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Original Article

Short-term effects of splenectomy on serum fibrosis indexes in liver cirrhosis patients

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Abstract: Objective: To determine the changing patterns of 4 liver fibrosis markers pre and post splenectomy (combined with pericardial devascularization [PCDV]) and to examine the short-term effects of splenectomy on liver fibrosis. Methods: Four liver fibrosis markers of 39 liver cirrhosis patients were examined pre, immediately post, 2 days post, and 1 week post (15 cases) splenectomy (combined with PCDV). Results: The laminin (LN) level decreased immediately post surgery compared with the preoperative LN level ($P < 0.05$). The type IV collagen level decreased immediately post surgery compared with that pre surgery ($P < 0.05$), it significantly increased ($P < 0.05$) 2 days post surgery and significantly decreased 1 week post surgery ($P < 0.05$). Hyaluronic acid and the procollagen III N-terminal peptide levels increased significantly 2 days post surgery compared with that pre and immediately post surgery, they significantly decreased 1 week post surgery compared to 2 days post surgery ($P < 0.05$). Conclusions: In the short-term, the 4 liver fibrosis markers and the FibroScans post splenectomy showed characteristic changes, splenectomy may transiently initiate the degradation process of liver fibrosis.

Keywords: Splenectomy, liver cirrhosis, 4 liver fibrosis markers

Introduction

For a long period of time, splenectomy combined with pericardial devascularization (PCDV) have been widely used to treat recurrent esophageal varices bleeding in liver cirrhosis patients. A recent study [1] reported that besides the effects from treating and preventing upper gastrointestinal hemorrhage and correcting hypersplenism, the liver functions of patients who underwent splenectomy showed certain improvements. In addition to this phenomenon, we also found that FibroScan values in over 90% of patients significantly decreased post splenectomy. Since liver fibrosis can be reversed by treatments targeting the causes [2], is it possible that splenectomy can also reverse liver fibrosis in liver cirrhosis patients? In this study, we examined 4 liver fibrosis markers (hyaluronic acid [HA], laminin [LN], procollagen III N-terminal peptide (PIIINP), and type IV collagen [IV-Col]) at different time points in 39 liver cirrhosis patients who underwent splenectomy and analyzed the results. For the first time, the changing patterns of each marker pre, immediately post, 2 days, and 1 week post splenectomy were observed. In addition, the short-term effects of splenectomy on liver fibrosis in liver cirrhosis patients were determined using FibroScan results.

Information and methods

General information

Thirty-nine liver cirrhosis patients (average age, 44.8 ± 11.1 years) (**Table 1**) who underwent splenectomy combined with PCDV between May 2013 and December 2013 in the Department of Hepatobiliary Surgery at Beijing Youan Hospital, Capital Medical University were included.

Inclusion and exclusion criteria

The inclusion criteria were liver cirrhosis with various causes, combined portal hypertension

Table 1. Patient demographics

Categories		Number of cases	Proportion%
Sex	Male	22	56.4
	Female	17	43.6
Underlying liver diseases	Hepatitis B cirrhosis	23	59.0
	Hepatitis C cirrhosis	3	7.7
	Autoimmune liver disease	5	12.8
	Alcoholic cirrhosis	4	10.3
	Idiopathic portal hypertension	2	5.1
	Budd-Chiari syndrome	1	2.6
	Spherocytosis	1	2.6
Surgical methods	Simple splenectomy	8	20.5
	Splenectomy with devascularization	31	79.5

and gastro-esophageal varices (with or without upper gastrointestinal bleeding), combined hypersplenism (white blood cell count < 3 × 10^9/L; platelet count < 50 × 10^9/L), liver function (Child-Pugh score < 10 points), elevated transaminase (< 2 times the normal value, < 80 umol/L), hepatitis B patients with negative hepatitis B virus deoxyribonucleic acid, no other recent antifibrotic therapies, and no significant heart, brain, lung, and kidney complications. The exclusion criteria were patients undergoing liver cancer surgery with existing liver disease activities, significantly elevated preoperative transaminase, massive intraoperative and postoperative transfusion, and secondary surgery due to postoperative hemorrhage.

Surgical method

Under general anesthesia, free portal pressure was measured through intubation via the right gastroepiploic vein from an incision under the left costal margin. Opened the gastrocolic ligament, ligated the splenic artery, seperated the perisplenic ligaments, ligated the vessels entering and exiting the spleen, and sutured the splenic vein at the splenic hilum. Portal pressure was measured again post splenectomy. Whether to conduct selective PCDV was based on portal pressure, conditions of the esophageal-gastro varices, preoperative gastroscopy, and hemorrhage history [3]. The portal pressure of patients who underwent PCDV was measured again post surgery. A routine liver biopsy and conventional drainage of the splenic fossa were performed.

