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CLINICAL, DIAGNOSTIC AND MOLECULAR GENETIC FEATURES OF CHRONIC MIXED-HEPATITIS B + C IN UZBEKISTAN

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The study established the frequency of detection of antigens and antibodies to HBV and HCV in mixed hepatitis B+C. The highest rates of detection of serological markers were observed in the identification in the blood the HBV genotype D and HCV genotype 1b. This phenomenon can be explained by the high replicative and antigenic activity of viruses in these genotypes, resulting in a high viral load in blood, compared with HBV genotypes A and C, HCV genotypes 3a and 2a. In this regard, the use of ELISA technique in combination with PCR diagnosis and genotyping of viruses is considered as desirable for the etiological interpretation of viral hepatitis. This approach also will enhance achieving the correct diagnosis and adequate choice of treatment programs.

Keywords: HBV, HCV, chronic mixed-hepatitis, genotype, pathological process.

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Introduction

The problem of associated hepatitis is relatively young and, therefore, not sufficiently studied (Aryamkina et al., 2001). Among the various combinations of agents that cause viral hepatitis, mixed HBV and HCV infection take the first place (Shkurko, 1998; Zhdanov, 1996; Zaika et al., 2001). The first reports of single observations of patients with mixed hepatitis B+C appeared in the early nineties. Afterwards, close attention of both scientists and practitioners to the mixed B+C infection has been increasing every year (Gorbakov et al., 2001). However, information submitted by various authors is sometimes contradictory. There is no clear understanding of the mutual influence of hepatotropic viruses when they are simultaneously or sequentially implanted in the human body. Some authors note more severe course of mixed hepatitis B + C in comparison with mono HBV and HCV infection, on the contrary, other researchers indicate slight course of mixed hepatitis (Korochkina et al., 1996; Liaw et al., 1994; Mimms et al., 1993; Sato et al., 1994). There are varied and contradictory notions about the dominant activity of the virus when HBV and HCV infection is combined. The data of some authors indicate that HCV infection plays the leading role in the pathogenesis of active hepatitis in most patients with combined HBV and HCV infection (Shkurko, 1998; Serfaty et al., 1997). On the contrary, other authors suppose that HBV infection is dominant. Thanks to modern advances and the development of molecular-genetic methods, it became possible to examine the existing notion of the clinical course and laboratory diagnostic characteristics of mixed hepatitis B+C (Sheen et al., 1992). There are insufficient data about the prognosis and disease outcome depending on various serological and molecular genetic markers in mixed hepatitis B+C. There are serious difficulties in the diagnosis of viral infection; literature data on the natural course of combined viral hepatitis B and C are highly controversial and require further detailed study.

The aim of our study was to investigate clinical and laboratory features of mixed hepatitis B+C, depending on the genotype of viruses.

Materials and methods

94 patients with mixed hepatitis B+C were under our observation. Laboratory examination noted that spectrum of serological and molecular genetic markers had significant differences. All patients in the dynamics were studied using ELISA for the presence of serum antigens and antibodies. We investigated serological markers of HBV infection: HBsAg, anti-HBs, HBeAg, anti-HBe, anti - HBcor Ig M, anti - HBcor Ig G. HCV specific markers were defined: anti-HCV IgM and anti-HCV IgG.

To determine the activity of the pathological process and the degree of fibrosis in liver puncture biopsy was performed in patients with chronic viral hepatitis B (CVHB), viral hepatitis C, mixed HBV+HCV hepatitis. We used a method of aspirating a blind percutaneous needle biopsy of the liver with Menghini needle. Morphological assessment of the degree of activity in the liver of patients was carried out by semi-quantitative evaluation scores using the criteria of histological activity index (FSA) by Knodell method (Knodell et al., 1981).

Molecular analysis of markers of hepatitis B+C and identification of mutant variants of the genome structure were investigated using PCR. Blood tests for detection of HBV DNA and HCV RNA using PCR and genotyping were carried out in the Reference Laboratory Ministry of Health of Uzbekistan, laboratory of molecular diagnostics in Ruzybakieva Institute of Immunology, genotyping laboratory in Nagoya City University Medical School.

