

Full Length Research Paper

Molecular detection of hepatitis B virus (HBV) among voluntary blood donor's HBsAg positive in shahrekord, Iran

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Hepatitis B virus, a common cause of viral hepatitis, consumes a large portion of health resources in developing countries. Hepatitis B virus (HBV) prevalence has been decreased dramatically in Iran population during the last decade, and now it is classified as having low endemicity for hepatitis B infection. Improvement of the people's awareness about HBV risk factors, national vaccination program since 1993 for all neonates, and vaccination of high risk groups could be the cause of this decrease. In this investigation, we announce the detection of HBV- DNA sequence from 90 HBsAg positive blood donors in shahrekord by nested PCR, using specific primers of the surface antigen region of the HBV genome. Of the 11472 volunteer blood donors admitted to Shahrekord blood transfusion organization center in Iran during 2007 - 2008, 90 specimens were positive for HBsAg. From 90 HBsAg positive serum samples, 75 specimens were positive in PCR.

Key words: Blood donors, HBsAg positive, Hepatitis B, Iran, PCR, Shahrekord.

INTRODUCTION

Hepatitis B virus infection (HBV) is one of the most common viral infections worldwide and leading to morbidity and mortality in the developing countries (Liu et al., 2006; Masroor et al., 2007). HBV carrier rate varies widely from 0.01 to 20% through the world (Alter et al., 1994). HBV is the major cause of liver disease and hepatocellular carcinoma (HCC) (Tabarestani et al., 1977; Bagheri et al., 1999; Shamszad et al., 1982). A large number of causes are seen in eastern Asia and Sub-Saharan Africa, where two of the most important health problems are chronic liver disease and liver cancer (Gust et al., 1996; Lok et al., 2002). Up to 80% of liver

cancers are believed to result from this viral infection which is the most important cause of cancer mortality worldwide after smoking. The prevalence of hepatitis B carriers varies in different parts of the world, ranging from less than 1 to 15%. In the Middle East, the endemicity is intermittent, with a carrier rate of 2 to 7% (Gust et al., 1996; Lee et al., 1997; Mahoney et al., 1999; Lok et al., 2002). Infection with HBV is preventable, but as yet, there is no long-standing efficient for chronic carriers of the virus (Kidd et al., 2004).

Approximately 2 billion of world's population has been infected by HBV. 400 million, making 5% of the world's population, have chronic infection and 600,000 die each year from HBV related liver disease (Shapard et al., 2006; Masroor et al., 2007).

The clinical manifestation of HBV infection range in severity from asymptomatic subliminal (70%),

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Table 1. Primers used for nested PCR.

| Use | Polarity | Sequence |
|---------|------------|-----------------------------------|
| 1st PCR | Sense | 5' TCGTGTTACAGGCGGGGTTT(192-211) |
| | Anti sense | 5' CGAACCCTGAACAAATGGC (704-685) |
| 2nd PCR | Sense | 5' CAAGGTATGTTGCCCGTTTG (455-474) |
| | Anti sense | 5'GGCACTAGTAACTGAGCCA(687-668) |

Table 1. Statistic data of the tested specimens.

| Characteristics | |
|-------------------------------------|-----------|
| Number of subjects | 11472 |
| Age-year(range) | 41(18-64) |
| Male/Female | 9602/1870 |
| Married/single | 8849 /26 |
| Tattooing/Untattooing | 400/11072 |
| Sexual contact / Non sexual contact | 40/11432 |

symptomatic hepatitis (30%), fulminate sever disease with liver failure (0.1 - 0.5%), following to exposure to HBV. Up to about 10% of the patients will progress to hepatitis B, which is defined as persistent of the infection for more than a month (Loh et al., 2006; Khouiril et al., 2004). Chronic HBV infection is the major problem on healthy delivery system in our country. HBV is the leading cause of chronic hepatitis, cirrhosis and mortality in Iran (Maynard, 1990; Margolis et al., 1991; Zali et al., 1996).

