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MORPHOLOGICAL FEATURES OF LIVER EFFECT IN PATIENTS WITH CHRONIC HEPATITIS B WITH HIV-COINFECTION

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Abstract

The article presents modern views on morphological disturbances in liver tissue at HBV/HIV patients. It was shown that "matte-glass-like" hepatocytes, inflammatory cell lymphocytic infiltrate of varying degrees and liver fibrosis are specific signs of HBV/HIV patients. Specific morphological signs of liver damage in co-infected patients confirm that HIV-infection worsens histological structure as well as functional balance. Therefore, development of pathohistology method of diagnostics becomes more and more necessary in patients with complicated liver injuries. New findings can give opportunity for early screening HBV-infected patients for HIV-markers, prescribing antiviral treatment at early stages. The research will build new approach for avoiding fast fibrogenesis.

Key words: **HBV/HIV co-infection, "matte-glass-like" hepatocytes, liver fibrosis.**

BACKGROUND

Viral hepatitis B (HBV) and HIV infection remain one of the most pressing health problems in the world [1, 2]. According to WHO, about 1% of HBV-infected people also have HIV infection, in turn, the prevalence of HBV infection among HIV-infected people is 7.4%. Sub-Saharan African countries are at the crossroads of high endemicity of these infections [2, 3, 4]. Every year in the world about 686 thousand people die from hepatitis B,

which is usually the result of inflammatory-necrotic destruction of hepatocytes with subsequent fibrosis and hepatic insufficiency. The development of hepatocellular carcinoma in such patients increases the risk of fatal events [13].

At present, the study of the peculiarities of the influence of coinfections on the architecture of the liver becomes especially relevant. Despite a significant number of works devoted to this topic, the issue of pathogistological rearrangement of hepatocytes [8-13] under the influence of hepatotropic viruses, autoimmune damage and hepatic-cellular insufficiency due to immunosuppression remains controversial.

In chronic hepatitis B in liver biopsy, dystrophy and necrosis of hepatocytes, inflammatory cellular infiltration and fibrous changes in lobules [5] and portal tract [4] are detected. Very characteristic hydropic dystrophy of hepatocytes, less often - its extreme form - balloon dystrophy are present [10]. Damage and destruction of hepatocytes are due to the action of immune mechanisms involving T-lymphocytes, products of decay of cells, in turn, are carried away by macrophages [9]. Therefore, cells of necrosis of parenchyma, as a rule, contain lymphoid-macrophage infiltrate (necro-inflammatory activity).

The presence of HBV / HIV co-infection greatly complicates the course of both diseases. HIV infection enhances the replication of the HBV virus while simultaneously inhibiting seroconversion (production of ab-HBeAg), which results in rapid liver fibrosis. HBV / HIV co-infection leads to loss of immune tolerance [5, 6]. In patients with HBV / HIV co-infection, the activity of the necro-inflammatory process in the liver is generally weaker, but higher HBV replication leads to more severe hepatic fibrosis, an increased (4.2-fold) risk of cirrhosis and a more rapid development of the terminal stage of liver injury [7].

AIM: To establish the morphological features of liver damage in patients with HBV/HIV co-infection.

MATERIALS AND METHODS:

We examined 53 samples of hepatobiliopaths in patients with HBV / HIV co-infection who were on outpatient and in-patient treatment at the Lewanika General Hospital (Zambia) hepatology department. Liver tissue samples were obtained by conducting a puncture biopsy of the oven 18-G 150mm tru cutting needle (MDL Srl Via Tavani 1 / A Delebio (So), Italy, 23014 info@mdlsrl.comwww.mdlsrl.com) and intraoperatively (during the conduct of planned or operative laparotomy for diseases of the organs of the abdominal cavity-3 samples). Further morphological study of hepatobiliopaths was conducted at the department of pathological anatomy National Pirogov Memorial Medical University (Vinnytsia, Ukraine, head of the department, Professor Havrylyuk A. O.).

For pathohistological, histochemical, immunohistochemical studies, columns of trepanobioptates of the liver were fixed in 10% of formalin buffered, carried out through isopropyl alcohol of increasing concentration and poured into paraffin. From the paraffin blocks on the rotational microtome AMOS ERM 3100, serial sections of thickness of 4-5 μm were used for standard pathogistological and immunohistochemical studies. For immunohistochemical studies, serial paraffin sections were used, placed on the "Super Frost Plus" adhesive substrates of the firm "DAKO" Denmark.

