Immunity level to diphtheria in beta thalassemia patients

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Abstract

Introduction: Beta thalassemia major which is very common is a principal health problem in Iran. These patients are more often affected by several infections. The aim of the study was to determine the immunity of patients with beta thalassemia major, to diphtheria.

Methods: In this case-control study, anti-diphtheria toxin antibody concentration and serum ferritin levels were compared in 224 patients with thalassemia major and in 224 sex and age matched healthy subjects as control group. The serum concentrations of antibody and ferritin were determined by ELISA and CLIA methods, respectively. Subjects who had diphtheria antibody level >0.1 IU/ml were seen to have complete protection, between 0.1 and 0.01 IU/ml as partial protection and <0.01 IU/ml as no protection. For the analysis we used SPSS version 15 software. A two sided P-value less than 0.05 was considered statistically significant.

Results: The mean serum anti diphtheria antibody level was lower in patients with beta thalassemia major than in healthy subjects (1.51 ± 1.60 vs 2.10 ± 1.86, p<0.001). Seventy percent and 20.0% of patients and 87.9% and 12.1% of healthy subjects had complete and partial protective serum anti diphtheria level, respectively (p<0.001). Only 24.1% of anti-diphtheria antibody (IgG) was dependent to serum ferritin level in patients group (P< 0.001). Thus serums anti diphtheria antibody decreased 0.001IU/ml, when serum ferritin increased 1ng/ml.

Conclusion: In conclusion, patients with beta thalassemia major had lower anti-diphtheria antibody level than healthy subjects. Thus monitoring immunization status and recommendations for vaccine are essential for increased serum anti-diphtheria antibody concentration.

Key words: diphtheria, antibody, thalassemia, immunity

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Introduction

Beta thalassemia major which is very common (1), is a central health problem in Iran (2) with at least 800 new cases every year (1). These patients are more often affected by several infections. It is suggested a chief defect in the host defense can be caused by iron overload, splenectomy and repeated transfusion (3).

Susceptibility to bacterial infections increased in splenectomized subjects (4). Lifetime risk of developing an overwhelming post splenectomy infection and mortality rates is 1–5% and 40-70%, respectively. The spleen plays a role in the maintenance of a pool of memory B cells involved in the protection against bacterial infections (5).

According to the latest figures released by the World Health Organization, 4,530 cases of diphtheria have been diagnosed worldwide and 28 of those cases occurred in Iran in 2015 (6). The diphtheria surveillance in Iran was done according to national protocol (7, 8).

In Iran, every subject under 7 years old routinely receives 5 doses of diphtheria-pertussis-tetanus (DPT) vaccine (at 2, 4, 6 and 18 months and 4 years of age) and patients and the healthy subjects have been vaccinated against diphtheria consistent with this program.

Although survival of children with beta thalassemia major has improved, both medical therapy and the disease causes immunodeficiency (9). But others have indicated that there is no significant change in humeral immune markers in patients with beta thalassemia major (10, 11). One of the most beneficial and cost effective measures for prevention of infectious diseases, especially diphtheria, is immunization (12). Immunity to diphtheria decreases with advancing age (13). Kruger et al (14) and Xu et al (15) showed that the antibody levels of diphtheria decreased over time.

Median antibody level for diphtheria was higher in patients with acute lymphoblastic leukemia than in the control group (0.202 IU/ml vs 0.071, $p<0.001$) (16). In a study, only 56.8% of patients with hematologic disorders had completely protective levels of diphtheria antibody, that was lower than healthy children (88.3%) (17). Also, more than 50% patients after anti neoplastic therapy (18) and more than 83% dialysis patients (19) had lacked protective immunity for diphtheria.

Previous studies described that 56.8% of patients with hemato-oncologic illnesses (17), 17% of patients with acute lymphoblastic leukemia (20), 82% of pediatric patients with sarcoma after antineoplastic therapy (21) and 70% in Indian pre-school children (22) had completely protective immunity. Loss of immunity to previous vaccinations, necessity and timing of re-immunization remains controversial.

Antibody level of diphtheria decreased over time and also the subjects with hematologic diseases especially thalassemia major does not respond well to immunization, because of iron overload. Thus the aim of this study was to compare diphtheria antibody levels in patients with beta thalassemia major and healthy subjects.

The general objective of the current study was to investigate the immunity of patients with beta thalassemia major to diphtheria. The specific objectives were to determine the immunity of patients with beta thalassemia major to diphtheria and healthy people according to the age, gender, serum ferritin level, splenectomy and non-splenectomy status and post splenectomy time.

