MORPHOLOGICAL CHANGES OF THE HEPATIC PORTAL TRACTS IN EXPERIMENTALLY INDUCED CHOLESTASIS

Kikalishvili L., Jandieri K., Turmanidze T., Jandieri L.

Tbilisi State Medical University, Department of Clinical Anatomy and Operative Surgery, Georgia

Difficulties in the diagnostics and treatment of pathological changes in the liver during biliary obstruction are one of the important challenges in hepato-biliary surgery. The last few decades have seen an increase in the number of liver diseases with impaired bile secretion, which has affected the average life expectancy [2].The prolonged biliary obstruction and bilirubinemia cause severe impairment of all liver functions and development of liver failure [1,3,4].

The above-mentioned causes the multiple studies conducted in different experimental animals. Common bile duct ligation (CBDL) in rodents is one of the widely used model for studying of different features of biliary obstruction. CBDL induces the cholestasis, biliary hypertension and bilirubinemia (followed by mechanical jaundice) causing significant changes in the bile ducts and liver tissue. The acute cholestasis induces proliferative processes in the liver tissue. This phenomenon which has been confirmed by many researches [5,7-9] starts as early as on the 2nd-3rd days and lasts for several weeks. The proliferation of cholangiocytes first appears in the large bile ducts (>15 μ) and later in the small ones (<15 μ) [6,7].

The proliferative processes in the liver caused by CBDL represents the specific type of ductular reaction (DR) – the process that accompanies different acute and chronic liver pathologies in humans or experimental models and reveals with the increased number of ductular profiles on the histological slices of liver tissue [9,12,16,17,19-21].

The dynamics of morphological changes developed in liver and bile ducts in biliary obstruction are well studiedin the models with CBDL [4,10,13-15]. However, the data on the morphology of the portal tracts and interrelationship of their components are scarce.

The aim of the research was to study the structure of the portal tracts and interrelationship of their structural components in experimental biliary obstruction.

Material and methods. Experimental cholestasis was induced with CBDL of 15 albino Wistar rats weighing 200-250 g.

CBDL was performed in accordance with technique described by Lee (laparotomy and dissection of CBD between two ligatures. 5 rats served as a control underwent the sham operation (only laparotomy). All interventions were performed under general ether anesthesia.

The livers of animals with CBDL were studied on the 3rd, 6th and 12th days after operation (5 animals on each term).In every case the adequacy of biliary obstruction was confirmed by the presence of an enlarged and tensed common bile duct filled with congested bile.

The histological, histochemical and immunohistochemical investigation of the 4 μ sections of liver tissue fixed in 12% neutral formalin,embedded into paraffin and stained with Hematoxylin& Eosin, Masson's trichrome (№C 970D37, Sigma-Aldrich Inc.) and marked with Kertain-8 (KRT8) (catalog N MBS8510691) MyBiosourse) antibodywere performed.

Results and discussion. In a normal liver, the elements of a large-caliber portal triad are surrounded by a well-distinguishable connective tissue sheath, the partitions of which extend between the single elements, creating certain compartments for each of them. As the caliber of the portal tracts decreases, the expression of the connective tissue covering decreases, and the portal triad elements become more closely related to each other. Thelumen of the portal vein is accompanied by 2-3 ductular profiles, the calibers of which may vary. The bile ducts are located at the periphery of the portal vein interfacing with the derivate of Laennec's capsule and bordering plate of lobular parenchyma; the arterial branches are located between the bile ducts and the portal vein branch.In addition, in some interlobular portal tracts, the arteries can not be detected at all. On the 3rdday after CBDL, the common bile duct appeared to be macroscopically enlarged and filled with bile. The wall of the duct was tensed. The liver was also slightly enlarged and tensed. No noticeable changes were observed in other organs.

Because of enlarged bile ducts in the portal tracts of big caliber, the interrelationship among the portal triad elements and thus, the construction of portal tracts in whole differs from the control. Besides, the diameters of small bile ducts do not undergo notable changes. In all portal tracts, the increase of the number of connective tissue fibers with the expansion of the portal area is expressed (Fig. 1).

Both inside the portal tract and at the border of the portal tract and parenchyma, multiple ductular profiles of small caliber are observed, which, like the bile ducts, express CK8 well (Fig. 2).

