Molecular Epidemiology of Hepatitis B Virus in Yakutia

Gerasimova V.V., Maksimova N.R., Levakova I.A., Mukomolov S.L.

ABSTRACT

Heterogeneity of hepatitis B virus and its link with clinical course of infection and its outcomes remains one of priority directions of researches in Republic Sakha (Yakutia), as the region with high incidence of this disease. Blood serum samples with chronic hepatitis, registered in dispensary of various regions of the Republic Sakha (Yakutia), have been investigated. On the basis of results of the research by means of molecular - biological methods in the republic a genotype D was considered as the dominating genotype at patients with CHB, and sufficiently a large number of patients are infected simultaneously by two genotypes, most intensively circulating in the territory of Republic (A, D, C). For adequate therapy of such diseases it is necessary to consider characteristics of excreted isolates.

Keywords: hepatitis B, genotypes, viral load, mutations, drug resistance.

INTRODUCTION

The Republic Sakha (Yakutia) is considered to be the region of high prevalence of parenteral viral hepatitis B [1,4,7]. This unfavorable epidemiological condition is connected with many factors, including climatic and geographic features of the Far North. Severe course of parenteral viral hepatitis, its further chronicity are connected with presence of immunodeficiency, the incidence considerably increasing in unfavorable ecological conditions. It is particularly characteristic for the Republics Sakha (Yakutia) [2,3,5,6].

The viral hepatitis B (HBV) is hepatitis B pathogen virus, refers to a family *Hepadnaviridae* [8] and is one of the most changeable DNA-containing viruses that is caused by complex replication cycle, including a stage of RnK-pregenom reverse transcription [11]. Despite very limited possibilities of coding, the virus is capable to be protected from patient's immune system and to remain during all life in infected hepatocytes. When using the reverse transcriptase during the replication, it can be modified during the selection of viral mutants, for example, by means of immune system or antiviral therapy. Besides, viral genomes of wild and mutant types are stably stored in a core of infected hepatocytes as an episomal DNA that provides isolation from cell replication or integration in patient's genome [9]. Such factors as HBV genotype, viral load and specific viral mutations can cause the disease progression. Among them, HBV genotype not only predicts clinical outcomes, but also causes the effectiveness of

treatment with interferon. At present 8 HBV genotypes and some subtypes have been identified, they having distinctive geographic location. Individuals with genotypes A and C are characterized by higher frequency of infection chronicity in comparison with individuals with genotypes B and D. Patients with genotypes A and B have lower indicators of spontaneous seroconversion (HBeAg) – anti-HBe as compared with patients with genotypes C and D. Isolates HBV of genotype C have higher frequency in basic core promoter (BCP) A1762T/G1764A , and are characterized with higher viral load, than the isolates of genotype B. the same findings have been revealed in genotype D with its higher prevalence (BCP) A1762T/G1764A, as compared with genotype A. Such supervisions allow revealing important distinctions between HBV isolates, referring to different genotypes. Particularly, patients with HBV of genotypes C and D suffer from more severe liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). Patients with HBV genotypes A and B are better treatable with interferon, as compared with genotypes C and D [10].

One of priority directions of the research is studying of HBV heterogeneity and its influence on the clinical course of the infection. In particular, the information concerning prevailing genetic HVB types, frequency of viral mutant forms in various territories of Russia is insufficient and inconsistent.

The purpose of this research was definition of HVB genotypes, detection of mutations in pre-core and (BCP) of HVB genome, as well as mutations in the field of polymerase gene, responding for drug resistance of the pathogen virus at patients with chronic hepatitis B (CHB), not receiving therapy in Republic Sakha (Yakutia).

MATERIALS AND RESEARCH METHODS

The sampling was carried out on the bases FBEH “Center of hygiene and epidemiology in RS (Y)” (the head physician Ushkareva O. A) in each region of the republic. The detection of viral load, genotyping and sequencing were carried out in the laboratory of viral hepatitis of the Federal budgetary establishment of science of the St.-Petersburg scientific research institute of epidemiology and microbiology named after Pasteur (the Head of laboratory Dr. of Medicine Mukomolov S.L.). In the laboratory aliquots of samples were prepared, the part was applied for molecular-biological researches, and the rest was stored as a bank of samples of blood serum at -80 º C.

