International Journal of Clinical and Experimental Medical Sciences
2019; 5(2): 41-45
http://www.sciencepublishinggroup.com/j/ijcems
doi: 10.11648/j.ijcems.20190502.13
ISSN: 2469-8024 (Print); ISSN: 2469-8032 (Online)

Morphological Peculiarities of Apoptosis' Course in Hepatic Cells

Asomiddin Faizovich Sadriddinov[1, 2, *], Khushvakt Beknazarov Joraevich[1], Muborak Asomidinovna Sadriddinova[1], Elena Nikolaevna Guzacheva[1]

[1]Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan

[2]Department of Histology and Pathologic Anatomy, Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan

Email address:
asom_sad_23@mail.ru (A. F. Sadriddinov)

[*]Corresponding author

To cite this article:
Asomiddin Faizovich Sadriddinov, Malika Ilhamovna Kamalova, Elena Nikolaevna Guzacheva, Muborak Asomidinovna Sadriddinova. Morphological Peculiarities of Apoptosis' Course in Hepatic Cells. *International Journal of Clinical and Experimental Medical Sciences.* Vol. 5, No. 2, 2019, pp. 41-45. doi: 10.11648/j.ijcems.20190502.13

Received: March 19, 2019; **Accepted**: May 16, 2019; **Published**: July 12, 2019

Abstract: The course dynamics of apoptosis in hepatic cells of experimental rabbits (n = 16) and white rats (n = 18) was researched by morphological methods. In natural death (apoptosis) the hepatic cells were divided into two parts. At the first type of apoptosis the hepatocyte's cytoplasm became clearing, the little vesicles occurred, therefore this type was called as "boiling cytoplasm". It was more often in rats. The volume of cell increased and became round, the nucleus got peaknotized, and, finally, the apoptotic bodies became formed and eliminated in sinusoids. The second type of apoptosis was characterized with homogenization and acidophilic colour of cytoplasm, the cellular nucleus was decomposed into large conglomerations and kariorexis occurred, so it was marked as "nuclear catastrophe". At the end of both types the hepatocyte cellular membrane got ruptured, and cytoplasm's content separated in lumen of vessel. Sometimes old, not being undergone to apoptosis "longliving" hepatocytes became separated from hepatic plates and wholly extrused in lumen of sinusoid. Conclusion: by the cytological course, depending on mainly damage of hepatocyte' cytoplasm or nucleus two types of apoptosis were divided. Except apoptic death in the liver the extrusion display of "longliving" hepatocytes were revealed.

Keywords: Liver, Morphology, Apoptosis, Hepatocyte Extrusion

1. Introduction

Liver as organ of epithelial origin have cellular composition that is constantly renovating in terms of portovenous gradient [1]. The gradient is formed with hepatic plates, being located between portal tract and central vein. In the gradient the hepatic cells are multiplied by mitosis, then after performing complex functions, they are undergone to natural death [2]. But concerning the ways of natural cellular death there are many unexpected, sometimes, contradictory opinions. Some researchers considered that apoptosis was fleeting process, so it was impossible to identify it with histological preparations [3]. Though, for exact identification of apoptosis, the immunocytochemical method was worked out, but the cytologic characteristics of apoptosis on lighting optical and ultrastructural level was not clear up to the end. As mitosis presents itself like general fundamental biological notion, the apoptosis after specification it's details, should be considered as the necessary part of cells and tissues' development in biology.

The aim of study: Comparative lighting optical, electronic and microscopic study cytological peculiarities of apoptosis' course at some laboratory animals: mongrel rabbits and white rats.

2. Materials and Methods of Study

The livers of pubertal rabbits (n = 16) and white rats (n = 18) of both sexes, mass from 2, 5 to 3 kg, being kept in usual conditions of vivarium, were examined. With the following ethic norms under light ether narcosis the animals were killed with decapitation. A pieces of liver tissue for histological studies were fixed in 12 % solution of neutral formalin for 24

hours. After general histological treatment they were poured in paraffin. The sections for lighting optical study were stained with hematoxylin-eozin, and, then they were examined on immersion objectives (x100) microscope model DN-300, with figure camera. For electron microscopic studies the liver tissue was examined on microscope JEM-100S.

