SAKARYA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ DERGİSİ

Sakarya University Journal of Science
SAUJS

e-ISSN 2147-835X | Period Bimonthly | Founded: 1997 | Publisher Sakarya University |
http://www.saujs.sakarya.edu.tr/en/

Title: Experimental 70% Hepatectomy Model: Apoptotic Index, Proliferative Index and Mitotic Index

Authors: Şamil ÖZTÜRK, Latife Ceyda İRKİN

Recieved: 2021-02-24 18:57:13

Accepted: 2021-05-03 19:19:00

Article Type: Research Article

Volume: 25
Issue: 3
Month: June
Year: 2021
Pages: 788-799

How to cite
Şamil ÖZTÜRK, Latife Ceyda İRKİN; (2021), Experimental 70% Hepatectomy Model: Apoptotic Index, Proliferative Index and Mitotic Index. Sakarya University Journal of Science, 25(3), 788-799, DOI:
https://doi.org/10.16984/saufenbilder.886245
Access link
http://www.saujs.sakarya.edu.tr/en/pub/issue/62736/886245

Sakarya University Journal of Science 25(3), 788-799, 2021

Experimental 70% Hepatectomy Model: Apoptotic Index, Proliferative Index and Mitotic Index

Şamil ÖZTÜRK *[1] Latife Ceyda İRKİN[2]

Abstract

Liver regeneration is known to begin after experimentally induced liver resection. At first 24-48 hours, DNA synthesis reaches the maximum level after resection and significantly regeneration known to occur on the first 10 days. In this study, we were examined the regeneration of liver on the 1st and 14th days after the resection.

In experiment 21 Wistar albino male rats were used. The rats were randomly divided into three groups. The first group was the control, the second group was sacrificed one day after hepatectomy, and the third group was sacrificed 14 days after hepatectomy. All experimentally groups realized midline incision with laparotomy for resection of liver. Pedicles of the left lateral and median lobes of the liver were applied 70% hepatectomy by 4/0 silk binding. At the end of 1st and 14th days, liver tissue removed for light misroscobic analysis.

No histopathological findings were found in the control group. The tissue of all experimentally groups were showed some histopatological changes such as sinuzoidal dilatation, vacuolization in the hepatocytes. This histopatological findings were seem to increase at grup II more than group III and group I with routin H&E staining.

Mitotic index, apoptotic index and proliferation index of values at group II was reached the maximum level. Group III dramatically reduced the value of these index and were seem to reach near to values of the control group. Relative liver weight that determined each of experimentally groups were statistically significant differences compared to the control group.

As a result, in this study, liver regeneration was shown in liver transplants without any agent that would contribute to the clinic.

Keywords: Partial hepatectomy, TUNEL, apoptosis, immunohistochemistry, PCNA.

1. INTRODUCTION

The liver is the largest organ of the body that secretes both endocrine and exocrine, plays a major role in regulating metabolic functions, and also weighs approximately 1,5 kg and has the largest gland feature in the body [1]. It has a central role in many fundamental

* Corresponding author: samilozturk16@hotmail.com
[1] Çanakkale Onsekiz Mart University, Vocational School of Health Services, Çanakkale, Turkey.
ORCID: https://orcid.org/0000-0002-9435-8139
[2] Çanakkale School of Applied Sciences, Çanakkale, Turkey.
E-Mail: latifeirkin@gmail.com
ORCID: https://orcid.org/0000-0001-6603-8413

physiological events such as bile acid synthesis and secretion, blood-glucose balance and lipoprotein synthesis, storage of vitamins (A, D, E, K, and B12), biotransformation, detoxification and expression of endogenous and exogenous compounds [2]. A disfunction that may occur in the liver affects all systems of the body. Many factors such as drugs, chemicals, accidents, alcohol, liver tumors, liver diseases of viral origin and surgical interventions (partial hepatectomy) can cause damage in the liver tissue. When the liver is damaged due to various reasons, replication and proliferation may begin to complete its functional mass [3,4]. This feature is extremely important for hepatocytes that normally divide rarely. This proliferative capacity and adaptation ability is maintained against different metabolic conditions. These events reveal the loss and increase of liver tissue [3]. The liver has a tremendous interaction between cells and a complex mediator network that can repair itself in a few weeks in situations such as significant tissue loss, and is the only organ that goes to tissue regeneration quickly after resection or injury [5].

