IRSTI 76.29.34 https://doi.org/10.26577/ijbch.2020.v13.i2.13

A.K. Martusevich[1,2*], O.B. Zhdanova[3,4]

[1]Privolzhsky Research Medical University, Nizhny Novgorod, Russia
[2]Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia
[3]Institute of Parasitology – Branch of Scientific Center "All-Russian Institute of Experimental Veterinary named after Ya.R. Kovalenko and K.I. Skryabin", Moscow, Russia
[4]Kirov State Medical University, Kirov, Russia
*e-mail: cryst-mart@yandex.ru

Dynamics of some physical and chemical properties of biological fluids in surgical treatment of alveolar hydatid disease of liver

Abstract. The aim of the current work was to clarify the dynamics in crystallogenic and initiating activity of patients' biological fluids in alveococcosis during surgery and in the late postoperative period. The material of the study included samples of saliva of 22 patients treated for alveococcosis. Sampling of biological fluids was managed at the admission and prior to discharge from the hospital. Subsequently slides were prepared using teziocrystalloscopy technique that combines the study of the crystal forming activity of mixed saliva (classical crystalloscopy) and its initiating properties considering 0.9% sodium chloride solution as basis substance (comparative teziography). Criterial evaluation of the results of crystalloscopic and tezigraphic tests was carried out using own system of parameters. The data of visual morphometry of microslides of dehydrated saliva was supplemented with spectrophotometric analysis of crystalloscopic and tezigraphic facies performed on PowerWave XS microplate spectrophotometer (USA) at wavelengths of 300, 350 and 400 nm. It was noted that surgical treatment leads to partial normalization of physical and chemical parameters and composition of patient's biological fluids during hospital discharge.
Key words: alveococcosis, alveolar hydatid disease, surgical treatment, saliva, crystallization.

Introduction

Significantly large group of patients with focal liver diseases was considered to be radically "inoperable" based on the data of exploratory laparotomy and palliative invasions [1-5].

Recently, in repeated operations on liver, doctors limited themselves to excision or curettage of fistulas, dissection of purulent cavities and injecting parasitotropic drugs [6-9]. There seems to be a real possibility to perform repeated radical operations on patients previously considered as "inoperable" [2-5, 9-11].

It is generally assumed that the first radical dissection of liver after exploratory laparotomy due to alveococcosis was performed by S.S. Yudin (1929) [1]. According to Merzlikin et al. (2011) and Błaszkowska, Góralska (2016), the development of surgery contributed to the fact that the majority of patients with focal liver lesions were not operated in specialized medical institutions [8,9]. Simultaneously, many of them due to technical complications and incorrect assessment of the operability of the lesion focus are limited to palliative surgery or exploratory laparotomy, thus dooming patients on disability and poor outcome [1, 4, 10-12].

Since 1964, a number of works of Russian surgeons dedicated to repeated operations, mainly on the liver alveococcosis, has been published [1-5]. Papers of Zhuravlev V.A. and other researchers showed that there is a serious problem of providing a radical means to "inoperable" patients with focal lesions of the liver, which occurred in parallel with advent of different skills in surgical treatment of patients with focal lesions of the liver [1-4, 8, 12-14].

On the other hand, it is important to support the full diagnostic guiding of alveococcosis surgery. The use of modern instrumental methods for study of liver morphology (X-ray diagnostics with contrasting, CT and magnetic resonance imaging, ultrasonography) enables evaluating with a high degree of accuracy the nature, depth and severity of structural damage of the organ associated with the development of the disease [10, 13, 15-19]. Intensity of the immune response to the presence of the parasite can be verified using latex agglutination reactions, and a specific ELISA alveococcosis diagnosticum [20]. Furthermore, alveococcus scolexes in some cases

can be found in the sputum. At the same time, the features of the metabolic changes, including changes in the physical and chemical properties of biological fluids in alveococcosis, surgical treatment of it as well as treatment within postoperative period were insufficiently studied previously. In this regard, we have shown that the presence of alveococcus significantly and directionally changes the crystallogenic properties of biological substrates, and the nature of this transformation leads to similar shifts in crystallostasis of saliva and urine [21-23]. In connection with the above, clarifying the dynamics of crystallogenic and initiating activity of patients' biological fluids in alveococcosis during surgery and in the late postoperative period, designating current research, has a great scientific and practical interest.

