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MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructure of Hepatorenal Cell Populations in Patients with HCV and HBV Infection Markers. Hepatorenal Associations

G. I. Nepomnyashchikh, M. A. Bakarev, D. L. Nepomnyashchikh, A. V. Yudanov, V. I. Kapustina, O. A. Postnikova, E. V. Vinogradova, S. G. Rusinova, and T. A. Telegina

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Hepatorenal cell populations were studied in patients with HCV and HBV infection markers and renal dysfunction. Pronounced mosaicism of ultrastructural changes in hepatocytes was associated with polymorphic cytopathic effects caused by RNA-genome hepatitis C virus and DNA-genome hepatitis B virus. The destructive component of the tubular compartment predominated in renal biopsy specimens from patients, with subsequent degeneration of the tubular epithelium associated with progressive interstitial fibrosis. Immunohistochemical studies detected HCV NS3Ag and HBcAg structural marker in the tubular epitheliocytes. An appreciable part of the structural and functional changes in the liver in patients with HCV and HBV infections was caused by the therapeutic complex, including programmed hemoperfusion.

Key Words: *chronic HCV and HBV infections; hepatopathy; nephropathy; immunohistochemistry; polymerase chain reaction*

The variety of "liver-kidney" relationships and mechanisms mediating the effect of the liver on the kidneys determine different clinical morphological variants of hepatic nephropathy. The major of these are the hepatorenal syndrome, glomerulonephritis, hepatic glomerulosclerosis, renal tubular acidosis, pyelonephritis [8,14].

One of the most incident extrahepatic manifestations of HBV and HCV infections is renal pathology presented by chronic glomerulonephritis or rarely by tubulointerstitial nephritis [10]. Renal involvement develops in 20-50% patients with HCV infection with mixed cryoglobulinemia; 10-20% of these patients develop membranous proliferative glomerulonephritis without detectable cryoglobulins, and less than 10% patients develop membranous nephropathy [7]. HCV in these patients is directly involved in the pathogenesis of vascular abnormalities in cryoglobulinemic vasculitis, which is proven by the presence of virus antigens in the glomerular vascular structures, mesangial cells, and interstitial vessels [13].

Detection of HCV proteins in the renal tissue can be a result of not only their delivery with circulating immune complexes, but also local replication of the virus. The use of highly specific PCR modifications and *in situ* hybridization have proven HCV replication in many organs, including the blood mononuclears, bone marrow cells, oral mucosa, heart, pancreas, in-

Research Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. G. I. Nepomnyashchikh

testine, lymph nodes, adrenals, thyroid, spleen, and kidneys [2].

The data on the incidence of renal involvement in patients with HBV infection are contradictory, which is most often explained by the latent course of nephropathy. Renal involvement often determines the disease prognosis in chronic HBV and HCV infections [12]; moreover, renal status cannot be neglected when creating the methods for adequate therapy of chronic hepatites.

We studied structural characteristics of hepatorenal cell populations in patients with HCV and HBV infections and renal dysfunction.

MATERIALS AND METHODS

Clinical morphological studies were carried out in 31 patients (20 men and 11 women aged 16-52 years) with renal dysfunction and markers of HCV (*N*=14) and HBV infections (*N*=13) and with mixed HCV+HBV infection (*N*=4). Comprehensive studies were carried out in all patients, including analysis of blood biochemistry, serological markers of viral hepatitis, HCV and HBV replication markers in the blood and liver, evaluation of viremia, levels of infected hepatocytes, and HCV and HBV genotypes.

Examination of puncture biopsy specimens of the liver and kidneys included, in addition to light (paraffin and semithin sections) and electron microscopy, immunohistochemical analysis of HCV NS3Ag and detection of hepatic fibrogenic cell populations by the expression of smooth muscle α-actin. Immunodetection was carried out by the standard immunohistochemical method with monoclonal antibodies.