It is one of the most important stages in modern surgery that liver regeneration has become a common and controllable procedure. For cancer treatment or transplantation, 60-70% of the liver volume can be safely removed to be used as a liver donor graft [6]. Today, in studies conducted with methods such as computed tomography, angiography and scintigraphy, it has been shown that the liver reaches its original size in 3-6 months in adults and less than 3 months in children after liver resection. In the presence of cirrhosis, this period can be up to 9-15 months [7,8]. It has been reported that the human liver can tolerate even resections up to 80-85% [9]. Regeneration occurs even if the resection is less than 10% [10]. It has been shown that regeneration in the remaining liver tissue after partial hepatectomy starts from the first day and DNA synthesis reaches its maximum in the first 24-48 hours after hepatectomy [11]. Hepatocytes normally show very rare mitosis activity. However, active cell replication begins within 24 hours after partial hepatectomy and continues until the organ reaches its normal weight. Significant regeneration occurs within the first 10 days and this event is completed in 4-5 weeks. The excised lobes do not take the same shape. Regeneration mostly occurs in the form of new lobules and the growth of residual lobules [12,13]. Endocrine, paracrine and autocrine interactions are required for regeneration and liver restructuring after experimental partial hepatectomy in humans and animals [14,15]. Necessary stimuli for hepatic regeneration are the pancreas, other extrahepatic organs and humoral factors originating from the regenerating liver itself [12,13].

It has been observed that hepatocyte proliferation increases in cases such as surgical removal of a part of the liver lobes or damage of hepatocytes from viruses or chemicals. However, studies have shown that after liver resection, the regenerative activity is increased as a result of giving a more stimulus. In addition, various drug applications are used to heal the medically damaged liver more quickly. However, as with many chemical drug treatments, side effects are inevitable in these studies. Therefore, we conducted this study in order to reveal the regeneration occurring as a result of hepatectomy in the liver tissue without any stimulus or chemical medication on the days of 1 and 14 with immunohistochemical staining and TUNEL method.

2. MATERIAL AND METHOD

This study, 21 adult male Wistar Albino rats in Trakya University Experimental Animal Research Unit, weighing between 250-300 g and having the same biological and physiological characteristics were used. During the duration of the experiment, all our subjects were fed daily drinking water and pelleted feeds (Purina) containing 21% crude protein under optimum laboratory conditions (22 ± 1^0C, 12 hours light/dark cycle). Cage maintenance was done regularly. A total of 3 groups were created in the experiment.

Approval for the study was obtained from Trakya University Ethics Committee on 09.06.2011.

2.1. Hepatic resection method

Ketamine (Ketalar®, 10ml, 50mg/ml, Pfizer, USA) (25mg/kg, intramuscular) 50mg/ kg/ip, xylazine (Rompun® 50ml, 23,32mg / ml, Bayer, Germany) 5mg/kg/General anesthesia was applied with a rope. Before laparotomy in order to prevent bacterial translocation intramuscularly at 25 mg/kg of cefazolin vial (MN Pharmaceutical Inc., Istanbul, Turkey) were performed [16]. Laparotomy was performed with an upper midline incision. The left lateral and median lobe pedicles of the liver were tied with 4/0 silk and 70% hepatectomy was performed as defined by Higgins and Anderson [17]. After the surgical procedure, the fascia was closed with 3/0 vicryl and the skin with 4/0 silk and cleaned with povidone iodine. Oral intake of water and diet was allowed from the 24th post-operative hour [18].

2.2. Experimental design

The rats were divided into 3 groups, one control and two experiment groups, with 7 animals in each group.

Group I: Control group (n=7): The rats were not applied to the hepatectomy.

Group II: Study group (1 day) (n=7): The rats in this group were sacrificed 1 day after hepatectomy.

Group III: Study group (14 days) (n=7): The rats in this group were sacrificed 14 days after hepatectomy.

After resection, the subjects of each group were sacrificed as planned and the livers of the rats were completely removed. Liver samples taken were detected in Bouin fixative (75 cc picric acid + 25 cc formalin + 5 cc Acetic acid) for light microscope and immunohistochemical examination.

2.3. Relative liver weight

The remaining liver weight after partial hepatectomy was subtracted from the liver weight at autopsy and the ratio of this value to the whole liver weight was calculated. The liver regeneration rate was found by multiplying the obtained value by 100 [19]. Whole liver weight was accepted as 3-4% of the rat weight [20]. Results are expressed as per.

Relative liver weight = [liver weight at autopsy- (whole liver weight-resected liver weight)/whole liver weight]×10

2.4. Histopathological parameters

2.4.1. Mitotic index

After fixing in Bouin **fixative** for 4 days, liver tissue was blocked in paraffin after routine tissue follow-up and stained with Hematoxylin-Eosin (H&E) Mitotic index**:** The number of hepatocytes and total hepatocytes showing mitotic activity at 30 high-power fields was calculated and expressed as their ratio per 1000 cells [21].

Mitotic index = (number of mitotic cells)/(total number of cells)×100

2.4.2. Proliferation index

After fixing in Bouin fixator for 4 days, liver tissue was blocked in paraffin after routine tissue follow-up and stained with proliferated cell nuclear antigen (PCNA) from immunohistochemical stains. Proliferation index; PCNA stained cell number and total hepatocyte count at 30 high power fields were calculated. It was then defined as the ratio per 1000 cells [21].

