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STEATOUS CHANGES IN HEPATOCYTES IN PATIENTS WITH CHRONIC HEPATITIS C

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Background. The main consequences of progressing of a chronic hepatitis C are a cirrhosis of a liver and hepatocellular carcinoma. Considering that for last years number of cases of CHC disease among population of the world has essentially increased, creation of adequate methods of an estimation of morphological and metabolic shifts in a liver tissue at CHC is the important clin-ical problem.

Aim: to determine the morphological features of steatosis in patients with CHC and assess its effect on the progression of hepatitis.

Materials and methods. Under supervision there were 46 CHC patients in the age of 19–66 years, 36 men and 10 – women. Liver biopsy was carried out under the control of ultrasonic research with local anesthesia.

Conclusion. Among CHC patients steatosis of a liver is observed in 60, 9% of supervised. Expressiveness of steatosis on Hornboll is distributed: 1-st degree at 13,0% of patients, 2-nd – 28,3%, 3-rd – 19,6%, absence of steatosis- at 39,1%. At initial stages of steatosis in hepatocytes of peripheral zone of segments the significant amount lysosomes, which look like lipofuscin granules which contain lipide, electronically-dense, small-sized granular, pigmentary component, safety of structure of organelles, hyperplasia mitochondrions. At expressed steatosis dense lipid vacuoles which borrow the most part of cytoplasm of hepatocytes, displace organelles, deform a kernel of cells, damage superficial membranes mitochondrions are observed. In periportal zone, on border of contact of hepatocytes which contain lipid granules, and lymphomonocytic infil-trate, the expressed activation of Kupfer cells, insufficiency of granules in cells of ITO, their transformation in fibroblasts, the expressed adjournment of collagenic fibers is observed.

Key words: chronic hepatitis C, liver, steatosis, electron microscopy.

Background. The main consequences of progressing of a chronic hepatitis C are a cirrhosis of a liver and hepatocellular carcinoma [1, 2]. Considering that for last years number of cases of CHC disease among population of the world has essentially increased, creation of adequate methods of an estimation of morphological and metabolic shifts in a liver tissue at CHC is the important clinical problem [3]. The estimation of expressiveness of factors which authen-tically influence progressing of a hepatitis is significant at clinico-laboratory inspection of CHC patients. It is necessary to ascribe to such factors lympho-monocytic infiltrate in portal and periportal zones of hepatic segments, ad-journment of hemosiderin at a syndrome of an overload iron [4], increase in concentration of proinflammatory cytokines [5], prevalence of a Th2-variant of the immune answer, activation of processes peroxide oxidations of lipids [6], activation of Kupfer and ITO cells [7], etc. Among possible factors which pos-sess profibrogenic action, is steatosis of a liver. In a number of researches it has been established, that steatosis at CHC disease is the independent factor which assists progressing fibrosis in a liver tissue [8]. There are individual works in which prevalence of steatosis and pathogenesis its actions is analyzed at CHC disease [9, 10], nevertheless because definition of granules of lipids in a liver tissue is not included into standard morphological methods, there are not enough scientific materials on this problem.

Aim: to determine the morphological features of steatosis in patients with CHC and assess its effect on the progression of hepatitis.

To research problems have entered:

1) The analysis of prevalence steatosis at CHC patients;
2) Definition of structure of expressiveness and

localization of ad-journment lipid granules in a liver tissue at CHC disease by means of standard morphological and computer morphometrical methods;
3) Research of electronically-microscopic features of adjournment li-pid granules at CHC patients;
4) The analysis of possible correlative communications between stea-tosis, histologic activity and a stage of fibrosis at CHC.

MATERIALS AND METHODS

Under supervision there were 46 CHC patients in the age of 19–66 years, 36 men and 10 – women. Diagnosis of CHC has been established on the basis of a complex of clinico-biochemical parameters, proved to be true by definition of antibodies-anti-HCV and polymerases chain reaction – HCV-RNA, carrying out ultrasonic researches of organs of a abdominal cavity with definition of the sizes of a liver, its ultrasonic density, a condition of vessels, bile ducts. To ex-cept virus hepatitis of another etiology research of HbsAg, HbeAg, anti - HbeAg, anti- HbcIg M, anti - Hbc total, anti - Hbs, anti - HDV eas carried out. Liver biopsy was carried out under the control of ultrasonic research with local anesthesia. The size of a fragment of a liver tissue which has been received at biopsy, was more than 10 mm. For colouring histologic samples we used such methods of colouring: with hematoxylin and eosine, pirofuxine according to Van-Geeson, definition of liver steatosis by a technique. The stage of fibrosis was defined on METAVIR [11], an index of histologic activity on Knodell R.G. et al. [12].