In the Middle East, the endemicity is intermediate, with a carrier ratio of 2 to 7%. Iran is an area of low endemicity for hepatitis B virus in the Middle East. The area of high endemicity has the highest death rate due to HCC in comparison with other areas.

Epidemiological studies have shown that over 35% of Iranians have been exposed to HBV and about 3% are chronic carriers, ranging from 1.07% in Fars province to over 5% in Sistan and Baluchistan province (Farzadegan et al., 1980; Ghavanini et al., 2000). In Iran, 46% of patients with hepatocellular carcinoma and 51% of those with cirrhosis are reported having hepatitis B surface antigen (HBsAg) positive. HBV is also recognized as the most frequent cause (70 - 80%) of chronic hepatitis in the country (Alizadeh et al., 2006). Similar studies suggest that HBV is the most common cause of cirrhosis and HCC in Iran (Alavian et al., 2006). The primary risk categories for infection by HBV are blood transfusion, blood derivatives, and dialysis, needle accident among health-care professionals, intra venues drug abuse and unprotected sexual contact. Vertical transmission is common in endemic regions such as Africa and Southeast Asia (Khouiril et al., 1990).

Despite the availability of an effective vaccine, HBV still continues to be a major health problem. It is necessary to

find the important transmission routes of hepatitis to prevent the disease in every country, specifically in endemic regions. The importance of programs to control risk factors becomes clear since many patients who are infected with hepatitis are asymptomatic.

MATERIAL AND METHODS

Serum sampling and DNA extraction

An analytic cross-sectional study on 11472 volunteer blood donors admitted to shahrekord blood transfusion center in Iran was carried out in 2007 - 2008. For detected HBV infection, hepatitis B surface antigen (HBsAg) was determined using commercially available Enzyme linked Immuno Sorbent Assay (ELISA) kits (Enzygnost HBsAg 5.0 DADE BEHRINC). HBsAg positive samples were tested by conformation test to detect the HBV DNA; nested PCR was performed using the DNA extracted from serum samples. All HBsAg positive samples were examined by Nested PCR using the method of Seong et al. (2007).

DNA extraction

DNA was extracted from serum using DNPTM kit (Cinna Gen Inc) according to the manufacturer's instructions.

