

Immunity to Pneumococcal Vaccine in Splenectomized β -Thalassemia Patients

¹Abdolreza Sotoodeh Jahromi,
¹Mohammad Reza Farjam, ²Alireza Makarem,
²Mohammad Yasin Karami, ²Aida Amanat and ³Abdoulhossein Madani
¹Department of Immunology, Faculty of Medicine,
²Department of Epidemiology, Student Research Committee,
Jahrom University of Medical Sciences, Jahrom, Iran
³Department of Epidemiology, Faculty of Health,
Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Abstract: Problem statement: Splenectomy is accompanied by a lifelong risk of overwhelming post splenectomy infection, mainly caused by encapsulated bacteria such as *Streptococcus pneumoniae*. The mortality rate in those infected patients remains high. Therefore the pneumococcal polysaccharide vaccine has been recommended. **Approach:** To obtain information on the immunity levels of pneumococcal antibody, in splenectomized β -thalassemia patients in south of Iran. **Patients and methods:** This descriptive and cross sectional study was carried out on 263 splenectomized β -thalassemia patients in south of Iran, June 2007-September 2008. Anti-pneumococcal vaccine antibody concentrations were measured by the enzyme-linked immunosorbent assay method. The patients were divided into two groups based on antibody concentrations: Group 1: Hyporesponsiveness patients or non-immune with less than 250 mU mL⁻¹ antibody concentrations and group 2: Immune patients or responder with equal or more than 250 mU mL⁻¹ antibody concentrations. **Results:** The results showed that 33.80 and 66.20% of the patients were immune and non-immune to pneumococcal infections, respectively. There was an inverse significant between Immunity level with the period after pneumococcal vaccination ($r = -0.683$, $p = 0.0001$). **Conclusion:** These results suggest that a high percentage splenectomized β -thalassemia patients are poor responders to pneumococcal vaccination. Therefore evaluations immunity levels of pneumococcal vaccine and re-vaccination for pneumococcus are recommended in these patients.

Key words: Immune response, pneumococcal infection, splenectomy, thalassemia, vaccination

INTRODUCTION

The term thalassemia (derived from the Greek “Thalassa”, which means “the sea” stating to the Mediterranean-and “emia”, meaning “related to blood”) designates a diverse group of genetic disorders of hemoglobin synthesis characterized by a disturbance of the making of globin chains, leading to anemia, ineffectual erythropoiesis (Weatherall and Clegg, 2001), particularly in some parts of the world like the Mediterranean area, north and west of Africa and the Middle East. Its' worldwide incidence is about 2 per 1,000 newborns while its' prevalence in Iran is 8-4 per 1,000 newborns and frequency of thalassemia gene in Iran is 4-10% (Ali *et al.*, 2008).

Health wealth is insufficient in most of the countries where the disease is common (Karnon *et al.*, 1999). In most of the countries where β -thalassemia is common, there is not a realistic situation for these patients. The blood transfusion resource was basic at best and desferrioxamine supply was inconsistent. Full facial and clinical features of the disease from the ineffective bone marrow expansion were presented in most of the patients. Splenomegaly was seen in patients early in their lives and splenectomy was done at a relatively young age. Splenectomy is necessary in β -thalassemia patients when the spleen develops hyperactive, leading to extreme destruction of erythrocytes and thus growing the need for regular blood transfusions, which in turn outcomes more iron

Corresponding Author: Mohammad Reza Farjam, Department of Immunology, Faculty of Medicine,
Jahrom University of Medical Sciences, Jahrom, Iran

accumulation. As a result, splenectomy is commonly done (Hoe *et al.*, 1994). Infectious diseases are the second most common cause of mortality and a main cause of morbidity in β -thalassemia patients (Cadili and Gara, 2008). Some immunological disorders have been reported in β -thalassemia patients (Gharagozloo *et al.*, 2009) and asplenia is one of the essential causes of immunodeficiency in patients with β -thalassemia (Ricerca *et al.*, 2009).

