

# The inverse correlation between serum levels of anti-pneumococcal and ferritin after pneumococcal vaccination in splenectomised beta thalassemia major

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Splenectomy is necessary in beta thalassemia major patients when the spleen becomes hyperactive, leading to extreme destruction of erythrocytes. This study assessed the ferritin effect on serum pneumococcal antibody response following pneumococcal vaccination, in patients with beta thalassemia major after splenectomy. In this case series study, convenience sampling was used to recruit 347 splenectomised beta thalassemia patients under the auspices of Jahrom University of Medical Sciences. Demographic data such as age, sex, and time after splenectomy were recorded by a questionnaire. All participants had been splenectomised and received a dose of Pneumovax<sup>®</sup> 23 vaccine 14 days before surgery. The IgG antibody responses to pneumococcal vaccine and levels of serum specific ferritin were determined by commercial enzyme immunoassay kits. For the analysis, SPSS software version 16 was used. A p-value less than 0.05 was considered statistically significant. Most of the participants (63.4%) were hypo-responders to pneumococcal vaccine. Also, serum anti-pneumococcal IgG antibody was related to post splenectomy duration and serum ferritin ( $p < 0.001$ ), but not to gender ( $p > 0.05$ ). An important result was a relation of serum anti-pneumococcal IgG antibody to serum ferritin according to post splenectomy duration groups. Therefore, in three groups of post splenectomy duration, the serum ferritin was higher in hypo-responder than in good responder subjects. Our results indicate that serum anti-pneumococcal IgG antibody decreased with increment of serum ferritin and post splenectomy duration. Thus, 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.

**Keywords:** pneumococcal vaccines; ferritins; splenectomy; beta thalassemia; Iran.

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## Introduction

Thalassemia is an inherited condition involving an abnormal form of hemoglobin, which leads to disturbances in globin chain. Active erythropoiesis and anemia are among the consequences of this disorder. Although thalassemia occurs across the globe, it is most prevalent in North Africa, the Mediterranean area and Western Asia. Newborn babies are affected by the disease by a rate of two in 1,000 across the globe.<sup>(1)</sup> In Iran, however, the rate is twice as high, with the frequency of thalassemia gene being 4-10%.<sup>(2)</sup>

Splenectomy is necessary in beta thalassemia major (BTM) patients when the spleen becomes hyperactive, leading to extreme destruction of erythrocytes and thus growing the need for regular blood transfusions, which in turn outcomes more iron accumulation. Splenectomy, the aim of which is to reduce blood consumption, is the major therapy for iron overload among transfusion-dependent patients with thalassemia.<sup>(3)</sup> Splenectomy as well as changes in immune system seem to be the major causes of infectious diseases in these patients. Infections are the second leading cause of mortality and main cause of morbidity among patients with

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beta thalassemia disorder.<sup>(4)</sup> Spleen is essential for the elimination of non-opsonized bacteria, which are highly encapsulated organisms. Thus, the absence of spleen makes the immune system ineffective in eliminating such bacteria and opens the way to sepsis and overwhelming post splenectomy infection (OPSI) accounting for over 70% of cases of OPSI, while under normal conditions only 5% of sepsis are caused by these bacteria.<sup>(5)</sup> This infection was often caused by capsular polysaccharide of *Streptococcus pneumoniae* and sometimes by *Hemophilus influenzae* type b (Hib).

An increase of immune children to Hib with advancing age was found, indicating an increase in children's contact with this microorganism during childhood.<sup>(6)</sup> Thus, it is highly recommended vaccination against such encapsulated bacteria especially in children with BTM who need to receive vaccination prior to splenectomy.<sup>(7)</sup> In Iran this procedure is carried out 14 days before splenectomy.

