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ORIGINAL ARTICLE

Evaluation of immune status against hepatitis B in children with thalassemia major in Egypt: A single center study

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ABSTRACT

Objectives: Thalassemic children with repeated blood transfusion are at higher risk of suffering transfusion related infections including hepatitis B virus (HBV). HBV vaccine immunogenicity in several studies showed variable response rates. The aim of this study is to evaluate the immunogenic effect of hepatitis B vaccine in thalassemic children at different age groups.

Materials and methods: After ethical approval and informed parent consent, 125 diagnosed thalassemic patients were recruited from the Hematology/Oncology Unit, Pediatric Department, Tanta University Hospital. Patient's transfusion, and vaccination history, clinical data, and blood samples were obtained. Patient's sera were stored at -20°C till tested for Anti-hepatitis B surface (anti-HBs) by ELISA. Patients with titers <10 IU were tested for HBs-Ag.

Results: Although none of our cases had hepatitis B virus infection, only 20.8% had a protective anti-HBs titer (>10 IU/L). Significantly higher percentage of protected patients (40.1%) were younger than 3 years of age, while age groups above 3years showed a significant trend towards having non protective titers (p=0.003). Anti-HBs titers weren't correlated to age, ferritin, liver enzymes, and duration of transfusion or number of transfused packs.

Conclusion: Protective Anti-HBs titer was reduced after age of 3 years in our patients. So, we recommend screening of thalassemic patients at age of 3 years to evaluate the need of a booster dose. *J Microbiol Infect Dis 2012; 2(2): 44-49*

Key words: Thalassemia, HBV, vaccination, immunogenicity

Mısır'da talassemi major hastası çocuklarda hepatit B'ye karşı gelişen immünitenin araştırılması: Tek merkezli bir çalışma

ÖZET

Amaç: Talassemili çocuklar tekrarlayan kan transfüzyonlarından dolayı Hepatit B virusu (HBV) dahil transfüzyonla ilişkili enfeksiyonlara daha fazla maruz kalırlar. Bu çalışmanın amacı değişik yaş gruplarındaki talassemili çocuklarda hepatit B aşısının immünojenik etkisinin araştırılmasıdır.

Gereç ve yöntem: Tanta Üniversitesi Pediatri Departmanı Hematoloji/Onkoloji Ünitesinden talassemili çocuklar çalışmaya alındı. Hastaların transfüzyon ve aşılanma hikayeleri, klinik verileri ve an örnekleri alındı. ELISA ile anti-HBs çalışılacağı zamana kadar hastaların serumları -20°C'de saklandı. Antikor titreleri <10 IU olan hasta serumlarında HBs-Ag arandı.

Bulgular: Hastaların hiç birinin hepatit B virus enfeksiyonu olmamasına karşılık sadece %20,8'i koruyucu anti-HBs titresine sahipti. Üç yaşından daha büyük olanlarda koruyucu antikor titresi düşme eğilimindeydi ve üç yaşından küçük olan hastalarda anlamlı şekilde daha yüksek koruma yüzdesi vardı (%40,1) (p=0,003). Anti-HBs titreleri, yaş, ferritin, karaciğer enzimleri ve transfüzyon süresi veya transfüzyon paket sayısıyla doğru orantılı değildi.

Sonuç: Hastalarımızda koruyucu anti-HBs titreleri üç yaşından sonra düşüş gösterdiler. Bu nedenle talassemili hastaların üç yaşında taranmasını ve ek aşı dozuna ihtiyaç yönünden değerlendirilmesini öneririz.

Anahtar kelimeler: Talassemi, HBV, aşılama, immünojenite

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INTRODUCTION

Thalassemia syndromes are the most common single gene disorder worldwide; about 3% of world population carries ß- thalassemia genes.[1] In Egypt, ß-thalassemia is the most common genetically determined chronic hemolytic anemia with a carrier rate not less than 9%.[2] Beta thalassemia major (β-TM) is an inherited hemoglobin disorder characterized by failure of production of beta hemoglobin chains; resulting in severe chronic hemolytic anemia requiring regular lifelong blood transfusions and daily iron chelation therapy, or stem cell transplantation to survive.[3]