As the spleen is more effective at eliminating non-opsonized bacteria, mostly encapsulated organisms, asplenia can lead to immune defects to encapsulated micro-organisms (Cadili and Gara, 2008) and splenectomized β -thalassemia patients are more at risk of developing sepsis by these encapsulated bacteria. Overwhelming Postsplenectomy Infection (OPSI) is a serious complication of asplenia and is usually caused by encapsulated bacteria (Waghorn, 2001). Over 70% of all cases of OPSI are due to *Streptococcus pneumoniae*, even though no more than 5% of other septic episodes are attributable to this bacterium (Cadili and Gara, 2008). To prevent OPSI, a pneumococcal vaccine with purified capsular polysaccharide from the pneumococcal types that commonly cause infection has been recommended (Austrian, 1977).

Iran splenectomized β -thalassemia patients are vaccinated with Pneumococcal vaccine (Pneumovax) to prevent OPSI 14 days before splenectomy.

Unfortunately, none of the Iranian splenectomized β -thalassemia patients have been assessed for immunity level to *Streptococcus pneumoniae* and there is no monitoring for this immunity in these patients in IRAN. This was the first study to determine the antibody response to vaccination for *Streptococcus pneumoniae* with Pneumococcal vaccine (Pneumovax) in Iranian splenectomized β -thalassemia patients.

MATERIALS AND METHODS

Patients and methods:

Patients: A total of 263 β -thalassemia patients who underwent splenectomy and vaccinated with Pneumovax, who were referred to thalassemia centers of in the south of Iran, were enrolled to this descriptive and cross sectional study.

Exclusion criteria for all the patients included: Any immune suppressive therapy in the prior six months and a history of immunodeficiency and Acquired Immunodeficiency Syndrome (AIDS). This study was carried out from June 2007 to September 2008.

The study protocol was reviewed and approved by the Research Ethics Committee of Jahrom University of Medical Sciences and informed consent was obtained from each patient.

Methods: A questionnaire was designed to collect demographic information and medical history of the patients by reviewing the patient's records. All patients vaccinated intramuscularly with a single dose of 0.5 ml of Pneumovax 14 days before splenectomy. Blood samples were taken from the patients and serum samples were stored at -70°C until assayed for antibody measurements. Specific antibodies against Pneumococcal Capsular Polysaccharide (PCP) were measured using the protocol of the third-generation enzyme-linked immunosorbent assay format (Wernette *et al.*, 2003; Mahmoodi *et al.*, 2009).

The assay has been designed to measure the IgG antibody responses to pneumococcal vaccine incorporating 23 polysaccharides isolated from *Streptococcus pneumoniae*. These polysaccharides correspond to approximately 80% of the commonly encountered, virulent serotypes. The levels of serum specific anti-PCP IgG antibodies were determined by an enzyme immunoassay kit (Pneumococcus IgG Immunopotency level/E-DG-MZ-001/04-04, ZenTech, Belgium). In this assay, microwaves were pre-coated with the PCP antigen. Serum samples were tested in accordance with the manufacturer's instructions. Serum samples were diluted 1:510. The titers of anti PCP IgG antibodies were determined with reference to the standard serum included in the kit and were expressed as mili unit/ml (mu/ml). Some reference plasmas with assigned values of anti PCP IgG were used as Calibrators to draw standard curve. The sensitivity of this kit was 6.9 mU/mL. Serum samples were tested in duplicate.

Corresponding anti PCP IgG antibodies in mu/ml are obtained by interpolating the observance of each sample on the calibration curve. According to directions on this kit the result Interpretation was done as follows:

- Lower than 250 mU mL^{-1} = Deficient in anti PCP IgG antibodies or hyporesponsiveness
- Equal to or more than 250 mU mL^{-1} = good response or Immune to pneumococcal infection
- All patients were categorized according to their serum anti-pneumococcal IgG titers

Statistical analysis: Data were analyzed using an SPSS 14.0 software package. Specific antibody titers were expressed as the geometric mean. T test and correlation test were performed for analysis.