Proliferation index= (number of PCNA stained cells) / (total number of cells)×100

2.4.3. Apoptotic index

After fixing in Bouin fixator for 4 days, liver tissue was blocked in paraffin after routine tissue follow-up and stained with TUNEL kit,

which is an apoptosis marker. Apoptotic index; TUNEL stained cell count and total hepatocyte count were calculated in 30 large magnification fields. It was then defined as the ratio of every 1000 cells (114) [21].

Apoptotic index= (apoptotic cell number) / (total cell number) × 100

2.5. Light microscopic inspection

For this purpose, the liver tissues were fixed in Bouin fixator for 4 days, and then the washing process was started. The tissues were washed in 70% alcohol for 2 days and the dehydration process was started. Tissues were kept for 1 hour in increasing alcohol series (70, 90, 96, 100%). After the dehydration stage, the tissues were treated with toluol for 3 series 15 minutes for the transparency step. Before embedding, the tissues were kept in soft paraffin for 1 night. The next day, liver tissues were removed from soft paraffin and kept in liquid hard paraffin for 1 hour and blocked. 5 μm thick sections were taken from these blocks using a Leica RM-2245 cylinder microtome. The sections taken were stained with H&E (Sigma-Aldrich). in order to reveal the histological structural changes in the liver. Photographs were taken by a light microscope (Olympus CX31-Japan).

2.6. Immunohistochemical analysis

The sections lowered into water were boiled for 20 minutes in the microwave oven in antigen retrival. After allowing to cool for 20 minutes at room temperature, the sections were washed with PBS. After this step, it was treated with 3% hydrogen peroxide (H_2O_2) prepared in methanol (Riedel-de Häen 24229) for 20 minutes to remove the hydrogen peroxidase activity. Sections were washed with phosphate buffer solution (PBS; pH 7.6) by rinsing in distilled water. 1% Preimmune rabbit serum (Ultra V Block, LabVision, TA-015-UB) was applied to sections to block non-specific antibody binding. The sections were then incubated with primary antibody diluted 1/100 in the moist chamber for 1 hour. The antibody used was mouse monoclonal anti-PCNA antibody (MS-106-B, Thermo LabVision, USA). Sections were kept in secondary antibody solution (Biotinylated Goat Anti-Mouse, LabVision, TM-015-BN) for 20 minutes after washing with PBS 3 times. Streptavidin peroxidase solution (Streptavidin Peroxidase, LabVision, TS-015-HR) was applied to the sections washed 3 times in PBS for 20 minutes. After washing the sections 3 times with PBS, 3-amino 9 ethyl carbazole (AEC) chromogen solution (LabVision, TA-002-HAC) was applied for 10 minutes.

Proliferation index; the number of PCNA stained cells in 30 high-power fields and the total number of hepatocytes was calculated and defined as the ratio per 1000 cells (μm^2), and the average number of PCNA positive cells was determined.

2.7. Statistical analysis

All data are expressed as mean (±) standard deviation (SD). The differences in the results between the groups were evaluated by Kruskal-Wallis analysis of variance. For comparisons between groups with significant differences, Mann-Whitney U test was used. If $p < 0.05$, the difference was considered statistically significant. In addition, hepatocyte vacuolization and sinusoidal dilatation numbers were determined semiquantitatively in all groups. Semi-quantitative evaluation was done as follows; none (-), rare (±), low (+), medium (++), too much (+++), too much (++++).

2.8. TUNEL staining

The 5 μm sections taken from the paraffin blocks on the slide were kept in an oven at 37°C for a night, then kept in toluol for 3x5 minutes and then passed through the decreasing alcohol series (100%, 95%, 70%) for 3 minutes and lowered into distilled water. Proteinase K (20 μg/ml, Chemicon, 21627) was applied to the sections kept in distilled water for 5 minutes at room temperature for 15 minutes for antigen recovery. Sections washed with distilled water were kept in 3% H_2O_2 prepared in methanol for 5 minutes to block

endogenous peroxidase. After shaking with distilled water and PBS, the pool around the sections was drawn with hydrophobic pen (Zymed, 00-8899) and a pool was created for 5 minutes at room temperature with equilibration buffer. Then the sections were kept at 37°C in terminal deoxynucleotidyl transferase (TdT) enzyme for a hour, then rinsed with stop/wash buffer for 15 seconds and kept at room temperature for 10 minutes. Conjugate of antidigoxigenin was applied to sections washed in PBS 3 times and kept at room temperature for 30 minutes. After washing the sections 3 times with PBS, diamino benzidine (DAB) chromogen solution (LabVision, TA-002-HAC) was applied for 10 minutes. After washing the sections with distilled water, contrast staining was done by applying methyl green for 10 minutes. Sections rapidly passed through distilled water were also rapidly passed through 100% N-Butanol. After dehydrated sections were kept in toluol for 3x2 minutes, the closure solution was placed and closed with coverslip and evaluated under a light microscope.

3. RESULTS AND DISCUSSION

3.1. Relative liver weight results

The remaining liver weight after partial hepatectomy was subtracted from the liver weight at autopsy and the ratio of this value to the whole liver weight was calculated. The liver regeneration rate was found by multiplying the obtained value by 100. Whole liver weight was accepted as 3.4% of the rat weight.

