MOLECULAR-GENETIC STUDIES FOR HCV INFECTION

B.M. Tajiev
_Tashkent Pediatric Medical Institute, Uzbekistan_, ndm2@mail.ru

F.A. Rashidov
_Tashkent Pediatric Medical Institute, Uzbekistan_.

M.M. Alimov
_Tashkent Pediatric Medical Institute, Uzbekistan_.

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Tashkent Pediatric Medical Institute, Uzbekistan.
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Tajiev B.M., Rashidov F.A., Alimov M.M.

Tashkent Pediatric Medical Institute

Abstract

Background. HCV infection is a dangerous disease that can lead to cirrhosis and hepatocellular carcinoma. There are several genotypes and many subtypes of the virus. Yet, the prevalence of each type and subtype is largely varies throughout the world. Furthermore, the sensitivity for the treatment and aggressiveness of one type of the virus widely differs compared to another one.

Methods. 120 patients were examined with chronic viral hepatitis C hospitalized from 2002 to 2009 at the clinic of the Research Institute of Virology and at the 5th City Clinical Infectious Disease Hospital. All participants of the study experienced a comprehensive clinical and laboratory examination during the dynamic observation.

Results. The obtained research results showed that 1b and 1a genotypes cause a more pronounced necrotic inflammatory process in hepatocytes. This fact proves the more aggressive antigenic and pathogenic properties of the 1b and 1a genotypes in comparison with the 2a, 3a and 1b + 3a genotypes of HCV.

Conclusion. Specific characteristics of the HCV were observed in the patients with different genotypes, which was reflected in clinical and laboratory changes in HCV infection.

Keywords. Clinical and laboratory features, viral hepatitis C, genotypes of viral hepatitis C.

BACKGROUND

Among the most important problems of practical health care and scientific medicine, viral hepatitis is one of the leading health issues nowadays. According to data presented by WHO, about 50 million people in the world infected with viral hepatitis annually, and up to 2 million people die. Recently, physicians all-around the world have been attracted by viral hepatitis C (HCV) (Nematova&Fayziboev, 2016; and Sokolova, 2003). Latent course with frequent transition to a chronic form and subsequent development of severe complications creates a serious problem for on time diagnosis and treatment of such patients (Ignatova&Serov, 2001; and Orlova et al, 2002).

Chronic HCV infection can lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma. HCV is classified into 7 genotypes and 67 subtypes (Smith et al., 2014). In North America, the majority of HCV infections are caused by genotype 1, 2,
and 3 (Zein et al., 1996). Although not well known for the scientific society, highly effective, direct acting antivirals against HCV are available today.

In addition to serious consequences for the patients, in the absence of treatment, they become a source of infection for others. Significant morphological rearrangement of the liver in chronic hepatitis C, up to fibrosis, occurs even with a latent course of the process, which is only clinically manifested in the terminal stage (Abdukadyrova, 2002; Pokrovsky et al, 2003; and Mirtazaev et al, 2003). Currently, many aspects of the studies and treatment of HCV remain unresolved. The dependence of clinical manifestations on the genotype of the virus has not been fully studied yet. A deeper study and comparison of clinical, virological and morphological changes in HCV at the present stage is required depending on the molecular genetic variants of HCV.

HCV can be genotyped using various methods including the gold standard - sequencing, real-time reverse transcriptase - PCR, and reverse hybridization line probe assays (LiPA) (Verbeeck et al., 2008, Larrat et al., 2013) (cited in Olmstead et al, 2017).

A real-time RT-PCR, being a laboratory based test for identification the genomes, genotyping assay was explored as an approach for low-cost, high-throughput screening of major HCV genotypes and subtypes. Various primer/probe combinations targeting the HCV Core, E1, and NS5B genomic regions were tested. A paired, duplex real-time RT-PCR assay that targets HCV Gt 1a and 3a in one reaction and Gt 1b and 2 in another reaction was ultimately validated using samples genotyped by LiPA (Olmstead et al, 2017).

