Genotypes of hepatitis B virus in Central Anatolia, Kayseri, Turkey

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ABSTRACT

الأهداف: التحقق من انتشار البنية الوراثية لفيروس التهاب الكبد ب بين المرضى المصابين بالتهاب الكبد ب المزمن في تركيا.

الطريقة: أُجريت هذه الدراسة في قسم الأحياء الدقيقة بجامعة إيرسيس، القيصرية، وفي معمل أيونتيك، اسطنبول، تركيا وذلك خلال الفترة من يناير 2005م إلى أكتوبر 2007م، وشملت الدراسة 110 مريضاً مصاباً بالتهاب الكبد ب المزمن. لقد قمنا باختبار الحمض النووي في الفيروس بواسطة التفاعل التسلسلي المبلمر ذو التوقيت الفعلي، حيث تم استخلاص الحمض النووي من ما مقداره 200 ميكرولتر من مصل الدم وذلك باستخدام عدة الكشف (QIAamp DNA minElute)، فيما تم تحضير خليط التفاعل بواسطة (Fluorion HBV QNP 2.0).

النتائج: أشارت نتائج الدراسة إلى اكتشاف البنية الوراثية د في 107 مريضاً من أصل 110 مريض (97.2%)، غير أن التنوع الجيني قد فشل في 3 مرضى (2.7%)، كما لم يتم العثور على بنيات وراثية أخرى.

خاتمة: تسلط هذه الدراسة الضوء على انتشار البنية الوراثية د بين مجموعة كبيرة من المرضى الأتراك المصابين بالتهاب الكبد ب المزمن.

Objectives: To investigate the distribution of hepatitis B virus (HBV) genotypes among patients with chronic hepatitis B in Kayseri, Turkey.

Methods: The study took place in the Department of Microbiology, Erciyes University, Kayseri, and Iontek Laboratory, İstanbul, Turkey, from January 2005 to October 2007. One hundred and ten patients with chronic hepatitis B were included in this study. Hepatitis B virus DNA in sera were investigated by using the real-time polymerase chain reaction. Viral DNA was extracted from 200 µL of serum using the QIAamp DNA minElute kit (Qiagen, Hilden, Germany). Reaction mixture was prepared by Fluorion HBV QNP 2.0 (Iontek, Istanbul, Turkey).

Results: Genotype D was detected in 107 of 110 (97.2%) patients, however, genotyping failed in 3 patients (2.7%). No other genotypes were found.

Conclusion: The vast majority of Turkish patients with chronic hepatitis B have genotype D.

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The hepatitis B virus (HBV) is one of the major causes of liver disease. Approximately 400 million people throughout the world are chronically infected while one million die every year due to liver failure.[1] Hepatitis B virus is the prototype member of the genus *Orthohepadnaviridae* of the family *Hepadnaviridae* and the viral genome is approximately 3.2 kb long. It circulates in the serum as a dane particle which is a round structure consisting of an envelope and an inner core of nucleocapsid protein, enclosing both a polymerase and the partly double stranded circular viral DNA.[2] Genetic variability of this virus is reflected by the 8 different genomic groups or genotypes (A-H) described so far, which show geographical differentiation.[3] The worldwide HBV genotype prevalence is the result of the distribution of humans in different continents.[4] They display an 8% inter-group divergence in the complete nucleotide sequence of HBV and differences in the nucleotide homology of the surface gene, which result in different hepatitis B surface antigen (HBsAg) serotypes.[5] The most cosmopolitan genotypes A and D are predominant in Europe, Africa and North America, and in Mediterranean and Middle East

countries. Genotypes B and C are located in the East and Southeast Asia, and genotype E in the West Africa. Genotypes F and H are restricted to Central and South America and North and Central America, respectively.[6] Hepatitis B virus infection is associated with a broad spectrum of clinical manifestations, ranging from acute or fulminant hepatitis, to diverse forms of chronic infection, including asymptomatic carrier (ASC) state, chronic hepatitis B (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC).[7] Reports indicate that HBV genotypes are related with the severity of liver disease. Hepatitis B virus genotype C has been associated with the development of liver cirrhosis and hepatocellular carcinoma.[8-10] In addition, HBV genotypes are related with the response to antiviral therapy. Asian studies have shown that compared to HBV genotype B, genotype C has a lower response to interferon alpha (IFN-α) therapy. A recent European randomized trial showed that patients infected with HBV genotypes A and B respond significantly better to pegylated IFN-α-2b alone or in combination with lamivudine than patients infected with HBV genotypes C and D.[11] Co-infection with 2 different genotypes has been known since serological typing was developed. However, mixed HBV infection is not often detected in HBV carriers, partly because of the genotyping methods used, some of which cannot detect all the HBV strains.[12] There are few reports in the literature on genotype study for patients with CHB in our country. The present study aims to investigate the prevalence of distribution of genotypes in patients with CHB in Kayseri, Turkey.

