Splenectomy for patients with β-thalassemia major: long-term outcomes

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Background/aim
The use of splenectomy for thalassemia major is restricted over concerns of its long-term outcome. The aim of this study was to assess the long-term outcomes of splenectomy for patients with β-thalassemia major.

Patients and methods
This study included 70 patients with β-thalassemia major. Patients were classified into two groups: 35 patients underwent splenectomy (S group) and 35 patients did not undergo splenectomy (NS group). Patients were assessed by review of medical records, assessment of medical history, and a clinical examination. In addition to complete blood count, liver function tests and serum ferritin were performed. Assessment of lymphocyte populations was carried out by flow cytometry. These investigations were performed at least 2 years after splenectomy in the S group.

Results
The mean age of the patients who underwent splenectomy was 6.68 ± 2.54 years and the mean postoperative follow-up period was 6.26 ± 3.03 years. Splenectomy improves anemia, but does not reduce iron burden; more patients were found to be on regular iron chelation after splenectomy. Hematocrit and red blood cell indices were significantly increased after splenectomy. Platelet count increased significantly in the S group (644.700 ± 299.400/mm^3). There were no significant differences in T-lymphocyte populations between both groups. IgM memory B lymphocytes were lower in the S group compared with the NS group. No overwhelming postsplenectomy infection was reported in this series. Postsplenectomy portal vein thrombosis was reported in one (2.9%) case.

Conclusion
With long-term follow-up after splenectomy for treatment of thalassemia major, thrombocytosis and the risk of thromboembolic persist. Splenectomy improves anemia, but does not reduce iron burden or the requirement for blood transfusion. Proper preoperative vaccination can reduce the risk of overwhelming postsplenectomy infection.

Keywords:
complications, hematologic disorders, immune system, lymphocytes, splenectomized

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Introduction

Thalassemia is a group of disorders of the red blood cell (RBC) protein, hemoglobin, which is the primary carrier of oxygen in the blood. β-Thalassemia major is one of the most prevalent disorders in Mediterranean regions, caused by mutations in the gene responsible for producing β-globin chain on the chromosome 11 [1]. Worldwide, β-thalassemia is believed to affect about one in 100 000 live births [2].

The treatment of thalassemia major has traditionally included transfusion of RBCs, iron chelation, and splenectomy. The need for splenectomy in thalassemia major is more likely where the disease is not suppressed efficiently by transfusion treatment.

Yet, even after decades of experience, important questions on splenectomy for thalassemia major remain unresolved, such as long-term hematological and immunological response. The aim of this study was to evaluate the long-term outcomes of splenectomy for the treatment of β-thalassemia major.

Patients and methods

This study was carried out at Assiut University Hospitals and included 70 patients with β-thalassemia major. An informed written consent in accordance with Assiut University Ethical Committee guidelines was obtained.

Patients were classified into two groups: 35 patients who had previously undergone splenectomy (S group) and 35 patients with β-thalassemia who had not undergone splenectomy (NS group). Splenectomy was performed from January 2004 to December 2011. The indications for splenectomy were a hugely enlarged spleen with blood transfusion requirement more than 250 ml/kg/year and any clinically significant

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complications such as pancytopenia. Preoperative vaccination against *Streptococcus pneumoniae*, *Haemophilus influenza* type B, and *Neisseria meningitides* was administered to all patients who underwent splenectomy 2–4 weeks before surgery. There was no regular prophylactic antibiotic administration, parenteral or oral, because of poor patient compliance. Antiplatelet was administered if the platelet count exceeded 1000 000/mm^3. Patients with known diabetes, cardiac, renal, infectious, inflammatory, or pulmonary diseases, and newly diagnosed β-thalassemia cases yet to receive a blood transfusion were excluded from the study.

All children in the study were assessed by review of medical records, assessment of medical history, and clinical examinations. In addition to complete blood count, liver function tests and serum ferritin were performed. Assessment of lymphocyte populations was carried out by flow cytometry using Cell Quest software (Becton Dickinson Biosciences, San Jose, California, USA). In the S group, these investigations were performed at least 2 years after splenectomy.

Monoclonal antibodies against surface markers were used. In immunofluorescence staining of the antibodies, cells were incubated with antibodies conjugated to a fluorochrome (e.g. fluorescein isothiocyanate). The percentages of CD19$^+$ (total B lymphocytes), CD19$^+$ CD27$^-$ (naive B cells), CD19$^+$ CD27$^+$ (total memory B cells), CD19$^+$ CD27$^+$ IgM$^+$ (IgM memory B cells), CD19$^+$ CD27$^+$ IgM$^-$ (switched memory B cells), CD3$^+$ (T lymphocytes), CD4$^+$ (T-helper cells), and CD8$^+$ (T-cytotoxic cells) were assessed. The data of flow cytometry were saved on a computer and plotted on a graph called a histogram (Fig. 1).