**PURPOSE OF THE STUDY**

To conduct molecular-genetic studies in HCV infection in order to elucidate the genetic type of the virus in different forms of HCV infection.

**MATERIAL AND METHODS**

**STUDY POPULATION**

We examined 120 patients with chronic viral hepatitis C hospitalized from 2002 to 2009 at the clinic of the Research Institute of Virology and at the 5th City Clinical Infectious Disease Hospital. All patients underwent a comprehensive clinical and laboratory examination during the dynamic observation.

**INCLUSION AND EXCLUSION CRITERIA**

The diagnosis of the disease was verified by detection of RNA of the HCV by PCR. Furthermore, the virus was genotyped. Patients with markers of other viral hepatitis were excluded from the analysis.

All examined patients were classified according to the international classification of chronic hepatitis, adopted in 1994 in Los Angeles. Patients with LC were diagnosed according to the Child-Pugh predictive scale, improved by A.I. Khazanov and N.N. Nekrasova and were included in classes A, B and C.
RESULTS

All patients were found to have anti-HCV. Among the examined patients, 61 (50.8%) were female, and 59 (49.2%) were men. The main age of patients was 41.4 ± 1.3 years (ranged from 17 to 70). In the age aspect, 3 (2.5%) patients aged from 14 to 20 years old, 19 (15.8%) aged from 21 to 30 years old patients, from 31 to 45 years old (41.7%) patients, from 46 to 60 years of age, 37 (30.8%) and over 60 years old -11 (9.2%) patients (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Women</th>
<th>Men</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abs</td>
<td>%</td>
<td>Abs</td>
</tr>
<tr>
<td>14-20</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>21-30</td>
<td>7</td>
<td>5.8</td>
<td>12</td>
</tr>
<tr>
<td>31-45</td>
<td>29</td>
<td>24.2</td>
<td>21</td>
</tr>
<tr>
<td>46-60</td>
<td>18</td>
<td>15.0</td>
<td>19</td>
</tr>
<tr>
<td>60 and more</td>
<td>7</td>
<td>5.8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>50.8</td>
<td>59</td>
</tr>
</tbody>
</table>

The results of an epidemiological history showed that 34 (28.3 ± 4.1) patients noted a previous acute form of the disease. During the observations of 120 patients with chronic viral diseases of the liver, the developed liver cirrhosis (LC) was diagnosed in 20 (16.7%). Among the remaining 100 (83.3%) patients, chronic viral hepatitis C (CVHC) of varying degrees of activity of the pathological process was determined. In most cases, the disease progressed in latent form in 67 (55.8%) patients, in 28 (23.3%) in a sluggish form, and in 25 (20.9%) patients the disease proceeded in an active form. Chronic hepatitis of minimal activity (CHMinA) was diagnosed in 18 (15.0%) patients, chronic hepatitis of moderate activity (CHModA) - in 52 (43.3%) patients, average chronic hepatitis (CHAA) - in 22 (18.3%) patients and 28 (23.4%) have high activity chronic hepatitis (CHHA).

As a result of the studies revealed the circulation of 3 genotypes of HCV. Molecular genetic variants of circulating viruses were distributed as follows; among the patients, 1b HCV-y genotype was diagnosed in 67 (55.8%) patients, 1a in 8 (6.7%), 2a in 15 (12.5%), 2c in 2 (1.7%), 3a 20 (16.7%) and mixed genotype 1b + 3a in 8 (6.7%) patients (Figure 1). Subsequently, clinical, laboratory, instrumental and pathomorphological studies were carried out depending on the HCV genotype. In patients with the genotype 1b in 10.5% of cases, the clinical course was defined as CHMinA, CHModA was observed in 44.8% of patients, CHAA in 13.4% and in 31.4% of patients. LC among patients with the 1b genotype was diagnosed in 20.9% of cases. Among patients with the genotype 1a, 12.5% were diagnosed with CHMinA, in 37.5% of cases of CHModA. CHAA in 25.0% and in 25.0% of CHHA.LC
formation was detected in 25.0% of patients. An analysis of the activity of the process in patients with genotype 2a revealed CHMinA in 26.7% of patients, in 46.6% of CHAA, in 20.0% of CHModA and in 6.7% of CHHA. LC was formed in 13.3% of patients.