Occasionally, the sites of necrosis are found in the vicinity of the portal areas, which are sharply demarcated by the surrounding normal parenchymal structures and connective tissue of a given portal tract. On the 6th day after CBDL the common bile duct appeared to be markedly enlarged, tensed, and overfilled with bile. All organs including the slightly enlarged liver had a yellowish tinge.

Large caliber bile ducts were further enlarged and put pressure on the portal vein, causing the deformation of its lumen. Dilatation of the bile ducts was less noticeable in the small-caliber portal tracts. The amount of connective

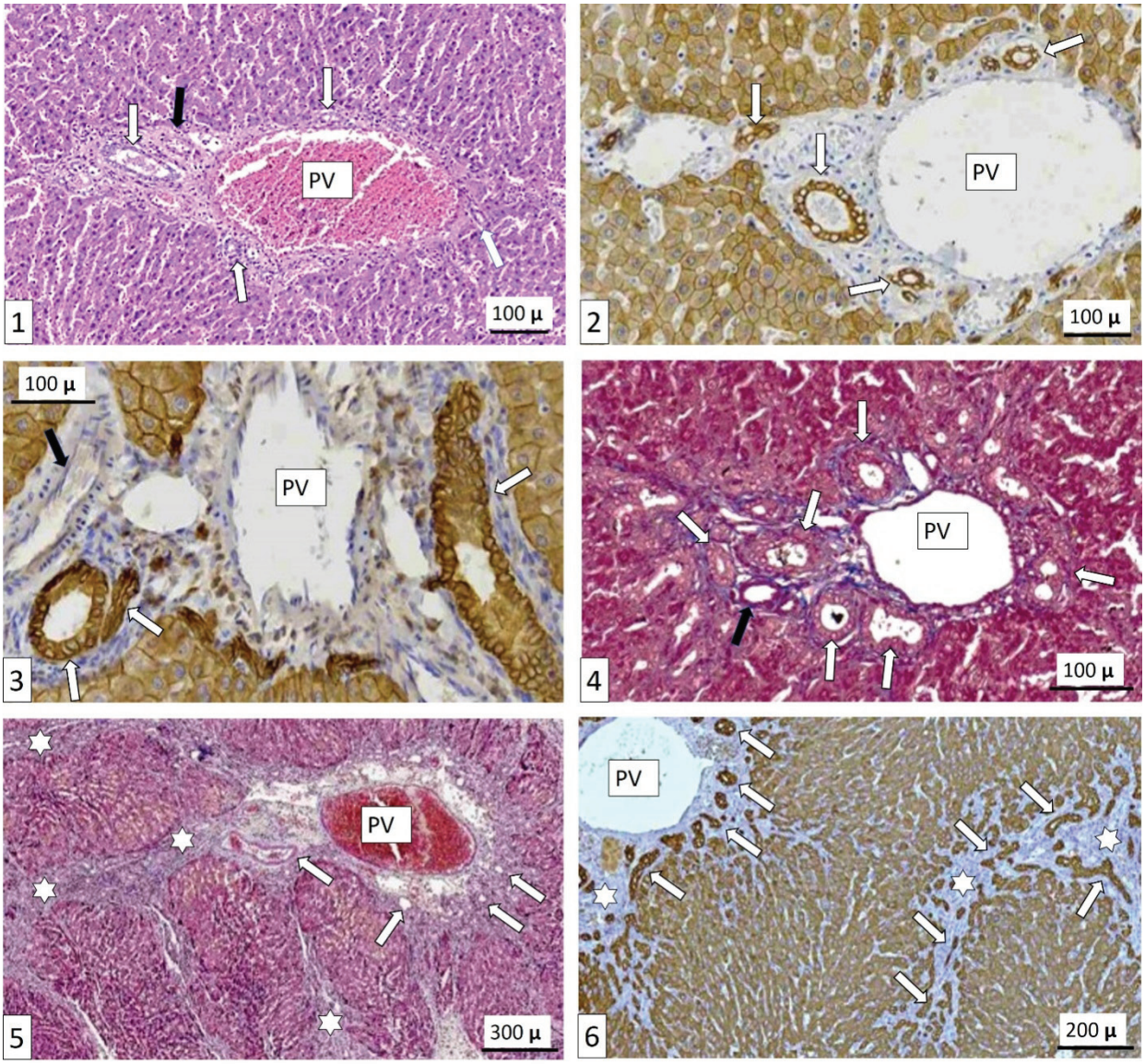

Fig. 1. Portal tract on the 3rd day after CBDL. PV- portal vein; white arrow – bile duct; black arrow – hepatic artery. H&E.
Fig. 2. Portal tract on the 3rd day after CBDL. PV- portal vein; white arrow – bile duct. CK8.
Fig. 3. Portal tract on the 6th day after CBDL. PV- portal vein; white arrow – bile duct; black arrow – hepatic artery. CK8.
Fig. 4. Portal tract on the 6th day after CBDL. PV- portal vein; white arrow – bile duct; black arrow – hepatic artery. Masson's trichrome.
Fig. 5. Portal tract on the 12th day after CBDL. PV- portal vein; white arrow – bile duct; star – fibrous sept. Masson's trichrome.
Fig. 6. Portal tract on the 12th day after CBDL. PV- portal vein; white arrow – bile duct; star – fibrous sept. CK8

tissue in both types of portal tracts was increased (Fig. 3).

On the 6th day after biliary obstruction, there is an increase in a number of the ductular profiles in both large and small portal tracts, but especially in the periportal areas of smaller portal tracts and in connective tissue septs formed between the neighboring portal tracts, which gives the impression that adjacent lobules are delimited by the complex of ductules (Fig. 4).

In addition, the ducts gradually begin to penetrate into the lobules of the liver. On histological preparations, they are visualized as round or oblong ductular profiles, the walls of which are formed by cholangiocytes.

