DETERMINATION OF HEPATITIS B VIRUS GENOTYPE IN UZBEKISTAN USING 3RD GENERATION SEQUENCING

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ABSTRACT

Determining the DNA sequence of the hepatitis B virus in the territory of Uzbekistan will greatly help to find a solution to this disease and will greatly help researchers and researchers in the treatment of the virus, which currently has no cure or vaccine.

Objects and research objects. DNA of hepatitis B extracted from human serum

Research methods. Method of isolating DNA, PCR real time and 3rd generation sequencing

Level of scientific novelty of research. determination of hepatitis b virus genotype in population of Uzbekistan.

Obtained samples.

The analysis of the final qualifying work, the results obtained and their analysis were carried out in Scientific and Diagnostic Center "IMMUNOGEN TEST" at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan.

Relevance of the study.

Hepatitis B virus (HBV) is the prototypical virus of the Hepadnaviridae family, an unusual family of approximately 3.2 kb long partial double-stranded (ds) DNA viruses that encode four genes, including a reverse transcriptase. encoded in circularly overlapping reading frames. genome [1, 2]. To date, 9 genotypes (A-I) and a putative 10th genotype (J) of HBV have been described [3]. Viruses show relatively high variability for a dsDNA virus [4], and virus genotypes are divided into more than 30 subgenotypes, many of which have distinct geographic and clinical associations [2].

One of the most important health problems in the world is hepatitis B virus (HBV) infection. There are about 400 million people chronically infected with this virus. Chronic carriers are at risk of developing complications from hepatitis B infection, resulting in cirrhosis, hepatocellular carcinoma, liver failure, or death [3]. HBV genotype is one of the most important factors affecting the outcome of infection [4]. Based on genome-wide sequence divergence, HBV has been classified into at least 8 genotypes (A–H) that differ by >8% at the nucleotide level. Recently, an additional provisional genotype (I) [5] and a variant isolated from Southeast Asians were also nominated as the tenth genotype J [6]. Most genotypes can be divided into subgenotypes that differ by >4%, such as HBV-A1 and HBV-A2 [7,8].

Aim and research tasks.

Hepatitis B virus (HBV) is a diverse, partially double-stranded DNA virus with 9 genotypes (A-I) and a presumed 10th genotype (J), and information on the prevalence of these polymorphisms in the Uzbek population is limited. Uzbekistan is a country with 36 million inhabitants. For this reason, we set ourselves the goal of determining the genotype of the hepatitis B virus found in Uzbekistan.

Introduction.

Hepatitis B virus (HBV) causes more than 257 million chronic infections worldwide and causes more than 880,000 deaths annually from HBV-related complications such as hepatocellular carcinoma (HCC) Although effective prophylactic vaccines and antiviral drugs are available, chronic HBV infection is still not curable [11].

Currently, HBV genotyping is not routinely performed in most clinical settings because it is not considered a common practice for patient management. However, as more treatment data become available and improvements are made with clinical stratification of patients, guidelines may change to reflect different genotype-specific recommendations. This has been exemplified in the management of hepatitis C virus (HCV) infection and a similar approach is beginning to emerge for HBV, with the European Association for the Study of the Liver (EASL) recently recommending different HBV treatment guidelines. suggests stopping. Points of non-response to treatment in genotypes A-D. Evidence supports the role of HBV genotype in influencing disease progression, including the risk of developing chronic infection, electron antigen seroconversion, mode of transmission, and development of hepatocellular carcinoma. Studies often refer to "wild-type" virus, but wild-type for one genotype may not reflect the consensus for other genotypes. Understanding the diversity of HBV strains circulating globally and their association with disease allows for a more precise and accurate approach to analysis [9].

HBV genotyping is becoming increasingly important as specific clinical associations with each genotype are becoming increasingly apparent. It has been shown that HBV origin, course of infection, disease severity, prognosis and response to antiviral treatmen, viral serological reactivity and replication pattern are mainly genotype. dependent Thus, understanding the genotype distribution is important for epidemiologic characterization of HBV prevalence and infection control [10].