In a previous study we found that immunity level significantly decreased with longer period after pneumococcal vaccination in splenectomised beta thalassemia (SBT) patients. Also, 66.2% of patients were non-immune to pneumococci.<sup>(8)</sup> *Hemophilus influenzae* type b antibody level was lower in SBT patients than non splenectomised beta thalassemia.<sup>(9)</sup>

Iron is an important material for the growth of all human cells including white blood cells, but large amounts of iron and iron overload lead to iron toxicity.<sup>(10)</sup> In BTM, red blood cell destruction causes iron accumulation in the body, which is called iron overload. Patients suffering from iron overload, in addition to cell damage, also increase the risk of infection. Iron overload is the main cause of immune abnormalities.<sup>(11)</sup>

Aleem et al., suggested higher counts of circulating B-cells and IgG immunoglobulin after reduction of iron overload with deferasirox therapy among 17 beta thalassemia patients.<sup>(12)</sup> On the other hand, iron overload creates an antigen exhibition blockage.<sup>(13)</sup> Nevertheless, Pardalos et al., reported higher serum ferritin levels among BTM patients than in control subjects, but no different for serum immunoglobulin levels between both groups.<sup>(14)</sup>

Due to adverse effect of iron overload on immune response, this study assessed the ferritin effect on antibody response following pneumococcal vaccination in patients with BTM after splenectomy.

## Materials and Methods

### Study design

In this case series study, convenience sampling was used to recruit 347 SBT patients under the auspices of Jahrom university of Medical Sciences. The patients who had received immune suppressive therapy prior the research were excluded from the research. The research project commenced on January 1<sup>st</sup>, 2016 and terminated on June 3<sup>rd</sup>, 2016. The study was approved by the local ethical committee (ethical code: IR.JUMS.REC.1400.057) and all participants were asked to complete an informed consent. Demographic data such as age, sex, and time after splenectomy were recorded by a questionnaire. No participants had primary immune deficiency or acquired immune deficiency syndrome. All participants were splenectomised and received a dose of Pneumovax<sup>®</sup> 23 vaccine, 14 days before surgery. The research instruments included data collection form aimed at collecting demographic data and medical history of the participants in the research.

### Sampling

The research process started with the collection of blood samples from the participants to be assayed for antibody and ferritin measurements. The IgG antibody responses to pneumococcal vaccine were assayed to measure pneumococcal capsular polysaccharide (PCP) IgG. To that end, 23 polysaccharides, which accounted for 80% of the virulent serotypes, were isolated from *Streptococcus pneumoniae*. Prior to testing serum samples, anti-PCP IgG antibodies were controlled by pneumococcus IgG immunopotency level/E-DG-MZ-001/04-04, ZenTech, Belgium, in which PCP antigen was used to pre-coat. After achieving 1:510 dilutions, a standard curve was drawn using reference plasmas as calibrators with sensitivity 6.9 mU/mL. Each sample on the curve was interpolated to anti PCP IgG antibodies in mU/mL as specified by the kit. According to directions on this kit, the result interpretation was done as follows:<sup>(5)</sup>

- Lower than 250 mU/mL: deficient in anti PCP IgG antibodies or hypo-responder.
- Equal to or more than 250 mU/mL: good responder or immune to pneumococcal infection.

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

The SBT patients were divided into three groups according to duration after splenectomy: group 1 included post splenectomy duration (PSD) till 36 month (PSD36), group 2 included PSD 37-120 month (PSD120) and group 3 included PSD over 120 months (PSD over 120).

### Statistical analysis

Continues variables are presented as mean  $\pm$  standard deviation and categorical variables are presented as number and percent. We used the independent t-test and One-way ANOVA to compare the means and chi square to compare the proportions according to age, sex and duration after splenectomy. Also, the dependency of antibody titers to serum ferritin was analyzed by linear regression test. For the analysis we used SPSS software version 16 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

### Results

A total of 347 SBT patients participated, 50.1% were male. Mean age of patients was 18.8 ( $\pm$  5.5) years, with average 10-38 years, which was not statistically different in both genders ( $p= 0.065$ ). Average PSD was 12-336 months (mean, 106.8  $\pm$  67.6). The mean duration after splenectomy was longer in male subject in comparison

to female ( $p<0.001$ ). In all of subjects, the mean anti pneumococcal IgG antibody (APA) and serum ferritin levels was 206.7  $\pm$  70.8 mU/mL and 2134.7  $\pm$  1614.1 ng/mL, respectively. Ferritin levels were significantly higher in male patients ( $p= 0.045$ ). Although, the mean antibody levels were higher in female patient, there were no statistical differences ( $p= 0.069$ ) (Table 1).

