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Original Article

# Histological and Ultrastructural Changes in the Liver of Albino Rat after Partial Hepatectomy

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## ABSTRACT

**Background:**
Understanding of the mechanisms governing liver regeneration may be useful for designing treatments for patients with hepatitis, liver injury, or liver transplant. The aim of the present study was to investigate the ultrastructural changes and BCL-2 protein expression associated with partial hepatectomy (PHx).

**Materials and Methods:**
This study was carried out on 30 male albino rats assigned to three experimental groups. PHx was done for all the 30 animals and liver tissues were collected to become control tissues. The rats were sacrificed in 24 h (group A), 48 h (group B), and 72 h (group C) after PHx. Liver specimens were processed for light and transmission electron microscope. BCL-2 protein expression was evaluated in all groups.

**Results:**
Hepatocytes of groups A and B showed several apoptotic changes. BCL-2 protein expression was not detected in the hepatocytes of controls. 48h after PHx, the nuclei of the hepatocytes showed abundant heterochromatin with indistinct nucleoli. The cytoplasms showed numerous electron lucent vacuoles, and swollen mitochondria compared with the controls. Moreover, dilated bile canaliculi with absent microvilli were observed. Meanwhile, 72 h after PHx, the general architecture of liver was retained nearly to normal.

**Conclusion:**
In conclusion, during the 48 h after PHx, the hepatocytes underwent proliferative and apoptotic changes followed by regaining their normal structure and organization.

**Keywords:** Hepatectomy, BCL-2, Liver, Regeneration

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## INTRODUCTION

Liver regeneration is known for a long time to occur in animals and humans. Hepatic regeneration following a two-thirds partial hepatectomy (PHx) in rat is a well-defined phenomenon, which starts within a few minutes and leads, after a latent phase (16 hours), to an increased synthesis of DNA followed by the first wave of mitosis. The mechanisms initiating and controlling this regenerative process, which restores quite precisely the original liver mass, remain poorly understood despite numerous studies performed in vivo and in vitro (1-4). Within a short period after PHx, hepatocytes, under

the effect of reduction of the liver mass, should be modified to become more responsive to various growth factors produced at a higher rate or, at least, present at a greater concentration (5).

B-cell lymphoma 2 (BCL-2), a gene located at chromosome 18q21, blocks programmed cell death without affecting the cellular proliferation. It was first described in B cell leukemia and later was detected in other malignant tumors (6). Many of the BCL-2 related proteins occur within intracellular membranes, especially those of the mitochondria. Phosphorylation of BCL-2 is required to exert the mitochondrial functions, including changes in calcium and energy flux within mitochondria, alteration of the redox state and inhibition of caspases (7).

The whole process of the liver regeneration lasts 5 to 14 days. Hepatocyte proliferation starts in the periportal areas and then the liver reconstitute itself. However, there is evidence supporting the existence of reserve liver stem cells, which proliferate in response to more severe injuries, impairing the replication capacity of hepatocytes. Such injuries can be induced by hepatotoxic chemicals, metabolic intermediates, or hepatotropic virus infections. In such circumstances the liver is rescued by the activation and proliferation of potential liver stem cells, giving rise to progenitors, classically named oval cells. These cells are supposed to have both clonogenic and bipotential capacity, meaning the ability to proliferate and differentiate into cells of either hepatocyte or biliary epithelial cell lineage (8).

The regenerative liver is a well-established experimental model for studying the process of cell junction disappearance and reappearance. PHx in experimental animals leads to marked changes in cell junction structure and function (9,10). After PHx, there is a rapid fall in the number and size of cell junctions between hepatocytes in the remaining lobes. However, the hepatocytes retain their plasma membrane domains (polarity) during regeneration (11). Mitochondrial bioenergetic impairment has been found in the organelles isolated from rat liver during the pre-replicative phase of liver regeneration. Mitochondrial swelling and release of both glutamate dehydrogenase and aspartate aminotransferase isoenzymes from mitochondria with an increase in the mitochondrial Ca2+ content were also observed (12).

The aim of the present study was to investigate the ultrastructural changes and BCL-2 protein expression associated with partial hepatectomy (PHx).

