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Original Article

Immune status of splenectomized patients following vaccination against encapsulated bacteria

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Abstract

Aim: The current study aimed to observe the effectiveness of a pneumococcal vaccine and a *Haemophilus influenzae* vaccine in splenectomized patients by quantification of serum antibody and complement levels, analysis of T and B lymphocytes with flow-cytometry and determination of the percentage of Natural Killer cells and the CD4+ to the CD8+ lymphocyte ratio.

Material and Methods: The study included 22 adult patients who had undergone splenectomy between May 1999 and December 2006. The Pneumo-23 vaccine was administered for protection against *Streptococcus pneumonia* to all patients, whereas the Act-HIB vaccine was administered for protection against *Haemophilus influenzae* type B to 12 patients after January 2002.

Hemogram, IgG and IgM antibody concentrations, serum levels of Complement protein 3 and Complement Protein 4 were obtained and lymphocytes were analyzed whether they were CD3+ and CD19+ by flow cytometry. At the second time point, in 2009, CD4+/CD8+ T-cell ratio and the percentage of CD3/CD16+CD56 Natural Killer cells were evaluated in addition to aforementioned parameters.

Results: All of the study patients had normal IgG levels. Three patients had low levels of IgM. C3 and C4 values were normal. Ten patients showed a low CD3+ T-cell distribution at 5 years. The percentage of CD19+ B-lymphocytes returned to normal. Sustained suppression of CD19 level was observed only in one patient with immune thrombocytopenic purpura. CD8 distribution was normal in all patients. At 5 years, reduced CD4 percentage was detected in 5 patients with immune thrombocytopenic purpura, 3 of whom had suppressed levels of Natural Killer cells concomitantly. Normal levels of IgG, C3, C4 and CD19+ B-cell percentages were found at the final laboratory assessment for all patients.

Conclusion: Our findings indicate that immunological functions are restored following administration of the Pneumo-23 and Act-HIB vaccines. Further studies are needed to obtain a prognostic immune profile index that could help predict patients at high risk for post-splenectomy sepsis.

Keywords: Bacteria; splenectomy; vaccine

INTRODUCTION

While splenectomy has an important role in the management of certain hematological diseases [e.g. hereditary spherocytosis, immune thrombocytopenic purpura (ITP)], it is remains controversial that it can overcome potential infectious complications over the long term despite vaccination (1-3). Since the spleen is the largest lymphoid organ, harmful events caused by splenectomy are alarming in these patients. (4) Encapsulated microorganisms (e.g. *pneumococcus, influenza type B virus* and *meningococcus*) may cause fatal infections in patients undergoing splenectomy (2,5). The effectiveness of pneumococcal polysaccharide vaccination has been shown in healthy adults as well as in patients with chronic obstructive pulmonary, diabetes, and asplenic patients (6). Nevertheless, protective antibody titers formed after S. *pneumoniae* vaccine administration currently is not clear (7).

Thus, additional markers are needed to detect the immune response. Vaccine-induced immunologic activity is closely correlated with serum concentrations of IgG and M antibody titers and IgA antibodies, T-cell activation, the levels and the opsonophagocytic activity of the cells producing antibody (8-11). In the present study, antibody titers of IgG and M antibody titers and C3, C4 levels were measured. The immune response and the opsonization T and B cell were analyzed by CD3 (T lymphocytes) and CD 19(B lymphocytes) flow-cytometry. Additionally, Natural Killer (CD3/CD16+CD56+) cells to helper/suppressor lymphocyte (CD4+/CD8+) ratio were determined for examination of Natural Killer-aided cytotoxicity and to assess the immune status in the asplenic patients. The purpose of the current study was to observe the effectiveness of a pneumococcal vaccine and a H. *influenzae* vaccine in splenectomized patients. An additional purpose of the study was to attempt constructing an immune

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profile index that could enable monitoring the long-term efficacy of the Pneumo-23 vaccine.

