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# Natural Immunity Against *Haemophilus influenza* Type B in Splenectomised Beta-thalassaemia Children

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**Abstract:** Patients with beta-thalassaemia major and asplenia have an increased risk of encapsulated bacterial infections. The aim of this study was to determine the *Haemophilus influenza* type b (Hib) antibody concentrations in beta-thalassaemia patients with or without spleens. The Hib antibody concentrations were investigated in 850 patients with thalassaemia major, of whom 437 had undergone splenectomy. Hib antibody levels equal or greater than 1.0 μg $mL^{-1}$ were classified as long-term protection, those between 0.15 and less than 1.0 μg $mL^{-1}$ as short term protection and those less than 0.15 μg $mL^{-1}$ as no protection. The mean Hib antibody level was lower in asplenic subjects than in non splenectomised subjects (0.39±0.5 vs. 1.08±0.55 μg $mL^{-1}$, $p<0.001$). The protective antibody level prevalence in asplenic patients was significantly lower than that in patients with spleens (32.3% vs. 85.7%, $p<0.001$). Protection against Hib decreased as the time interval after splenectomy increased from 57.2% at a less than 60 months interval to 10.8% at a greater than 120 months interval ($p = 0.001$). Nearly 30% of the 437 splenectomised subjects had long-term protection against Hib, whereas 64.4% of the 413 non splenectomised subjects had long term protection ($p<0.001$). Asplenic subjects had lower Hib antibody levels than non splenectomised subjects. Additionally, the antibody levels decreased as the time interval increased after splenectomy. A Hib vaccine recommendation for splenectomised thalassaemia major seems essential.

**Key words:** Haemophilus influenza, splenectomy, thalassemia major, immunity

## INTRODUCTION

Thalassaemia is among the most common genetic diseases worldwide (Quirolo and Vichinnky, 2007) and constitutes a major health problem. This disorder is common in Mediterranean countries, the Middle East, Central Asia, India, Southern China and the Far East as well as countries along the northern coast of Africa and in South America (Galanello and Origa, 2010; Sheikha *et al*., 2007; Faramarzi *et al*., 2010). Also, beta-thalassaemia major is very common and is an important health problem in Iran (Samavat and Modell, 2004), with more than 20,000 cases (Azizi *et al*., 2004).

Patients with beta-thalassaemia major (Cooley anaemia) have an increased risk of serious infections (Eigenberger *et al*., 2007). Kadymova suggested that the functional activities of segmental neutrophils decreased after splenectomy but increased to baseline levels within the first year after the splenectomy (Kadymova, 2009).

Asplenic subjects, especially those with blood disorders (Thomsen *et al*., 2009) have a higher risk of infection than do healthy subjects (Eigenberger *et al*., 2007; El-Alfy and El-Sayed, 2004; Wasserstrom *et al*., 2008). Factors that contribute to infections include lower specific antibody response, impairments in the complement-dependent opsonisation pathway and the phagocytosis of un-opsonised particulate matter (Ram *et al*., 2010; Ricerca *et al*., 2009). Such infections can be caused by encapsulated bacteria, such as *Streptococcus pneumoniae* (Smets *et al*., 2007) and *Haemophilus influenzae* type b (Hib) (Webb *et al*., 2006). Asplenic individuals have a 200- fold higher risk of sepsis, compared to subjects with spleen (Beytout *et al*., 2003). Overwhelming Post-Splenectomy Infection (OPSI) is a long-term risk in asplenic patients (Faramarzi *et al*., 2010; Coignard-Biehler *et al*., 2008). Therefore, adequate antibody concentrations play an important role in preventing infections caused by these pathogens.

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Splenectomised patients must be included in guidelines to protect against former infections. Eigenberger *et al.* (2007) in a study of splenectomised subjects suggested that patients with haematological disorders developed less effective immune responses to the Hib vaccine than did patients who were splenectomised after trauma (Eigenberger *et al.*, 2007). Additionally, Stanford *et al.* (2009) and Mikoluc *et al.* (2008) demonstrated effective responses to a pneumococcal vaccine in asplenic individuals. The blood levels of anti-Hib immunoglobulin G (IgG) indicate a patient's level of immunity against this infection. In Iran due to some limitations, the vaccination of splenectomised individuals against Hib is not done. In a study conducted in Iran, 84.5% of children <5 years of age exhibited natural immunity against Hib infections (Jahromi and Rahmanian, 2012). This result suggested a very high rate of contact to with Hib during childhood. The aim of our study was to compare Hib antibody levels in beta-thalassaemic patients with and without spleens.