Fig. 1. Molecular-genetic variations of HCV

Among patients with the 2b genotype, 50% were determined as CHModA and in 50% as CHAA, CHMinA and CHHA and the formation of LC in patients of this group was not recorded. Among patients with the 3a genotype, CHMinA was observed in 20.0% of cases, in 40.0% of CHAA, in 25.0% of patients with CHModA, in 15.0% of CHHA. LC formation was noted in 10.0% of patients. With a mixed genotype 1b + 3a-in most cases, 37.5% was determined by CHAA, in 25.0% of CHModA, in 25.0 patients with CHAA and in 12.5% of patients with CHHA. It should be noted that the formation of LC is diagnosed among patients with 1b, 1a, 2a, for HCV genotypes. Symptoms characteristic of LC were not observed in patients with mixed 1b + 3a genotype and 2b HCV genotype. An analysis of clinical manifestations showed that 50 (74.6%) patients of the first group with the 1b genotype more often complained of asthenovegetative syndrome, which was manifested by weakness and rapid fatigue. Patients with genotype 1a and patients with genotype 3a of HCV complained of similar complaints in 5 (62.5%) cases in 9 (45.0%) cases. Patients in whom the mixed 1b + 3a genotype was detected in 5 (62.5%) cases also complained of weakness and fatigue. In 6 (40.0%) cases, patients with genotype 2a complained of asthenovegetative symptoms. For liver enlargement, which was accompanied by pain in the right hypochondrium, 38 (56.7%) patients with genotype 1b were treated. Patients with genotype 1a pain in the right hypochondrium were noted in 4 (50.0%) cases, patients with genotype 3a were noted in 9 (45.0%) patients in 3 (37.5%) patients with mixed HCV genotype 1b + 3a and 7 (46.7%) patients with genotype 2a also noted an unpleasant sensation and pain in the right hypochondrium. Jaundice in the form of ictericity of the skin and mucous membranes of different intensities was observed in 11 (16.4%) patients. 1b genotype, 1 (12.5%) with 1a genotype, 5 (25.0%) with 3a genotype, 1 (12.5%) with 1b + 3b genotype and 2 (13.3%) with 2a with the HCV genotype. In observations, a symptom of cholestasis in the form of skin itching was observed in 8 (11.9%) patients with genotype 1b, 2 (25.0%) with genotype 1a, 3 (15.0%) with genotype 3a, and 1 (12.5%) 1b + 3a genotype. In the observed patients with genotype 2a, in 1 (6.7%) patient.
Symptoms like mucosal bleeding were detected in 15 (22.4%) patients, and 12 (17.9%) patients with genotype 1b also complained of sleep inversion. In patients with the genotype 1a, hemorrhagic disturbances were revealed in 2 (25.0%) patients and in 1 (12.5%) sleep inversion. The same disorders were detected in 3 (15.0%) patients and in 2 (10.0%) patients with the 3rd type of HCV genotype. In patients with mixed 1b + 3a genotype, bleeding from the mucous membranes was noted in 12.5% of cases, and sleep inversion was noted in 2 (25.0%) patients. 2 (13.3%) patients complained of hemorrhagic disorders, and 1 (6.7%) patients with HCV genotype 2a complained of sleep inversion. 14 (20.9%) patients with 1b genotype, 2 (25.0%) patients with 1a genotype, 2 (10%) patients with 3a genotype, 2 (13.3%) patients with 2a genotype complained of weight loss. Patients infected with 1b + 3a of the virus genotype did not complain of this nature. 6 (9.0%) patients with 1b genotype complained of abdominal enlargement, 2 (25%) with 1a genotype, 1 (5%) with 3a genotype and 1 (6.7%) patient with a 2a genotype. One of the main symptoms of chronic liver damage is hepatomegaly. 40 (59.7%) patients with a genotype 1b complained of an increase in liver size, 5 (62.5%) patients with a genotype 1a, 8 (40.0%) patients with a genotype 3a, and 3 (37.5%) patients with 1b + 3a genotype and 6 (40.0%) patients with 2a HCV genotype. Palmar erythema was observed in 38 (56.7%) patients with genotype 1b, in 4 (50.0%) patients with genotype 1a, in 8 (40.0%) with genotype 3a, in 5 (33.3%) patients with 2a genotype and in 2 (25%) patients with 1b + 3 and the HCV genotype. Teleangiectasias and spider veins on the chest and anterior abdominal wall were detected in 14 (20.9%) patients with the 1b genotype, in 2 (25.0%) patients with the 1st genotype, in 2 (20.0%) with the 3rd genotype, in 2 (13.3%) patients with a 2a gene type. Splenomegaly was determined in 13 (19.4%) patients with the 1st genotype, in 2 (25.0%) patients with the 1st genotype, in 2 (10.0%) patients with the 3rd genotype and in 1 (6.7%) 2a with the HCV genotype. It should be noted that in patients of these groups in 12 cases (60.0%), class B and C LCs were diagnosed, and in the remaining 8 (40.0%), class A was revealed according to the Child-Pugh classification - in patients with 1b genotype in 10 (14.9%) cases, vein expansion of the lower third of the esophagus was observed. The same changes were observed with 1a genotype in 12.5% of patients with 3a genotype in 5.0% of cases and in patients with 2a genotype in 6.7% of cases. The formation of ascites was observed with 1b genotype in 8 (11.9%) patients, with 1a genotype in 2 (25%) patients, with 3a genotype in 1 (5.0%) cases and with 2a genotype in 1 (6.7 %) of the patient.

