Original Article
Subtotal splenectomy for splenomegaly in cirrhotic patients

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Abstract: Background: In recent years, the spleen has become to be recognized as the “control center” of the immune-metabolic-endocrine network. However, it is controversial that splenomegaly due to portal hypertension is treated by subtotal splenectomy. The aim of this study was to evaluate the distribution of fibrous tissue, morphology of cells as well as splenic size, hemodynamics, hematological and immunological indexes in the residual spleen after subtotal splenectomy. This information may help finding the basis for the operation of subtotal splenectomy. Methods: Ten cases of splenomegaly due to portal hypertension were investigated. Two groups were created: Splenomegaly and Residual spleen. Control group was 10 cases of trauma-induced splenic rupture. Samples were sliced, and morphological changes were observed under light microscopy and electron microscopy. Indexes of splenic size, hemodynamics, hematology and immunology of the spleen were measured. Results: Under light microscopy, the number of collagen fibers and elastic fibers was increased, and the number of reticular fibers was decreased in the residual spleen and splenomegaly groups. Under electron microscopy, the ultrastructure of endothelial cells, lymphocytes, macrophages, and reticular cells in the residual spleen group were noticeably improved more than in the splenomegaly group. Flow volume in the residual spleen and portal vein decreased obviously, with number of platelet rising to normal, and there was no significant difference in the indexes of immunology. Conclusion: After subtotal splenectomy, the residual spleen will not experience a high-pressure environment, and the fibrosis of splenic tissue and remodelling of corpuscular morphology will cease.

Keywords: Histopathology, cytomorphology, fiber tissues, residual spleen, cirrhosis

Introduction

The spleen is the largest secondary lymphoid organ in the human body. With its location in the circulatory system and with the unusual structure and function of its compartments, the spleen is a unique organ. The spleen also contains about one-fourth of the body’s lymphocytes and initiates immune responses against blood-borne antigens [1-3]. Various immune cells ensure the complex function of the spleen as a filter of the blood as well as a lymphoid organ [4, 5].

In recent years, the spleen has become to be recognized as the “control center” of the immune-metabolic-endocrine network [6]. The prevalence of infection and mortality after splenectomy is 3.2% and 1.4%, respectively [5]. The asplenic state or hyposplenism may be important features of low immune function [7, 8]. The duration of overwhelming post-splenectomy infection can range from < 1 week to a much longer period [4]. Splenomegaly due to portal hypertension is treated by subtotal splenectomy, which is a challenge to conventional splenectomy. How the fibrous tissue and cellular morphology of the residual spleen are changed upon carrying out this treatment is controversial [9-11]. Advocates for splenectomy believe that, with fibrosis and little immune function in splenomegaly, recurrence of splenomegaly and hypersplenism may happen in residual spleen [12]. However, advocates inclined to preserve spleen suggest that subtotal splenectomy may reduce portal venous pressure, correct hyper-
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Splenectomy and retain the immune function of spleen— which means killing two birds with one stone [13]. Besides, incidence of portal venous thrombosis in patients after subtotal splenectomy for portal hypertension was obviously high, and this may affect the flow of blood into liver [14, 15]. Subtotal splenectomy includes two ways, i.e., with preserving the upper pole and the lower pole of the spleen supplied by the gastro-splenic vessels and splen-omentum and splen-colon vessels [13, 16]. Currently, subtotal splenectomy was mainly performed in giant splenomegaly patients with hereditary spherocytosis and portal hypertension due to cirrhosis [17, 18]. In order to decrease the portal pressure and reserve the splenic function, we have operated subtotal splenectomy of preserving the lower splenic pole (residual spleen about 11 × 7 × 4 cm), for splenomegaly owing to portal hypertension since 1984. Our surgical method is that, via transthoracic approach, residual spleen (supplied by splenic omentum and splenocolic vessels) and partial omentum were fixed with left lung, which formed porto-pulmonary shunt; or by transperitoneal approach, residual spleen (supplied by splenic omentum and splenocolic vessels) and partial omentum were fixed with left retroperitoneal, forming portosystemic shunt.

