ORIGINAL ARTICLE

Natural Immunity against Neisseria meningitides in beta Thalassemia Major: A Descriptive Study in a South-West of Iran

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ABSTRACT

Aim: Natural immunity to Neisseria meningitides(NM) was evaluated in patients with beta thalassemia. Then, the proportion of natural protective immunity was compared between splenectomised and non splenectomised patients.

Methodology: All patients with beta thalassemia major were enrolled in our descriptive study that was done in Thalassemia Ward of the academic hospital at Motahari Hospital, Jahrom, Iran. There was not any vaccination against Neisseria meningitides in Iranian National Vaccination schedule. All patients were categorized to groups A (non splenectomised) and B (splenectomised). Demographic data such as age and sex, and time after splenectomy (month) were recorded by a questionnaire. The levels of serum ferritin and serum anti meningococcalpolysaccharide capsule antigen IgGwere determined by enzyme-linked immune sorbent assay method. An IgG antibody level of 2µg/ml or higher was named as protective immunity (natural immunity) against N. meningitides. We used the independent student t, One-way ANOVA and chi square test to compare the means and ratios between study groups. For the data record and analysis, we used SPSS software version 16 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 were considered as statistically significant.

Results: Overall, 14.4% of patients had natural protective MN specific IgG antibody (≥ 2µg/ml). The trend of this protective immunity was insignificantly decreased with advancing age. Sex, post splenectomy duration and serum concentration of ferritin was no significant differences between immune and un- immune patients. The proportion of natural protective immunity was 17% and 11.7% in non splenectomised and splenectomised cases, respectively.

Conclusions: Our results showed a lower natural protective immunity in patients with beta thalassemia major that it was similar in splenectomised and non splenectomised patients. Thus seems to be necessary that these susceptible patients vaccinate against NM.

Keywords: Neisseria meningitides, protective immunity, beta Thalassemia, Splenectomy

INTRODUCTION

Beta thalassemia disorder produced through a defect in beta globin chain and is one of the hemolytic anemia disorders.It is prevalent in Mediterraneancountries, Central Asia and Middle East[1]. Also, it is an important health problem in our country with frequency of 3-10% in some regions[2,3]. Spleen hyper-activation(hypersplenism) in beta thalassemia major causes massive destruction of red blood cells that increase need to recurrent blood transfusions[4]. Thus, one of approaches that was done for these patients is splenectomy[5].

Spleen act as a defense against infections[6]. Thus, loss of spleen (asplenia and splenectomy) increases the risk of overwhelming infections[7,8] particularly in hematologic diseases as thalassemia major[9]. Also, it caused impaired immune response[10]. The prevalence of septicemia is 200 fold in subjects without spleen than in ones with spleen[5]. These infections are followed by encapsulated bacteria in 50-80% of cases[8] specially by the Streptococcus pneumonia, Hemophilus influenza type b and Neisseria meningitides (NM)[11]. If splenectomy was done during first life years, the risk of infection is higher[12]. Infectious morbidity and mortality is 4.4% and 2.2% in subjects without spleen before 16 years old, respectively[12]. In one study, investigators reported 20% of these infections after splenectomy despite pre operation vaccination with pneumovax[13].Consequently, immunization against pneumococci and NM is one of approaches for prevention of these infections.

In addition, beta thalassemia subjects exposed to iron overload due to ineffective erythropoiesis and repeated blood transfusion[14]. Free radical making in cells propagate fallowing of the excess iron, which can damage to cells. Also, iron overload may changes the T helper and cytotoxic balance[15], promote microbial growth and can suppress immune function. Iron overload caused chemo-taxis and phagocytosis failing and decreased activity of natural killer cells that these processes triggered susceptibility to infections[16].

In a case-control study, we suggested that serum anti-tetanus[17] and anti-diphtheria[18] titers was lower in beta thalassemia major than healthy subjects but these were insignificantly differences between splenectomised and nonsplenectomised patients. In another study, we suggested a lower natural antibody titers against haemophilus influenza type b in splenectomised beta thalassemia major than in patients without spleen[19]. Also, we showed that Iranian children under 5 years old were exposed to Hemophilus influenza type b infection during childhood[20].Spoulou et al[21] in 2011 in Greece reported insignificantly a lower natural immunity against NM in unvaccinated splenectomised thalassemia patients than in vaccinated patients and in healthy individuals. Also, they suggested that only 63% unvaccinated patients with

negative baseline status achieved protective IgG concentration at one month, compared to 100% and 90.1% for vaccinated patients and healthy control group, respectively[21].

