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SPLENIC AUTOTRANSPLANTATION "EXPERIMENTAL AND APPLIED STUDY"

By

Gamal I. Moussa, M.D.; Osama H. El Khadrawy, M.D;Said E. Asker, M.D. ♆,Ahmed Issa, M.D.; Mostafa A. Eltablawy, M.D.; Osama A. Baza, M.D.; S. Abdel Aziem, M.D. and Mahmoud M. El Shiekh, M.D.*

Department of Surgery, Faculty of Medicine, Tanta and Menoufiya♆ Universities

Department of Clinical Pathology, Faculty of Medicine, Tanta University.

Background: ***After splenectomy, patients have an increased risk of overwhelming infection. The value of human spleen autotransplantation after splenectomy because of trauma has long been questioned. The aim of this work is to study (experimental and applied) the effect of partial splenic autotransplantation (PSA) on haematological changes and some immunological splenic functions.***

Methods: ***The present study was conducted on 45 patients and 60 rabbits divided into groups underwent either total splenectomy only, total splenectomy with intraperitoneal partial splenic autotransplantation (IPPSA) or total splenectomy with extraperitoneal partial splenic autotransplantation (EPPSA). Another group of 15 rabbits underwent total splenectomy with intramuscular partial splenic autotransplantation (IMPSA). Haematological changes and serum immunoglobulins (IgM, IgA, IgG) were studied on days 1, 30 and 90 postoperatively.***

Results: ***Both experimental and applied groups underwent PSA showed preservation of the splenic immunological functions and insignificant haematological changes irrespective of the site of autotransplantation. The vascularity of the autotransplanted splenic slices depends on the optimum size of the slice, (5 mm in thickness).***

Conclusion: ***With extensive splenic trauma, total splenectomy with PSA can be performed safely with insignificant haematological changes and preservation of splenic immunological functions.***

Key words: autotransplantation, spleen, applied, experimental.

INTRODUCTION

A successful splenic salvage following trauma to the spleen was first reported in 1902. Since that report, a variety of splenorrhaphy techniques has been documented and recently reviewed [1]. The search for salvage techniques is continued because splenectomy is known to increase the risk of overwhelming bacterial infection with an associated mortality rate more than fifty percent [2,3]. The introduction of new techniques and materials (as splenic haemostasis, splenic suturing, partial splenectomy and mesh splenic wrapping), have made splenic conservative procedures, a viable concept in modern surgery [4].

If the spleen is irreparable after injury, nor is it possible to resect a portion of it safely, partial splenic autotransplantation (PSA) will solve the problem of splenic preservation for maintaining its normal functions in these complex situations as well as during resection of the tail of the pancreas for trauma or tumor [5] the amount of blood supply per gram of splenic tissue is the major determinant of successful preservation of the function of the autotransplanted splenic tissue, regardless which vessels carry that amount of blood [6].

PATIENTS AND METHODS

The present study was conducted on 45 patients, and an experimental work on 60 New Zealand Albino rabbits, at the Gastroenterology Unit, Department of General Surgery, Tanta University Hospitals.

The applied part of the study was done on 45 patients with grade V splenic trauma according to the spleen injury scale selected from 168 patients presented with abdominal trauma during the period from May 1998 to Marsh 2000. There were 33 males and 12 females and their ages ranged from 9 to 43 years with a mean age 24.8 years. These patients were randomly classified into three groups: group A (15 patients): underwent total splenectomy, group B (15 patients): splenectomy with IPPSA and group C (15 patients): splenectomy with EPPSA.

All the patients were subjected preoperatively to history taking, thorough clinical examination, radiological and laboratory investigations as: complete blood count, pocked cell "notched RBCs" Test and determination of Immunoglobulins (IgM, IgA and IgG).

Operative technique of applied part:

- Under general endotracheal anaesthesia, through upper midline incision, exploration of the abdomen was done with grading of the trauma according to the splenic injury scale as grade V (completely torn vascular splenic pedicle in 7 patients and completely shattered spleen in 38 patients).