Materials and methods

Samples of saliva of 22 patients treated for alveococcosis at the Kirov Zonal Centre of Liver and Biliary Tracts were studied. The diagnosis was verified with standard instrumental (ultrasound, CT and/or MRI) and laboratory (latex agglutination, ELISA) tests. All the patients have successfully undergone surgery (partial hepatectomia, lobectomia, hemihepatectomia).

Sampling was managed at the admission and prior to hospital discharge. Within 3 hours before the study, patients did not engage in physical activity, neither they were in a state of psycho-emotional stress. Before collection of biological fluids, patients rinsed their mouths thoroughly with approximately 100 mL of dH_2O for 5 min. Afterwards 1 mL of oral fluid was collected by spitting into clean and dry test tubes.

Subsequently slides were prepared using teziocrystalloscopy technique that combines the study of the crystal forming activity of mixed saliva (classical crystalloscopy) and its initiating properties considering 0.9% sodium chloride solution as basis substance (comparative teziography) [21-23].

Criterial evaluation of the results of crystalloscopic and tezigraphic tests was carried out, using own system of parameters [21, 23]. It allows estimating specialties of crystallization (initiation potential – in teziography) of the biological substrate, severity of the individual zones of the facia, degree of destruction of crystalline and amorphous components, the uniformity of their distribution on the texture of the sample, etc.

The data of the visual morphometry of microslides of dehydrated saliva was supplemented with spectrophotometric analysis of crystalloscopic and teziographic facias, performed on PowerWave ™ XS microplate reader (BioTek, USA) at the wavelengths of 300, 350 and 400 nm. To neutralize the influence of the characteristics of glass to the results of spectrometric studies of biological crystals we calculated the level of optical density of facies by subtracting the optical density of pure glass from the total value of the indicator.

The study was approved by the local ethical committee of Kirov State Medical University (19.09.2016, No. 16).

Statistical data analysis was done using the Microsoft Excel 2007 spreadsheets along with Primer of biostatistics version 4.03 program.

Results and discussion

Considering that saliva is the most convenient noninvasive marker of modification of physical and chemical properties and composition of the blood and also biological substrates secreted in the digestive tract. Last one is locus morbi of alveococcosis and therefore advantageously reflects its functional and metabolic status, consequently current analysis of crystallogenic activity of the biological fluid was of particular scientific and practical interest. Based on the studies it was found that the dynamics of varying parameters is observed in all major morphometric parameters of crystalloscopic facies of patients' saliva (Figure 1).

In particular, the multidirectional changes recorded in respect of the indicators of dehydration structuring of biological medium – the structure index (SI) and crystallizability (Cr) of biological substrate components. So, initially in patients with alveococcosis on the slides of oral fluid there are more complex by the nature of the organization elements, fern-like, dendrite crystals with branching order of 3.4 or higher, which is reflected in the growth of structure in relation to the level of the index microslides of saliva of nearly healthy people ($p<0.05$). The enlargement of the main structural elements of the saliva facies of patients with alveococcosis leads to a decrease in the density of the crystals (Figure 2), being registered as a parameter "crystallizability" ($p<0.05$). In turn, the pathological character of the detected shifts of crystalogenic potential of considered patients is clearly manifested in the dynamics of facies destruction, reaching almost 2 relative units (rel.un.), which corresponds to the formation of severe disturbance of crystal elements formation while maintaining their differentiation by types.