Paraffin sections for light microscopy were stained with hematoxylin and eosin with Pearls' reaction, by van Gieson method with poststaining of elastic fibrils with Weigert resorcin-fuchsin and PAS reaction. Liver and kidney specimens for electron microscopy and immunohistochemical analysis were fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.2-7.4). Semithin sections were stained with Schiff reagent, azure II, and Congo red for detecting amyloid depositions in kidney specimens. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope (Jeol) at accelerating voltage of 80 kV. The diagnostic complex included clinical, biochemical, and immunoserological methods. The HBsAg, HBeAg, HBcAb, HBsAb, HBeAb, summary HCVAb, antibodies to HCV Core and NS antigens were tested. HCV RNA was detected by PCR in the blood sera and mononuclear cells, in native liver tissue; HBV DNA was detected in the blood, liver, and kidney specimens. The duration of infection was determined by the initial detection of HCV and HBV infection serological markers, serum viremia level was evaluated using the test system manufactured by Research and Production Laboratory of Central Research Institute of Epidemiology (Moscow); the linear range of the test system was 10^3 to 3×10^6 genome copies of HCV RNA/ml serum.

Analysis of morphological changes in liver biopsy specimens was carried out using the Los Angeles Classification of Chronic Hepatites (with the etiological factor as the leading characteristic); the activity of the infectious process was evaluated.

RESULTS

The majority of patients with HCV and HBC infection markers exhibited no obvious clinical manifestations of chronic hepatitis; the pain syndrome, hepatomegalia, and high levels of aminotransferases were observed in only some cases. Renal involvement more often manifested by the nephrotic syndrome combined with arterial hypertension (*N*=20), the rest patients developed urinary syndrome, also mainly concomitant with arterial hypertension.

Structural reactions of the liver in combined hepatorenal disease. Analysis of liver biopsy specimens from patients with HCV and HBV infection markers showed less pronounced phonotypical heterogeneity intrinsic to the hepatocyte population. This indicated reduced reactivity of the organ as a result of not only chronic renal disease, but also drug-induced pathomorphosis. The main pathomorphological changes found in liver biopsy specimens were hepatocyte degeneration, cellular infiltration, and fibrosis of different degree.

Among degenerative changes, polymorphic lipid infiltration of hepatocytes predominated, mainly medium- and large-vesicular, or small-vesicular subplasmolemmal in HCV infection. An appreciable population of hepatocytes had signs of cellular involutive degeneration: slightly eosinophilic "devastated" cytoplasm (Fig. 1, *a*) with sites of electron transparent cytoplasmic matrix, observed at the ultrastructural level, which was explained by the absence of membrane organelles, free ribosomes, polysomes, and glycogen together with preserved perinuclear compartment.

Polymorphic mitochondria with significant destruction of the cristae were located perinuclearly and diffusely in the hepatocyte cytoplasm (Fig. 1, *b*). Just few hepatocytes contained peroxisomes, multivesicular and residual bodies (osmiophilic membranes and tubulovesicles; Fig. 1, *c*), which was indicative of the cytopathic effects of the virus.

Intracellular cholestasis with predominant location in the pericentral hepatocytes was worthy of note. The hepatocyte cytoplasm contained large granules with