- Total splenectomy was done for 15 patients (group A).

- Splenectomy with IPPSA was done for another 15 patients (group B) by taking transverse slices from the removed spleen weighing one third of normal splenic weight (from 30 gm. to 50 gm. according to the age of the patient). Each slice was 5 millimeters in thickness. These slices were reimplanted to the same patient in an omental pouch, which wrapped them completely. Three to four simple stitches (Dexon 2-0) were used to close the omental pouch (Fig. 1).

- Splenectomy with EPPSA was done for another 15 patients (group C) by taking similar slices as in group B. These slices were reimplanted extraperitoneally in a pocket in the anterior abdominal wall in the umbilical region to the left side of the abdominal incision. Closure of the pocket was done by continuous stitches using Dexon 2-0 (Fig. 2).

- A tube drain in the splenic bed was used in all patients.

One and three months postoperatively, the patients of all groups were subjected to the following: clinical evaluation, complete blood count, pocked cell test, determination of Immunoglobulins (IgM, IgA &IgG) and abdominal ultrasonography with duplex scanning.

In the experimental part, the animals used were 60 New Zealand Albino rabbits, which were housed in the central animal house, Department of Physiology, Faculty of Medicine, Tanta University, which maintained their environment at a controlled temperature, relative humidity, and 12 hours light / dark cycle. The rabbits were quarantined for one day before use and all were males weighing from 2000 to 2100 grams with a mean of 2060 grams. The rabbits were randomly classified into equal four groups (15 for each), group A: totally splenectomized group, group B: splenectomized with IPPSA, group C: splenectomized with EPPSA and group D: splenectomized with IMPSA.

All the rabbits were subjected preoperatively to complete blood count, pocked cell test and determination of Immunoglobulins (IgM, IgA & IgG).

Operative technique of experimental part:

Animals were anaesthetized with 50mg. /kg. Ketamine subcutaneously, and placed in the supine position on the operating table. The surgical region was cleansed with povidone iodine 10% (Betadine). An upper midline incision was done for all rabbits. In addition, oblique incision in the thigh was done to rabbits in group D (IMPSA). Exploration of the abdomen was performed for detection of splenules, which were excised when found. Total splenectomy was done for 15 rabbits (group A).

Splenectomy with IPPSA was done for another 15 rabbits (group B) as in the applied part of the study using only one splenic slice. (Normal weight of the spleen is about 15 gm.) (Fig. 3).

Splenectomy with EPPSA was done for another 15 rabbits (group C) by a similar technique as in the applied part using only one splenic slice (Fig. 4)

Splenectomy with IMPSA was done for another 15 rabbits (group D) by taking a similar transverse slice and reimplanting it in a pocket between the thigh muscles. Closure of the pocket was done by continuous stitches using Dexon 2-0 (Fig. 5).

In all groups, the rabbits were subjected (one month postoperatively) to the following: complete blood count, pocked cell test, determination of Immunoglobulins (IgM, IgA, and IgG) and histological study to the part which was transplanted; for studying the splenic architecture, the viability of the lymphoid follicles and patency of the blood vessels.

RESULTS

In the applied part of the study the age of the patients ranged between 9 and 43 years with a mean age 24.8 years. The number of males was 33 patients constituting 73.33%.

As regard to the cause of trauma, motor car accident in 40 patients (88.9%), falling from height in 4 patients (8.9%) and only one patient (2.2%) from group A was due to a stab wound. Thirty patients (66.6%) had associated extraabdominal injuries; as chest trauma in 26 patients (57.7%), simple fractures in 6 patients (13.3%) and brain concussion in 4 patients (8.88%). Forty-three patients (95.56%) had associated intraabdominal injuries; 18 from group A, 16 from group B and 9 from group C while 2 patients (4.44%) had pure splenic trauma.

The operative time ranged between 35 and 50 minutes, with a mean time 43 minutes in group A (total splenectomy), from 39 to 62 minutes with a mean time 51 minutes in group B (IPPSA) and from 41 to 65 minutes with a mean time 52 minutes in group C (EPPSA).