The group of CHC patients has been distributed on 2 groups according to attributes of activity of process. To group with minimal activity of CHC (scores on Knodell 1–8 points) have entered 27 (58, 7%) men, with the moder-ate activity (scores on Knodell 9–15 points) – 19 (41, 3%). Among 46 surveyed the stage of fibrosis F0 – was at 4 patients (8, 7%), F1 – at 14 (30, 4%), F2 – at 11 (23, 9%), F3 – at 10 (21, 7%), F4 – at 7 (15,3%). Expressiveness of steatosis was defined by Hornboll P technique., 1982 [13] where 1-st degree of steatosis – if fatty vacuoles are defined in 1/3 hepatocytes, 2-nd degree - fatty vacuoles are defined more than in 1/3 hepatocytes and 3-rd degree – fatty vacuoles take place more than in 2/3 hepatocytes.

Also for an estimation of expressiveness of steatosis at CHC patients the specialized package of applied programs "Hepatomorpholog" (by Pinsky L.L., Ovcharenko V.V. has been used; the certificate on registration of the copyright 10060). The data of given morphometrical researches gained as a result of work were exported to program Excel for the further statistical processing. In each received picture of a histologic preparation such parameters were defined: the specific area of adjournment of lipid granules (LG) in histologic cuts of a liver of CHC patients (SALG), quantity of hepatocytes in the surveyed site of segments, the average area of lipid granules per 1 hepatocyte (AALG). SALG has been received by division of the area of adjournment of LG in an investi-gated site of a liver tissue into a total area of this site. AALG has been calculat-ed by division of area of LG into quantity of hepatocytes in the same site of a liver. All morphometrical researches were done in 3 zones of segments: centri-lobular, peripheral and portal.

Statistical processing of the obtained results was carried out using the method of variance and discriminant analysis using the software package SPSS v.22, Excel 2016 in the Windows 10 environment. All samples were assessed for the level of normality of distribution according to the Kolmogorov-Smirnov scale and Shapiro-Wilk criteria and, in In the absence of this correspondence even in one of the groups, nonparametric statistics methods were used (Mann-Whitney, Wilcoxon tests) [14].

RESULTS AND DISCUSSION

As a result of made by us researches it has been established, that fatty and albuminous dystrophy of hepatocytes, polymorphism of their kernels, focal and step necrosis, focal infiltration segments and portal pathes can be ob-served in liver tissue of CHC patients. The portal and sine wave sclerosis, for-mation connective-tissue septs also took place. In the surveyed group the min-imal histologic activity (1–8 points on Knodell) the moderate histologic activi-ty (9–15 points on Knodell) – at 19 patients (41, 3%) has been established at 27 patients CHC (58,7%).

In the general group of CHC patients steatosis of a liver has been verified at 28 of 46 patients (60,9%). On stages of steatosis according to Hornboll the group of CHC patients has been distributed thus: 1-st degree – at 6 patients (13,0%), 2-nd degree – at 13 (28,3%), 3-rd degree – at 9 (19,6%), absence of steatosis – at 18 sick (39,1%). In group with the minimal histologic activity (1–8 points on Knodell) steatosis was at 14 of 27 patients (51,9 %), in group with the moderate activity – at 14 of 19 (73,7%).

At microscopic research it has been established, that the initial degree of steatosis is accompanied by adjournment of lipid mainly in centrilobular zone of segments. At progressing steatosis small-sized granular steatosis in centri-lobular zone is transformed into large granular, and in periportal zone there are centers of small-sized granular steatosis. At increase to 3-rd degree of steatosis its character varies on large granular not only in centrilobular zone, but in periportal. Destruction of hepatic beams which takes place at a stage of fibro-sis F4 (cirrhosis) is accompanied by reduction of adjournment of lipid vacuoles in hepatocytes all zones.

At electronically-microscopic research of biopsy-piece a liver of patients with steatosis. It has been established, that in many hepatocytes, mainly, of 3-rd zone are observed numerous vacuoles of fats which have the different sizes, from small-sized granular up to large granular and different electronic density (fig. 1).

In hepatocytes fatty drops can freely be in cytoplasm or in tanks of endo-plasmatic netting. In a significant amount of hepatocytes, dense lipid vacuoles borrow the majority of cytoplasm, displace organelles, deform a kernel of a cell, have contact with mitochondrions. In a place of this contact mitochondri-ons get attributes of damage of the superficial membrane (fig. 2).

Meanwhile, a part of hepatocytes of periportal zone has the same struc-ture of organellas, hyperplasia of mitochondrions and granular endoplasmatic networks, occurrence of lipofuscine granules which contain lipid, an electroni-cally-dense pigmentary component and matrix of moderate electronic density which testifies presence of compensating reparative processes with attributes of endocellular degradation of fatty granules.