There was not any vaccination procedure against Neisseria meningitides in Iranian National Vaccination schedule. In Iran, splenectomised patients are vaccinated with pneumococcal vaccine (pneumovax) 14 days before splenectomy but they are not vaccinated against NM.

Therefore, the aim of study was to compare the natural immune response to NMinbeta thalassemia major patients. Then, we compared the anti-meningococcal protective status in splenctomised and non splenctomised cases.

MATERIALS AND METHODS

This descriptive study was done in Thalassemia Ward of the academic hospital at Motahari Hospital, Jahrom, Fars province, Iran. All subjects with diagnosed of beta thalassemia major were enrolled in our study.

The study was approved by the local ethical research committee (ethical research number: IR.JUMS.REC.1394.030,1394.03.27) and all participants were asked to complete an informed consent.

Demographic data such as age and sex, and time after splenectomy (month) were recorded by a questionnaire. Not any participants had primary immune deficiency or acquired immune deficiency syndrome.

All patients were categorized to groups A and B accordingto splenectomy history. Group A patients had been non splenectomised whereas group B patients had been splenectomised. Serum meningococcal IgG against polysaccharide capsule antigen was determined in the same serum samples obtained from thalassemia subjects. Serawere separated, frozen and stored at -20 ºC until tested.

Anti-Neisseria Meningitides Immunity was determined by enzyme-linked immune sorbent assay method using commercial kits manufactured by Diapro Italy, Ref. code: MENG.CE. The cut-off value of anti-meningococcal polysaccharide capsule antigen IgG determined by calculating the mean OD450nm value of the Negative Control (NC) and then applying "NC + 0.250 = Cut-Off" formula. Samples with an OD450nm lower that the Cut-Off value were considered not reactive for anti-meningococcal polysaccharide capsule antigen IgG or not protective. Samples with an OD450nm higher than the Cut-Off value were considered positive for anti-meningococcal polysaccharide capsule antigen IgG or protective.

The levels of serum specific ferritin were determined by an enzyme immunoassay kit (Human ferritin Elisa, Biovender, Czech Republic, Cat. No.: RCD012R).

Quantitative and qualitative data are presented as mean±standard deviation or number and percent, respectively. We used the independent student t, One-way ANOVA and chi square test to compare the means and ratios between study groups. For the data record and analysis, we used SPSS version 16. A two side p-value less than 0.05 were considered as statistically significant.

RESULTS

The mean age of all participants was 18.80 ± 5.59,and 121cases (45.6%) were in age group of 10-17 years, 130 cases (49.4%) were male, and 95 cases (36.1%) had ferritin levels under 1000 ng/ml (Table 1). ANMI: Anti-Neisseria Meningitides Immunity, SD: Standard Deviation, SP: Splenectomised

Out of 263 patients with beta thalassemia major, 128 (48.7%) subjects had undergone splenectomy (group B). Sex, age and age groups, and serum ferritin level and percent of ferritin level of less than 1000 ng/ml were similar between groups A and B.The prevalence of natural immunity against N. meningitides was 14.4 in all patients. Although the prevalence of natural immunity was lower in splenectomised patients in compare to non splenectomised cases but it was no significantly difference (11.7% vs. 17.0%, p= 0.220).

The patients with protective and non-protectiveanti N. meningitides antibody were similar according to age, age groups, sex, serum ferritin level and post splenectomy duration (PSD) (Table 2).

The immune patients were younger than non-immune ones but this difference was not significant. The trend of naturally immunity to N. meningitides insignificantly was decreased through advancing age (16.7% in 10-15 years to 11.4% in 21-38 years, p= 0.663). While, patients withPSD less than 65 months had lesser natural protective immunity in compare to others (7.5% vs. 13.6%), but there was no significant different (p= 0.338).

