Journal of Advances in Medicine and Medical Research

29(5): 1-7, 2019; Article no.JAMMR.21415
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Histological Changes of Spleen Autotransplanted in the Omentum of Rats

Mohammad Reza Javadi[1*], Ali Reza Monsef Esfahani[2], Nazila Hassanzadeh Ghavifekr[3] and Amin Niayesh[3]

[1]Department of General Surgery, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.
[2]Department of Pathology, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.
[3]Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2019/v29i530089
Editor(s):
(1) Dr. Fuhong Su, ICU Laboratory, Erasme Hospital, Free University Brussels, Brussels, Belgium.
Reviewers:
(1) Somchai Amornyoti, Siriraj Hospital Mahidol University, Thailand.
(2) Abrão Rapoport, São Paulo University, Brazil.
Complete Peer review History: http://www.sdiarticle3.com/review-history/21415

Original Research Article

Received 11 September 2016
Accepted 15 December 2016
Published 05 April 2019

ABSTRACT

Introduction: The spleen is a lymphoid organ that plays an important role in the body's defense against organisms, participation in blood filtration process, phagocytosis and immunoglobulin production. When splenectomy due to severe splenic trauma or hematological disorder is inevitable, spleen autotransplantation may be the only option to preserve the spleen. It has not been determined the structural rearrangements after transplanting in comparison with the amount of transplanted spleen. The aim of this study was to evaluate the histological changes of spleen autotransplanted in the omentum of rats.
Materials and Methods: In this experimental study, 16 male Wistar rats underwent splenectomy and transplanting three pieces of splenic tissue consists of 10-15% of the greater omentum. The rats were divided to two equal groups; Eight caseswere randomly separated and followedfor 6 months (Group Au-6) and 8 other cases also were separated for 12 months' follow-up (group Au-

**Corresponding author: E-mail: javadim52@yahoo.com;*

12). At the end of follow-up period, after the re-operation of autotransplanted rats, the splenic tissues underwent the macroscopic and microscopic examination and two groups were compared together.

Results: After laparotomy, splenic tissues were detected in all cases (8/8, 100%) of Au-6 group and 7 cases (87.5%) of Au-12 group. It's observed no significant difference between two groups in the capsule around splenic tissue, organized structure, fibrosis and revascularization (P>0.05). The hemosiderin pigmentation was significantly higher in Au-12 group (P=0.03).

Conclusions: The results of this study showed that autotransplanting about 15 percent of splenic tissue were associated with a high success rate in tissue structure rearrangement. Therefore, spleen autotransplantation even in small sizes was highly recommended in cases of unavoidable splenectomy.

Keywords: Histological changes; spleen autotransplantation; omentum; rat.

1. INTRODUCTION

The spleen is a lymphoid organ that plays an important role in the body's defense against organisms, participation in blood filtration process, phagocytosis and immunoglobulin production [1]. The spleen is commonly injured after blunt abdominal trauma and penetrating trauma to the left upper quadrant of abdomen. By identifying immunological function of the spleen, the operation was focused on preserving it. After the success of preserving spleen in children, non-surgical approachto splenic injuries became the preferred method. However, still in severesplenic injuries, splenectomy is proposed as a treatment more common than splenorrhaphy [2]. Early and late complications after splenectomy were evaluated already in several clinical trials, but promoting knowledge in the field of late complications such as loss of function of the spleen and various infections was done only according to recent studies and based on trials comparing surgery to preserve the spleen and splenectomy [3-6].

In clinical trials, Immunoprophylaxis, chemoprophylaxis and spleen preserving surgery such as resection of the spleen and splenic autotransplantation along with informing and training patients prevented many serious complications [7]. Some studies have suggested the autotransplantation of homogenized spleen tissue. Because, preserving the spleen filtration performance while maintaining its structure is better. This issue is also discussed that given the priority of life saving the smaller pieces of transplanted tissue, regeneration of the parts is done in less time than larger pieces. Also about the site of transplantation, different locations have been investigated including greater omentum, peritoneal cavity, spleen, retroperitoneum, intraportal, abdominal muscles and under the skin of abdominal wall. Among them, the greater omentum has several advantages such as proper vascularization and possibility of blood exchange on a large area [1]. In spleen preservation method using greater omentum, the sheets of spleen named "*chip*" will be replaced between two layers of omentum to prevent adhesion formation [8-11].