Results and discussion

Results produced simultaneous HBV+HCV infection detection in 33 (35.1%) patients (group I). Viremia HCV, with no detection of DNA HBV in blood, found in 25 (26.6%) patients (group II). Viremia HBV was noted in 21 (22.3%) patients - group III. Serological markers of mixed infection without viremia HBV DNA and HCV RNA were indicated in 15 (16.0%) patients - group IV.

Subsequently, we conducted genotyping of all patients. Genotype HBV A of was revealed in 25 (26.6%) patients, genotype HBV C - in 18 (19.2%) patients and genotype HBV D - in 51 (54.3%) patients. Genotyping revealed HCV genotype 1b in 52 (55.3%) patients, genotype 3a - in 23 (24.5%) patients, and genotype 2a - in 19 (20.2%) patients.

Viral load in the blood serum of patients with mixed hepatitis was 6.5 x 10^6 copies of HBV DNA/ml for A genotype, 4.7x10^5 HBV DNA copies/ml for genotype C, 5x10^7 copies of HBV DNA/ml for genotype D. This suggests that the replicative activity of the D genotype in mixed hepatitis B+C is higher than in genotypes HBV A and C.

The next step was an analysis of frequency of serological markers occurrence depending on the virus genotype. Analysis of the results showed that HBsAg was detected in 10 (40.0%) patients with genotype A, in 6 (33.3%) patients with C genotype, and in 30 (58.8%) patients with D genotype. HBeAg was detected in 9 (36.0%) patients with genotype A, in 23 (45.1%) patients with genotype D, it was not detected in patients with genotype C HBeAg. Anti-HBcorIgM was found in 9 (36.0%) patients with genotype A, 4 (22.2%) patients with genotype C, and in 21 (41.2%) cases with genotype D. Anti-HBe was detected in 34 (66.7%) patients with genotype D, in 6 (24.0%) patients with genotype A, in 13 (72.2%) cases with genotype C. Anti-HBcorIgG was revealed in 51 (100%) patients with genotype D, 23 (92.0%) patients with genotype A and 14 (77.8%) patients with genotype C.

Viral load HCV RNA was 9.1x10^8 HCV RNA copies/ml for genotype 1b, it made 6.5x10^6 HCV RNA copies/ml for genotype 3a, and 5x10^5 HCV RNA copies/ml for genotype 2a. Anti-HCV IgM was found in 27 (51.9%) patients with genotype 1b, in 6 (26.1%) cases with genotype 3a, and in 6 (31.6%) cases with genotype 2a. Anti-HCV IgG was detected in 42 (80.8%) patients with genotype 1b, in 12 (52.2%) patients with genotype 3a, and the marker was found in 9 (47.4%) patients in cases with genotype 2a.

In further study we determined the occurrence frequency for certain HBV and HCV genotypes in mixed hepatitis B+C. HCV genotype 1b was detected together with HBV genotype D in 50.0% (26) of cases, genotype 3a was combined with D genotype in 34.8% (8) cases, and genotype 2a had a tropism to the D genotype in 15.8% (3) of cases. HBV genotype A was found together with HCV-2a in 76.0% (19) of cases; it was found together with HCV-1b in 24.0% (6) of cases. HBV genotype C had certain tropism. C genotype was noticed in combination with genotype 1b in 50.0% (9) of cases; it was combined with genotype 2a in 22.2% (4) of cases and with HCV genotype 3a in 27.8% (5) of cases.

Patients in group I with HBV+HCV infection were detected with genotype 1b in 23 (69.7%) cases, with genotype 3a - in 7 (21.2%) patients, with genotype 2a - in 3 (9.1%) patients. Analysis of laboratory parameters depending on the genotype identified specific features. ALT made 2.4±0.7 mmol/ml for genotype 1b; respectively, γ-globulin made 22.6±4.9%, IgG - 20.7±2.9 g/l, indicators histological activity index - 8-12 points. For genotype 3a these parameters were the following: ALT - 1.9 ± 0.9 mmol/ml, γ-globulin - 18.7±3.1%, IgG - 16.1±3.2 g/l, and histological activity index - 4-8 points. For genotype 2a theses parameters were correspondingly: ALT - 1.4±0.5 mmol/ml, γ-globulin - 15.3±5.2%, IgG - 15.9±3.9 g/l, histological activity index - 3-8 points.

