Abstract:
Context: Transfusion transmittable infections (TTIs) continue to be a major threat to safe transfusion practices. Blood is one of the major sources of transmission of infectious diseases viz. human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, malaria, and many other infections in India. Screening assays for the infectious diseases with excellent sensitivity and specificity helps to enhance the safety of the blood transfusions reducing the diagnostic window period as much as possible. Aims: The present study was designed to determine the seroprevalence of TTIs viz., HIV, HCV, and HBV, among the blood donors in Max Super Specialty Hospital, New Delhi, India based on dual testing strategy using high sensitive screening assays such as enhanced chemiluminescence assay and nucleic acid testing (NAT). Materials and Methods: A total of 41207 blood units collected from the donors (both voluntary and replacement donors) were screened for the TTIs viz., anti HIV 1 and 2 antibody, anti HCV antibody, anti HBcore antibody, and HBsAg by enhanced chemiluminescence assay on VITROS® ECIQ immunodiagnostics system. NAT was performed using Roche Cobas® TaqScreen MPX assay, which can detect simultaneously HIV 1 (groups M and O), HIV-2, HCV, and HBV on Roche Cobas® s201 system. Results: The seroprevalence of HIV, HBsAg, anti HBcore antibody, and HCV based on enhanced chemiluminescence assay was found to be 0.25, 0.2, 7.06, and 0.7%, respectively. A total number of 6587 samples from July 2010 to December 2010 were tested on NAT, of which 3 samples were reactive for HBV in NAT; this was missed by enhanced chemiluminescence assay. Conclusions: Based on the seroprevalence study of infectious diseases viz., HIV, HBV, and HCV, we conclude that screening of blood and blood components by dual testing strategy using high sensitivity serological assay like enhanced chemiluminescence technology and NAT helps in detecting the potentially infectious blood units in all phases of infection, which aids in enhancing the safety of blood transfusion and reducing the potential risk of post-transfusion infection.

Key words: Seroprevalence, seroprevalence in blood donors, transfusion transmittable infections

Introduction

Blood safety is major concern globally going by the increasing incidence of transfusion transmittable infections (TTIs). Safe transfusion of blood and blood components saves millions of lives, but unsafe transfusion practices put millions of people at risk of TTIs. Blood is one of the major sources of transmission of infectious diseases viz. HIV, HBV, HCV, syphilis, and many other infections in India. With an estimated population of 1.21 billion, India has the world’s third largest population suffering from HIV/AIDS. The estimated adult HIV prevalence was 0.31% in 2009.[4] India has intermediate endemicity of hepatitis B with HBsAg prevalence of 2–10% among the study population. It has been estimated that up to 40 million people out of the 350 million hepatitis B chronic carriers worldwide arise in India.[5] HCV is a leading cause of chronic liver diseases, viz., hepatic fibrosis, cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). In India, there are about 12–13 million HCV carriers and modeling data predict that the burden of disease could soon increase substantially.[5]

Despite implementation of various screening assays for detection of TTIs, occasional cases of post-transfusion infections are common. Majority of these problems are due to prevalence of asymptomatic carriers in the society as well as due to blood donations during the window period of infections. The hazards of transfusion were minimized by proper selection of donors and screening for infectious diseases by a high sensitivity screening assay. World Health Organization (WHO) recommends an integrated strategy to improve blood transfusion safety by establishment of well-organized blood transfusion services, prioritization of blood donation from voluntary non-remunerated donors, screening of donated blood for at least four major TTIs with quality assured system, rational use of blood and implementation of effective quality control systems.[6]

The objective of this study was to determine the seroprevalence of HIV, HBV, HCV, and syphilis infections in blood donors of the Max Super Specialty Hospital, New Delhi, India by dual testing strategy using high sensitivity screening assays like enhanced chemiluminescence technology and nucleic acid testing (NAT).
Materials and Methods

A total of 41,207 units of blood were collected from donors (voluntary and replacement donors) from May 2006 to December 2010 at Max Super Specialty Hospital (A unit of Devki Devi Foundation), Saket, New Delhi, India. Donors were selected by following strict Donor selection criteria and taking history and clinical examination to eliminate professional donors. All selected donors were screened for HIV, HBV, and HCV infection by both serological tests and NAT.

Serological screening for HIV, HBV, and HCV infections

All serum samples were screened for the presence of anti HIV 1 and 2 antibody, anti HCV antibody, anti HBcore antibody, and HBsAg using enhanced chemiluminescence technology in VITROS® EG IQ system, following manufacturer’s instructions. All samples that showed “initial reactive” or “borderline” reactive were retested either after re-centrifugation or by using fresh samples.

Screening for anti HBs antibody

In a pilot study, some of the serum samples that showed anti HBcore antibody alone reactive were tested for the presence of anti HBs antibody using VITROS® Anti HBs Antibody–quantitative assay based on enhanced chemiluminescence technology in VITROS® EG IQ system.

NAT

NAT was performed for all initial 5000 donor samples from 1st July, 2010 using Roche Cobas® TaqScreen MPX assay, which can detect simultaneously HIV-1 (groups M and O), HIV-2, HCV, and HBV on the Roche Cobas® s201 system. The minipool samples that showed “Pool reactive” in Cobas TaqScreen MPX assay were retested individually and then the sample that were positive were sent for discrimination. Discrimination was outsourced to Roche Diagnostics for Max Super Specialty Hospital.

Quality control

Internal quality controls were performed daily by using both positive and negative controls from the manufacturers. In case of any deviation, the root cause analysis was carried out and the corrective action was taken before analyzing the samples.

Results

Of the 41,207 blood donors screened for the TTIs, viz., HIV, HBV, and HCV, the incidence of sero-reactive samples for anti HIV antibody, HBsAg, anti HBcore antibody, and anti HCV antibody are shown in Table 1.

