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Original article

Antibody response to hepatitis b immunization in Egyptian children with sickle cell disease

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***Background**: Despite improvement in the safety of blood products, sickle cell disease (SCD) and thalassemic patients are at greater risk than the general population for hepatitis B infection and chronic liver disease, making hepatitis B immunization especially important for this population. This study was conducted to evaluate and follow up the antibody response to hepatitis B vaccination, in patients with SCD, after 1-15 years of vaccination. **Methods**: participants were 30 SCD and 30 thalassemic patients attending the Hematology Department, Children's Hospital, Cairo University as well as 30 ages and sex matched normal controls. They were subjected to clinical evaluation, complete blood count, and measurement of liver transaminases, serum bilirubin, and serum ferritin levels as well as estimation of anti-HBs titer by enzymatic immunoassay. **Results**: Anti-HBs titers in SCD patients ranged between 5.6 and 381 IU/L (54.83 ± 15.30), while the levels of thalassemic patients ranged between 16 and 343 IU/L (93.4 ± 30) and those of the control group ranged from 10 to 523 IU/L (83.4 ± 28.1) which revealed statistically significant decrease in SCD patients compared to thalassemic and healthy controls (p =0.0317). Out of the 30 SCD patients, 40% showed anti-HBs titer below 10 IU/L (non-protective titer), while none of the thalassemic patients or the control group revealed the same. Achievement of a protective titer had no correlation with sex, consanguinity, or any of the clinical or laboratory data tested. **Conclusion**: Immune dysfunction in thalassemia is not playing a major role in response to hepatitis B vaccination. However, SCD children should have their anti-HBs titer measured after routine hepatitis B immunization to ensure that they achieved protective titer, then after 1 year of vaccination and repeated every 5 years and those who do not seroconvert should receive additional doses. Booster HBV vaccination of unprotected SCD patients seems mandatory.*

Key words: sickle cell, immunity, hepatitis B, immunization.

INTRODUCTION

Most patients with SCD will be transfused with red blood cells during their lives for the treatment or prevention of SCD related complications. Despite improvement in the safety of blood products, SCD patients are at greater risk than the general population for hepatitis B infection and chronic liver disease, making hepatitis B immunization especially important for this population[1]. In addition, SCD is associated with impaired humoral responsiveness caused by splenic hypofunction[2]

Hepatitis B virus (HBV) infection acquired during infancy and early childhood is a major cause of liver disease and liver cancer worldwide[3]. The World Health Organization has recommended that all infants receive hepatitis B vaccination[4,5]. In Egypt, vaccination against HBV is compulsory since 1992 by three intramuscular injections into the deltoid muscle of 0.5 ml of vaccine at age of 2, 4 and 6 months. Substantial reductions in the prevalence of chronic infection and the incidence of acute hepatitis B have been demonstrated among children in populations where routine infant hepatitis B vaccination has been implemented[6-9]. However, the duration of protection after infants are vaccinated is unknown[10,11].

The argument about the need of revaccination against HBV at 5 years interval encouraged us to conduct this work to explore the validity of the protective titer and the need for booster dose in such immunodeficient patients. The aim of this study was to evaluate and follow up the antibody response to hepatitis B vaccination in these pediatric SCD patients after 1 to 15 years of starting obligatory vaccination schedule.

METHODS

Patients were consecutively enrolled among sickle cell disease and thalassemic patients previously diagnosed by complete blood count and HB electrophoresis and attending the Hematology Department, Children's Hospital, Cairo University after fulfilling the inclusion criteria. All candidates had received hepatitis B vaccine according to the compulsory schedule of vaccination in Egypt, by three intramuscular injections of 0.5 ml of the recombinant vaccine at 2, 4 and 6 months.

Inclusion criteria: All SCD patients were in a quiescent state.

Exclusion criteria: SCD patients suffering from vaso-occlusive crisis, acute infection, dehydration and those on immunosuppressive medications for treatment of any associated pathology at the time of study recruitment .

Participants were enrolled after obtaining an informed consent from each child's parent/guardian.

The study included 30 pediatric sickle cell disease patients. Their age ranged from 2 to 15.6 years (7.8±0.7), they were 14 males (46.7%) and 16 females (53.3%). They were compared to 30 thalassemic patients (mean age 9.2±1.5) and 30 age and sex matched subjects as a control group. Children's medical charts including birth records were reviewed to ascertain receival 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, clinical examination, complete blood counting with manual differential, and estimation of liver transaminases, serum bilirubin, and serum ferritin levels as well as measurement of anti-HBs titer.