The red blood cells, white blood cells, haemoglobin level and haematocrite values showed insignificant changes ($P > 0.05$) in the studied groups between the samples studied on day 1 and day 90 postoperatively. The platelets count among group A (total splenectomy) showed a significant statistical decrease ($P < 0.05$) in the day 90 than in day 1 but in group B (IPPSA) and group C (EPPSA) showed insignificant statistical changes ($P > 0.05$) between the samples studied on day 1 and day 90. As regard the comparison of the notched RBCs count from day 1 to day 90, there was statistically significant increase ($P < 0.05$) in group A (total splenectomy). While in group B (IPPSA) and group C (EPPSA), there was statistically insignificant increase ($P > 0.05$) (Table 1).

Serum IgM levels from day 1 to day 30, showed statistically insignificant decrease ($P > 0.05$) in all groups. From day 30 to day 90, there was statistically significant decrease ($P < 0.05$) in group A (total splenectomy) while in the other groups there was statistically insignificant decrease ($P > 0.05$) (Table 2).

Serum IgA & IgG levels from day 1 to day 90, showed statistically insignificant increase ($P > 0.05$) in group B (IPPSA) and group C (EPPSA), while in group A (total splenectomy), there was statistically significant increase ($P < 0.05$) (Table 3 & 4).

Ultrasonography with duplex scanning in group B (IPPSA) and C (EPPSA) showed that the size of the splenic slices did not change along the 90 days of the study, with detection of the arterial flow and color signals along the splenic slices, also venous flow was detected from the periphery of the slices after 90 days (Fig. 7).

In the experimental Part, the red blood cells, white blood cells and haemoglobin level showed insignificant changes ($P > 0.05$) in the studied groups between the samples studied on day 1 and day 30. The platelets count among group A (total splenectomy) showed that there is a significant statistical decrease ($P < 0.05$) in the day 30 than in day 1 and the platelets count in groups B (IPPSA), C (EPPSA) and D (IMPSA) showed insignificant statistical changes ($P > 0.05$) between the samples studied on day 1 and day 30.

Notched RBCs count from day 1 to day 30, showed statistically significant increase ($P < 0.05$) in group A(total splenectomy). While in groups B (IPPSA), C (EPPSA) and D (IMPSA), there was statistically insignificant increase ($P > 0.05$) (Table 5).

Serum IgM levels from day 1 to day 30, showed statistically significant decrease ($P < 0.05$) in group A (total splenectomy) and insignificant decrease ($P > 0.05$) in all other groups (Table 6).

Serum IgA & IgG levels from day 1 to day 30 showed, statistically significant increase ($P < 0.05$) in group A (total splenectomy) and insignificant increase ($P > 0.05$) in the other groups (Table 7 & 8).

Histological study of autotransplanted slices in groups B (IPPSA), C (EPPSA) and D (IMPSA) showed that there was preservation of the splenic architecture, the viability of the lymphoid follicles and patency of the blood vessels after 30 days of autotransplantation (Fig. 7&8).

Table (1)*: Notched erythrocytes levels in different groups of applied study*

	GROUP (A)		*GROUP (B)*		*GROUP (C)*	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	1.40	0.51±	1.33	0.49±	1.53	0.52±
Day 30	*7.87	1.39±	1.73	0.39±	1.73	0.90±
Day 90	*18.73	0.80±	1.97	1.13±	2.04	0.98±

*P less than 0.05 when compared to values at day 1.

Table (2): *IgM (mg/dl) levels in different groups of applied study*

	GROUP (A)		GROUP (B)		GROUP (C)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	164.46	26.9±	166.8	32.7±	153.86	33.7±
Day 30	*152.6	28±	162.46	33.4±	149.86	32.8±
Day 90	*119.4	16.8±	158.6	34.3±	146.53	34.1±

*P less than 0.05 when compared to values at day 1.