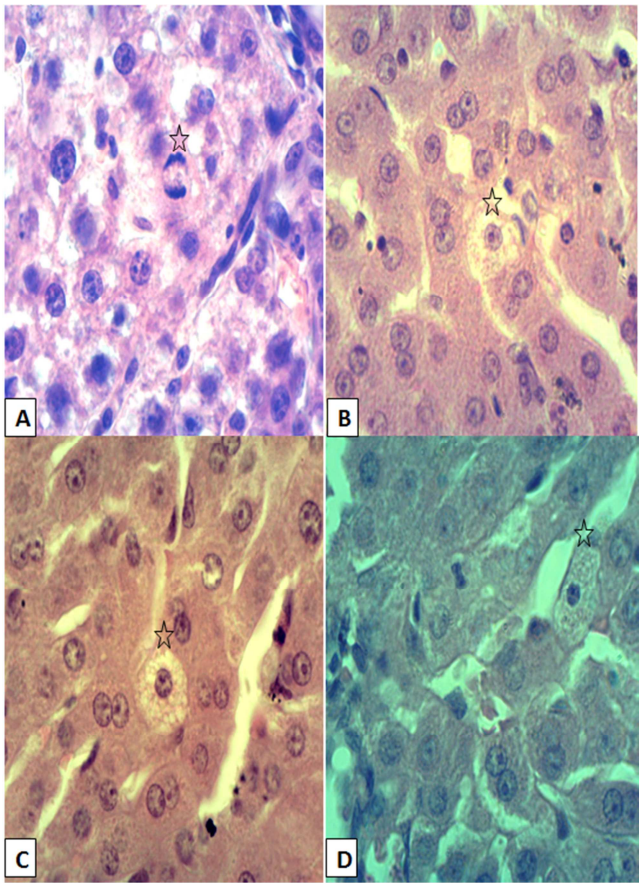

Figure 1. *Morphological patterns of mitotic division and the first type of apoptosis in the porto-venous gradient of the liver. Liver of rat (1a), liver of rabbit (1b-d). Coloring hematoxylin-eosin. Immersion (x100), ocular 15.*

A. Mitotic division of the hepatocyte in the periportal part of the gradient. B. Enlightenment of hepatocyte cytoplasm (apoptosis of the first type). C. "Boiling cytoplasm", apoptosis of the first type (AFT). D. Cytoplasmic vesiculation and pycnosis of the nucleus (AFT).

3. Results of Study

The radially orientated fragment of rabbits and white rats' hepatic lobules formed portovenous gradient. The calculations of cells, forming the gradient, were from 15 to 24 cells. The main functional elements of that gradient were hepatocytes, but the number of multiplying and dying cells were quite little in the lobule. Mitotically multiplying hepatocytes were 1: 2500 and they were revealed on periphery of gradient (Figure 1a). In opposition to it, the extrusing and apoptotically dying cells were found out in perivenous, sometimes, intermediate parts of gradient. The study cells' state of gradient allowed to determine, that the last 3 series of hepatocytes, being terminally located, were undergone to extrusion, whereas the apoptosis' deaths were found in cells under 8-10 positions from perivenous ones in portovenous gradient. According to apoptosis' cytological picture, what part of the cell was more changed (cytoplasm or nucleus) two types of apoptosis were divided. At apoptosis of the first type (AFT) the clarification of cytoplasm and shrinkage of nucleus took place, and, at apoptosis of the second type (AST) the cytoplasm became thickening and nucleus was decomposed into fragments. The single hepatocytes were usually undergone to apoptosis, and, they were sharply differed from surrounding ones with light cytoplasm (Figure 1b). Besides, the primary stage of AFT was characterized by occurrence of 2 - 3 large vacuoles in cytoplasm of hepatocyte, the number of those were gradually increased, occupying all cytoplasm, and in nucleus the nucleolus disappeared, the chromatin was concentrated in little lumps, the nucleus' volume was decreased and the peaknosis occurred (Figure 1d). At the next stage the apoptotic cell increased in volume, the cytoplasm became foamy and round, but the intercellular contacts with surrounding cells were still kept (Figure 1c). Then the cellular nucleus became shriveled and large vacuoles of cytoplasm turned out in little light cyst vesicles (Figure 1c, d), in connection with it, the given type was called "boiling plasm".