When the relative liver weights determined in the all groups were compared, a statistically significant difference according to $p < 0.05$. When the groups II and III were compared (11.46 ± 2.21, 38.45 ± 7.11), a statistically significant difference was found as $p < 0.001$ (Figure 1).

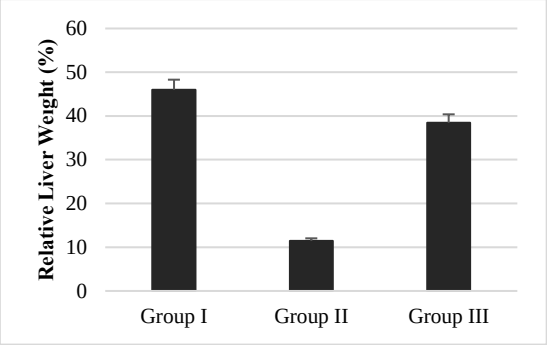


Figure 1 Relative liver weight values of experimental groups

Groups	Group I (control)	Group II (1st day)	Group III (14th day)
Hepatocyte vacuolization	-	++++	+
Sinusoidal dilatation	-	+++	±

Table 1 Semi-quantitative evaluation of hepatocyte vacuolization and sinusoidal dilatation in experimental groups

PV: Portal ven, long arrow: hepatocyte vacuolization, short arrow: sinusoidal dilatation

3.2. Light microscopic results

When the H&E stained liver sections of the rats in the control group were examined under a light microscope. It was observed that the liver sections were in normal histological appearance. It was observed that hepatocytes, which are liver parenchymal cells, were placed regularly around the central veins to form hepatocyte cords. Branch of the portal vein, hepatic arteriole and bile duct were observed in the portal areas around the hepatic lobules. In the sinusoid wall, endothelial cells were distinguished by the flat-shaped and dark staining of their nuclei, while Kupffer cells were distinguished by their nuclei larger than the endothelial cell nuclei, oval or triangular shapes. Hepatocyte nuclei were one or two large and round in shape, and their cytoplasm showed eosinophilia.

In all groups, hepatocyte vacuolization and sinusoidal dilatation counts were determined semiquantitatively. It was observed that the hepatocytes in the liver sections of animals

belonging to groups II**,** and III had common mitosis at various stages and was noticeable in hepatocytes vacuolization and sinusoidal dilatation. In group II hepatocyte vacuolization and sinusoidal dilatation were noticeable too much (++++) (Figure 2b-c, Table 1). In the sections belonging to the group III, it was observed that hepatocyte vacuolization decreased very much and became similar to the control group, decreasing in less (+) and sinusoidal dilatation as rare (±) (Table 1). When the sections belonging to all groups were evaluated, it was observed that the classical liver lobule structure was preserved (Figure 2). MI value of the group I, group II and group III were evaluated. The statistically significant difference between the groups were evaluated according to $p<0.05$. Statistically significant difference was observed between the control group and the other experimental groups. When the group I and II were compared, a statistically significant difference was found at the $p<0.001$ level. When the group I and III were compared, a statistically significant difference was found in $p<0.01$ (Figure 5).

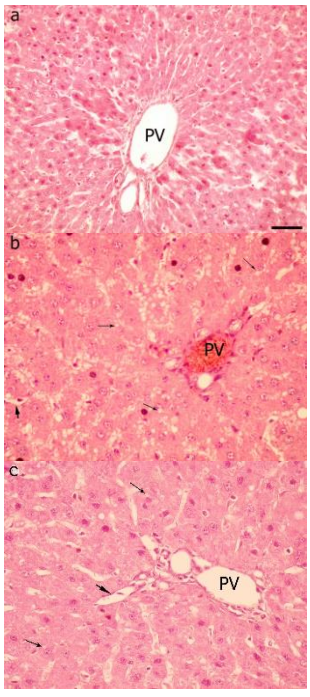
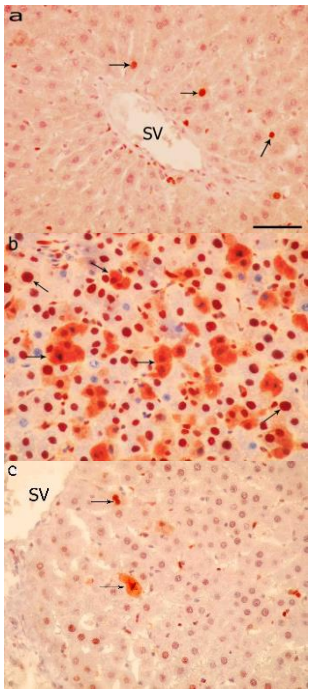


Figure 2 a) Normal histological appearance of the liver (group I), b) the liver section after 1 day of resection-intense vacuolization is seen around the portal areas (group II), c) the liver section after 14 day of resection-vacuolization is seen around the portal areas (group III) (X400), H&E staining.