Specimen collection: The blood specimen collected by venipuncture, allowed clotting. Serum was then separated by centrifugation at 1500 rpm and kept frozen (-20 degree or below) until used. Grossly haemolyzed samples as well as those containing particulate matter or exhibiting obvious microbial contamination were excluded.

Quantitative determination of serum ferritin level was carried out for patients and controls by Abbot Axsym system (Abbot diagnostic division. Longford Co., Ireland). Quantitative Estimation of anti-HBs antibody titer done by enzyme immunoassay technique (EIA) (AUSAB; Abbott Laboratories, Abbott Park, IL, USA)

Principle of enzyme immunoassay: The technique is used for quantitative determination of anti-HBs titer in serum. Anti-HBs from sample is added to a well coated with HBsAg, incubated for 2 hours at 37 degree then washed, after washing enzyme tracer is added, incubated for 1 hour at 37 degree then washed. The presence of anti-HBs allows the enzyme tracer to bind to the solid phase. The enzyme activity is therefore proportional to the concentration of anti-HBs present in sample or calibrators. Enzyme activity is measured by adding a colorless chromogen/substrate solution. The enzyme action on it produces a color which is measured with a photometer.

Interpretations of results: It is generally accepted that an anti-HBs concentration above 10 IU/L is indicative of a protective titer or positive response to vaccination. Antibody concentration below 10 IU/L is indicative of absence of acquired immunity[12].

Statistical analysis: Statistical analysis was conducted using Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science). Data were statistically described in terms of mean ± standard error (± SE) and frequencies (percentages) when appropriate. Comparison of quantitative variables between the study groups were performed using the Mann Whitney U test and (ANOVA) tests. For comparing categorical data, Chi-square (χ^2) test and Yates correction equation were used. A probability value (p value) less than 0.05 considered statistically significant.

RESULTS

Descriptive and frequencies of clinical data of sickle cell and thalassemic patients are illustrated in Table (1, 2) respectively.

Concerning iron chelation, deferoxamine was used only in 5 sickle cell (16.7%) and 6 thalassemic patients (20%) while deferiprone was used in 2 sickle cell (6.6%) and 18 thalassemic (60%) patients**.**

Anti-hepatitis B surface (Anti-HBs) titers in sickle cell patients ranged from 5.6 to 381 IU/L (54.83 ± 15.3), while in thalassemic patients it ranged between 16 and 343 IU/L (93.4 ± 30) and in the control group it ranged between 10 and 523(83.4 ± 28.1). Comparison between the 3 groups shows statistically significant decrease ($p<0.05$) in SCD patients as compared to the other 2 groups (Table 4).

Out of our 30 sickle cell patients, 12 (40%) were below the protective level against hepatitis B infection i.e. below 10 IU/L. while none of the thalassemic patients or the control group revealed the same **.**

In our study, the frequency of protected SCD patients tended to increase with age (Table 5).

Table 1. Descriptive clinical data of sickle cell and thalassemic patients

Variables	Sickle cell patients			Thalassemic patients		
	Mean	SE	SD	Mean	SE	SD
Age (yr)	7.8	0.7	4.1	9.2	1.5	4.8
Age at presentation (yr)	1.8	0.3	1.4	1.4	0.4	1.1
Age at first blood transfusion(yr	2.2	0.3	1.7	1.4	0.4	1.1
No. of blood transfusion / year	5.3	0.7	3.7	9.4	1.2	3.8
No. of crisis / year	3.8	0.5	2.7			
No. of attacks of fever / year	3.4	0.4	2.1			
No. of hospital admission	3.3	0.6	2.9			

Table 2. Frequencies of clinical data of sickle cell disease and thalassemic patients.

Variables	Percentage of sickle cell patients	Percentage of thalassemic patients
Sex:		
- Male	14	18
- Female	16	12
Consanguinity	21 (70%)	12(40%)
Family history	15 (50%)	15(50%)
Spleen		
- Non-palpable	19 (63.3%)	3 (10%)
- Splenomegaly	5 (16.7%)	6 (20%)
- Splenectomized	6 (20%)	21 (70%)
Hepatomegaly	22(73.3%)	24 (80%)
Vasocclusive crisis		
- Acute abdomen	6 (20%)	
- Back pain	11 (36.7%)	
- Dactilitis	18 (60%)	
- Bony aches	25 (83.3%)	
Causes of Hospitalization		
- Painful crisis	21 (70%)	
- Fever	9 (30%)	

Table 3. Descriptive laboratory data of sickle cell and thalassemic patients.