Table 1 Demographic characteristic, serum ferritin level and naturally immunity to N. meningitides in all beta thalassemia major patients and also according to splenectomy

Variables	Group	All patients	Non SP (group A)	SP (group B)	P
		No: 263	No: 135	No: 128	
Age, year	Mean (SD)	18.80 (5.59)	18.81 (5.58)	18.78 (5.62)	0.946
Age group, years	10-15, n (%)	72 (27.4)	37 (27.4)	55 (27.3)	1.000
	16-20, n (%)	121 (46.0)	62 (45.9)	59 (46.1)	
	21-38 n (%)	70 (26.6)	36 (26.7)	34 (26.6)	
Sex	Male, n (%)	130 (49.4)	70 (51.9)	63 (49.2)	0.669
Protective ANMI	≥ 2µg/ml, n (%)	38 (14.4)	23 (17.0)	15 (11.7)	0.220
Ferritin, ng/ml	Mean (SD)	2167.9 (1602.3)	2136.3 (1595.4)	2201 (1615.2)	0.749
Ferritin group, ng/ml	< 1000, n (%)	95 (36.1)	50 (37.0)	45 (35.2)	0.751
	≥ 1000, n (%)	168 (63.9)	85 (63.0)	83 (64.8)	

Table 2 Demographic characteristics, serum ferritin and post splenectomy duration in naturally immune and non-immune patients with beta thalassemia major

Variables	Non Protective ANMI	Protective ANMI	p
Age, year, Mean (SD)	19.0 (5.8)	17.6 (3.8)	0.396
Age group, n (%), 10-15 years	60 (83.3)	12 (16.7)	0.663
16-20 years	103 (85.1)	18 (14.9)	
21-38 years	62 (88.6)	8 (11.4)	
Sex, male, n (%)	113 (50.2)	20 (52.6)	0.783
Ferritin, ng/ml, Mean (SD)	2174.5 (1643.3)	2128.8 (1352.8)	0.580
Ferritin group, n (%), < 1000 ng/ml	82 (86.3)	13 (13.7)	0.791
≥ 1000 ng/ml	143 (85.1)	25 (14.9)	
PSD, month, Mean (SD)	107.4 (69.6)	104.8 (51.8)	0.923
PSD group, n (%), < 65 months	37 (92.5)	3 (7.5)	0.388
≥ 65 months	76 (86.4)	12 (13.6)	

ANMI: Anti-Neisseria Meningitides Immunity, PSD: Post Splenectomy Duration, SD: Standard Deviation, SP: Splenectomised

DISCUSSION

Totally, 14.4% of patients with beta thalassemia major were naturally immune to N. meningitides. It was 11.7% for splenectomised patients that was lesser than in non splenectomised group (17%) but there was no significant different. Also, the trend of immunity was insignificantly decrescendo through advancing age. Sex, age and serum ferritin level was similar in two groups A and B (non splenectomised and splenectomised patients). Immune and non-immune patients were similar as age, sex, serum concentration of ferritin and PSD.

The serum bactericidal antibody titers and proportion of protective levels against N. meningitides serogroup W was significantly no different in adolescents (10-15 years old) and middle aged adults (50-65 years old)[22] that it was in line to our resultwhere we found that the naturalprotective immunity was parallel in three agegroups.Moreover, similar to our results, in general population, the positive meningococcal serogroup C-specific antibody diminished from 20% to 13.4% through age groups of 2-6, 9-12 and 13-19 years[23]. In the Sudan, serum concentration of serogroup A N. meningitides anti-capsular antibody decreased through1 month and 5 years after vaccination[24]. Conversely, Trotter et al in a sample of urban residents of Burkina Faso aged 1month to 59 years[25] showed an increased serogroup A-specific IgG with advancing age groups till age 20-24 years where this determinate sustained for other age groups. Similar to this study in Burkina Faso, Yaro et al showed an increase meningococcal serogroup A IgG level of 1.6µg/ml for children 12-23 months to 9.82µg/ml for subjects 20-35 years[26].

Only 12% of asplenic patients were naturally immune against N. meningitides[27] that it was in line of our result. But in study by Spoulou et a[21], 19.3% unvaccinated splenectomised beta thalassemia major had positive Meningococcal C-specific IgG antibody. In subjects aged 2-19 years, the antibody levels against meningococcal sero group C were protective in 15.1% of participants[23] that was similar to our result in all patients (14.4%). In a sample of general population in Burkina Faso, the meningococcal anti-A IgG concentration ≥ 2µg/ml found in 27% of children under 5 years of age[25]. Natural immunity to Neisseria meningitides serogroup C insignificantly was lower in splenectomised patients compared to healthy subjects[21]. Despite the unimportantamount of patients naturally developed immunity tomeningococciin splenectomisedindividuals with beta thalassemia; however, a second dose of meningococcilooks to be suitable in this high-risk group.Similarly, Balmer et al suggested 2 doses of meningococciin subjectswho had splenectomised for medical reasons[28].