Extrahepatic manifestations in the form of autoimmune thyroiditis were observed in 13 (19.4%) patients with 1b genotype, in 1 (12.5%) patient with 1a genotype, in 2 (10.0%) patients with 3a genotype, in patients with mixed genotype 1b + 3, and autoimmune thyroiditis was observed in 2
(25%) cases and in 3 (20%) patients with the 2a virus genotype. Symptoms of arthritis were diagnosed in 14 (20.9%) patients with 1b genotype, in 2 (25%) patients with 1a genotype, in patients with 3a genotype - in 4 (20.0%) cases, with 1b + 3a - in 1 (12.5%) patient and in 1 (6.7%) patient with genotype 2a. Renal disorders in the form of glomerulonephritis were diagnosed in 5 (7.5%) patients with 1b genotype and in 1 (6.7%) patient with 1b + 3a HCV genotype.

The ALAT index as an indicator of cytolysis was: for genotype 3a, 2.8 ± 0.4 μmol/l, for genotype 1a, the indices were 1.5 ± 0.6 μmol/l, 3.2 ± 0.5 μmol/l was determined at 1b + 3a genotype, with 1b and 2a genotype, 1.6 ± 0.7 μmol/l and 1.3 ± 0.4 μmol/l, respectively. Indicators of ALAT at 3a and mixed 1b + 3a genotype significantly differed from the indicators of other groups (P <0.05) (table 2).

<table>
<thead>
<tr>
<th>Laboratory parameters of different genotypes of HCV</th>
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<tbody>
<tr>
<td><strong>Indexes</strong></td>
</tr>
<tr>
<td>ALAT μmol/l</td>
</tr>
<tr>
<td>Thymoltest Units</td>
</tr>
<tr>
<td>Alkalinephosphatase Units/l</td>
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<tr>
<td>PTI %</td>
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</tbody>
</table>

* The difference is significant in comparison with other groups.