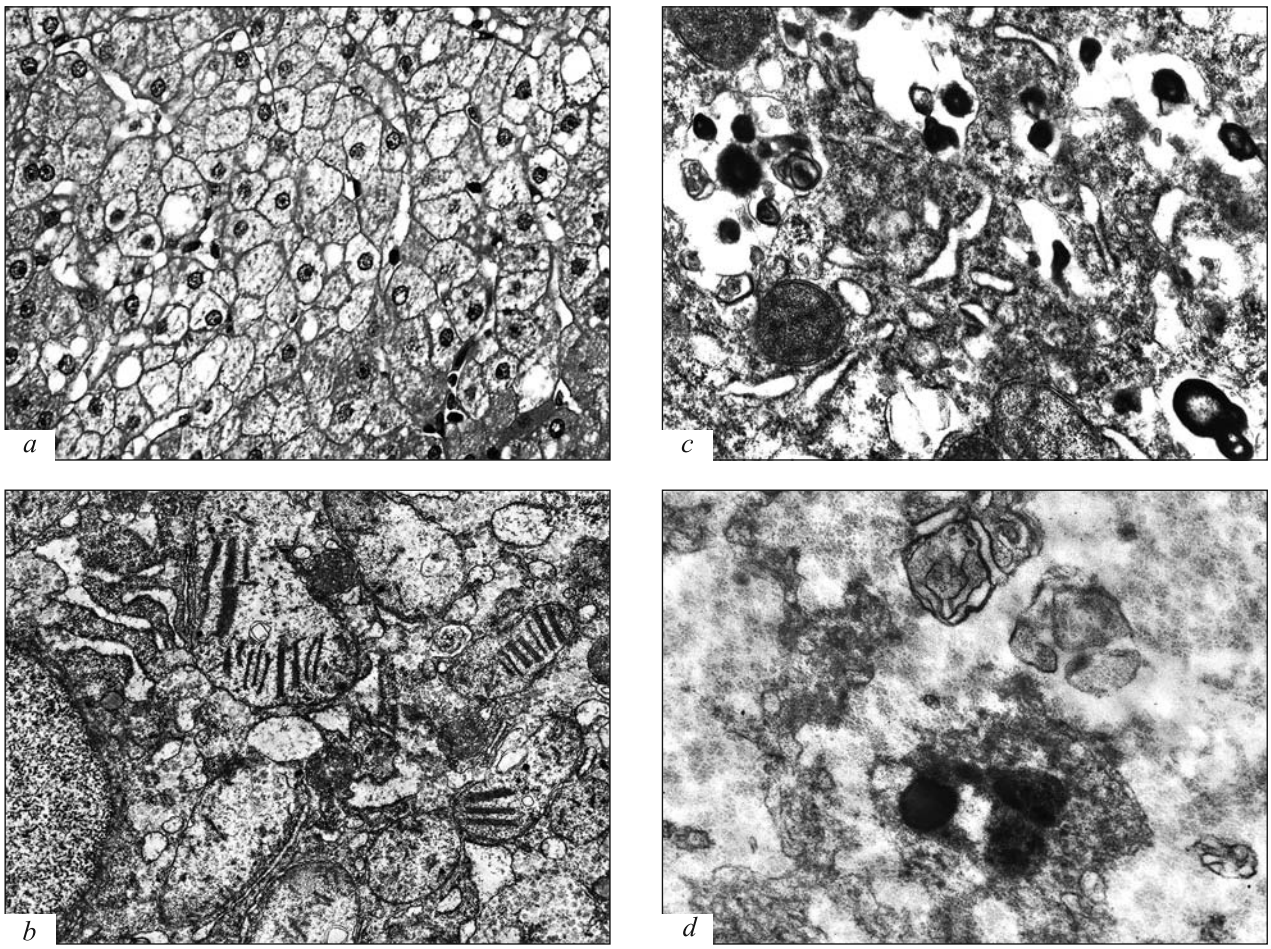

Fig. 1. Pathomorphology of liver biopsy specimens from patients with renal dysfunction and serological markers of HCV+HBV (*a*, *d*) and HCV infection (*b*, *c*). Hematoxylin and eosin staining (*a*), electronograms (*b-d*). *a*) Involutive degeneration of hepatocytes, ×400; *b*) hepatocyte fragment: destruction of mitochondrial cristae, ×15,000; *c*) osmiophilic residual bodies and HCV infection pathognomonic trabecular structures in the hepatocyte cytoplasm, ×15,000; *d*) hepatocyte fragment: bile components involved in the formation of lipofuscin osmiophilic granules and residual bodies, ×20,000.

heterogeneous floccular contents; they were located by the biliary poles and diffused in the cytoplasm. If evacuation of the bile-containing granules was disordered, secondary phagosomes formed with participation of lysosomes (large osmiophilic conglomerations) and heterogeneous residual bodies (Fig. 1, *d*). Marked hemosiderosis was found in the liver biopsy specimens from patients receiving programmed hemoperfusion: hemosiderin granules were found in many hepatocytes, particularly in the periportal zone, and in the siderophages.

