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Immunity to tetanus in major beta thalassemia patients

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Purpose: Patients with beta thalassemia major are at increased risk for bacterial infections specially splenectomized patients. The aim of this study was to determine the anti-tetanus antibody concentration among patients with beta thalassemia major.

Materials and Methods: The anti-tetanus antibody concentration was investigated in 224 patients with thalassemia major and 224 healthy subjects matched for age and gender. Tetanus antibody and ferritin serum level were determined by enzyme-linked immunosorbent assay method using commercial kits. Subjects who had antibody level ≥ 0.1 IU/mL was defined as complete protection, 0.01 to < 0.1 IU/mL as partial protection and < 0.01 IU/mL as no protection. For the analysis, we used SPSS version 11.5 software. A two-sided p-value less 0.05 was considered statistically significant.

Results: In patients with beta thalassemia major, antibody level against tetanus was inversely dependent about 29.3% to serum ferritin level. Thus, when serum ferritin increased 1 ng/mL, serum antibody against tetanus decreased 0.002 IU/mL. Mean anti-tetanus (IgG) antibody titers was lower in thalassemia patients compared to healthy subjects (1.53 ± 1.71 vs. 2.02 ± 2.05, p = 0.007) that was no significantly associated to age and gender in both study groups. All of participants had serum antibody level 0.01 IU/mL or greater. The complete protective level of anti-tetanus antibody was lower in thalassemia subjects in compare to healthy persons (71% vs. 87.9%, p < 0.001).

Conclusion: Patients with thalassemia had lower anti-tetanus antibody level than healthy subjects. Thus the vaccine recommendation seems essential for patients with beta thalassemia major.

Keywords: Tetanus, Thalassemia, Immunity, Antibodies

Introduction

Tetanus infection is a serious but a preventable disease that caused by *Clostridium tetani*. This microorganism enters the human body through a wound. This agent produces toxin in wound that spreads via blood and lymph and affects the neuromuscular system of the body. The incidence of tetanus infection is about one million per year in the world [1]. Some subjects with sarcoma [2], immune deficiency disorders [1], HTLV1 infected [3], hemodialysis patients [4,5] had no protective antibody level against tetanus or don't respond sufficiently to tetanus vaccination. In a study conducted by Kruger et al. [6] suggested that 63% of the unprotected hemodialysis patients against tetanus did not respond to a single tetanus vaccination. But Ercan et al. [7] in children treat-

ed for acute lymphoblastic leukemia and Zengin and Sarper [8] in patients with acute lymphoblastic leukemia showed that antibody responses to tetanus vaccination was sufficient.

In Iran, 91% of hemodialysis patients and 83% of peritoneal dialysis children had no protective anti-tetanus antibody and the mean anti-tetanus antibody was low in two groups than control healthy children [9]. Also, Alavi et al. [10] indicate that chemotherapy for hematologic malignancies has no effects on vaccine-induced antibody protection against tetanus.

Thalassemia is one of the most common genetic diseases in the world and it is a major health problem [11]. Beta thalassemia major is one of the most common types of thalassemia. Beta thalassemia is very common and an important health problem in Iran [12]. The patients with beta thalassemia major (Cooley anemia) are at increased risk for infections that it has important role for morbidity and mortality [13,14]. Several factors which are expected to be involved are anemia, splenectomy, iron overload and alterations in immune responses [15-17]. Thalassemia subjects may have impaired immune response to vaccination by reason of iron overloading [17]. In a study through in Iran, from 99 subjects with thalassemia major who were vaccinated with three doses against hepatitis B virus, 10.1% were non-responders [11]. Also, Li Volti et al. [18] showed that a high percentage of thalassemia patients who candidate for bone marrow transplantation had anti-tetanus antibody levels below the protective levels. Also after revaccination, the majority of patients achieved to sufficient protective levels of anti-tetanus antibody.

According to reduced response to immunization in thalassemia subjects [19], increased immunity to tetanus after immunization and necessitate of assessment of immunity to tetanus in beta thalassemia subjects, the aim of study was to compare anti-tetanus antibody levels in beta thalassemia patients with healthy subjects.

Materials and Methods

This case-control study conducted in academic hospital of Jahrom University of Medical Science in Jahrom, southwest of Iran. Two hundred and twenty four children with beta thalassemia major (114, 50.9% female), aged 1-9 years, were enrolled as case group and 224 healthy subjects who matched for age, gender and previous tetanus dose reception were enrolled as control group.

Patients and controls had been previously vaccinated according to the Iranian National Vaccination schedule. The current program includes active universal immunization against tetanus at 2-, 4-, 6-, 18-month-old and 6-year-old, 0.5 mL intramuscular then repeated every 10 years [20].