Material and Methods

Patients and controls had been previously vaccinated according to the Iranian national vaccination program. During 2010-2011, two hundred and twenty-four patients with major beta thalassemia referred to thalassemia ward of hospitals in Jahrom and in Bandar Abbas, Iran, enrolled in this study. Patients included 109 subjects with spleen and 115 individuals without spleen. Also, 224 healthy subjects that were similar for sex and age were considered as the control group.

Blood samples for determination of diphtheria antibodies and ferritin were collected from both groups. Five milliliters of venous blood were obtained from participants. Serum samples were stored at -80 degree centigrade until analyses were performed.

Serum antibodies against diphtheria toxin were determined using an ELISA kit (IBL, Germany). Results are expressed as international unit (IU). Serum diphtheria antibody levels greater than 0.1 IU/ml were considered as complete protection, titers 0.01 to 0.1 IU/ml were considered as partial protection and titers less than 0.01 IU/ml were interpreted as non-protection (23). Serum ferritin levels were also measured using CLIA method (Chemi-Luminescent Immunoassay Technology, Liasion, Italy, REF 313, 551).

The information and data about the patients were extracted without name by using codes and were kept confidential. This study was approved by the Research Ethics Committee of Jahrom University of Medical Sciences (ethic code: JUMS.REC.1389.65.1).

Data are presented as median ± standard deviation and percent. We used the independent-samples t-test, One-way ANOVA and chi square test to compare the means and percent in the two groups (beta thalassemia patients and healthy subjects). For the relation of antibody titers with age and serum ferritin, we used backward linear regression test. Also, the backward linear regression analysis was used for relation of antibody with ferritin and age. For the analysis we used SPSS version 15 software. A two sided P-value less 0.05 was considered statistically significant.

Results

Totally 50.9% (114) of healthy people and 50.9% patients were females. Also, the mean age of subjects between two groups was no different (p= 0.633, Table 1). Among the thalassemia group, 56.5% (65) of non-splenectomized subjects and 45.0% splenectomized subjects were female (p=0.083). The splenectomized subjects (6.85 ± 1.69 years) were younger than non-splenectomized subjects (7.37 ± 1.71 p= 0.023).

Serum ferritin level in healthy subjects was much lower than patients (p<0.001, Table 1), but there was no significant difference between the two patient groups, splenectomized (832.11 ± 568.08) and non-splenectomized (981.37 ± 592.99, p= 0.056). Serum anti-diphtheria antibody level in the healthy group was higher than in patients group (p< 0.001, Table 1).

Table 1: The mean and standard deviation of age, serum anti diphtheria and ferritin level in the two study groups

Variables	Healthy subjects, 224		Beta thalassemia major, 224		p value
	Mean	SD	Mean	SD	
Age, year	7.04	1.64	7.12	1.72	0.633
Anti-diphtheria antibody, IU/ml	2.10	1.88	1.51	1.59	< 0.001
Serum ferritin (ng/ml)	64.47	65.41	908.74	584.49	< 0.001

Although, serum antibody level against diphtheria was higher in splenectomized patients (1.60 ± 1.76) than in non-splenectomized ones (1.43 ± 1.43), there were no significant difference (p=0.448).

All participants had protective serum anti diphtheria antibody level (partial or completely protected). One hundred and ninety-seven (87.9%) of healthy individuals were completely protected against diphtheria which was significantly higher than patients (71.0%, p< 0.001) (Table 2).

Table 2: The percent of protective immunity level against diphtheria in the two study groups

Variables		Healthy subjects, 224		Beta thalassemia major, 224		p value
		Number	Percent	Number	Percent	
diphtheria immunity level groups	Partial protection (0.01 to 0.1 IU/ml)	27	12.1	65	29.0	< 0.001
	Complete protection (> 0.1 IU/ml)	197	87.9	159	71.0	

Although, the percent of complete anti-diphtheria protection was higher (75.2%) in splenectomized patients than in non-splenectomized patients (73.9%), there was no significant difference (p= 0.821).

Table 3 shows the relation between diphtheria antibody level with age and serum ferritin by backward linear regression analysis. In this model, age did not affect the relationship.

Table 3: Relation of anti-diphtheria antibody titers to serum ferritin levels in study groups

Groups		R Square	Adjusted R Square	B Coefficients		
				Constant	Ferritin	p value
Healthy subjects		0.018	0.014	2.35	-0.004	0.046
Beta thalassemia major	All patients	0.241	0.237	2.73	-0.001	< 0.001
	Non splenectomized	0.308	0.302	2.75	-0.001	< 0.001
	Splenectomized	0.191	0.183	2.72	-0.001	< 0.001

This analysis showed that only 24.1% anti-diphtheria antibody (IgG) alterations was dependent to serum ferritin level in the patients group (p<0.001), while, in healthy subjects only 1.8% of anti-diphtheria antibody was dependent to serum ferritin (p=0.045). The level of anti-diphtheria antibody decreased with intensification of ferritin level. Thus, when serum ferritin increased 1 ng/ml among patients, serum diphtheria antibody decreased 0.001 IU/ml. This model is better predicted in non- splenectomized patients; with about 31% of the serum anti-diphtheria antibody which is predictable by knowing the serum ferritin level (p<0.001).