There is no comprehensive agreement in the size of transplanted tissue in experimental studies. The results of researches suggest that the regeneration of transplanted tissue occurs in the size of 1 mm to a thickness more than 1 cm. Thicker parts are degenerated before starting the regeneration process [1]. In previous studies, it was determined that splenic chips including 10-15 percent of total splenic mass successfully survive for several months in the omentum and at the end of second months, the splenic chips will be regenerated histologically in rats in the early days [12-15]. One of the important points is that various studies believe that about 25-30 percent of spleen tissue is required for a suitable replacement of function. After a high-grade splenic injury including $4^{th}$and 5^{th} grades called "*Shattered spleen*" can be transplanted only a small part around 10-15 percent of normal spleen tissue structure [14,15]. Therefore, this study was performed to transplant about 10-15 percent of splenic tissue and evaluate its histological effects.

2. MATERIALS AND METHODS

2.1 Animal Models

This experimental study was performed on rats and animal specimens prepared from Animal Research Center of Faculty of Medicine of Hamedan University of Medical Sciences

(Hamedan, Iran). For this purpose, 16 male Wistar rats weighing 200-250 g were used. The animals were kept for a week in order to adapt to the environment. The animals were kept in steel cages covered with wood dust at 22-23°C temperature and 12 hours' light and dark cycle and had free access to drinking water and eating standard food. At all stages of study, all conditions of maintenance and tests on the animals were performed, based on guidelines approved by the Ethics Committee of University. After one week, the rats were randomly divided into 2 groups and were kept in separate cages; Group 1: Autotrans planted rats at 6th month=Au-6, and Group 2: Autotrans planted rats at 12th month=Au-12.

2.2 Procedure

Within one week, the rats were anesthetized by intraperitoneal injection of ketamine at a dose of 35 mg/kg and 1 mL of lidocaine 1% and then underwent surgery in clean conditions. The rats were splenectomized with a midline incision, and then splenic tissue autotransplantation was performed using Furka's splenic chip technique on the omentum. For performing Furka's method, 3 small pieces of spleen as chips after splenectomy with approximate dimensions of 2×3×4 mm were prepared and placed in normal saline solution at room temperature. After creating 3 nests on the omentum by opening its first layer, the pieces were placed between two layers of greater omentum and then fixed with PDS 0.5 threads. Before closing the abdominal cavity, 1 ml of sterile normal saline was administered intraperitoneally and then abdomen was repaired again in two layers of muscles and skin. The autotransplanted rats underwent again laparotomy and the transplanted splenic tissue was sent for pathological study.

2.3 Histopathological Examination

At first, tissue samples obtained from the omentum were put in 0.9 percent solution of sodium chloride to be isolated additional blood cells and then were dried and tissue fragments were measured by a ruler on millimeter. After initial macroscopic examination, the obtained samples were diluted in formalin and pathological preconditioning. The presence of fat tissue and fibrous at the site of adhesions and remaining tissue structure were evaluated. Splenic tissue was examined in structure rearrangement and the samples were divided to two groups; "*organized*" and "*low or disorganized*". Active lymphatic follicles consisting of germinal centers in all sections were examined. In tissue sections prepared from transplanted spleen, hemosiderin pigment and activated macrophages versus normal spleen sections were examined and compared with normal spleen, the numbers of 0 to 4 were assigned to each: 0=No, 1=low, 2=medium but less than normal, 3=high but less than normal, 4=abundant in the extent of normal tissue.

2.4 Statistical Analysis

Histological changes in splenic tissue were compared using examination of tissue slides in two groups of Au-6 and Au-12. The collected data were analyzed using SPSS-17 statistical software. The mean and standard deviation were used to describe data. For normally distributed data analysis, ANOVA was used and if the results were significant, Dunett test was used to compare two groups. The significance level for all tests was considered as 0.05.

3. RESULTS

In this study, 16 rats were studied in two groups. In Au-6 group, spleen tissue was detectable in all cases (100%) after laparotomy and all three pieces of transplanted tissue were identified and examined pathologically. In Au-12 group, spleen tissue was detectable in 7 cases (87.5%) after laparotomy. In the investigation of omentum, in one case, a fibrous tissue about 1 mm was found at the site of spleen transplantation that was not well known. In this group, the samples were also taken for further pathological investigation (Images 1 to 4 and Fig. 1).

In microscopic examination of spleen samples, presence or absence of capsule around the spleen tissue in Au-6 and Au-12 groups were studied. Presence of splenic capsule was seen in 7 cases (87.5%) in Au-6 group, and in one case, the capsule was not well known. In Au-12 group, 7 cases of splenic capsule were detected (87.5%) and only in one case, the spleen was very small and had no clear capsule. The difference between two groups in presence of capsule was not statistically significant (P=0.75) (Fig. 1).