On the 12thday after CBDL, the common bile duct was more enlarged in comparison with the duct on the previous term, tensed, and overfilled with bile. All organs including the slightly enlarged liver had a yellowish tinge, the intensity of which was also increased compared to the previous term.

The lumens of the bile duct In large-caliber portal tracts are even more enlarged than before. Their pressing on the portal vein branch causes both deformation (flattening) of the portal vein lumen and a change in the architectonics of the entire portal tract. This is facilitated by pronounced fibrotic events.

The fibrous changes in the small portal tracts and surrounding parenchyma are so pronounced that their ratio is equal to that of the parenchymal one (Fig. 5). The hepatocytes, which show virtually no signs of damage, appear as separate clumps in areas sharply demarcated by fibrous tissue. Fibrous tissue is practically completely saturated with ductal profiles and their accompanying blood vessels and inflammatory cells (ductular reaction) (Fig. 6).

Conclusion. The CBDL-induced biliary obstruction triggers a systemic reaction of the bile ducts and a complex of accompanying reactions that is heterogeneous, depending on the caliber of both the bile ducts themselves and the portal tracts containing them.

CBDL causes dilation of the large bile ducts, their pressure on the portal veins with the deformation of their lumens, changes in the architectonics of the portal tracts, and expansion of the portal areas accompanied by increasing portal fibrosis and ductular reaction.The small bile ducts are less prone to dilation under CBDL conditions but actively proliferate and penetrate widely into the parenchyma of the liver lobules. Based on this ductal reaction, fibrosis of increasing intensity develops, which connects the adjacent portal tracts as well as the portal tracts and the connective tissue sheaths of the thin tributaries of hepatic veins. In the conditions of such fibrosis, it is difficult to identify the individual portal tracts of small caliber and, moreover, to clarify the relationship between their elements.