## MATERIALS AND METHODS

### Animals

This study included 30 male albino rats, weighing 250-300 g. The animals were housed in stainless-steel cages, with 12-h dark/light cycles. They were maintained on standard laboratory diet with tap water ad libitum throughout the experiment, except for an overnight fasting before surgery. The rats were divided randomly into three groups (each consisting of 10 rats) based on the duration after PHx. Groups A, B, and C were sacrificed 24 h, 48 h, and 72 h after PHx, respectively. The liver tissues resected from animals before surgery were considered as controls. The experiment was conducted in Animal House, Faculty of Medicine, Zagazig University. All institutional and national guidelines for the care and use of laboratory animals were followed. Albino rats were used in this experiment as they have a close analogue genetic and physiological match to humans. Moreover, they are calm and easy to handle. previous studies investigating liver regeneration were conducted using albino rats (9).

### Surgical procedure

The surgical procedure was performed under strictly sterile conditions, using ether anesthesia. A midline incision (4-5 cm in length) was done in the upper abdomen. The median and left lateral lobes of the liver were excised according to a previous protocol (13). Subsequently, the abdominal incision was closed. Then, the rats were kept on a standard diet until they were killed. The animals were assigned randomly to three groups and sacrificed 24 h (group A), 48 h (group B), and 72 h (group C) after PHx. The liver samples were collected and processed for all parameters.

### Histopathological Examination

The liver samples were fixed in 10% neutral formalin. The fixed liver tissues were embedded in paraffin, stained with hematoxylin and eosin (14), and examined for histological findings.

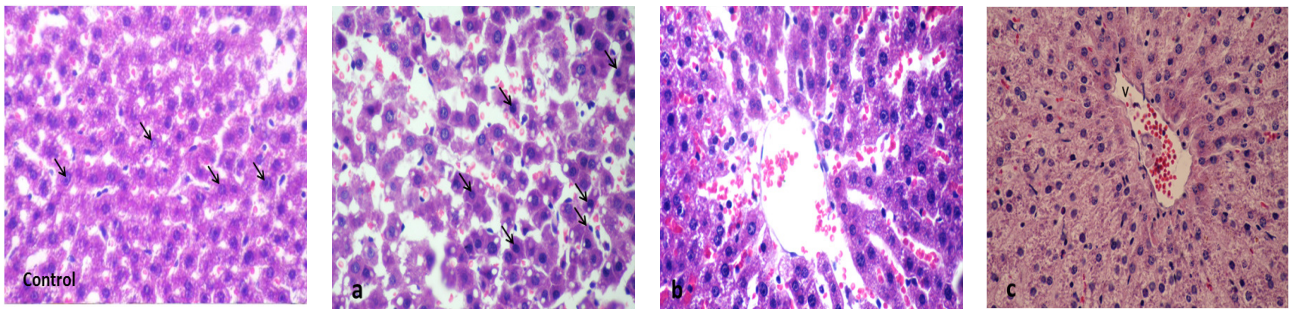


**Fig.1: (Control) showing hepatocytes with light-stained nuclei and prominent nucleolus (arrow) and liver sinusoids. (a) 24 h after PHx, showing hepatocytes with dark stained small nuclei (arrows), cytoplasmic vacuoles, and dilated liver sinusoids. (b) 48 h after PHx, showing hepatocytes with dark stained small nuclei and dilated liver sinusoids. (c) 72 h after PHx, showing hepatocytes with dark stained small nuclei and other hepatocytes with light stained large nuclei an d central vein (v) filled with blood cells. H&E, x400**

### Immunohistochemistry

The detection of cells that express BCL-2 protein relied on immunohistochemistry based on a streptavidin-biotin peroxidase method (Biogenex, San Ramon, USA). Briefly, four µm thick tissue paraffin sections were added into xylene and hydrated through graded concentrations of ethyl alcohol. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 15 min. Sections were then processed in a microwave oven twice for 5 min each time at high power and subsequently stained with polyclonal antibodies to BCL-2 (Santa Cruz, USA, at a dilution of 1:40). The sections were washed by Tris-Buffered Saline (TBS). Diaminobenzidine (Sigma Fast DAB tablets, USA) was used as a chromogen. All incubations were performed for 30 min at room temperature between the steps. For the negative control, the same streptavidin-biotin technique was used on tissue sections in which 1% Bovine Serum Albumin (BSA) in Phosphate Buffer Saline (PBS) substituted the primary antibody.