## METHODOLOGY

**Patients:** Eight hundred and fifty subjects with beta-thalassaemia major who were referred to the thalassaemic wards of the Motahhari hospitals in Jahrom (Fars Province) and Bandar Abbas (Hormozgan Province), located in southern Iran, including 413 non splenectomised subjects and 437 asplenic subjects were enrolled in this study, 2009-2011. The active Hib infections rule out by paediatrics. Also, if patient referred repeatedly, she/he only enrolled in study first time. Ethical approval was obtained from the Ethics Committee of Jahrom University. Informed-consent from patients or his/her parents was obtained.

**Laboratory evaluations:** Blood samples (3 mL) were collected from both groups to determine the Hib antibody levels. The tests were conducted in the central laboratory at the Jahrom Medical University. No any subjects (beta-thalassaemic subjects with or without spleen) received Hib vaccines because the national guidelines are lacking in this regard and the Hib vaccine is not included in the routine childhood immunization schedule in Iran.

Serum antibodies against Hib determination was measured by using the VaccZyme Human Anti-Hib Enzyme Immunoassay kit (MK016, The Binding Site Ltd, Birmingham, England). Limits of quantification for the assay were 0.11-9.00 μg $mL^{-1}$. Anti-Hib antibody titres of greater than 0.15 and equal or greater than 1.0 μg $mL^{-1}$ have been correlated with minimum and long-term protective immunity, respectively also an Anti-Hib antibody titres under 0.15 μg $mL^{-1}$ gives insufficient protection against *Haemophilus influenzae* type B. (Kayhty *et al.*, 1983; Peraza *et al.*, 2004). Additionally, two groups (long term and short term protection) mention as protective group (Arvas *et al.*, 2008; Ocaktan *et al.*, 2004; Redwan and Elsawy, 2005).

**Statistical analysis:** Data are presented as Mean±Standard deviations or percentage. We used the independent Student's t test and chi-square tests to compare the means and ratios of the two groups (beta- thalassaemic subjects with or without spleen) and in groups alone or one-way ANOVA to compare the mean of antibody titre for time after splenectomised in splenectomised subjects. We used SPSS 11.5 software (SSPS Inc., Chicago, IL, USA) for this analysis. A p-value <0.05 was considered statistically significant.

## RESULTS

Two hundred and nineteen of the splenectomised subjects (49.9%) and 169 (59.1%) of the subjects with spleen were female and this difference was significant (p = 0.007, Table 1). Non splenectomised subjects were younger (4.19 years) than those who were splenectomised (p<0.001).

There were differences in the Hib antibody levels between the groups of subjects with and without spleen. The mean antibody level was 1.08±0.55 μg $mL^{-1}$ in non splenectomised subjects and was 0.39±0.5 μg $mL^{-1}$ in asplenic subjects (p<0.001). The mean Hib antibody levels were equal among the female and male subjects in both groups (p>0.05).

The mean Hib antibody titre was no significant differences for gender in both non splenectomised and splenectomised subjects (p>0.05, Table 2). In non splenectomised subjects, the mean serum antibody concentration in subjects greater than 15 years old was insignificantly higher than that of subjects equal or less

Table 1: Some characteristics of enrolled beta-thalasseamic subjects

| | Non-splenectomised | | Splenectomised | | |
|---|---|---|---|---|---|
| Variables | No. (413) | (%) | No. (437) | (%) | p-value |
| **Sex** | | | | | |
| Male | 169 | 40.9 | 219 | 50.1 | 0.007 |
| Female | 244 | 59.1 | 218 | 49.9 | |
| **Age (year)** | | | | | |
| ≤15 | 265 | 64.2 | 115 | 26.3 | <0.001 |
| >15 | 148 | 35.8 | 322 | 73.7 | |
| **Time after splenectomised (month)** | | | | | |
| ≤60 | - | - | 152 | 34.8 | - |
| 61-120 | - | - | 119 | 27.2 | |
| >120 | - | - | 166 | 38.0 | |
| Mean age (±SD) | 13.72±5.13 | | 17.91±4.14 | | <0.001 |