REFERENCES

1. Борисенко В.Б., Сорокина И.В., Горголь Н.И. «Патоморфологическая характеристика холедоха при экспериментальном холестазе». Вестник проблем биологии в медиц. 2014-4, том3 (115) стр. 261-266.
2. Даценко Б.М., Борисенко В.Б. Механическая желтуха, острый холангит, билиарный сепсис. Новости хирургии, том 21, №5 2013
3. Демешко Н.Д., Распространение крупных желчных протоков в печени человека. Дисс., Воронеж, 1955.
4. Кикалишвили Л.А. Влияние на организм временного выключения печени из кровоснабжения при нормотермии, застое желчи, гипотермии при условиях лечения препаратом “Плаферон-ЛБ”. Автореферат дисс. доктора медицинских наук. Тб.: 1999; 96.
5. Ахаладзе ГГ. Гнойный холангит: вопросы патофизиологии и лечения. Журнал доказ. медицины для практ.врачей Том 05/N 4/2003
6. Alpini, G., J.M. McGill N.F. Larusso (2002). «The pathobiology of biliary epithelia.» Hepatology 35(5): 1256-1268.
7. Aslamazishvili, T., D. Kordzaya, G. Shaishmelashvili, J. Pharcakhashvili and I. Kureli (2007). Proliferation of biliary epithelial cells in early stage of cholestasis. Experimental and Clinical Medicine 4(37): 4.
8. Azmaiparashvili, E., Kordzaia D., Dzidziguri D. Biliary hypertension as the cell proliferation trigger in bile duct ligated rats. Georgian Med News 2009 (168): 111-116.
9. Azmaipharashvili E., Berishvili E., Jangavadze M., Kordzaia D. Study on the Origin of «Newductules» Appearing in the Rat Liver in Several Hours After Common Bile Duct Ligation. Acta MorpholAnthropol. 2012;18:8–13.
10. Chanukvadze I. Portacaval Fibrous Connections: Little Known Anatomical Structures of Liver. Rom Med J. 2017;64 (1):43-48
11. Chanukvadze I., Archvadze V., Soreli M. Bilio-vaskular architecture of main magistral portal trakts. Georgian Critikal Care Medicine Institute Tbilisi. New Steps in Critikal Care Medicine. Materials of Tbilisi Fourt International Conference. Tbilisi, Georgia - Tel-Aviv, Israel. 9-10. 2012; 77-81.
12. Desmet, V.J. (2011). «Ductal plates in hepatic ductular reactions. Hypothesis and implications. I. Types of ductular reaction reconsidered.» Virchows Arch 458(3): 251-259.
13. Esrefoglu, M., M. Gul, M. H. Emre, A. Polat and M. A. Selimoglu (2005). «Protective effect of low dose of melatonin against cholestatic oxidative stress after common bile duct ligation in rats.» World J Gastroenterol 11(13): 1951-1956.
14. Falkowski, O., H.J. An, I. A. Ianus, L. Chiriboga, H. Yee, A. B. West and N.D. Theise (2003). «Regeneration of hepatocyte ‘buds’ in cirrhosis from intrabiliary stem cells.» J Hepatol 39(3): 357-364.
15. Georgiev, P., A.A. Navarini, J. J. Eloranta, K. S. Lang, G.A. Kullak-Ublick, A. Nocito, F. Dahm, W. Jochum, R. Graf and P.A. Clavien (2007). «Cholestasis protects the liver from ischaemic injury and post-ischaemic inflammation in the mouse.» Gut 56(1): 121-128.
16. Georgiev, P., W. Jochum, S. Heinrich, J.H. Jang, A. Nocito, F. Dahm and P.A. Clavien (2008). «Characterization of time-related changes after experimental bile duct ligation.» Br J Surg 95(5): 646-656.
17. Glaser S.S., P. Onori, C. Wise, F. Yang, M. Marzioni, D. Alvaro, A. Franchitto, R. Mancinelli, G. Alpini, M. K. Munshi and E. Gaudio (2010). «Recent advances in the regulation of cholangiocyte proliferation and function during extrahepatic cholestasis.» Dig Liver Dis 42(4): 245-252.
18. Kiss, A., J. Schnur, Z. Szabo and P. Nagy (2001). «Immunohistochemical analysis of atypical ductular reaction in the human liver, with special emphasis on the presence of growth factors and their receptors.» Liver 21(4): 237-246.
19. Kordzaia D, Chanukvadze I, Jangavadze M. FunctionalAnatomy of Intrahepatic Biliary System (Clinical and Experimental Data). In: Miguel Ángel Mercado, editor. Bile Duct: Functional Anatomy, Disease and Injury Classification and Surgical Management. Nova Science Publishers, Inc; 2014. p. 1–87.
20. Yin, L., D. Lynch, Z. Ilic and S. Sell (2002). «Proliferation and differentiation of ductular progenitor cells and littoral cells during the regeneration of the rat liver to CCl4/2-AAF injury.» Histol Histopathol 17(1): 65-81.
21. Yoshioka, K., A. Mori, K. Taniguchi and K. Mutoh (2005). «Cell proliferation activity of proliferating bile duct after bile duct ligation in rats.» Vet Pathol 42(3): 382-385.