A genotype was detected in 27.3% (9) during analysis of the frequency of HBV genotypes in the group I; D genotype - in 51.5% (17) and C genotype - in 21.2% (7) patients. Thus, higher activity of necrotic-inflammatory syndrome was observed under the D genotype circulation than in cases of HBV genotypes A and C circulation. ALT level in genotype D was 2.6±1.2 mmol/ml against 2.1±0.9 mmol/ml in genotype A, histological activity index - 8-12 points against the 5-8 points, respectively. The level of γ-globulin in genotype D was - 19.6±2.1%, IgG - 16.4±3.6 g/l, while the ones in genotype A were -17.1±3.1% and 14.6±3.3 g/liter respectively. Viral load in the blood was higher in genotype D - $6x10^7$ HBV DNA copies/ml against $5x10^5$ HBV DNA copies/ml in genotype A. Indicators of the necrotic-inflammatory syndrome in patients with HBV genotype C: ALT - 1.5±0.5 mmol/ml, level of γ-globulin - 23.3±3.7%, IgG - 17.6±4.2 g/l, histological activity index - 8-15 points. This could indicate on a higher pathogenicity of this genotype in comparison with HBV genotypes A and D in mixed hepatitis B+C. Viral load in genotype C was $5x10^4$ HBV DNA copies/ml. It might be also caused by mutation development in the virus genome, resulting in reduced activity of the virus and increase of pathogenic properties.

It should be emphasized that HBV genotypes D and P in patients of the group I caused more pronounced liver damage and inflammatory reaction in comparison with genotype A. Relatively low levels of viral load at genotypes A and C could indirectly indicate on a more pronounced inhibitory effect on HCV genotypes A and C than in the HBV genotype D.

Genotype 1b was detected in the majority of patients with chronic hepatitis C with HCV mixed-monoviremia - in 80.0% (20) patients; genotype 3a was diagnosed in 12.0% (3) patients, and genotype 2a was found in 8.0% (2) patients. Chronic mixed hepatitis of high activity was diagnosed more frequently with detection of genotype 1b than in cases of HCV genotypes 3a and 2a. ALT in genotype 1b was 2.2±1.3 mmol/ml, histological activity index - 7-10 points, γ-globulin - 20.3±4.6%, IgG - 19.1±2.9 g/l; while in genotype 3a, ALT level was - 1.7±0.8 mmol/ml, histological activity index - 6-9 points, γ-globulin-18.4±3.6%, IgG - 17.5±3.4 g/l respectively. These data for genotype 2a virus were the following: ALT - 1.4±0.6 mmol/ml, histological activity index - 3-7 points, γ-globulin - 16.2±4.5%, IgG - 15.6±3.3 g/l. It should be noted that higher levels of cytolytic syndrome and viral load were detected in genotype 1b than in cases of genotypes 3a and 2a.

Research of HBV genotypes in group III identified genotype C in 24.0% (6) of patients and genotype D in 76.0% (19) of patients. It should be noted that HBV genotype A in this group was not detected. Lack of genotype A detection can be explained by the fact that

this genotype is less prone to mutations: of HCV infection dominance eliminates genotype A. This may explain also the absence of HBV viremia.

Comparative analysis of laboratory data also revealed differences related with HBV genotype. In patients with genotype D, ALT was 2.5±1.1 mmol/ml, histological activity index - 8-12 points, the level of γ-globulin 19.2±2.3%, IgG - 17.1±2.1 g/l. Indicators for genotype C: ALT - 1.6±0.5 mmol/ml, histological activity index - 4-8 points, the level of γ-globulin 20.5±1.8%, IgG - 16.7±3.4 g/l. Given the fact that the replicative activity of HBV decreases rapidly with the development of mutations in precore/core-zone, it could be assumed that mutations HBV genotypes A and C result in a significant suppression of its replicative activity. It is known that structural features of genotypes A, C and D are different.