SD: Standard deviation

Table 2: Mean±SD Hib antibody concentrations (μg $mL^{-1}$) in enrolled beta-thalasseamic patients

| | Non-splenectomised (413) | | Splenectomised (437) | |
|---|---|---|---|---|
| Variable | Mean | SD | Mean | SD |
| **Sex** | | | | |
| Male | 1.12 | 0.650 | 0.420 | 0.51 |
| Female | 1.05 | 0.460 | 0.360 | 0.49 |
| p-value | 0.226 | 0.193 | | |
| **Age (year)** | | | | |
| ≤15 | 1.04 | 0.530 | 0.550 | 0.53 |
| >15 | 1.14 | 0.570 | 0.330 | 0.48 |
| p-value | 0.100 | <0.001 | | |
| **Time after splenectomised (month)** | | | | |
| ≤60 | - | 0.660 | 0.540 | |
| 61-120 | - | 0.300 | 0.480 | |
| >120 | - | 0.170 | 0.350 | |
| | p-value | - | <0.001 | |

SD: Standard deviation

Table 3: *Haemophilus influenza* type b (Hib) antibody classification in participant subjects

| | Non- splenectomised (No. %) | | | Splenectomised (No. %) | | |
|---|---|---|---|---|---|---|
| Variable | LTP | STP | NP | LTP | STP | NP |
| **Sex** | | | | | | |
| Male | 105 (62.2) | 31 (18.3) | 33 (19.5) | 70 (32.0) | 8 (3.7) | 141 (64.3) |
| Female | 161 (66.0) | 57 (23.4) | 26 (10.6) | 63 (28.9) | 0 (0.0) | 155 (71.9) |
| p-value | 0.032 | 0.011 | | | | |
| Age (year) | | | | | | |
| ≤15 | 158 (59.6) | 71 (26.8) | 36 (13.6) | 49 (42.6) | 8 (7.0) | 58 (50.4) |
| >15 | 108 (73.0) | 17 (11.5) | 23 (15.5) | 84 (26.1) | 0 (0.0) | 238 (73.9) |
| p-value | 0.001 | <0.001 | | | | |
| **Time after splenectomised (month)** | | | | | | |
| ≤60 | - | 86 (56.5) | 1 (0.7) | 65 (42.8) | | |
| 61-120 | - | 29 (24.4) | 7 (5.9) | 83 (69.7) | | |
| >120 | - | 18 (10.8) | 0 (0.0) | 148 (89.2) | | |
| p-value | - | <0.001 | | | | |
| Total | 266 (64.4) | 88 (21.3) | 59 (14.3) | 133 (30.4) | 8 (1.8) | 296 (67.8) |

LTP: Long term protection, STP: Short term protection, NP: No protection

than 15 years old (p>0.05). However, among asplenic subjects, the mean antibody concentration was higher in subjects equal or less than 15 years old than in subjects greater than 15 years old (p<0.001). Subjects who underwent splenectomy equal or less than 60 months earlier had higher antibody levels than did subjects who underwent splenectomy, 61-120 months or greater than 120 months earlier (p<0.001).

Table 3 shows the classified Hib antibody levels according to some subject characteristics. Among the non splenectomised subjects, 85.7% had protective antibody levels against Hib equal or less than 0.15 μg $mL^{-1}$, whereas among asplenic subjects, this rate was 32.2% (p<0.001). Also, a protective antibody level was associated with age in both the non splenectomised and asplenic subjects (p<0.05). Protection against Hib decreased as the time interval after splenectomy increased from 58.2% with a 60 month or lower interval to 10.8% with a greater than 120 months interval (p<0.001). Therefore, 89.2% of splenectomised subjects who underwent splenectomy greater than 120 months ago did not have protective antibody levels.

Nearly 30% of the splenectomised subjects had long-term protection against Hib (antibody level equal or less than 1.0 μg $mL^{-1}$) compared to 64.4% of non splenectomised subjects (p<0.001).

