STUDIES OF THE BILE CRYSTALLIZATION STRUCTURE FOR PATIENTS WITH LIVER PATHOLOGIES

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The polarization structure of crystallized samples of bile of practically healthy people as well as of those ill with hepatitis, cirrhosis of a liver and patients with small doses of ionizing radiation is studied. The found changes in the bile pictures of crystallization structures can be used for primary diagnostics of liver pathologies. The absorbance and photoluminescence spectra of bile were measured. The possibilities of using this data as additional during traditional liver diagnostic are shown.

The present paper is devoted to the studies of the specific features of liver and bladder bile crystallization and determining the diagnostic possibilities of photometry studies of bile.

Bile for investigation was taken by duodenal probing with intravenous injection of cholecystokinin at laparotomic cholecystectomy [1]. The bile was put between two glass platelets and then incubated at 37°C until spontaneous crystallization. Solid bile was studied by polarization microscopy using Carl Zeiss NU-2E microscope. Native bile absorption spectra were measured on a two-beam Hitachi 356 spectrometer. At the luminescence studies of bile the optical scheme, described in [2], was used. The measurements were carried out using a Carl Zeiss SPM-2 monochromator-based photoelectric set-up.

While studying the liver bile of 17 practically healthy people of the test group the bile was found to be a uniform mycellar solution whose crystallization process follows the dendrite mechanism. The liver bile is a well-defined, saturated, ordered fractal structure which can be characterized as a "fern leaf" (Fig. 1a). For the bladder bile the ordered location of micelles is broken due to the change of concentration. The crystallization structure of the bladder bile at the microscopic studies appeared to be glass-like (Fig. 1b). Such crystallization structure of the liver and bladder bile is attributed to type I.

In case of the inflammation process in the bile-secreting system the mycellar equilibrium of bile is violated, resulting in the changes in the crystal structure. We have studied the crystallization structure of bile in 79 patients, 53 of them with chronic persisting hepatitis and 26 - with liver cirrhosis. For those suffering from chronic persisting hepatitis typical changes in the liver bile crystallization structure were found (Fig. 2a): an ordered structure in the form of "larch leaf" or "palm leaf". The inclusions of integrated lipid anisotropic crystals were observed. In the bladder bile the typical feature of the crystallization consisted in a glass-like structure with ordered anisotropic inclusions of integrated lipids was observed in the form of quadrangular crystals (Fig. 2b). Such crystallization structure of the liver and bladder bile is attributed to type II.

For the patients with cirrhosis in the liver bile the changes in fern structure were observed (Fig. 3a), the bile crystallized in accordance with type IV. The fern structure is fine, against its background small anisotropic branch-shape inclusions (cholesterol ethers), bright irregular-shaped inclusions of bile acids, small rectangular or rhombic cholesterol monohydrate crystals were found. In the bladder bile anisotropic crystals and liquid crystal phase, considered the predecessor of cholelithiasis, were observed (Fig. 3b). Many crystals of cholesterol monohydrate were observed in the peripheral area of the preparation, necklace-shaped aggregations often being formed (type III of bladder bile crystallization).



Fig. 1. Crystallooptical structure of bile (COSB) of the test group: (a) - C, (b) - B. Magnification ×1200.

For the patients suffering from chronic alcoholism (27 people) in the liver bile fractal structure (fern leaf) was found, being slightly distorted (type III of liver bile crystallization). The bladder bile of these patients was unstructured, totally isotropic, without optically active inclusions.

Thus, the studies of bile crystallization structure is one of the approaches to primary diagnostics of various diseases of hepatobiliary system. The crystallooptical method is simple, easily available, rather informative,

promising for primary detection of liver pa thology.



Fig. 2. COSB of the patients with chronic persisting hepatitis: (a) -C, (b) -B. Magnification ×1200.

In order to elucidate the specific features of intermolecular interaction in bile we have performed the studies of absorption and luminescence spectra of native bile and solidstate bile (lyophized at special conditions) in 23 patients suffering from chronic persisting hepatitis before and after hospital treatment and 17 practically healthy people. Essential differences have been found in the absorption spectra. For the patients of the control two absorption bands have been found. In the spectra of patients with chronic persisting hepatitis the intensity of the absorption bands was almost 3 times lower; besides, for the

patients of this group an additional absorption band was observed with less pronounced resolution. The bile absorption spectral range is 380+500 nm, where usually two absorption bands - 410.0 ± 4.0 nm and 460.0 ± 4.0 nm - are observed, their presence reflecting the content of direct (the shortwavelength band) and indirect (the longwavelength one) bilirubin. As concerning the absorption band at 220.0±4.0 nm, it is related to the light absorption by the bile lipoproteide complexes.



Fig. 3. COSB of the patients with liver cirrhosis: (a) -C, (b) - B. Magnification ×1200.

The luminescence spectra of the bile of the patients under investigation also had differences. The control spectra contain three bands: a broad intense one centered at λ = 510.0±5.0 nm and two bands of different intensities at 625.0±5.0 nm and 675.0±5.0 nm.

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Contrary to this spectrum, that of the patients with chronic hepatitis contains two bands at $\lambda = 460.0 \pm 5.0$ nm and $\lambda = 510.0 \pm 5.0$ nm instead of one at $\lambda = 510.0\pm 5.0$ nm. As concerning the long-wavelength part of the spectrum, in the case of the patients with chronic hepatitis one emission band with a maximum near $\lambda = 660.0 \pm 5.0$ nm is observed. Probably, the position and intensity of this band depend on the complexity of the disease. In the course of curing variation of its position and intensity was observed. Perhaps, such changes in the bile luminescence spectra after curing are the evidence for the structural changes in the lipoprotein complex, responsible for the observed spectral features.

The observed spectral features (changes in the absorption and luminescence band intensity ratio, maximum wavelength shift) of bile seem promising for the diagnostics of diseases due to high sensitivity and simplicity of the measurements. The traditional analyses of bile should be complemented by spectral characteristics.

References

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КРИСТАЛООПТИЧНІ ДОСЛІДЖЕННЯ СТРУКТУРИ ЖОВЧІ ХВОРИХ З ПАТОЛОГІЄЮ ПЕЧІНКИ

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Вивчено поляризаційну структуру кристалізації жовчі практично здорових людей та пацієнтів, хворих гепатитом, цирозом печінки, а також пацієнтів, що отримали малі дози іонізуючого випромінювання. Знайдені відмінності картин структури кристалізації жовчі можуть бути використані для первинної діагностики захворювань печінки. Зроблено вимірювання спектрів поглинання та люмінесценції жовчі. Показано можливість використання цих даних як додаткових при традиційному діагностуванні захворювань печінки.