Spleen was organized in 3 cases (37.5%) in Au-6 group and was low organized in 5 cases (62.5%). The splenic tissue was organized in 6 cases (75%) in Au-12 group and was disorganized in one case. It was not found a significant

difference between two groups in presence of organized structure of spleen (P=0.31). The revascularization in Au-6 group was occurred in 6 cases (75%). Also, in Au-12 group, revascularization was observed in 6 cases (87.5%). Fibrosis in transplanted splenic tissue in Au-6 group was found in 2 cases (25%) that had moderate fibrosis. In Au-12 group, severe fibrosis in remained splenic tissue was observed in one case (12.5%). The difference between two groups in terms of fibrosis and revascularization in splenic tissue was not statistically significant (Fig. 1). Hemosiderin pigmentation in splenic tissue in Au-6 group was measured from 0 to 4 based on a quantitative scale compared to normal tissue. The mean hemosiderin concentrations in Au-6 and Au-12 groups were 1.50±0.75 and 2.50±0.92 mg/ml, respectively. Hemosiderin pigment in Au-12 group was significantly higher than Au-6 group (P=0.03).

The formation of lymphoid follicles in white pulp in Au-6 was observed in 4 cases (50%). In Au-12 group, formation of lymphoid follicles was observed in 6 cases (75%). The formation of red pulp in tissue sections of transplanted spleen was high in 6 cases (75%) in Au-6 group. In Au-12 group, red pulp formation was good in 7 cases (87.5%) but in one case (12.5%) was low. The difference between two groups in the formation of red pulp, white pulp and lymphoid follicles were compared that the difference between two groups was not significant (P>0.05) (Fig. 2).

Image 1. Pieces of splenic tissue at 12 months after transplantation (Au-12)

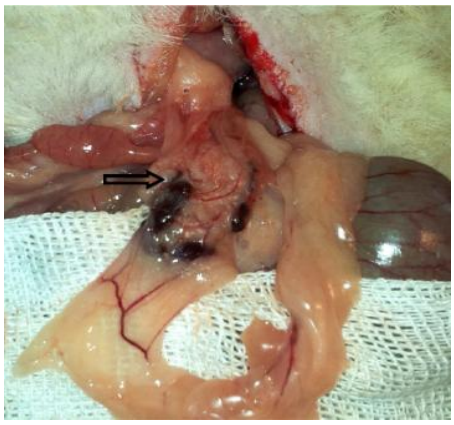

Image 2. Pieces of splenic tissue at 6 months after transplantation (Au-6)

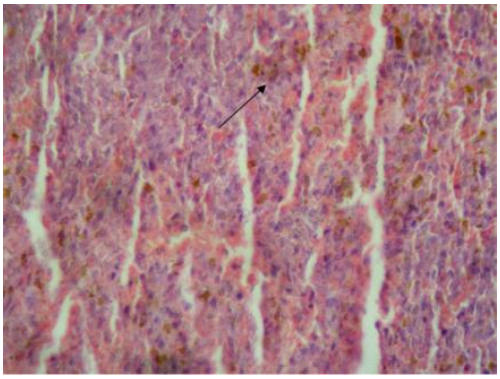

Image 3. Red pulp in transplanted splenic tissue in Au-12 group (Arrow: Hemosiderin pigment)

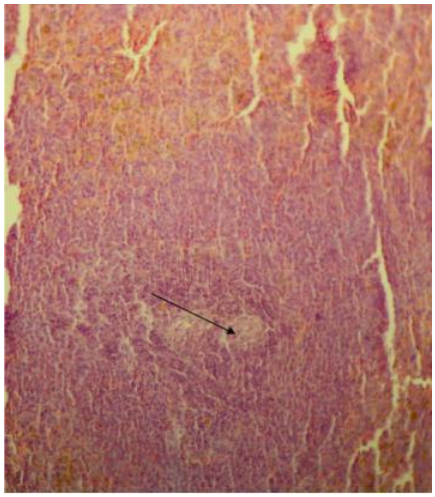

Image 4. White pulp with lymphoid follicle in transplanted splenic tissue in Au-12 group (Arrow: germinal centers)