Dystrophic changes in hepatocytes, apoptosis, Kaunsilmen's bodies, cylindrical hepatocyte cytolysis, were determined by light microscopy of paraffin sections stained with hematoxylin and eosin.

The main pathogistological changes in the liver in fibrosis and cirrhosis were studied in serial paraffin sections of biopsy stained with hematoxylin and eosin, using the Van Gyzon method, as well as using immunohistochemical techniques.

Immunohistochemical studies using monoclonal antibodies were performed in paraffin sections of the liver tissue. After deparafinization and rehydration of cuts, temperature de-masking was performed (by heating in a water bath in citrate (pH = 6.0) or in Tris-EDTA buffer with pH = 9.0), suppressed the activity of endogenous peroxidase with a 3% solution of hydrogen peroxide and applied blocking whey. After incubation with primary antibodies, the corresponding antigens were detected using the EnVision FLEX +, Mouse, High pH "DAKO" visualization system with diaminobenzidine.

The presence of hepatocytes in hepatitis B virus against the background of HIV infection was determined in paraffin sections by indirect immunoperoxidase method using antibodies to Hep Par 1 clone OCH1E5.2.10 «DAKO» and EnVision FLEX +, Mouse, high pH «DAKO» with diaminobenzidine.

Kupfer cells of peri-sinusoidal liver regions were immunohistochemically determined using monoclonal antibodies CD34 Clone QBEnd 10 and Vimentin, Clone V9 (DAKO, Denmark) and EnVision FLEX +, Mouse, High pH «DAKO» with diaminobenzidine.

For identification of bile duct cells, monoclonal antibodies to Cytokeratin7 (Clone OV-TL 12/30) DAKO and EnVision FLEX +, Mouse, High pH «DAKO» with diaminobenzidine were used.

The degree of liver steatosis was measured in dasgs stained with hematoxylin and eosin in a standardized area of the histological section of the liver, which was its digital image in a Canon DS 126311 (Japan) camera, shot by a microscope Erma EZ 122F (Japan) with an

increase of x200. By graduation E.M. Brunt, D.E. Kleiner (2005) we identified lightweight S1, moderate S2, and severe S3 degree liver steatosis.

In paraffin sections stained for van Gizon, according to a modified graduation E.M. Brunt, D.E. Kleiner, L.A. Wilson et al. (2011) we determined peri-sinusoidal-pericellular or portal-Z1-3 perisinusoidal type of liver fibrosis, as well as its severity: light - F1, moderate - F2, severe F3 fibrosis and F4 cirrhosis of the liver.

RESULTS:

As a result of the conducted researches it was established that in the liver of patients with chronic hepatitis B with HIV infection, non-specific pathomorphological lesions of hepatocytes, manifested by the polymorphism of hepatocyte nuclei with the presence of picnotial and vacuolized nuclei, protein degeneration of hepatocytes in combination with small and large fatty degeneration of hepatocytes, the presence in Kaunsilmen's cells and cytokaryolysis of hepatocytes, nonspecific focal mononuclear infiltration of portal tract, lack of neutrophils and plasmacytes in intracellular and portal cell infiltrates. Characteristically, periportal immune cells infiltrated at different depths and intensities formed so-called "stepped necroses," and portal-centrolobulular-portal cell infiltration formed "bridge-like" necrosis, which caused the development of different intensity of the fibrosis of portal tracts, centrolobular veins and portal-limb fibrous septa. Suspicion of viral damage to the liver was possible by the presence of "matte-glass" hepatocytes in preparations stained with hematoxylin and eosin or by the van Gizon method. These are large hepatocytes with a pale-eosinophilic fine-grained cytoplasm and a nucleus displaced to the cytoplasmic membrane and separated from the cytoplasm by a light rim. According to histochemical studies of the liver, "matte-glass" hepatocytes reflect uneven glycogen content and RNA in different hepatic cells. (Fig.1)

