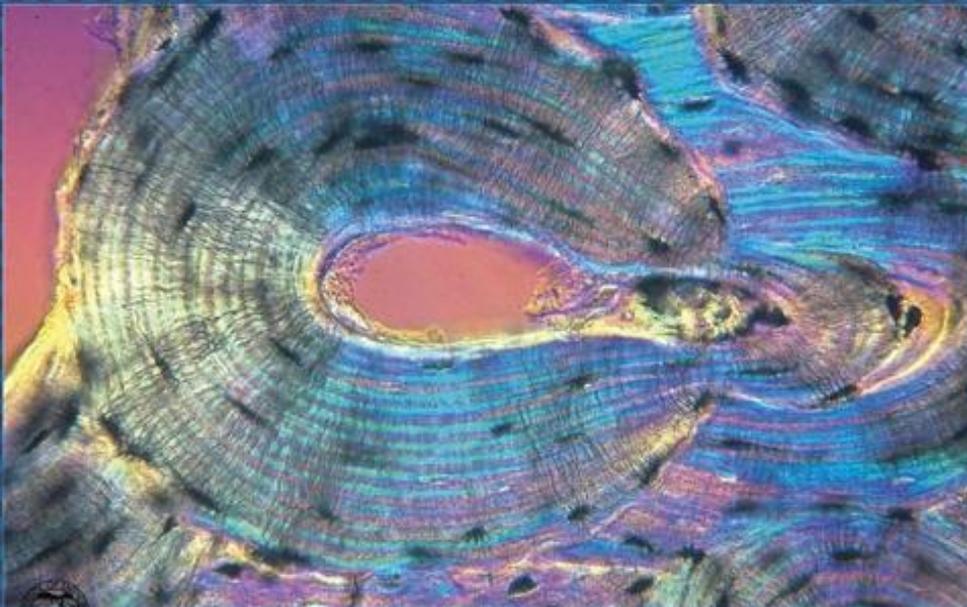




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The Early Changes in Splenic Lymphocyte and Macrophage Populations Following Major Liver Resection in Rats.

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ABSTRACT

Background and aim of the work: Splenic hypertrophy occur after major hepatectomy (HTX). The spleen was suggested to inhibit hepatocyte proliferation and consequently, splenectomy was considered. The present study aims to examine the influence of 70% partial hepatectomy (HTX) on the splenic histological structure, T, B lymphocyte and macrophage populations and to discuss the functional correlation. **Methods:** The rats were assigned to two groups; Sham group, and 70% Partial hepatectomy (HTX) group; which was further subdivided into 2 subgroups; sacrificed 24 or 48h, after HTX; Groups (HTX 24h) and (HTX 48h); respectively. H & E as a routine stain, iron staining by Prussian blue, as well as immunohistochemical detection of splenic CD3; a marker for T lymphocyte; CD20; a marker for B lymphocyte and CD68; a marker for macrophage; were done. **Results:** HTX 24h and HTX 48h groups exhibited enlargement of splenic follicles, no expansion of red pulp, no increase in apoptosis, mild increase in the number of melanomacrophages. CD3 expression increased significantly in HTX groups as compared to Sham group. However, CD3 expression in HTX 24h and HTX 48h groups exhibited insignificant difference. CD20 expression showed no significant difference among the studied groups. CD68 expression and Prussian blue staining showed a non-significant increase in HTX 24h group and significant increase in HTX 48h group. **Conclusion:** During the first two days after HTX, there was a rapid increase in splenic T- but not B-lymphocytes with subsequent increase in splenic macrophages. The splenic changes may explain a role of in liver regeneration.

INTRODUCTION

Crosstalk between liver and spleen in anatomical and physiological terms is clear in various liver diseases (hepatitis, liver fibrosis). However, the pathways underlying this communication, still need more elaboration, e.g. the effect of spleen on the normal liver hasn't been studied until recently, Elchaninov *et al.* (2020) found that splenectomized rats exhibited increased expression for hepatocytic Ki67; a proliferative marker, at 24 h after splenectomy, the finding interpreted by the fact that the spleen is a chief producer of Transforming Growth factor b1 (TGFb1) a major hepatocyte growth inhibitor; meaning that the spleen is an inhibitor of hepatocyte proliferation even in the basic hepatic state.

The spleen is a large lymphoid organ with a fundamental role in modulating the immune system, differentiation and activation of T and B cells, destruction of worn-out red blood cells and clearance of the circulating apoptotic cells. Splenic macrophages with the endothelium of marginal sinus, form a blood-spleen barrier. The interactions, between the blood macrophages and lymphocytes, are responsible for the regulation of the lymphocyte entry into white pulp (Torimura, 2016).

Splenic hypertrophy usually occurs after major hepatectomy (HTX). It was thought that this was due to the reduced portal bed and hepatic outflow. However, the hypertrophy of both spleen and liver exhibited linear correlation suggesting other underlying mechanisms for splenic hypertrophy after HTX e.g. the effect of hepatic growth factors on spleen or the reorganization of reticuloendothelial cells (Petrovai *et al.*, 2013).

Chemokine activity-related genes and immediate early response genes were upregulated in the spleen after HTX. Therefore, spleen was suggested to have a harmful impact on the liver during the post-HTX phase (Arakawa *et al.*, 2014). Moreover, the spleen was found to display a suppressor effect on hepatic regeneration through upregulation of TGF- β 1 and its receptor RII with downregulation of Hepatocyte Growth factor (HGF) and its receptor; c-Met in the liver. Consequently, splenectomy was suggested as a therapeutic choice for successful hepatic regeneration (Lee *et al.*, 2015). Furthermore, a significantly high preoperative volume of spleen volume/ surface area of the body was suggested to be a strong predictor of post-hepatectomy liver failure (Bae *et al.*, 2020).

Hypothesis:

The aim of current study is to examine the influence of 70% partial hepatectomy (HTX) on the splenic T, B

lymphocyte and macrophage populations by evaluation of the immunohistochemical reaction for CD3, CD20 and CD68, during the first 2 days after HTX and to discuss the functional correlation between this immune response and the liver status and regeneration in the post-HTX phase.