The study was approved by the local ethical committee and all participants were asked to complete an informed consent. Demographic data such as age, sex, and tetanus dose reception and time after last tetanus vaccination were recorded by a questionnaire. No participants had primary immune deficiency or acquired immune deficiency syndrome.

Serum tetanus antibody (IgG) and serum ferritin level were determined in the same serum samples obtained from test and control groups. Serum level of tetanus IgG and also and serum ferritin level were determined by enzyme-linked immunosorbent assay method using commercial kits manufactured by IBL company, catalog No. RE56901 and catalog No. DB59111 (IBL Company, Hamburg, Germany), respectively.

The results were articulated as IU/mL. An IgG antibody level of 0.1 IU/mL or greater was considered as complete protection, between 0.01 to less than 0.1 as partial protection and less than 0.01 as no protection [21].

Data are presented as mean±standard deviation. We calculated the ratio of immunoglobulin concentration or titers. We used the independent student t-test, One-way ANOVA and chi square test to compare the means and ratios in the two groups (beta thalassemia persons and healthy subjects) or three groups (beta thalassemia persons with or without spleen and healthy subjects). Also, the dependency of antibody titers of patients and controls to serum ferritin were analyzed by linear regression test. For the analysis we used SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA). A two sided p-value less 0.05 was considered statistically significant.

Results

Mean age of the participants were 7.12 (±1.71) years and 7.04 (±1.64) years in patients and healthy controls, respectively. One hundred and fourteen subjects of case and control groups (50.9%) were females. One hundred and fifteen (51.4%) of beta thalassemia subjects were non-splenectomized (Table 1). Although, healthy subjects insignificantly was younger than thalassemia patients, but in thalassemia subjects, splenectomized patients were significantly younger than non-splenectomized subjects (p=0.020).

Mean anti-tetanus (IgG) antibody titers was lower in thalassemia patients compared to healthy subjects (1.53±1.71 vs. 2.02±2.05, p=0.007). In thalassemia subjects, splenectomized

Table 1. Demographic variables and serum anti-tetanus IgG and ferritin level of case and control groups

Variable		Controls (n = 224)	Beta thalassemia major (cases) (n = 224)		p-value
			Non-splenectomized (n = 115)	Splenectomized (n = 109)	
Sex (female)		114 (50.9)	65 (56.5)	49 (45.0)	0.224
Anti-tetanus IgG group (IU/mL)	Partial protection (0.01 to < 0.1)	27 (12.1)	36 (31.3)	29 (26.6)	< 0.001
	Complete protection (≥ 0.1)	197 (87.9)	79 (68.7)	80 (73.4)	
Age (yr)		7.04±1.64	7.37±1.71	6.85±1.69	0.060
Anti-tetanus IgG (IU/mL)		2.02±2.05	1.41±1.71	1.66±1.70	0.015
Serum ferritin (ng/mL)		64.47±65.41	981.37±592.99	832.11±568.04	< 0.001

Values are presented as number (%) or mean±standard deviation.

Table 2. Anti-tetanus IgG levels in case and control groups by gender

Variable		Control		Beta thalassemia major (cases)			
				Non-splenectomized		Splenectomized	
		Female	Male	Female	Male	Female	Male
Mean±SD		2.27±2.30	1.75±1.72	1.48±1.75	1.31±1.67	1.74±1.73	1.58±1.68
p-value		0.056		0.614		0.639	
Anti-tetanus IgG groups (IU/mL)	Partial protection (0.01 to < 0.1)	8 (7.0)	19 (17.3)	21 (32.3)	15 (30.0)	15 (30.6)	14 (23.3)
	Complete protection (≥ 0.1)	106 (93.0)	91 (82.7)	44 (67.7)	35 (70.0)	34 (69.4)	46 (76.7)
p-value		0.018		0.791		0.392	

Values are presented as mean±standard deviation or number (%).

Table 3. Relation of anti-tetanus IgG titers to serum ferritin levels in study groups

Variable		r	r^2	Adjusted r^2	B Coefficients		p-value
					Constant	Ferritin	
Controls		0.096	0.009	0.005	2.213	–0.003	0.152
Beta thalassemia major (cases)	Non-splenectomized	0.563	0.317	0.311	3.009	–0.002	< 0.001
	Splenectomized	0.516	0.266	0.259	2.994	–0.002	< 0.001

patients had higher anti-tetanus level than non-splenectomized patients, without significant difference (Table 1). But the mean of antibody levels was significantly lower in non-splenectomized thalassemia patients than healthy subjects (p=0.005).

Antibody level of ≤0.01 IU/mL wasn't reported in any case and control subjects. The complete protective level of anti-tetanus antibody was lower in thalassemia subjects in compare to healthy persons (71% vs. 87.9%, p<0.001). Although splenectomized patients had higher complete protective level of anti-tetanus antibody than non-splenectomized patients, but it was no statistically significant (p<0.439).