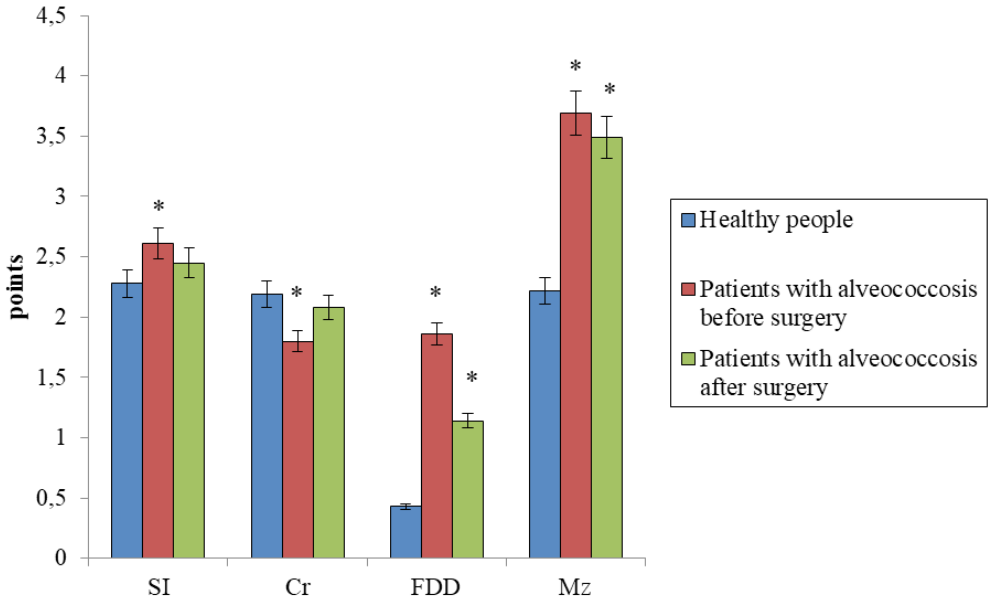
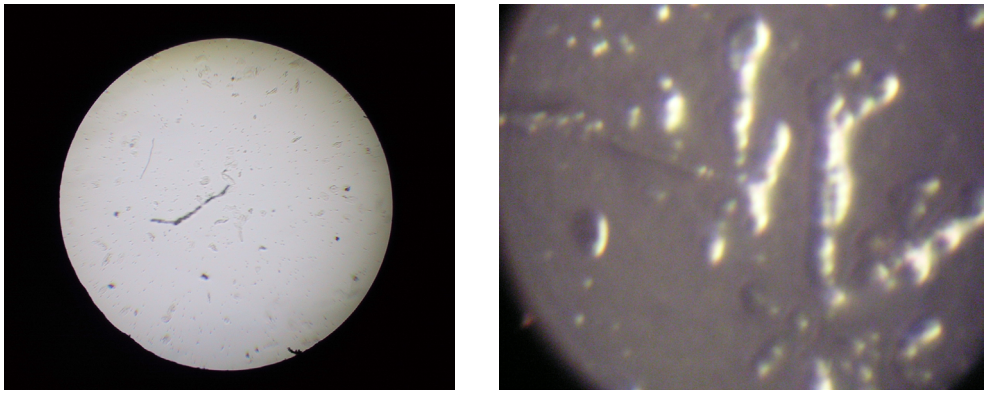


Figure 1 – The result of parametric analysis of own crystallization of patients' saliva during surgical treatment of alveococcosis (SI – structure index, Cr – crystallizability, FDD – facia destruction degree, Mz – clearity of marginal zone; "*" – value of statistical differences to the level of healthy people is $p<0.05$)



A. In healthy person

B. In patient with alveococcosis

Figure 2 – Crystallosopic facias of the saliva of healthy person and patient with alveococcosis (magnification x56)

It is known that in the pathogenesis of alveococcosis not only mechanical properties of the developing liver and other organs of the parasite, but the immunological reactions to its presence are manifested. Therefore, in the blood, and to a lesser extent in other biological substrates an increased concentration of immunoglobulins was noted that leads to an increase in their protein levels. Data for markers of metabolic shifts in saliva crystallogram serves as a manifestation of the marginal zone of the slides (parameter Mz), where the protein macromolecules of dehydration are concentrated. It was found that this index in patients with alveococcosis is registered on high value, and it is significantly below normal level ($p<0.05$). In general, prior to the surgery crystallogenic properties of saliva significantly differ from crystalloscopic "pattern" of a healthy person.