Spleen is fundamental for prevention of infection due to clearing encapsulated bacteria such as pneumococci, hemophilus influenza and N. meningitides[29]. Thus asplenic patients have an infection risk due to absence of splenic macrophage and immunoglobulin M production. Asplenic cases with invasive pneumococcal disease have a more severe infection, need mechanical ventilation and admission to the intensive care unit than those with a spleen[30]. Of 349 episodes of sepsis in splenectomised cases,3.7% of infections were caused by N. meningitides[31]. The important matter about post splenectomy infections are the lifetime incidence of 5%[32] with more risk in the first 2 years after splenectomy and the death rate of 38-70% even with sufficient therapy. Furthermore, this risk was higher in cases that splenectomy was performed for a hematologic disorder such as thalassemia[29]. The Center for Disease Control and Prevention recommends a 23-valent pneumococcal vaccination for asplenic patients[32] that must revaccinated every 3-6 years.For patients without spleen, vaccination against Streptococcal pneumonia, Hemophilus influenza type b and Neisseria meningitides are hardly suggested. These vaccines have to administered at least two weeks formerly elective surgery or next emergency surgery of spleen removal[33].

CONCLUSIONS

Although, our result showed a lower natural protective immunity in patients with beta thalassemia major (14.4%) that it was similar in splenectomised (11.7%) and non splenectomised (17.0%) subjects, but suggested the existence of contact with NM in our community. According to, there was not any vaccination procedure against NM in Iranian National Vaccination schedule and also, no vaccination against NM in splenectomised people in Iran, thus seems to be necessary that these susceptible patients vaccinate against NM.However, more studies in this field are recommended on other Iranian splenectomised and non splenectomised beta thalassemia people.