### Electron Microscopic Examination

The liver specimens were fixed using 4% glutaraldehyde and then in 0.1 m sodium cacodylate buffer (pH 7.4) for 4 h at 4°C. After fixation, the specimens were washed in sodium cacodylate buffer at 4°C, postfixed with 1% osmium tetroxide in sodium cacodylate buffer for 1 h at 4°C, dehydrated in alcohol and embedded in Araldite resin. Semi-thin sections (1µm) were taken for light microscope inspection. Ultra-thin sections were mounted on copper mesh grids, stained with uranyl acetate and lead citrate, according to a previous protocol (15), then examined with a Joel TEM 1010 electron microscope. All techniques were carried out at the Department of Histology, Faculty of Medicine, Zagazig University.

## RESULT

### Histopathological examination

Control cells showed normal hepatic lobules consisting of hepatocyte plates separated by blood sinusoids radiating from the central vein. The blood sinusoids were empty or sometimes possess a few erythrocytes. Individual hepatocytes were polygonal in shape, possessing one or two large, round, and euchromatic (pale-stained) nuclei with dark prominent nucleoli (figure 1, control). 24 h and 48 h after PHx, (groups A and B, respectively), the hepatocytes were observed to be arranged in clusters with tortuous dilated blood sinusoids. Furthermore, dark-stained nuclei and numerous cytoplasmic vacuoles were detected (figure1, a &b). Findings from liver sections obtained from group C (72 h after PHx) showed that the histological and cytological features of the liver returned to the preoperative conditions (control group) (figure1, c).

### Immunohistochemistry

In the control cells, BCL-2 protein was not detected in the hepatocytes (Figure 2, control). 24 h after PHx, BCL-2 protein was expressed in most of the cells in the form of diffuse cytoplasmic brown granules (figure 2, a). 48 h after PHx, BCL-2 protein expression was detected. However, it was mainly observed in the prevascular areas (figure 2, b). Meanwhile, 72 h after PHx, the BCL-2 protein was not detected (figure 2, c).

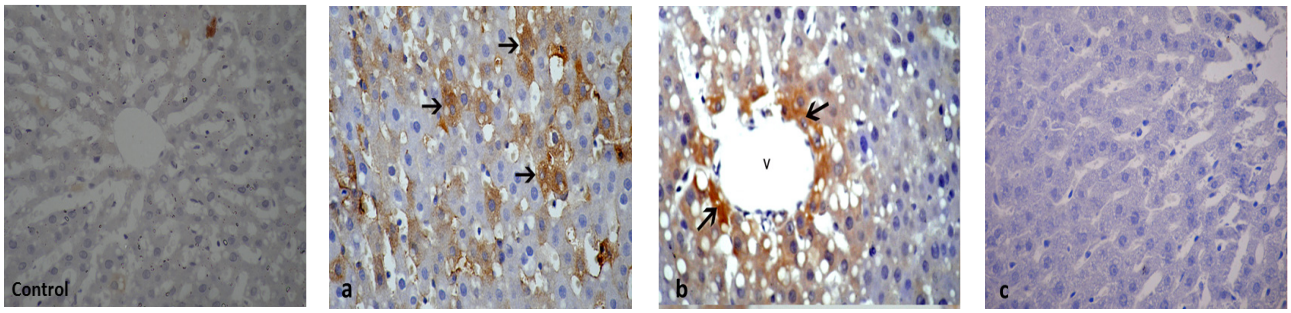


**Fig.2: (Control) showing a negative immunohistochemical reaction for BCL-2 protein. (a) 24 h after PHx, showing a positive immunohistochemical reaction for BCL-2 protein (brown colour) (arrows). (b) 48 h after PHx, showing a positive immunohistochemical reaction for BCL-2 protein (brown colour) (arrow), most extensive in the perivascular hepatocytes. (c) 72 h after PHx, showing a negative immunohistochemical reaction for BCL-2 protein. Bcl-2 protein and Hx counter stain, x400**