The patients with thalassemia major had very higher mean serum ferritin than healthy subjects (908.74±584.48 ng/mL vs. 64.47±65.41, p<0.001) (Table 1).

Table 2 showed anti-tetanus antibody in study groups by gender. Mean anti-tetanus antibody titers in control and case groups were no significant difference between female and male. In healthy subjects, 93% of females had complete protection level against tetanus that were higher than in male (82.7%, p=0.018). But the percent of complete protection were no significant between genders and splenectomized and non-splenectomized patients.

With linear regression analysis (Table 3), 31.1% and 25.9% of anti-tetanus antibody (IgG) was dependent to serum ferritin level in non-splenectomized and splenectomized patients, respectively (p<0.001). In healthy subjects only 0.5% of anti-tetanus antibody was dependent to serum ferritin, but without statistical significant. In all participants, 11.2% of anti-tetanus antibody was dependent to serum ferritin levels (p<0.001), but that was 29.3% of thalassemia patients (p<0.001). Totally with entered of variables such as gender and serum ferritin in

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linear regression, this model predicted 12.1% of anti-tetanus antibody changes and that was predicted 29.9% in thalassemia patients ($p<0.001$).

Discussion

Infectious complications are one of the most common cause of mortality and a main cause of morbidity in beta thalassemia. Although survival beta thalassemia major has enhanced, but treatment modalities and the underlying disease may result in impair immune system. Recent studies on immune competence in beta thalassemia have discovered numerous quantitative and functional defects such as involving T and B lymphocytes, immunoglobulin production, chemotaxis, and phagocytosis, as well as the complement system. Also, iron overload is thought to be the main precipitating mechanism, due to the important immune-regulatory properties of iron and its binding proteins; iron excess may disturb the immune balance supporting the growth of infectious organisms [17].

In the present study, levels of protective anti-tetanus antibody were 100% in patients and controls. Also, 29% of the thalassemia patients were partial protective level of IgG against tetanus and they may susceptible to tetanus. Adversely, in one study conducted in beta thalassemia patients (aged 5-17 years) that submitted for bone marrow transplantation, a high percentage of subjects had anti-tetanus antibody levels below the protective levels [18]. But in study in Iran, there were no significant changes in humeral immune markers in the patients with beta thalassemia major compared to the controls, except in the case of IgA which was higher in the beta thalassemia patients [22].

In another diseases, Aminzadeh et al. [23] found a non protective level of IgG against tetanus in most of the hemodialysis patients. Complete protection anti-tetanus antibody found in our study is more than that Aminzade et al. (4.5%) [23], Modarresi et al. [9] and Kruger et al. (44%) [6] in hemodialysis patients; and Kwon et al. [5] in hematology and oncology patients, but less than that Zengin and Sarper (83.3%-100%) [8] in patients groups with acute lymphoblastic leukemia.

In our study, there was no difference in tetanus antibody level between female and male subjects in both thalassemia and control groups, similar to the survey conducted by Kwon et al. in Korea [5], Modarresi et al. in Iran [9], Chatchatee et al. in Thai population [24], and Paulides et al. [2].

In our study, anti-tetanus antibody insignificantly was higher in asplenic than non-splenectomized patients. Also, Rosado et al. [25] found that anti-tetanus specific IgG secreting B cells were in normal range in asplenic children. The mean concentrations of serum IgG and IgA were similar in splenectomized and non-splenectomized thalassemia children as compared to healthy controls [26].

In present study, the serum anti-tetanus antibody titers was dependent to serum ferritin that with each unit increment in serum ferritin; the serum antibody decreased 0.002 ng/mL. Thalassemic subjects may have impaired immune response to vaccination by reason of iron overloading [17]. Ghaffari et al. [22] indicated that patients with serum ferritin level >3,000 ng/mL had lower C4 and CH50 levels.

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

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References

1. Thomas PB. Clostridium tetani. In: Mendel GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 5th ed. Philadelphia: Churchill Livingstone; 2000. p.2537-72.
2. Paulides M, Stohr W, Laws HJ, et al. Antibody levels against tetanus and diphtheria after polychemotherapy for childhood sarcoma: a report from the Late Effects Surveillance System. Vaccine 2011;29:1565-8.
3. Souza AS, Amorim CF, Carvalho N, Santos SB, Carvalho EM. Impairment of humoral and cellular immune response to tetanus toxoid in HTLV-1 infected individuals. Retrovirology 2014;11(Suppl 1):P66.
4. Kruger S, Muller-Steinhardt M, Kirchner H, Kreft B. A 5-year follow-up on antibody response after diphtheria and tetanus vaccination in hemodialysis patients. Am J Kidney Dis 2001;38:1264-70.
5. Kwon HJ, Lee JW, Chung NG, Cho B, Kim HK, Kang JH. Assessment of serologic immunity to diphtheria-tetanus-