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REFERENCES

1. Galanello, R. and R. Origa, *Beta-thalassemia.* Orphanet Journal of Rare Diseases, 2010. **5**: p. 11.
2. Bazi, A. and E. Miri-Moghaddam, *Spectrum of -thalassemia Mutations in Iran, an Update.* Iran J Pediatr Hematol & Oncol, 2016. **6**(3).
3. Shahraki-Vahed, A., et al., *Lived experiences of Iranian parents of beta-thalassemia children.* J Multidiscip Healthc., 2017. **10**: p. 243-251.
4. Vento, S., F. Cainelli, and F. Cesario, *nfections and thalassaemia.* Lancet Infect Dis, 2006. **6**: p. 226–233.
5. Rahav, G., et al., *Severe infections in thalassaemic patients: prevalence and predisposing factors.* Br J Haematol, 2006. **133**(667-674).
6. Cadili, A. and C. de-Gara, *Complications of Splenectomy.* The American Journal of Medicine, 2008. **121**: p. 371-375.
7. Di Sabatino, A., R. Carsetti, and G.R. Corazza, *Post-splenectomy and hyposplenic states.* Lancet, 2011. **378**: p. 86–97.
8. Cadili, A. and C.D. Gara, *Complications of splenectomy.* American Journal of Medicine, 2008. **121**: p. 371-275.
9. Bisharat, N., et al., *Risk of infection and death among postsplenectomy patients.* J Infect, 2001. **43**: p. 182-186.
10. Sotoodeh.Jahromi, A., et al., *Immunity to Pneumococcal Vaccine in Splenectomized β-Thalassemia Patients.* American Journal of Immunology, 2012. **8**(2): p. 33-37.
11. Waghorn, D.J., *Overwhelming infection in asplenic patients: Current best practice preventive measures are not being followed.* J Clin Pathol, 2001. **54**: p. 214-218.
12. Beytout, J., O. Tournilhac, and H. Laurichesse, *Asplenia and hyposplenism.* Presse Med, 2003. **32**(28 SUPPL): p. S5-9.
13. TalaeiZadeh, A.H., S. AskarPoor, and K.M. Zandian, *Outcomes of splenectomy in Thalassemic patients.* Scientific Medical Journal of Ahwaz University of Medical Sciences, 2004. **40**: p. 31-38.
14. Berdoukas, V., et al., *Tissue iron evaluation in chronically transfused children shows significant levels of iron loading at a very young age.* Am J Hematol, 2013. **88**: p. E283-285.
15. Cunningham-Rundles, S., et al., *Effect of Transfusional Iron Overload on Immune Response.* The Journal of Infectious Diseases, 2000. **182**(Suppl 1): p. S115-121.
16. Porto, G. and M. De Sousa, *Iron overload and immunity.* World J Gastroenterol, 2007. **13**(35): p. 4707-4715.
17. Sotoodeh-Jahromi, A. and K. Rahmanian, *Immunity to tetanus in major beta thalassemia patients.* Clin Exp Vaccine Res, 2015. **4**: p. 1-5.
18. Sotoodeh.Jahromi, A., et al., *Immunity level to diphtheria in beta thalassemia patient.* WORLD FAMILY MEDICINE/MIDDLE EAST JOURNAL OF FAMILY MEDICINE, 2017. **15**(8): p. 7-11.
19. Sotoodeh.Jahromi, A., et al., *Natural Immunity Against Haemophilus influenza Type B in Splenectomised Beta-thalassemia Children.* Pakistan Journal of Biological Sciences, 2014. **17**(11): p. 1190-1194.
20. Sotoodeh-Jahromi, A.R. and K. Rahmanian, *Natural Immunity to Hemophilus influenza Type b in Children, South of Iran: Need for Vaccination.* Pak J Biol Sci, 2012. **15**(3): p. 160-163.
21. Spoulou, V., et al., *Natural and vaccine-induced immunity to Neisseria meningitidis serogroup C in asplenic patients with beta-thalassemia.* Vaccine, 2011. **29**: p. 4435–4438.
22. van-der.Heiden, M., et al., *Lower antibody functionality in middle-aged adults compared to adolescents after primary meningococcal vaccination: Role of IgM.* Experimental Gerontology, 2018. **105** p. 101–108.
23. Mitchell, L.A., et al., *Analysis of Meningococcal Serogroup C-Specific Antibody Levels in British Columbian Children and Adolescents.* The Journal ofInfectious Diseases, 1996. **173**: p. 1009-1013.
24. Flitter, B.A., et al., *Group A Antibody Persistence Five Years After Meningococcal Polysaccharide Vaccination in the Sudan.* Human Vaccines, 2006. **3**(4): p. 135-139.
25. Trotter, C.L., et al., *Seroprevalence of Bactericidal, Specific IgG Antibodies and Incidence of Meningitis Due to Group A Neisseria meningitidis by Age in Burkina Faso 2008.* PLoS ONE, 2013. **8**(2): p. e55486.
26. Yaro, S., et al., *Antibody Persistence at the Population Level 5 Years After Mass Vaccination With Meningococcal Serogroup A Conjugate Vaccine (PsA-TT) in Burkina Faso: Need for a Booster Campaign?* Clinical Infectious Diseases, 2019. **68**(3): p. 435–443.
27. Meerveld-Eggink, A., et al., *Impaired antibody response to conjugated meningococcal serogroup C vaccine in asplenic patients.* European Journal of Clinical Microbiology and Infectious Diseases, 2010. **30**(5): p. 611-618.
28. Borrow, R., P. Balmer, and E. Miller, *Meningococcal surrogates of protection—serum bactericidal antibody activity.* Vaccine, 2005. **23**(17-18): p. 2222-2227.
29. Sinwar, P.D., *Overwhelming post splenectomy infection syndrome – Review study.* International Journal of Surgery, 2014. **12**(12): p. 1314-1316.
30. Marrie, T.J., et al., *Asplenic patients and invasive pneumococcal disease—how bad is it these days?* International Journal of Infectious Diseases, 2016. **51**: p. 27-30.
31. Holdsworth, R.J., A.D. Irving, and A. Cuschieri, *Postsplenectomy sepsis and its mortality rate: actual versus perceived risks* Br. J. Surg., 1991. **78**: p. 1031–1038.
32. Davidson, R.N. and R.A. Wall, *Prevention and management of infections in patients without a spleen.* Clin Microbiol Infect, 2001. **7** p. 657-660.
33. Bonanni, P., et al., *Recommended vaccinations for asplenic and hyposplenic adult patients.* Human Vaccines & Immunotherapeutics, 2017. **13**(2): p. 359–368.