3.3. Immunohistochemical results

PCNA immunostaining of group I liver section was weak, group II (1 day after resection) PCNA positive cell density was strong, group III PCNA positive cell was weak immunoreactivity (Figure 3). When the PCNA values detected into all groups were compared, a statistically significant difference was found between the group I and in all study groups (Figure 5). PI value of group I, group II and group III were evaluated. When the group II and group I were compared, statistically significant differences was found ($p<0.0001$). When the control and group III were compared, the statistically significant difference was low ($p<0.05$) (Figure 5).

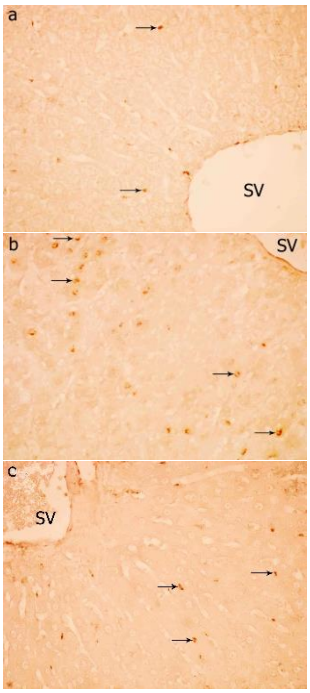


SV: Central ven, arrow: PCNA positive cells

Figure 3 a) PCNA immunostaining of (group I) liver section, b) PCNA immunostaining of the (1 day after resection)-PCNA positive cell density is seen (group II), c) PCNA immunostaining of the (14 days after resection)-PCNA positive cell density is seen (group III).

3.4. TUNEL results

A statistically significant difference was found between group I and experimental groups (group II, III) when the apoptotic index (AI) values obtained as a result of the evaluation of cells whose apoptotic nuclei were stained as a result of TUNEL staining performed into all groups (Figure 4). AI value of the group I, group II and group III were evaluated. The statistically significant difference between the groups were evaluated according to $p<0.05$. When the group II and group I were compared, a statistically significant difference was found ($p<0.001$). When the group I and group III were compared, the statistically significant difference was found low ($p<0.05$, Figure 5).



SV: Central ven, arrow: TUNEL positive cells

Figure 4 a) The liver section of group I, b) TUNEL positive cell high density was seen in group II, c) TUNEL positive cell low density was seen in group III (X400), TUNEL staining

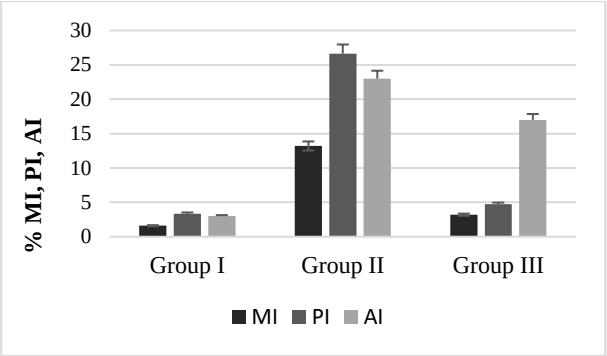


Figure 5 Mitotic, proliferative and apoptotic index values of control and experimental groups

3.5. Discussion

The liver exhibits a distinct feature from other organs with its regeneration capacity. The liver regulates many basic mechanisms such as biotransformation, regulation of metabolic functions and immunological events. However, it has many functions that have not been fully illuminated yet [22,23].

The existence of the ability to replenish the tissue mass after the loss or damage of an organ is defined as regeneration [3]. Regeneration is a complex process in which cytokines, hormones, transcription factors and oxidative stress products play a role [5]. Studies have shown that the liver has the ability to repair itself in significant tissue losses, thanks to intercellular interaction and a complex mediator network [24, 25]. Most studies on liver regeneration have been studied on the pre-existing damaged liver in experimental animals, or liver damage has been caused by using chemicals or a partial hepatectomy [26]. Palmes and Spiegel [26] described the model used in partial hepatectomy studies by stating the ratio of each liver lobe to the total mass of all liver lobes in rats. According to the total liver mass, the right lobe contains 38%, the left lobe is 30%, the tail lobe is 8%, and the quadrat (quadrilateral) lobe, which is two-part, contains 10%. It has been stated that the most suitable model for partial hepatectomy studies in rats is resection of 68-70% of the liver [24].

Partial hepatectomy is one of the most frequently used surgical procedures today and

is an inevitable surgical treatment option in primary liver tumors, trauma, liver metastases of gastrointestinal tumors and liver transplantations [5, 25]. Liver resection has become safer with the development of diagnostic methods, improvement and improvement in surgical techniques, and improvement in postoperative care, especially in the last 25 years [27]. Postoperative mortality and morbidity in liver resection are directly related to preoperative liver function and the functional and regenerative capacity of the remaining liver after postoperative resection. It is a very important support mechanism for replacing the functional hepatic mass in a short time after regeneration of liver tissue with normal parenchyma, tissue injuries and hepatocellular necrosis. Active cell replication starts within 24 hours after partial hepatectomy and this replication continues until the organ reaches its first weight [8].