RESULTS

A total of 263 splenectomized β -thalassemia patients, (133 males and 130 females), aged 18-64 years (mean, 41 ± 13 years) and with time post pneumococcal vaccination 12-336 mounts (mean, 106.77 ± 67.62) mounts were evaluated for antibody response to PPV23.

Table 1: Demographic and serum anti-pneumococcal IgG titers for the patients

Sex	Age (years)	Time post vaccination (months)	Anti-pneumococcus IgG (mU/ml)
Female N=130 (49.40%)	18.39±4.55	94.85±59.00	212.67±68.72
Male N=133 (50.60%)	19.25±6.40	118.42±73.46	199.00±74.50
P-value	0.214	0.004	0.123

Table-2: Comparison of Hypo-responsive group Vs responder group

Groups	Age (years)	Time after vaccination (months)	Anti-pneumococcus IgG (mU/mL)
Hypo-responsive	19. 17±5.00	133.88±60.71	167.30±52.15
Responder	18. 16±6.53	53.76±45.52	280.94±37.42
P-Value	0. 165	0.0001*	0.0001*

There were found inverse significant correlations between anti-pneumococcal IgG titers and time post vaccination ($r = -0.683$, $p = 0.0001$)

Demographic and serum anti-pneumococcal IgG titers (mU/mL) for all patients were summarized in Table 1. All patients were categorized according to their serum anti-pneumococcal IgG titers (mU/mL) to two groups:

- Group-1 (hypo-responsive): 174 patients (66.2%) with mean 167.30±52.15 mU/mL serum anti-pneumococcal IgG
- Group-2 (immune or responder): 89 patients (33.8%) with mean 280.30±52.15 mU mL⁻¹ serum anti-pneumococcal IgG

There were significant differences between serum anti-pneumococcal IgG titers and time post vaccination in two groups. The characteristics of two patient groups (hypo-responsiveness Vs immune or responder) according to anti-pneumococcal IgG titers are presented in Table 2.

DISCUSSION

Splenectomized β -thalassemia patients have a high risk of Streptococcus pneumoniae infections, which could be due to the reduced antibody response to bacterial polysaccharide antigens in these patients due to splenectomy (Zarina *et al.*, 2010).

Although pneumococcal immunization in Splenectomized β -thalassemia patients is recommended (Sheikha *et al.*, 2007), there are few reported studies involving the evaluation of the antibody response to polysaccharide antigens in these patients or the efficacy of pneumococcal vaccination in these patients (Sakran *et al.*, 2010).

Sufficient antibody response to pneumococcal antigens (equal to or more than 250 mU mL⁻¹) in Splenectomized β -thalassemia patients can show the

significance of vaccination in order to protect them against pneumococcal infections (Cadili and Gara, 2008; Waghorn, 2001; Austrian, 1977).

The result of the present study indicated that 66.2% of Splenectomized β -thalassemia patients were hypo-responsive to pneumococcal antigens, which is in arrangement with the study performed by Rao *et al.* (1995) showing that 42% of the vaccinated Splenectomized β -thalassemia patients were protective to Streptococcus pneumonia. This indicates that measurement of post-vaccination antibody levels can be used to identify the poor responders who most likely are at increased risk of pneumococcal infection.

In the present study, mean anti-pneumococcal IgG titers in male patients did not differ from female patients. This data shows that anti-pneumococcal IgG titer is independent of sex in these patients.

