Short Report

Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital

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ABSTRACT

Background. In July 2010, we started universal individual donor nucleic acid testing (ID-NAT) at our blood bank. This test simultaneously detects human immunodeficiency virus-1 (HIV-1), hepatitis B virus (HBV) and hepatitis C virus (HCV) in samples of donor blood. We continued to do the enzyme-linked immunosorbent assay (ELISA) test for these agents, as per the guidelines of the Drug Controller General of India. We assessed the impact of ID-NAT in preventing transfusion-associated transmission of viruses.

Methods. We used fourth generation ELISA to screen blood samples of all voluntary and replacement blood donors. ID-NAT was done by transcription-mediated amplification (TMA).

Results. Of the 18,356 donors, ID-NAT could not be performed on 2 samples which were inadequate. Of the 18,354 donors tested by both ID-NAT and fourth generation ELISA, 7 were found to be NAT-positive but ELISA-negative (NAT yield) for HBV and HCV. The prevalence of NAT yield cases among routine donors was 1 in 2622 donations tested (0.038%). Since we supply blood as components (packed red cells, fresh frozen plasma and platelet concentrate), these 7 units of blood would have yielded 21 components and hence 21 patients could have been infected with HBV and HCV viruses.

Conclusion. In the vast majority of blood units tested, the results of ELISA and ID-NAT for HIV-1, HBV and HCV were concordant. ID-NAT did detect the presence of viruses missed by ELISA in some blood units. It widespread use in blood banks would ensure safer blood transfusion.

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INTRODUCTION

The inspection and licensing of blood banks in India is covered under the Drugs and Cosmetics Rules of the Ministry of Health and Family Welfare, Government of India. These rules stipulate that before transfusion, mandatory testing should be done of each sample of donor blood for hepatitis B surface antigen (HBsAg), antibodies to HIV, antibodies to hepatitis C virus (HCV), syphilis and malaria.

The seroprevalence of anti-HIV-1, HBsAg and anti-HCV among Indian blood donors is 0.5%, 1.4% and 0.4%, respectively. We started screening donated blood for HIV-1 and -2 and hepatitis B in 1989, and for antibodies to HCV in 1995 by serological testing (enzyme-linked immunosorbent assay [ELISA]). Evaluation by individual donor nucleic acid testing (ID-NAT) was started in 2009 as a pilot study and routine screening with ID-NAT was started from July 2010.

The introduction of second- and third-generation screening assays for HIV-1 and -2, for hepatitis C antibodies and for HBsAg has significantly reduced the risk of transmission. Nevertheless, there remains a residual risk for transmission of these viruses. This is mostly due to the ‘window period’ (pre-seroconversion seronegativity). NAT detects viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) by the amplification method. Early in the course of an infection, NAT detects low levels of viral genetic material present in the blood. NAT is thus able to detect viruses during the ‘window period’ or the time between donor exposure to the virus and the appearance of antibodies. This allows for earlier detection of infection and further decreases the possibility of transmission via transfusion. NAT also detects mutants and occult cases. Blood banks in several countries use NAT, which is currently used in conjunction with serological tests. Serology, when combined with ID-NAT, provides the most sensitive and specific screening platform for blood screening.

ID-NAT detects infection at an earlier time point than serology. The window period for detection by NAT is 5.6 days for HIV-1, 4.9 weeks for HCV and 24.6 days for hepatitis B virus (HBV). The corresponding window periods for serological tests are 15–22 days, 2–26 weeks and 50–150 days, respectively.

METHODS

Study design and settings

All voluntary and replacement blood donors at the Main Blood Bank, All India Institute of Medical Sciences (AIIMS), and those donating in the outdoor voluntary blood donation camps organized by us in and around Delhi between July 2010 and January 2011 were included in the study. Samples from the donated blood units were tested in our NAT laboratory.

Participants

All healthy blood donors between 18 and 60 years of age, weighing >45 kg and with a haemoglobin level of >12.5 g/dl were included in the study. Donors were asked to fill up a simple form providing demographic data and health-related information. A doctor reviewed the donor’s history and did a physical examination. Demographic details of each donor were noted. Written consent was obtained after explaining the procedure as well as the tests to be done on the donated blood.