The apoptosis of the second type was characterized with thickening and homogenization of cytoplasm into fine-grained material of acidophilic colour, the nucleus became at once decomposed into large fragments (Figure 2a). The formation of thick and formless nuclear condensates were well seen in picture 2d, in connection with that, it was marked as "nuclear catastrophe", besides, the given cell began separating from neighbouring cells. These two kinds of apoptosis really taking place in the liver were clearly demonstrated on little part of parenchyma (Figure 2b), where one cell had light cytoplasm (AFT) and the other was with lump like decomposition of nucleus and dark cytoplasm (AST) (Figure 2c). To the apoptosis were undergone both one-nuclear and two-nuclear cells, in apoptosis' case of the two-nuclear cells, both nuclei were pouring out together forming large conglomerate. Therefore, in both cases, to the full disorganization were undergone both the hepatocyte's nucleus and cytoplasm. At the final stage both apoptosis of cellular membrane became ruptured and cellular content was separated in lumen of sinusoid (Figure 2c, d). The integrity of hepatic cytoarchitecture plate was restored on the account of replace for neighboring cells. Our lighting optical data were proved with electron microscopic researches. The primary stage of the first type apoptosis was characterized with sharp swelling and rounding of cytoplasm, because the cell looks like "hypertophic one" (Figure 3).

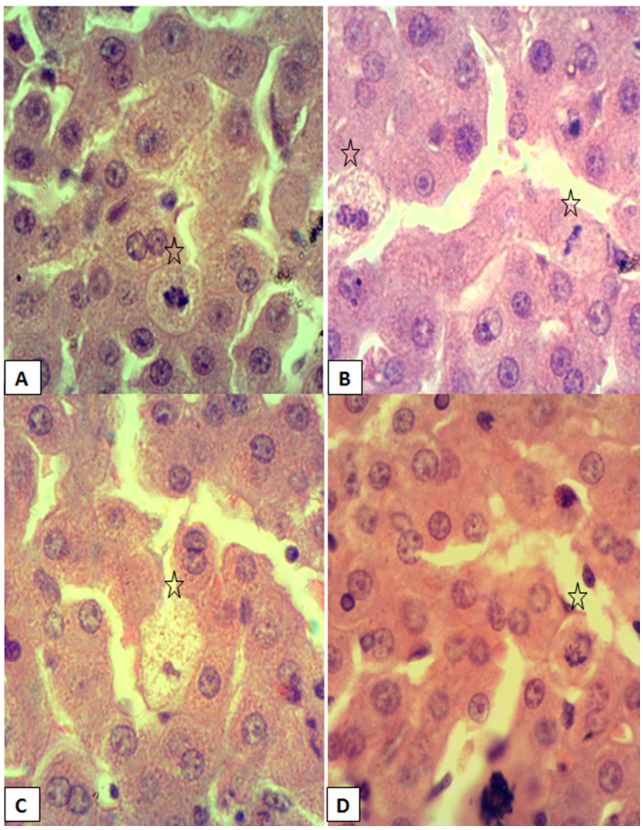

Figure 2. *Morphological picture of the first and second type of apoptosis. Rabbit liver.*

A. Homogenization of cytoplasm and chromatin nucleus lumps (apoptosis of the second type). B. Cytological picture of the first and second type of apoptosis. C. The final stages of the (AFT) and the elimination of apoptotic cell into the lumen of sinusoid. D. The final stage of (AST), was rupture of membrane and leaching of the cell into the lumen of sinusoid.