Testing methods of the 4 liver fibrosis markers

Serum samples of patients were collected pre, immediately post, 2 days post, and 1 week post splenectomy. Four liver fibrosis markers were tested (HA, LN, PIIINP, and IV-Col). Among all included patients, 39 were tested for the 4 liver fibrosis markers 2 days post surgery, and 15 were tested 1 week post surgery. The experimental method included using of the sensitized chemiluminescence immunoassay detection system (JETLIA-96/2; China Medical Technologies, Beijing, China).

FibroScan method

Continuous FibroScan (Echosens, Paris, France) examinations were performed in the areas between the 7th, 8th and 9th rib from the right anterior axillary line to the midaxillary line. Ten successful examinations were performed by 2 physicians, and the median was used as the final examination result, which was expressed as elasticity (kPa).

Observation indexes

There were variations in the 4 liver fibrosis markers between the pre and post splenectomy, and there were variations in the FibroScan values in the experimental group between the 1 month post and pre splenectomy.

Statistical treatment

The data of the 4 liver fibrosis markers, if normally distributed, were expressed as means ± standard deviation. The paired t test was used to compare the 2 time points pre and post surgery, and a repeated measurements analysis of variance was used to compare multiple time points pre and post surgery. If not normally distributed, the data were expressed as medians (quartile). The paired rank sum test was used to compare the 2 time points pre and post surgery. A difference of $P < 0.05$ was considered statistically significant. SPSS, version 18 software was used for statistical analysis.

Table 2. The changes in 4 liver fibrosis markers at different time points in liver cirrhosis patients (ug/L)

Sampling time	LN (n=39)	HA (n=39)	PIIINP (n=39)	IV-Col (n=39)
Pre surgery (n=39)	62.38 ± 64.94	44.33 ± 28.40	3.41 ± 1.76	67.05 ± 37.54
Immediately post surgery (n=39)	56.21 ± 59.68*	44.59 ± 21.77	2.97 ± 1.83	50.09 ± 32.94*
2 days post surgery (n=39)	52.09 ± 53.10	146.38 ± 117.44*	7.02 ± 3.67*	121.60 ± 76.50*
1 week post surgery (n=14)	62.49 ± 61.26	77.07 ± 30.83*	3.51 ± 1.06*	49.75 ± 19.77*

Note: *indicates that there was a statistical significance when compared with the value of a previous time point ($P < 0.05$). LN: laminin; IV-col: type IV collagen; HA: Hyaluronic acid; PIIINP: procollagen III N-terminal peptide.

Table 3. Changes in the FibroScan (kPa) and liver blood flow (mL/min) pre and at 1 month after splenectomy in liver cirrhosis patients

Examination time	FibroScan (n=16)	Hepatic arterial blood flow (n=24)	Portal blood flow (n=24)
Pre surgery	17.03 ± 8.99	165.6 ± 66.6	1334.9 ± 459.4
1 month post surgery	14.08 ± 7.26	225.7 ± 105.1	891.2 ± 425.1
P	0.006	0.012	< 0.001

Results

The immediate post splenectomy LN level (56.21 ± 59.68 ug/L) decreased compared with that pre surgery (62.38 ± 64.94 ug/L) ($P < 0.05$). The LN level 2 days post surgery (52.09 ± 53.10 ug/L) continued to decrease, but the difference was not statistically significant compared with that immediately post surgery. The LN level 1 week post surgery (62.49 ± 61.26 ug/L) slightly increased compared with that at 2 days post surgery (52.09 ± 53.10 ug/L) with no statistical significance (**Table 2**).

The immediate post splenectomy IV-Col level (50.09 ± 32.94 ug/L) decreased compared with that pre surgery (67.05 ± 37.54 ug/L) ($P < 0.05$). The IV-Col level 2 days post surgery (121.60 ± 76.50 ug/L) significantly increased ($P < 0.05$). The IV-Col level 1 week post surgery (49.75 ± 19.77 ug/L) significantly decreased compared with that at 2 days post surgery (121.60 ± 76.50 ug/L) ($P < 0.05$) (**Table 2**).

The HA level (146.38 ± 117.44 ug/L) 2 days post splenectomy significantly increased compared with that pre (44.33 ± 28.40 ug/L) and immediately post surgery (44.59 ± 21.77 ug/L) ($P < 0.05$). The HA level 1 week post surgery (77.07 ± 30.83 ug/L) decreased with statistical significance compared with that at 2 days post surgery (**Table 2**).

The PIIINP level (7.02 ± 3.67 ug/L) 2 days post splenectomy significantly increased compared with that pre (3.41 ± 1.76 ug/L) and immediately post surgery (2.97 ± 1.83 ug/L) ($P < 0.05$). The PIIINP level 1 week post surgery (3.51 ± 1.06 ug/L) significantly decreased compared with that at 2 days post surgery ($P < 0.05$) (**Table 2**).