In a pilot study, the samples that showed anti HBcore antibody alone without HBsAg were subjected to anti HBs antibody quantification study to verify whether the donors had resolved infection or continued to have chronic infection without detectable HBsAg. The obtained results are shown in Table 2. In this study, 41% of anti HBcore antibody reactive donors without detectable HBsAg had high level of anti HBs antibody (>100 mIU/mL), indicating resolved infection. About 31% of the anti HBc antibody reactive donors had anti HBcore antibody alone without detectable HBsAg and anti HBs antibody and such donations may be potentially infectious for transfusion.

Since July 2010, all donor samples were subjected to NAT testing in order to enhance the safety of the blood issued for transfusion and to identify any sample missed by serological screening as the donor may be in the early window phase of infection. Initial 5000 samples were tested simultaneously on NAT and enhanced chemiluminescence assay. Later, we decided to run only sero-negative samples on NAT in order to enhance the safety of the blood or blood components for transfusion. A total of 6587 samples were tested on NAT until December 2010, of which 3 samples showed reactivity for HBV in NAT, which were missed by the enhanced chemiluminescence assay. Based on the dual testing strategy, the prevalence of HBV was increased by 0.045%.

Discussion

Technological advancements have led to the development of more sensitive methods to detect various infectious disease markers, e.g., viral specific antigens, antibodies and nucleic acids in order to enhance the safety of blood transfusion. However, early detection of infection remains elusive goal due to the existing problem of “Window period,” false negative results due to the limitation in the screening assays, genetic modifications in viral strains, and laboratory errors.

This study was undertaken to study the prevalence of infectious disease markers in the donor population attended in the blood bank in the tertiary care hospital based on dual testing strategy. Since ours is a hospital-based blood bank, majority of the blood units are collected from the replacement donors and very few are voluntary donors. Since the transfusion transmitable diseases screening was carried out for transfusion safety, we followed the WHO® and NACO® testing strategy 1 to maximize safety of the blood for transfusion.

In our study, the overall seroreactivity based on enhanced chemiluminescence assay, was 0.25% for HIV and 0.7% for HCV. In 2009, it was estimated that 2.4 million people were living with HIV in India, which equates to a prevalence of 0.3%.7 Mukhopadhyya® reported the HCV prevalence in blood donors in different parts of India range from 0.5% to 1.85%. Meena et al.,8 revealed that the prevalence of HCV infection among blood donors showed a significant increasing trend from 0.18% in 2005 to 0.82% in 2009. Their study was based on the sero-reactivity in anti HCV ELISA-based assay.

| S. No. | Year | No. of Donors | Anti HIV Ab (%) | HBsAg (%) | Anti HBc Ab (%) | Anti HCV Ab (%) |
|-------|------|---------------|----------------|-----------|----------------|----------------|
| 1     | 2006 | 4977          | 13 (0.26)      | 7 (0.14)  | 308 (6.2)      | 20 (0.41)      |
| 2     | 2007 | 9122          | 12 (0.13)      | 19 (0.21) | 685 (7.51)     | 86 (0.94)      |
| 3     | 2008 | 7889          | 18 (0.23)      | 8 (0.1)   | 559 (7.08)     | 43 (0.54)      |
| 4     | 2009 | 9049          | 30 (0.33)      | 20 (0.22) | 639 (7.06)     | 74 (0.81)      |
| 5     | 2010 | 10170         | 31 (0.31)      | 28 (0.27) | 717 (7.05)     | 65 (0.63)      |
| Total |      | 41207         | 103 (0.25)     | 81 (0.2)  | 2908 (7.06)    | 287 (0.7)      |
In the course of HBV infection HBsAg is the first sero-marker to indicate active HBV infection followed by anti HBcore antibody. In our study, the blood donors were screened for both HBsAg and anti HBcore antibody (both IgM and IgG) to enhance the safety of blood. The sero-reactivity of HBsAg was 0.2% and that of anti HBcore antibody was 7.06%. NAT testing on sero-negative samples yielded 3 more reactive samples for HBV out of 6587 samples screened, which may be in the pre-seroconversion period. By dual testing strategy using both serology and NAT, the HBV reactivity rate increased by 0.045%. Anti HBcore antibody are markers of acute, chronic or resolved HBV infection and remain detectable for life. This can be present in the absence of both HBsAg and anti HBs antibodies during convalescent period following acute hepatitis B before the appearance of anti HBs antibodies, or in patients with resolved infection but lost detectable anti HBs antibodies. In our study, among anti HBcore antibody reactive donors, 31% showed the presence of only anti HBcore antibody in the absence of HBsAg and anti HBs antibodies which may be potentially infectious. Behzad-Behbahani et al., reported highest rate of HBV-DNA in individuals positive for anti HBc antibody D negative but negative for anti HBs antibody and HBsAg.

Based on the seroprevalence study among blood donors by dual testing strategy using high sensitivity serological assay and NAT testing, our study reveals serious concerns regarding the HIV, HBV, and HCV infections among the blood donors and the safety of the blood supply in our country. Considering the vast population of the country, even low prevalence amounts to large number of infected people. If high sensitivity serological assays are not used, the safety of the blood for transfusion may become a big concern. Stringent measures in donor screening including better donor recruitment, promoting voluntary blood donation, screening of blood and blood products using dual testing strategy with high sensitivity serological Assays and NAT, inclusion of anti HBcore antibody screening in blood donors and other infectious diseases markers would considerably improve the current screening procedure for blood donation and enhance the safety of the blood intended for transfusion.

We conclude that dual testing strategy using enhanced chemiluminescence technology and NAT helps to detect potentially infectious blood units in all phases of infection, which in turn helps in enhancing the safety of the blood and blood components for transfusion.

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