Within the early postoperative period crystallogenic properties of studied biological fluid are transformed significantly (Figure 1). Thus, there is almost complete normalization of the parameters characterizing the complexity and density of

structure-building crystal elements (SI and Cr, respectively). It should be noted that both of these parameters do not significantly vary in relation to control (pre-surgery) level, while greatly differ from the baseline ($p<0.05$). The main parameter describing the "correctness" of crystallogenesis – the facia destruction degree (FDD) – is also reduced significantly compared with the preoperative crystalloscopic "pattern" ($p<0.05$) but does not reach the physiological level.

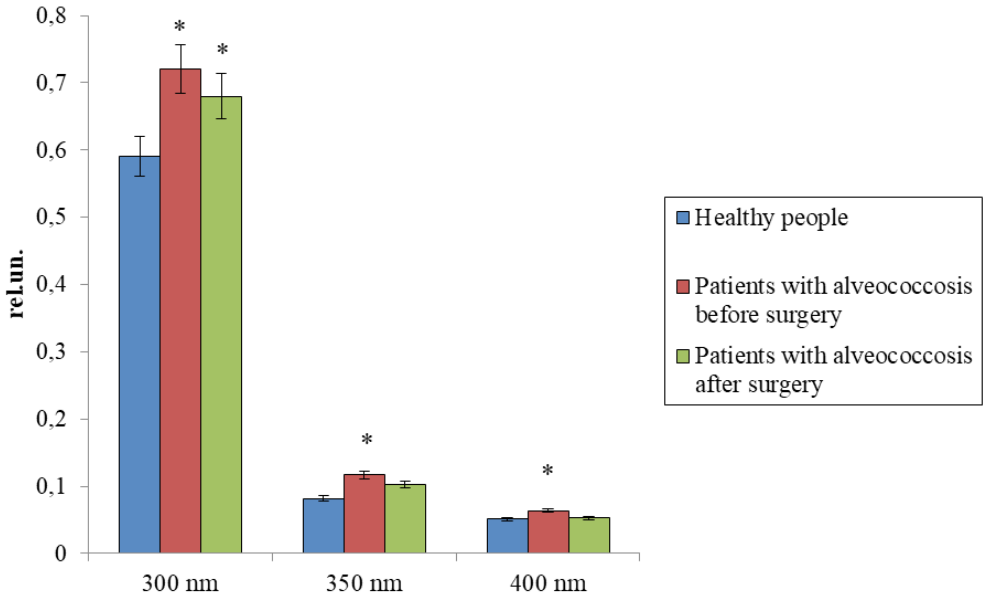


Figure 3 – Spectrometric analysis of saliva crystalloscopic facias of patients with alveococcosis during surgery ("*" – value of statistical differences to the level of healthy people is $p<0.05$)

It is interesting that the severity of the marginal zone of saliva microslides after the operation changes at a minimum degree. This trend is perhaps related to the persistence of high concentrations of anti-alveococcus antibodies in the blood and, therefore, in other biological fluids. This, in turn, has a corresponding impact on the parameter Mz, being directly dependent on the level of a protein. Upon completion of surgery partial normalization of crystallogenic properties of saliva is observed in patients with alveococcosis.

Such data is fully confirmed by a verifying method – spectroscopic study of crystalloscopic facies (Figure 3). Considering the fact that the prevalent trend of the shifts of crystallogenic properties of saliva in alveococcosis is enlargement of crystal elements along with the complexity of their construction, the increase of the optical density of the sample as a whole, for all wavelengths used in the preoperative period, seems to be logical from control values ($p<0.05$). The presence of such shifts was first shown in this study.

After the surgery, the level of the total optical density of crystalloscopic facies of saliva decreases but remains significantly higher than the values which are typical for healthy individuals (Figure 3). It should be noted that this dynamic is most clearly seen in the spectroscopic study at $\lambda = 300$ nm, but is less pronounced at $\lambda = 350$ nm, and at the maximum $\lambda = 400$ nm, as a result of rather low absolute values, it is almost eliminated.