Table (3): *IgA (mg/dl) levels in different groups of applied study*

	GROUP (A)		GROUP (B)		GROUP (C)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	199.33	43.4±	190	64.8±	196.66	59.3±
Day 30	*222	46.3±	192.66	73.2±	202.66	66.8±
Day 90	*251.33	43.5±	195.33	68.2±	212	64.2±

*P less than 0.05 when compared to values at day 1.

Table (4): *IgG (mg/dl) levels in different groups of applied study*

	GROUP (A)		GROUP (B)		GROUP (C)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	1258.6	378±	1225.3	478±	1200	536±
Day 30	*1393.3	387±	1235.3	456±	1212.6	479±
Day 90	*1546	416±	1246.6	440±	1226	520±

*P less than 0.05 when compared to values at day 1.

Table (5): *Notched erythrocytes levels in different groups of rabbits*

	GROUP (A)		GROUP (B)		GROUP (C)		GROUP (D)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	1.36	1.48±	1.76	1.09±	1.11	0.81±	1.94	1.12±
Day 30	*12.13	1.04±	1.81	0.87±	1.45	0.59±	2.03	0.87±

*P less than 0.05 when compared to values at day 1.

Table (6): *IgM (mg/dl) levels in different groups of rabbits*

	GROUP (A)		GROUP (B)		GROUP (C)		GROUP (D)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	163.73	30.2±	161.4	29.9±	161	31.8±	163.86	29.8±
Day 30	*142.66	31.9±	159.93	31.3±	157.46	30.1±	159.6	31.3±

*P less than 0.05 when compared to values at day 1

Table (7): *IgA (mg/dl) levels in different groups of rabbits*

	GROUP (A)		GROUP (B)		GROUP (C)		GROUP (D)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	192	31.8±	194.66	33.6±	188.66	31.4±	186.66	31.8±
Day 30	*221.33	30.2±	196	28.2±	192	32.7±	192	31.7±

*P less than 0 05.when compared to values at day 1.

(Table 8): *IgG (mg/dl) levels in different groups of rabbits*

	GROUP (A)		GROUP (B)		GROUP (C)		GROUP (D)	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Day 1	1230.6	422±	1204	437±	1226.6	391±	1212.6	446±
Day 30	*1331.3	399±	1220	452±	1244	418±	1215.3	458±

*P less than 0.05 when compared to values at day 1.

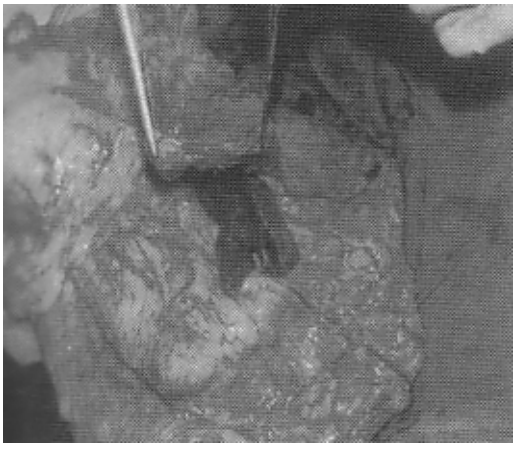

Fig. (1): folding the greater omentum on the splenic slice to perform omental pouch.

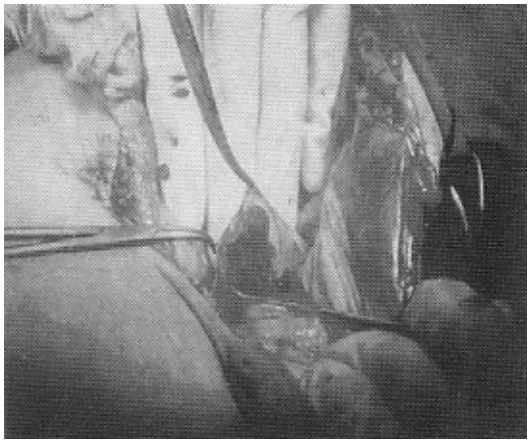

Fig. (2): Implantation of the splenic slice in the extraperitoneal space before closure of the pocket.