In the present study, the distribution of fibrous tissue, morphology of cells, splenic size, hemodynamics, hematological and immunological in the residual spleen was contracted. This information may help finding the basis for the operation of subtotal splenectomy.

Materials and methods

Ethical approval of the study protocol was obtained from the Human Research Ethics Committee of Weifang Medical University (Weifang, China). All individuals provided written informed consent before induction into the study.

Patients and methods

Our studies relating to subtotal splenectomy started in 1984. Up to 2012, we have conducted 752 operations of subtotal splenectomy (preserving the lower pole, normal spleen size of splenic tissue, splenic omentum and splenocolic vessels for the purpose of blood-supply), with 117 cases for splenic trauma and 635 for splenomegaly due to portal hypertension. Ten tissue samples were randomly collected from patients in the 89th Hospital of the People’s Liberation Army (Weifang, China). Ten cases (6 males and 4 females; mean age, 32 (27-38) years) with splenomegaly due to portal hypertension who had received subtotal splenectomy (with preservation the upper pole) plus fixation of the posterior sternal omentum majors were included in this group. Selection criteria: (1) Hepatitis-B infection patients (HBV-DNA < 5 × 10² copy/ml) who were confirmed to have cirrhosis, and were classified as Grade A or B according to the Child-Pugh classification. (2) Cirrhosis was accompanied by hypersplenism with moderate or severe varicose veins of the lower esophagus, plus a history of digestive tract hemorrhage. (3) The fibrosis level in the spleen was II [19]. (4) All patients were followed up continually. Spleen organization obtained by operating formed splenomegaly group. Patients who had spleen puncture to check for spleen organization 6 years after surgery formed the residual spleen group. Ten cases (7 males 3 females; mean age, 31 (26-35) years) who underwent splenectomy without splenomegaly formed the control group. Guided by color Doppler ultrasound, hollow-needle biopsy was used to obtain samples of residual splenic tissue [20].

Thirty tissue samples were collected. Specimens were fixed in 10% formalin, dehydrated, embedded and sliced. Each specimen was made into 15 slices (5 slices per group). After staining (elastic-van Gieson (EVG), Masson, and improved ammonia silver staining), slices were observed under light microscope (BX51; Olympus, Tokyo, Japan) for histomorphological analyses. A total of 1 mm³ of fresh specimens from the splenomegaly group and residual spleen group were taken. They were fixed in 3% glutaraldehyde for 24-48 h, dehydrated, embedded and dried. Specimens were then made into ultra-thin (70 nm) slices. Slices were cleansed with water, and soaked in a saturated aqueous solution of uranyl acetate. They were then cleaned with double-distilled water and soaked in lead citrate solution. The histomorphology of splenic cells was observed using a Hitachi H-7500 Transmission Electron Microscope (Hitachi, Tokyo, Japan).

Quantitative analyses of fibers [21]

The percentage composition of the three fibers in each sample was calculated under high mag-
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In addition, patients underwent complete physical examination before and after the operation, which included abdominal ultrasonography (splenic size: splenic length, splenic thickness, splenic square area; hemodynamics: splenic artery flow volume, portal venous diameter, portal venous flow volume), abdominal CT scan, splenic scintigraphy with $^{99m}$Tc sulfur colloid, laboratory hematomal (platelets) and immunological (IgA, IgM, IgG and Tuftsin) evaluation.

