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Full Length Research Paper

Investigation of the HBV DNA in isolated hepatitis B core antibody positive blood donors

Fahriye KÖTEN[1], A. Yasemin ÖZTOP[2]*, Aynur ENGİN[3]

[1] Institute of Health Sciences, Cumhuriyet University, Sivas, Turkey.
[2] Departments of Microbiology, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey.
[3] Departments of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Cumhuriyet University Sivas, Turkey.

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The aim of this study was to determine the prevalence of anti-HBc positivity and presence of HBV DNA from anti-HBc positive found donations that are living in the city of Sivas, in Turkey. A total of 987 voluntary blood donors, admitted to blood centre of Cumhuriyet University Hospital Sivas, (Turkey) between February to June 2008 were included in this study. Presence of Anti-HBc was screened in all serum samples. Positive samples for Anti-HBc were tested for the presence of anti-HBs then anti-HBs-positive samples were investigated for HBcIgM, HBeAg and anti-HBe. All of the HBV serological markers were tested in all serum samples using ELISA method. The presence of HBV-DNA was determined quantitatively in serum samples of cases with isolated anti-HBc by real-time PCR. Anti-HBc total was established as positive 209 (21.1%) of the 987 blood donors. The isolated anti-HBc positivity was 15.8% and HBV DNA positivity in the blood donors was 0.1%. In conclusion, the risk of HBV transmission by transfusion in blood centers can reduce by routine screening for anti-HBc in addition to HBsAg screening and then investigating of HBV DNA in anti-HBc positive samples.

Key words: Blood donors, isolated anti-HBc, HBV DNA.

INTRODUCTION

Hepatitis B caused by the Hepatitis B virus (HBV) is one of the serious public health problems that affect the people in our country and around the world. Our country is in the moderate endemic area for this infection. It is known that in Turkey approximately 4 million people are said to be carrier for the HBV (Ökten, 2003).

HBV may cause acute infection, chronic infection, cirrhosis and hepatocellular carcinoma. Serologic markers of HBV are used to identify HBV infection status. During acute infection, the first serologic marker is hepatitis B surface antigen (HBsAg). It is a protein on the surface of HBV. The presence of HBsAg for longer than 6 months after acute infection indicates chronic infection.

*Corresponding author. E-mail: ayoztop@gmail.com Tel: 0346 2191010/1987. Fax: 0346 2191155.

HBs antibody (Anti-HBs) can usually be detectable after disappearance of HBsAg. The presence of anti-HBs is interpreted as indicating recovery and immunity from HBV infection. Hepatitis B core antigen (HBcAg) is internal core antigen of HBV (Ponde et al., 2010). Total anti-HBc test detects both IgM and IgG anti-HBc (Hollinger, 2008) While clinical symptoms develop, IgM antibody to hepatitis core antigen (anti-HBc IgM) can be detectable. Its positivity indicates acute infection. Anti-HBc IgM decreases to undetectable levels within 6 months whereas the anti-HBc IgG persist for life. The presence of anti-HBc indicates previous or ongoing infection with HBV. Therefore, anti-HBc is widely employed in HBV screening (Ponde et al., 2010).

The "isolated anti-HBc" HBV serological pattern refers to the detection of antibodies to the anti-HBc without detectable HBsAg or anti-HBs. This occurs around 10 to 20% of people with detectable anti-HBc in low endemic

Table 1. Distribution of anti-HBc total positive among age.

Anti-HBc	Group I (18 to29) (%)	Group II (30 to 39) (%)	Group III (40 to 49) (%)	Group IV (50 to 61) (%)	Statistical analysis
Anti-HBc total+	40 (9.2)	75 (22.9)	68 (36.1)	26 (66.6)	
Anti-HBc total -	393 (90.8)	252 (77.1)	120 (63.9)	13 (33.4)	X^2= 111/p= 0.01
Total	433 (100)	327 (100)	188 (100)	39 (100)	

Table 2. Distribution of anti-HBc total positive among sex**.**

Anti-HBc	Male (%)	Female (%)	Total (%)	Statistical analyze
Anti-HBc total +	200 (22.2)	9 (10.2)	209 (21.1)	
Anti-HBc total -	699 (77.8)	79 (89.8)	778 (78.9)	X^2 6.89/p= 0.002
Total	899 (100)	88 (100)	987 (100)	

areas (Grob et al., 2000). Isolated anti-HBc can interpreted some possibilities: resolved infection, false positive anti-HBc, low level chronic infection, resolving acute infection or infection by HBV mutants (Ponde et al., 2010). Anti-HBc alone with HBV DNA has been associated with infectivity of blood products or organs. A proportion of individuals with this serological pattern are carriers of HBV and may transmit HBV by blood or organ donation to both immunocompetent and immunosuppressed recipients (Liu et al., 2006).