MATERIAL and METHODS

The study included 22 adult patients undergoing splenectomy between May 1999 and December 2006. Vaccinations were given 8 days (on average) before elective surgeries. The Pneumo-23 (Aventis-Pasteur), a 23-valent polysaccharide vaccine, was applied for protection against S. *pneumoniae.* The Act-HIB (Aventis-Pasteur) against *influenza* type B was administered to 12 patients after January 2002.

Hemogram, IgG and M titers and serum levels of complements proteins C3 and 4 levels were measured and flow-cytometric analysis for CD3+ and CD19+ lymphocytes was carried out postoperatively immediately after vaccination. Five years later, in 2009, new blood samples were collected from the patients and CD4+/CD8+ T-cell and CD3/CD16+CD56 Natural Killer-cells ratios were examined in addition to aforementioned parameters.

Becton-Dickinson Simultest CD4/CD8, CD3/CD16+CD56 and CD3/CD19 (BD Biosciences 2350 Quine Drive San Jose, CA 95131 1807 USA) assays were applied to determine the percentages of Natural Killer cells, helper/inducer and suppressor/cytotoxic lymphocytes and T and B lymphocytes. Lymphocyte subpopulations were identified using fluorochrome-labeled monoclonal antibodies. Then, stained cells were immediately examined with the flow cytometer.

IgG and IgM measurements were performed with the aid of IMMAGE Immunochemistry Systems (Beckman Coulter) using the immunoassay technology. C3 and C4 levels were determined by nephelometry.

Statistical analyses for the study were conducted using the Statistical Package for the Social Sciences software (SPSS 11.0 for Windows III, IBM, Chicago, USA). The study datas were expressed as arithmetic mean ± SD and a p value less than 0.05 was considered statistically significant. Only the Student's t-test was used to determine differences in the parametric data between the two periods (postoperative 6 months vs. 5 years) for all patients.

RESULTS

22 patients (11 females) in total were included in the study. The average age of the patients was 47.5± 2.65 years (range, 21-67). The follow-up duration was between 29 to 120 months with an average of 65.2 months. The characteristics of the patients in the study are summarized in Table 1.

The diagnoses of the 22 patients were as follows; 11 had immune thrombocytopenic purpura and 4 had autoimmune hemolytic anemia (AHA). Additionally, isolated splenic hydatid cyst was detected in 3 patients, traumatic splenic rupture in 3 patients and spleen hamartoma in 1 patient.

The operative morbidity rate was 3%. A female patient with AHA developed post-operative hemorrhage in the splenic lodge and was reoperated. The mean duration of hospitalization was 12.7 days and ranged between 5 and 53 days. Two patients (1 with ITP and 1 with AHA) were lost due to cardiac and cerebrovascular events during follow-up. The overall mortality rate was 9%.

Table 1. Demographic characteristics of the study sample

Splenectomy Indication	ITP& OHA	Trauma & Splenic Mass
Number of patients	15	7
Gender (M/F)	7/8	4/3
Pneumo-23 vaccination		
Pre-splenectomy /Post-splenectomy	13/2	8/0
ACT-HIB vaccination		
Pre-splenectomy /Post-splenectomy	8/0	4/0
Comorbidities	6*	2**
Morbidity	1	0
Mortality	2/15	0

*Diabetes mellitus (n=2), Hypertensive atherosclerotic cardiac disease (n=2), Kidney transplantation (n=1), psoriasis (n=1)
** COPD (n=1), Diabetes mellitus (n=1)
ACT-HIB vaccination, ITP& AHA (Immune Thrombocytopenic Purpura & Autoimmune Hemolytic Anemia)

The immunologic profiles of 22 patients were collected within the first 6 months after splenectomy and over the next 5 years of follow-up (Table 2).

All of the patients in our series had normal IgG levels in both periods. The mean IgG values were at 6 months and 5 years following the operation were 1417.4 ± 353.4 mg/dl and 1360.5±387.8 mg/dl; respectively. None of the patients showed reduced levels of IgG (<751mg/dl). The mean IgM values at postoperative 6th months and 5th years were 103.9±61 mg/dl at 6 months and 95.3±71.6 mg/dl; respectively. A low IgM level (38 mg/dl) was detected in one patient with ITP at 6 months showed no improvement during the follow-up (38.1 mg/dl at 5th year). In addition, 2 patients [ITP(n=1) and AHA(n] showed IgM values which were either low or at the lower limit of normal at 5 years (45 and 38 mg/dl) respectively. Despite low IgM levels, none of the 3 patients developed a serious infection during follow-up.