Statistical analysis

Data analyses were carried out using SPSS ver17.0 (SPSS, Chicago, IL, USA). All values are mean ± standard deviation (SD). Study groups

Figure 1. (A) Hyperplasia of collagen fibers were seen in the cord of the red pulp and surrounded the central artery of the white pulp. (B) Collagen fibers of the splenic cord showed “cord-like” or “small pieces” hyperplasia. The central artery was surrounded by hyperplastic collagen fibers. The white pulp, like the red pulp, was seen. (C) The central arterial wall in white pulp was defined clearly, but collagenous fibers were not observed around (A-C, Masson staining, × 100). (D) The number of elastic fibers around the central artery increased. These fibers were lamellar or loose; capillary change was observed. (E) Hyperplastic elastic fibers were around the central artery, which demonstrates “white pulp vascularization”. (F) The elastic fibers of central arterial wall were clear, but elastic fibers were not observed around. (D-F, EVG staining, × 100). (G) The number of reticular fibers in the splenic cord decreased and they arranged like threads. (H) The number of reticular fibers in the splenic cord decreased, and they showed as irregular filaments. (I) Reticular fibers around endothelial cells in sinus lienis spread to splenic cord, and sinus lienis were separated like mesh and presented like wire netting (red pulp: WP; central artery: arrow, reticular fiber: arrowhead) (G-I, silver staining, × 100).
were compared with the control group by the rank-sum test and Nemenyi test. P < 0.05 (two-sided) was considered significant.

**Results**

*Images of splenic tissue under light microscope in residual spleen, splenomegaly and normal spleen*

**Collagen fibers:** In residual spleen group, hyperplastic collagen fibers with slender collagen fibers around blood vessels were clearly observed. Collagen fibers surrounding the central artery in the white pulp extended all around (Figure 1A). Hyaline degeneration of the splenic capsule was also found due to the hyperplasia of collagenous fibers. In splenomegaly group, collagen fibers hyperplasia in splenic cord were “cord-like” or “small pieces”. The central artery surrounded by hyperplasia of collagen fibers made the white pulp look like the red pulp (Figure 1B). Elastic fibers were replaced by collagen fibers in the spleen capsule, the hyaline change occurred, in other words, “rock candy spleen” happened. In control group, the central arterial wall in the white pulp was clearly defined, and collagenous fibers were not observed (Figure 1C).
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Elastic fibers: In residual spleen group, slender and successive intimas were observed in the white pulp. The number of elastic fibers around the central artery increased, and they were lamellar or loose (i.e., capillary change was seen; Figure 1D). In splenomegaly group, the elastic fibers of the central artery in the white pulp demonstrated a sparse layer change, and the elastic membrane was irregular and not successive. The elastic fibers of the arterial wall were piled up and arranged irregularly. Hyperplastic elastic fibers were clearly observed, which showed white pulp vascularization (Figure 1E). In control group, the elastic fibers of the central arterial wall were clear, but elastic fibers around the wall were not observed (Figure 1F).

Reticular fibers: In residual spleen group, the number of reticular fibers in the splenic cord was decreased and arranged like threads (Figure 1G). In splenomegaly group, the number of reticular fibers in the splenic cord was reduced and appeared like irregular filaments; distinguishing between the splenic cord and sinus lienis was difficult (Figure 1H). In control group, the reticular fibers around the endothelial cells in the sinus lienis spread to the splenic cord; the sinus lienis was separated like mesh and looked like wire netting (Figure 1I).

Images of cellular morphology under electron microscope in residual spleen and splenomegaly

Endothelial cells: In residual spleen group, the endothelial cells had a spindle-shaped and their basilemmas were clear with fenestrations. The structures of mitochondria in the cytoplasm were approximately normal (Figure 2A). In splenomegaly group, endothelial cells lost their spindle-shaped, were decreased in number, and had a shrinking volume. In addition, the cytoplasm and nucleoplasm were scarce; the basement membrane was not clear and most of the fenestrations had disappeared. Vacuolar degeneration was observed in the mitochondria (Figure 2B). In control group, the endothelial cells had a spindle-shaped with well-developed golgiosome and rough endoplasmic reticula in the cytoplasm.

Lymphocytes: In residual spleen group, medium-sized and small lymphocytes were observed with approximately normal morphologies (Figure 2C). In splenomegaly group, large, medium-sized and small lymphocytes were observed along with plasmocytes with abundant rough endoplasmic reticula and high secretion. However, medullary degeneration of some mitochondria was observed in the plasmocytes (Figure 2D). In control group, lymphocytes were oval with big, round and deeply dyed nuclei.