Detection of HBV-DNA by nested PCR

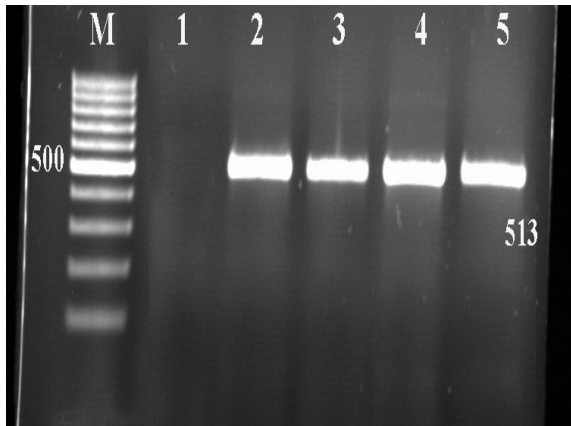
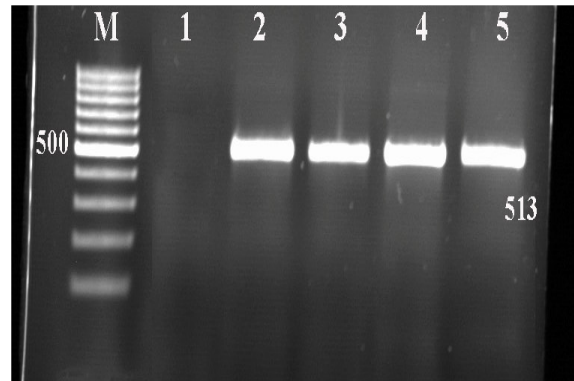
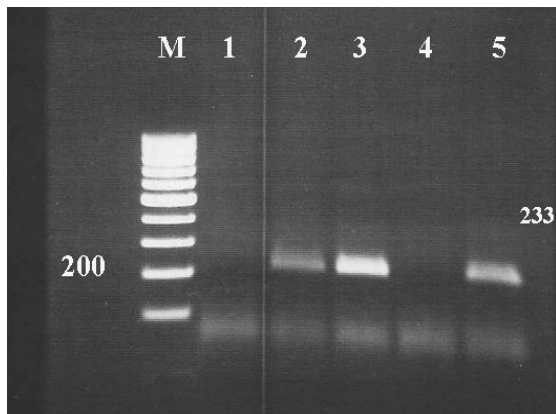
Nested PCR was performed using specific primers of the surface antigen region of the HBV genome according to the method previously reported with Seong et al. (2007). Sequence of the primers was shown in Table 1. In brief, the first round PCR was carried in final volume of 50 µl containing 5 µl of 10X PCR buffer, 4 µl of 10 mM dNTP, 3 µl of 25 mM MgCl₂, 25 pmol of each primer, 1.25 units of Taq polymerase (Roche applied science) and 5 µl DNA template. The amplification was carried out for 35 cycles (1 min at 94°C, 1 min at 55°C, 1 min at 72°C) after initial denaturation for 2 min. A final extension step was performed for 10 min at 72°C. The second round PCR was carried out using 5 µl of the first PCR product under the same condition as the first PCR except 25 pmol of each internal primer was used. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1X TBE buffer and evaluation by ethidium bromide visualization in gel electrophoresis by gel documentation (Uvitec UK company). 1000 bp DNA marker (fermentase) was used to distinguish DNA fragment bands in lanes. Water was used as a negative control.

RESULTS

The base-line characteristics of subjects were shown in Table 2. The median age of 11472 subjects enrolled in

Table 2. Positivity of HBsAg and HBV- DNA from Shahrekord blood donor.

| Characteristics | HBsAg positivity | HBV-DNA positivity |
|-------------------------------------|------------------|--------------------|
| Male/Female | 70/20 | 60/15 |
| Married/Unmarried | 30/60 | 23/52 |
| Tattooing/Untattooing | 65/25 | 60/15 |
| Sexual contact / Not sexual contact | 40/50 | 40/35 |

**Figure 1a.** Ethidium bromide stained agarose gel of 1st PCR. DNA 100bp markers (lane M), Negative control (lane 1), DNA samples from volunteer blood donors (lanes 2, 3, 4, 5).**Figure 2.** Ethidium bromide stained agarose gel of 1st PCR. DNA 100bp markers (lane M), Negative control (lane 1), DNA samples from volunteer blood donors (lanes 2, 3, 4, 5).**Figure 1b.** Ethidium bromide stained agarose gel of 2st PCR. DNA 100bp markers (lane M), Negative control (lane 1), DNA samples from volunteer blood donors (lanes 2, 3, 4, 5).

our study was 41 years (between 18 - 64). 9602 (83.69%) subjects were male and 1870 (16.3%) were female. 8849 (77.13%) subjects were married and 2623 (22.86%) subjects were unmarried. 400 (3.48%) subjects had tattooing precedence, and 40 (0.34%) subjects had sexual contact precedence. Of the 11472 subjects, 90 specimens (0.78%) were positive in anti-HBs, and from

90 HBsAg positivity subjects, 75 (0.65%) specimens were positive in PCR assay.

The positivity of anti-HBs were 70/11472 (0.61%) in male, 20/11472 (0.17%) in female, 30/11472 (0.26%) in married subjects, 65/11472 (0.56%) in the subjects with the tattooing precedence and 40/11472 (0.34%) in the subject with sexual contact precedence.

HBV-DNA was detected in 75 of 90 HBsAg positive subjects by using primers mentioned in materials and methods. The existence of 233 bp fragment in samples showed positive PCR assay. Of the 90 DNA samples from blood donors, 75 specimens (0.65%) contained 233 bp DNA fragment bands in PCR assay. The results are shown in Figures 1 and 2.