Discussion

Although survival of subjects with beta thalassemia major has improved, both treatment modalities and the underlying disease may effect in secondary immunodeficiency. Thus these patients are at risk of attaining a variation of infectious diseases. We therefore assessed the serologic immunity against diphtheria in patients with beta thalassemia major and compared to healthy subjects.

Our study found that the mean of serum diphtheria antibody was lower in patients with beta thalassemia major than age and sex-adjusted healthy subjects. But for ferritin level it was the reverse, in other words, the level of serum ferritin was much higher in healthy subjects than in patients. Also, less percent of patients had complete antibody protection against diphtheria as compared to healthy subjects.

From the 1980s serological research showed that a high percentage of subjects become susceptible to diphtheria with advancing age. This may be due to a decrease in the level of antibody in individuals over time. Chatchatee et al in the Thai population demonstrated that subjects aged between 5 and 9 years had the highest titer of tetanus antibody, and subjects above 60 years of age had the lowest titer (24). In the present study to eliminate the effect of time and sex on the antibody levels; we used sex and age-adjusted healthy subjects as controls for patients.
We found no similar research that compared diphtheria antibody among patients with beta thalassemia major and healthy subjects.

Our study showed that the diphtheria antibody level and the percent of complete protection antibody were lower in patient groups than controls. Jahromi and Rahmanian (25) reported that mean anti-tetanus antibody titers (1.53 ± 1.71 vs 2.02 ± 2.05) and the complete protective level of anti-tetanus antibody (71% vs 87.9%) were lower in patients with beta thalassemia major in comparison to healthy persons. Also Modarresi et al reported that the patients on dialysis had less protective levels of anti-diphtheria than normal populations (19).

In the present study seventy-one percent of beta thalassemia patients were completely protected against diphtheria. Our finding is in contrast with other published results. Kown et al (17) reported that 31.5% of 146 Korean children with hematologic malignancies aged 1-17 years were completely protected against diphtheria. Also, van der Hardt et al (18), Ek et al (20) and Small et al (26) found that less than 50% patients against diphtheria, 17.0% of ALL patients against diphtheria and less than 70% of peripheral blood stem cell transplantation recipients against pertussis had complete immunity, respectively. Similarly, complete protection anti-diphtheria antibody was found in our study to be more than anti tetanus titers that were reported by Aminzade (27), Modarresi et al (19) and Kruger et al (28) in dialysis patients; but less than that Zengin and Sarper (83.3-100%) in patients with acute lymphoblastic leukemia (16).

One of the reasons for this difference in patients with beta thalassemia major may be related to high level of serum iron. Iron overload, a primary complication of both thalassemia itself and transfusion therapy, is thought to be the main causing mechanism of immune incompetence in beta thalassemia major (3). Patients with thalassemia major who had serum ferritin level more than 3000 ng/ml had lower C4 and CH50 levels (10). Recent studies on immune competence in beta-thalassemia have revealed numerous quantitative and functional defects, involving T and B lymphocytes and immunoglobulin production (3). Also, Alavi et al (29) indicated that chemotherapy has independent adverse effects on vaccine-induced antibody protection against diphtheria. In the present study, serum ferritin levels were found to be much higher in patients than in healthy subjects.

In our study, only 25.4% of the thalassemia patients had a partial protective level of IgG against diphtheria and they may susceptible to infection. Adversely, in one study conducted in beta thalassemic patients (aged 5-17 years) who were submitted for bone marrow transplantation, a high percentage (83%) of subjects had anti-diphtheria antibody levels below the protective levels (30). Zengin and Sarper (16) in their study showed that 11.1% of subjects with acute lymphoblastic leukemia had protective level for diphtheria after chemotherapy. Also, Alavi et al (29) reported that chemotherapy in hematologic malignancies caused failure to achieve protective levels of antibodies against diphtheria. In another study, Aminzadeh et al found a non-protective level of IgG against tetanus in most of the hemodialysis patients (27). This alteration seems to be due to difference in mean age of study participants.

Conclusion

Our study indicated that increased serum iron levels in beta thalassemia patients decreased the level of antibody against diphtheria. Therefore, lowering the serum levels of iron may prevent further reduction of antibody levels as compared to healthy people over time. It is suggested that further studies are done.

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