Variables	Sickle cell patients				Thalassemic patients			
	Range	Mean	SE	SD	Range	Mean	SE	SD
RBCs x million /ul	2-4.5	2.8	0.1	0.6	2.5-3.6	3.1	0.1	0.4
Hb /dl	5.1-11	7.6	0.3	1.4	6-8	6.9	0.2	0.6
HCT (%)	16.4-30	22.8	0.6	3.2	18-27	22.5	0.9	2.7
MCV (fl)	60.9-113	83.4	1.9	10.6	53-68	61	1.9	5.9
WBCs/thousand/ul	3.5-33.3	12.3	1.2	6.8	3.9-11.2	7.4	0.7	2.2
Neutrophils %	35.5-75	53.1	1.8	9.7	32-60	45.5	3	9.5
Lymphocytes %	18-60	39.7	1.7	9.3	37-64	50.7	2.9	9.2
Platelet thousand/ul	130-698	320.3	29.2	160.1	250-1250	688.8	97.6	308.8
Serum ferritin/ug/dl	134-3060	1072.2	312.7	1188.2	530-3480	1639	280.3	886.4
Total bilirubin/mg/dl	0.6-3.7	1.8	0.2	1.1	0.8-4.8	2.6	0.4	1.4
Direct bilirubin (mg/dl)	0.04-2.07	0.8	0.3	1.4	0.2-0.8	0.5	0.07	0.2

HCT: hematocrit; MCV: mean corpuscular volume; RBCs: red blood cells; WBCs: white blood cells; SD: standard deviation; SE: standard error.

Table 4. Comparison between anti-HBs titer of SCD patients, thalassemic patients and controls.

	SCD patients No. = 30 Mean ± SE	Thalassemic patients No. =30 Mean ± SE	Control No. = 30 Mean ± SE	P-value
Anti-HBs titer	54.83 ± 15.3	93.4 ± 30	83.4 ± 28.1	0.0317

P< 0.05

Table 5. Relation between age of SCD patients and anti-HBs titer.

	Below 5 years No.=11	5 to 10 years No.=11	Above 10 years No.=8
Protected Anti-HBS ≥10 IU/L	5 (45.4%)	7 (63.6%)	6 (75%)
Unprotected Anti-HBS <10 IU/L	6 (55.6%)	4 (36.4%)	2 (25%)

There were a tendency towards a positive correlation between anti-HBs titer (IU/L) in sickle cell patients and neutrophilic count, and negative ones between anti-HBs titer (IU/L) in these patients and age of first blood transfusion, number of attacks of fever per year, number of hospital admission per year and serum ferritin level (ug/dl). However, these correlations did not reach statistically significant so they were denied.

DISCUSSION

Our results indicate that after starting obligatory vaccination, the antibody response to hepatitis B vaccine is significantly lower in patients with SCD, compared to thalassemic patients and the normal population. This is demonstrated in two ways. Statistically significant low anti-HBs titer was observed in SCD patients compared to thalassemic patients and control groups p=0.0317 (Table 4). In addition, out of our 30 sickle cell patients, 12 (40%) patients lie below the protective level against hepatitis B infection i.e. below 10 IU/L. while none of the thalassemic patients or the control group demonstrated such finding.

In SCD, the presence of additional factors contributing to immune deficiency may offer an explanation. The immunological response in SCD patients may be different; this disease is associated with frequent and often severe infections because of immune function impairment and functional asplenia[13]. This immunologic dysfunction associated with SCD is documented in literature. The most common two diseases associated with the development of hyposplenism are sickle cell anemia and celiac disease. Hyposplenism is relatively easy to recognize by typical changes observed on the peripheral blood smear; including Howell-Jolly bodies, monocytosis, lymphocytosis, and increased platelet counts. Diagnosis can be confirmed by 99Tc-labelled radio colloid scan of the spleen; wherever available[14].