Frequent blood transfusions for patients with thalassemia have improved not only their survival, but also their quality of life. However, it carries a definite risk of being infected with blood borne viruses. Screening for hepatitis viruses was added to the blood donation battery in Egypt (HBV in 1985 and HCV in 1993).[4] But, even in countries where the blood supply is screened for viral hepatitis, the risk of parenteral infection is still significant.[5]

The WHO maps Egypt as an intermediate region for HBV infection (HBV prevalence 2% -7% of the population.[6,7] Studies in Egypt have found HBsAg prevalence to be 4.3%.[8] Hepatitis B virus (HBV) infection acquired during infancy and early childhood is a major cause of chronic liver disease and liver cancer worldwide. Active immunization by administration of hepatitis B vaccination before exposure to the virus is the most effective way to prevent infection and related hepatocellular carcinoma.[9-10] However, there is a large argument about the duration of vaccine protection.[11-12] In Egypt, vaccination against HBV was added to compulsory vaccines in 1992. It is given as three intramuscular injections at ages of 2, 4 and 6 months to all infants.[13] Three doses of recombinant HBV vaccine had been found to induce a protective response (>10 IU/L anti-HBs) in more than 90% of healthy adults and children.[14] Although this vaccine regimen was highly effective and didn't necessitate a booster dose in healthy children, its efficacy was not the same when evaluated in high risk groups.[15] Saberifiroozi et al. studied health care workers as a high risk group and reported decline in their sustained immunity after 10 years and recommended their revaccination.[16] Also Saraswat et al. found up to 50% of repeated transfusion patients to show undetectable levels of the anti-body after vaccination and recommended that they need to be revaccinated.[17] The argument about the need for revaccination against HBV in high risk patients encouraged the conduct of this study to evaluate the immunogenic effect of hepatitis B vaccine in thalassemic children at different age groups.

Materials and methods

One hundred and twenty five patients (74 male and 51 female; with age ranging from 12 months to 16 years) were sequentially enrolled over the period from March 2011 to September 2011. All were thalassemic patients previously diagnosed by complete blood count and hemoglobin electrophoresis, registered at the Hematology/Oncology Unit, Pediatric Department, Tanta University Hospital, and regularly attending the unit for repeated packed cell transfusion. All candidates had received hepatitis B vaccine according to the compulsory schedule of vaccination in Egypt, by three intramuscular injections of 0.5 ml recombinant vaccine at 2,4 and 6 months. Children's medical charts including official child vaccination records were reviewed to ascertain receiving of the primary immunization schedule and to assure that no additional hepatitis B vaccine doses had been received.

All patients were subjected to: History taking with special stress on age at diagnosis, age at start of regular transfusions, inter-transfusion interval, history of splenectomy and complete physical examination. A 5ml venous blood sample was obtained for performance of the following investigations; complete blood counting, estimation of liver transaminases, serum bilirubin, and serum ferritin levels. A blood sample of 2ml was centrifuged at 3000 RPM for at least 20 minutes at room temperature then stored at -20°C till tested for hepatitis B s Ag and antibodies titer.

ELISA kits for hepatitis B surface antigen (anti-HBs) in human serum or plasma samples (Produced by G.B. Lippincott Company, USA) were used for quantitative estimation of anti-HBs titer.

Interpretation of results: According to manufacturer instructions, patients with a titer >10IU/L were interpreted as protected, while patients with a titer <10 IU were considered risky for HBV infection so they were tested for HBs-Ag to detect positive cases which would need confirmation

with PCR. Hepatitis B surface antigen (HBsAg): done by ELISA technique to detect HBsAg in human serum or plasma samples (Produced by G.B. Lippincott Company, USA).

Interpretation of results: According to manufacture instructions, titer<0.9 is negative, >1 is positive, and 0.9-1 is borderline necessitating a confirmatory test.

Statistical Analysis

Statistical Package for Social Sciences (SPSS, ver.10.0) software was used. Chi square for linear trend was used to compare the levels of anti-HBs titer in all age groups, Pearson correlation was used to detect association between anti-HBs and other parameters. For all used tests, P value <0.05 was considered statistically significant.