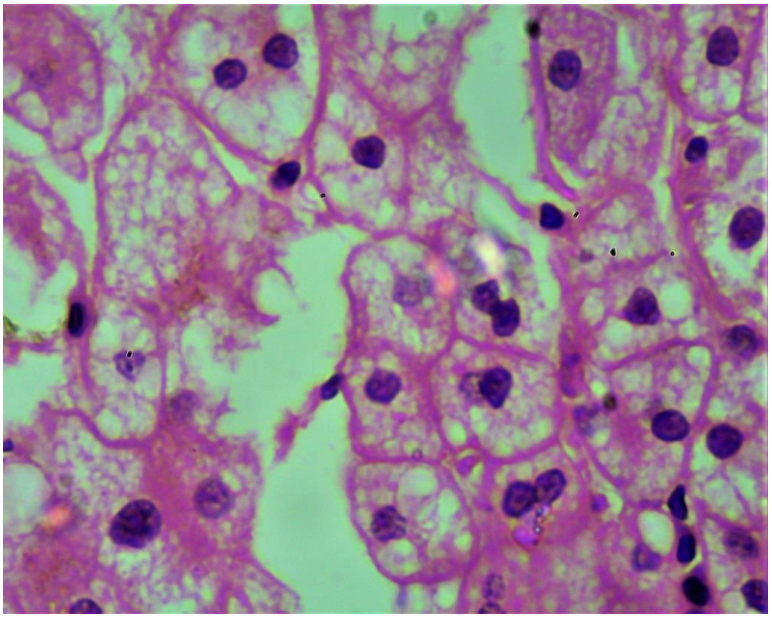

Fig.1. Hydropic dystrophy of hepatocytes, coloration by hematoxylinum. x400

Hepatocytes in the state of apoptosis were reduced in size, had a round-oval form, eosinophilic cytoplasm and hyperchromic nucleus. At microscopy around an apoptotic hepatocyte, an optically clear circular slit was observed, which is a macrophage process. (Fig. 2)

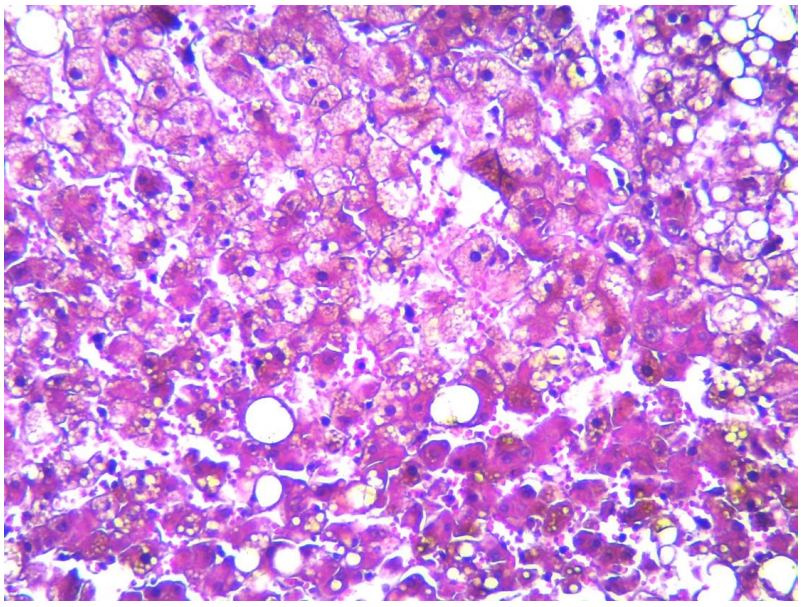

Fig. 2. Hepatocytes in the state of apoptosis, hyaline-droplet and small, - and that of giant fatty dystrophy of hepatocytes, intracellular cholestasis. Coloring with hematoxylin and eosin. x200

In patients with chronic hepatitis B virus, in the background of HIV infection in the liver biopsy, small centers of necrosis of certain areas of the liver slugs were found, in which necrosis covers not only hepatocytes with steatosis, but also cells of perisinusoidal spaces. Such hepatocytes had classical morphological signs of cell necrosis: lysis of the cytoplasm, nucleus picnose, and their disintegration.

In addition, in the liver of patients revealed small formations of non-nuclear hepatocytes, whose cytoplasm has the form of aggregates of medium and small lipid vesicles without the presence of a cytoplasmic membrane. The performed histological studies showed that in the hepatocyte cytoplasm lipid drops were determined as optically empty vacuoles of the appropriate size (from 4 to 40 micrometers in diameter) when staining paraffin sections with hematoxylin and eosin, with the nucleus of hepatocyte shifted to the periphery. (Fig.3)