MATERIALS AND METHODS

Research Design:

Twenty-two male Sprague-Dawley rats (weighing 250-300 gm) were used in the present study. The rats were obtained from Urology Centre Mansoura University where housing and experimental surgical procedures were performed. The experimental design, used in the current study, was approved by the institutional research board (IRB) of the Faculty of Medicine, Mansoura University, Egypt (proposal code R.21.03.1234). The rats were housed in cages with firm day/night cycles, humidity and temperature, under aseptic conditions with *ad libitum* free access to water and food. Two weeks prior to the start of the experiment, the animals were housed for acclimatization. The animals were then assigned randomly to three groups; Group 1 (Sham; n=6), group 2 (HTX 24h; n=6): sacrificed 24 h after 70% HTX and group 3 (HTX 48h; n=6): sacrificed 48 h after 70% HTX (as four rats died during hepatectomy). At the assigned time, the animals were sacrificed through cervical dislocation, followed by abdominal dissection with rapid harvesting of the spleens.

Surgical Procedures:

Rats were anesthetized by intraperitoneal injection of 75 mg/kg ketamine and 5 mg/kg xylazine. After midline laparotomy, the left and median lobes of the liver of HTX groups were ligated with 3/0 vicryle sutures and resected according to Higgins and Anderson's method (Glanemann *et al.*, 2009) to achieve 70% liver resection. The rats of Sham group underwent only midline laparotomy. The abdomen for all rats

was closed with continuous stitches using a 3/0 vicryle and cleaned with povidone iodine.

Histopathology:

A portion from the splenic tissue was fixed by formaldehyde (10%) then kept in paraffin (Bisen, 2014), to assess the histological changes. Consequently, 7 μ m thick splenic tissue sections were stained by hematoxylin and eosin (H&E) as a routine stain, meanwhile the other sections were stained by Prussian blue to evaluate iron deposition. The slides were examined with Olympus Microscope and SC100 camera.

Immunohistochemical Techniques:

Splenic tissue sections (3 μ m thick) were processed for immunostaining according to the method, described by Elsayed *et al.*, (2021a). The splenic Sections were kept with the primary rabbit polyclonal antibody for CD3 (Genemed 61-0011; 1:100 dilution), mouse monoclonal antibody for CD20 (Genemed 60-0010; 1:100 dilution) and rabbit monoclonal antibody for CD68 (Genemed 61-0184; 1:100 dilution), at 4°C, overnight. Consequently, the slides were kept for 30 min with the mouse-rabbit polydetector (BSB 0268, Bioscience). The slides were then washed through PBS to evaluate the binding of the primary antibody. Lastly, washing, dehydration and examination of the slides were done followed by their examination under a light microscope (Ramos-Vara and Miller, 2014).

Morphometric Analysis of Histopathological and Immunohistochemical Results:

Morphometric analysis was performed utilizing ImageJ and Fiji ImageJ programs (Schneider *et al.*, 2012 & Schindelin *et al.*, 2012). We measured the area percentage of Prussian Blue, CD3, CD20 and CD68 positive (in x100). The morphometric analysis was done on random non-overlapping fields from the splenic tissues.

Statistical Evaluation:

Data were submitted to tabulation and analysis utilizing SPSS program (V. 25). Quantitative normally distributed data were tabulated as means \pm standard error. One-Way ANOVA and Post-hoc LSD tests were used to compare the data. When the p-value is ≤ 0.050 , the results were considered as significant.

RESULTS

Effect of 70% PH on Histopathological Structure of the Spleen:

The spleens of Sham group demonstrated a normal structure of white pulp, splenic follicles, red pulp and blood sinusoids (Figs. 1A, 2A). Additionally, HTX 24h group exhibited an enlargement of splenic follicles of the white pulp, no increase in apoptotic cells, no congestion or expansion of red pulp, normal appearance of sinusoids with no dilatation or congestion, mild increase in haemosiderin deposition and in the number of melanomacrophages, and no change in the number or size of megakaryocytes (Figs 1B, 2B). Moreover, HTX 48h group exhibited marked enlargement of splenic follicles, no increase in apoptotic cells, no congestion or expansion of red pulp, normal appearance of sinusoids with no dilatation or congestion, a moderate increase in haemosiderin deposition and in the number of melanomacrophages, and no change in the number or size of megakaryocytes (Figs. 1C and 2C). Prussian blue-stained sections exhibited moderate reaction for iron in the Sham group (Figure 3A), and in HTX 24h group (Figure 3B) with a strong reaction in HTX 48h group (Figure 3C).

Results of The Immunohistochemical Study:

Splenic tissues of Sham group showed moderate immunoreactivity for CD68, CD3 and CD20 (Figs. 4A, 5A and 6A). Noticeably, spleens of HTX 24h group showed an increased CD3 immunoreactivity with moderate immunoreactivity for CD20 and CD68 (Figs. 4B, 5B and 6B). Moreover, HTX 48h group exhibited strong

immunoexpression for CD3 and CD68 with moderate immunoreactivity for CD20 (Figs. 4C, 5C and 6C).

Results of The Morphometric Evaluation of Percentages for Prussian Blue, CD3, CD20 and CD68 Stained Areas:

Percentage of Prussian blue, CD3, CD20 and CD68 reactive areas, exhibited a significant difference among all groups ($p < 0.0005$), while CD20 expression exhibited insignificant difference among the groups ($P = 0.232$). Post-hoc tests demonstrated a

significantly increased reaction of CD3 and insignificantly increased reaction for CD20, CD68 and Prussian blue in HTX 24h group as compared to the Sham group. Moreover, there was a significant elevation in Prussian blue, CD68, CD3 and an insignificant increase in CD20 positive areas in HTX 48h as compared to the Sham group. Furthermore, there was a significant elevation in Prussian blue, CD68 but not in CD3 and CD20 positive area in HTX 48h group when compared to HTX 24h group (Table1 and Fig. 7).

Table 1: Results of morphometric analysis of immunohistochemical results

Parameter	Group			F value	P value
	Sham (n=6)	HTX 24h (n=6)	HTX 48h (n=6)		
Prussian Blue stained area percentage	2.99 ± 0.21 A	3.80 ± 0.40 A	6.01 ± 0.44 B	18.234	<0.0005
CD3 immunopositive area percentage	19.01 ± 0.94 A	28.33 ± 1.22 B	30.09 ± 1.35 B	25.286	<0.0005
CD20 immunopositive area percentage	13.60 ± 0.54 A	14.23 ± 0.62 A	14.89 ± 0.41 A	1.498	0.232
CD68 immunopositive area percentage	7.80 ± 0.30 A	8.57 ± 0.36 A	10.95 ± 0.60 B	13.977	<0.0005

Results are tabulated as mean ± Standard error. (different letters = significant different). Significant p values (≤ 0.05).