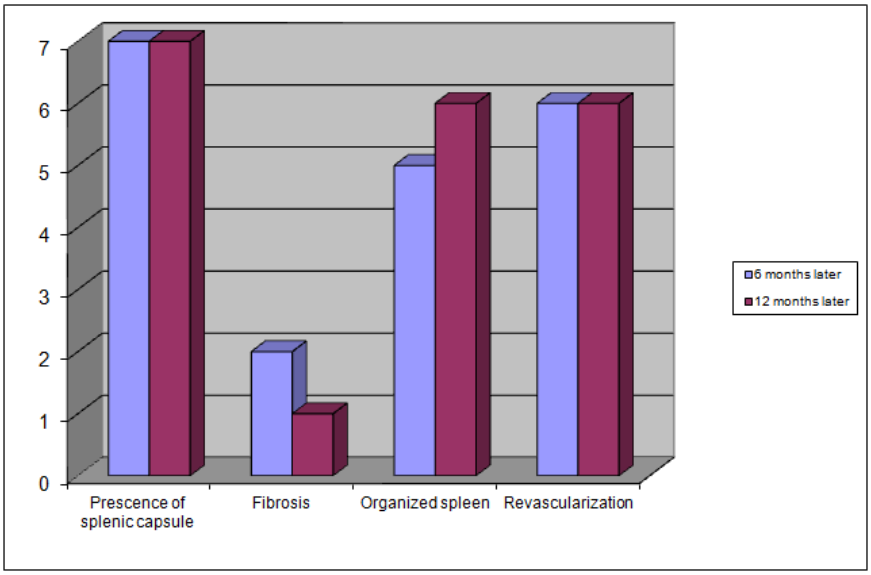

Fig. 1. Comparing structure of transplanted splenic tissue between two groups in follow-up period

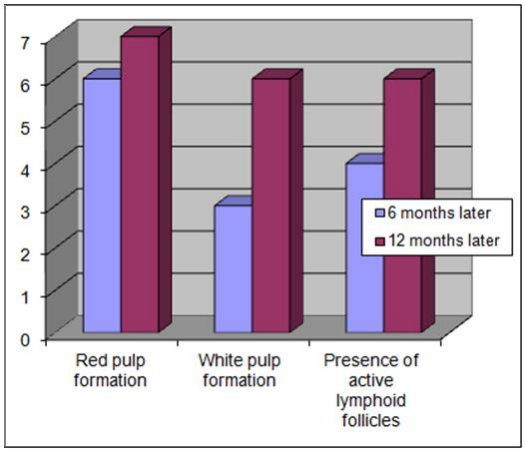

Fig. 2. Comparing rearrangement of tissue components of transplanted spleen between two groups in follow-up period

4. DISCUSSION

In severe splenic trauma and hematological disorders, splenectomy is inevitable and in these cases, heterotopic autotransplantation of spleen is the only possible option to preserve spleen function, because, preserving function of splenic tissue has a great importance to maintain immunological status and defense versus organisms, especially in children [16-19].

The evidence suggests that autotransplanted tissue is degenerated and then regenerated over a period of 12 weeks. The first time in 1988, Steely introduced the optimal rate for survival and coping with streptococcal sepsis about 80 percent [20]. Of course, the amount of spleen regarding severity of trauma leading to splenectomy, is high that is not always available. The study of Marques, et al. showed that the minimum size of spleen for developing phagocytic function of macrophages in adult rats is 26 percent of total splenic tissue [1]. In this study, only about 15% of splenic tissue was autotransplanted that it was less clear than transplanted size in previous studies. However, it was shown that low volume of transplanted tissue in this study was higher and even comparable with higher splenic volumes in the formation of an organized splenic structure over a long-term follow-up. This issue when is valuable that in cases of severe splenic injury leading to splenectomy, saving even 10-15 percent of spleen tissue is often possible and in terms of time and technical aspects, transplanting this amount is possible.

It seems that according to the present study, the benefits of spleen transplantation even low volume compared to splenectomy alone is clearly evident. Some studies support this finding of present study. Braga, et al. in their study splenectomized 20 male Wistar rats that in first group, 25 percent of spleen size and in second group, 30 percent of spleen size were transplanted in the omentum and the results

were evaluated macroscopically and microscopically after 8 weeks. The results showed that in both groups, white pulp by forming follicles and lymphoid tissue and the red pulp were maintained. In second group, white pulp was less organized and red pulp was hemorrhagic. Interesting result was that regeneration of autotransplanted spleen tissue in first group with lower volume was better [19].