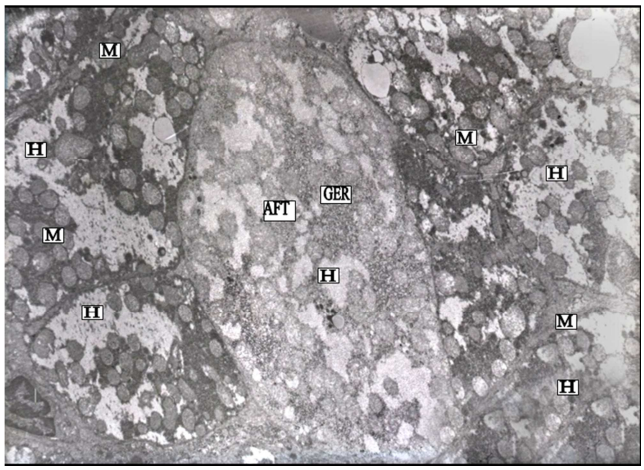

Figure 3. *Ultrustructure of apoptically decomposing hepatocyte. Rat's liver. There is "hypertrophic" hepatocyte by apoptosis, start of cytoplasm's vesiculation (AFT) n. 5600;*

H-hepatocytes; AFT-apoptosis of the first type;
M- mitochondria; GER-granular endoplasmic reticulum.

The subcellular elements became loose and presented with little vesical structures. Due to disintegration and organelle's fragmentation the apoptotic bodies were formed, being composed from hardly noticeable mitochondria, the canaliculi of endoplasmatic reticulum, Golgi complex, and, also, lysosomes and phagosomes. The residual of cellular nuclei were revealed as chromatin's lumps between apoptotic bodies, and, the wedged fragments of erythrocytes between them, that witnessed on rupture of cellular membranes and taking out the apoptotic bodies to sinusoid lumen (Figure 4). The moment of extrusioning cell's rupture and simultaneously decomposition of hepatocyte's cytoplasm at the secondary type of apoptosis were demonstrated in figure 5, where the fragment of hepatocyte with still little part of cytoplasm had the contact with neighboring one, and, close to it, there were the elements of cellular decomposition (cellular detritus AST). Around the fragments of these dying cells the reticular fibers, cellular residue, and, also contacting macrophage or it's fragments with lysosomes and phagosomes, were found out. All these structures, the presence of endothelial lining and erythrocytes in lumen of vessel proved that it was the perivenous part of portovenous gradient where more often marked the apoptotic death or hepatocytes' extrusion.

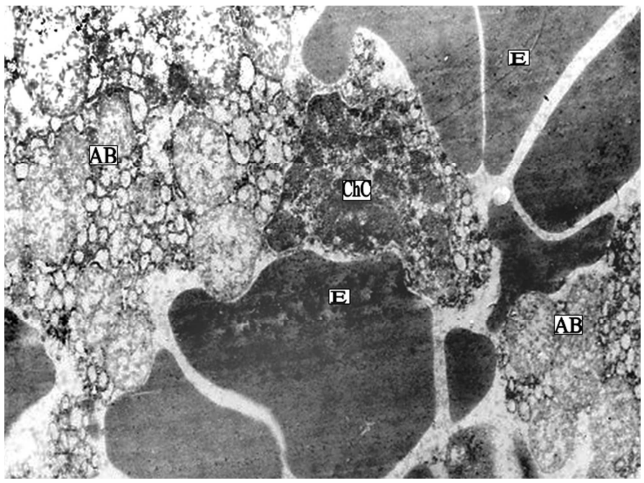

Figure 4. *Apoptosis of the second type. Cell disintegration into apoptotic bodies. Rat's liver. E-Erythrocyte; ChC- chromatin clumps; AB-apoptotic body.*

At lighting optical study extrusion there was typical disorder of cell's contact with surroundings and replacement to the vessel lumen. The boundaries of such cell were diffused, and, sometimes, the nucleus was revealed as slight shadowing, and, in other cases it was like decomposed chromatin's fragments, and the cell obviously "crawled out" in lumen of central vein or sinusoid.

The cells extrusion was more clearly revealed at electron microscopic study. The separated hepatocyte had irregular form, well-preserved subcellular elements, and, it's poles were easy differed. The biliary pole was characterized with desmosome's residue and presence of many secretory granules, but sinusoid pole had short microvilli and large amount of less changed organelles (Figure 5). Unlike apoptosis at extrusion the nucleus of cell was swelling, the chromatin's condensates were absent but the nucleoli's structure was sharply changed and had little sizes, and,

instead of granular and fibrillary elements it was presented with dense filamentous structures. But, finally, the concrete sign of extrusion was in direct contact of given cell, intravascular located erythrocytes and absence of endothelial lining.