SUMMARY

MORPHOLOGICAL CHANGES OF THE HEPATIC PORTAL TRACTS IN EXPERIMENTALLY INDUCED CHOLESTASIS

Kikalishvili L., Jandieri K., Turmanidze T., Jandieri L.

Tbilisi State Medical University, Department of Clinical Anatomy and Operative Surgery, Georgia

The dynamics of morphological changes developed in liver and bile ducts in biliary obstruction are well studied in the animal models of biliary obstruction, however,the data on the morphology of the portal tracts are scarce.

The aim of the research was to study the structure of the portal tracts and interrelationship of their structural components in experimental biliary obstruction.

The investigation was conducted on albino Wistar rats weighing 200-250 g, undergone biliary obstruction by common bile duct ligation (CBDL). The histological, histochemical and immunohistochemical investigation of the liver tissue stained with Hematoxylin& Eosin, Masson's trichrome and marked with CK8antibody were performed on the 3rd, 6th and 12th days after CBDL.

The CBDL-induced biliary obstruction triggers a systemic reaction of the bile ducts and a complex of accompanying reactions that is heterogeneous, depending on the caliber of both the bile ducts themselves and the portal tracts containing them.CBDL causes dilation of the large bile ducts, their pressure on the portal veins with the deformation of their lumens, changes in the architectonics of the portal tracts, and expansion of the portal areas accompanied by increasing portal fibrosis and ductular reaction.

The small bile ducts are less prone to dilation under CBDL conditions but actively proliferate and penetrate widely into the parenchyma of the liver lobules. Based on this ductal reaction, fibrosis of increasing intensity develops, which connects the adjacent portal tracts as well as the portal tracts and the connective tissue sheaths of the thin tributaries of hepatic veins. In the conditions of such fibrosis, it is difficult to identify the individual portal tracts of small caliber and, moreover, to clarify the relationship between their elements.

Keywords: liver, portal tracts, cholestasis.

РЕЗЮМЕ

МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ПОРТАЛЬНОМ ТРАКТЕ ПЕЧЕНИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ХОЛЕСТАЗЕ

Кикалишвили Л.А., Джандиери К.Д., Турманидзе Т.Р., Джандиери Л.А.

Тбилисский государственный медицинский университет, департамент клинической анатомии и оперативной хирургии, Грузия

При обструкции желчных протоков динамика развившихся изменений в печени хорошо изучена у экспериментальных животных, однако данные о морфологии портального тракта незначительные.

Целью исследования явилось определение структурных элементов портального комплекса и их взаимоотношений при экспериментальном холестазе.

Эксперименты проведены на белых крысах породы Wistar, весом 200-250 г. После окклюзии общего желчного протока на 3-ий, 6-ой и 12 дни гистологическое, гистохимическое и имунногистохимическое изучение ткани печени проведено методами окраски гематоксилин-эозином, по Массону - трихром и СК8 маркером.

Окклюзия общего желчного протока вызывает системную и сопутствующую реакцию желчных протоков, которая гетерогенна, и зависит как от калибра желчных протоков,так и от калибра портальных трактов.

Окклюзия общего желчного протока вызывает расширение крупных желчных протоков с последующим давлением на воротные вены с их деформацией, изменением архитектоники портального тракта и расширением портальных участков, заканчивающихся фиброзом и дуктулярной реакцией.

При окклюзии общего желчного протока внутрипеченочные желчные протоки малого калибра расширяются в меньшей степени, однако активно происходит их пролиферация и внедрение в печеночные дольки. В связи с этим развивается интенсивный фиброз, объединяющий близлежащие портальные тракты между собой, а также связывающий их с соединительнотканными оболочками мелких притоков печеночных вен. При таком фиброзе сложно идентифицировать портальные тракты малого калибра и тем более распознать взаимоотношение между их элементами.