We do not know exactly the regulatory mechanisms, changes that occur in liver regeneration and their interrelationships. What we know for sure is that the liver knows when to start regeneration and when to stop [3]. Loss of liver tissue initiates regeneration [10]. The sensitive point that allows regeneration to stop is the relationship between body mass and liver mass. When the liver reaches the size to meet the functional needs of the body and carry out metabolism, regeneration stops [28-30]. Interestingly, when a large liver tissue transplant is performed after transplantation relative to the recipient, liver mass decreases until the optimal liver to body mass ratio is achieved [23].

Liver regeneration rates can be calculated by evaluating the liver mass taken in damages caused by partial hepatectomy and the masses of the whole liver taken after a certain time after hepatectomy. They reported that the remaining liver weight doubled 48 hours after 2/3 partial hepatectomy in rats and reached full weight within 7-10 days. In our study, it was found that the liver weights regenerated after 72 hours after partial hepatectomy nearly doubled in the partial hepatectomy groups [14, 31].

In some studies, liver age weights and their amounts of 68% and 32% were used to determine the regeneration rate by placing it in the Child's formula [14, 31]. In our study, the relative liver weight defined by Fishback [19]. The remaining liver weight after partial hepatectomy was subtracted from the liver weight at autopsy and the ratio of this value to the whole liver weight was calculated. The liver regeneration rate was found by multiplying the obtained value by 100 [20]. In the values we obtained, after 70% partial hepatectomy, relative liver weight increased significantly in all study groups starting from the group II.

Partial hepatectomy was performed in this study. Determination of mitotic index and more importantly PCNA index has been used frequently in studies on liver regeneration and played an important role in interpretation of regeneration [32, 33-35]. Hou et al. [25] Mitotic index and PCNA index data were also used to determine the effects of an organic compound named FR167653 on liver regeneration in rats with partial hepatectomy. In their study, they determined that the mitotic index that they determined simultaneously from the liver sections of the control group rats they underwent partial hepatectomy was higher than the PCNA index. The mitotic index and PCNA index data were in parallel with each other. According to the mitotic index and PCNA index results, group II and group III was high. Both mitotic index and PCNA indexes were found to be higher on 1st day compared to 14th day. By decreasing mitotic and PCNA index values in the group III approached to the control group. When the PCNA index results were evaluated in this study, results was in parallel with the previous studies. Proliferation index values between the groups were found to be statistically significant. Studies have shown that the mitotic activity peaks in the resected liver tissue and the tissue has an intense proliferating activity. It was determined in this study and previous studies that the

proliferation activity depends on the time after resection.

Akcan [36] reported that one of the opposite mechanisms seen after hepatectomy is apoptosis and that cell apoptosis begins when regeneration reaches its peak. In addition, it showed that apoptosis accompanies cell proliferation, overgrown cells are eliminated and new tissue formation is successfully completed. Sowa et al. [37] showed that the TUNEL index increased significantly after resection. Although apoptosis decreased over time, the TUNEL index continued to increase compared to control groups. Thus, these data showed that in 70% hepatectomy first apoptosis and correspondingly regeneration occurs. In addition, it has been shown that more tissue damage in the early postoperative phase increases the number of apoptotic cells. In contrast, a slight increase in the TUNEL index was observed at the beginning of 90% hepatectomy. However, 1 day after the operation, apoptosis was observed to increase strongly, which means that the regeneration in tissue damage was highest after 14 days. The TUNEL index supports the results. In this study, it was observed that apoptosis was at the maximum level in the group II, and apoptosis gradually decreased in the group III in the following days.

4. CONCLUSION

In this study, we planned the histological examination of liver regeneration after liver resection. It was observed that regeneration due to the relative weight of the liver increased significantly over time. In histopathological examination performed with light microscopy, it was observed that vacuolization of hepatocytes and dilatation in sinusoids occurred first after partial hepatectomy, and increased mitotic activity, which is an indicator of regeneration increase, in the following days.

Vacuolization, sinusoidal dilatation, apoptosis and mitosis activities were at the highest levels in hepatocytes in group II. In Group III, it was observed that these values reached values close to Group I. It was observed that the mitotic index reached the highest value in group II, and decreased in time in group III. In immunohistochemical staining of liver tissue, it was observed that PCNA positive cells were concentrated in group II and the number of positive cells in group III decreased. It was observed that the time spent in resection directly affected the proliferative process.

Apoptotic cells were observed as positive in staining with the TUNEL method. It was observed that the apoptotic index values reached the maximum level after 24 hours (group II). It was observed that TUNEL positive cells decreased in group III and positivity close to group I.