Slight mononuclear infiltration of the portal tracts was found in all biopsy specimens; in some cases, it involved the parenchyma. Fibrosis was moderate in the majority of cases, central fibrosis always predominating, perisinusoidal less so, and portal and periportal the least. Reconstruction of ultrathin sections detected a unique ultrastructural phenomenon – restructuring of the sinusoidal hepatocyte pole with plasmalemma formation of numerous microvilli and folds with concentration of numerous mitochondria in these regions, paralleled by phagocytosis of collagen fibril fragments. Hence, the expression of macrophageal properties of hepatocytes reflected plasticity of differentiated parenchymatous liver cells.

The presence of serological markers of HBV infection in the liver biopsy specimens was associated with more manifest damage to the nuclear compartment – significant anisokaryosis, solitary cells with ring-shaped and vacuolated nuclei, and binuclear hepatocytes; Counsilman's bodies were detected in several cases. Lymphoid aggregations and follicles formed in 4 cases with positive reaction to HCV RNA. Rare detection of morphological markers of HCV and HBV infection in hepatic tissue can be explained by extrahepatic replication of hepatitis viruses and/or immunosuppression; latent HBV infection was also probable [1].

The degenerative changes in the liver biopsy specimens from patients with mixed infection were deeper and more disseminated than in HCV monoinfection,