RESULTS

Among 125 Egyptian thalassemic children investigated, 74 were males (59.2%) and 51 females (40.8%). Their ages at time of data analysis ranged between 12 months and 16 years with a mean age of 7.6 ±4.4 years. Consanguinity was reported in 40% of patients. Depending on patient's individual response, the average blood transfusions in these children was 4-24 times per year.

Clinically, 96% of our cases were jaundiced. Hepatomegaly was present in 99 (79.2%), splenomegaly in 100 (80.0%) and splenectomy had been done in 20 (16.0%). None of our cases had features of liver cell failure or extrahepatic manifestations of HBV infection. The number of children with anti- HBs positive titer >10 was 26 (20.8%) (Tables 1 and 2).

As regards the protective immune response, children aged from 1-3 years showed a higher percent of protection (42.1%). The level of protection varies with age to reach 13.0% for age group 3-6, 17.4% for age group 6-9, and 21.6% for age group 9-16, with a significant trend for decrease of immune protective titer above the age of 3 years (Table 3).

There was no meaningful association between anti-HBs response and age, hemoglobin, ferritin, liver enzymes, duration of transfusion, or total number of transfused packs (Table 4).

Table 1. Clinical and laboratory parameters of studied patients (n=125)

Range	Mean ± SD	Parameter
1-16	7.55±4.4	Age (years)
5-60	16.2±13.49	Age at 1st transfusion (months)
15-90	37.19±13.12	Transfusion interval (days)
4-24	10.73±4.29	Transfused packs (200ml) /year
5-360	67.19±64.11	Total no. of transfused packs (200ml)
550-8000	2671.22±1620.42	Ferritin (ng/ml)
19-107	46.01±16.729	ALT (IU/ml)
18-112	45.11±17.31	AST (IU/ml)

Table 2. Percentage of clinical presentations and laboratory data in the patients (n=125)

Parameter	Frequency	Percentage
Consanguinity	50	40%
Jaundice	120	96%
Hepatomegaly	99	79.2%
Splenomegaly	100	80%
Splenectomy	20	16%
Anti-HBs protective titer	26	20.8%

Table 3. Immune response of thalassemic patients with hepatitis B vaccination (n=125)

Age groups	Total	Non protected (Anti-HBs <10IU/L)	Protected (Anti-HBs >10 IU/L)
	No.(%)	No. (%)	No. (%)
1-3 years(GI)	19 (15.2)	11 (57.9)	8 (42.1)
3-6 years(GII)	46 (36.8)	40 (86.96)	6 (13.04)
6-9 years(GIII)	23 (18.4)	19 (82.61)	4 (17.39)
9-16 years(GIV)	37 (29.6)	29 (78.37)	8 (21.63)
Total	125 (100)	99 (79.2)	26 (20.8)

χ^2=8.700, p=0.003*

Table 4. Correlation of anti-HBs titer to patients' parameters (n=125)

Anti-HBs titer	p	r
Age	0.479	-0.0639
Hemoglobin	0.709	0.0337
Ferritin	0.205	-0.114
ALT	0.149	0.13
AST	0.420	0.07
Duration of transfusion	0.754	0.0283
Number of packs	0.575	0.0506

DISCUSSION

The universal HBV vaccination in infancy has significantly reduced the burden of the disease in high-prevalence populations.[18] Three doses of recombinant HBV vaccine induce a protective response (>10 IU/L anti-HBs) in more than 90% of healthy adults and children.[14] The annual decay rate of hepatitis B surface antibody (Anti-HBs) has been estimated to be 10.2% in healthy children who did not receive a booster dose.[18]

Multiple studies regarding immunity level and duration of acquired immunity from hepatitis B vaccination have been performed in high risk groups with controversial results. Seroconversion reached 80% after 6 years of vaccination in vaccinated thalassemic children in one study,19 and 85% in high risk infants in another study.[20] But, haemodialysis patients have low persistence of the protective titer with only 38% seroprotected 10 years after vaccination.[21] In a study by Azarkeivan in 2009 a booster dose of vaccine increased the protection level among thalassemia patients from 46.9% to 69.4%22. Thus, the matter of boosting remains questionable, especially in high risk groups.