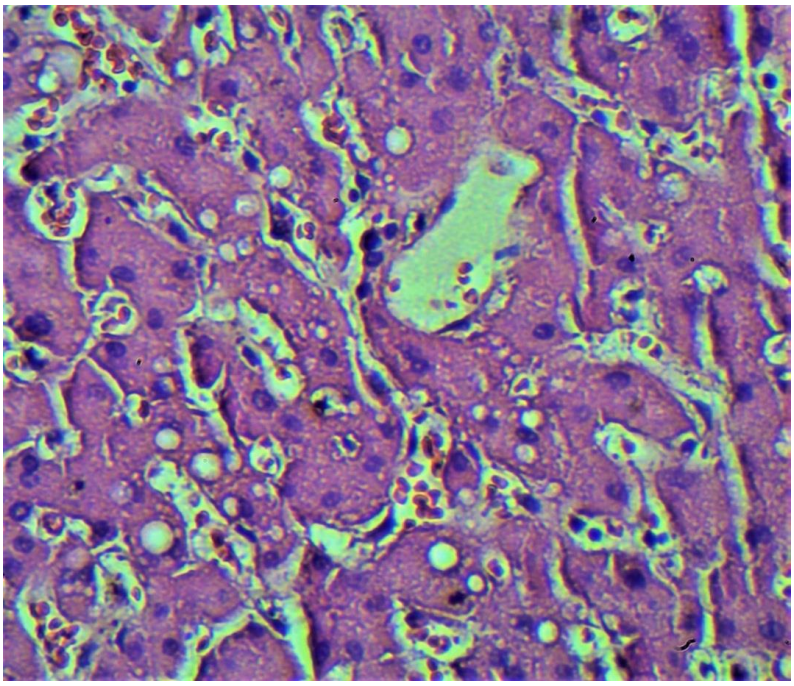

Fig. 3. Fractional cartilage fatty degeneration of hepatocytes. Coloring with hematoxylin and eosin. x200

We performed parallel pathologist studies that proved that one of the hallmarks of chronic hepatitis B virus in the background of HIV infection is the presence of inflammatory cell lymphocytic infiltrate of various degrees and liver fibrosis, which in the final stages leads to the formation of liver cirrhosis. By the progression of the fibrosis of the portal pathways and their fibrous thickening leads to further fibrous transformation of common immune-cellular peripartal necrosis. n the presence of hepatotoxicates in patients with chronic hepatitis

B in the common and long-term periportal immune-cell infiltrates, with a thorough analysis of histological liver preparations stained with hematoxylin and eosin and for van Gizon, one and the same patient may be able to detect a gradual decrease in immune cells in peripartal zones and their replacement with fibrous tissue. The fibrous transformation of periportal immune-cell infiltrates in such patients is also confirmed by histological liver preparations stained for van Gizon. In the periportal zones of portal liver pathways, with the decrease of immune cells, simultaneous formation of portal-perissinusoidal connective tissue septum and expansion of periporateral fibrosis is observed. (Fig.4)

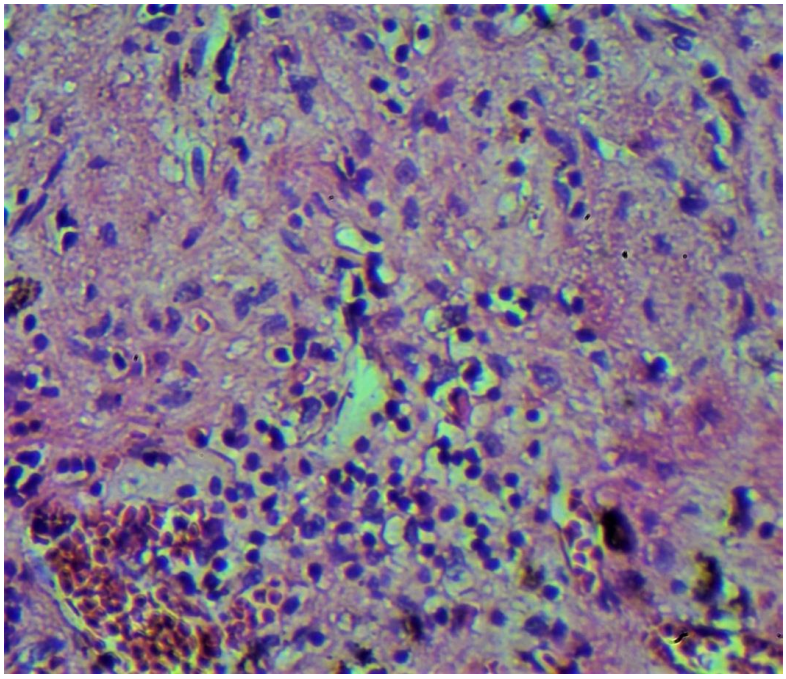

Fig. 4. Inflammatory cell mononuclear infiltrate in the periportal zone, activation of fibroblasts in fibrosis of the liver. Coloring with hematoxylin and eosin. x200

It has been established that patients with chronic hepatitis B virus in the background of HIV infection in paraffin sections of the liver colored by Van Giason dominated portal-perissinusoidal fibrosis of the liver. The thin layers of the connective tissue cover individual hepatocytes and hepatic cell groups. (Fig. 5-7).

