negative (60)	26 (92.9)	34(65.4)	0.005*
Positive (20)	2 (7.1)	18 (34.6)	
HBV- DNA:			
Negative (54)	24(85.7)	30 (57.7)	0.009*
Positive (26)	4 (14.3)	22 (42.3)	
ANA :			
Negative (46)	20 (71.4)	26 (50)	0.05*
Positive (34)	8 (28.6)	26 (50)	

P-value is significant if < 0.05*

Table 4. Comparison between HCV-RNA, HBV-DNA and ANA of thalassemic patients included in the study in relation to sex

Variables	Males (54) N (%)	Females (26) N (%)	P value
HCV- RNA :			
Negative (60)	36 (66.7)	24 (92.3)	0.01*
Positive (20)	18 (33.3)	2 (7.7)	
HBV- DNA:			
Negative (54)	30 (55.6)	24 (92.3)	0.001*
Positive (26)	24 (44.4)	2 (7.7)	
ANA :			
Negative (46)	26 (48.1)	20 (76.9)	0.01*
Positive (34)	28 (51.9)	6 (23.1)	

P-value is significant if < 0.05*

Table 5. Comparison between laboratory data of thalassemic patients included in the study in relation to residence

Variables	Urban Mean ± SD	Rural Mean ± SD	P value
Age (yrs)	7.2 ± 3.3	7.6 ± 3.0	0.5
AST (U/L)	33.3 ± 6.1	33.7 ± 12.2	0.3
ALT (U/L)	20.8 ± 7.2	27.5 ± 10.5	0.001*
Total billirubin	1.7 ± 1.0	1.8 ± 1.3	0.9
Direct bilirubin	0.3 ± 0.3	0.7 ± 1.7	0.9
Albumin	4.0 ± 0.2	4.1 ± 0.3	0.06
AFP	6.4 ± 1.8	7.5 ± 1.56	0.01*

responders and non-responders. This study showed that the prevalence of occult hepatitis B infection is relatively high in multitransfused thalassemic patients, which refutes the results of previous studies that depend on ELISA detection of hepatitis B surface antigen and show that there was a minor risk for HBV infection in patients with thalassemia [12]. The present results are in agreement with those of Lee *et al*. [13, who concluded that the estimated risk of acquiring hepatitis infection in children receiving multiple blood transfusions is surprisingly higher than generally accepted.A study conducted in Turkey [12] demonstrated that there was a serious risk for HCV infection and a minor risk for HBV infection in patients with thalassemia. Meanwhile, the results of the current study also highlighted the high risk of occult HBV infection that is overlooked by the ELISA test that is used worldwide to denote HB infection.

A recently published study on Egyptian transfusion-dependent beta-thalassemia major children showed that HCV-PCR in liver biopsy was positive in 64% of patients [14]. Others reported 45% of their thalassemia patients positive for HCV-RNA [15]. However, we found only 25% of our group positive for HCV by RT-PCR. This may be attributed to the exclusion of patients with positive hepatitis B surface antigen who are more likely to also have HCV infection. However, our results are similar to those of Singh *et al*. [9], who reported twelve children from seventy (17.1%) had antibodies to HCV. These results differ from those of Rezvan *et al*. [16], who demonstrated that HCV is the most prevalent of transfusion-transmitted infections.

By analyzing the relationship of hepatitis B and C infection (proved by PCR tests) with the autoantigenicity represented by the presence of antinuclear antibody, we found a highly positive association. Ghoneim *et al*. [17] stated that some hepatotropic viruses (HBV and HCV) are capable of triggering autoimmune phenomena and concluded that autoantibodies are commonly found among patients with HCV infection. Gregorio *et al*. [18] also reported anti-nuclear autoantibodies (ANA) levels reached 6% to 21% in patients with viral hepatitis.

In an Iranian multicenter study, 19.3% of their B-thalassemia patients were HCV positive, which coincides with our results [4]. The investigators further concluded that the prevalence of HCV infection is much higher than that of HBV among Iranian beta-thalassemic patients, but they used HB surface antigen to indicate HBV infection, ignoring patients with occult HBV infection, who were the main concern in our study. Other investigators have reported much lower prevalences of anti-HCV in haemophilics (54.5%) and in thalassaemics (5%). HBsAg was detected in 9.09% haemophilics and 5% thalassaemics [19].

Arababadi *et al*. [15] reported that HBV-DNA was not seen in HCV-infected patients. However, they also reported that none of the samples were HBsAg positive but 33% of HCV-RNA positive patients were anti-HBc positive and 40.7% were positive for anti-HBs. The presence of anti-HBs antibodies could be explained by previous immunization; however, the core antibodies indicate previous infection. Occult HBV infection was reported to be highly prevalent in Egyptian chronic HCV adult patients and a significant number of patients with anti-HBc had detectable levels of HBV-DNA in the serum, with HBV-DNA detected in the serum of 22.5% of anti-HBc-positive patients [20].

On the other hand, hepatitis C infection was reported to be very high in thalassemic Iranian

patients, whereas HBV-DNA in these patients could not be detected. This observation is differs from our results, and the discrepancy may possibly be due to the difference in prevalence of these infections in the donating blood pool population as patients from countries highly endemic for HBV are more likely to develop occult HBV infections [21].

Our results showed an increased prevalence of occult HBV infection in patients older than six years of age, who have had a higher number of blood transfusions than younger patients, and these observations are similar to those of many other reports [9,4,14]. In our study, the majority of the patients were males, representing 67.4% of the subjects, which is similar to observations of male preponderance in such infections in other investigations [7,4]. Allain [22] concluded that anti-HBc screening identifies most occult HBV infection but not all, which is similar to our results that showed that HBc antibody has the highest sensitivity and negative predictive value for the presence of HBV-DNA.

Libanore *et al*. [23] stated that the number of chronic hepatitis cases in thalassemic patients was 19.7% following hepatitis B, and data were statistically significant only with regard to differences from ALT. Ragab *et al*. [14] reported a significant positive correlation between mean serum ferritin level (representing iron load from multiple transfusions) and mean levels of ALT and AST.

Comparing variables between rural and urban patients, infection with hepatitis B e antigen was significantly higher in patients from rural areas. Also, ALT and AFP were significantly higher in patients from rural areas although AFP levels were still within normal range.

We conclude that the estimated risk of acquiring hepatitis B and C infection is surprisingly high for children who receive multiple blood transfusions. Moreover, occult hepatitis B infection is a considerable risk. Screening such at-risk patients with surface antigen only is not enough and PCR detection is recommended. Also, blood donors screening for HBsAg reduces, but does not abolish, the risk of transfusion-transmitted HBV infection

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Corresponding author
Olfat Shaker
Faculty of Medicine
Cairo University
Cairo, Egypt
Telephone/Fax: 002-01227449192
Email: olfatshaker@yahoo.com

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