The patients were divided into two groups based on their serum APA titers (mU/mL): hypo-responders and good responders. While 220 patients (63.4%) fell into the first category, the rest, 127 patients (36.6%) fell into the second. Patients in good responder group were more female ( $p= 0.009$ ) and younger ( $p= 0.024$ ), and had lower ferritin level ( $p<0.001$ ) and a shorter duration after splenectomy ( $p< 0.001$ ) than hypo responder group (Table 2).

As shown in Table 3, the age and serum level of ferritin increased with PSD increment ( $p<0.001$ ). On the contrary, the serum level of APA declined with increasing PSD ( $p< 0.001$ ). The immunity to *Streptococcus pneumoniae* decreased with time after splenectomy ( $p< 0.001$ ).

Among PSD36 group, the ferritin level was higher in hypo-responder subjects compare to good responder ones (3251.0  $\pm$  0.0 vs. 464.5  $\pm$  128.9,  $p< 0.001$ ). Also, in PSD120 and PSD over 120 patients, the ferritin level was higher in hypo-responder subjects compare to good

**Table 1.** The percent or mean of studied variables in all participants and by sex of patients.

Variables	All	Female	Male	P
Number (%)	347 (100.0)	173 (49.9)	174 (50.1)	-
Age, year: mean (SD)	18.8 (5.5)	18.2 (4.4)	19.3 (6.4)	0.065
Post splenectomy duration, month: mean (SD)	106.1 (66.6)	93.4 (58.2)	118.7 (71.9)	< 0.001
Anti-pneumococcal IgG antibody, mU/mL: mean (SD)	206.7 (70.8)	213.7 (66.7)	199.8 (74.3)	0.069
Ferritin, ng/mL: mean (SD)	2134.7 (1614.1)	1960.6 (1449.3)	2307.8 (1749.7)	0.045

SD: Standard deviation.

**Table 2.** Comparison of studied variables between hypo-responder and good responder group.

Variables	Hypo-responder	Good responder	P
Number (%)	220 (63.4)	127 (36.6)	-
Sex, male: number (%)	122 (55.5)	52 (40.9)	0.009
Age, year: mean (SD)	19.3 (4.9)	17.9 (6.3)	0.024
Post splenectomy duration, month: mean (SD)	134.2 (60.3)	57.3 (45.3)	< 0.001
Ferritin, ng/mL: mean (SD)	3025.6 (1362.2)	591.4 (377.0)	< 0.001

SD: Standard deviation.

**Table 3.** Age and serum concentration of anti-pneumococcal antibody and ferritin according to post splenectomy duration.

Post splenectomy duration	1-36 month	37-120 month	>120 month	P
Number (%)	72 (27.7)	130 (37.5)	145 (41.8)	-
Age, year: mean (SD)	15.8 (4.5)	16.7 (2.7)	22.2 (6.1)	< 0.001
Anti- pneumococcal IgG antibody, mU/mL: mean (SD)	283.3 (47.9)	208.5 (58.0)	167.1 (58.3)	< 0.001
Hypo-responder, number (%)	7 (9.7)	77 (59.2)	136 (93.8)	< 0.001
Ferritin, ng/mL: mean (SD)	735.4 (840.3)	1761.6 (1158.9)	3164.1 (1592.7)	< 0.001

SD: Standard deviation.

**Table 4.** Relation of anti-pneumococcal IgG antibody with sex, age, duration after splenectomy and serum ferritin level according to regression analysis.