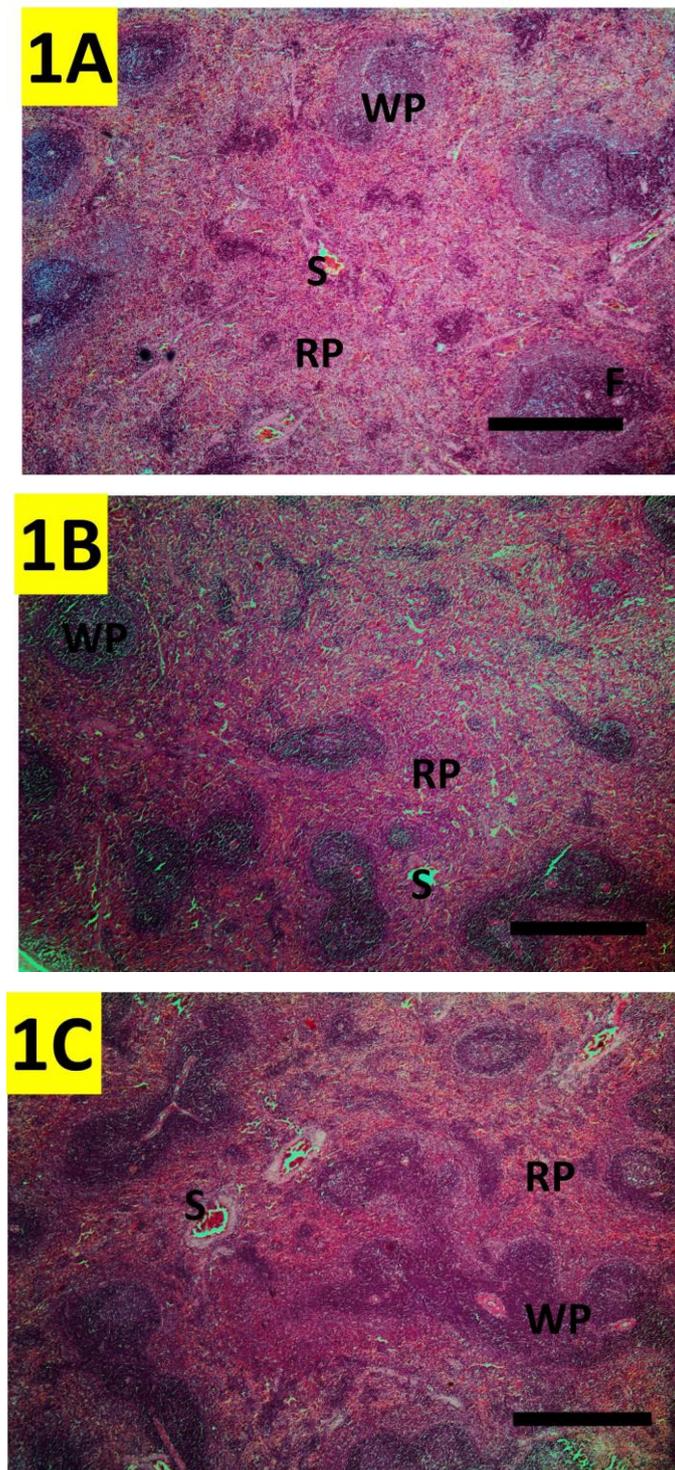


Fig. 1A: A photomicrograph of splenic tissue of sham group demonstrating normal splenic architecture with a normal white pulp (WP), containing follicles (F) and red pulp (RP) containing blood sinusoids (S). H&E X40. Scale bar: 250 μ m.

1B: A photomicrograph of splenic tissue of HTX 24h group demonstrating marked enlargement of splenic follicles of the white pulp (WP), no expansion or congestion of the red pulp (RP) and normal appearance of sinusoids (S) with no dilatation or congestion. HTX 24h: sacrificed 24h after partial hepatectomy. H&E X40. Scale bar: 250 μ m.

1C: A photomicrograph of splenic tissue of HTX 48h group demonstrating marked enlargement of splenic follicles of the white pulp (WP), no expansion or congestion of the red pulp (RP) and normal appearance of sinusoids (S) with no dilatation or congestion. HTX 48h: sacrificed 24h after partial hepatectomy. H&E X40. Scale bar: 250 μ m.