The geographic distribution of HBV genotypes by regional host population and endemicity has been widely reviewed. Accordingly, HBV/B and HBV/C predominate in most parts of Asia, including China and Japan. Genotypes A, D, and F are common among the five geographic regions of Brazil. Genotype A is the most common in the United States, while genotype B is the most common in Canada. Genotypes E and F are restricted to Africa and the Americas. HBV/G has a global distribution, and HBV/H was first identified in Central America. HBV genotypes I and J have been reported sporadically in Asia and Japan, respectively. Genotype D also predominates in the Middle East, including Iran [10].

Method.

Extraction dna from sample. We extracted DNA from plasma with "ROSSAmed MagSorb Plasma" reagent kit designed for the extraction of nucleic acids from the plasma of the ROSSA company. this process is a necessary step in preparation for the PCR step. The step of extracting nucleic acids is carried out on a magnetic stand. Nucleic acids were isolated in this sequence using the company's kit instructions.

PCR real time method. We can prepare the DNA extracted samples for PCR real time method. In this case, we use "ROSSAmed HBV-DNA" reagent kit for detection of hepatitis B virus of ROSSA company and prepare the sample for PCR according to the company's instructions and carry out the PCR stage. This method helps us to determine whether the sample contains hepatitis B virus or not.

Sequencing. the 3rd generation MinION (1-figure) sequencer of the oxford nanopore technology company was selected for sequencing. The sequencing process is considered to be very complicated; first DNA is extracted from the sample and then it is prepared for the sequencer using a special kit produced by Oxford Nanopore Technology. The results were analyzed using this company's EPI2ME lab program.

Figure-1. MinION sequencer from oxford nanopore technology.

Result. 10 samples were selected for sequencing and passed through all the above steps. 5 of those selected are women and 5 are men. Demographic characteristics are shown in Table 1. The results of the sequencing method showed that B genotypes were detected in all of these 10 HBV positive samples (figure-2).

Demographic characteristics of patients with positive PCR for DNA		
gender	male	5(50%)
	Female	5(50%)
Age (years)	20-30	4(40%)
	30-40	6(60%)
Vaccination status	vaccinated	3(30%)
	Unvaccinated	7(70%)