Post splenectomy duration	1-36 month	37-120 month	>120 month	P
Post splenectomy duration, month	298.93	- 0.288	- 0.271	< 0.001
Serum ferritin, ng/mL		- 0.029	- 0.658	< 0.001

SD: Standard deviation.

responder individuals ( $2467.4 \pm 922.5$  vs.  $736.2 \pm 527.8$ ,  $p < 0.001$ ;  $3330.1 \pm 1502.4$  vs.  $655.2 \pm 144.0$ ,  $p < 0.001$ , respectively). The serum APA level was related to PSD (constant: 282.59, B: - 0.715,  $p < 0.001$ ) and ferritin (constant: 283.83, B: - 0.036,  $p < 0.001$ ) inversely when variables entered separately in linear regression analysis model. When the variables sex, age, PSD and ferritin level were entered together in linear regression model, APA was related to both PSD and ferritin level (Table 4).

## Discussion

*Streptococcus pneumoniae* infections were found to be a high risk factor to SBT patients, which may be as a result of the reduced antibody response to bacterial polysaccharide antigens attributable to splenectomy.<sup>(15)</sup> Meanwhile, though preventive measures such as pneumococcal vaccination can be adopted, their efficacy needs to be proved.<sup>(16)</sup> Nowadays, pneumococcal vaccination in SBT individuals is mentioned.

The result of this study demonstrated that 63.4% of SBT patients were hypo-responders to pneumococcal antigens. Similar results have been reported by Rao et al, who reported that 42% of the immunized SBT patients were defensive to *Streptococcus pneumoniae*.<sup>(17)</sup> This indicates that measurement of post-vaccination antibody levels can be used to identify poor responders who are likely to be at increased risk of pneumococcal infection. Also, the results of our study showed that 93.8% and 59.2% of SBT patients who belong to PSD over 120 and

PSD 120 were hypo-responders, respectively, compare to patients among PSD 36 group that 9.7% were hypo-responders. Higher PSD and ferritin level were found for hypo-responder SBT patients. Furthermore, patients with prolong PSD had higher ferritin level and lower immunity to *Streptococcus pneumoniae*. This indicates that higher level of ferritin and prolongation of time after splenectomy and pneumococcal immunization may cause immunity deterioration. This idea was shown by the linear regression model indicating that the level of PSD and ferritin has a significant reverse effect on serum APA. An important result of our study was the higher level of ferritin in the hypo-responders compared to the good responders in each PSD categories.

A low number of T suppressor cells ( $CD_8$ ) without change of T helper cells ( $CD_4$ ) was reported in patients with hereditary hemochromatosis (HH) compare to healthy controls.<sup>(13)</sup> Also, a decrease in the  $CD_8$  count and an increase in the  $CD_4/CD_8$  ratios were observed in HH patients; there was a positive correlation between  $CD_4/CD_8$  ratios and amount of phlebotomy.<sup>(13)</sup> This result is in line with our study where 90.3% of SBT patients were immune against pneumococcal pneumonia until 36 month after vaccination. In contrast to our study, the increment of absolute numbers of  $CD_8$  cells in HH patients and an antigen presentation obstacle time and dose dependent in iron overload status was suggested.<sup>(13)</sup>

The results obtained here suggest that an immunization policy with pneumococcal polysaccharides might help

to protect the majority of SBT patients against invasive pneumococcal diseases; therefore, it is suggested that the vaccine be administered. The significant inverse relation between mean anti-pneumococcal IgG titers and time post splenectomy that was found in our study indicates a decline of anti-pneumococcal IgG titer along the time. Thus, with progression of time after splenectomy, antibodies against polysaccharide antigens will decrease and patients will remain at significant risk for such infections. Published data indicate that vaccinated immune compromised patients, who had a nearly normal antibody response to pneumococcal vaccine and immune patients to streptococcus pneumonia, will require revaccination to maintain their immunity.<sup>(15,18)</sup>

It is notable that some factors such as chronic kidney disease and hemodialysis can reduce the immune response to vaccines;<sup>(19,20)</sup> renal function tests and immunity to the pneumococcal vaccine were not investigated in this research.