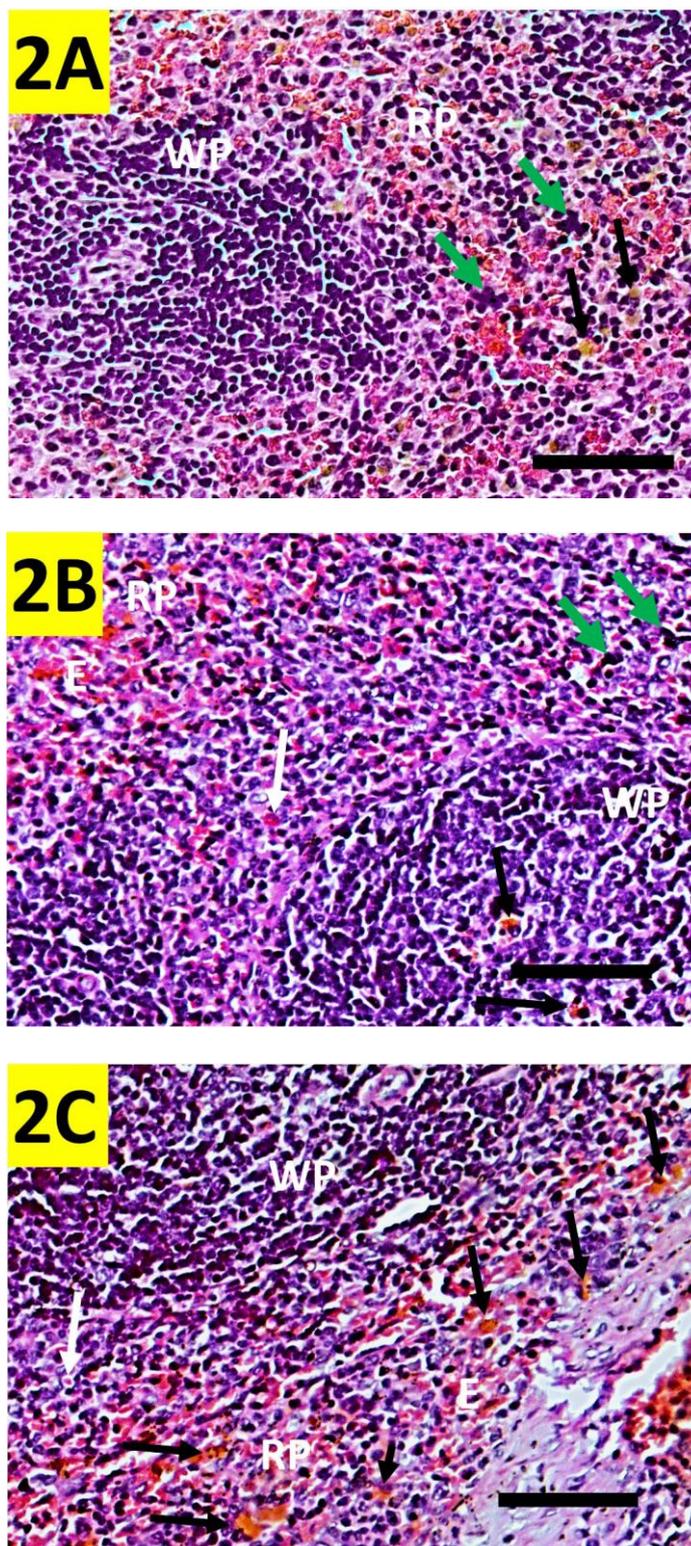


Fig. 2A: A photomicrograph of splenic tissue of sham group demonstrating normal splenic architecture; a normal white pulp (WP) containing follicles of lymphocytes, and red pulp (RP) containing blood sinusoids. Few megakaryocytes (Green arrows) and melanomacrophages (Black arrows) are seen. H&E X200. Scale bar: 50 μm.

2B: A photomicrograph of splenic tissue of HTX 24h group demonstrating few apoptotic bodies (White arrow), few megakaryocytes (Green arrows) and few melanomacrophages with haemosiderin deposition (Black arrows). RP: red pulp, WP: white pulp. HTX 24h: sacrificed 24h after partial hepatectomy. H&E X200. Scale bar: 50 μm.

2C: A photomicrograph of splenic tissue of HTX 48h group demonstrating few apoptotic bodies (White arrow), few megakaryocytes (Green arrows) with a large number of melanomacrophages with haemosiderin deposition (Black arrows). RP: red pulp, WP: white pulp. HTX 48h: sacrificed 48h after partial hepatectomy. H&E X200. Scale bar: 50 μm.

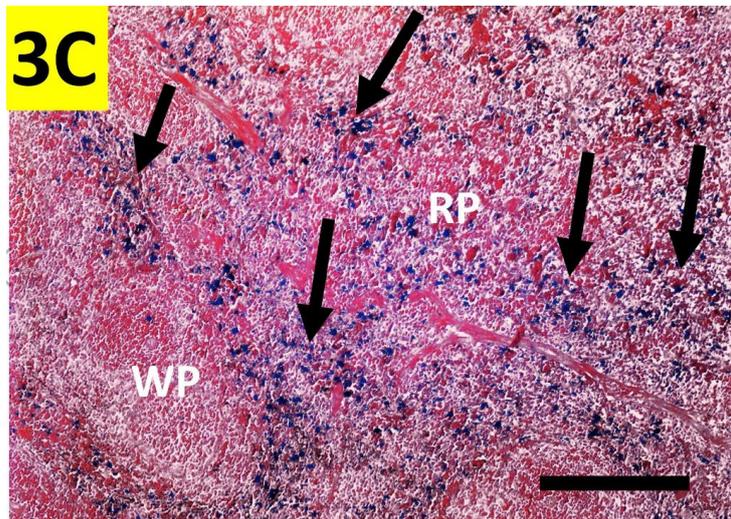
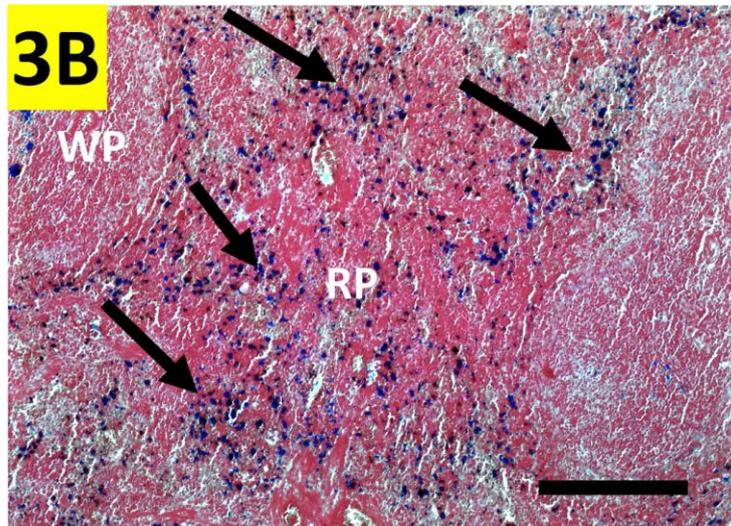
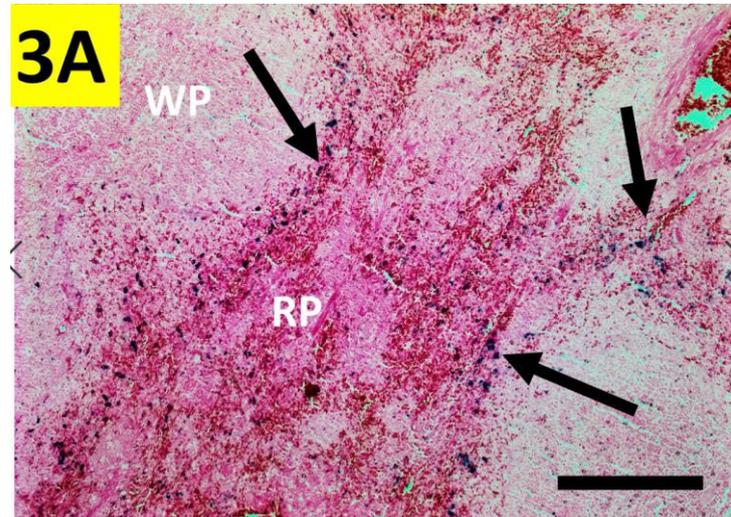


Fig. 3A: A photomicrograph of splenic tissue of sham group demonstrating moderate reaction for iron (Arrows). RP: red pulp, WP: white pulp. Prussian Blue X200. Scale bar: 50 um.

3B: A photomicrograph of splenic tissue of HTX 24h group demonstrating moderate reaction for iron (Arrows). HTX 24h: sacrificed 24h after partial hepatectomy. RP: red pulp, WP: white pulp. Prussian Blue X200. Scale bar: 50 um.