Analysis of virus genotyping of infected patients in group III revealed HCV genotype 1b in 9.5% (2) of patients, genotype 3a - in 42.9% (9) of patients, and genotype 2a - in 47.6% (10) of patients. Necrotic-inflammatory indicators in this group were characterized by distinctive features. The level of ALT at genotype 1b was 1.6±0.5 mmol/ml, histological activity index - 5-8 points, the level of γ-globulin 19.2±2.1%, IgG - 15.3±2.8 g/liter. In genotype 3a, ALT was 1.2±0.4 mmol/ml, histological activity index - 4-7 points, the level of γ-globulin - 17.6±2.2%, IgG - 15.4±3.28 g/l. In genotype 2a, ALT level was 1.0±0.3 mmol/ml, histological activity index - 3-5 points, the level of γ-globulin 15.1±1.9%, IgG - 12.6±1.6 g/liter.

Detecting of HBV genotypes in group III revealed genotype A in 76.2% (16) of patients, genotype D - in 19.0% (4) of patients, and genotype C - in 4.8% (1) of patients. Such ratio of genotypes can be explained with HBV monoviremia: high frequency of genotype A detection is connected with its low disposition to mutations. This may explain the high frequency of HBV DNA detection. Study of inflammatory processes in the liver revealed that ALT level in genotype A was 1.4±0.6 mmol/ml, histological activity index - 3-5 points, the level of γ-globulin - 17.2±2.8%, IgG - 16.6±1.6 g/liter. In genotype C, ALT was 2.3±0.6 mmol/ml, histological activity index - 5-8 points, the level of γ-globulin - 18.4±3.1%, IgG - 17.7±2.3 g/liter. In genotype D, ALT was 2.3±1.2 mmol/ml, histological activity index - 6-9 points, the level of γ-globulin - 18.7±3.4%, IgG - 17.9±3.1 g/liter. Comparative analysis showed higher activity of the inflammatory process in genotypes D and C than in genotype A.

Analysis of group IV patients showed HCV genotype 1b in 46.6% (7) of patients, genotype 3a in 26.7% (4) of patients, and HCV genotype 2a in 26.7% (4) of patients. The level of ALT at genotype 1b was 1.7±0.3 mmol/ml, histological activity index - 5-8 points, the level of γ-globulin - 17.2±2.1%, IgG - 15.4±2.2 g/liter. In genotype 3a, ALT was 1.5±0.2 mmol/ml, histological activity index - 3-7 points, the level of γ-globulin - 16.5±2.1%, IgG - 15.1±1.9 g/l. lastly. And genotype 2a indicators were: ALT - 1.2±0.3 mmol/ml, histological activity index - 3-5 points, the level of γ-globulin 14.1±2.6%, IgG - 14.3±2.2 g/liter.

Genotype A was not detected in HBV genotypes in patients of group IV. Genotype D was found in 73.3% (11) of patients, and HBV genotype C - in 26.7% (4). Indicators of inflammatory processes in genotype C were the following: ALT was 1.4±0.3 mmol/ml, histological activity index - 4-7 points, the level of γ-globulin - 18.2±2.6%, IgG - 14.2±1.2 g/l. In genotype D, ALT was 1.5±0.2 mmol/ml, histological activity index - 5-8 points, the level of γ-globulin - 17.1±2.7%, IgG - 15.1±2.4 g/liter. HBV virus in genotypes C and D showed similar trends in activity of the pathological process.

Conclusion

Thus, the obtained data showed differences in detection of markers of viruses with mixed HBV+HCV infection. Use of both ELISA and PCR methods was highly informative for etiological deciphering of disease. Comprehensive survey revealed that the simultaneous detection of HBV DNA and HCV RNA in blood was observed in 35.1% (33) of patients.