Statistical analyses

Statistical analyses were carried out using the statistical package for the social sciences (version 16.0; SPSS Inc., Chicago, Illinois, USA). Data are expressed as mean ± SD for continuous variables and percentages for categorical variables. An independent sample *t*-test was used to analyze continuous variables, whereas the χ^2-test was used to analyze categorical variables. The *P*-value was considered statistically significant when less than 0.05.

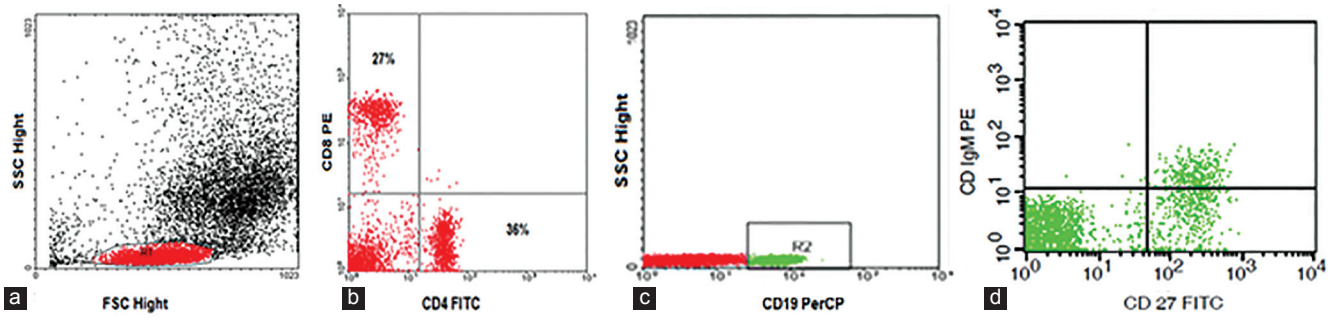
Results

The mean age of the patients at splenectomy was 6.68 ± 2.54 years. After a mean postoperative follow-up period of 6.26 ± 3.03 years, the mean white blood cells and platelet counts were 16.490 and 644.700/mm^3, respectively (Table 1). RBC count, mean hemoglobin level, hematocrit, and RBC indices were significantly elevated after splenectomy. Splenectomy did not reduce iron burden; more patients were found to be on regular iron chelation after splenectomy. Total and indirect bilirubin were significantly higher in the NS group compared with the S group (Table 2).

Splenectomized patients showed a significant increase in total lymphocytes and B lymphocytes. However, IgM memory B lymphocytes were significantly lower in the S group compared with the NS group. There was no significant difference in total T lymphocytes, CD4 helper T lymphocytes, CD8 cytotoxic T lymphocytes, or CD4$^+$/CD8$^+$ ratio among both groups (Table 3).

There was no history of overwhelming postsplenectomy infection (OPSI) in this series. An 18-year-old man had liver abscess; the abscess was in the left lobe of the liver and developed 8 years after splenectomy. The liver abscess was treated by pigtail percutaneous drainage and antibiotics. The culture and sensitivity of the aspirated pus showed Gram-negative bacilli, *Klebsiella* spp., which was sensitive to amikacin and ciprofloxacin.

Figure 1



Representative flow cytometric analysis of lymphocyte subsets in thalassemia patients. (a) Forward and side scatter histogram was used to define the lymphocyte population (R1). (b) The expression of CD4$^+$ and CD8$^+$ in T lymphocytes. (c) CD19$^+$ cells were then gated for further analysis. (d) The expressions of IgM and CD27 in B cells were detected. FITC, fluorescein isothiocyanate; FSC, forward scatter; perCP, peridin chlorophyll protein; PE, phycoerythrin; SCC, side scatter.

Postsplenectomy portal vein thrombosis was reported in an 8-year-old female patient (2.9%). The preoperative platelet count was 410 000/mm^3. One year after the operation, she developed severe abdominal pain with fever. Laboratory investigation showed thrombocytosis with a platelet count of 1 000 000/mm^3. Portal vein thrombosis was diagnosed by abdominal ultrasonography and confirmed by color

Table 1 Characteristics of splenectomized and nonsplenectomized thalassemia patients

