Short Communication

Investigation of Mutant Hepatitis B Virus in Core Antibody Seropositive Cases of Blood Donor Population

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This study was carried out to determine the prevalence of isolated anti-HBe among blood donors in this province and its impact on rejection of collected blood units. Isolated hepatitis B core positivity was found 15% in blood center but in this population we have found no HBV-DNA positivity. We proposed that in order to detect mutant hepatitis B viruses in blood donor population, multi-center studies must be done in this country.

Key words: Isolated core positivity, mutant HBV, pooled sera PCR, blood transfusion

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INTRODUCTION

Successes in preventing transmission of viral infections during the last 10 to 20 years have led to very low incidence rates and estimated residual risk for transfusion-transmitted viral infections (Dodd et al., 2002). This reduction was primarily achieved by a careful medical selection of the donors improved sensitivity of serological tests and the introduction of NAT in minipools for HCV and HIV (Roth et al., 2002a; Eiras et al., 2003; Stolz et al., 2003). In many studies pooled or single sample NAT for HBV is advised to confirm safe blood transfusion in high prevalence areas (Kuhrs and Busch, 2006; Matsumoto et al., 1997; Kleinman et al., 2005).

Enzyme linked immunosorbent assay is the most preferred method in detecting hepatitis B surface antigen (HBsAg). This method is based mainly on capture of antigens by antibodies attached on solid phase and recapture them by using signalled antibodies which detected by an enzymatical reaction (Hofnagle and Di Bisceglie, 1991; Hofnagle, 1999).

Post-transfusion hepatitis B is still a relevant subject in spite of high performance of immunoassays using in hepatitis virus screening (Kojima et al., 1991).

False sero-negative has three common causes in traditional methods. First of all, HBsAg titer may below the range in chronic carrier status. Anti-HBe may be the only marker detected in low level carriers (Jilg et al., 1995). Second reason is mis-detection of antigenic arrangement of variant virus type by antibodies on solid phase of assay. In various geographic regions, vaccine escape mutants may be selected under pressure of active immunisation and found as dominant strains by means of national vaccination programs. Detection of vaccine escape mutant strain is more difficult than wild type virus by traditional methods (Howard and Allison, 1995). Finally, decreased production of hepatitis B surface antigen in variant strains may cause false sero-negative (Carman and Mimms, 1997; Smith and Wu, 2002).

Hepatitis B virus is a common cause of viral hepatitis in worldwide. More sensitive assays has been developed for screening of blood donors to prevent transfusion associated hepatitits. Anti-HBc tests are being used by some countries to detect low level viremia in chronic carriers. Because of low level viral load in chronic isolated anti-HBc carriers, PCR tests from plasma pools may give negative results. Screening for anti-HBc may decrease this risk (Jilg et al., 1995). Seropositivity of anti-HBc is the major cause of rejection for blood donation. Testing for HBsAg alone is not fully protective and anti-HBc remains necessary as a screening test. The presence of anti-HBs is not always indicative of absence of the replicative virus.

The major aim of this study is to find frequency of Hepatitis B virus mutants in special blood donor groups (HBsAg weak positive, isolated anti-HBc) and its importance in rejection of blood donation.

MATERIALS AND METHODS

Blood samples from 174 blood donors were collected between October 2005 and October 2006 in Çanakkale State Hospital Clinical Laboratory Unit. All samples were stored at -10°C until the study procedure. This samples were tested for HBsAg, anti-HBc IgM and anti-HBs by using Beckman Coulter (USA) Access 2 immunanalysis system. The study designed in 3 groups:

- Low level HBsAg positivity (below 5 IU mL⁻¹) (N = 53)
- Isolated anti-HBc positivity without anti-HBs seropositivity (N = 109)
- Anti-HBc positivity with low level anti-HBs seropositivity (below 10 IU mL⁻¹) (N = 22)

We proposed to investigate mutant hepatitis B virus and false negative HBsAg test results in our blood donor population. In order to detect false negative results in our laboratory, control group is selected from serum samples of 180 HBsAg positive cases from outpatient clinics of Çanakkale State Hospital with cut-off levels higher than 100 IU mL⁻¹. Serum specimens stored at -20°C and later studied by using Abbott Murex, AxSYM V2.

The AxSYM HBsAg V2 assay is based on microparticle immunoassay technology. Briefly, the sample (150 μL), anti HBs (one monoclonal antibody)-coated microparticles and biotinylated anti-HBs (poly-clonal antibody) are combined and inebated in one reaction vessel. Because of poly-clonal antibody detection, this assay was found most sensitive in recognition of mutant HBV antigenic series by many authors (Weber et al., 2003; Taylor et al., 2004; Thoai et al., 2006).

Finally a pooled sera sample prepared by taking 20 μL serum from each blood samples, stored at -20°C and later studied PCR by using ABI PRISM® 5700 DNA sequencer, TaqMan® 1000 RXN PCR Core Reagents. DNA extraction was achieved by using Nucleospin DNA isolation kit. The quantitation range of HBV-DNA was accepted as 3×10⁴-3×10⁶ copy mL⁻¹ sera.

