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Original Research Article

Prevalence of Window Period-associated Transmission of HBV, HCV, and HIV in Blood Donors

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ABSTRACT

Background and Objectives: Blood donors undergo screening for infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) utilizing enzyme-linked immunosorbent assay (ELISA) methodology. This study aims to assess seronegative plasma from blood donors using nucleic acid testing (NAT) approach.

Material and Methods: A total 567 blood donor plasma samples were initially screened using ELISA, followed by NAT conducted on pools comprising five blood plasma samples each. Subsequently, 100 minipools of donor plasma were subjected to NAT.

Results: Positive results were observed in 2.91% of HBV minipools, 2.95% of HCV minipools, and 2.96% of HIV minipools by NAT.

Conclusion: Prevalence of window period-associated transmission of HBV, HCV, and HIV in blood donors was found to be around 3%. Our study underscores the utility of NAT in identifying occult HBV and reducing the window period for HCV and HIV in seronegative plasma from blood donors.

Key Words: Blood transfusion, widow period, HBV, HCV, HIV, Virus

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INTRODUCTION

Blood transfusion safety can be significantly enhanced by improving the sensitivity of screening assays for donated blood. Traditionally, serological tests have served as the cornerstone for screening transfusion-transmitted diseases. However, these tests exhibit limitations in detecting infections during the pre-seroconversion window period (PWP), in cases involving donors with genetic or immunovariant viral strains, and in instances of immunosilent infections. The efficacy of such tests is evident only after the donor's immune system responds to the pathogens in question. Over the past two decades, the introduction of advanced techniques, such as nucleic acid testing (NAT), has played a pivotal role in reducing the viral "window period." [1-3]

NAT, a molecular technique employed for the detection of viral nucleic acids, demonstrates heightened sensitivity and specificity in screening donated blood. This amplification technique targets specific regions of viral RNA or DNA, enabling the early detection of viruses compared to conventional screening methods. Notably, NAT significantly narrows the window period for HIV, HBV, and HCV infections and addresses false reactive results encountered in serological methods [1-3].

A global survey on NAT testing of blood donations conducted from 1999 to 2009, screening over 300 million blood donors for HCV and HIV-1 and approximately 100 million blood donors for HBV, revealed over 2000 NAT-reactive donations with serology-negative results. These donations, if not for NAT, could have been transfused, underscoring the importance of NAT in enhancing blood safety. The infection rates for HCV, HIV, and HBV were found to be 1:447,000, 1:111,000, and 1:66,000, respectively. However, the cost of NATs remains a challenge, being 5–10 times higher than that of the most expensive enzyme immunoassay. To address the cost issue associated with NATs, two strategies have been proposed. One involves the use of pooled plasma samples, reducing the number of tests required for screening large sample sizes. The other strategy employs multiplex PCR assays, capable of detecting several viruses simultaneously in a single reaction tube [4-6].

Despite serological tests being the current standard for screening donated blood [7-10], questions arise regarding their adequacy in ensuring the safety of blood or plasma for transfusion. To address this concern, we designed this study to shed light on various critical aspects of blood transfusion. The study aims to ascertain the prevalence of positive HBV, HCV, and HIV in donated blood within a sample of Indian donors. Furthermore, the study aims to evaluate the transmission rates of HBV, HCV, and HIV during the seroconversion window.

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MATERIAL AND METHODS

Approximately 20 milliliters of blood were collected from seemingly healthy Indian blood donors using EDTA tubes. The subsequent plasma isolation was achieved through centrifugation at 4000 revolutions per minute for a duration of 20 minutes. State-of-the-art serological kits were employed for the detection of HBsAg through ELISA, the identification of antibodies to the hepatitis C virus using an advanced ELISA kit, and the determination of the presence of HIV-1/2 antibodies and/or HIV-1p24 antigen in plasma through EIA.

A total of 567 blood donors who tested seronegative for HBV, HCV, and HIV were enrolled in this investigation. These samples were divided into two categories: 280 plasma samples collected after 6 hours from blood withdrawal and the remaining 287 collected after 12 hours from blood withdrawal. Subsequently, 1 milliliter of plasma was pipetted into 1.5 milliliter microcentrifuge tubes and stored at -80 degrees Celsius until utilization.

Pooling of plasma from blood donors for nucleic acid extraction and nucleic acid amplification testing (NAT) was carried out. Pre-determined positive samples with concentrations of 10^3 copies/ml for HBV and HCV, and 10^4 copies/ml for HIV, were utilized to validate the pooling system. This validation involved the mixing of positive samples with negative samples.