The FibroScan of 16 patients 1 month post surgery (14.74 ± 7.39 kPa) decreased with statistical significance compared with that pre surgery (16.93 ± 9.06 kPa) (**Table 3**).

Discussion

The 4 liver fibrosis markers (HA, LN, PIIINP, and IV-Col) are used to clinically detect the occurrence and progression of liver fibrosis in patients with liver diseases. Among them, LN and IV-Col are mainly involved in forming the basement membrane in sinusoidal capillaries, indicating fiber deposition in the liver. The HA and PIIINP are markers of liver fibrotic activities, indicating fibrotic activities in liver. In this study, the 4 markers were all within the normal ranges for most of the 39 patients in the experimental group pre surgery, indicating that in the stage of liver cirrhosis, liver fibrosis in patients can be in a substantially steady state without progressions of primary diseases such as activated hepatitis B virus. However, performing splenectomy (combined with PCDV) disrupted this steady state. A decreased portal pressure and portal blood flow rate were observed immediately post splenectomy, as well as an increased hepatic arterial flow (**Table 3**). This blood flow remodeling in the liver can result in changes in a series of cytokines and induce the liver to progress toward liver cell proliferation, improved liver functions, and fibrosis reversion [1, 4, 5]. These changes are also reflected in the changes of the 4 liver fibrosis markers, and

our study found short-term characteristic changes in the 4 liver fibrosis markers post splenectomy. First, the LN and IV-Col levels immediately decreased post splenectomy while the HA and PIIINP levels showed no significant variations. The decrease in the LN and IV-Col levels indicated a reduced fiber deposition in the patients' livers. However, with respect to time, liver fibrotic changes do not occur immediately post splenectomy. Hence, we believed that the immediate decreases in the LN and IV-Col post splenectomy were due to a relatively more abundant LN and IV-Col in the removed spleen [6]. The LN level continued to decrease 2 days post surgery, suggested a continuous decrease in extracellular matrix (ECM) deposition. Since the spleen had already been removed, this change should occur in the liver. In other words, 2 days post splenectomy, the deposition of ECM in the liver was decreasing. The IV-Col was cross-linked and deposited through the LN and other ECM components [7]. After the LN degraded, the IV-Col was de-cross-linked and released into the blood, and its level was significantly elevated 2 days post surgery. Similarly, the HA and PIIINP were released through the depolymerization of deposited ECM in liver 2 days post surgery, and significant increases were shown in the test results. At 1 week post surgery, the HA, IV-Col, and PIIINP levels showed significant decreases, while the IV-Col and PIIINP levels decreased to levels that were even lower than those pre surgery, indicating that the obvious degradation of ECM occurred within 1 week post splenectomy. The long-term changes of liver fibrosis post splenectomy still need to be studied further and verified. In addition, there is no evidence showing that short-term liver cell regeneration post splenectomy affects the testing results of the 4 liver fibrosis markers [7].

The FibroScan (transient elastography) is a method for testing liver tissue fibrosis. It evaluates the degree of liver fibrosis by measuring liver stiffness, and it has a high efficiency, accuracy, and other advantages, as it can partially replace liver biopsy [8]. In the FibroScan examinations of 16 patients, we found that the FibroScan values in most patients 1 month post surgery were lower than those pre surgery, indicating that the liver stiffness of patients was reduced post splenectomy. Reduced liver stiffness can be related to hepatic blood flow changes, liver cell proliferation, and other factors. However, the reduction of liver fibrosis has a more significant influence on the FibroScan results. Hence, we can also believe that the liver fibrosis was reduced post splenectomy. This is consistent with previously mentioned test results of the 4 liver fibrosis markers.

To determine the degrees of liver cirrhosis and fibrosis, pathological examinations should be the golden standard. However, due to ethical reasons, we were unable to obtain liver samples from the patients during the pathological examinations at the postoperative follow-ups. Thus, this study only used serologic indexes and the liver stiffness index to indirectly determine the changes in liver fibrosis pre and post splenectomy. Theoretically, all 4 liver fibrosis markers should be normally distributed. The poor normality of some LN data in this study may be attributed to the small sample size; hence, different statistical methods were applied. In addition, the formation and progression of liver cirrhosis are long-term dynamic processes, and the indexes observed 1 week post surgery in only some patients were not complete. Besides, a series of changes occurred post splenectomy, including liver blood flow remodeling, liver fibrosis reversion, and liver function improvement, and their related mechanisms still require further study. Therefore, we have continued to accumulate cases and conduct a series of studies on several cytokines, including matrix metalloproteinases, tissue inhibitors of metalloproteinases, hepatocyte growth factor, interleukin (IL)-6, tumor necrosis factor-α, etc. [9], macrophage phenotype and functional changes [10], changes of differential proteins in serum post splenectomy, and a cohort study of long-term survival to further illustrate the series of changes in the liver and their mechanisms post splenectomy.

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Disclosure of conflict of interest

None.

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