The present study indicates a significant reverse relation between serum APA and serum levels of ferritin. Also in a study, thalassemia patients who was on the iron chelation therapy showed no differences in immunoglobulin concentrations compare to healthy subjects.<sup>(21)</sup> BTM patients who receive multiple blood transfusions and express a decreased T4+ cell activity should be considered as a high risk population for the development of clinical infection. The evident association between iron overload and T cell abnormalities observed in another study suggests that correct and continuous regulation of annual iron balance in BTM patients is an important factor in minimizing this immunological disturbance.<sup>(14)</sup>

## Conclusion

Our results indicate that serum anti-pneumococcal IgG antibody decreased with increment of serum ferritin and PSD. Thus, 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 and analytic studies are recommended for this approach.

## Conflict of interest

The authors declare that there is no conflict of interest.

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## Author's contributions

Karamatollah Rahmanian: conception of the study and participated in the statistical analysis.

Masihollah Shakeri: contributed to the writing of the manuscript.

Vahid Rahmanian: design and management of the study, writing the manuscript and implementing all comments from the reviewers.

Shahriyar Najafi-Tireh Shabankareh: translation of the manuscript.

Fatemeh Sotoodeh Jahromi: contributed to the writing and translation of the manuscript.

Abdolreza Sotoodeh Jahromi: conception, design and management of the study, writing the manuscript and implementing all comments from the reviewers.

All authors read and approved the final manuscript.

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## **Correlación inversa entre los niveles séricos de anticuerpos antineumocócicos y ferritina después de la vacunación antineumocócica de pacientes con beta talasemia mayor esplenectomizados**

### **Resumen**

La esplenectomía es necesaria en pacientes con beta talasemia mayor cuando el bazo se vuelve hiperactivo, lo que lleva a una destrucción extrema de los eritrocitos. Este estudio evaluó el efecto de la ferritina sobre la respuesta de anticuerpos antineumocócicos en suero después de la vacunación antineumocócica, en pacientes con talasemia beta mayor a los que se les realizó esplenectomía. En este estudio de serie de casos, se utilizó un muestreo de conveniencia para reclutar a 347 pacientes con beta talasemia esplenectomizados bajo los auspicios de la Universidad de Ciencias Médicas de Jahrom. Los datos demográficos como la edad, el sexo y el tiempo después de la esplenectomía se registraron mediante un cuestionario. Todos los participantes fueron esplenectomizados y recibieron una dosis de la vacuna Pneumovax® 23, 14 días antes de la cirugía. Las respuestas de anticuerpos IgG a la vacuna neumocócica y los niveles de ferritina sérica específica se determinaron mediante estuches comerciales de inmunoensayo enzimático. Para el análisis se utilizó el programa SPSS versión 16. Un valor de p inferior a 0,05 se consideró estadísticamente significativo. La mayoría de los participantes (63,4%) resultaron hiporrespondedores a la vacuna antineumocócica. Además, el anticuerpo sérico antineumocócico IgG se relacionó con la duración de la esplenectomía y la ferritina sérica ( $p < 0,001$ ), pero no con el género ( $p > 0,05$ ). Un resultado importante fue la relación del anticuerpo sérico IgG antineumocócico con la ferritina sérica según los grupos de duración postesplenectomía. Por lo tanto, en tres grupos de duración posterior a la esplenectomía, la ferritina sérica fue mayor en los sujetos con hiporrespuesta que en los sujetos con buena respuesta. Nuestros resultados indican que el anticuerpo sérico IgG antineumocócico disminuyó con el incremento de la ferritina sérica y la duración posterior a la esplenectomía. Por lo tanto, existe la necesidad de volver a abordar el enfoque hacia la revacunación en este grupo de pacientes inmunocomprometidos mediante la administración de una vacunación antineumocócica de refuerzo en un intento por recuperar la inmunidad y reducir la morbilidad.

**Palabras clave:** vacunas neumocócicas; ferritinas; esplenectomía; talasemia beta; Irán.