Throughout the study, C3 and C4 values exhibited a normal distribution. Additionally, C3 levels showed a significant improvement from 108.1±23.7 mg/dl to 133.5±37.3 mg/dl at the end of the follow-up period (p=0.002).

The CD3+ percentage dropped from 63.3 ± 11.4% to 57.5 ± 12.3%. The CD3+ T lymphocytes were between 45 and 55% as detected in the blood samples drawn at 6 months for 5 patients (4 with ITP and 1 with AHA). While the percentage of CD3+ T lymphocytes returned to normal in AHA patients

over time, it decreased gradually in all ITP patients. As a result, a total of 10 patients (9 with ITP, 1 with traumatic splenic injury) showed a subnormal distribution of CD3+ T lymphocytes at 5 years. In contrast a continuous fall in CD3+ T cells, CD19+ B lymphocyte percentage gradually improved and returned to reference range at 5 years in 12 of 13 patients (10 with ITP or OHA and 3 with traumatic injury) that had a level less than 7% at 6 months. Sustained suppression of CD19 level was observed in 1 patient with ITP who had a CD19 value of 5% at both time points.

The distribution of lymphocyte populations at postoperative 5 years were as follows: CD4 (range: 16-56%, mean: %30.0±2.34), CD8 (range: 19-47%, mean: 35.8±2.7%) and Natural Killer cells (range: 3-57%, mean: 21.2±3.1%). CD8+ cytotoxic T lymphocytes were normal in all patients at both time points. At 5 years, reduced CD4 percentage was detected in 5 ITP patients, 3 of whom had suppressed levels of Natural Killer cells concomitantly. Despite this, IgG, C3, C4 and CD19+ B-cell distribution were normal in the final assessment for the study sample as a whole.

Table 2. Immunological variables at 6 months and 5 years post-operatively (mean ± standard deviation)

Variables	6 months	5 years	P
IgG	1417.4±353.4	1360.5±387.8	0.618
IgM	103.9±61.0	95.3±71.6	0.439
CD3	63.3±11.4	57.5±12.3	0.118
CD19	8.8±7.8	12.7±6.1	0.096
C3	108.1±23.7	133.5±37.3	0.002
C4	22.7±5.2	23.7±6.7	0.523
WBC count	17649±6834	10996±5321	0.000
Neutrophil count (%)	77±10	52.5±11	0.000
CD4	-	30.0±2.34	
CD8	-	35.8±2.7	
CD3-CD16+/CD56+ (NK cells)	-	21.2±3.1	

Normal range of opsonins, immunoglobulins, NK cells and T and B lymphocytes: C3 (79.0-152.0 mg/dl) , C4 (16.0- 38.0 mg/dl), IgG antibody level (751-1560 mg/dl), IgM antibody level (46.0-304.0 mg/dl), CD3 distribution (61-85%), CD19 distribution (7-23%), CD4 distribution (28-58%), CD8 distribution (19-48%), NK cells (6-29%)

DISCUSSION

The spleen and lymph nodes are the primary sites of IgM synthesis in humans. IgM is produced as a response to antigens and allows for the clearance of encapsulated microorganisms. Secondly, CD4+ T-cells residing white pulp in spleen resemble the cells found in the paracortical zones in lymph nodes. Thus, IgM and CD4+ T-cells which are depleted after splenectomy may be partly or fully restored by lymphoid tissues.