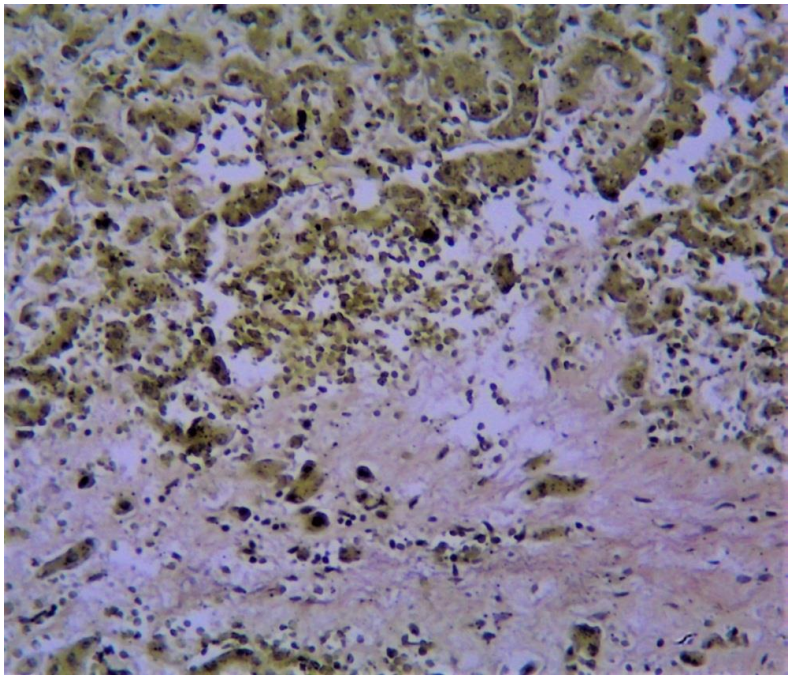

Fig. 5. Formation of port centrifugal connective tissue septum. Coloring for Van Gizon. x100

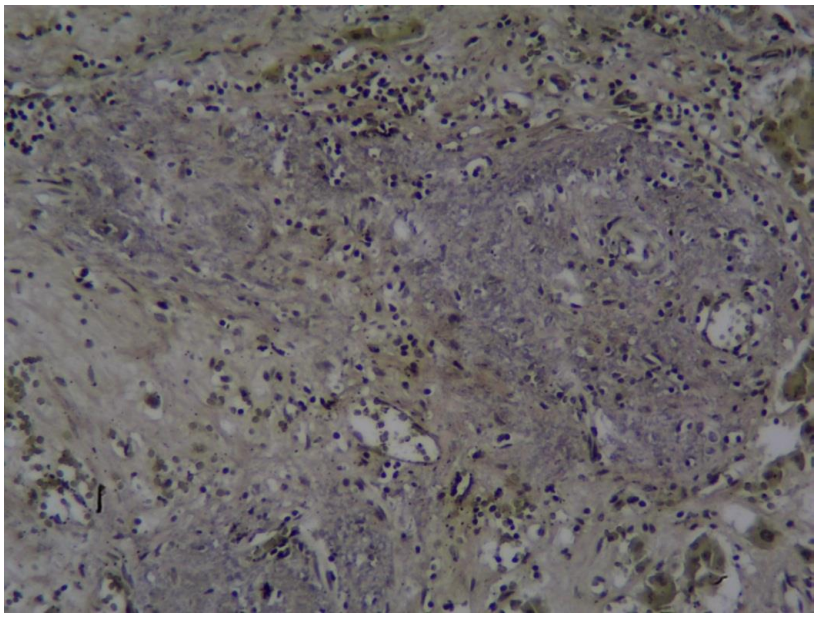

Fig.6. Expressed fibrosis due to the growth of coarse fibrous connective tissue. Coloring for Van Gizon. x100.