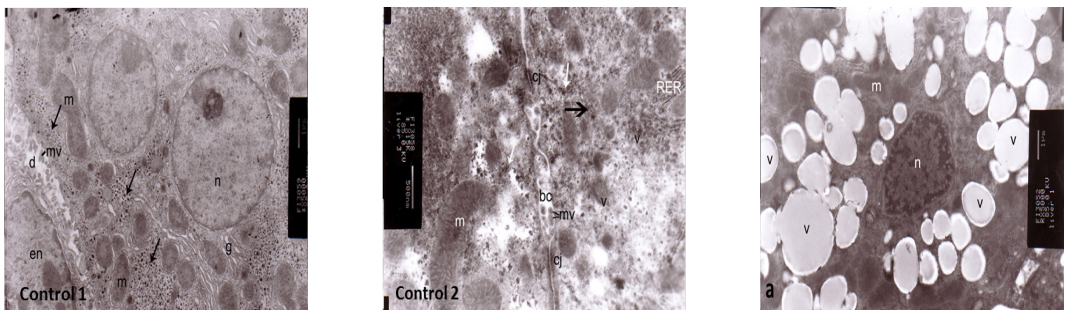


**Fig.3: (Control 1) showing hepatocyte with double nuclei (n), prominent nucleoli, mitochondria (m), well developed Golgi complex (g), numerous glycogen granules (arrows), Space of Disse (d) microvilli (mv), endothelium (en) TEM, x5000. ((Control 2) showing prominent cell junctions between two adjacent hepatocytes; mitochondria (m), rough endoplasmic reticulum (RER), glycogen granules (arrows), bile canaliculus (bc), microvilli (mv), and endothelium (en) TEM, x15000. (a) 24 h after PHx, showing a hepatocyte with numerous cytoplasmic vacuoles (v), electron-dense nucleus (n) with peripheral heterochromatin, and swollen mitochondria with absent clear cristae (m) TEM, x5000.**

### Electron Microscopic Examination

Hepatocytes of control showed rounded single nucleus or double nuclei with a prominent nucleolus, abundant euchromatin, and well defined nuclear envelope. The cytoplasm showed numerous mitochondria with easily recognizable cristae, well developed endoplasmic reticulum, Golgi apparatus, and glycogen granules (figure 3, control). Meanwhile, hepatocytes of group A and B showed marked apoptotic changes. The nuclei showed peripherally distributed extensive heterochromatin and absent nucleoli. The cytoplasm showed an excessive number of electro-lucent vacuoles. The mitochondria showed non-prominent cristae with electron-dense matrix. Moreover, glycogen granules were not detected in the cytoplasm of hepatocytes. Wide elongated bile canaliculi were observed. Blood sinusoids showed overcrowded erythrocytes in their lumen along with absent microvilli (figure 3, a & figure 4, b1-b2). On the other hand, 72 h after PHx, hepatocytes tend to resemble that of the control except for the heterochromatin, which was relatively increased (figure 4, c1-c2).

## DISCUSSION

The liver is one of the few organs that retain the capacity to regenerate after degenerative stimuli such as partial resection, toxic injury, ischemia, and transplantation. It is thus an excellent model for the study of growth regulation. Following PHx, the remaining mature hepatocytes enter a complex process, known as liver regeneration, which after an initial pre-replicative phase, reconstitute the original mass of the liver (16,17). The residual hepatocytes re-enter the cell cycle while the normal homeostatic mechanisms that prevent the cells to re-enter the cell cycle are suspended (18). The present study showed that 24 h after surgical removal of two-thirds of the