RESULTS AND DISCUSSION

There have been found no HBsAg seropositivity by using Abbott Murex, AxSYM V2 in totally 174 blood samples although all 180 control sera samples were positive for HBsAg. There is no false negative results and no difference found in three study group.
After than, pooled sera sample tested with HBV DNA real-time polymerase chain reaction (ABI PRISM® 5700 DNA sequencer, TagMan® 1000 RXN PCR Core Reagents), Pooled sera was found negative for HBV. We determined the isolated core seropositivity as 15% (179/1196) in our blood center for study interval period.

The replication of HBV is ongoing in a substantial proportion of healthy blood donors who have anti-HBc. Blood from such donors may contain very low levels of HBV free of immune complex formation and should be excluded for transfusion (Jongerius et al., 1998; Levelnic-Stezinar, 2004).

Collectively, around 30 to 35% of HBsAg-negative subjects with chronic hepatitis B or HBsAg mutant investigation should be considered when unusual serologic profiles occur, e.g., for (i) individuals with isolated anti-HBc reactivity, (ii) patients with discordant results between HBsAg assays, (iii) patients seronegative for HBsAg but positive for HBeAg and (iv) individuals with the presence of both HBsAg and anti-HBs (mostly at low titer of 100 mIU/mL) (Alhababi et al., 2003).

A study from Canada reports that 3.25% of anti-HBe positive samples (38/1169) were found to be positive for the presence of HBV DNA in blood (Chevrier et al., 2007).

In another study from Canada, the proportion of potentially infectious donations intercepted by anti-HBe screening has been found 1 in 17,800 cases (O’Brein et al., 2007).

In a study from Europe, isolated anti-HBe reactive 104 patient was comparatively investigated by Elecsys HBsAg and Murex HBsAg assays. Only 1 sample found (0.96%) to be repetitively reactive by the Murex HbsAg, suggesting that a mutant form of HbsAg was responsible for the isolated anti-HBe reactivity, however neutralisation assay was not interpretable and HBV DNA PCR was negative (Weber et al., 2001). In another study from Germany, after screening 3.6 million donor samples, 6 HBV PCR-positive, HBsAg-negative donations were identified (0.0016%) and 1 of them was found chronic anti-HBe positive low-level HBV carrier. Authors of this study states that, minipool PCR was sensitive enough to identify HbsAg-negative occult HBV infection in blood donor population (Roth et al., 2002b).

Another study from Pakistan states that isolated anti-HBe reactivity in HBsAg-negative blood donors is 17.28% (167/966) and of them 2.95% (5/167) has detectable HBV-DNA which presumptive of occult HBV infection (Bhatti et al., 2007).

In a large study from Japan, 308 samples from 16 million blood donors (0.01%) which HBsAg negative has been found anti-HBe positive (Tomono et al., 2002).

Turkey is a medium endemic area about hepatitis B prevalence found 2.7% in many studies (Balik, 1994). Studies in our country showed that HBV-DNA positivity rates is 11.3-41% in HBsAg positive cases. But in this studies authors found no HBV-DNA positivity in HBsAg negative cases (Sahin et al., 2001; Heper et al., 1999; Ozbilge et al., 2005). In another study from Turkey, no HBV-DNA positivity was found in isolated anti-HBe seropositive chronic hepatitis cases (Sonmez et al., 1992).

We have found no HBV DNA showing that there is no mutant hepatitis B in this serum samples studied.

And also found that there is 15% isolated HBV core antibody positivity in our blood center, showing high seroprevalence of hepatitis B. Although there is no transfusion transmitted acute hepatitis B case for the last ten years in our province (Health Statistics for Basic Health Services, Turkish Ministry of Health). This finding may be explained by strict donor selection by blood centers and high serum antibody titers found in blood donor population in our province.

Some studies proved that in HBeAg negative subjects, there is a strong correlation between the serum HBV-DNA and alanine aminotransferase (ALT) levels; ALT level is usually normal if the samples tested showed an HBV-DNA level less than 10(5) mL(-1) and monitoring of ALT is of value in assessing hepatocellular damage in patients with chronic hepatitis B virus infection (Sakugawa et al., 2001; Yalcin et al., 2003).

Many researchers states that isolated core-positive donors may potentially infectious for HBV so recommends to exclude core-positive donations. However in countries with high hepatitis B seroprevalence, exclusion of isolated core antibody positive donors may result difficulty in donation programs. But for making donations safer this is obviously logical decision. The anti-HBe test is not obligatory in Turkish Blood Banks controlled by Health Minister.

In conclusion we advice screening for HBsAg and anti-HBe with transaminases to make safer donations. The PCR technology has been used as a routine clinical test in Turkey for only last 10 years. Nucleic acid assays is not cost beneficial because of low detection rate in isolated core positive cases. Clinicians should be educated by transfusion safety programs, because there is no guarantee for safe blood transfusion especially in high seroprevalence areas.

REFERENCES


