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Auto-Transplantation of Splenic Fragments After Total Splenectomy in Patients with Severe Splenic Trauma Lesions: A Clinical Study

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Abstract

Background: The known early and late complications following splenectomy increase the tendency to preserve splenic tissue after splenic trauma.

Objectives: This study was conducted to determine the safety and feasibility of auto-transplantation of splenic fragments in patients with severe splenic injury.

Methods: Nineteen patients with severe splenic blunt trauma, who underwent total splenectomy and autotransplantation of splenic fragments at Besat hospital, Hamadan University of Medical Sciences, Iran during years 2015 and 2016, were enrolled in the study. Complete blood counts, blood smear for Howell-Jolly bodies, and phagocytic function tests were performed just before, at 3 months, and 6 months after surgery.

Results: The means of red blood cell counts increased significantly at both 3 and 6 months after surgery (P = 0.01 and 0.049, respectively) and the means of hemoglobin, hematocrit, and the percentage of lymphocytes increased significantly at 3 months after surgery (P = 0.001, 0.046, and 0.01 respectively) while this increase was not significant 6 months after surgery (P = 0.52, 0.15, and 0.34, respectively). The Howell-Jolly bodies were present in 5 patients (26.3%) at 3 months after surgery, which was significantly reduced to 3 (15.8%), at 6 months after surgery (P = 0.042). The median of splenic phagocytic function significantly increased from 3 $\pm$ 1.2 hotspots (range = 2 to 6) at 3 months after surgery to 4 $\pm$ 1.4 (range = 2 to 8) at 6 months after surgery (P = 0.044).

Conclusions: Auto-transplantation of splenic fragments may be feasible and safe and a suitable option for splenic tissue salvage in patients with severe splenic lesions, who require total splenectomy.

Keywords: Splenectomy, Trauma, Autotransplantation

1. Background

The spleen is one of the intra-abdominal organs most frequently found to be damaged, particularly in young patients with blunt abdominal trauma (1). Some early and late complications following splenectomy are thrombocytosis, leukocytosis, decrease in serum IgM level, overwhelming post-splenectomy infection (OPSI), recurrent infections, and atherosclerosis (2-4). Hence, currently non-operative management of blunt splenic trauma lesions is the most common approach of management in hemodynamically stable patients and in patients with lower grade injuries (5,6). Failure of non-operative management, hemodynamic instability, and high-grade injuries are the current indications for operative management (5). Even at times when operative management is indicated, total splenectomy is not always performed and different techniques for preservation of splenic tissue and function, such as application of hemostatic agents, splenorrhaphy, infrared coagulation, and partial splenic resection, are sought because of increased recognition of immunological function of the spleen (7). In conditions when total splenectomy is inevitable, including hemodynamic instability, damage control, and complete avulsion of the spleen from its hilum, auto-transplantation of splenic fragments may be of interest.

To the best of our knowledge, there is limited clinical experience available on autotransplantation of splenic fragments. The clinical and immunological benefits and viability of autotransplantation of splenic fragments has yet to be proven. We performed this prospective study in order to find safety and feasibility of autotransplantation of splenic fragments in patients with severe splenic injury

and evaluate the viability and function of the autotransplanted splenic tissue.

2. Methods

This prospective study was conducted after obtaining approval of the ethics committee of Hamadan University of Medical Sciences. Nineteen patients, including hemodynamically unstable patients with complete avulsion of the spleen from its hilum and patients with injury to the upper pole of the spleen due to its separation from the diaphragm with an age of older than 12 years old, were enrolled in the study. All patients were referred to the emergency department of Besat hospital, Hamadan University of Medical Sciences during years 2015 and 2016, and were planned for total splenectomy. All patients were conscious before transfer to the operating room and necessary information was provided to them or their legal guardians. An informed consent was obtained from all patients or their legal guardians. Patients with severe concurrent injuries and medical history of diabetes mellitus, idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, hereditary spherocytosis, autoimmune hemolytic anemia, and malignancies affecting the spleen, such as Hodgkin lymphoma, were excluded from the study.

A 2-mL blood sample was collected from each patient just before the surgery was started and sent for blood smear and complete blood counts, including red blood cells, hemoglobin, hematocrit, platelets, leukocytes, and lymphocytes in an ethylene diamine tetra-acetic acid tube. Blood smears were stained by the May-Grunwald-Giemsa method for Howell-Jolly bodies. After general anesthesia, a midline incision was made above and on the left of the umbilicus in order to avoid any injury to the veins of the hepatic round ligaments. All 4 quadrants were packed, and then the packs were removed to expose the injured spleen. In order to mobilize the spleen, the peritoneum was divided laterally by retracting the spleen posteromedially to expose the retroperitoneal attachments. After opening the peritoneum laterally, a blunt plane was created posterior to the spleen in a medial direction, extending behind the tail of the pancreas. While avoiding the greater curve of the stomach, the short gastric vessels were ligated and divided. Finally, the spleen was removed after clamping and ligating the splenic artery and the splenic vein, being sure not to injure the tail of the pancreas. The spleen was placed in a container with 0.9% saline solution then several 1-cm thick spleen sections were obtained and cut to 10 smaller fragments, measuring approximately $1 \times 1 \times 1$ cm each. The fragments were attached to the greater omentum with 2 to 3 sutures, using chromic suture and the greater omentum was folded over the autotransplanted splenic fragments. Size and weights of the fragments were chosen using protocols in relevant literatures (8,9).

