Original Article

Real Time Polymerase Chain Reaction for Hepatitis B Screening in Donor Corneas in the Central Eye Bank of Iran

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Abstract

Purpose: The aim of this study was to report the results of the use of real-time polymerase chain reaction (PCR) for the diagnosis of hepatitis B virus (HBV) infection in cornea donors at the Central Eye Bank of Iran.
Methods: Between 2014 and 2016, all cornea donors that had negative screening serologic results for hepatitis B (HB) surface antigen, HB surface antibody (Ab), hepatitis C virus Ab, human immune deficiency virus Ab, human T-cell leukemia virus Ab, and syphilis, and positive serology for HB core Ab were subjected to real-time PCR with a detection limit of 400 IU/mL to identify HBV DNA. Positive results for HBV DNA were considered occult HBV infections in these donors.
Results: Over the 3-year period, 122 out of 10448 cornea donors had negative screening serologic tests outside of HB core Ab. Of which, 90 cases were subjected to real-time PCR. Occult HBV was detected in 11 cases (12.2%), resulting in the rejection of the corresponding corneas. The remaining 79 cases (87.8%) had negative results for HBV DNA and the corresponding corneas were used for transplantation.
Conclusion: Implementation of PCR for the detection of occult HBV in cornea donors is necessary to not only increase the security level of cornea donation but also minimize the rejection rate of donors that have isolated HB core Ab reactivity.

Keywords: Cornea Donors; Eye Bank, Hepatitis B Virus; Occult Hepatitis B Virus; Real Time Polymerase Chain Reaction

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INTRODUCTION

Hepatitis B (HB) is one of the most important public health challenges, affecting about 400 million people worldwide.[1,2] The estimated prevalence of this infection

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in Iran, as an intermediate endemic area for hepatitis B virus (HBV), is 1.7% of the general population.[3] Occult HBV infection, is defined as the presence of hepatitis B virus DNA in blood or tissue outside of the window period while HBV surface antigen (HBS Ag) is undetectable, disregarding HB core antibody (HB core Ab) or HBS antibody (HBS Ab) reactivity.[4-6] Occult infections were reported in healthy blood donors, patients with chronic liver disease and hepatocellular carcinoma, viral reactivation following immune suppression, and transmission through allograft tissue transplantation or blood transfusion.[7-9] By implementing high-sensitivity molecular techniques, HBV DNA has been detected in 1.6% to 38% of blood donors that were HBS Ag-negative and HB core Ab-positive.[7,10-12] Although, adding an HB core Ab screening test has led to the rejection of large numbers of blood and tissue donors, it definitely excludes HBV-infected donors and decreases disease transmission to recipients.[13] Using real time PCR, Said et al,[14] could detect HBV DNA in 17.2% of blood donors that were HBS Ag-negative and HB core Ab-positive, among which 21.2% were HBS Ab-negative. In other words, in these cases, more than 80% of blood donors' samples were wasted without performing real time PCR.[14]

The incidence of HBV transmission through human tissue and cell transplantation is unknown and varies depending on the type of tissues.[15] Given the reports of documented cases of HBV transmission following corneal transplantation, screening of cornea donors for HBV is mandatory in all eye banks.[15-17] In accordance with the medical standards of the American Eye Bank Association and the European Eye Bank Association,[18-20] donor selection and performing screening serologic tests are necessary for preventing disease transmission from donors to recipients of allograft corneas in the Central Eye Bank of Iran (CEBI). The first line for HBV screening is enzyme-linked immunosorbent assay (ELISA) for HBS Ag;[21] in positive cases, the corresponding donated ocular tissues cannot be released for transplantation.[19,20] Figure 1 illustrates the algorithm for HBV screening of cornea donors' sera used by the CEBI until 2013. Accordingly, occult HBV infections were suspected in cornea donors when HBS Ag-negative cases were positive for HB core Ab but negative for HBS Ab. Given the lack of HBV DNA detection using real time PCR at that time, all of the corresponding ocular tissues were rejected for transplantation. The annual statistics of the CEBI reveals that 513 donor eyes were disposed due to the possibility of occult HBV in 2013 (unpublished data). Since the majority of these cases might have had negative results for HBV DNA, and therefore, could have been releasable for transplantation.[22] the CEBI began to perform real time PCR on donors' sera suspected of occult HBV in 2014. In this study, we report the results of real time PCR for the detection of occult HBV infection amongst cornea donors to the CEBI.

METHODS

The investigation was performed on donors' sera between May 2014 and June 2016 at the CEBI. Research approval was obtained from the Institutional Review Board of the Central Eye Bank of Iran and the Ethics Committee of the Ophthalmic Research Center at the Shahid Beheshti University of Medical Sciences in Tehran, Iran.