In this study, from 125 vaccinated Egyptian thalassemic children between 1-16 years of age, only 26 (20.8%) had a protective anti HBs levels (titer >10 IU/L), 52 (41.6%) had a titer <1 IU/L, and 47 (37.6%) had titer between 1-10 IU/L. Our results were consistent with an Iranian study done in Kerman on 215 children with major thalassemia, where 34.8% were non responders and the remaining were either low or good responders.[23] Sharifi et al. 2010 in another study reported 89.9% anti-HBs positivity in thalassemic children.[24] However, their results were qualitative. So it doesn't reflect the actual percentile for patients with anti-HBs protective titer.

In an Egyptian study, 48 children subjected to repeated blood transfusion due to different diseases (Thalassemia, sickle cell anemia and leukemia) were tested. Their response to HBV vaccine was significantly lower than response reported for age and sex matched healthy controls. The same study reported 39.6% of those children to have a less than protective titer of Anti-HBs.[25]

In the present study, the highest percentage of immune protected patients (42.1%) was reported in children below 3 years of age. There was a sharp decline in percentage of protected children among patients aged between 3-6 years (13.0%), but this decline was attenuated with the advancement of age to 21.6% having a protective titer in age group 9-16 years. This sharp decline was previously reported even in healthy children as protective serum Anti-HBs titer was reported in 75% of children within 2 years of vaccination and decreased to 48.2% 7 years post vaccination in Chinese children [26]. In Alaskan children receiving recombinant HBV vaccine (same used in Egypt), Anti-HBs titer >10 IU was detected in 29% of children versus 14% of adolescents before booster dose administration.[27]

An Egyptian study performed in Ismailia governorate found the percent of good responders (Anti-HBs titer>100 IU) to decline from 75.3% one month after vaccination to 28% after one year follow up of the same patients. The protective titer (>10 IU) in that study was reported in 93.7% after one month versus 82% after one year follow up.[28] This antibody titer decline can explain the significant difference in antibody titer reported in our study between children less than 3 years of age and other age groups.

The limitation of our results is lack of records for the patient's initial response just after vaccination. So we could not avouch the exact cause of lacking protective level after the age of 3 years. This low titer may be due to decline of previously formed anti bodies or initial post vaccination poor response.

Most of our patients lacked protective anti-HBs titers. However, none of our patients was HBsAg positive. Our results were in accordance with results reported by Sharifi et al. 2010 who reported 1.0% of thalassemic Iranian patients to be

HBsAg positive but PCR negative for HBV DNA despite more than 10% of their patients having negative Anti-HBs.[24] Also, Samandari et al., who reported no case of HBV infection among 166 Alaskan vaccinated children and 138 vaccinated adolescents despite the presence of a non-protective Anti-HBs titer in 71%, and 86% of them respectively.[27] Our results are in accordance with those of Ocak et al. who reported only 3 out of 399 Turkish thalassemic patients to be HBsAg positive (0.75%).[29]

In Egypt, there has been a decline in the incidence of HBV infection in multiple-transfused patients in recent years due to some precautions taken, such as improvement of blood screening procedures, use of disposable equipment for invasive procedures and initiation of free routine immunization for all infants. Relevant studies conducted in the Hematology Clinic, New Children's Hospital, Cairo University Egypt, on thalassemic patients detected HBsAg positivity in 16.5% in 1993, but only one out of 50 thalassemic patients (2%) was HBsAg positive in year 2009.[30] However, our results were different from those of Mokhles et al. who reported 31.3% positivity for HBs-Ag in repeatedly transfused patients.[25] This variation may be related to heterogeneity of their patients as some of them had hematological malignancies, and it is not mentioned if those patients were or were not immune suppressed due to chemotherapy at time of study.

Except for this variant, our results were consistent with Mokhles et al., who concluded that high risk groups of children with repeated blood transfusion proved to become significantly risky for HBV infection secondary to loss of Anti-HBs protective titer after a variable period of time. And we can add that this significant drop occurs above the age of three years.[25]

From this study, we can conclude that: HBV infection is not common among vaccinated thalassemic patients in Egypt, even in those with titers below 10 IU/L. Both the percentage of protected children and protective titer levels are reduced in patients above 3 years of age. So we recommend screening of thalassemic patients for Anti-HBs titer at age of 3 years to evaluate the need of a booster dose.

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Conflicts of interest: None declared.

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