Patients were visited during their hospital stay and were discharged after they had attained good general condition, including stable hemodynamics, having no signs and/or symptoms of infection or intraabdominal abscess, and no early surgical site complications, such as bleeding and hematoma. Later, they were visited at 3 months and 6 months after surgery and the same lab tests performed prior to the surgery plus phagocytic function test were performed. The phagocytic functions of the autotransplanted splenic fragments in all patients were qualitatively evaluated using 20 millicurie of technetium-99 m colloidal sulfur, injected intravenously, followed by gamma camera scintigraphic images recorded 1, 2, and 3 hours after injection. The absorption of the isomer by the autotransplanted splenic fragments was determined in the frontal and dorsal incidence and was recorded as the number of hotspots indicative for phagocytic function.

Quantitative and qualitative variables were compared using nonparametric tests, including Wilcoxon signed rank tests and Cochran's Q test. Statistical significance was determined as a $P \leq 0.05$. All statistical analysis was performed using the SPSS software (version 19.0, SPSS Inc., Chicago, Illinois).

3. Results

During the study period, 19 patients, who underwent total splenectomy with a median age of 38 ± 19 years old (range = 2 - 6), including 14 (74%) males, were enrolled in the study. All patients had isolated spleen injuries except for 2 patients with superficial laceration of liver, which were sutured and a left renal subcapsular hematoma, which was managed conservatively. The means (range) of complete blood counts, including hemoglobin, red blood cell, hematocrit, platelets leukocytes, and lymphocytes just before the surgery were 11.6 (9.3 to 14.3) (g/dL), 428 (347 to 582), 38% (28% to 50%), 291 (167 to 507), 9.5 (5 to 14), and 29% (15% to 51%), respectively. As shown in Table 1, the means of red blood cells increased significantly at both 3 and 6 months after surgery (P = 0.01 and 0.049, respectively) and the means of hemoglobin, hematocrit, and the percentage of lymphocytes increased significantly at 3 months after surgery (P = 0.001, 0.046 and 0.01, respectively) whereas the increase was not significant at 6 months after surgery (P = 0.52, 0.15, and 0.34, respectively). The other blood counts were not changed significantly at 3 or 6 months from surgery.

The graph of trends of complete blood counts over time after surgery is depicted in Figure 1.

Table 1. Complete Blood Counts Just Before and at Three and Six Months After Surgery

	Just Before Surgery	Three Months After Surgery	Six Months After Surgery	P Value (Just Before vs. 3 Months After Surgery)	P Value (Just Before vs. 6 Months After Surgery)
Hemoglobin (g/dL)	11.6 ± 1.4[a]	13.4 ± 1.7	12.2 ± 1.7	0.001	0.52
Red Blood Cell ($\times 10^{12}$/liter)	428 ± 81	489 ± 57.1	479 ± 54	0.01	0.049
Hematocrit (%)	38 ± 6	43 ± 6	41 ± 5	0.046	0.15
Platelets ($\times 10^{9}$/liter)	291 ± 103	308 ± 107.4	289.5 ± 103	0.57	0.78
Leukocytes ($\times 10^{9}$/liter)	9.5 ± 3.3	9.1 ± 3.1	9.7 ± 3.8	0.54	0.84
Lymphocyte (%)	29 ± 1	38 ± 9	33 ± 8	0.01	0.34

[a]Data is shown as mean ± standard deviation.

The presence of Howell-Jolly bodies significantly changed over time (P value = 0.042), such that just before the surgery, no Howell-Jolly body was present in any patient. At 3 months after surgery, 5 Howell-Jolly bodies were present in each red cell field (26.3%) and was significantly reduced to 3 (15.8%) at 6 months after surgery.

The median of splenic phagocytic function significantly increased from 3 ± 1.2 hotspots (range = 2 to 6) at 3 months after surgery to 4 ± 1.4 (range = 2 to 8) at 6 months after surgery (P value = 0.044).

The detailed data of demographics of the patients, presence of Howell-Jolly bodies, and phagocytic function over time after surgery is shown inTable 2.