რეზიუმე

ღვიძლის პორტული ტრაქტის მორფოლოგიური ცვლილებები ექსპერიმენტული ქოლესტაზის პირობებში

ლ.კიკალიშვილი,ქ.ჯანდიერი,თ.თურმანიძე, ლ.ჯანდიერი

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, კლინიკური ანატომიის და ოპერაციული ქირურგიის დეპარტამენტი, საქართველო

ღვიძლში და ნაღვლის სადინრებში ობსტრუქციის დროს განვითარებული მორფოლოგიური ცვლილებების დინამიკა კარგად არის შესწავლილი ექსპერიმენტულ ცხოველებში, თუმცა მონაცემები პორტული ტრაქტის მორფოლოგიაზე მწირია.

კვლევის მიზანს წარმოადგენდა პორტული ტრაქტების სტრუქტურისა და მათი სტრუქტურული ელემენტების ურთიერთდამოკიდებულების შესწავლა ექსპერიმენტული ქოლესტაზის პირობებში.

კვლევა ჩატარდა ვისტარის ჯიშის თეთრ ვირთაგვებზე, რომელთა წონა იყო 200-250 გ. ნაღვლის საერთო სადინრის გადაკვანძვიდან მე-3, მე-6 და მე-12 დღეს ჰემატოქსილინ-ეოზინით, მასონის ტრიქრომის შეღებვის მეთოდით და CK 8 ანტისხეულის მარკერის საშუალებით ჩატარებული იყო ღვიძლის ქსოვილის ჰისტოლოგიური, ჰისტოქიმიური და იმუნოჰისტოქიმიური კვლევა.

ნაღვლის საერთო სადინრის გადაკვანძვა იწვევს სანაღვლე სადინრების სისტემურ რეაქციას და თანმხლები რეაქციების კომპლექსს, რომელიც ჰეტეროგენურია, რაც დამოკიდებულია როგორც თავად სანაღვლე გზების, ასევე პორტული ტრაქტების კალიბრზე. ნაღვლის საერთო სადინრის გადაკვანძვა იწვევს ნაღვლის მსხვილი სადინრების გაფართოებას, მის ზეწოლას კარის ვენებზე, რის შედეგადაც ვითარდება სანათურების დეფორმაცია, პორტული ტრაქტის არქიტექტონიკის ცვლილება და პორტული უბნების გაფართოება, რაც, თავის მხრივ, იწვევს კარის ფიბროზსა და სადინრების რეაქციის ზრდას.

ნაღვლის საერთო სადინრის გადაკვანძვის პირობებში ნაღვლის მცირე სადინრები ნაკლებადაა მიდრეკილი გაფართოებისკენ, მაგრამ აქტიურად ხდება მათი პროლიფერაცია და ფართოდ შეღწევა ღვიძლის წილაკების პარენქიმაში. ამ სადინრების რეაქციის საფუძველზე ვითარდება მზარდი ინტენსივობის ფიბროზი, რომელიც აკავშირებს მომიჯნავე პორტულ ტრაქტებს ერთმანეთთან და აგრეთვე აკავშირებს პორტულ ტრაქტს და ღვიძლის ვენების წვრილი შენაკადების შემაერთებელი ქსოვილის გარსებს. ასეთი ფიბროზის პირობებში ძნელია მცირე კალიბრის ცალკეული პორტული ტრაქტის იდენტიფიცირება და უფრო მეტიც, მათ ელემენტებს შორის ურთიერთობის გარკვევა.

EFFECTS OF IONIZING RADIATION ON COGNITIVE PARAMETERS IN WHITE MICE

Kalmakhelidze S., Museridze D., Gogebashvili M., Lomauri K., Gabunia T., Sanikidze T.

Tbilisi State Medical University; Beritashvili center of Experimental Biomedicine, Georgia

Ionizing radiation has multiple effects on brain functioning, behavior, and cognitive function. These changes are largely dependent on the radiation dose. Studies revealed that ionizing radiation affects the functions of the central nervous system what results in behavior and memory changes; these changes occur as a result of a direct effect of irradiation of the central nervous system and also its indirect effects, resulted of response to irradiation of other organ systems [6].

The central nervous system is considered a radiosensitive system, and the degree of its dysfunction can be evaluated by electrophysiological, biochemical, and behavioral parameters. Impairments of these parameters can be observed after local, also total irradiation of the whole body [14]. Nowadays, there is increasing evidence literature date that the response of the central nervous system to radiation is a continuous and interactive process. Particular attention is paid to apoptotic cell (neuronal) death, as well as, mechanisms of cells' damage and death induced by secondary injury [9].