pertussis after treatment of Korean pediatric hematology and oncology patients. J Korean Med Sci 2012;27:78-83.
6. Kruger S, Seyfarth M, Sack K, Kreft B. Defective immune response to tetanus toxoid in hemodialysis patients and its association with diphtheria vaccination. Vaccine 1999; 17:1145-50.
7. Ercan TE, Soycan LY, Apak H, et al. Antibody titers and immune response to diphtheria-tetanus-pertussis and measles-mumps-rubella vaccination in children treated for acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2005;27:273-7.
8. Zengin E, Sarper N. Humoral immunity to diphtheria, tetanus, measles, and hemophilus influenzae type b in children with acute lymphoblastic leukemia and response to re-vaccination. Pediatr Blood Cancer 2009;53:967-72.
9. Modarresi M, Gheissari A, Sattari M. Protective status of end-stage renal disease children against tetanus and diphtheria vaccination. Int J Prev Med 2013;4:420-4.
10. Alavi S, Rashidi A, Arzanian MT, Shamsian B, Nourbakhsh K. Humoral immunity against hepatitis B, tetanus, and diphtheria following chemotherapy for hematologic malignancies: a report and review of literature. Pediatr Hematol Oncol 2010;27:188-94.
11. Sharifi Z, Milani S, Shooshtari MM. Study on efficacy of hepatitis B immunization in vaccinated beta-thalassemia children in Tehran. Iran J Pediatr 2010;20:211-5.
12. Samavat A, Modell B. Iranian national thalassaemia screening programme. BMJ 2004;329:1134-7.
13. Eigenberger K, Sillaber C, Greitbauer M, et al. Antibody responses to pneumococcal and hemophilus vaccinations in splenectomized patients with hematological malignancies or trauma. Wien Klin Wochenschr 2007;119:228-34.
14. Ezer U, Gulderen F, Culha VK, Akgul N, Gurbuz O. Immunological status of thalassemia syndrome. Pediatr Hematol Oncol 2002;19:51-8.
15. Wiener E. Impaired phagocyte antibacterial effector functions in beta-thalassemia: a likely factor in the increased susceptibility to bacterial infections. Hematology 2003;8: 35-40.
16. Kadymova EA. The features of immune status of major beta-thalassemia patients during various periods after splenectomy. Georgian Med News 2009;(174):39-44.
17. Farmakis D, Giakoumis A, Polymeropoulos E, Aessopos A. Pathogenetic aspects of immune deficiency associated with beta-thalassemia. Med Sci Monit 2003;9:RA19-22.
18. Li Volti S, Mauro L, Di Gregorio F, et al. Immune status and immune response to diphtheria-tetanus and polio vaccines in allogeneic bone marrow-transplanted thalassemic patients. Bone Marrow Transplant 1994;14:225-7.
19. Weatherall DJ. Toward an understanding of the molecular biology of some common inherited anemias: the story of thalassemia. In: Wintrobe MM, editor. Blood, pure and eloquent. 5th ed. New York: McGraw-Hill; 1980. p.373-80.
20. Moradi-Lakeh M, Esteghamati A. National Immunization Program in Iran: whys and why nots. Hum Vaccin Immunother 2013;9:112-4.
21. Galazka AM. The immunological basis for immunization series. Module 3. Tetanus. Global Programme for Vaccines and Immunization. Expanded Programme on Immunization. Geneva: World Health Organization; 1993.
22. Ghaffari J, Vahidshahi K, Kosaryan M, Soltantooyeh Z, Mohamadi M. Humoral immune system state in ss thalassemia major. Med Glas (Zenica) 2011;8:192-6.
23. Aminzadeh Z, Yaghmaei F, Poorkazemi A, Gachkar L. Tetanus antitoxin levels and cutaneous anergy in hemodialysis patients in two university hospitals in Iran. Iran J Clin Infect Dis 2006;1:31-4.
24. Chatchatee P, Chatproedprai S, Warinsathien P, et al. Seroprevalence of tetanus antibody in the Thai population: a national survey. Asian Pac J Allergy Immunol 2007;25: 219-23.
25. Rosado MM, Gesualdo F, Marcellini V, et al. Preserved antibody levels and loss of memory B cells against pneumococcus and tetanus after splenectomy: tailoring better vaccination strategies. Eur J Immunol 2013;43:2659-70.
26. Ahluwalia J, Datta U, Marwaha RK, Sehgal S. Immune functions in splenectomized thalassaemic children. Indian J Pediatr 2000;67:871-6.