3C: A photomicrograph of splenic tissue of HTX 48h group demonstrating strong reaction for iron (Arrows). HTX 48h: sacrificed 48h after partial hepatectomy. RP: red pulp, WP: white pulp. Prussian Blue X200. Scale bar: 50 um.

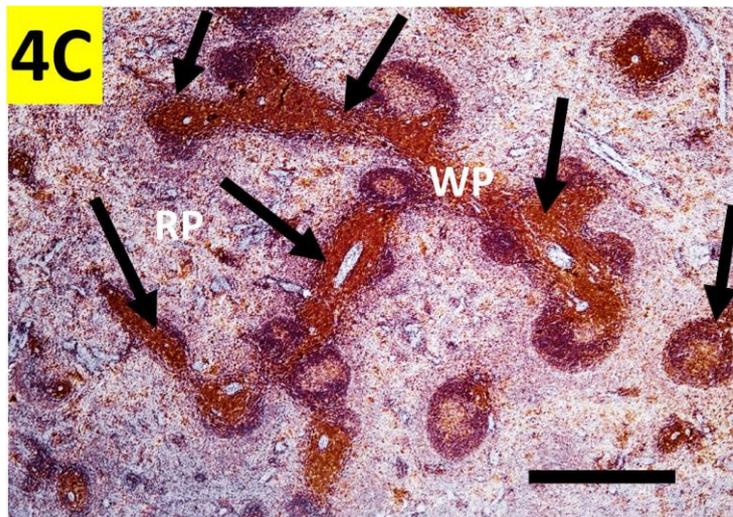
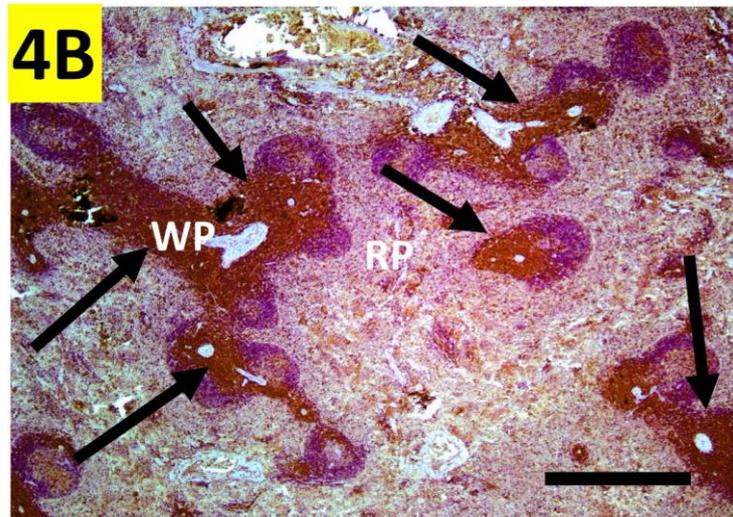
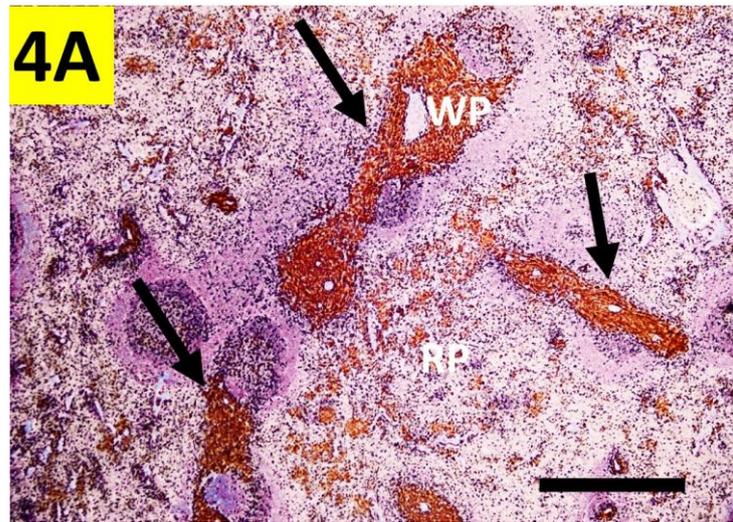


Fig. 4A: A photomicrograph of splenic tissue of sham group demonstrating moderate expression for CD3 (Arrows). RP: red pulp, WP: white pulp. Immunohistochemistry for CD3. X40. Scale bar: 250 um.

4B: A photomicrograph of splenic tissue of HTX 24h group demonstrating strong expression for CD3 (Arrows). RP: red pulp, WP: white pulp. HTX 24h: sacrificed 24h after partial hepatectomy. Immunohistochemistry for CD3. X40. Scale bar: 250 um.

4C: A photomicrograph of splenic tissue of HTX 48h group demonstrating strong expression for CD3 (Arrows). RP: red pulp, WP: white pulp. HTX 48h: sacrificed 48h after partial hepatectomy. Immunohistochemistry for CD3. X40. Scale bar: 250 um.