A prospective study conducted in Qatif (Eastern Province of Saudi Arabia) to study the splenic function among (SCA) patients, using (99m)Tc stannous colloid liver-spleen scan during the steady state proved that: 70% of patients had evidence of splenic hypofunction, and most of them (83%) had severe hyposplenism[15]. In addition, splenic function studied in 72 Omani SCD patients according to their colloid uptake revealed that, 20 (28%) had severe hyposplenism; and 26 (36%) patients had functional asplenia[16]. A Kuwaiti study demonstrates that the phagocytic function of the spleen, which is tested by colloid uptake, is the first to be lost while the filtration function, tested by denatured RBC uptake, persists for much longer[17].

This sequence of loss of spleen function may explain the tendency of hyposplenic subject to develop fatal invasive disease. Overwhelming pneumococcal sepsis accounts for most cases of mortality in hyposplenic subjects[14]. Inhibitors of normal T lymphocytes appear to be present in a significant percentage of SCD sera, which inhibits normal lymphocyte response to phytohemagglutinin (PHA) in vitro even during the steady state of disease[18].

Similar to our results is a study conducted in the Children's Hospital Medical Center of Akron, Ohio on 150 children with SCD. It observed that 89% of SCD children are seroprotected in comparison to 97% in the general population (P=0.002). The authors concluded that children with SCD do not respond to hepatitis B immunization as efficiently as the general population. They were unable to clearly

demonstrate a correlation between age, sex, or SCD subtype and humoral responsiveness[19].

On the contrary, a study on the immunization status of 30 SCD patients, 5 years post hepatitis B vaccination, was conducted by the University of West Indies in St Augustine, Trinidad and Tobago. It revealed that there was no significant difference between SCD patients and normal controls in the levels of antibody or in the numbers that required booster vaccination[20].

Our study revealed that thalassemic patients and normal children could maintain a protective level of anti-hepatitis B surface antibody. These results are supported by relevant studies conducted in the Hematology Clinic, New Children's Hospital, Cairo University on thalassemic patients. In 1993, the HBsAg positivity detected was 16.48%[21] while recently in 2009 a study that included 50 thalassemic patients revealed that hepatitis B virus infection occurred in one patient only (2%)[22]. These results reflect the protective impact of the obligatory hepatitis B vaccination, which started in Egypt since 1992.

An Egyptian study assessing the long-term immunity to hepatitis B among 720 healthy 10 years children, who were vaccinated in infancy, concluded that up to 10 years, booster doses are unnecessary possibly due to protective anamnestic response to antigenic challenge[23]. An Italian study noted that, a strong immunological memory persists more than 10 years after immunization of infants and adolescents with a primary course of vaccination. Booster doses of vaccine did not seem necessary to ensure long-term protection[24]. A study conducted on 1355 Saudi students showed the excellent efficacy of the HBV vaccination program in Saudi Arabia 18 years after its launch[25].

On the contrary, another recent Egyptian study assessed the long-term immunity to hepatitis B among 242 Egyptian children aged 6-12 years who had received a full vaccination course in infancy. It showed that only 39.4% had protective hepatitis B surface antibodies titer[26]. Also, a study conducted in Taiwan that involved 5875 new university entrants concluded that a booster dose of HB vaccine, given 18 years following HB vaccination, perhaps even earlier, should be considered[27].

However, certain percentage of vaccinees will be non responsive or hypo responsive to HBV vaccination; this is specially true for patients with chronic liver disease[28], renal disease or haemodialysis (HD), since these patients lose hepatitis B immunity both after natural infection or vaccination[29]. In children with cancer, the immune response maintains a protective level of anti-HBs in about 60% of cases after vaccination, despite immunosuppression[30]. In this study, it is surprising that the SCD patients who were vaccinated since 5 years showed a higher incidence of low titer (unprotected) compared to those vaccinated since 10 years or more (Table 5). This might be explained by the fact that repeated exposures to HBV through multiple blood transfusion stimulated immune memory by natural boosting[31] which is more likely common with increasing age, however the factors that stimulate the immune mechanism in some children and not in others are still the field of extended studies. The findings are also limited by the sample size.

In Conclusion, Sickle cell disease patients need follow up of their immune response to HBV vaccination; since in our study up to 40% did not achieve protective titer against HB. On the other hand, the immune dysfunction in thalassemia does not seem to play a major role in response to hepatitis B vaccination.

We recommend that, anti-HBs titer should be measured after routine hepatitis B immunization then after 1 year of vaccination and every 5 years in SCD patients. Those who do not seroconvert should receive additional doses of the hepatitis B vaccine. Revaccination of the unprotected patients is mandatory and must be followed carefully till the protective immune level is achieved.

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