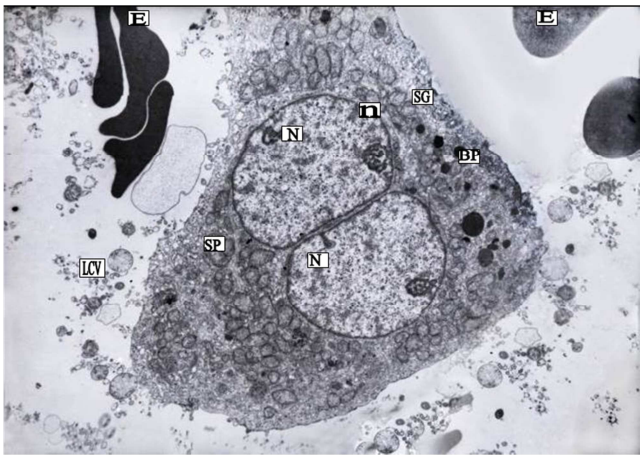

Figure 5. *Electrone microscopic study of rabbit's liver. Extrused hepatocyte and contacting erythrocytes with it (explanation in the text).*

N-nucleus; n-nucleolus; E– erythrocytes; SG-secretory granules; LCV-lumen of central vein; M-mitochondria; BP –biliary pole; SP-sinusoid pole of hepatocyte.

4. Discussion of Taken Results

Morphological analysis of portovenous gradient allowed to reveal the single figures of mitotic cellular multiply, mainly, in periportal zones of hepatic lobule. These results were coordinated with researches [4] which showed that at introduction H 3- timedin the less differentiated hepatocytes were localized in terms 200 mcm from portal tracts. Probably, in this zone the cellular proliferation was formed, which being differentiated, replaced to the central vein. The topographic analysis of hepatocytes showed that separate cells of intermediate, and, mainly, central zone of hepatic lobule, were undergone to apoptosis. The taken data proved the studies [5], which were also described the apoptosis of the latest 2 series for hepatic cells, being located around the terminal hepatic venule and sometimes between 3-5 series. Two types of apoptosis' development were divided into: clarification of cytoplasm, shrinkage of nucleus being replaced with cytoplasm's vesiculation (it was obviously according to apoptosis bodies) and peaknosis of nucleus (the first classical sign of apoptosis), thickening cytoplasm, nuclear fragmentation. At the end of apoptosis the elimination of apoptotic bodies to lumen of vessels occurred in both cases. The first signs both apoptosis and extrusion were apparently the changes of nucleolus. At apoptosis the nucleolus disappears among the chromatin's residues of nuclei, but at extrusion it's structure becomes undergone to deep changes, that is, granular and fibrous elements turn out into thickened filamentous structures. The primary changes of hepatocyte at apoptosis were, obviously, connected with changes of nuclear structure that led fast to it's thickening, probably, on the base of it there was the proteolytic action of caspase [6]. The cytoplasm's clarification joined the nuclear shrinkage, being transient to vesicular decomposition. At the second type of apoptosis it was marked the cytoplasm's thickening with simultaneously lump decomposition of nucleus, but some authors consider that it was necrosis [7]. It was supposed that cytoplasm's clarification at apoptosis was caused by disorder of osmotic pressure, occurring because of change calcium ions's concentration [8]. Between apoptotic bodies or vesicular elements the chromatin's lumps were electron microscopically revealed, that was the main sign of programmed cellular death [9]. Some researchers showed that at the end the apoptotic bodies were caught by close located cells or macrophages [10], but the studies showed that in the liver the apoptotic bodies were eliminated in lumen of sinusoid, and, not often, if it occurred with terminal hepatocytes they became phagocytized with close located macrophages. In the liver, together with apoptosis, like epithelium of other organs, the extrusion displays were revealed when the whole cell was separated from hepatic plate with less changed form and subcellular structures. It can be supposed that such cell can be as "longliving" among other groups of hepatocytes, those were undergone to extrusion. The results of our study proved the existence of portovenous gradient in the liver, in terms of occurred new formation of young hepatic cells and death of old hepatocytes. Besides, the hepatocytes gradually replaced from periportal zones to perivenous ones, where they, achieving their final goal, i.e. terminal hepatic venules, were undergone to extrusion or programmed death. According to data the hepatic cells were transferred from periportal zone in direction of central vein with the speed 1, 44 mcm/day, and, the cycle of cellular development from 200 days to 1 year [4]. If to take for the base the average number of cells, composing portovenous gradient, it was equal to 17-18 cells, the middle diameter of separate hepatocyte was 22-25 mcm, therefore, the general length of portovenous gradient was 18 x 25 = 450 mcm, and, for moving cell it was necessary 450: 1, 44 = 312, 5 days. So, for full renovation of gradient's cells it took about a year (312, 5 days). Coming from it, it can be concluded that the liver is slowly renovating organ, and, it's cells are relatively "longliving" [11].