However, poor responders proved to be unresponsive to booster doses of polysaccharide antigens (Rodriguez-Barradas *et al.*, 1996; Petrasch *et al.*, 1997). Purified polysaccharide antigens result in T cell-independent, type-2 antibody formation and do not elicit memory B cells. T cell-independent antibody responses cannot be boosted to produce secondary responses. It is possible that conjugate vaccines (capsular polysaccharides conjugated to carrier proteins) might improve the immune response in non-responders to PPV: the 7-valent pneumococcal conjugate vaccine has improved the response rate to PPV23 in patients with selective antibody deficiency (Zielen *et al.*, 2000).

The results obtained here suggest that an immunization policy with pneumococcal polysaccharides might help to protect the majority of Splenectomized β -thalassemia patients against invasive pneumococcal diseases; therefore, we suggest that the vaccine be administered. Nevertheless, some subjects who cannot mount an adequate antibody response to polysaccharide antigens will remain at significant risk for such infections and should be offered other, prophylactic methods such as immunization with pneumococcal conjugate vaccines.

According to an inverse significant correlation between mean anti-pneumococcal IgG titers and time post splenectomy which was found from this study, indicates a decline of anti-pneumococcal IgG titer along the time. Published data indicate that vaccinated immunocompromised patients, who had a nearly normal antibody response to pneumococcal vaccine and immune patients to Streptococcus pneumonia, will require revaccination to maintain their immunity (Zarina *et al.*, 2010; Orthopoulos *et al.*, 2009).

It is notable that some factors such as chronic kidney disease and haemodialysis can reduce the immune response to vaccines (Jahromi *et al.*, 2009; 2011) and it has been not investigated between kidney function tests and immunity to pneumococcal vaccine in this research.

CONCLUSION

Based on the results of this study, there is a need to re-address the approach towards revaccination in this immune-compromised group of patients by administering a booster pneumococcal vaccination in an attempt to recover immunity and reduce morbidity. Further researches with larger samples from other areas of IRAN are recommended for this approach.

ACKNOWLEDGMENT

This research was funded by the vice-chancellor for research of Jahrom University of Medical Sciences and Health Services. The authors are appreciative to all the patients and their families for their kind cooperation in this research.