Methods. One hundred and ten consecutive Turkish patients with chronic HBV infection were studied prospectively (65 men and 45 women; mean age, 38.39 ± 12.90 years; 30 were in the <30-year group and 80 in the ≥30-year age group). These patients had been treated at the Hospital of Erciyes Medical University, Kayseri, Turkey from January 2005 to October 2007. Serum samples were collected from all in-patients and out-patients with different stages of HBV-linked hepatic diseases and stored at -20ºC until analysis. All patients were positive for HBsAg, but negative for HBs, HCV, HDV, and HIV antibodies. The patients met the following criteria: 1) positive for HBsAg for at least 6 months to establish chronic HBV infection; and 2) exclusion of other concomitant causes of liver disease (hepatitis C or D, HIV infection, alcohol consumption >60 g/day) and relatively rare liver diseases (autoimmune hepatitis and metabolic liver disease).[7] The presumed sources of HBV infection were also identified, with information provided by physicians, based on the clinical history and responses to a questionnaire, including at least one incidence of intravenous drug use, blood transfusion, occupational exposure, iatrogenic exposure (namely, endoscopy or colonoscopy examination, history of surgery, acupuncture, or hemodialysis), or unknown. Erciyes University Hospitals Research Ethics Committee approved this study. All patient gave informed consent.

The levels of serum alanine aminotransferase (ALT) and aspartate transaminase (AST) were determined with routine automated techniques (upper limit of normal: 40 IU/L). Hepatitis B virus markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and anti-HBcAb IgM) were measured at a virological laboratory using the enzyme-linked immunosorbent assay (DiaSorin, Saluggia, Italy). Hepatitis B virus DNA in sera were investigated by the real-time PCR assay according to the manufacturer's instructions. Viral DNA was extracted from 200 mL of serum using the QIAamp DNA minElute kit (Qiagen, Hilden, Germany). Reaction mixture was prepared by Fluorion HBV QNP 2.0 (Iontek, Istanbul, Turkey). Five microliters of extracted DNA were added to the plate containing 20 μL of the reaction mixture. The amplification profile was performed as follows: 30 minutes at 95ºC, 30 seconds at 95ºC, 1.30 minutes 54ºC with 50 cycles, and 10 second at 22ºC. Amplification and detection were performed by ICycler detection system (Biorad, USA). The quantification range of real time PCR was 10^3-10^7 copy/mL. The analytical detection limit for real-time PCR was 200 copy/mL. No amplification results were reported as <200 copy/mL. The results between 200-1000 copy/mL were reported as <1000 copy/ml. The results greater than 10^7 copy/mL were reported as >10^7 copy/mL.

Hepatitis B virus genotypes were determined by DNA sequencing in Iontek Laboratory in Istanbul, Turkey. The S gene was amplified, and concentrated products were sequenced directly with the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, New Jersey, USA). The sequencing products were analyzed on an ABI Prism 310 Genetic Analyzer (Perkin Emler, abi Prism, Foster City, CA, USA). The data were analyzed using a standard statistical software package for Microsoft Windows (release 11.0.0, standard version, SPSS Inc., 2001). A p-value of <0.05 was considered statistically significant.

Results. ***Demographic characteristics.*** The study included 110 patients with CHB, 65 (59%) were male and 45 (41%) were female. The mean age was 38.39±12.90 years. Eighteen (16%) were HBeAg positive anti-HBe negative compared to 90 patients (82%) being HBeAg negative anti-HBe positive; one female patient was recorded as both HBeAg and HBe antibody positive and one male patient recorded as both HbeAg and Hbe antibody negative. Of 19 HBeAg

positive patients, 10 were males and 9 were females, while 55 of 91 anti-HBe positive patients were males and 36 were females (**Table 1**). The cause of infection could be identified in 98 of 110 patients (**Table 1**). According to given values, dental care was a dominant risk regardless of group.

Serological patterns. Most patients did not express HBeAg (82.7%). However, the mean level of alanine aminotransferase (ALT), and aspartate aminotransferases (AST) were significantly higher in the HBeAg positive group (99.6 versus 52.2 and 71.8 versus 46.9, respectively) (**Table 2**).

Genotype outcomes. Genotype D was detected in 107 of 110 (97.2%) patients, however, genotyping failed in 3 patients (2.7%). No other genotypes were found. Since all the patients in this study were genotype D, it is not possible to comment on the altered clinical outcomes in relation to genotype.