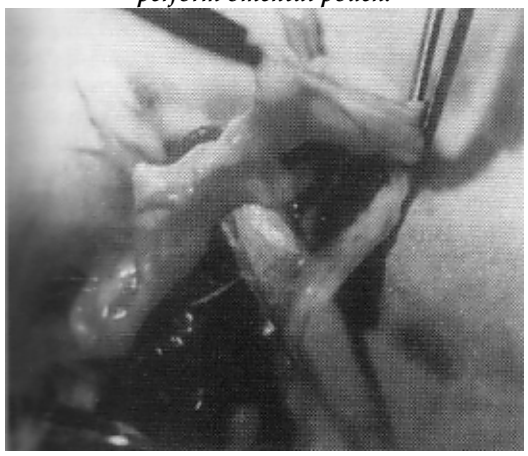

Fig. (3): Folding the greater omentum on the splenic slice to perform omental pouch (experimental group).

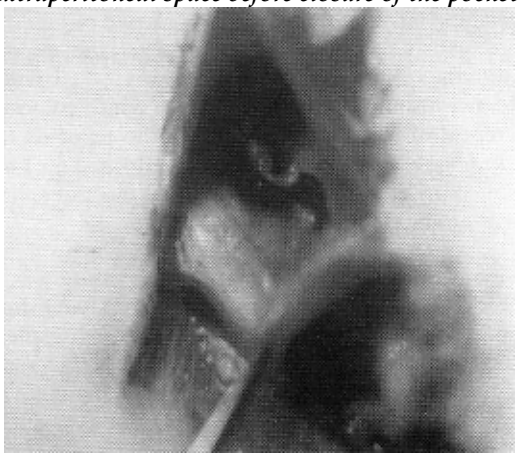

Fig. (4): Implantation of the splenic slice in the extraperitoneal space (experimental group).

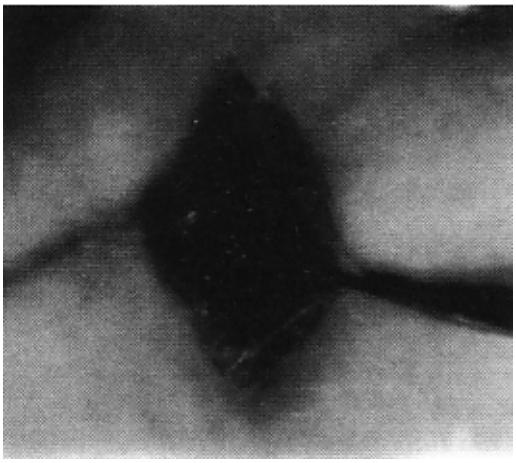

Fig. (5): Implantation of the splenic slice in the thigh pocket (experimental group).

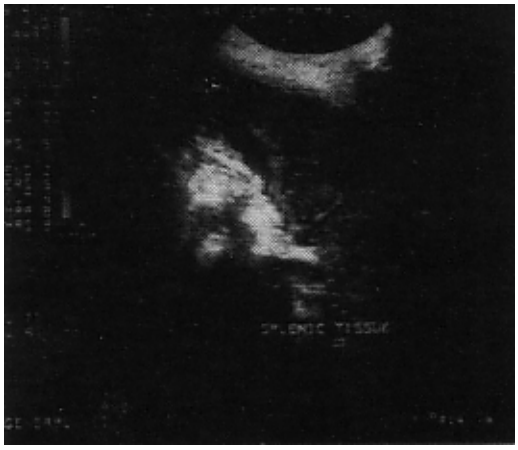

Fig. (6): Ultrasonography with duplex scanning for patient in group B.