For simultaneous purification of viral RNA and DNA from plasma, serum, and cell-free body fluids, the Latest virus spin kit was employed. The viral nucleic acid extraction from pooled blood donor's plasma was performed according to the manufacturer's instructions. Subsequently, the concentration and purity of all extracted samples were assessed using a fluorometer. The DNA extracts (eluent) were stored at -20 degrees Celsius. The detection of HBV, HCV, and HIV in the plasma of pooled blood donors was accomplished through multiplex qualitative Real-Time RT PCR tests. Results were deemed acceptable if the positive amplification and negative amplification controls, in addition to the negative and positive controls of extraction, passed. A sample was considered positive for HCV or HIV or HBV if the value of Ct was lower than 35, in accordance with the manufacturer's instructions.

RESULTS

A total of 567 plasma samples exhibiting sero-negativity were amalgamated into 113 distinct mini-pools (MPs), each comprising 5 samples. These combined specimens underwent screening via NAT tests utilizing a commercial multiplex PCR kit designed for the detection of HBV, HCV, and HIV. The outcomes of the multiplex PCR analysis disclosed that approximately 2.94 out of 100 (2.94%) plasma samples in MP5, initially identified as sero-negative, demonstrated positivity for HBV, HCV, and HIV, as delineated in Table 1.

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The comparison of the HBV, HCV, and HIV detection rates at 6 hours versus 12 hours post-withdrawal exhibited marginal disparities. Notably, no statistically significant distinction was observed in the positive rates of HBV, HCV, and HIV between the blood samples collected at 6 hours and those collected at 12 hours, as elucidated in Table 2.

Table 1: Plasma samples detected positive for viruses by multiplex PCR

Viruses detected by PCR	Positive detection %
HBV	2.91
HCV	2.95
HIV	2.96

Table 2: Comparison of Initial 6 Hours and 12 Hours after Blood Withdrawal

Viruses detected by PCR	Positive detection %		p value
	6 hrs	12 hrs	
HBV	2.15	4.11	0.35
HCV	5.95	0	0.06
HIV	5.92	0	0.06

DISCUSSION

The imperative for serological screening holds significance in diminishing the risk of transfusion-transmitted infections and enhancing donation selection. In the absence of nucleic acid testing (NAT) application, serological screening remains a steadfast and established method for blood screening assays. This present study was conducted on 567 seronegative plasma samples from Indian blood donors. The positive rates of HBV, HCV, and HIV in these seronegative plasma samples were assessed using real-time PCR. Additionally, the study explored the viability of screening pooled samples through multiplex PCR. Consequently, 113 mini-pools of plasma were subjected to multiplex PCR screening, revealing a 2.94% positivity rate for HBV, HCV, and HIV in MP5. These research findings closely align with prior investigations conducted on donor databases [11-14].

In Western countries, NAT testing has become mandatory over the years, employing commercial NAT systems through multiplex PCR for the detection of HBV, HCV, and HIV genomes on automated platforms. The United States employs minipool nucleic acid testing (MP-NAT) by pooling 6 to 16 specimens, while some other countries opt for individual donation testing. Initially, many countries performed NAT testing in larger pools of 96–16 samples; however, a recent trend has emerged towards smaller pools of 6 to individual donations (ID) to enhance testing sensitivity [15, 16].

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This study also sought to assess the impact of the duration between blood phlebotomy and screening assays. Categorizing plasma samples at 6 hours and 12 hours post-withdrawal, the study observed higher detection rates for HCV and HIV after 6 hours compared to 12 hours, with no such observation for HBV. Consistent with prior studies, the stability of HBV DNA over a longer period was attributed to the inherent stability of DNA compared to the more labile nature of RNA, influencing HCV and HIV [17-19].

In conclusion, despite NAT's ability to detect transfusion-transmitted infectious (TTI) viruses during the window periods earlier than serological screening assays, a comprehensive approach involving both serological and NAT assays is recommended. This study underscores the estimated risk of HCV, HIV, and HBV transmission through blood transfusion, even in seronegative donated blood samples. The risk is attributed to the window periods of these viruses, with NAT screening assays demonstrating greater sensitivity than serological screening assays in TTI virus detection [20, 21].

CONCLUSION

Prevalence of window period-associated transmission of HBV, HCV, and HIV in blood donors was found to be around 3%. Our study underscores the utility of NAT in identifying occult HBV and reducing the window period for HCV and HIV in seronegative plasma from blood donors. However, larger sample size studies need to be done to further validate these findings.

CONFLICT OF INTEREST - Nil

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