B-cell follicles of the spleen and the lymph node are different because memory B-cells has CD19 and CD20 antigens on their surface. After exposure of antigen, plasma cells that release antibody are transformed from memory B-cells (12). In addition, the spleen is considered as the main source of major opsonins designated as C3, C4, C3b and C4b (13). Elimination of encapsulated microorganisms is achieved through their opsonization by IgG antibodies. These antibodies aid accumulation of complement opsonins on the surface of the encapsulated pathogens (14). In general, bacteria coated with opsonins are phagocytosed and eliminated by macrophages and neutrophils. Theoretically, patients that underwent splenectomy are susceptible to due to lack of proliferating antigen-presenting memory B-cells and opsonization capacity. However, the clinical and immunological profile of our study sample contradicts with the previous reports. The mature B lymphocytes with CD19 levels were in normal limits in all patients except in 1 patient. The recovery of these lymphocyte subpopulations causes a doubt regarding the source of production of memory B-cells with CD19 and CD20; such as the follicles in white pulp of the spleen. Together with the increase in CD19+ lymphocyte levels to a normal range, all patients showed normal IgM levels at postoperative 6th month and 5th year. Furthermore, C3 and C4 were in normal levels in all patients. This caused some doubts about the opsonization capacity loss of the immune system after splenectomy. Only 3 patients with hematologic disease had normal or low IgM levels at designated time points and all the other patients had stable values which were in normal limits during the follow-up period. Ruben et al demonstrated satisfactory humoral responses following immunization with a one dose of a polysaccharide vaccine for meningococcus

in patients who had splenectomy for splenic trauma or non-lymphoid tumors. The seroconversion rate in IgG, A and M was similar with control patients (who were also vaccinated). The only exception was the IgM levels which remained stable in patients who had non-lymphoid tumors of nonlymphoid origin (15).

The incidence of fulminant sepsis was found to range between 1.5 and 2.0% over a follow-up period of 8 years in an adult patient population undergoing splenectomy due to trauma or a benign hematologic disease in an era in which routine vaccination was not available. The most common pathogen was *Streptococcus pneumoniae* which was isolated from 76% of the patients, with a reported mortality rate of 56% (16). Patients with different etiologies carry a total risk of sepsis in 7% of the cases in 10 years and the risk of sepsis is highest within the first 3 years following splenectomy. The Advisory Committee on Immunization Practice (ACIP) recommend that pneumococcal vaccine (23-valent polysaccharide type) be administered to all patients 2 weeks prior to elective splenectomy (17). In the majority of the studies using the ELISA methodology for titration of polysaccharide antibodies, a serum pool against 8 to 10 antigenic serotypes was created and vaccine dosage was recommended based on the antibodies that are less produced in the organism, ignoring the remaining 10-15 serotypes (18). The British Committee for Standards in Hematology (BCSH) currently recommends a routine re-immunization schedule for asplenic patients every 5 to 10 years; however, BCSH also recommends that a second dose of the vaccine be given immediately regardless of the previous vaccination to patients in whom low, non-protective antigen-specific antibody titers are detected (19).

CONCLUSION

Our findings demonstrated that we could reach a satisfactory seroconversion for IgG in all patients and also for IgM in more than 85% of the patients with hematologic diseases and traumatic injury to spleen. Also, C3 and C4 levels were within reference range in all patients. Moreover, the titers of CD3+ cytotoxic T-cells and CD19+ mature plasma cells were close to normal range in all discrete time points in the study. None of the patients developed an infection related to Streptococcus pneumonia or *Haemophilus influenzae.* Furthermore, neither of the patients died due to sepsis. Furthermore, booster dose of the vaccine was not needed in any patient after initial immunization. The results of our study indicated that immunological functions were restored following administration of the Pneumo-23 and Act-HIB vaccines and suggest that this improvement was most probably driven increased T and B lymphocyte activation and C3, C4 and IgG, IgM antibody synthesis in distant lymphoid tissues. Since none of the study patients died or experienced a severe infection, these immune responses may be considered as prognostically relevant. Further studies with a similar design and prolonged follow-up are needed to obtain an immune profile index which has prognostic implications and could also aid in predicting patients with high-risk for sepsis post-splenectomy and to ascertain the specific prognostic capability of the immunological parameters explored in the current study.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study is based on the dissertation titled "Immune profiles of asplenic patients following a single or double-dose vaccination" (Izmir Bozyaka Research and Training Hospital, Izmir 2009).

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