Analysis of the initiating properties of saliva of the considered cohort of patients during surgery (Figure 4) demonstrated initially significantly higher level of the basic indicator of the initiating potential of biological fluid – tezigraphic index (TI), when compared to healthy subjects ($p<0.05$). Upon completion of surgical treatment, this parameter is moderately decreased, but these changes are not even statistically significant. A similar pattern was registered respectfully to the complication marker of structure-building elements – crystallinity (C). However, the postoperative level of the indicator does not significantly differ from both the pre-surgery and normal tezigraphic pattern of saliva. The minimal variation among the basic tezigraphic indicators (Figure 4) is uncovered for the belt coefficient (BC), which is the

criterion of scattering of molecular weight of biological substrate components.

The effectiveness of surgical treatment is most clearly manifested in the dynamics of the principal tezigraphic criterion of "correctness" of the processes of structure formation – the facia destruction degree, as well as the clearity of the marginal zone of the slides (Figure 4). These figures after the completion of surgery were significantly reduced relative to the preoperative level, but without reaching the structures which are typical for healthy individuals ($p<0.05$). This trend is an indirect evidence of the safety of alveococcus-associated metabolic shifts of the composition and physical-chemical properties of biological fluids and after performing radical surgery.

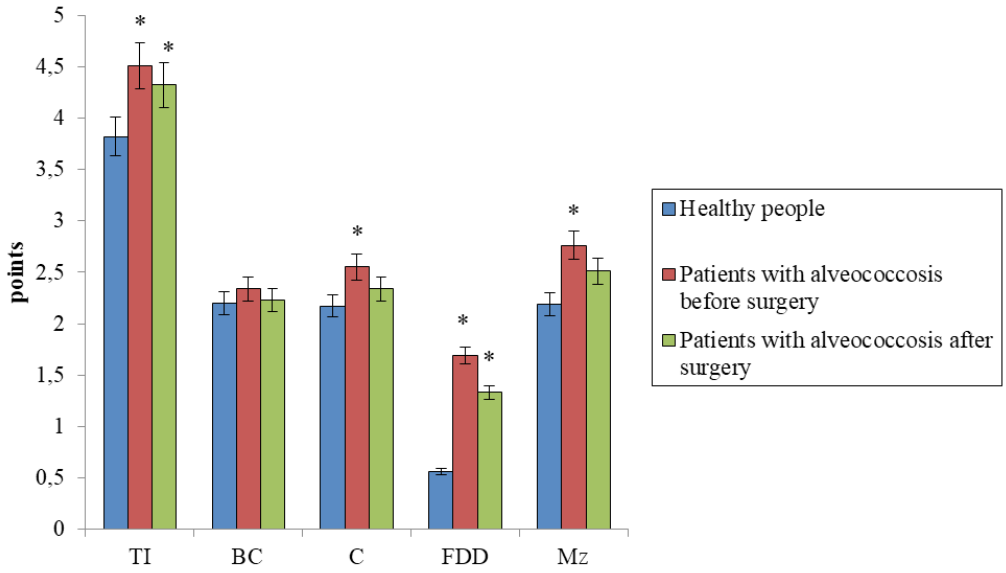


Figure 4 – The data of tezigramms parametric analysis of saliva during surgical treatment of alveococcosis (TI – teziographic index, BC – belt coefficient, C – crystallinity, FDD – facia destruction degree, Mz – clearity of marginal zone; "*" – value of statistical differences to the level of healthy people is $p<0.05$)