In periportal zone of segments at the minimal histologic activity and weakly expressed steatosis of hepatocytes, one can observe lipocytes with great outgrons, great kernels, mainly of oval form, insignificant quantity of or-ganellas, well developed lamellar complex, separate lipid inclusions.

At acute steatosis and moderate histologic activity of CHC, in periportal hepatocytes the quantity of phagolysosomes increases, degranulate and dila-tion of tanks of granular endoplasmatic network swelling, an enlightenment of matrix of mitochondrions, disorganization of their cristas, occurrence of paracrystal and lipid inclusions is observed.

At the analysis of interaction between immunocompetent cells of a portal zone and hepatocytes which contain lipid granules, we established

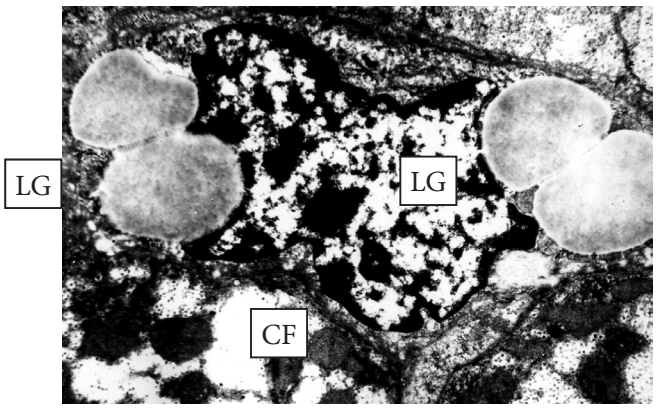

Fig.1. Initial manifestations of collagen formation (CF) with concomitant stea-tosis (Electrogram of the patient's liver tissue, CF – collagen formation, LG – lipid granules, x12000)

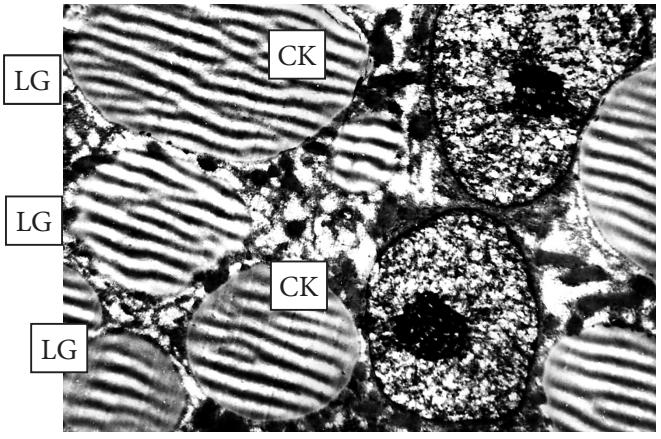

Fig.2. Significant deposits of lipid granules (LG) in hepatocytes (Electron-nogram of liver tissue of the patient, B., CK – cellular kernel, x12000)

presence of peripolesis. And after an output into a gleam of sinusoid and space Disse lym-phocytes had contact with cytoplasmatic membrane of hepatocytes, that was accompanied by significant accumulation of lipid granules near a pole of hepatocytes where there was a contact with lymphocytes (fig.3). Presence of this feature of interaction between lymphocytes and lipidcontained hepatocytes at steatosis demands the further studying.

At expressed steatosis in sinusoids in a zone of contact of hepatocytes with lymphomonocytic infiltrate, substantial growth of destructively changed organelles of hepatocytes, lipid granules, significant activation of Kupfer cells (swelling of kernels, separation of KC from a wall a sinusoid, activation of phagocytosis), disappearance from cells ITO of granules, their transformation in fibroblasts is observed. Mature fibroblasts have expressed granular endo-plasmatic reticulum, the developed lamellar complex, elements contracted fila-ments, takes place expressed perisinusoid and pericellular fibrosis with ad-journment of dense bunches fibrils (fig.4).

CONCLUSIONS

1. Among CHC patients steatosis of a liver is observed in 60, 9% of supervisied. Expressiveness of steatosis on Hornboll is distributed: 1-st degree at 13,0% of patients, 2-nd – 28,3%, 3-rd – 19,6%, absence of steatosis- at 39,1%.
2. At initial stages of steatosis in hepatocytes of peripheral zone of segments the significant amount lysosomes, which look like lipofuscine granules which contain lipid, electronically-dense, small-sized granular, pigmentary component, safety of structure of organellas, hyperplasia mitochondrions.
3. At expressed steatosis dense lipid vacuoles which borrow the most part of cytoplasm of hepatocytes, displace organellas, deform a kernel of cells, damage superficial membranes mitochondrions are observed. In periportal zone, on border of contact of hepatocytes which contain lipid granules, and lymphomonocytic infiltrate, the expressed activation of Kupfer cells, insufficiency ofgranules in cells of ITO, their transformation in fibroblasts, the expressed adjournment of collagenic fibres is observed.