Table-1. Demographic characteristics of patients with positive PCR for DNA

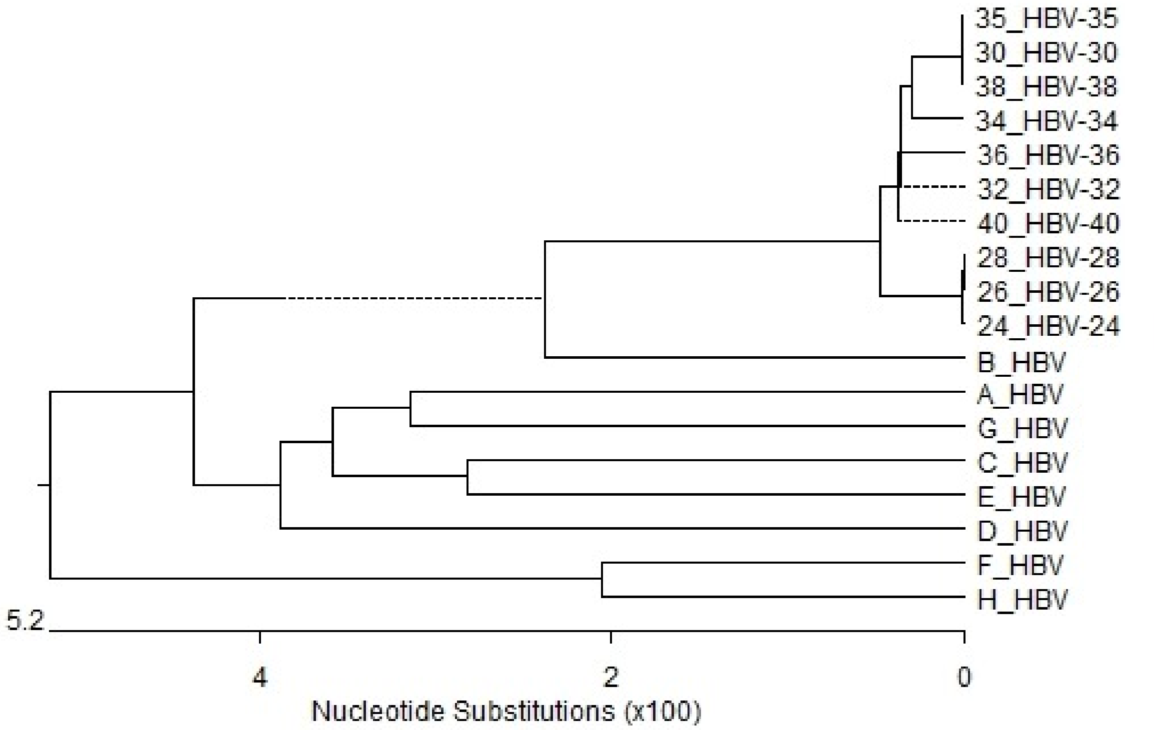

Figure-2. Phylogenetic tree of 348 bp fragments from 10 HBV isolated in the studied population and 8 standard genotypes from NCBI database. Tree constructed with DNAstarmegalign software. Genotype of samples is closed with HBV genotype B.

Conclution.

The results of this study may provide useful regional epidemiological information. However, since these results cannot be generalized to the entire population of Uzbekistan, a large-scale, nationwide study should be conducted to determine HBV genotypes in the general population of Uzbekistan

References:

1. 1. Locarnini S, Zoulim F. Molecular genetics of HBV infection. Antivir Ther. 2010;15:3–14. doi: 10.3851/IMP1619. [PubMed] [CrossRef] [Google Scholar]

2. 2. McNaughton AL, D'Arienzo V, Ansari MA, Lumley SF, Littlejohn M, et al. Insights from deep sequencing of the HBV genome—unique, tiny, and misunderstood. Gastroenterology. 2019;156:384–399. doi: 10.1053/j.gastro.2018.07.058. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

3. Günther S. Genetic variation in HBV infection: genotypes and mutants. J ClinVirol. 2006;36:S3–S11. [PubMed] [Google Scholar]

4. Schaefer S. Hepatitis B virus taxonomy and hepatitis B virus genotypes. World J Gastroenterol. 2007;13(1):14–21. [PMC free article] [PubMed] [Google Scholar]

5. McMahon BJ. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. Hepatol Int. 2009;3(2):334–42. [PMC free article] [PubMed] [Google Scholar]

6. Yu H, Yuan Q, Ge SX, Wang HY, Zhang YL, Chen QR. et al. Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I". Plos One. 2010;5(2):e9297.

[PMC free article] [PubMed] [Google Scholar]

7. Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T. et al. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. J Virol. 2009;83(20):10538–[PMC free article] [PubMed] [Google Scholar]
8. El IA, Khaled A, Mahmoud OM, Saleh AF, Baioumi EA. Prevalence of HBV genotypes in Egypt among hepatitis patients. J Am Sci. 2010;6(11):185–90. [Google Scholar]
9. Analysis of genomic-length HBV sequences to determine genotype and subgenotype reference sequences J Gen Virol. 2020 Mar; 101(3): 271–283. Published online 2020 Mar 5. doi: 10.1099/jgv.0.001387
10. Sequence-based genotyping of hepatitis B virus in general population Med J Islam Repub Iran. 2015; 29: 165. Published online 2015 Jan 27.
11. The Genotype (A to H) Dependent N-terminal Sequence of HBV Large Surface Protein Affects Viral Replication, Secretion and Infectivity Front. Microbiol., 09 July 2021 Sec. Virology