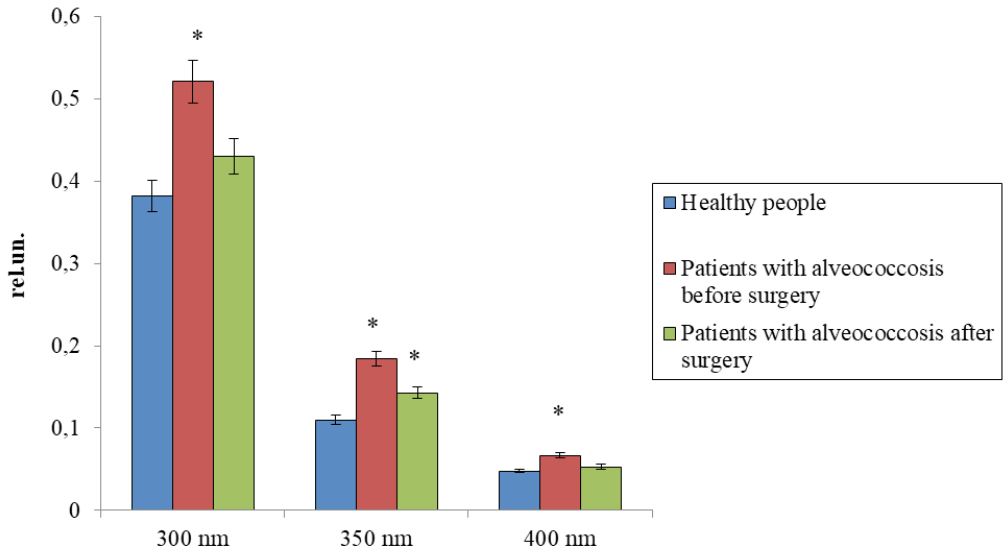


Figure 5 – Results of spectrometry of saliva tezigraphic facias of patients with alveococcosis during surgery ("*" – value of statistical differences to the level of healthy people is $p<0.05$)

These variations are fully verified by the results of spectroscopic study of tezigraphic facies of patients' saliva in the dynamics of surgical treatment (Figure 5). In this case the dynamics of the total optical density of tezigrams is evident at all wavelengths used.

Acknowledgment

Authors thank Dr. Vladimir Yanchenko (from Kirov State Medical University, Russia) for advance in patients' selection.

Conclusion

Conducted crystalloscopic studies allowed us to establish that in alveococcosis of the liver there are significant transformations of the crystallogenic properties of saliva. They are manifested in an increase in the structure index, the severity of the sample marginal zone and the degree of destruction of individual structures, as well as in a decrease in crystallizability. Such shifts lead to an increase in the optical density of facias. After surgical treatment, most of the parameters are normalized, while only the degree of facias destruction and the clarity of the marginal zone of the samples are preserved at elevated values. At the same time, the optical density of micro-preparations remains elevated only at a wavelength of 350 nm. Identification of such dynamics of crystalloscopic indicators allows us to consider them as an additional indicator of the effectiveness of treatment of alveococcosis of the liver. Further verification of the diagnostic information content of the technology used by us can become the basis for developing a method for rapid testing of the usefulness of liver resection in alveococcosis. In addition, our data complement the understanding of the metabolic changes that are formed in the body of patients with alveococcosis of the liver.

References

1 Alperovich B.I. (1997) Alveokokkoz [Alveococcosis]. Yakutsk. 254 p.

2 Panteleev V., Nartaylakov M., Mustafin A. et al. (2019) Surgical treatment of liver echinococcosis and alveococcosis. Infez Med. vol. 27, no. 4, pp. 422-428.

3 Novozhilov A.V., Movsisyan M.O., Grigoriev S.E., Magolina O.V., Kleimenova N.S. (2019) Kombinirovannaya levaya gemigepatektomiya v lechenii mul'tiorgannogo al'veokokkoza [Combined left hemihepatectomy for multiple organ alveococcosis]. Khirurgiia (Mosk). no. 9, pp. 52-57. doi: 10.17116/hirurgia201909152.

4 Veronsky G.I. (1997) Surgical treatment of liver alveococcosis. Annals of surgical hepatology, no. 2, pp. 15-19.

5 Zhuravlev V.A. (1997) Liver alveococcosis. Annals of surgical hepatology, no. 2, pp. 9-14.

6 Gawor J. (2011) Potential risk factors for alveolar echinococcosis in humans in Poland. Przegl. Epidemiology. vol. 65, no. 3, pp. 465-470.