The blood serum samples of 1304 patients with chronic hepatitis have been investigated, of them 819 (62,8 %) women and 485 (37,2 %) men, middle age of all patients has made 43,2 years. All patients were included in dispensary registration of different areas of the Republic Sakha (Yakutia): Aldansky-72, Amginsky-96, Bulunsky-37, Verkhoyansk-19, Vilujskiy-100,

Zhigansky-30, Lensky-90, Megino-Kangalassky-5, Mirniy-100, Nerjungrinsky-129, Njurbinsky-61, Suntarsky-103, Tattinsky-64, Tomponsky-34, Khangalassky-100, Churapchinsky-59, Yakutsk - 205 patients. The serum was investigated on HBsAg by a method of immune-ferment analysis (IFA) by means of test systems manufactured by Joint-Stock Company "Vector-best" (Novosibirsk), according to the firm-manufacturer instruction. 700 blood serum samples of patients with HBV were selected on storage at-80^{0}S.

Of 700 samples 345 blood serum indices have shown positive viral activity. The viral activity and viral load were revealed by means of PCR-TEST SYSTEM taking into account results in on-line mode «AmpliSensHbV-Monitor-Fl" ("InterLabServis", Moscow). Of the samples containing DNA HBV, 31 indices from citizens of various regions of Yakutia (Churapchinsky, Mirninsky, Suntarsky, Verkhoyansk, Bulunsky, Hangalassky, Njurbinsky, Amginsky, Tomponsky, Tattinsky, Aldan, Viljujsky, Nerjungrinsky улусы, Yakutsk) with CHB diagnosis concerned genotype A virus in 87 - mutations in pre-core and P genome with the use of test systems INNO-LIPAHBV Genotyping, INNO-LIPAPreCore, INNO-LIPAHBVMulti-DR (Innogenetics, Belgium). Besides, quantitative content HBsAg in blood serum by means of Alcor-Bio Test System (Saint Petersburg) was revealed at 87 patients.

RESULTS AND DISCUSSION

In Yakutia 3 genotypes(A,D,C) have been revealed at patients with CHB. At 77,8 % surveyed HBV isolates concerned genotype D, genotype A at 3,7 % and C at 3,7 %. In samples of 14,8 % patients simultaneous presence of two genotypes of the virus has been revealed: A+D (11,1 %), D+C (3,7 %) (Fig. 1). The domiciliary distribution of virus genotypes has shown the genotype D in all regions, the genotype A in Verkhoyansk and Yakutsk, the genotype A+D in Bulunsky, the genotype C+D- in Tattinsky regions.

At all surveyed patients the isolates with mutations of basic core promoter and pre-core (BCP+PC-28 codon) were at 37 %, this parameter being more frequent as compared to PC-28 codon (25,4 %) and BCP (5,3 %). Only 28,6% isolates were of wild type without mutations in the field of C gene.

In Yakutia the mutation BCP+PC28 is the most spread, it makes 37.3±4,3 % and is extended in the category of people aged 50 years and more. While the virus of wild type noted in 28,6±4,0 is diagnosed among 15-29 years (43,5 %) and middle aged (37,3 %). The wild type is noted more often at men of 15-29 years, at women the widespread mutation is BCP+PC-28 and makes 50 % (Table 1). The mutation PC-28 codon has higher prevalence both at men and women of more senior than 50 years (Fig.2). By analyzing the prevalence of mutations CHB in the field C gene depending on age of patients the mean age of patients without mutations in this

field of genome (wild type virus) was estimated on 37±5 years. While the corresponding age characteristics of HBV patients with mutations in BCP and PC-28 codon has amounted 67±6 years. It is necessary to note that HBeAg was detected at patients with wild type HBV, i.e. without mutations in the field of genome C.

The mutations in HBV polymerase gene responding for drug resistance were revealed at two CHB patients. In one case the patient from Aldan region had the mutation in position L-173 with combination of gene C (BCP+PC codon 28). In the second case the patient from Suntarsky region had the same mutation in T-194 with combination of gene C (BCP+PC codon 28) as well. In both cases the patients with mutations of resistance had no specific antiviral treatment, so the mutations can be characterized as primary resistance, it being of lower frequency (2,2%).

No distinctions in the distribution of levels of viral load depending on age were revealed. At 55, 7 % of men and 53,7 % of women the viral load appeared less than 150 ME\ml. The relatively higher viral load 10^5 at 5,6% men and 4,5% women (Table 1). The viral load correlated with quantitative content HBsAg. The lowest content HBsAg in serum – 261ME/ML was detected in the viral load lower than 150 ME/ml. When the viral load increased, the average concentration HBsAg (ME/ML) increased proportionally as well. Thus the concentration of surface antigene in samples with viral load 10^4 ME/ML and higher, it amounting 459 ME/ML (Table 2). The higher concentration HBsAg is found out at patients having mutations BCP and PC28 simultaneously.