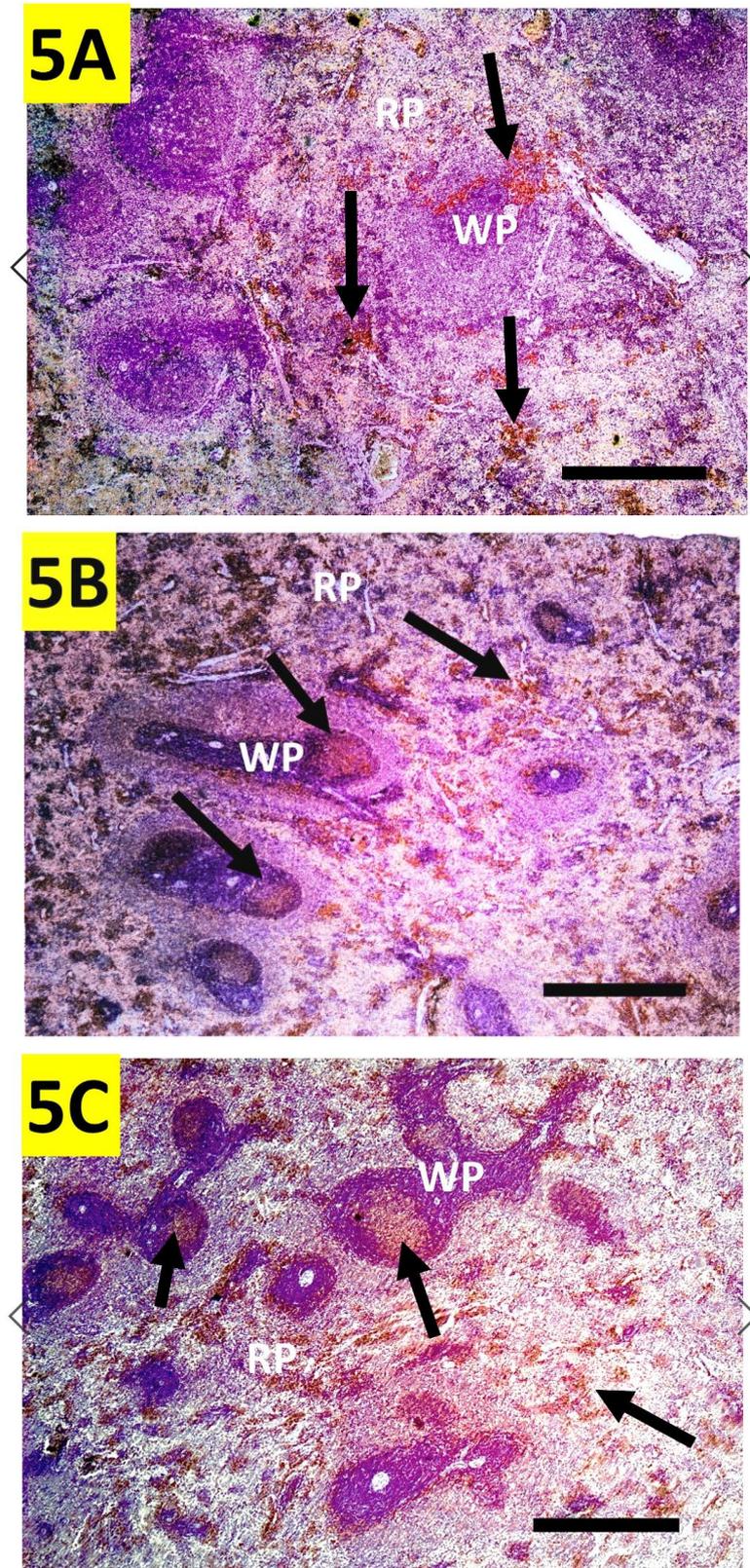


Fig. 5A: A photomicrograph of splenic tissue of sham group demonstrating moderate expression for CD20 (Arrows). RP: red pulp, WP: white pulp. Immunohistochemistry for CD20. X40. Scale bar: 250 um.

5B: A photomicrograph of splenic tissue of HTX 24h group demonstrating moderate expression for CD20 (Arrows). RP: red pulp, WP: white pulp. HTX 24h: sacrificed 24h after partial hepatectomy. Immunohistochemistry for CD20. X40. Scale bar: 250 um.

5C: A photomicrograph of splenic tissue of HTX 48h group demonstrating moderate expression for CD20 (Arrows). RP: red pulp, WP: white pulp. HTX 48h: sacrificed 48h after partial hepatectomy. Immunohistochemistry for CD20. X40. Scale bar: 250 um.

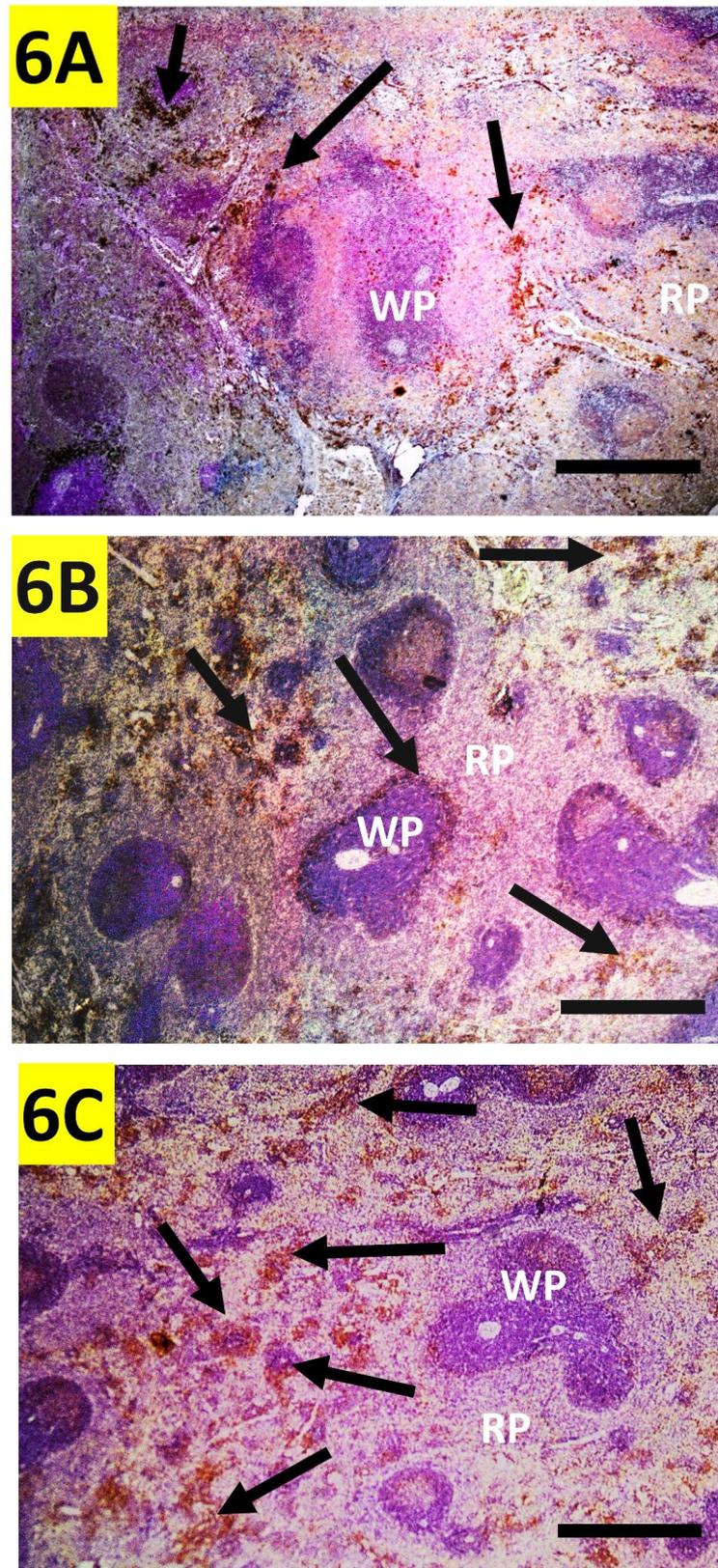


Fig. 6A: A photomicrograph of splenic tissue of sham group demonstrating moderate expression for CD68 (Arrows). RP: red pulp, WP: white pulp. Immunohistochemistry for CD68. X40. Scale bar: 250 μm.