The study population was divided into three age groups: 18–30 years, 31–45 years and 46–60 years. Donors with a history of high-risk behaviour such as promiscuity and intravenous drug abuse were ineligible to donate blood.
Donor recall policy
Donors found to be reactive for any of the viral markers were contacted telephonically or by post and asked to come to the blood bank where a repeat sample was obtained for re-testing. If the repeat sample of the donor was found to be reactive by ELISA and NAT, then the donor was referred either to the Integrated Counselling and Testing Centre (for HIV) or to the Department of Gastroenterology (for HBsAg/HCV), AIIMS.

Procedure of blood collection
Blood was drawn from a vein in the antecubital fossa using an aseptic technique. Unique donor identification stickers were pasted on the primary, the satellite blood bags and also on the donor form. The 5 ml pilot tubes (vacutainer) used for sample collection were also given the same identification number (bar coded). Three pilot tubes were used—one each for blood grouping, testing by ELISA for transfusion transmitted infections (TTI) and NAT.

Bags with a capacity of 350 ml were used for collecting blood from donors who weighed <55 kg and 450 ml sized bags were used for those who weighed ≥55 kg. After collecting the required volume of blood and removing the needle from the donor’s vein, the pilot tubes were filled up. Thereafter, the pilot tube meant for NAT testing was transported to the NAT laboratory.

Method of doing ID-NAT
ID-NAT was done using the Procleix® Ultro® Assay (Novartis, Emeryville, CA) by transcription-mediated amplification (TMA) technology for simultaneous detection of HIV-1, HCV-RNA and HBV-DNA. This system is a semi-automated modular NAT platform with high throughput and is easy to use. The TMA assay has three main steps: (i) target capture of viral nucleic acid; (ii) TMA; and (iii) hybridization protection assay (HPA) and dual kinetic assay (DKA).

All three tests can be done using a single tube. Each of these steps incorporates an internal control to validate each reaction. Since ID-NAT is a multiplex assay which provides simultaneous detection of HIV-1 RNA, HBV-DNA and HCV-RNA, samples found to be reactive in this assay were further analysed to distinguish HIV-1, HBV and HCV using discriminatory assay reagents. These discriminatory assays utilize the same steps as the multiplex assay (target capture, TMA, HPA and DKA) but use HIV-1, HBV and HCV specific probes.

Serological tests were done using Bio-Rad Genscreen plus HIV Ag-Ab, Bio Rad Laboratories, Steenvoorde, France; BioMerieux Hepanostika HBsAg Ultra, BioMerieux Hepanostika HCV Ultra; M/s BioMerieux, B.V. Boseind, The Netherlands.

Data collection and processing
Results of ELISA and NAT tests were compiled every day at 5 p.m. Units of donated blood which were found to be reactive by any of the tests were segregated and kept in a separate quarantine area till they were sent for disposal using biohazard labels.

Statistical methods
Data were compiled in a Microsoft Excel spreadsheet. Statistical analysis was done using the Pearson Chi-square test and Fisher exact test as appropriate (SPSS version 15, SPSS Inc. Chicago). The study was approved by the institutional ethics committee.

RESULTS
A total of 18,356 samples were collected but 2 could not be processed as these were inadequate. Of the 18,354 samples, 188 (1.02%) were reactive by ID-NAT. Discriminant assays found 17 (0.09%) to be reactive for HIV-1, 38 (0.21%) to be reactive for HCV and 133 (0.72%) for HBV. Of these 188 ID-NAT reactive samples, 7 (0.04%) were ID-NAT reactive but ELISA non-reactive (NAT yield). These samples had been collected within the first 7 months of ID-NAT implementation. Of these 7 samples, 3 were reactive for HCV and 4 for HBV.

Due to inadequate sample volume of these 7 samples, further confirmatory tests could not be done. All 7 samples were from replacement donors who did not come to the hospital for giving another sample despite repeated attempts to contact them. None of the samples was serology reactive but NAT non-reactive.

The study population consisted of 11,934 (65%) donors aged 18–30 years, 4,650 (25.4%) in the 31–45 years age group and 1,770 (9.65%) aged 46–60 years. All 7 NAT yield samples came from men. Two of them were aged 18–30 years and 5 were aged 31–45 years. There was no relation between donor age group and NAT yields using Fisher exact test.