CONCLUSION

Thus, according to this research in the republic the genotype D is the dominating type at CHB patients. For the first time it has been revealed that sufficiently large number of patients is infected simultaneously by two genotypes of the pathogen virus, most intensively circulating in the Republic territory (A, D, C). Among most of the investigated patients with the genotype D HBV mutation in pre-core (PC28) and BCP genome has been confirmed. The higher concentration HBsAg is found out at patients having simultaneous mutations BCP and PC28. It is important that the frequency of primary resistance to nucleoside analogues was extremely low and made up 2,2 %.

The results testify to necessity for surveying patients with chronic forms HB by means of molecular - biological methods in order to make decision for adequate therapy, considering features of excrete isolates.

REFERENCES

1. Alekseeva M. N. Virusnye gepatity v Respublike Sakha (Yakutiya) [Viral hepatitis in the Republic Sakha (Yakutia)]: dissertatsiya … doktora meditsinskikh nauk [diss. … Dr. of medical sciences]. Saint-Petersburg, 2002, P. 285.

2. Alekseeva M. N. Grinenko L.E. Timchenko A.V. [et.al.] Virusnye gepatity v Respublike Sakha (Yakutiya) Virusnye gepatity i drugie aktualnye infektsii: Sb. nauch. tr. [Viral hepatitis and other actual infections: Coll. scient. works]. Saint - Petersburg, 1997, V. 2., P. 164-166.

3. Alekseeva M. N. Meltser I.M. Rafailova M. A. [et.al.]. Osobennosti techeniya gemokontaktnykh gepatitov v Respublike Sakha (Yakutiya) [Features of the course of haemocontact hepatitis in the Republic Sakha (Yakutia)] Mat. russko-yaponskogo med.simpoziuma: sb. nauch. trudov [Mat. of Russ.-Jap. Med. Symp.], Khabarovsk, 1998, P. 408.

4. Ivanov P.M. Tomskiy M.I. Chabyeva L.G. [et.al.]. Gepatologiya Severa [Hepathology of the North]. The Yakut scientific centre of CMP SB RAMS, Medical Institute NEFU named after M.K.Ammosov, Yakutsk: LTD «Publishing House Sphera», 2012, P.304.

5. Petrova P. G. Volozhin A.I. Ekologiya cheloveka v usloviyakh Severa [Ecology of a person in the conditions of the North]. Yakutsk, 1996, P. 182.

6. Petrova P.G. Ekologiya, adaptatsiya i zdorove [Ecology, adaptation and health].Yakutsk, 1996, P. 262.

7. Sleptsova S.S. Semenova V.K. Nikitina S.G. [et.al.]. Khronicheskie virusnyy gepatity [Chronic virus hepatitis]. The Yakut Medical Journal, 2013, № 1, P.52-55.

8. Shahildjan I.V. Mihailov M.I. Onishchenko G.G. Parenteralnye virusnye gepatity (epidemiologiya, diagnostika, profilaktika) Parenteral viral hepatitis (epidemiology, diagnostics, prophylaxis).Moscow: GOU VUNMC MZ RF, 2003, P.384.

9. Glebe. D. The molecular virology of hepatitis B virus / D. Glebe, C. Bremer // Semin.Liver.Dis.-2013. – Vol.33 (2).-P.103-12.

10. Lin C. The clinical implications of hepatitis B virus genotype: Recent advances / C. Lin, J.Kao//GastroenterolHepatol.- 2011.-Suppl 1.- P.123-130.

11. Naturally occurring escape mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen / K.Yamamoto, M.Horikita, F.Tsuda et al.//J.Virol.-1994.-Vol.68.-P.2671-2676

The Authors:

Gerasimova Vilena Vasilevna- research assoc. FSAEE HPE NEFU named after M.K.Ammosov, res. assoc. FSBE YSC CMP SB RAMS virlab@mail.ru, Yakutsk, Russia;

Maksimova Nadezhda Romanovna – Dr. of Medicine, Prof., Chief of Scientific laboratory «Genome medicine» FSAEE HPE NEFU named after M.K.Ammosov, FSBE YSC CMP SB RAMS

Levakova Irina Aleksandrovna – research assoc. FBES St.-Petersburg scientific institute of epidemiology and microbiology named after Pasteur, St.-Petersburg;

Mukomolov Sergey Leonidovich - Dr.of Medicine, Prof., Chief of Laboratory of viral hepatitis FBES St.-Petersburg scientific institute epidemiology and microbiology named after Pasteur, St.-Petersburg.