We think that the data obtained from our study will constitute a very important reference for liver transplantation. Because transplants are inevitable in organ failure and transplant failures pose an important problem. The data we have obtained is of a nature to shed light on the clinic, and transplants can be made according to the course of both mitotic activity and apoptotic activity.

Funding

"This study is supported by Trakya University Scientific Research Projects Coordination Unit. Project Number: 2011-125."

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

In the study, the first author contributed 60% and the second author contributed 40%.

The Declaration of Ethics Committee Approval

Approval for the study was obtained from Trakya University Ethics Committee on 09.06.2011.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the article and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCES

[1] Aktümsek, "Anatomi ve fizyoloji insan biyolojisi," Nobel Yayınları, Ankara, 3th edt, pp. 366-367, 2006.

[2] T. E. Andreoli, "Liver," In M. B. Fallon, B. M. McGuire, G. A. Abrams, M. R. Arguedas (ed), Cecil essentials of medicine, 5th edt. WB Saunders Company, Philadelpia, pp. 365-398, 2001.

[3] N. Fausto, "Liver regeneration," *Journal of Hepatol*, vol. 32, pp. 19-31, 2000.

[4] M. Basoglu, A. A. Balık, R. Kavak, C. Gundogdu, Z. Erman, R. Yıldırgan, D. Oren, "Effects of growth hormone on hepatic regeneration," *Turk J Med Sci.*, vol. 30, pp. 529-534, 2000.

[5] Z. G. Ren, J. D. Zhao, K. Gu, J. Wang, G. L. Jiang, "Hepatic proliferation after partial liver irradiation in Sprague-Dawley rats," *Mol. Biol. Rep*., vol. 39, pp. 3829-3836, 2012.

[6] E. A. Pomfret, J. J. Pomposelli, F. D. Gordon, N. Erbay, L. Lyn, W. D. Lewis, "Liver regeneration and surgical outcome in donors of right-lobe liver grafts," *Transplantation*, vol. 76, pp. 5-10, 2003.

[7] T. Nakamura, S. Mizuno, "The discovery of hepatocyte growth factor (HGF) and its significance for cell biology, life sciences and clinical medicine," *Proc Jpn Acad Ser B Phys Biol Sci*., vol. 86, no:6, pp. 588-610, 2010.

[8] S. Perek, S. Kapan, "Cerrahi Gastroenteroloji," In U. Değerli, Y. Bozfakıoğlu (ed), İstanbul, Nobel Tıp Kitabevleri, vol. 5, pp. 194-208, 2000.

[9] G. Garcea, G. J. Maddern, "Liver failure after major hepatic resection," *J Hepatobiliary Pancreat Surg*., vol. 16, pp. 145-155, 2009.

[10] G. K. Michalopoulos, M. C. DeFrances, "Liver regeneration", *Science*, vol. 296, pp. 60-66, 1996.

[11] L. Chen, H. L. Sun, L. Yang, H. J. Du, W. An, "Transfection of human hepatic stimulator substance gene could protect BEL-7402 cells against hepatotoxins," *Zhonghua Gan Zang Bing Za Zhi.*, vol. 12, no:2, pp. 99-101, 2004.

[12] H. Y. Hsu, M. C. Yu, C. W. Lee, "RAM score is an effective predictor for early mortality and recurrence after hepatectomy for hepatocellular carcinoma," *BMC Cancer*, vol. 17, no:1, pp. 742, 2017.

[13] S. Iwatsuki, B. W. Shaw, T. E. Jr Starzi, "Experience with 150 liver resections," *Ann Surg.*, vol. 197, pp. 247-252, 1983.

[14] M. Canbek, M. Uyanoglu, S. Canbek, E. Ceyhan, A. Ozen, B. Durmus, O Turgak, "The effect of geraniol on liver regeneration after hepatectomy in rats," *In Vivo*, vol. 31, no: 2, pp. 209-213, 2017.

[15] M. Bouattour, E. Raymond, S. Qin, A. L. Cheng, U. Stammberger, G. Locatelli, S. Faivre, "Recent developments of c-Met as a therapeutic target in hepatocellular carcinoma," *Hepatology*, vol. 67, no: 3, pp. 1132-1149, 2018.

[16] M. J. Xu, D. Feng, H. Wu, H. Wang, Y. Chan, J. Kolls, N. Borregaard, B. Porse, T. Berger, T. W. Mak, J. B. Cowland, X. Kong, B Gao, "Liver is the major source of elevated serum lipocalin-2 levels after bacterial infection or partial hepatectomy: a critical role for IL-6/STAT3," *Hepatology*, vol. 61, no: 2, pp. 692-702, 2015.

[17] R. Bönninghoff, K. Schwenke, M. Keese, R. Magdeburg, H. Bitter-Suermann, M. Otto, T. Hasenberg, S. Post, J. Sturm, "Effect of different liver resection methods on liver damage and regeneration factors VEGF and FGF-2 in mice," *Can J Surg.*, vol. 55, no: 6, pp. 389-393, 2012.