6B: A photomicrograph of splenic tissue of HTX 24h group demonstrating moderate expression for CD68 (Arrows). RP: red pulp, WP: white pulp. HTX 24h: sacrificed 24h after partial hepatectomy. Immunohistochemistry for CD68. X40. Scale bar: 250 μm.

6C: A photomicrograph of splenic tissue of HTX 48h group demonstrating strong expression for CD68 (Arrows). RP: red pulp, WP: white pulp. HTX 48h: sacrificed 48h after partial hepatectomy. Immunohistochemistry for CD68. X40. Scale bar: 250 μm.

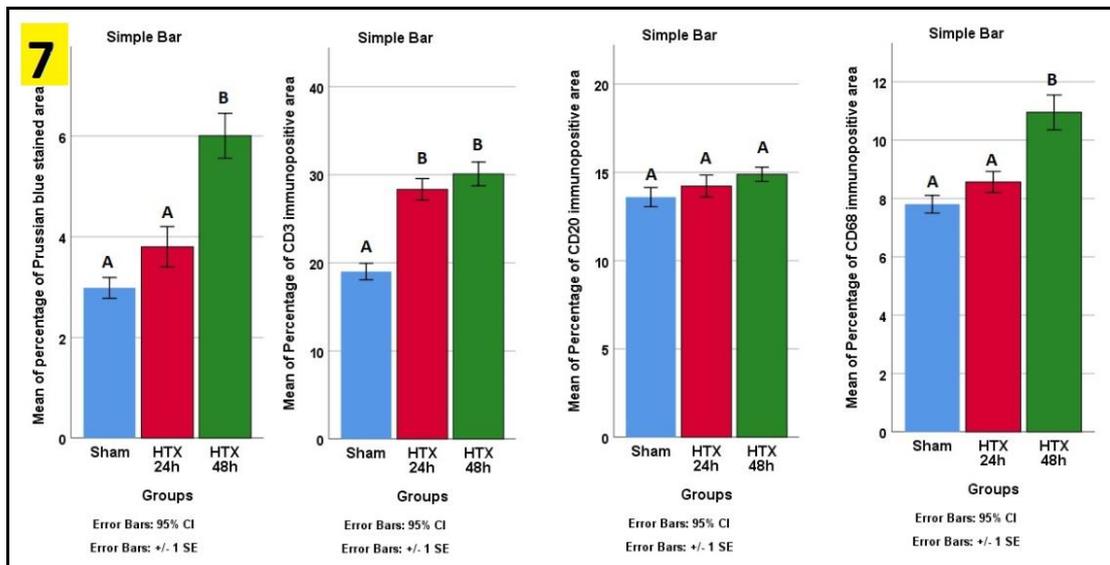


Fig. 7: Morphometric analysis of the mean percentage (%) of Prussian blue, CD3, CD20 and CD68 positive area in the studied groups (A, B, C and D, respectively). Results are mentioned as mean \pm standard error. Different letters = significant different (P value ≤ 0.05). HTX 24h: sacrificed 24h after partial hepatectomy. HTX 48h: sacrificed 48h after partial hepatectomy.

DISCUSSION

The aim of the current study was to examine the influence of 70% partial hepatectomy (HTX) on the splenic histological structure, T, B lymphocyte and macrophage populations 24h and 48h after HTX in male SD rats through investigating CD3, CD20 and CD68 expressions, respectively, and to discuss the functional correlation between the splenic structural alteration and the liver status in the post-HTX phase.

The sham group showed normal splenic architecture; white and red pulps, with moderate staining for iron, CD3, CD20 and CD68 (Elsayed *et al.*, 2021b).

Hepatectomy increased the expression of CD3 (The total T-lymphocytes marker) in both HTX 24h and HTX 48h groups in the splenic white pulp, and this may indicate the regulatory role of T-lymphocytes on liver regeneration after hepatectomy in accordance with the reports indicating that T lymphocytes extracted from the splenic tissues of the mice after HTX, enhanced the proliferative activity of hepatocytes and Kupffer cells in livers of non-operated recipients (Babaeva *et al.*, 1980), moreover, mice having

deficiency of T cells exhibit a reduction in hepatic regeneration after HTX. Furthermore, surface lymphotoxins, secreted by T cells, are significant for hepatic regeneration. The mice with specific deficiency of these lymphotoxins exhibited severe hepatic damage and decreased ability to synthesize DNA following HTX, meaning that the adaptive immune system can regulate hepatic regeneration directly through T cell-lymphotoxins (Tumanov *et al.*, 2009), furthermore, IL-17A derived from splenic T lymphocytes was found to be an enhancer of liver regeneration after HTX (Furuya *et al.*, 2013).

Hepatectomy didn't cause a significant change in CD20 expression (The B-lymphocytes marker) either in HTX 24h or in HTX 48h group in the splenic white pulp and this may indicate no role of B-lymphocytes in the early phase of hepatic regeneration following HTX in accordance with the reports indicating that the B lymphocytes extracted from the splenic tissues of mice after HTX, didn't show any enhancing effect on the mitotic activity of hepatocytes and Kupffer cells when compared to B lymphocytes from non-operated mice (Babaeva *et al.*, 1980).

Hepatectomy increased the expression of CD68 (The macrophage marker) in HTX 48h but not in HTX 24h and this may indicate the regulatory role of macrophage on liver regeneration after hepatectomy. This role is like a double-edged weapon, showing both positive and negative regulatory roles on liver regeneration. On one hand, the increased CD68; the macrophage marker mainly in HTX 48h group might explain the positive regulatory role of splenic macrophage on liver regeneration in post HTX phase; e.g., the spleen can promote hepatic regeneration through a preferential increase in splenic macrophage-derived heme oxygenase 1 and reduction of tumor necrosis factor-alpha (Arakawa *et al.*, 2009). Moreover, mRNA expression for splenic macrophage-derived Hypoxia-inducible factor (HIF-1alpha), vascular endothelial growth factor (VEGF) and HGF, which are well-known to have a hepatic regenerative effect, were up-regulated during liver regeneration. Furthermore, portal VEGF was found to be significantly increased when compared to the systemic VEGF during liver regeneration. These results support the positive regulatory role of splenic macrophages in hepatic regeneration (Yamamoto *et al.*, 2010).