Conflict of interests. The authors of this manuscript claim that there is no conflict of interest during the research and writing of the manuscript.

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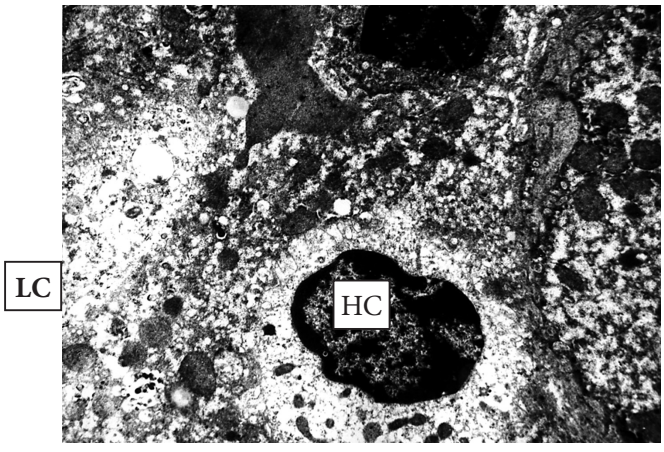

Fig.3. Lymphocytic infiltration of liver tissue (Electronnogram of a liver tissue of the patient M., LC – Lymphocyte, HC – hepatocyte, x12000)

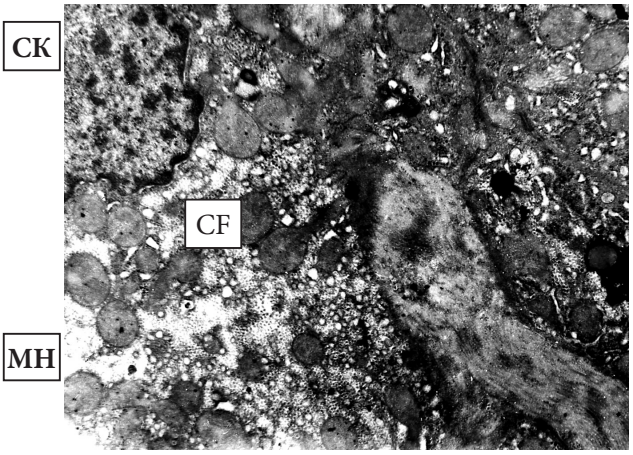

Fig.4. A significant stage of fibrosis with widespread deposits of collagen fibers (CF) in the periportal zone (Electronogram of the liver tissue of patient C., CK - cel-lular kernel, MH - mitochondria, x12000)

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СТЕАТОТИЧНІ ЗМІНИ ГЕПАТОЦИТІВ У ХВОРИХ НА ХРОНІЧНИЙ ГЕПАТИТ С

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Актуальність. Основними наслідками прогресування хронічного гепатиту С є ци-роз печінки та гепатоцелюлярна карцинома. Враховуючи, що за останні роки кількість випадків ХГС серед населення світу істотно зросла, створення адекватних методів оцінки морфологічних і метаболічних зрушень у тканині печінки при ХГС є важливою клінічною проблемою.

Ціль: визначити морфологічні особливості стеатозу у хворих на ХГС та оцінити йо-го вплив на прогресування гепатиту.

Матеріали та методи. Під наглядом перебувало 46 хворих на ХГС віком 19 - 66 років, 36 чоловіків і 10 - жінок. Біопсію печінки проводили під контролем ультразвуково-го дослідження з місцевою анестезією.

Висновки. Серед хворих на ХГС стеатоз печінки спостерігається у 60,9% спостере-жених. Вираженість стеатозу по Hornboll розподіляється таким чином: 1-а ступень у 13,0% хворих, 2-а – 28,3%, 3-а – 19,6%, відсутність стеатозу – у 39,1% хворих. На початкових стадіях стеатозу в гепатоцитах периферійної зони сегментів значна кількість лізосом, які мають вигляд ліпофусцинових гранул, які містять ліпідний, електронно-щільний, дрібно-зернистий, пігментний компонент, збереженість структури органел, гіперплазовані міто-хондрії. При вираженому стеатозі спостерігаються щільні ліпідні вакуолі, які займають більшу частину цитоплазми гепатоцитів та витісняють органели, деформують ядро клітин, ушкоджують поверхневі мембрани мітохондрій. У перипортальній зоні, на межі контакту гепатоцитів, які містять ліпідні гранули, і лімфомоноцитарного інфільтрату спо-стерігається виражена активація купферівських клітин, недостатність гранул в клітинах ІТО, їх трансформація у фібробласти, виражене відкладання колагенових волокон.

Ключові слова: хронічний гепатит С, печінка, стеатоз, електронна мікроскопія.