Variable	Splenectomized thalassemia patients (n = 35)	Nonsplenectomized thalassemia patients (n = 35)	P-value
Age (years)	12.94 ± 2.44	5.54 ± 3.01	0.000
Sex			0.21
Male	24 (68.6)	19 (54.3)	
Female	11 (31.4)	16 (45.7)	
Weight (kg)	29.85 ± 6.04	16.82 ± 5.43	0.000
Height (cm)	131.94 ± 11.27	102.42 ± 17.51	0.000
BMI (kg/m^2)	17.05 ± 2.15	15.86 ± 2.25	0.017
Age at first transfusion (months)	9.62 ± 5.25	10.23 ± 7.52	0.680
Patient on regular chelation	24 (68.5)	15 (42.9)	0.030
Frequency of blood transfusion (duration between each transfusion in days)	34.26 ± 8.18	31.60 ± 13.91	0.316
Liver size (cmBCM)	7.09 ± 3.25	4.35 ± 2.13	0.000

Data are represented as means ± SD or n (%); cmBCM, centimeter below the costal margin; $P \leq 0.05$ was considered significant.

Table 2 Some hematological parameters and liver function tests in splenectomized and nonsplenectomized thalassemia patients

Variable	Splenectomized thalassemia patients (n = 35)	Nonsplenectomized thalassemia patients (n = 35)	P-value
WBCs (10^9/l)	16.49 ± 11.01	9.62 ± 3.40	0.000
RBCs (10^6/μl)	3.10 ± 1.2	3.4 ± 0.64	0.000
Hemoglobin (g/dl)	6.74 ± 1.27	5.75 ± 1.02	0.000
MCV (fl)	73.2 ± 8.5	62.45 ± 8.7	0.000
HCT (%)	22.70 ± 5.42	18.80 ± 5.3	0.000
MCH (pg)	21.7 ± 4.5	19.17 ± 2.6	0.000
MCHC (g/dl)	30.5 ± 1.8	29.6 ± 2.2	0.000
Platelet count (10^9/l)	644.700 ± 299.4	340.700 ± 160.9	0.000
Total bilirubin (μmol/l)	23.73 ± 7.59	39.56 ± 42.60	0.010
Indirect bilirubin (μmol/l)	17.67 ± 6.02	32.89 ± 48.84	0.029
Albumin (g/dl)	3.56 ± 0.64	3.97 ± 0.56	0.002
ALT (IU/l)	81.27 ± 27.02	49.19 ± 52.48	0.001
AST(IU/l)	112.38 ± 66.50	49.87 ± 29.58	0.000
Serum ferritin (mcg/l)	2893.2 ± 1409.0	1700.4 ± 1648.4	0.001

Data are represented as means ± SD; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC,white blood cell; $P \leq 0.05$ was considered significant.

Table 3 Lymphocytes subsets in splenectomized and nonsplenectomized thalassemia patients

Variable	Mean percentage ± SD		P-value
	Splenectomized thalassemia patients	Nonsplenectomized thalassemia patients	
Total lymphocytes	67.05 ± 10.48	58.06 ± 12.37	0.026
B lymphocytes (CD19$^+$)	16.27 ± 2.42	13.83 ± 2.06	0.001
Naive B lymphocytes (CD19$^+$ CD27$^-$)	76.69 ± 10.61	67.65 ± 11.66	0.044
Total memory B lymphocytes (CD19$^+$ CD27$^+$)	23.31 ± 10.67	32.25 ± 11.96	0.034
IgM memory B lymphocytes (CD19$^+$ CD27$^+$ IgM$^+$)	8.66 ± 3.23	17.09 ± 6.23	0.000
Switched memory B lymphocytes (CD19$^+$ CD27$^+$ IgM$^-$)	15.06 ± 7.11	15.77 ± 5.74	0.921
T lymphocytes (CD3$^+$)	63.89 ± 10.45	60.31 ± 14.53	0.686
CD4$^+$ (%)	42.02 ± 7.10	36.96 ± 8.01	0.091
CD8$^+$ (%)	25.47 ± 6.24	23.24 ± 6.16	0.505
CD4$^+$/CD8$^+$ ratio	1.70 ± 0.43	1.66 ± 0.44	0.929

CD, cluster of differentiation.

Doppler ultrasound and computerized tomography scan. Liver function tests, prothrombin time, partial thromboplastin time, bleeding time, and clotting time were within normal limits. The patient was treated by an anticoagulant and antiplatelet agent (low-dose aspirin). During regular follow-up at the outpatient clinic, the dose of warfarin was adjusted to maintain the international normalized ratio between 2 and 3.

Discussion

Since the first deliberate removal of a diseased spleen by Quittenbaum in 1826, splenectomy has become a well-established surgical procedure [3]. A spleenless existence was considered to be quite safe as the spleen was considered unnecessary for life until 1952, when King and Schumacher [4] drew attention to the risk of OPSI. Since then, interest in splenectomy has reduced. The spleen clearly serves very important hematological and immunological functions [5].