The positivity of PCR were 60/90 (0.52%) in males, 15/90 (0.13%) in females, 23/90 (0.20%) in married subjects, 52/90 (0.45%) in unmarried subjects, 60/90 (0.52%) in the subjects with tattooing precedence, 15/90 (0.13%) in the subjects with untattooing preceding and 40/90 (0.34%) in the subject with sexual contact precedence, 35/90 (0.30%) in the subject with non sexual contact precedence. The results are shown in Table 2.

DISCUSSION

Despite considerable advances in medical technology to cure the disease, viral hepatitis still remains as a major

public health problem with its worldwide high morbidity and mortality. Around one million people die each year of HBV infection, making it the 9th leading cause of death worldwide. It has been estimated that over 35% Iranians have been exposed to the HBV and about 3% were chronic carriers (Boag et al., 1991).

This is the first report of molecular evidence for HBV infection in ShareKord blood donors. Of the 11472 volunteer blood donors admitted to shahrekord blood transfusion center in Iran during 2007 - 2008, 90 specimens were positive for HBsAg, and of the 90 HBsAg positive serum samples, 75 specimens (0.65%) contained 233 bp DNA fragment.

The first published report about HBV infection in Iran was in 1972. In later studies, the rate of HBV infection was reported 1 to 1.2% in 1977. While further reports stated higher rates (between 3.5 and 2.49%) in both voluntary blood donors and general population from 1980 to 1993 (Sadi et al., 1972).

In the 1980s, almost 3% population was affected, differing from prevalence rate of 1.7% in the Fars province to 5% in Sistan-Baluchestan province. However, the recent studies showed that the rate of hepatitis B carriers in different provinces of the country varied between zero and 3.9% (Zali et al., 2005).

In this study significant statistical relationships were observed in the prevalence rate of hepatitis B carrier based on several factors. Statistical analysis by SPSS, Version 13 and with the X^2 test (P value = 0.0005), showed significant statistical relationships between the tattooing precedence, sexual contact precedence and HBV-DNA positivity.

But statistical analysis with the X^2 test did not show significant statistical relationships between the sex (P value = 0.384), married spouse (P value = 0.106) and HBV-DNA positivity.

The prevalence of HBsAg in the other countries are variable, for example, among blood donors in Turkey and Kuwait the prevalence of HBsAg reported 4.19% and between 1.1 and 3.5% respectively (Gurol et al., 2006; Ameen et al., 2005).

The prevalence of HBsAg from Afghan refugees living in the camps of Baluchistan province, Pakistan in 2003, was reported 8.3%. It has been suggested that it is advisable to monitor the changing patterns of HBV infection in countries with large immigrant population (Quddus et al., 2006).

In Yemen, prevalence of HBsAg ranges from 8 to 20% (Shamahy et al., 2000). Seroprevalence of HBsAg in pregnant women in Oman, Qatar and United Arab Emirates (UAE) was 7.1, 1 and 1.5% respectively (Awaidy et al., 2006).

In Asia and most of Africa, chronic HBV infection is common and usually acquired prenatally or in childhood (Lavanchy et al., 2004). The risk ranges from 70 to 90% for infants born to mothers who are positive for both HBsAg and HBeAg, in contrast to the 10 to 40% risk in

infants born to mothers who are positive for HBsAg but negative for HBeAg (Toukan et al., 1990).

Comparatively, East Asia has been found to have a higher prevalence of HBeAg positive mothers and a greater risk of prenatal transmission from HBeAg positive mothers than sub-Saharan Africa (Edmunds et al., 1996).

In Iran mass vaccination of neonates HBV infection started from 1993 as a national program in routine neonates care universal vaccination significantly has decreased the carriers among young children. It seems that the average age of the infected individuals has increased (Alavian et al., 2007).

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