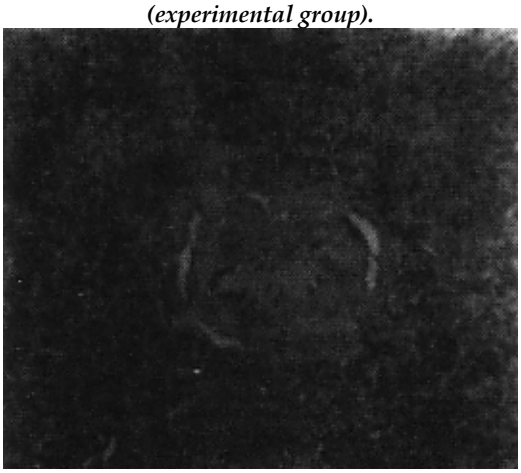

Fig. (7): Histopathological picture of autotransplanted splenic slices in group C rabbits.

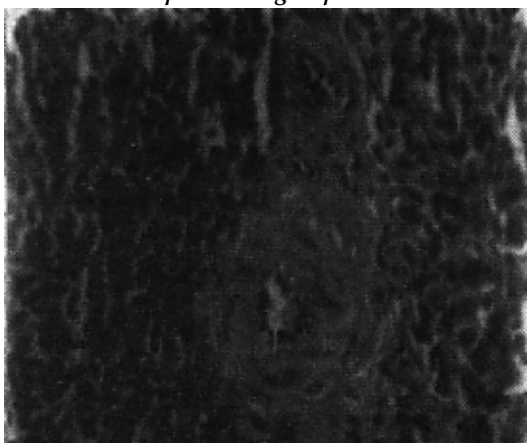

Fig. (8): Histopathological picture of autotransplanted splenic slices in group D rabbits.

DISCUSSION

Despite the fact that this study was carried out on little number of patients with splenic injury yet the male to female ratio among the studied patients was 2.8 : 1 with a peak incidence around the age of 21years, which coincided with the report of Richardson et al., [7] who reported that the male to female ratio ranging from 2 : 1 to 3.6 : 1 and the work of Pearl et al., [8] who found that most of splenic injuries was around the age of 20 years.

The main cause of splenic injury in our study was by motor vehicle (88.9%), falling from height (8.9%) and direct stab wound (2.2%). In a study reported 413 cases of splenic trauma, 52% were due to motor vehicle accidents, 22.5% due to falling from height, 16.5% due to direct blow to the abdomen, 5% due to stab wound trauma and 4% due to iatrogenic operative injury [9].

The mean operative time in our applied study was 43 minutes in group A (total splenectomy) which was 8 minutes less than the mean operative time in group B (IPPSA) and 9 minutes less than the mean operative time in group C (EPPSA). So, no significant difference was noted in operative time between performing splenectomy alone or performing splenectomy with any type of PSA in both applied and experimental studies. In a study on 148 patients, reported that the difference in the mean operative time did not exceed 12 minutes in comparison between the IPPSA and EPPSA [10].

In our study, the red blood cell count, white blood cell count and haemoglobin level showed no statistically significant changes in the studied groups (experimental and applied). There was significant decrease in platelets one month after the operation in totally splenectomized patients and rabbits, while there was insignificant changes in the

platelets count in other groups with PSA. This could be explained by the release of collagen and adenosine diphosphate from the red pulp of the autotransplanted splenic tissue that helps the formation of platelets again. This explanation was suggested by Shokouh et al., [11] who found the same results in 44 patients divided into two groups, underwent either splenectomy alone or splenectomy with IPPSA.

In our study the changes of the pocked cell (notched RBCs) count were statistically significant in group A (total splenectomy) when compared with the other groups either in the applied study or in the experimental study ($P < 0.05$). There is marked increase in group A (total splenectomy) at the day 90 in applied study and at the day 30 in the experimental study. These findings coincided with the results obtained by Shokouh et al. [11] who attributed these changes to the absence of splenic tissue necessary to remove the circulating pocked cell. Some authors concluded that the pocked cell count may be helpful as a screening test, but it reflects a nonspecific function of the spleen, and should be used as a diagnostic test for evaluation of the amount of autotransplanted splenic tissue only [12-14]. Another study found that the pocked cells start its appearance in the blood of the patients immediately one day after splenectomy and become significantly increased at the day 14 and persist for years [15].