Elective splenectomy is indicated in the management of a wide variety of medical disorders [6,7]. In patients with thalassemia, there is an increased rate of RBC removal by the spleen; therefore, RBCs, hemoglobin, hematocrit, and RBC indices are elevated after splenectomy. Transfusion frequency, and its subsequent complication, is expected to be reduced after splenectomy. In this study, despite the significant increase in the hemoglobin level after splenectomy, the reduction in blood transfusion frequency did not reach statistical significance. In splenectomized patients, blood transfusion is needed every 34 days on average.

The primary underlying pathology of red cell dysfunction persists after splenectomy; the risk of damage to different organs because of iron overload still remains significant. Aydinok *et al.* [8] reported that splenectomized patients had a higher incidence of myocardial siderosis (48%) compared with those with an intact spleen (28%). In the present study, more patients were found to be on regular chelation after splenectomy because of the significantly higher ferritin level, suggesting that splenectomy does not alleviate the iron burden in patients with thalassemia. After splenectomy, the transaminases were higher and serum albumin was lower compared with nonsplenectomized patients. A recent observational study found that the rates of iron overload-related organ damage in splenectomized patients were comparable with those who had not been splenectomized [9].

In the present study, a high leukocytic and lymphocytic count was found in splenectomized thalassemic patients, mainly B lymphocytes. The significant increase in lymphocytes after splenectomy may suggest that the spleen could play a role in the control of lymphocyte counts and may act as a reservoir for lymphocytes. Our results are consistent with other studies in which splenectomized thalassemia patients show a large increase in the number of B lymphocytes [10,11].

Splenectomized thalassemia patients had lower IgM memory B cells than nonsplenectomized patients. IgM memory B cells express IgM; it develops in the marginal zone of the spleen, and requires the spleen for survival and/or generation [12–14]. Opsonized bacteria are removed efficiently by macrophages in the spleen and liver. However, poorly opsonized bacteria, such as encapsulated bacteria, are only cleared by the spleen. For removal of these bacteria during the course of initial infection, natural antibodies are needed, which are pentameric IgM that can facilitate phagocytosis either directly or through complement deposition on the capsule [12,15,16].

Patients with thalassemia major are predisposed to infection by altered complement activation and immunoglobulin levels, cardiopulmonary disease, and hematochromatosis. Splenectomy further increases the risk of infection [17]. The term OPSI defines fulminating sepsis, meningitis, or pneumonia mainly caused by encapsulated bacteria, such as pneumococci, meningococci, and *H. influenza* type b. Characterized by evolution in a just a few hours, in association with hypotension, alteration of consciousness, or shock, OPSI is a major concern after splenectomy, with a high mortality risk of ~40–50% [18]. Preventive measures (new protein conjugate vaccines, antibiotic prophylaxis, increased awareness, and patient education) are believed to considerably reduce the risk of OPSI. In the present study, with strict application of preoperative vaccination, there were no reported cases of OPSI.

Thrombosis following splenectomy had been reported in the literature [19–21]. Splenectomy leads to immediate reactive thrombocytosis and an increase in circulating microparticles, with an increased risk of subsequent venous thromboembolism particularly within the splenoportal system [18,22–25]. In the literature, the incidence of postsplenectomy portal vein thrombosis ranges from 0.7 to 8%; patients usually present with fever and abdominal pain [26–28]. With long-term postsplenectomy follow-up (mean 6.26 years) in this study, the thrombocytosis persisted. One (2.9%) patient developed portal vein thrombosis 12 months after splenectomy. A high index of suspicion, early diagnosis, and prompt anticoagulation are keys to a successful outcome [29].

In thalassemia, splenectomy is reserved for patients with marked symptoms related to the extent of splenomegaly, increased transfusion requirements, and complications such as pancytopenia. Splenectomy should not be undertaken lightly and the risks should be weighed against the potential benefits in each individual case. Most of this risk seems to be because of the underlying splenectomy indication and not splenectomy alone [5].

This study had some shortcomings. Only patients with severe thalassemia major underwent splenectomy. It cannot, therefore, be excluded that the high serum ferritin level and low serum albumin in the splenectomized group were caused by severe disease before the splenectomy. Moreover, there was a discrepancy between both groups in age, body weight, and height.

Conclusion

With long-term follow-up after splenectomy for the treatment of thalassemia major, thrombocytosis and the risk of thromboembolic remain. Splenectomy improves anemia, but does not reduce iron burden or blood transfusion requirement. Proper preoperative vaccination can reduce the risk of OPSI. Splenectomized β-thalassemia patients had a significantly high absolute lymphocytic count and significantly low level of IgM memory B cells; the abnormal distribution of lymphocytes does not affect T-cell subgroups.

Acknowledgements

Conflicts of interest

None declared.

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