HBV is the most important cause of hepatitis by blood route. After beginning to investigate of HBsAg in blood donors the risk of infection is reduced. But transmission of HBV infection through transfusion of HBsAg-negative, anti-HBc-positive blood has been documented in studies. HBV DNA has been detected in the serum of blood donors with isolated anti-HBc (Zanetti et al., 2006; Gibney et.al., 2008). HBsAg screening of blood donations is mandatory while anti-HBc is not in our country. Therefore, the prevalence of isolated anti-HBc profile in blood donations and these blood donations infectious risks are unknown in our blood centre. The aim of this study was to determine the prevalence of anti-HBc positivity and presence of HBV DNA in isolated anti-HBc positive donations.

MATERIALS AND METHODS

This study was performed in 987 random blood samples collected from donor volunteers, who were referred to Cumhuriyet University Hospital Blood Center in Sivas city (central Anatolia) in Turkey during a five month period (between February to June, 2008). Routine screening tests for these blood donors, HBsAg, anti-HIV I/II, anti-HCV, the venereal disease research laboratory test (VDRL) were negative. The collected serum samples were stored in two separate aliquots at 20°C for subsequent testing.

Detection of anti-HBc total was done on these samples using ELISA. All anti-HBc-positive samples were re-tested with the same assay for confirmation of anti-HBc positive results. Anti-HBs presence was investigated in all anti-HBc–positive serum samples using ELISA. Then, anti-HBc total positive and anti-HBs negative samples were tested for the presence of anti-HBc IgM, HBeAg and anti-HBeAg using ELISA. The commercial enzyme immunoassay kits used were as follows: Anti-HBc total, anti-HBs, anti-HBc IgM, HBeAg and anti-HBe (Abbott Murex, Dartfort, UK).

The presence of HBV-DNA was determined quantitatively in plasma samples of cases with isolated anti-HBc (HBsAg-negative, anti-HBs-negative, and anti-HBc positive) by real-time PCR (Bal et al., 2009). HBV DNA was extracted from serum samples using the Mag Attract M48 kit on BioRobot M48 (Qiagen, Germany). HBV-DNA was determined quantitatively using the artus HBV RG PCR kit on the Rotor-Gene 3000 real-time thermal cycler.

Statistical analysis was performed using the SPSS statistical package, version 13 (SPSS, Chicago, IL) and the chi-square test was used for the analysis of categorical variables.

RESULTS

Distribution of the total 987 blood donors according to the sex showed that, 899 (91.1%) were male while only 88 (8.9%) female were observed. Age of these blood donors was ranged between 18 and 61 years old. For convenience, we have classified them into four groups according to ages (Table 1). Among all the 987 blood donors enrolled in this study, 209 (21.1%) were found to be anti-HBc total-positive; in which, 200 (20.2%) were male, while 9 (0.9%) were female. Differences in positive anti-HBc total were statistically significant (X^2=6.89, $p<0.05$) according to the sex (Table 2).

Distribution between the prevalence of anti-HBc total positivity and the four age groups was found to be statistically significant (X^2=111, $p<0.05$). The highest prevalence (66.6%) was observed in the group IV: 50 to 61 years old, while the lowest one (9.2%) was reported in the group I: 18 to 29 years old (Table 1).

Among the 209 anti-HBc positive serum samples, 33 (15.8%) were anti-HBs-negative, while 176 (84.2%) were positive. These 33 isolated anti-HBc-positive donors (anti-HBs-negative) were tested for HBeAg, anti-HBe and anti-HBc IgM. Our results showed that 7 were found to be

anti-HBe- positive whereas all of them were HBc IgM and HBeAg-negative.