Only HCV RNA was detected in 26.6% (25) of patients with mixed hepatitis B+C, HBV monoviremiya was detected in 22.3% (21) of patients. 16.0% (15) of patients had negative detection for molecular genetic HBV-HCV markers and in blood, but in the presence of serological markers of these viruses. Comparative analysis of clinical manifestations showed that chronic mixed hepatitis high activity was more often diagnosed with the simultaneous detection of HBV DNA and HCV RNA in 39.4% (13) of patients; formation of liver cirrhosis in this group was revealed in 12.1% (4) of patients. Patients of group II with HCV monoviremia had a high activity of hepatitis in 24.0% (6) and the formation of liver cirrhosis in 24.0% (6). In group III, the high activity of chronic hepatitis was found in 14.3% (3) of patients, and the formation of liver cirrhosis was established in 23.8% (5) of patients. Group IV patients were characterized with the diagnosis of chronic mixed hepatitis of high activity in 26.7% (4), and the formation of liver cirrhosis was diagnosed in 6.7% (1) of the patients.

In summary, it can be suggested that the frequency of detection of antigens and antibodies to HBV and HCV in mixed hepatitis B+C had the specific characteristics and depended on the virus genotype. The highest rates of detection of serological markers were observed in presence of HBV genotype D and HCV genotype 1b. This phenomenon can be explained by the high replicative and antigenic activity of viruses in these genotypes, resulting in a high viral load and blood, compared with HBV genotypes A and C, HCV genotypes 3a and 2a.

References

Aryamkina, O., Klimov, N., Kovalenko, E. et al., 2001. "Clinical characteristics of patients with diffuse chronic liver diseases of viral HBV-and HCV-etiology," Rus. Journal of Gastroenterol. Gepatol. Koloproktol., No1(12), p.5.

Gorbakov, V., Blokhina, N., Khazanov, A. et al., 2001. "The frequency of detection of HBV-DNA in serum HBsAg carriers and patients with simultaneous detection of anti-HCV and HBsAg," Rus. Journal of Gastroenterol. Gepatol. Koloproktol., No1(12), p.9.

Knodel, R., Ishak, K., Black, W., 1981. "Formulation and application of numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis," Hepatology, 1(1), pp.431-35.

Korochkina, O., Sobolevskaya, O., 1996. "Virus specific T-lymphocytic reaction in predicting the threat of chronic mixed hepatitis B+C," Rus. Journal of Gastroenterol. Gepatol. Koloproktol., No4(3), p.178.

Liaw, Y., Tsai, S., Chang, J. et al., 1994. "Displacement of hepatitis B virus by hepatitis C virus as the cause of continuing hepatitis," Gastroenterology, 106, pp.1048-1053.

Mimms, L., Mosley, J., Hollinger, B. et al., 1993. "Effect of concurrent acute infection with hepatitis C virus on acute hepatitis B virus infections," British Medical Journal, 307, pp.1095-1097.

Sato, S., Fujiyama, S., Tanaka, M. et al., 1994. "Coinfection of hepatitis C virus in patients with chronic hepatitis B infection," J. Hepatology, 21, pp.159-66.

Serfaty, L., Chazouilleres, O., Poujol-Robert, A. et al., 1997. "Risk factors for cirrhosis in patients with chronic hepatitis C virus infection: Results of a case-control study," Hepatology, 26(3), p.776.

Sheen, I., Liaw, Y., Chu, C. et al., 1992. "Role of hepatitis C virus infection in spontaneous hepatitis B surface antigen clearance during chronic hepatitis B infection," J. Infect Dis., 165, pp.831-34.

Shkurko, T., 1998. "Clinical and diagnostic features of mixed HBV / HCV-infection in adults," Synopsis of doctoral dissertation.

Zaika, G., Gileva, R., Zakharova et. al., 2001. "Clinical and biochemical differences in hepatitis C and C + B in acute and chronic stages," Rus. Journal of Gastroenterol. Gepatol. Koloproktol, No1(12), p.11

Zhdanov, K., Lobzin, Y., Mukomolov, C., 1996. " Clinical and morphological study of subclinical forms of infection with hepatitis B and C in young age," Rus. Journal of Gastroenterol. Gepatol. Koloproktol, No4 (3), p.174.