Several studies have been conducted on transplanted tissue. In this study, transplanted splenic tissues in all cases except one were associated with severe fibrosis and decreased volume, but in other cases, it was well known. Fibrosis of spleen tissue was detected only in 3 cases. Revascularization rates in the omentum after transplantation were high in both follow-up groups at 6 and 12 months. The percentage of proper formation of red pulp and white pulp in 12-month follow-up group in this study was higher than 6-month follow-up group. The percentage of formation of germinal centers in white pulp was also higher. However, given the small sample size in 6 and 12-month follow-up groups, the results of comparison was not statistically significant. Hemorrhagia and hemosiderin concentrations in 12-month follow-up group were significantly higher than 6-month follow-up group. In the study of Sajtos, et al., autotransplanted splenic tissue was histologically similar to normal spleen tissue. The results of this study showed that autotransplanted spleen was gradually regenerated and its function was returned relatively that it is possible to prevent complications of splenectomy [7]. This improvement in performance even after transplanting 10-15 percent of spleen tissue was observed. The main limitations of this study were the unpredictable death of samples before the end of study and impossibility of full controlling the standard conditions for keeping the animals.

5. CONCLUSION

The results of this study showed that autotransplanting about 15 percent of splenic tissue were associated with a high success rate in tissue structure rearrangement. There was no significant different between two 6- and 12-month follow-up groups of autotransplanted rats in made of capsule around the splenic tissue, presence of organized structure, fibrosis and revascularization. The results of this study and other studies emphasize on the importance of effort to spleen autotransplantation regarding the vision of its advantages that is particularly relevant with size of transplanted splenic tissue and maintained tissue after transplantation. The final key finding is that the histological and functional aspects of spleen autotransplantation after splenectomy were significantly preserved that can be effective in reducing the incidence of splenectomy. This study was conducted on the animals and so, further investigations should be performed on humans to confirm the results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Marques RG, et al. Critical mass of splenic autotransplant needed for the development of phagocytic activity in rats. Clin Exp Immunol. 2012;170(1):77-85.
2. Forsythe R, et al. Blunt splenic trauma. Scandinavian J Surgery. 2006;95:146-51.
3. Feliciano PD, et al. A decision analysis of traumatic splenic injuries. J Trauma. 1992;33:340-348.
4. Malagó R, et al. Late histological aspects of spleen autologous transplantation in rats. Acta Cir Bras. 2008;23(3):274-81.
5. De Porto APNA, et al. Assessment of splenic function. Eur J Clin Microbiol Infect Dis. 2010;29(12):1465-73.
6. Bergmann L, et al. Quantitative and functional restorations and alterations of peripheral lymphocytes in patients with autologous spleen implantation. Arch Orthop Trauma Surg. 1990;109(2):102-5.
7. Sajtos E, et al. Long-term following-up of viability of spleen autotransplants in the Beagle canine model. Acta Cir Bras. 2012;27(2):95-101.
8. Tavassoli M, et al. Studies on regeneration of heterotopic splenic autotransplants. Blood. 1973;41(5):701-9.

9. Iinuma H, et al. Optimal site and amount of splenic tissue for autotransplantation. J Surg Res. 1992;53(2):109-16.
10. Marques RG, et al. Regeneration of Splenic autotransplants. Ann Hematol. 2002;81(11):622-6.
11. Boasquevisque EM, Portela MC. Bacterial clearance after total splenectomy and splenic autotransplantation in rats. Appl Radiat Isotopes. 2002;57 (6):767-71.
12. Furka I, et al. Heterotopic autotransplantation of the spleen in animal experiments. Khirurgija. 1989;9:125-7.
13. Furka I, et al. Autotransplantation of the spleen. Second world week of professional updating in surgery and in surgical and oncological disciplines of the University of Milan. Lecture Book Bologna: Monduzzi Editore; 1990. p. 767-9.
14. Miko I, et al. Spleen autotransplantation in mice: A novel experimental model for immunology study. Microsurgery. 2001; 21(4):140-2.
15. Miko I, et al. Hematological, hemorheological, immunological, and morphological studies of spleen autotrans-plantation in mice: Preliminary results. Microsurgery. 2003;23 (5):483-8.
16. Sipka S Jr, et al. Cellular and serological changes in the peripheral blood of splenectomized and spleen autotrans-planted mice. Transpl Immunol. 2006; 16(2):99-104.
17. Miko I, et al. Morphological and functional follow-up after spleen autotransplantation in mice: a research summary. Microsurgery. 2007;27(4):312-6.
18. Mikó I, et al. Can the injured spleen be preserved? Results of 20-year experiments. Magy Seb. 2005;58(2):69-73.
19. Braga AA, et al. Histological aspects of autologous transplantation of different fragments of the spleen in rats. Acta Cir Bras. 2012;27(12):880-4.
20. Steely WM, et al. Splenic autotransplantation: Determination of the optimum amount required for maximum survival. J Surg Res. 1988;45(3):327-32.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/21415