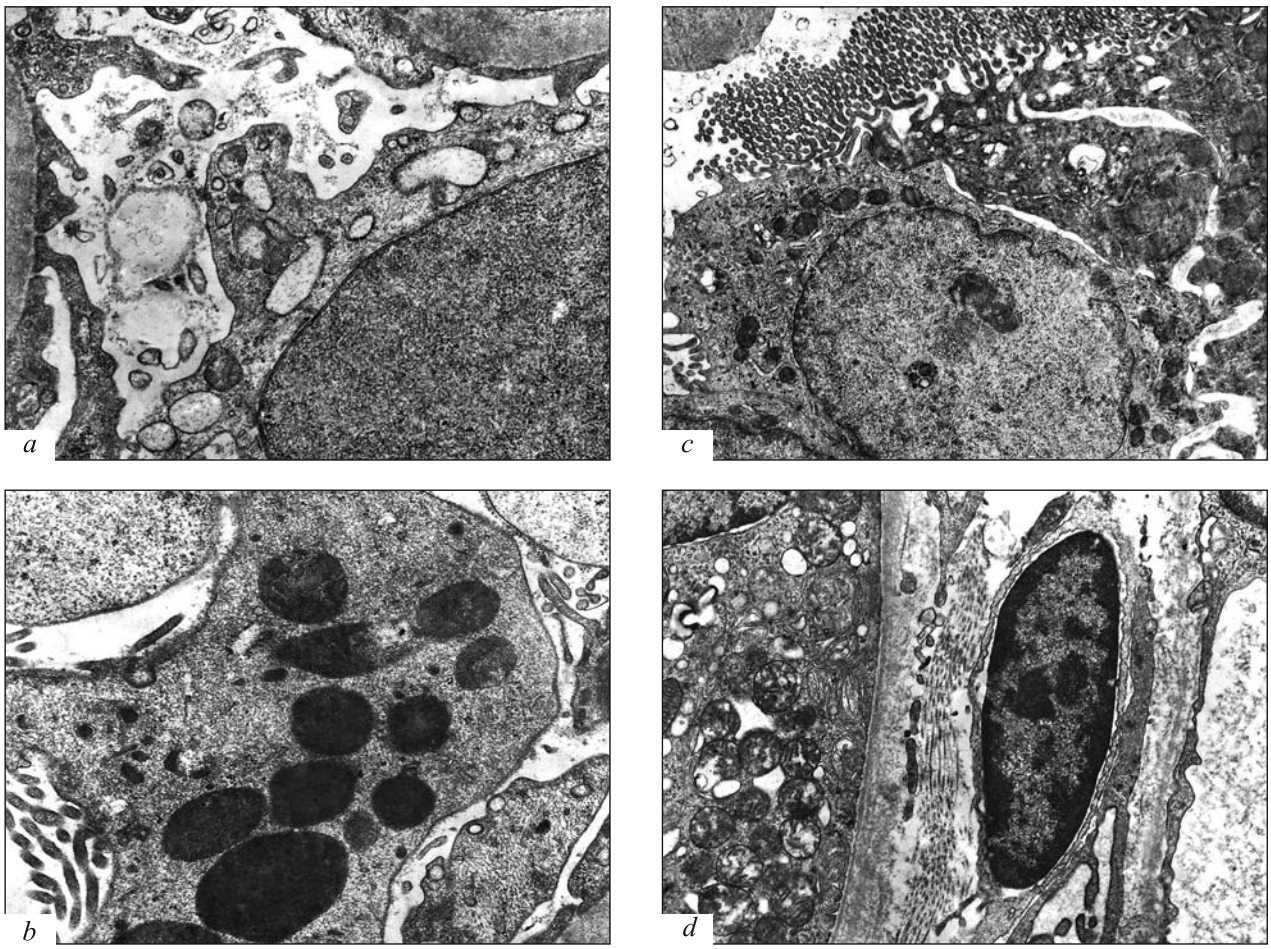

Fig. 2. Ultrastructural characteristics of renal cell populations in patients with renal dysfunction and serological markers of HBV (*a*, *b*) and HCV+HBV infection (*c*, *d*). *a*) Podocyte of a glomerular capillary: cytoplasm vacuolation, focal fusion of cytopodia, ×6000; *b*) podocyte cytoplasmic process with incorporation of electron-dense substance. Podocyte villous transformation, ×8000; *c*) epitheliocyte polymorphism in a proximal convoluted tubule, irregular location and focal disappearance of brush border microvilli, ×5000; *d*) peritubular bundles of collagen fibrils. Low pinocytous activity of interstitial capillary endotheliocytes, ×4000.

the mosaicism of ultrastructural changes in hepatocytes was more pronounced because of a variety of cytopathic effects of a complex viral exposure. The RNA-genome HCV destroyed mainly the cytoplasmic organelles, sparing the nuclei. The DNA-genome HBV caused degradation of the nuclear compartment, sparing, more or less, the ultrastructure of the cytoplasmic organelles [5]. Predominance of the pathogenic role of one of the viruses could be indirectly determined by predominance of this or that type of degenerative changes in the biopsy specimen.

Thus, an appreciable part of the structural and functional changes in the liver seemed to be caused by the complex of therapeutic measures, including immunosuppressive therapy and programmed hemoperfusion. This explained primarily the absence of phenotypical heterogeneity characteristic of the hepatocyte population, macrovesicular lipid infiltration, and intracellular cholestasis, causing direct destruction of hepatocytes and thus creating the vicious circle in the bile synthesis and excretion; hepatic hemosiderosis with subsequent destruction of hepatocytes as a result of hemoperfusions and use of iron-containing drugs, and central vein fibrosis and perisinusoidal fibrosis – universal markers of toxic exposure.

Structural reactions of the kidneys in combined hepatorenal disease. Structural changes in renal biopsy specimens involved the glomerular, tubular, and interstitial compartments. Lymphoid aggregations and follicles – typical morphological markers of chronic HCV infection – were detected in half of the specimens. Immunohistochemical analysis with monoclonal antibodies to HCV NS3Ag in these cases showed a clear-cut positive reaction in the tubular epitheliocytes [6]. Structural marker HBcAg – ring-shaped transformation of podocyte and tubular epitheliocyte nuclei – was detected in part of specimens from patients with serological markers of HBV infection (HBsAg).