## DISCUSSION

Our study clearly demonstrates a marked difference in the Hib antibody levels between beta-thalassaemic patients with and without spleens. Also, asplenic subjects had lower rates of long-term protection and protective Hib antibody levels than non splenectomised subjects. Hib immunity decreased as the time interval after splenectomy increased. Furthermore, Hib immunity and the mean antibody levels were not associated with the subjects, sexes and ages regardless of the splenic status.

There is good evidence that splenectomised subjects have an increased risk of *S. pneumoniae* and Hib infections (Moffett, 2009; Konradsen *et al*., 1997; Rubin, 1988). Several studies have indicated that the rates of serious bacterial diseases are higher in asplenic subjects with specific conditions such as thalassaemia

major (Moffett, 2009; Kyaw *et al*., 2002). Also, if an infection occurs, the mortality rates are high, ranging from 38-69% (Moffett, 2009). Therefore, the ability of patients to combat encapsulated organism-mediated infections is essential.

In our study of thalassaemia major subjects, the mean Hib antibody level was lower in asplenic subjects than in non splenectomised subjects. Only 32.2% (141) of the asplenic subjects had Hib antibody levels equal or less than 0.15 μg $mL^{-1}$, whereas in a study conducted by Konradsen *et al.* (1997), all splenectomised subjects had antibody levels greater than 0.15 μg $mL^{-1}$.

There were significant differences between the different time intervals after splenectomy groups with regard to the percentages of patients with Hib antibody levels less than 0.15 μg $mL^{-1}$, 0.15-1.0 μg $mL^{-1}$ or greater than 1.0 μg $mL^{-1}$. In contrast, Konradsen *et al.* (1997) suggested that there were no differences in the antibody levels with respect to the time interval after splenectomy.

Nearly 70% of asplenic subjects had Hib antibody levels less than 1.0 μg $mL^{-1}$. This indicated that at least 30% of asplenic subjects were currently protected against invasive Hib infection. Currently in Iran, there are no recommendations for human Hib vaccinations. However, studies have shown that the risk of acquiring lethal sepsis is higher in splenectomised subjects than in the general population (Konradsen *et al*., 1997). Therefore, Hib vaccination should be considered for all splenectomised thalassaemic subjects.

The Hib antibody levels decreased in asplenic subjects as the time interval after splenectomy increased. This agrees with a study of splenectomised thalassaemic subjects that showed reduced antibody concentrations over time passage after Hib vaccination (Cimaz *et al*., 2001). Heath *et al.* (2000) showed that the Hib antibody concentrations also decreased over time after Hib vaccination in healthy subjects.

In this study, 147 (35.6%) non splenectomised subjects had Hib antibody levels less than 1.0 μg $mL^{-1}$ (long-term protection). This is very similar to a result (44%) reported by Goldblatt *et al.* (1996) among sickle cell disease patients.

Immunisation at least 2 weeks before an elective splenectomy (when possible) is essential to prevent disease. Unimmunised patients should receive vaccines shortly after surgery. Re-immunisation is recommended every 5 to 10 years (Moffett, 2009).

In a meta-analysis, pneumococcal vaccination reduced the rate of definitive pneumococcal pneumonia by 71% and that of pneumonia-associated mortality by 32% in immunocompetent adults (Eigenberger *et al*., 2007) and the vaccination of beta-thalassaemic (Cimaz *et al*., 2001) and haematological and post-traumatic (Eigenberger *et al*., 2007; Konradsen *et al*., 1997) splenectomised patients is supported by the evidence of adequate antibody responses to Hib-conjugated vaccines.

Our study has some limitations. We did not record the other characteristics such as times and amounts of the blood transfusion from any patients. Also in Iran, the Hib vaccine don't use in national vaccination. The Hib infections rule out by history and physical examination, thus, it concealed asymptomatic Hib infection.

## CONCLUSION

Splenectomised subjects had lower Hib antibody levels than non splenectomised subjects. Also, the antibody levels decreased as the time interval after splenectomy increased. Therefore, a vaccine recommendation seems essential for beta-thalassaemic splenectomised patients in order to increase the serum Hib antibody concentrations.

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