On the other hand, the increased CD68; as a macrophage marker, mainly in HTX 48h group, might explain the negative regulatory role of splenic macrophage on liver regeneration in post-HTX phase; e.g., thromboxane B2 production by splenic macrophages was found to be increased after HTX and was linked to remnant hepatic dysfunction (Kitagawa *et al.*, 1999). Moreover, previous reports indicating that IL-10, secreted by splenic macrophages, shows increased splenic mRNA expression after HTX and that IL-10 was found to play a critical role in adversely regulating hepatic regeneration through reducing the inflammatory response and therefore lessening of the liver STAT3 activation

(Yin *et al.*, 2011). It is worth mentioning that other splenic macrophage-derived factors e.g. HGF activator-inhibitor (HAI) and endothelin-1 (ET-1) can also suppress hepatic regeneration (Torimura, 2016). In addition, the spleen was found to show a suppressor effect on hepatic regeneration through the upregulation of splenic macrophages-derived TGF-beta1 (a major hepatocyte growth inhibitor) and its receptor RII and suppressing HGF and its-related receptor c-Met in the liver. (Lee *et al.*, 2015). Moreover, a recent study found that splenectomized rats exhibited increased expression for hepatocytic Ki67; a proliferative marker, at 24 h after splenectomy, the finding interpreted by the fact that the spleen is a chief producer of Transforming Growth factor b1 (TGFb1), a major hepatocyte growth inhibitor, meaning that the spleen is an inhibitor of hepatocyte proliferation even in the basic hepatic state (Elchaninov *et al.*, 2020).

It seems that the negative role is more predominant than the positive role of spleen on liver regeneration. Similarly, HTX combined with splenic artery ligation, allowed portal flow modification, encouraging increased hepatocytic viability and regeneration, with no impairment of the function, possibly through causing a less obvious increase in oxidative stress markers through the first 48 hours (Carrapita *et al.*, 2016). Moreover, HTX combined with microwave ablation of spleen was found to be an effective and safe procedure for patients with hepatic cancer and hypersplenism (Han *et al.*, 2017). Furthermore, a significantly high preoperative volume of spleen/surface area of the body was a strong predictor of post-hepatectomy liver failure confirming the negative impact of spleen on liver regeneration (Bae *et al.*, 2020).

Limitations of The Study:

Sprague Dawley rats were applied in the current study as they

represent perfect models for investigating the immunity-related aspects of spleen and because they are similar to the human spleen (Haley, 2017). Moreover, a larger resected volume of the liver followed for longer durations may show different results. Furthermore, the lymphatic systems show sexual dimorphism in the immune response. Further research using different percentages of resected liver volumes, following the changes over longer durations after HTX and using different species of animals and different sex, is recommended.

Conclusion:

The target of the current study was to evaluate the influence of 70% partial hepatectomy (HTX) on the splenic histological structure, T, B lymphocyte and macrophage populations, after HTX. It was found that during the first two days after HTX, splenic T lymphocytes area was rapidly increased with subsequent increase in macrophage populations with no significant change in B lymphocytes area. The splenic changes may explain the double-edged weapon role of spleen in the regulation of hepatic regeneration through the post-HTX stage.

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Conflict of Interest: The author reports no conflict of interest.

Ethical Approval: This study was approved by the institutional research board (IRB) of the Faculty of Medicine, Mansoura University, Egypt (proposal code R.21.03.1234)

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ARABIC SUMMARY

التغيرات المبكرة في الخلايا اللمفاوية والبلعمية للطحال في الجرذان بعد استئصال جزء كبير من الكبد

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الخلفية العلمية والهدف من البحث:

يحدث تضخم الطحال عادة بعد الاستئصال الجزئي للكبد. وقد أخبرت بعض الأبحاث السابقة عن دور الطحال المثبط لتكاثر خلايا الكبد والذي قد يكون ضارًا بالكبد، وبالتالي تم النظر في استئصال الطحال لتحسين تجدد الكبد، فكان الهدف من هذه الدراسة هو فحص تأثير الاستئصال الجزئي للكبد بنسبة 70 % عند الجرذان على التركيب النسيجي للطحال، وعلى الخلايا اللمفاوية التائية والبائية والبلاعم ومناقشة الارتباط الوظيفي بين التغيير الهيكلي للطحال وحالة الكبد في مرحلة ما بعد الاستئصال الجزئي للكبد.

الطرق والأدوات المستخدمة:

تم تقسيم الجرذان لمجموعتين: مجموعة ضابطة، ومجموعة الاستئصال الجزئي للكبد والتي تم تقسيمها إلى مجموعتين فرعيتين؛ تم قتلهم بعد مدة 24 أو 48 ساعة، بعد الاستئصال الكبدي. تم استخراج الطحال وصبغه بصبغة الهيماتوكسيلين والايوسين كصبغة روتينية، وصبغة البروسي الزرقاء لصبغ الحديد المترسب بالأنسجة، والكشف المناعي الكيميائي لسي دي 3 كعلامة للخلايا اللمفاوية التائية؛ سي دي 20 كعلامة للخلايا اللمفاوية، وسي دي 68 كعلامة للبلاعم في الطحال، مع تحليل النتائج.

النتائج:

أظهرت صبغة الهيماتوكسيلين والايوسين تضخمًا في بصيلات الطحال في اللب الأبيض، وعدم وجود توسع أو احتقان في اللب الأحمر، وعدم وجود زيادة في موت الخلايا المبرمج، مع زيادة طفيفة في ترسب الهيموسيديرين وفي عدد الخلايا البلعمية الصبغية في مجموعات الاستئصال الجزئي للكبد. وقد زاد تعبير سي دي 3 بشكل ملحوظ في مجموعتي الاستئصال مقارنة بالمجموعة الضابطة مع عدم وجود فرق كبير بين مجموعتي الاستئصال. ولم يظهر تعبير سي دي 20 فرقًا قويا بين المجموعات المدروسة. كما أظهر تعبير سي دي 68 وصبغ البروسي الأزرق زيادة غير كبيرة في المجموعة التي تم قتلها بعد ساعة 24 من الاستئصال الجزئي للكبد، مع زيادة كبيرة فيهما عند المجموعة التي تم قتلها بعد ساعة 48 من الاستئصال الجزئي للكبد.

الاستنتاج:

خلال اليومين الأولين بعد الاستئصال الجزئي للكبد، كانت هناك زيادة سريعة في عدد الخلايا اللمفاوية الطحالية التائية، دون البائية، مع زيادة لاحقة في البلاعم الطحالية. وقد تفسر هذه التغيرات في نسيج الطحال دورًا في تجديد الكبد.