MORPHOLOGICAL FEATURES OF CHRONIC VIRAL HEPATITIS “B” DEPENDING ON THE VIRUS GENOTYPE

The paper studies morphological changes in the liver in different genotypes of chronic viral hepatitis “B” (HBV) in Uzbekistan. In examined patients with D genotype, 7.4% showed the minimum activity of pathological process, 48.2% - low, 25.9% - moderate and 18.5 % - the pronounced activity of pathological process. Results in patients with the C-genotype were following: low activity in 33.3%, moderate activity - in 16.7%, pronounced activity - in 50% of patients. Among patients with A-genotype of HBV 25% - revealed the minimal activity of pathological process, 50% - low, 12.5% - moderate and 12.5% - marked activity. It is necessary to notice that at patients with C genotype HBV disease proceeds more hard with more pronounced pathomorphological changes in comparison with D and A -genotypes.

Keywords: Morphological, genotype, pathological process, chronic viral virus hepatitis B (HBV).

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Introduction

At present, the problem of viral hepatitis occupies a leading position in infectious diseases in view of their widespread dissemination, as well as the manifestations of severe, and fulminant hepatic cirrhosis in patients with chronic forms of HBV (Aliev and Kushimov, 2002; Valiev et al., 2001; Khazanov et al., 2001).

Research on hepatitis B acquires special significance in Uzbekistan which territory, according to WHO criteria, belongs to a region with high prevalence of markers of HBV (Zakirhodzhaev, 2003; Nepomnyashchikh, 1994). These facts call hepatologists to conduct in-depth studies and to take anti-epidemic and prevention measures in the country, as well as to improve diagnostic procedures to reduce the patients with both acute and chronic disease course. Currently, there are significant advances in the study of many aspects of chronic viral hepatitis B - etiology, pathogenesis, clinics, diagnostic services in light of modern molecular genetic techniques, as well as mutational variants of HBV (Daminov et al., 2002; Podymova, 1996; Kidd-Ljunggren et al., 2002). Despite positive achievements, there are many issues that remain unresolved. In particular, the issues of clinical and morphological diagnosis of various versions of chronic viral hepatitis B require further examination considering the genotype and mutations of the virus. Unfortunately, even the application of clinical and serological and instrumental methods do not sufficiently provide the correct diagnosis, which is important for assigning the respective optimal therapeutic program, monitoring and predicting outcomes disease (Titov, 1996). In response to this, it becomes urgent the use of intravital liver biopsy with subsequent histological and immunomorphological examination of liver biopats to identify chronic viral hepatitis, its activity level and development stage, to evaluate the ongoing effectiveness of antiviral drugs, as well as to diagnose early stages of formation of cirrhosis (Serov et al., 1996).

Materials and methods

Purpose of the work was to study the morphological changes in the liver in different genotypes of HBV in Uzbekistan.

We have examined 121 patients aged 15 to 70 years old with chronic viral hepatitis B diagnosis. Average age was 38.5 ± 9.1 years. The diagnosis was made according to the directive of the Ministry of Health of the Republic of Uzbekistan on viral hepatitis.
(30th October, 2000, No560), as well as the classification adopted at the World Congress of Gastroenterology (Los Angeles, 1994) and it based on morphological study of liver biopsy with determination of the histological activity index.

Among the observed cases there were 121 men 78 (64.4%) and 43 women (35.6%), so men were significantly more than women (P <0.001). The surveyed patients had concomitant diseases in the form of chronic cholecystitis, chronic pyelonephritis which in turn had contributed to the late diagnosis of chronic viral hepatitis B. Patients were hospitalized in the clinic of Scientific Research Institute of Virology (SRIV) under the Public Health Ministry of Uzbekistan (PHM).

All patients’ diagnosis was determined through application the complex of clinical, epidemiological, anamnestic, biochemical, instrumental and morphological examinations. Morphological changes in liver biopsies performed in 41 patients with chronic viral hepatitis B. All 121 patients were identified as having HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBcor Ig G and anti-HBcor Ig M). Patients who had revealed markers of HCV, HDV and HAV were excluded from the study. Molecular and genotyping of HBV PCR method was carried out in the Laboratory of Molecular Biology, Nagoya University (Japan), Institute of Immunology (Uzbekistan Academy of Sciences) and Health Reference Laboratory (PHM). It should be noted that the majority of examined patients prior to admission to the SRIV clinic had repeatedly received treatment over chronic viral hepatitis B but antiviral therapy had not been performed.

Among the clinical signs observed there were presence of yellow skin and sclera, skin rash, palmar erythema, vascular stars - telangiectasias, the manifestation of collaterals on the anterior and lateral surface of the abdomen. There were determined the liver spleen dimensions; their density, state of edges and surface pain were taken into account.

Ultrasound examination also studied the state of the gall bladder, pancreas, and intestines. Degree of expansion of varicose veins in esophagus was determined using esophagogastroduodenoscopy method. During the examination, there were distinguished 3 degrees of severity of esophageal varicose veins:

- 1st degree - modest increase in varicose veins (diameter 1-2 mm). Esophageal mucous pale - pink;
- 2nd degree - mild varicose veins (diameter 3-4 mm). Esophageal mucous pale - hyperemic, puffiness, pale;
- 3rd degree - highly pronounced varicose veins (diameter 5 mm and more). Esophageal peristalsis - sluggish. Mucous membrane of vascular bundles - thinned, and numerous erosions are revealed in the distal esophagus.

Nature of urine, presence of ascitic fluid and the tension of muscles of the abdominal wall were examined.

Biochemical studies carried out in all patients in the disease dynamics. Total bilirubin with its direct and indirect fraction, hepatic-specific aminotransferases (AST and ALT) were determined. The serum concentrations of total protein and its fractions - albumin and globulin - also were established. In the dynamics of the disease, thymol test and blood coagulogram (prothrombin index, trombotest, etc.) were studied and general analysis of blood and urine was carried out.