Macrophages: In residual spleen group, apoptosis was observed in some macrophages. Chromatin gathered at the edge, karyopyknosis karyotheca had disappeared and “chromatin spillover” could also be observed (Figure 2E). In splenomegaly group, the electron concentration in the macrophage cytoplasm was lower; also, macrophages were seen to phagocytize erythrocytes (Figure 2F). In cytoplasm, the number of mitochondria increased along with an increase in the size of the matrix space. Medullary degeneration and vacuolar degeneration as well as cristae fragmentation were observed in some mitochondria (Figure 2G). In control group, the nuclei of macrophages with abundant cytoplasm were round or oval and deeply dyed.

Reticulocytes: In residual spleen group, the number of reticulocytes with large somas decreased; a considerable amount of nucleoplasm and clear intracellular tonofilament was observed. Mitochondria and other cytoplasmic organelles were not observed (Figure 2H). In splenomegaly group, the number of reticulocytes with enlarged somas, appreciable nucleoplasm, with various morphologies as well as protuberances increased. Karyoplasm and mitochondria increased, mitochondria crista ambiguity, and medullary degeneration was observed in some of them (Figure 2I). In control group, reticulocytes were oblong with protuberances, and the cytoplasm was abundant with a few pigmented granules.

Images of clinical data

Residual spleen was preserved about 11 × 7 × 4 cm on operation. It was alive that was confirmed by CT scan, and still had phagocytosis via splenic scintigraphy with 99mTc sulfur colloid after eight years (Figure 3A-E).

Measurement of collagenous, elastic and reticular fiber contents in spleen tissues

With respect to the content of collagenous and elastic fibers, a significant difference was not
observed between residual spleen groups and splenomegaly groups ($p > 0.05$). The collagenous and elastic fiber contents in residual spleen and splenomegaly groups were markedly increased compared with the control group, and a significant difference was observed ($p < 0.05$). Reticular fiber content in the residual spleen and splenomegaly groups was markedly decreased compared with the control group and a significant difference was observed ($p < 0.05$) (Table 1).

**Change of hematological and immunological indexes in residual spleen and splenomegaly**

The platelets in residual spleen were markedly increased compared with splenomegaly groups, and a significant difference was observed ($p < 0.001$). With respect to IgA, IgM, IgG, Tuftsin level, there was no significant difference between residual spleen groups and splenomegaly groups ($p > 0.05$) (Table 2).

**Change of splenic size and hematological indexes between the two groups**

With respect to splenic length, splenic thickness, splenic square area, splenic artery flow volume, portal venous diameter and portal venous flow volume, a significant difference was observed between residual spleen groups and splenomegaly groups ($p < 0.001$) (Table 3).

**Discussion**

It has been reported that 60-65% of patients with cirrhosis and portal hypertension may suf-
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Table 1. Comparison of splenic collagenous, elastic and reticular fiber contents among the three groups (n = 10, means ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Collagenous fibers (%)</th>
<th>Elastic fibers (%)</th>
<th>Reticular fibers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual spleen</td>
<td>7.76 ± 0.88*</td>
<td>8.17 ± 2.93*</td>
<td>4.83 ± 1.41*</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>7.81 ± 0.82**</td>
<td>9.02 ± 3.34**</td>
<td>4.91 ± 1.36**</td>
</tr>
<tr>
<td>Control</td>
<td>3.13 ± 0.55</td>
<td>1.63 ± 3.34</td>
<td>10.95 ± 0.94</td>
</tr>
</tbody>
</table>

Compared with the splenomegaly group, *P > 0.05; compared with the control group, *P < 0.05, **P < 0.05.