4. Discussion

Nowadays, there is an increasing tendency to preserve splenic tissue (10). Autotransplantation of splenic fragments were initially performed on experimental animal models, such as different species of rats, dogs, pigs and rabbits, and the results have been very promising. The main recommended location for autotransplantation of splenic fragments is the greater omentum (11). Many experimental studies have shown that animals that had underwent autotransplantation of splenic fragments experienced less infections in comparison to those that underwent total splenectomy alone (8,12). However, it should be stressed that the mere presence of splenic tissue as auto-transplantation does not necessarily imply normal immune function and that infection rates in the group with healthy normal spleens is significantly lower than the group in which total splenectomy and autotransplantation of splenic fragments was performed (13).

Meanwhile, some studies have been performed regarding atherosclerosis observations after splenectomy for splenic trauma, which suggest a possible role for the spleen in lipid metabolism and that auto-transplantation of splenic fragments may be protective in conditions with increased lipid levels (3,4). Likewise, other studies have revealed that the presence of Howell-Jolly-bodies was clearly reduced with increased regeneration and splenosis rate of auto-transplantation splenic fragments (14,15). The OPSI is reported to be the most important and frequently fatal complication of total splenectomy. The overall incidence of septicemia remains low, yet death rates for OPSI have been reported to be up to 600-fold greater than the general population, with an estimated lifetime risk for OPSI of approximately 5% (16). Improvement of the antibody response after total splenectomy vaccination is another advantage to autotransplantation of splenic fragments, especially in children. Hence, there was controversy regarding efficacy of vaccination after total splenectomy, and the literatures recommended performing further studies on comprehensive assessment of autotransplantation of splenic fragments significance in humans (17-19).

In the current study, the means of red blood cells increased significantly at both 3 and 6 months after surgery. Moreover, the means of hemoglobin, hematocrit, and the percentage of lymphocytes increased significantly at 3 months yet not 6 months after surgery. Some studies have reported a significant increase in number of lymphocytes, compared to other blood counts, after autotransplantation of splenic fragments (18,19). Sipka et al. (17) reported a non-significant increase in the number of lymphocytes at 8 months after total splenectomy and autotransplantation of splenic fragments in mice compared to those that underwent total splenectomy alone.

The Howell-Jolly bodies are basophilic nuclear remnants in circulating erythrocytes, which are normally filtered by the spleen from the blood. Hence, lack of presence of Howell-Jolly bodies indicates the regeneration and function of the autotransplanted splenic fragments. In the