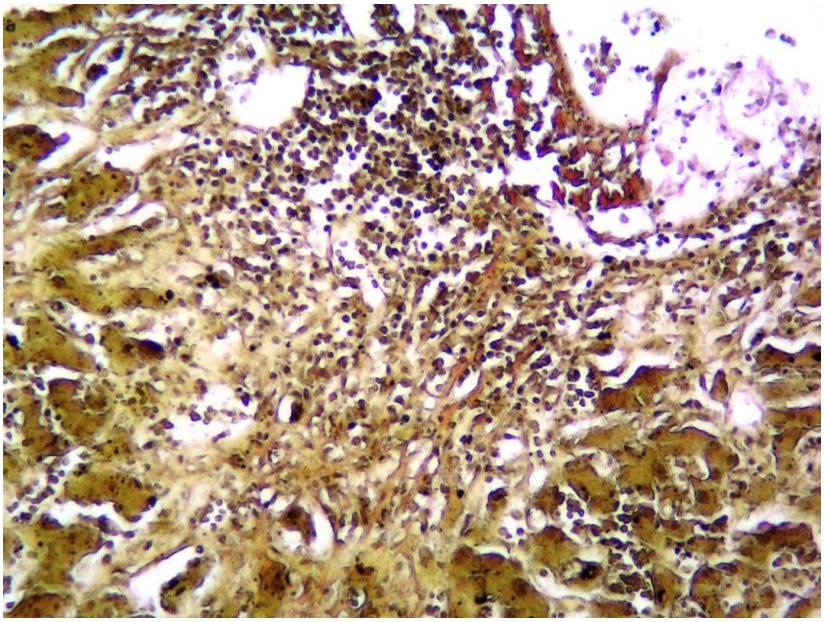

Fig. 7 The layers of the connective tissue cover individual liver cells and cell groups. Coloring for Van Gizon. x100.

And also local (isolated) peri bilirial fibrosis is detected, in which significant circular fibrosis is detected around small bile ducts localized between the liver lobes, which is not part of the portal triads. (Fig.8)

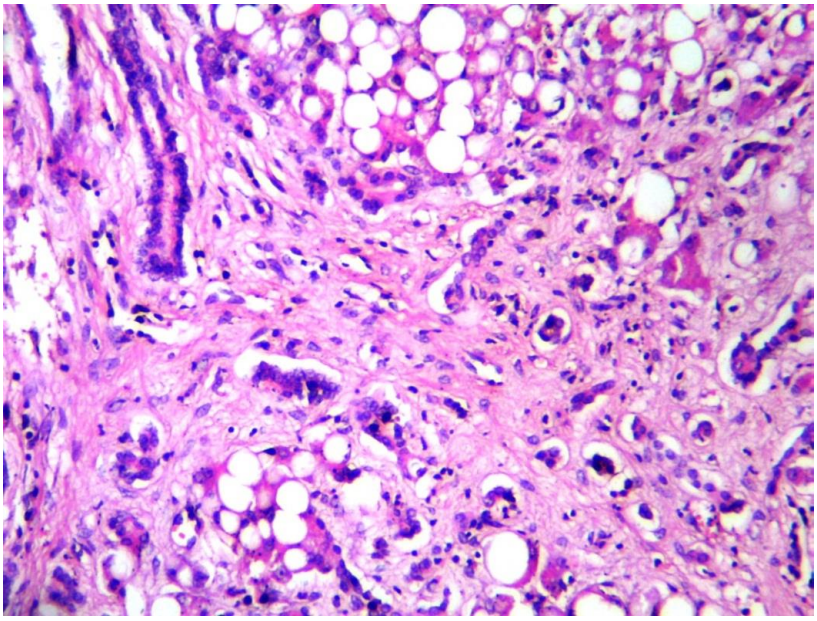

Fig. 8. Local peri bilar fibrosis of the liver. Coloring with hematoxylin and eosin. x100

CONCLUSIONS.

The morphological picture in the liver tissue in patients with chronic hepatitis B with HIV infection is characterized by non-specific pathomorphological damage to hepatocytes and the presence of periportal immune cell infiltrates of different depths and intensities. In the liver tissue, pathognomonic signs of HBV-infection in the form of "matte-glass-like" hepatocytes were detected, reflecting the unevenness of the content of glycogen and RNA in different hepatic cells. A characteristic feature of HBV/ HIV infection was the presence of inflammatory cell lymphocytic infiltrate of varying degrees and liver fibrosis.

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