REFERENCES

- Ali, D., K. Mehran and A.G. Moghaddam, 2008. Comparative evaluation of renal findings in Beta-thalassemia major and intermedia. *Saudi J. Kidney Dis. Transpl.*, 19: 206-209. PMID: 18310868
- Austrian, R., 1977. Pneumococcal infection and pneumococcal vaccine. *N. Engl. J. Med.*, 297: 938-939.
- Cadili, A. and C.D. Gara, 2008. Complications of splenectomy. *Am. J. Med.*, 121: 371-275. PMID: 18456028
- Gharagozloo, M., M. Karimi and Z. Amirghofran 2009. Double-faced cell-mediated immunity in beta-thalassemia major: Stimulated phenotype versus suppressed activity. *Ann. Hematol.*, 88: 21-27. PMID: 18690440
- Hoe, T.S., A. Lammi and B. Webster, 1994. Homozygous β -thalassaemia: A review of patients who had splenectomy at the Royal Alexandra Hospital for Children, Sydney. *Singapore Med. J.*, 35: 59-61. PMID: 8009282
- Jahromi, A.S., M. Pourahmd, S. Azhdari, G. Manshoori and A. Madani *et al.*, 2011. Immunity to diphtheria in haemodialysis patients. *Am. J. Infect. Dis.*, 7: 20-23. DOI: 10.3844/ajidsp.2011.20.23
- Jahromi, A.S., R. Raoofi, M. Sarikhani and A. Madani. 2009. Evaluation of anti-tetanus immunity in haemodialysis patients. *Am. J. Immunol.*, 5: 108-112. DOI: 10.3844/ajisp.2009.108.112
- Karnon, J., D. Zeuner, J. Brown, A.E. Ades and B. Wonke *et al.*, 1999. Lifetime treatment costs of beta-thalassaemia major. *Clin. Lab Haematol.*, 21: 377-385. PMID: 10671989
- Mahmoodi, M., A. Aghamohammadi, N. Rezaei, M. Lessan-Pezeshki and G. Pourmand *et al.*, 2009. Antibody response to pneumococcal capsular polysaccharide vaccination in patients with chronic kidney disease. *Eur. Cytokine Netw.*, 20: 69-74. PMID: 19541592
- Orthopoulos, G.V., M.C. Theodoridou, V.A. Ladis, D.K. Tsousis and V.I. Spoulou, 2009. The effect of 23-valent pneumococcal polysaccharide vaccine on immunological priming induced by 7-valent conjugate vaccine in asplenic subjects with beta-thalassemia. *Vaccine*, 27: 350-354. PMID: 19010369
- Petrasch, S., O. Kuhnemund, A. Reinacher, M. Uppenkamp and R. Reinert *et al.*, 1997. Antibody responses of splenectomized patients with non-Hodgkin's lymphoma to immunization with polyvalent pneumococcal vaccines. *Clin. Vaccine Immunol.*, 4: 635-638.
- Rao, S.P., K. Rajkumar, G. Schiffman, N. Desai and C. Unger *et al.*, 1995. Anti-pneumococcal antibody levels three to seven years after first booster immunization in children with sickle cell disease and after a second booster. *J. Pediatrics*, 127: 590-592. DOI: 10.1016/S0022-3476(95)70119-2
- Ricerca, B.M., A.D. Girolamo and D. Rund, 2009. Infections in thalassemia and hemoglobinopathies: Focus on therapy-related complications. *Mediterr J. Hematol. Infect. Dis.*, 28: e2009028-e2009028. PMID: 21415996
- Rodriguez-Barradas, M.C., J.E. Groover, C.E. Lacke, D.W. Gump and C.J. Lahart *et al.*, 1996. DisIgG antibody to pneumococcal capsular polysaccharide in human immunodeficiency virus-infected subjects: Persistence of antibody in responders, revaccination in nonresponders and relationship of immunoglobulin allotype to response. *J. Infect. Dis.*, 173: 1347-1353. PMID: 8648206
- Sakran, W., C. Levin, Y. Kenes, R. Colodner and A. Koren, 2010. Clinical spectrum of serious bacterial infections among splenectomized patients with hemoglobinopathies in Israel: A 37-year follow-up study. *Infection*, 40: 35-39. DOI: 10.1007/s15010-011-0178-5
- Sheikha, A.K., Z.T. Salih, K.H. Kasnazan, M.K. Khoshnaw and T. Al-Maliki *et al.*, 2007. Prevention of overwhelming postsplenectomy infection in thalassemia patients by partial rather than total splenectomy. *Can. J. Surg.*, 50: 382-386.

- Waghorn, D.J., 2001. Overwhelming infection in asplenic patients: Current best practice preventive measures are not being followed. *J. Clin. Pathol.*, 54: 214-218. DOI: 10.1136/jcp.54.3.214
- Weatherall, D.J. and J.B. Clegg, 2001. *The Thalassemia Syndromes*. 4th Edn., Blackwell Science, Oxford, ISBN-10: 0865426643, pp: 846.
- Wernette, C.M., C.E. Frasch, D. Madore, G. Carlone and D. Goldblatt *et al.*, 2003. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clin. Diagn Lab Immunol.*, 10: 514-519. PMID: 12853378
- Zarina, A.L., K.N. Norazlin, A. Hamidah, D.A. Aziz and S.Z. Zulkifli *et al.*, 2010. Spectrum of infections in splenectomised thalassaemia patients. *Med. J. Malaysia*, 65: 283-285. PMID: 21901946
- Zielen, S., I. Buhring, N. Strnad, J. Reichenbach and D. Hofmann, 2000. Immunogenicity and tolerance of a 7-valent pneumococcal conjugate vaccine in nonresponders to the 23-valent pneumococcal vaccine. *Infect. Immun.*, 68: 1435-1440.