From Cornea Donation to Eye Bank Serology Tests

Suitable cornea donors were selected considering past medical history, physical examination findings, social behavior, and available medical records. After obtaining 5 ml cadaveric blood, and immediate centrifugation, the resultant serum was subjected to serologic tests including HBS Ag (ELISA, Enzygnost, Siemens, München, Germany), HCV Ab (ELISA, Dia-Pro, Milan, Italy), HIV Ab (ELISA, Enzygnost, Siemens, München, Germany), HTLV Ab (ELISA, Dia-Pro, Milan, Italy), HB core Ab (ELISA, Enzygnost, Siemens, München, Germany), and syphilis (Immutrep PRP, Omega Diagnostics Ltd, Scotland, UK). When the serologic results were negative, the corresponding corneal/scleral tissues could be released for transplantation. In the case of positive HB core Ab, ELISA for HBS Ab (Dia-Pro) was performed; the positive results would lead to the release of corresponding ocular tissues. Cases with negative HBS Ab were subjected to real time PCR for the detection of HBV DNA and the corresponding whole eyes were either frozen at −70°C or cut and the resultant corneo-scleral rings were stored in Optisol-GS at 4°C.

Real Time Polymerase Chain Reaction

The HBV genome in HB core Ab-positive samples was investigated using a real time PCR technique. DNA was extracted from 150 µl of each serum sample using a Baharafshan kit (Baharafshan, Tehran, Iran) and quantitative real time PCR was performed using a homebrew Taqman MGB method.[23] The TaqMan MGB probe and primers were selected from a highly conserved region of the S gene to amplify all known HBV genotypes from A to H deposited in the GenBank database. Brome-mosaic virus (BMV) genome was used as a control to evaluate all phases from the initial separation of nucleic acids to the end phase of real time PCR. The limit of HBV DNA detection was 400 IU/ml. A negative control and a positive control (at certain concentrations) were added to each run. A data table was used to record the results. The results are presented as mean ± standard deviation, frequency, and percentage.

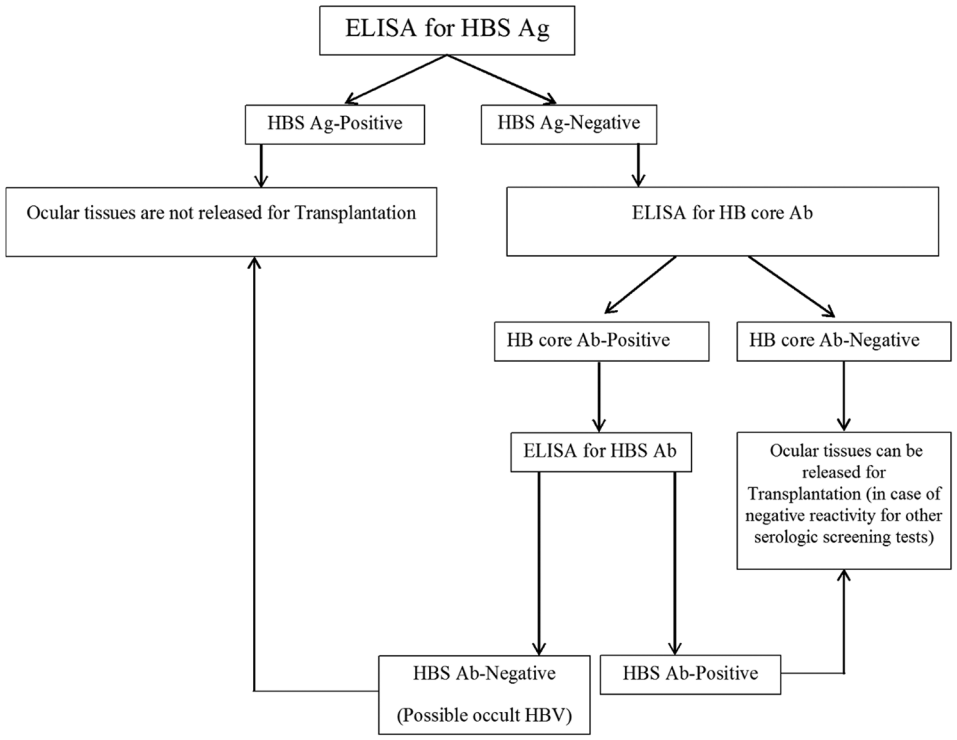

Figure 1. The algorithm for hepatitis B virus screening of cornea donors' sera in the Central Eye Bank of Iran up to 2013.

RESULTS

During the study period, 10448 donors' sera were investigated. The age of donors ranged from 3 to 80 years and 77.8% were male. Overall, 304 specimens (2.9%) were negative for HIV Ab, HCV AB, HBS Ag, HTLV Ab, and syphilis but positive for HB core Ab based on ELISA tests. Out of those 304 cases [Figure 2], 182 were HBS Ab-positive and their corresponding ocular tissues were released for transplantation. The remaining 122 cases were HBS Ab negative; 90 out of which were subjected to real time PCR for the detection of HBV DNA. The corresponding ocular tissues of the remaining 32 cases were disposed due to either poor corneal quality or insufficient serum for PCR. The HB core Ab-positive and HBS Ab-negative donors aged 17 to 71 years old (mean of 44.1 ± 14.2 years) and were predominantly male (87%).