Polymorphic changes in the glomerular structural components conformed to the picture of membranous

nephropathy and focal segmented glomerulosclerosis in the majority of cases and were associated with coarse disorders in the glomerular filter ultrastructure. Significant changes in the capillary loop endotheliocytes predominated in patients with markers of HCV infection: uneven distribution of fenestra, sharply increased electron density of the cytoplasmic matrix and caryoplasm with alteration of membrane organelles. In HBV infection the changes were more pronounced in the epithelial compartments – podocytes with focal or total fusion of cytopodias (Fig. 2, *a*), hyperplasia of the cytoplasmic fibrillar structures, villous transformation phenomena, emergence of vacuoles filled with electron-dense contents (Fig. 2, *b*).

Tubular changes, most significant in HBV infection, were a characteristic morphological sign. Tubular epitheliocytes were in a state of marked degeneration; the most severe destructive changes were smooth basal cytolemma, lesser size and number of mitochondria, destruction of the proximal tubular epitheliocyte brush border (Fig. 2, *c*), and tubular necrosis and intracellular regeneration of different degree. These changes were associated with peritubular edema and accumulation of collagen fibrils between the epithelial basal membrane and peritubular capillaries (Fig. 2, *d*), in which endotheliocyte degeneration developed. Numerous vacuoles with electron-dense centers were detected in the thickened basal membrane.

Pronounced fibrosis with the peritubular component predominated in biopsy specimens from patients with HBV infection markers (50% cases); in chronic hepatitis C it was found in 27% cases. However, fibrous changes in the interstitium were detected in all cases and they presumably made an important contribution to clinical manifestations of the renal involvement. Mononuclear infiltration was the minimum, focal, with a trend to formation of lymphoid aggregations. The arterial hypertension syndrome was associated with specific restructuring of the renal vessels with development of arterial myoelastofibrosis, paralleled by perivascular sclerosis.

Renal disease was associated with cryoglobulinemia in 7 (23%) patients (including 5 with HCV infection markers). However, no specific morphological changes characteristic of cryoglobulinemic glomerulonephritis (capillary clots, double basal membranes, renal vasculitis) were detected. Marked monocyte infiltration of the glomeruli was detected in just one case.

On the whole, the structural changes in renal biopsy specimens from patients with HCV and HBV infection markers can be interpreted as nephropathy of mixed origin (toxic, infectious, hypertensive), its evolution corresponding to the "dystrophy–atrophy–fibrosis" scheme. The glomerular compartment involvement plays the leading role in the pathogenesis of nephropathy, but it is essential that chronic renal insufficiency is unfolding in parallel with augmenting interstitial sclerosis and tubular degeneration under conditions of the minimum inflammatory cellular infiltration. Renal proteinuria in this case is determined by high permeability of the glomerular basal membranes (glomerular proteinuria), a lesser tubular reabsorption capacity, and desquamation of the tubular epithelium (tubular proteinuria).

High incidence of renal involvement in viral hepatitis can be explained by close ontophysiological, anatomical, functional, and humoral relationships between the liver and kidneys, common autonomic nervous system, and anastomoses between the portal and renal vein systems. Liver involvement necessitates elimination of toxins, which implies more intense work of the kidneys [3]. In turn, renal dysfunction and the relevant complex of therapeutic measures, primarily programmed hemoperfusion, augment the course of hepatic disease [11].

In addition to induction of systemic immune reactions with participation of cryoglobulins, immune complexes, and Toll-like receptors [15], hepatitis viruses can directly participate in the mechanisms of renal involvement in HCV and HBV infections. This is proven by regular detection of HCV proteins in endothelial, mesangial cells, and epitheliocytes of the tubules, association of these phenomena with higher proteinuria values [13], and detection of structural markers of HCV and HBV infection in renal biopsy specimens.

Hence, pathological changes in the kidneys of HCV and HBV infected patients can be interpreted within the framework of a systemic infectious process [4,9] as components of its patho- and morphogenesis, which is confirmed, among other things, by the data of parallel analyses of biopsy specimens of the liver and kidneys. Mutual effects of the pathological processes in these organs are essential, not only as regards their mutual pathomorphosis, but also for the development of adequate therapeutic methods [13].

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