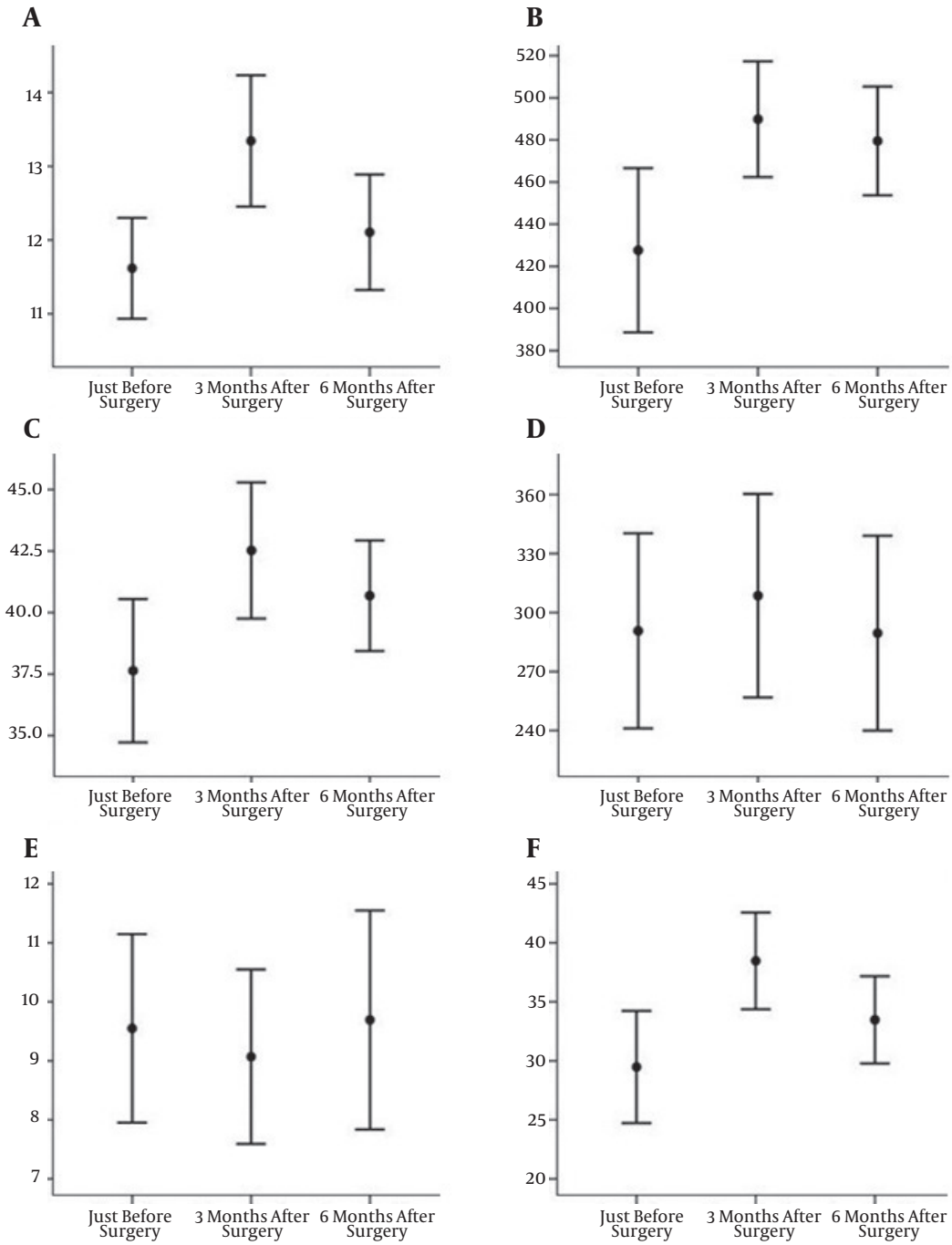

Figure 1. The Standard Error Bar Graphs of Complete Blood Counts Including A, Hemoglobin (g/dL); B, Red Blood Cell ($\times 10^{12}$/liter); C, Hematocrit (%); D, Platelets ($\times 10^{9}$/liter); E, Leukocytes ($\times 10^{9}$/liter) and F, Lymphocyte (%)

current study, the presence of Howell-Jolly bodies was significantly reduced over time after autotransplantation of splenic fragments. Similarly, Sajtos et al. (20) reported that from postoperative months 4 and 5, filtration function of autotransplanted splenic fragments in beagle dogs, showed particular restoration compared to ones that un-

Table 2. The Detailed Data of Patients on Demographics, Presence of Howell-Jolly Bodies, and Phagocytic Function Over Time After Surgery

No.	Gender	Age (Years)	Presence of Howell-Jolly Bodies			Phagocytic Function	
			Just Before Surgery	3 Months After Surgery	6 Months After Surgery	3 Months After Surgery	6 Months After Surgery
1	Male	49	No	Yes	No	2[a]	3
2	Male	61	No	No	No	4	8
3	Male	69	No	No	No	5	3
4	Male	38	No	Yes	Yes	2	4
5	Male	19	No	No	No	4	4
6	Male	35	No	No	No	3	4
7	Male	61	No	Yes	Yes	3	2
8	Male	53	No	No	No	3	3
9	Male	32	No	No	No	3	3
10	Male	35	No	No	No	3	4
11	Female	71	No	No	No	6	5
12	Male	24	No	No	No	4	4
13	Male	38	No	Yes	No	2	4
14	Male	81	No	No	No	3	5
15	Female	19	No	No	Yes	3	3
16	Female	18	No	No	No	6	7
17	Male	41	No	No	No	3	5
18	Female	32	No	No	No	5	5
19	Female	33	No	Yes	No	3	4

[a] Number of hotspots.

derwent total splenectomy alone. Marques et al. (21) presented that functional regeneration of splenic fragments occurred 8 months after autotransplantation and that when 22.5% of regenerated autotransplanted splenic fragments were reached, almost no Howell-Jolly bodies could be observed in the bloodstream, resembling a spleen in situ.

Similar to the current study, it has been reported that phagocytic function of autotransplanted splenic fragments is not regained in the early months after autotransplantation, due to inflammatory response, which improves relatively over time (3,8,22).

In summary, autotransplantation of the splenic fragments may be feasible and safe and a suitable choice for splenic tissue salvage in patients with severe splenic lesions, who require total splenectomy. However, the major limitation of the current study was the lack of splenectomized and/or non-splenectomized control group for comparison. The researchers decided to conduct a prospective pilot study rather than a randomized controlled one because of the limited number of patients with splenic injury, who needed urgent total splenectomy at the center, and limited number of clinical experience on autotransplantation of splenic fragments and its vague results. The researchers hope to conduct a powerful randomized controlled study with larger number of patients in the near future.

4.1. Ethics

This study was conducted after obtaining approval of the ethics committee of Hamadan University of Medical Sciences. All patients were conscious before transfer to the operating room and necessary information was provided to them or their legal guardians. An informed consent was obtained from all patients or their legal guardians.

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