Table 1 - Characteristics of the patients with chronic hepatitis B virus (HBV) infection.

Characteristics	n (%)	
Gender (male/female)	65/45	
Mean age (yr)	12.90 ± 38.39	
Serum ALT (±SD) IU/mL	65.1 ± 59.7	
Serum AST (±SD) IU/mL	58.7 ± 50.7	
Serum GGT (±SD) IU/mL	37.3 ± 44.1	
HBV DNA (copies/ml) n (%)		
≤1.0×10^3	3	(2.7)
>1.0×10^3-1.0×10^5	40	(36.3)
>1.0×10^5	67	(60.9)
HBeAg positive/HBeAb negative	18	(16)
HBeAg negative/HBeAb positive	90	(82)
HBeAg positive/HBeAb positive	1	(1)
HBeAg negative/HBeAb negative	1	(1)
Presumed source of HBV infection		
Family history	33	(30)
Transfusion history	8	(7.2)
Dental care history	81	(73.6)
Operation history	38	(34.5)
Unknown	12	(10.9)

ALT - Alanine transaminase, AST - aspartate aminotransferase, GGT - gamma-glutamyl transpeptidase, HBeAg - hepatitis B "e" antigen, HBeAb - Hepatitis B "e" antigen and antibody

Table 2 - Clinical and serological patterns of all 110 HBsAg positive patients

Criteria	HBeAg positive	HBeAg negative
HBeAb positive	1	90
HBeAb negative	18	1
Abnormal ALT	14	37
Abnormal AST	13	27
Abnormal GGT	4	13

ALT - Alanine transaminase, AST - aspartate aminotransferase, GGT - gamma-glutamyl transpeptidase, HBeAg - hepatitis B "e" antigen, HBeAb - Hepatitis B "e" antigen and antibody

Discussion. Hepatitis B virus can be classified into 8 genotypes A-H, and different HBV genotypes are dominant in various parts of the world.[13] There are few reports in the literature on genotype study for patients with CHB in Turkey. Multi-center national study including the cases of acute viral hepatitis B, genotype D was determined in 147 of 158 patients, and genotyping failed in 11 cases.[14] Yalcin et al[15] also found genotype D in 32 CHB patients and 12 inactive HBsAg carrier. Our results showed that also genotype D is the most prevalent genotype in Turkey. This is an important finding because, in Europe, most HBV infections are genotypes A and D and the likelihood of developing chronic liver disease is significantly higher in genotype A carriers compared to genotype D carriers.[15] There is accumulating evidence that patients with genotype D might achieve higher sustained viral response rate than patients with genotype A,[16] despite being less responsive to interferon treatment when compared to genotypes A and B.[17] It has been also reported that the response rate is higher in genotype A and B patients treated with interferon compared to genotype D and C patients.[18,19] (These data will oblige the organization of clinical trials for antiviral therapies to be based on genotype in the future).

Several methods have been developed and used for HBV genotyping including direct sequencing, PCR based restriction fragment length polymorphism, line probe assay, and enzyme-linked immunoassay.[20] In this study, genotyping was investigated by direct sequencing and genotype D was found in CHB patients. Mutations are possible in various sections of HBV genome. The most common is the mutation in precore region and HBeAg cannot be synthesized in such mutants. The persistent response to interferon is lower in precore mutant CHB patients compared to wild type HBV patients. Recently, many investigators in Europe assumed that CHB patients with HBeAg negativity are more than HBeAg positive patients.[19] Prevalence rates in the Mediterranean region are approximately 50-80%, with 40-55% in East Asia and at least 15% in South Asia.[21] In the present study, HBeAg positive disease made up only 17% of the 110 CHB patients studied. The remaining 83% were HBeAg negative/HBeAb positive, one patient was HBeAg negative/HBeAb negative and also one patient was HBeAg positive/HBeAb positive. This shows us the predominance of mutant HBV in our region. The relationship between HBV genotype and mode of transmission is not clear. The high prevalence of HBV genotypes B and C among Asians raises the possibility that HBV genotype may be related to the endemicity of HBV infection.[22] In our study, most of the patients had dental care history. Hepatitis B virus genotype D appears to be the only circulating type in

Turkish patients. This limitation makes it very difficult to draw firm conclusions regarding the effect of the genotype on the clinical courses of patients.

In summary, this study demonstrated the predominance of HBV genotype D in Turkish people with chronic hepatitis B. Awareness of these serologic and genotypic patterns might help in the formulation of management plans and in predicting clinical outcomes.

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