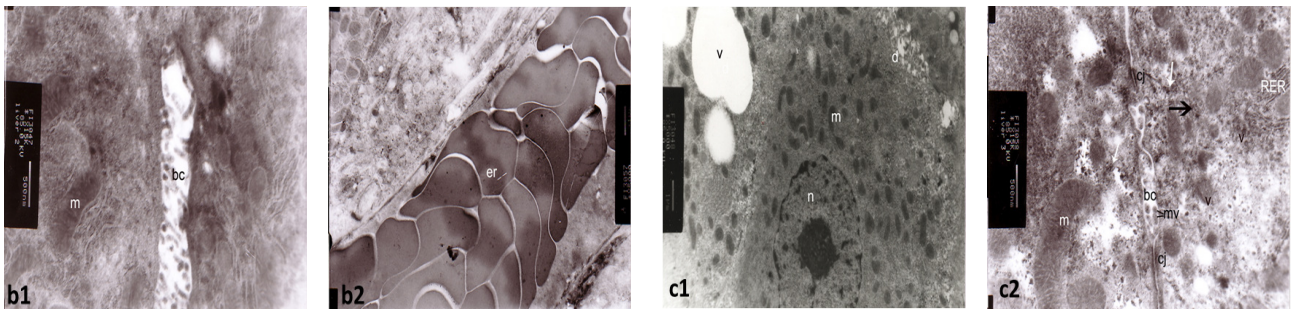


**Fig.4: (b1) 48 h after PHx, showing a bile canaliculus (bc) between two adjacent hepatocytes with microvilli and mitochondria with absent cristae (m) TEM, x15000. (b2) 48 h after PHx, showing a blood sinusoid filled with overcrowded erythrocytes (er). The absence of microvilli of the cell membrane was noted TEM, x5000. (c1) 72 h after PHx, showing a hepatocyte with an electron-lucent nucleus and central prominent nucleolus, cytoplasmic vacuoles (v), small mitochondria (m) TEM, x5000. (c2) 72 h after PHx, showing prominent cell junctions (cj) between two adjacent hepatocytes, mitochondria (m), rough endoplasmic reticulum (RER), glycogen granules (arrow). Dilated bile canaliculus (bc) with microvilli (mv) was noted TEM x15000.**

mass of rat liver, mitochondria in the remaining hepatocytes underwent marked ultrastructural changes. The electron microscopic study showed that the typical transverse alignment of the mitochondrial cristae was nearly absent in the mitochondria of the hepatocytes. This could be suggested as a leading cause of the decrease in the surface area of the inner membrane, thus, the decrease in the ATP synthesis rate, which was observed in mitochondria isolated during the prereplicative phase of liver regeneration (19).

Changes in mitochondria of the hepatocytes of the liver samples obtained 24 and 48 h after PHx were in agreement with Igbavboa and colleagues (20) who reported that the ultrastructural changes observed in liver mitochondria 24 h after PHx were consistent with the changes found in the membrane permeability properties of the mitochondria isolated from the residual liver mass. Moreover, it has been suggested that permeability of the inner mitochondrial membrane could be required for the turnover of matrix proteins following swelling of mitochondria isolated 24 h after PHx. This suggested an involvement of the inner mitochondrial membrane transition pore in the release of matrix enzymes in vivo. Similar findings were reported by previous studies, which proposed that both mitochondrial calcium ions accumulation and oxidative stress increased the probability of changes in the mitochondrial membrane permeability (21-23). Oxidative stress, $Ca^{+2}$ uptake, and the opening of the transition pore in mitochondria are considered as signals of cell death (24,25). On the other hand, 72h after PHx, mitochondria showed normal structure.

In the present study, glycogen depletion was reported 24 h after PHx. Previous studies demonstrated a depletion of glycogen granules 3 hours after hepatectomy (26). Moreover, It was observed that as soon as one hour after surgery, the glycogen depletion occurred (27). The depletion of glycogen may be due to changes associated with the initial stages of cell division (26). 48 h after PHx, the bile canaliculi showed dilatation and tortuosity with elongation of microvilli. These features gradually returned to normal in 72 h after PHx. These findings were in agreement with the morphometric analysis of the liver cells during regeneration after PHx which showed that the mean area and number of bile canaliculi has increased (28). This phenomenon suggested that enlargement of the area of bile canaliculi during liver regeneration may be due to the increase in membranous protein synthesis. Moreover, the increase in the number of bile canaliculi suggests that the proliferation of hepatocytes is associated with the formation of new branches of bile canaliculi.

Dilatation of sinusoids, the disappearance of the microvilli, and marked engorgement of sinusoids with red blood cells were observed 48 h after PHx. These findings were in accordance with a previous study (28), which suggested that a rise in portal pressure, as a consequence of PHx, might be related to the observed ultrastructural changes in the liver blood sinusoids. BCL-2 protein was not detected in control group. After PHx, BCL-2 expression was increased 24 h and 48 h after PHx. This suggests an activation of the apoptotic inhibitory mechanisms, which leads

partially to the activation of the proliferative process. Previous studies suggested that BCL-2 is an adaptive mechanism, which stimulates liver growth in order to resist the effect of bile salts (29). Furthermore, the decrease in cytoplasmic BCL-2 in group C indicates that the process of apoptosis became more active to reformat the architecture of the hepatic lobules and liver lobs. We suggest that the increase in BCL-2 expression was a protective mechanism against the apoptotic changes following the partial hepatectomy. It could be considered as a powerful mechanism to restore liver cell structure and functions after induced liver cell damage caused by PHx.

## CONCLUSION

The present study revealed that 24 and 48 h after PHx, the hepatocytes underwent several changes in the cell organelles and cell inclusions. Upregulation of BCL-2 protein directed the cell homeostasis towards the proliferation to rescuer the normal size of the liver. These changes seemed to be secondary effects and were not of direct importance by evidence that nearly all changes returned to normal 72 h after PHx.

## CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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