Investigation for the presence of HBV-DNA was made on samples obtained from the 26 donors Anti-HBc total positive only (the 7 isolated anti-HBc /anti-HBe positive donors were not investigated for HBV DNA), and showed that one donor (3.8%) was positive (61 copies/mL or 8.7 IU/mL). So, HBV DNA positivity in the HBsAg negative blood donors was (1/980) 0.1%.

DISCUSSION

The prevalence of anti-HBc in Turkey has been reported to vary between 16 to 38% in blood donors (Kaya et al., 2009; Bal et al., 2009; Durupınar et al., 1994; Işık et al., 1996; Fındık et al., 2007). It was reported that the anti-HBc positivity rate of healthy people varies from region to region in our country (Badur, 1991). In this study, it has been found that anti-HBc prevalence was 21.1% (209/987) among blood donors of Sivas. Findings of the present study indicated that the rate of anti-HBc was similar to those seen in the other regions of Turkey. While the rate of HBV positive men was higher than female in some of the studies. No difference has been found in HBV seroprevalence between sexes in other studies (Kaçar et al., 2003; Coşkun et al., 1996). However, in the present study, there was a significant difference (X^2 =0.74 $p > 0.005$) between the anti-HBc positivity rates and the sex. The higher anti-HBc positivity rate determined for man can be the result of the higher number of male donors in the study. In the previous study performed in our country, the HBV seropositivity was found at highest rate in age group of 50 to 54 years old (Pasha et al., 1999). In this study, while the highest prevalence of anti-HBc was in the age group IV: 50 to 61 years old, the lowest prevalence of anti-HBc was among the age group of 18 to 29 years old. Our results showed that the possibility of exposure to HBV increases with life time.

The HBV infectivity by only the presence of anti-HBc in blood donors has been described. Cases of transmission by anti-HBc without detection of anti-HBs were reported in some studies (Gerlich et al., 2007; Satake et al., 2007). Occult HBV infection is most frequently seen in patients with anti-HBc as the only HBV serological marker (Aghakhani et al., 2010). It was reported that isolated anti-HBc prevalence varies in different countries: 0.56% in the UK, 1.4% in Germany, 15.03% in Greece and 76% in Ghana (Sofian et al., 2010). Some studies performed in our country showed that the isolated anti-HBc positivity, rates 2% in 2500 blood donors (Kaya, 2009). Bal et al. (2009), reported that the isolated anti-HBc positivity prevalence was about 2.5% in 9107 blood donors and HBV DNA positivity in the HBsAg negative group was 0.011%. Of the 1000 blood donors enrolled in other study, 59 (5.9%) were established to be anti-HBc total positive/anti-HBs negative. HBV DNA did not establish in any of the donors observed with isolated anti-HBc positivity (Fındık et al., 2007). In this present study, isolated anti-HBc positivity rate has found to be 3.3% and this rate was in concordance with other observed in different city of Turkey.

As the anti-HBc-positive blood usually has a very low level of HBV DNA (Zayadi et al., 2008), only sensitive and standardized methods should be used to screen for HBV DNA in HBsAg-negative samples (Liu et al., 2006). It was suggested that target amplification assays, such as polymerase chain reaction assay make possible the detection of less than 6 IU per mL or as few as 25 to 35 HBV DNA copies per mL and rapidly approaching the level of a single HBV genome. Chevrier et al. (2007) reported that 1.05% donations at Canada were found to be positive for HBV-DNA at the level of 29 to 212 copies/mL. In Poland, among 250191 HBsAg negative bloods, 20 were found to be HBV DNA with viral loads that ranged between less than 10 to 390 IU per mL (Hollinger, 2008). Nantachit et al. (2007) reported that ten of the HBsAg- negative and anti-HBc-positive samples were negative for the presence of HBV DNA, but five of these were positive after the use of a more sensitive assay at levels ranging between 1.8 to 20.6 IU per mL. In our study, the HBV DNA positivity rate was found to be 0.1% in HBsAg-negative blood donors and viral load at a low level of 61 copies per mL.

Our results showed that, a blood donor with isolated anti-HBc has HBV in this study. Therefore, the risk of HBV transmission by transfusion in blood centers can be reduced by routine screening of anti-HBc in addition to HBsAg testing and then investigating of HBV DNA in anti-HBc positive samples.

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