Table 2. Comparison of hematological and immunological indexes between the two groups (n = 10, means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Splenomegaly (× 10⁹/L)</th>
<th>Residual spleen (× 10⁹/L)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>50.90 ± 6.66</td>
<td>219.60 ± 33.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IgA (g/L)</td>
<td>4.14 ± 0.39</td>
<td>4.34 ± 0.08</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>2.98 ± 0.25</td>
<td>3.27 ± 0.12</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>20.18 ± 1.52</td>
<td>21.14 ± 1.17</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Tuftsin (μg/L)</td>
<td>604 ± 165</td>
<td>664 ± 148</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

In present study, the distribution of collagen fibers and elastic fibers as well as reticular fibers in the residual splenic group had certain characteristics under light microscopy. The first was the obvious hyperplasia of collagen fibers surrounding the central artery and the splenic cord; reticular fibers proliferated and surrounded the splenic capsule. Vascular endothelial cells were scattered, and no regularity was associated with the expansion of the luminal diameter of the red pulp [30]. At present, fine-needle aspiration and hollow-needle aspiration biopsies are the main methods used for the diagnosis of splenic disease [31, 32]. However, few authors have compared the hematological changes between subtotal splenectomy and splenectomy [33]. Hence, it is necessary to observe whether remodeling happened in retroperitoneal residual spleen, and collect relevant data of splenic size, hemodynamic, hematology, immunology, these have important clinical guidance to recognition of the immune function in residual spleen.

In present study, the distribution of collagen fibers and elastic fibers as well as reticular fibers in the residual splenic group had certain characteristics under light microscopy. The first was the obvious hyperplasia of collagen fibers surrounding the central artery of the white pulp and the splenic cord of the red pulp; also, slender collagen fibers around the blood vessel were also observed. Secondly, slender and successsive intimas were observed in the artery of the white pulp. The number of elastic fibers around the central artery increased, and they were lamellar or loose. Thirdly, the number of reticular fibers of the splenic cord decreased and they were arranged like threads. With regard to the quantitative analysis of fibrosis, collagen fibers, elastic fibers and reticular fibers showed no significant difference between the residual splenic group and splenomegaly group. The content of collagen fibers, elastic fibers of the residual splenic group and the splenomegaly group were obviously increased compared with the control group. The cellular morphology of the residual spleen under elec-
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Table 3. Comparison of splenic size and hemodynamic indexes between the two groups (n = 10, means ± SD)

<table>
<thead>
<tr>
<th>Splenic size and hemodynamic indexes</th>
<th>Splenomegaly</th>
<th>Residual spleen</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic length (cm)</td>
<td>47.50 ± 5.48</td>
<td>11.10 ± 1.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Splenic thickness (cm)</td>
<td>10.30 ± 1.77</td>
<td>3.60 ± 0.52</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Splenic square area (cm²)</td>
<td>398.00 ± 111.67</td>
<td>32.32 ± 7.38</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Splenic artery flow volume (ml/kg•min)</td>
<td>7.51 ± 0.63</td>
<td>2.91 ± 0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Portal venous diameter (cm)</td>
<td>1.42 ± 0.05</td>
<td>1.15 ± 0.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Portal venous flow volume (ml/kg•min)</td>
<td>19.71 ± 0.42</td>
<td>16.45 ± 0.43</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The results of the present study showed that, splenomegaly was caused by congestion, with the formation of a high blood flow environment. This environment promotes the fibrosis of the spleen and changes in corpuscular morphology. If the spleen underwent surgery for grade I-II fibrosis, the structure of the residual spleen would be better. Reticular fiber content in residual spleen and splenomegaly groups was markedly decreased compared with that seen in the control groups. This phenomenon was related to the relative decrease in the number of reticular fibres.

Conclusions

Overall, we believe that, after subtotal splenectomy, the residual spleen does not experience a high-pressure environment (supplied by splen-omentum and splen-colon), and the fibrosis of splenic tissue and remodelling of corpuscular morphology will cease. Our results prove that high pressure may be one of the key factors promoting the splenic fibrosis, and the initiating factor inducing the remodeling of splenic tissue. We still need long-term clinical observation in large quantity cases before our findings can be adopted as one of the theoretical evidences for subtotal splenectomy due to splenomegaly and before our findings can be accepted by the majority of surgeons.

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Disclosure of conflict of interest

The authors indicated no potential conflicts of interest.

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