[18] R. Yang, T. Uchiyama, S. K. Watkins, X. Han, M. P. Fink, "Ethyl pyruvate reduces liver injury in a murine model of extrahepatic cholestasis," *Shock*, vol. 22, pp. 369-375, 2004.

[19] N. Ekici Günay, S. Muhtaroğlu, A. Bedirli, "Administration of Ginkgo biloba extract (egb761) alone and in combination with fk506 promotes liver regeneration in a rat model of partial hepatectomy," *Balkan Med J.*, vol. 35, no: 2, pp. 174-180, 2018.

[20] K. Kagure, Y. Q. Zhang, H. Shibata, I. Kojima, "Immediate onset of DNA synthesis in remnant rat liver after %90 hepatectomy by an administration of follistatin," *J Hepatol*., vol. 28, no: 3, pp. 977-984, 1998.

[21] M. Selzner, P.A. Clavien, "Failure of regeneration of the steatotic rat liver: Disruption at two different levels in the regeneration pathway," *Hepatology*, vol. 31, pp. 35-42, 2000.

[22] R. G. Kilbourn, D. L. Traber, C. Szabo, "Nitrik oxide and shock," Dis Mon., vol. 47, no: 5, pp. 277-348, 1997.

[23] H. Hayashi, M. Nagaki, M. Imose, Y. Osawa, K. Kimura, S. Takai, M. Imao, T. Naiki, T. Kato, H. Moriwaki, "Normal liver regeneration and liver cell apoptosis after partial hepatectomy in tumor necrosis factor-alpha-deficient mice," *Liver Int.*, vol. 25, no: 1, pp. 162-170, 2005.

[24] J. A. Ebrenfried, T. C. Ko, E. A. Thompson, B. M. Evers, "Cell cycle-mediated regulation of hepatic regeneration," *Surgery*, vol. 122, no: 5, pp. 927-935, 1997.

[25] R. Veteläinen, A. K. van Vliet, T. M. van Gulik, "Severe steatosis increases hepatocellular injury and impairs liver regeneration in a rat model of partial hepatectomy," *Ann Surg.*, vol. 245, pp. 44-50, 2007.

[26] D. Palmes, H. U. Spiegel, "Animal models of liver regeneration," *Biomaterials*, vol. 25, no: 9, pp.1601-1611, 2004.

[27] W. C. Meyers, R. S. Jones, "Anatomy," In W. C. Meyers, R. S. Jones (eds), Textbook of liver and biliary surgery, Press: JB Lippincott Company, Philadelphia, pp. 18-38, 1990.

[28] F. G. Court, S. A. Wemyss-Holden, R. A. Dennison, G. J. Maddern, "The mystery of liver regeneration," *Br J Surg.*, vol. 89, pp. 1089-1095, 2002.

[29] J. Kountouras, P. Boura, N. J. Lygidakis, "Liver regeneration after hepatectomy," *Hepatogastroenterology*, vol. 48, no: 38, pp. 556-562, 2001.

[30] N. Fausto, "Hepatic regeneration," D. Zakim, T. D. Boyer Eds., Press: Hepatology WB Saunders, Philadelphia, pp. 32-58, 1998.

[31] S. T. Fan, C. M. Lo, C. L. Liu, C. M. Lam, "Hepatectomy for hepatocellular

carcinoma toward zero hospital deaths," *Ann Surg.,* vol. 229, pp. 322-330, 1999.

[32] N. Assy, G. Y. Minuk, "Liver regeneration: methods for monitoring and their applications," *J Hepatol.,* vol. 26, pp. 945-952, 1997.

[33] Picard, L. Lambotte, P. Starkel, C. Sempoux, A. Saliez, V. V. D. Berge, "Steatosis is not sufficient to cause an impaired regenerative response after partial hepatectomy in rats," *J Hepatol., vol.*32, pp. 645-652, 2002.

[34] J. O. Castro-e-Silva, S. Zucoloto, F. S. Ramalho, L. N. Z. Ramalho, J. M. C. Reis, A. A. C. Bastos, M. V. H. Brito, "Antiproliferative activity of *Copaifera duckei* oleoresin on liver regeneration in rats," *Phytother Res.*, vol. 18, pp. 92-94, 2004.

[35] E. Kamer, A. Çöker, A. R. Sevinç, E. Özkara, E. Özer, T. Özzeybek, "Effect of intraperitoneal dministration of gemcitabine and paclitaxel on hepatic regeneration in rats," *Turk J Gastroenterol.,* vol. 14, no: 1, pp. 1-6, 2003.

[36] Akcan, "The effect of amrinone on liver regeneration in experimental hepatic resection model," *J Surg Res.*, vol. 130, pp. 66-72, 2006.

[37] J. P. Sowa, J. Best, T. Benko, M. Bockhorn, Y. Gu, E. M. Niehues, "Extent of liver resection modulates the activation of transcription factors and the production of cytokines involved in liver regeneration," *World J Gastroenterol.*, vol. 14, no: 46, pp. 7093-8100, 2008.