HBV DNA was detectable in 11 (12.2%) male donors with an age range of 17 to 59 years (mean of 41.6 ± 13.5 years). Figure 2 illustrates the laboratory processes along with the corresponding results. Evaluation of all donors' sera revealed that 161 donors (1.54%) were positive for HBS Ag; given the detectable HBV DNA in 11 cases, the rate of HBV-positive donors during the 3-year period was 1.64%. In 79 cases (87.8%) with undetectable HBV DNA, the corresponding 156 corneas were usable for graft and distributed for keratoplasty. Out of 90 serum specimens that were sent for real time PCR, HBV DNA was detectable in 7 out of 49 specimens in 2014 and 4 out of 13 cases in 2016. None of the 28 specimens

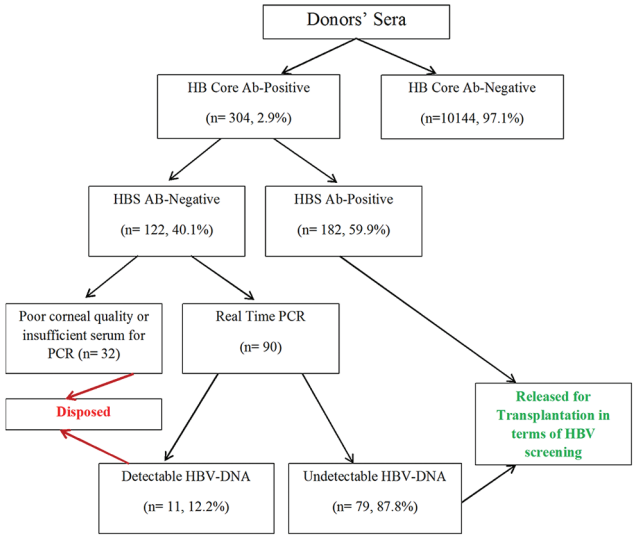

Figure 2. Results of hepatitis B core antibody (Ab), hepatitis B surface Ab, and hepatitis B virus DNA among the cornea donors in the Central Eye Bank of Iran (2014–2016).

in 2015 had positive results for HBV DNA. During the 3-year period, the rate of rejected donated eyes that had negative serologic tests except for HB core Ab, was 0.4% (86 out of 20630 eyes) among which 22 eyes from 11 donors had positive results for HBV DNA and 64 eyes from 32 donors had either poor corneal quality or insufficient serum for PCR, and were therefore not subjected to real time PCR.

DISCUSSION

The results of the current study demonstrate the importance of real time PCR in detecting occult HBV cases amongst cornea donors, and by exclusion of the corresponding ocular tissues the safety of distributed corneas in terms of lack of potential HBV transmission was increased. Furthermore, the ocular tissues from donors with undetectable HBV DNA could be released for transplantation, hence avoiding the rejection of these tissues. This was culturally and economically invaluable for the CEBI.

According to the annual report of the CEBI in 2013 (unpublished data), approximately 6% of donated eyes that had negative serology for HBS Ag, HBS Ab, HCV Ab, HIV Ab, HTLV Ab, and treponema were rejected due to the isolated positive serology for HB core Ab and the lack of implementation of molecular methods involving nucleic acid amplification for the detection of HBV DNA. This rate, as reported in this study, was decreased to 0.4% during the 3-year period in which the real time PCR was implemented.

Despite using the very sensitive HBS Ag test, there still is a low possibility for HBV transmission through corneal transplantation from apparently healthy donors. In such cases, the corresponding ocular tissues, are not safe for transplantation due to the insufficiency of routine screening tests for the detection of HBS Ag in the window period or in occult HBV cases.[19,24-26] By using complementary real time PCR test in the current study, similar to the study by Fornés et al,[22] the above-mentioned cases could be detected and rejected for transplantation.

In the current study, there was a decreasing trend for the number of donors that were seronegative for all screening eye bank tests excepting HB core Ab over a 3-year span. This might be explained by the high efficacy of a vaccination program on a large proportion of the population of Iran.[27,28] The decreased incidence of HBV infection after vaccination against the virus has been observed in many countries that were endemic for HBV.[29.30] It seems that a combination of HBV vaccination and improved public health may have caused the reduction of such cases in the CEBI.

By implementing nucleic acid amplification tests (NATs) for the diagnosis of HIV, HCV, and HBV in many eye and tissue banks in developed countries, the risk of infection transmission through eye and tissue transplantations has been reduced during the window period when viral proteins are not detectable.[22,31] In a few studies from blood banks, the window period of hepatitis B has been decreased from 38 days to 6-15 days by using NATs.[32,33] Our results show that the occult HBV cases could be detected amongst the group of interest by the method used in the current study. However, given that real time PCR was not performed on all the donors' sera at the CEBI, the test had no effect on reducing the hepatitis B window period in cornea donors.

The results of the current study exhibit the importance of performing real time PCR for the detection of occult HBV infection in all cornea donors with isolated HB core Ab reactivity. Implementation of this molecular test not only reduces the rejection rate of these donors but also increases the safety level of cornea donation. The use of real time PCR in our series could lead to the transplantation of large numbers of donated corneas that were previously rejected because of an isolated serologic profile of HB core Ab reactivity. This approach has both economic and cultural consequences for the eye bank.

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Conflicts of Interest

There are no conflicts of interest.

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