7 Kogan E.A., Nekrasova T.P., Lerner Y.V., Kukleva A.D. (2020) Sochetanie al'veokokkoza pecheni i gepatotselliuliarnoĭ kartsinomy (sektsionnoe nabliudenie) [Liver alveococcosis concurrent with hepatocellular carcinoma (autopsy observation)]. Arkh Patol. vol. 82, no. 1, pp. 47-51. doi: 10.17116/patol20208201147.

8 Merzlikin N.V., Alperovich B.I., Paramonova M.M. (2011) Repeated operations in localized liver diseases. Surgery. no. 8, pp. 51-57.

9 Błaszkowska J, Góralska K. (2016) Clinical cases of parasitoses and fungal infections important from medical point of view. Ann Parasitol. vol. 62, no. 4, pp. 255-265. doi: 10.17420/ap6204.61.

10 Dybicz M., Borkowski P.K., Padzik M., Baltaza W., Chomicz L. (2018) Molecular determination of suspected alveolar echinococcosis requiring surgical treatment in human cases from Poland. Ann Parasitol. vol. 64, no. 4, pp. 339-342. doi: 10.17420/ap6404.169.

11 Pielok Ł., Karczewski M., Cierach W. et al. (2020) Portal hypertension as a result of the incomplete surgically treated advanced alveolar echinococcosis: a case description. BMC Gastroenterol. vol. 20, no. 1, pp.176. doi: 10.1186/s12876-020-01320-0.

12 Scripenco O.G., Shatveryan G.A., Bagmet N.N. (2012) Liver alveococcosis: retrospective analysis of treatment of 51 patients. Surgery. no. 12, pp. 4-13.

13 Stefaniak J. (2007) Guidelines for diagnosis and treatment of liver alveococcosis caused by Echinococcus multilocularis. Wiad. Parazytologia. vol. 53, no. 3, pp. 189-194.

14 Tumolskaya N.I. (2010) Cases of alveococcosis in humans in European part of Russia. Medical parasitology and parasitic diseases. no. 3, pp. 45-47.

15 Cheremisinov O.V. (2003) Possibilities of radiological and magnetic resonance tomography in diagnostics of liver alveococcosis. Medical visualization. no. 4, pp. 46-52.

16 Gossios K.J. (1997) Uncommon locations of hydatid disease: CT appearances. European Radiology. vol. 7, no. 8, pp. 1303-1308.

17 Imankulov S.B., Fedotovskikh G.V., Zhampeissov N.K. et al. (2015) Treatment of liver alveo-

coccosis with high-intensity focused ultrasound. Ultrason Sonochem. vol. 27, pp. 707-711. doi: 10.1016/j.ultsonch.2015.05.022.

18 Patkowski W., Kotulski M., Remiszewski P. et al. (2016) Alveococcosis of the liver – strategy of surgical treatment with special focus on liver transplantation. Transpl Infect Dis. vol. 18, no. 5, pp. 661-666. doi: 10.1111/tid.12574.

19 Tsitouridis I., Dimitriadis A.S. (1997) CT and MRI in vertebral hydatid disease. European Radiology. vol. 7, no. 8, pp. 1207-1210.

20 Wnukowska N., Salamatin R., Gołab E. (2011) Human echinococcosis in Poland in 2003-2010 according to the serological tests results of NIPH-NIH. Przegl Epidemiology, vol. 65, no. 3, pp. 455-458.

21 Martusevich A.K., Grishina A.A., Bochkareva A.V. (2010) Crystallodiagnostics of some animals' helmintosis. Asian Pacific Journal of Tropical Medicine. vol. 3, no. 3, pp. 176-179.

22 Martusevich A.K., Zhdanova O.B., Yanchenko V.A. (2006) Pathogenic significance of biological fluids crystallization in alveococcosis. Annals of surgical hepatology. vol. 11, no. 3, pp. 50-51.

23 Martusevich A.K., Zhdanova O.B., Napisanova L.A. (2012) Biocrystalomics in parasitology: state-of-the-art, possibilities and perspectives. Russian Journal of Parasitology. no. 4, pp. 77-88.