Thymol test averaged 33.3±12.6; 22.6±4.6, 19.6±6.7; 14.4±3.7 and 7.0±2.0 units in 1b, 1a, 1b + 3a, 2a and at 3a genotype, respectively, and during dynamic observation tended to decrease. The activity of alkaline phosphatase increased significantly, the average indicators were 298 ± 56.6, 188 ± 17D 282.0 ± 65.2, 263 ± 19.7, and 254.5 ± 25.5 mmol/l at 1b. 1a, 1b + 3a, 2a and with 3a genotype, respectively. The average level of the prothrombin index was 77.3 ± 3.4%, 75.4 ± 2.7%, 77.3 ± 6.9%, 74.3 ± 1.9%, and 71.0 ± 2.5% with 1b, 1a, 1b + 3a, 2a and with the genotype, respectively.

DISCUSSIONS

Thus, the results of clinical studies have shown that the 1b and 1a genotypes cause a more pronounced pathological process, which indicates a more aggressive antigenic and pathogenic properties of these genotypes compared with 2a, 3a and 1b + 3a of the HCV genotypes. With mixed 1b + 3a genotypes, indicators of the degree of activity of the pathological process could be
compared with those of infected patients with the 1b genotype, which in turn indicates the leading role of the 1b genotype in HCV infection with mixed genotypes. The indicators of the necrotic inflammatory process in the liver with genotype 3a were not pronounced, which testified to the non-aggressive antigenic and pathogenic properties of this genotype. In our opinion, the minimal activity of the pathological process in the liver with the HCV genotype 2a is associated with less pronounced cytopathic and antigenic properties of this genotype in comparison with the above HCV genotypes.

In one very interesting research conducted in several provinces of China, in total, 4 HCV genotypes and 18 subtypes were identified among more than 32 000 patients. From them, 5 dominant subtypes were detected from 99% of the samples. Genotypes 4, 5 and 7 were not detected. No mixed infections with rare subtypes were found. Males, compared with females, showed higher HCV subtype diversity, a lower percentage of HCV1b and 2a and a higher percentage of rare subtypes and mixed infections. The analyses revealed the comprehensive distribution patterns of HCV genotypes in the general population of mainland China. HCV genotypic patterns were differentially distributed on the basis of geography, sex and age (Chen et al, 2017).

In the United States, on the other hand, HCV genotypes 1a and 1b are the most commonly found genotypes in patients with chronic HCV. Infection with HCV genotype 1b can be associated with more severe liver disease and may have a higher risk for the development of liver cancer including hepatocellular carcinoma. HCV genotype 2b is more likely to be the most sensitive and HCV genotype 1b was the least sensitive to interferon therapy (Zein&Persing, 1996).

According the results of research by Tsukiyama-Kohara et al. (2017), HCV genotypes distribution varies based on geography. Moreover, each genotype associate with different tolerance to interferon treatment. Recently-developed direct-acting antiviral (DAA) drugs, which target viral proteases or polymerases, mediate drastically better antiviral effects than previous therapeutics. Although treatment with DAAs has led to the development of drug-resistant HCV mutants, the most recently approved DAAs show improved pan-genomic activity, with a higher barrier to viral resistance.

**CONCLUSION**

Summarizing, it can be argued that specific characteristics of the virus were observed in patients with different genotypes, which was reflected in clinical and laboratory changes in HCV infection.

**STUDY LIMITATIONS**

The study was conducted on the patients with no information of their living area of our Republic, so we could not reveal the prevalence of different genotypes of the virus in different area of Uzbekistan. To uncover long-term outcomes of the HCV
infection and the results of treatment, more studies should be carried out on this topic.

ACKNOWLEDGEMENTS
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ETHICAL APPROVAL
The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

CONSENT
Written informed consent was obtained from all participants of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available for review by the authors.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

FUNDING
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