In our study, it was noticed that, in group A (total splenectomy) in both applied and experimental studies, there was gradual decrease in IgM level till it reached a significant reduction at the end of the study, while there was no significant change in IgM levels in the other groups. There were gradual increases in IgA and IgG levels till they reached to a significant increase at the end of the study in group A (total splenectomy), applied and experimental, while there was no significant changes in IgA and IgG levels in the other groups. This could be explained by the protective effect of the autotransplanted splenic tissue in groups B (IPPSA), C (EPPSA) & D (IMPSA) which preserved the immunological function of the native spleen. Immunological changes occurred after splenectomy and PSA in our study are coincided with what obtained by other series[2, 16, 17].

Keron[18] and Livingstone[19] suggested that splenosis resulting from intraperitoneal implantation of ruptured splenic tissue occurs at a significant rate, however the mass of the implanted splenic tissue (splenosis) is probably too small to restore complete splenic functions. This splenosis is responsible for insignificant variation in the immunoglobulin levels in the different literature [2,16,17] which had studied the immunological changes in the group that underwent splenectomy because of trauma.

Anderson[12] reported that IgM levels increased in the splenectomized group with fever postoperatively, while patients with an uncomplicated postoperative course, showed a decrease in IgM immediately after splenectomy, but one year later, all patients had lower IgM concentration than before splenectomy. An increase in the serum IgA concentration was seen immediately after splenectomy and one year later, the average serum IgA concentration was significantly higher than before the operation. An experimental work studied PSA in different locations in 286 pigs, reported that the IgM, IgA and IgG showed no significant changes among the IPPSA, IMPSA, subcutaneous or transhepatic partial splenic autotransplantation after nine months follow up [20] which is coincided with our results.

In our study transverse slices from the removed spleen, each 5 millimeters in thickness, weighing approximately one third of normal spleen were quite enough to maintain most of the normal splenic functions. Other studies concluded that more than 25% of the normal spleen must be preserved in traumatic rupture of the spleen by PSA to maintain normal immunological functions of the spleen [13&14]. Mizrahi et al., 21 found that 50 gm. of splenic tissue were quite enough to compensate the removal of the spleen in their study on 10 adult patients where splenectomy with IPPSA was done.

Ultrasonography with duplex scanning was done in our study to evaluate the vascularity and the size of the splenic slices in groups B (IPPSA) and C (EPPSA) of the applied study which showed that the vascularity was seen along the slices and the size of the splenic slices did not change in these sites along the time of study (2 years). Cahill[22] found that the vascularity of the autotransplanted splenic slices started to appear in the periphery in the first week and cover the entire slice within 10 days postoperatively. This study was done on 30 patients who underwent IPPSA.

Leemans et al., [23] performed PSA in 134 rats and found that the splenic slices were uptaken successfully with good blood supply after one month from the operation. Yamataka et al., [24] were in agreement with our results when they performed PSA in neonates, and found that the splenic slices were uptaken successfully after 3 weeks of the operation.

Romball,[25] found that the autotransplanted splenic slices completely disappeared after two months in 48 patients. The thickness of his slices was 10 millimeters, which was too thick to regain their vascularity by direct diffusion.

Histological study of the autotransplanted splenic slices in the experimental groups showed that the architecture is preserved with the white pulp formed of lymphoid follicles and the red pulp formed of patent blood

vessels in all groups.

In 45 rats re-explored after 15 days of PSA, Moxon and Schwartz [26] found that the remnants of the splenic slices were completely necrotic without blood supply in 33 rats, and this necrosis of the slices could be explained by the failure of thick slices taken to regain their vascularity by direct diffusion.

CONCLUSION

Dealing with extensive splenic trauma, the surgeon should remember splenic autotransplantation, to preserve the immunological splenic functions if other methods of splenic preservation could not be performed.

The technique of splenic autotransplantation is simple and does not require special skills and a safe method in adults and children.

Splenic slices equal to one third of normal splenic tissue are quite enough for autotransplantation to preserve most of splenic functions.

Autotransplanted splenic slices 5 millimeters in thickness are optimum to obtain their vascularity from the surrounding tissues by diffusion.

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