DISCUSSION
Safe blood implies blood or a blood component transfusion with no TTI. As TTIs such as HIV-1, HCV and HBV can be easily transmitted through infected blood, considerable effort has been made to reduce their transmission. In the mid-1990s, the risk of transfusion-associated HCV infection was estimated to be more than 1:5,000. Until the late 1990s, blood screening for TTIs depended entirely on serological assays. Except for HBV, where the virus can be detected using HBsAg assays, tests for the detection of other TTIs relied almost exclusively on antibody detection. However, these tests are associated with a relatively long window period because they detect the response of the immune system to an infection.

In the first Indian multicentre ID-NAT study, 12,224 donor blood samples were tested. Of these, 217 samples (1.78%) were found to have markers of infections, including 8 (0.07%) which had markers of only the viral genome without serological signs of infections. These 8 NAT yield cases consisted of 1 HIV, 1 HIV-HCV coinfection and 6 HBV.

Our observed NAT yield rate of ID-NAT among blood donors (NAT reactive, serological non-reactive cases) for all three viruses was 1 in 2622 samples tested. Seven potential TTIs were detected, thereby preventing 21 potential cases of infection through different blood components.

The NAT yield rate from another blood bank in New Delhi was 1 in 4,251 samples which is 1.62 times lower than our observed yield rate. Another blood bank in Chennai reported a NAT yield rate of 1 in 2,726 which is similar to our observed yield rate. We compared our NAT results with those from other South- east Asian countries. Blood banks in Singapore had a NAT yield rate of 1 in 24,567 samples, which is 9.37 times lower than our estimate. The NAT yield in blood banks in Hong Kong was reported to be 1 in 400,000, a figure much lower than ours. Reports from Thailand found a NAT yield of 1 in 25,000, nearly one-tenth of our study. The NAT yield reported from South African blood banks was 1 in every 19,790 samples, almost 7.5 times lower than our observed yield rate.

The higher NAT yield in our blood bank is possibly because of the higher prevalence of these viral infections in our population; 2.5 million patients with HIV as per the National AIDS Control Organization (NACO), 43 million with HBV and 15 million with HCV. Nearly half our blood donors are replacement donors who...
are known to have higher rates of infection compared to voluntary donors.13
13 In most developed countries, most blood donors are repeat voluntary donors. In India, voluntary donors constitute only 55% of all blood donors. At our blood bank, voluntary donors were 15% of all donors in 1989 and this increased to 53.4% in 2010. The seroprevalence among voluntary blood donors at our blood bank is 0.13%, 0.8% and 0.41% for HIV, HBV and HCV, respectively, compared with 0.34%, 1.93% and 0.56%, respectively, for replacement donors. One of the reasons for the higher prevalence is that replacement donors do not reveal their medical conditions for fear of being rejected as donors. The majority of Indian voluntary donors are first-time donors but first-time voluntary donors may not be safer than replacement donors.13

This could explain the higher NAT yields in India compared to some other Asian countries in spite of an increase in voluntary blood donations.

ID-NAT provides test results faster, since it does not require balanced sample pooling and resolution of positive/contaminated samples.13 Some recent data from South-east Asia and China illustrate this advantage. With an occult HBV infection frequency of 1/40 000, ID-NAT detected HBV-DNA in blood samples consistently at levels of <100 IU/ml. If instead of ID-NAT, screening had been done in mini pools of 6 with an analytical sensitivity of 5 IU/ml, 70.7% of samples would not have been detected. Recent reports show that in case of HBV, apart from the initial window period, a second window period exists at 75–85 days post-infection, when HBsAg is no longer detectable and anti-HBs is not yet detectable. However, HBV NAT is positive during this period.14–16

The benefits of ID-NAT are especially important in patients who receive multiple blood transfusions for diseases such as thalassaemia and haemophilia. Such patients need regular, repeated and life-long blood transfusions and are at higher risk of being infected with serious TTIs. In a survey by the National Thalassaemia Welfare Society among 551 multiply transfused patients with thalassaemia, 33 were HIV-positive, 89 were HCV-positive and 43 were HBV-positive.17

In summary, our study shows that ID-NAT can play an important role in further reducing TTIs. Within the first 7 months of using ID-NAT, we detected TTIs in seven samples of donated blood which were missed by serological tests. This translated into 21 patients being saved from infection due to blood components. In India, with a high prevalence of TTIs, it is essential that transfused blood is safe and free of viruses. Universal and routine use of ID-NAT for HIV, HBV and HCV by all blood banks would be an important step in this direction.

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