PREVALENCE OF HBV-DNA AMONG HBsAg POSITIVE PATIENTS IN SOUTHEASTERN REGION OF TURKEY BY PCR

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ABSTRACT
Background/Aims: Although hepatitis B virus (HBV) infections are a major health problem in Turkey, there is little information about prevalence of HBV-DNA that real indicator of infectious virion of HBV in Southeast of Turkey. The aim of this study was to investigate prevalence of HBV-DNA participated in Dane particle of HBV instead of HBsAg in our region. Methods: We studied with serum of 132 HBsAg positive patients. For detection of HBV-DNA in serum, high sensitive polymerase chain reaction (PCR) technique was used. Results: From the 132 HBsAg positive patients, HBV-DNA could be amplified in the serum of 40 patients (30.30%). Conclusion: Generally for hepatitis B infection prevalence, HBsAg marker has been used in our region. By this study HBV-DNA prevalence was exposed for our region and these results may give right information about circumstance of Hepatitis infection because of infectious virion.

Introduction
All over the world, hepatitis B remains an important public health problem affecting more than 300 million persons. The agent causing this disease is the hepatitis B virus (HBV), belonging to the Hepadnaviridae family. Infected person rate in Turkey population is 4-10% and this rate is 4-7% in only the Southeast of Turkey community that varios region for distribution of HBV. In Turkey, a region of intermediate endemicity, a recent survey identified the overall prevalence of chronic HBV infection around 3.5% in the western part and 7% in the eastern part of the country (1). In addition, one-third of the Turkish population is positive for anti-HBs antibody. It is estimated that 100,000 new HBV infections occur annually in Turkey (1). Therefore, HBV infections represent a major public health problem for the Turkish population (2).

HBV infection is associated with a wide spectrum of clinical manifestations, ranging from acute or fulminant hepatitis to various forms of chronic infection including asymptomatic carrier state, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (3). Hepatitis B virus is unusual among animal viruses in that infected cells produce multiple types of virus-related particles. Electron microscopy (EM) of partially purified preparations of HBV shows three types of particles: (a) 42 to 47-nm double-shelled particles (known as Dane particles, after their discoverer), (b) 20-nm spheres, usually present in a 10,000- to 1,000,000 fold excess over Dane particles, and (c) smaller quantities of filaments of 20-nm diameter and variable length. All three forms have a common antigen on their surface, termed hepatitis B surface antigen (HBsAg), which is present in enormous quantity in the serum of infected hosts, with concentrations ranging from 50 to 300 µg/ml. Most of the circulating pool of HBsAg is composed of 20-nm spheres,
which can reach titers as high as $10^{13}$/mL (4).

The Dane particle is the infectious virion of HBV; titers of Dane particles in the blood can range from less than $10^4$/mL to greater than $10^9$/mL. Its outer shell is a lipoprotein envelope containing the viral surface glycoproteins, originally detected serologically as HBsAg. These are the determinants against which neutralizing antibody (anti-HBs) is directed (5,6).

The genome of HBV, within virus particles or spherical virions (Dane particle), is composed of relaxed-circular, partially double-stranded DNA (rcDNA) (7). The long, full-length minus-strand is approximately 3200 nucleotides (nt) in length. The HBV genome includes four ORFs that encode at least seven translation products through the use of varying in-frame initiation codons. These translation products include three surface antigens (HBsAg): the envelope glycoproteins preS1, preS2, and S; core (C) and e antigens (HBeAg and HBeAg); viral polymerase (P); and the X protein (HBx) (8,9,10,11).

The objective of this study was to investigate HBV-DNA prevalence and was to study importance of this prevalence.

**Materials and Methods**

Forty patients who were found to be HBV-DNA positive by polymerase chain reaction (PCR) among randomly determined 132 HBsAg positive patients with chronic/acute HBV infections were determined. HBV-DNA samples were isolated by Heliosis isolation kit (Metis Biotechnology).

For PCR, 50µl reaction mixture was contained 5µl of PCR buffer, 1µl of dNTP (10mM) (Fermentas), 4µl MgCl$_2$ (25mM), 1µl primer (sense; 5'-CTAGACTCGTGGTTGACTTCTC-3' and antisense; 5'-AAATCCCAAAGACCCACCAAT-3') and 0.25µl Taq DNA polymerase (5U/µl) (Fermentas) and enough amount of ddH$_2$O to reach 40µl. To reach 50µl total volume, 10µl template DNA was added to the reaction mixture and then amplified in thermal cycler (Techne Genius FGENO5TD, England) for 30 cycles. Each cycle entailed denaturation at 94 °C for 20 s, primer annealing at 55 °C for 40 s and extension at 72 °C for 60 s with a final extension at 72 °C for 5 s. PCR products were analyzed by gel electrophoresis on a 2% agarose gel stained with ethidium bromide to determine HBV-DNA positive and negative samples. Specific primers were used that suitable for PCR for amplifying S gene region. Amplification product: 770bp. Nucletide position: 250-1019. 1kb digested molecular weight marker was used.

**Results and Discussion**

From the 132 HBsAg positive patients, HBV-DNA could be amplified in the serum of 40 patients (30.30%) especially in family members. All precautions were taken to avoid contamination (12,13) and each serum was studied by negative and positive controls. All bands were observed in expected size (770 bp.) (Fig. 2) for weak bands, studies were repeated. Thus, positive and negative samples were determined.

As seen at Fig. 1, HBsAg (+) and HBV-DNA (+) serums numbered and were investigated prevalences in some patient
groups (family members, dialise patients, hospital patients, donors, exp.). And while HBsAg was showing high prevalence in one group, HBV-DNA had been showed the lowest prevalence in same group.

Candan F. and et all. (14) investigated the Hepatitis B and C prevalence in Sivas, Turkey. And also, Dursun M. and et all.(15) studied prevalence of Hepatitis B infection Southeast of Turkey. Both of them reported HBsAg positivity not HBV-DNA positivity.

Bozdayı M.D. and et all. (16) reported 26 (61.90%) HBV-DNA positive patients among 42 HBsAg positivity in Turkey. In this study HBV-DNA rate was determined as 30.30%.

This study showed that the prevalence of HBV-DNA in Southeast of Turkey is at an intermediate level. Prevalence of real virion is possible by only fixation of HBV-DNA. HBsAg serological marker gived positive result in 132 patients but we found only 40 HBV-DNA positive serum samples in 132 HBsAg positive serums.

Dane particules cause hepatitis because of replicating in hepatocytes. Also three types of HBV have same surface proteins. HBsAg serological marker does not put forward real infection due to these same antigens on all particules.

Especially in acute phase, virion can leave from the host but HBsAg positivity can be continue. Polimerase chain reaction, is the most sensitive methods for HBV diagnosis.

In this study HBV-DNA positivity was found as 30.30%. But Bozdayı M.D. and et all. found 61.90%. These rates may be considered that HBV-DNA prevalence was higher in Western than Southeast of Turkey. We also observed HBV-DNA prevalence was higher in family members than the other groups in our region.

When we investigated prevalence both of HBV-DNA and HBsAg at Fig. 1, it was observed that HBsAg was maximum rate in one group and in same group HBV-DNA
was showing minimum rate. Generally in all groups HBsAg rates and HBV-DNA rates were not showing harmony. So there was no relation between HBsAg and HBV-DNA prevalence. Each of them indicated different distribution.

By this study prevalence of virion causing the hepatitis B was exposed in South-eastern region of Turkey. Previously only HBsAg positivity was mentioned but there has no report about HBV-DNA rates in this region. We determined HBV-DNA rates that the real indicator of hepatitis in our region.

REFERENCES