Thus, the taken studies showed that in periphery the little differentiated cells were undergone to multiply, and, on the way of cells' movement to the side of central vein their differentiation and specialization were carried out. Then at finishing definite time by becoming cells aging, they were undergone to apoptotic death, those by cytologic picture were divided in two types. The more strong "longlivings", reaching to perivenous areas, had to tear off from the "life" by the extrusion.

5. Conclusions

The portovenous gradient was in the liver, in it's periportal part the cells were multiplied by mitosis, and, in intermediate and perivenous part, they were undergone to apoptotic death

or extrusion.

The death of cells occurred by apoptotic way, it's two types were found out: the first type was cytoplasm's vesiculation and nuclear peaknosis; the second one was cytoplasm's thickening and lump like nuclear decomposition with the following elimination of cellular dentritus to the lumen of sinusoid.

It was determined that the liver refers to the slow renovating organs, the cellular cycle of hepatocytes was one year (312, 5 days).

References

[1] Aruin l. I. Apoptosis and pathology of liver., // Russian jornal Gastroenterology, Hepatology Coloproctology.-2008.-№2.- P. 6-10.

[2] Belushkina I. I. Molecular bases of apoptosis' pathology., //Archives of pathology.-2001.- V. 63. №1. - P. 51-60.

[3] Zalesskiy V. N., Velikaya N. V. Apoptosis mechanisms at hepatic diseases.,// Modern problems of toxicology.- 2002. - № 4. - P. 27-32.

[4] Arber N., Zajicek G., Arnicl J. The streaming liver // Liver-2008. - P. 80-87.

[5] Benedetti A., Jezugel A. M., Oriondi F. Preferential distribution of apoptotic bodies in acinar zone 3 of normal human and rat liver // J. Hepatol.- 2012.-7. –P. 319-324.

[6] Cande C., Cecconi F., Dessen P. Apoptosis-inducing factor: key to the conserved caspase- independent pathways of cell death? // Cell Sci. 2002. - V. 115-P. 4727-4734.

[7] Ivashkin V. T., Cellular and molecular biology of liver inflammation.,// Russian journal Gastroenterology, Hepatology, Coloproctology.- 2005.- W. 15.– P. 13-17.

[8] Vinogradov A. A. Influence of doxirubicine on the apoptosis' process in hepatic cells at experiment, // Ukrainian journal of experimental medicine.- 2008.- V. 9. №4. - P. 59-61.

[9] Kerr J. F. R., Willie A. H., Carrie A. R. Apoptosis: a basic biological phenomen with wide-ringing implications tissue kinetics // Brit. J. Cancer- 2012.-26. –P. 239-257.

[10] Colombono A., Ledda-Columbano G. M. Com G. et al. Occurrence of cell death (apoptosis) during the involution of liver hyperplasia // Lab. Invest. -2015-51.-P. 670-675.

[11] Sadriddinov A. F., Isaeva N. Z. Portovenous gradient is functional element of liver. /In mater. collect: International extra (заочная) research practical conferens "Scintific discussion: Medical questions`. Moscow, 2013, -P. 57-66.