Lifetime morphologic study of liver biopsy was done in 41 patients diagnosed with chronic virus hepatitis B. The aspiration method with a Menghini needle was applied. Resulting from liver biopsy the punctate was studied in the virus infections morphology laboratory of PHM. The liver biopsies were fixed in 10% neutral formalin (2-4 hours), Carnua fluid and embedded in paraffin. Slices of liver were stained with hematoxylin and eosin, as well as the method of Van Gisone. The degree of activity of pathological process in the liver was studied by semiquantitative assessment scores, histological activity index (HAI) by Knodell et. al. (1981).
Results

Studies have shown that the minimal degree of histological activity index (HAI) has been established in 20 (48.8%) patients, which corresponds to 1.3 magnitudes in Knodell score. Soft activity (4-7 points) detected in 8 (19.5%) liver biopsy specimens of patients with chronic viral hepatitis B. Accordingly, moderate activity (8-12 points) is diagnosed in 5 (12.2%) cases and the high one (13-18 points) in 8 (19.5%) cases.

Histologically examination of liver biopsy specimens, in patients with minimal activity chronic viral hepatitis B, marked inflammatory reaction in the stroma of the liver, resulting in a slight expansion of some portal tracts by mononuclear infiltration submitted by lymphocytes, isolated macrophages and plasma membranes. Small lymphocytic infiltrates were shown. Detected HBsAg were in form of dull and glassy cells with pale eosinophilic cytoplasm. Slight hypertrophy of the endothelial cells of liver was registered.

Biopsies of patients with mild HBV activity were characterized by isolated perportal necrosis and slight inflammatory infiltration of portal tract. In the study of biopsies in patients with moderate HBV activity, above described changes were more pronounced with the formation of focal necrosis with marked inflammatory cell infiltration of some portal tracts. There was expansion of sinusoids with hypertrophy of endothelial cells and within the lobules there were found dull glassy hepatocytes containing HBsAg. Studying biopsies of patients with high HBV activity indicated on increase of portal tracts by mononuclear infiltration, including lymphocytes and isolated macrophages, eosinophils, and plasma cells fibroplasty. Destroyed integrity of boundary plate is explained by increase in the proliferating interlobular connective tissue that penetrates between the lobules. All these changes lead to a stepped necrosis of hepatocytes. In some parts of the lobes the focal necrosis of hepatic cells were observed. Parenchyma infiltrated with lymphocytes. Hepatocytes have granular and hydropic degeneration, dull and glassy cells are centrolobular, as well as in periphery and in the middle of lobes, including some fibrous tissue. Endothelial cells of the liver are hypertrophic. Clearly defined the formation of liver fibrosis with marked sclerotic changes in the portal fields. 2 patients with high HAI showed signs of HBV transition to cirrhosis with beginning formation of false lobules.

The liver biopsy in patients with HBV enabled a more in-depth study of morphological changes in liver tissue, identification of HAI and stage of disease. The examination also allowed to link pathological changes with clinical disease manifestations, genotype of HBV, which in turn solves the problem of appointment antiviral therapy to patients.

In our further studies, we examined the genotypes of HBV in 41 patients with chronic viral hepatitis B. The liver biopsy and HAI were established in these patients. The study found HBV DNA in blood serum. Further, the genotyping determined D, C, A and mixed A + G genotypes of HBV. Studies on genotyping showed that the D-genotype was found in 27 (65.9%) patients, A-genotype - in 8 (19.5%) cases and C genotype of HBV - in 6 (14.6%) patients.

The next step was to identify the G genotype. It should be noted that the G genotype in all cases was identified, alongside with A, as mixed genotype accounted for 42% of the total number of patients with A-genotype. This is probably due to the fact that the sequence of nucleotides in the G-genotype genome is similar to the one in A-genotype genome. For differentiation, the additional enzyme was used which divided A-genotype genome in 251-255 n.s. and 530-534 n.s. and G-genotype in 482-486 n.s. Full transcript of the nucleotide sequences of the genome and amino Pre-Core and HBcoreAg showed that the G-genotype genome - is 3248 n.s. in average that is slightly longer than the genomes of six other genotypes, ranging in size from 3182 to 3221 n.s. Difference in genome length of G-HBV genotype is conditioned by 36 n.s. in the second codon of HBV core gene. Another feature of the genotype G is the presence, in positions 2 and 28 of pre-C region, two stop codons which block the synthesis of HBeAg.

In our further studies, we faced the question: whether the G-genotype is separate or represents a single defective virion which needs A-genotype for its vital activity? Sequence analysis of nucleotide order of A and G in comparison with data GenBank/EMBL showed that association was caused by the high-matched nucleotide sequences in the S-gene to 4%. This probably occurs because of their recombination.
Proceeding from the above the data of mixed A+G genotype were engaged in the same group.

**Conclusions**

Analysis of morphological changes in dependence on HBV genotype showed the following:

- Among patients with D genotype, the minimal activity of the pathological process was registered in 7.4% of cases, low activity - in 48.2%, moderate - in 25.9%, and pronounced activity - in 18.5% of patients;
- Among patients with genotype C, low activity was detected in 33.3% of patients, moderate activity - in 16.7%, and the pronounced activity - in 50% of patients;
- Among patients with A genotype, the minimal activity was registered in 25% of patients, low activity - in 50%, moderate activity - in 12.5%, and the pronounced HAI - in 12.5% of cases.

Thus, in observed patients the D-genotype of HBV dominates. However, it should be noted that patients with C-genotype HBV disease follows with more severe pathological changes compared with the D and A - genotypes.

**References**


