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Screening of blood donors by ELISA & Nucleic acid amplification testing along with notification to TTI reactive cases – A comprehensive measure for blood safety

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ABSTRACT

Background: Safe blood transfusion is essential requirement of present health care system and the transfusion safety begins with healthy donor. With inclusion of NAT testing besides the serological tests, there has been reduction in the window period of screening of transfusion transmitted infections. The present study aims at the analysis of the efficacy of MP-NAT testing, seroprevalence of transfusion transmitted infections (TTI) ,its role in improving blood safety and to determine the response rate following notification of reactive status to the donors.

Materials and method: Study was performed on 58,191 donations from July 2016 to October 2018 where all negative cases for anti-HIV, anti-HCV and HBsAg by ELISA were subjected to MP-NAT test. All reactive donors were retested (wherever possible) and notified of their status by telephone or letter. All initial reactive screens were followed over six months.

Result: Out of 57,918 of blood tested, 629 (1.086%) were seroreactive. Out of 57,289 sero-negative donors subjected to MP-NAT testing, 53 (0.092 %) were NAT reactive (NAT yield -1in1078). Of all the 682 donors who were notified of their reactive status only 534 donors could be contacted reported back to transfusion facility.

Conclusion: NAT has improvised the blood safety by detecting the virus in the pre-seroconversion, window period thereby providing much higher sensitivity as compared to newest generation serological tests. There is an urgent need to implement latest technology like NAT besides the routine serology and formulate the nationally acceptable guidelines for notification of all reactive donors for availability of safe blood supply.

Keywords: Donor notification; Nucleic Acid Testing; Transfusion Transmitted Infections.

INTRODUCTION

Throughout the world, annually, millions of people receive blood transfusions whose safety can be ensured through collection of blood from voluntary, non-remunerated blood donor and screening of all donations for viral markers by highly sensitive tests. Family members/relatives play a major role as replacement donors and voluntary donation is contributing to around 84% of the blood donation.[1] even after the combination of serological testing and policy decisions to make safe blood supplies, there is

still some risk of transfusion-transmitted infection (TTI) with blood transfusion such as human immunodeficiency virus (HIV), hepatitis B and hepatitis C. The traditional method for screening blood donations, known as immunoassay (or serology) testing is the mainstay for screening blood donors which detect antibodies to viruses or viral antigens, but is unable to detect in the window period (WP) which is the interval between the donor's exposure to a virus and production of antibodies against the virus . It is now well established that Nucleic Acid Testing (NAT) reduces the window period of Transfusion Transmitted Infection and helps improve blood safety by detecting the viruses missed by serological tests.[2] NAT can be done individually or in pools and is used in conjunction with serological tests.

Currently in India, the donors are informed only on the basis of their screening tests available in blood bank. As most of the donors do not expect to hear that they have reactive results they may become extremely distressed to hear this news.[3,4] On the other hand, a small minority of individuals appear to ignore notification and continue to donate blood elsewhere. Some of the donors even use blood donations as a means for free testing because of their high risk behavior (test seekers).[5]

The primary objective of this study is to analyze the efficacy of MP-NAT testing as additional donor screening programme, seroprevalence of transfusion transmitted infections (TTI) ,its role in improving blood safety and to determine the response rate following notification of reactive status to the donors.

MATERIALS & METHODS:

The study was conducted in the Department of Transfusion Medicine, Sriram Chandra Bhanja (SCB) Medical College & Hospital Cuttack, in the State of Odisha in India from July 2016 to October 2018. At the time of blood collection, donor samples were collected for all mandatory screening serological tests and MP-NAT Testing. The donors were requested to fill up the donor questionnaires, to determine whether they are eligible to donate as per the guidelines of World Health Organization (WHO), along with giving consent for the donation ,screening of the donated blood and disclosure to them in case of any unfavorable findings prior to the blood collection. Blood samples of three millilitres collected in a clean and dry test tube for the TTIs screening were centrifuged for serum and then tested for HBsAg (by ERBA LISA PICO HBsAg) and anti-HCV antibody (by ERBA LISA HCV), anti-HIV 1+2 (by ERBA LISA HIV 1+2 By TRANSASIA BioMedicare Pvt Ltd). Rapid kit tests were performed for Syphilis (by Carbogen TULIP Diagnostic Pvt Ltd) and Malaria antigen to Plasmodium Falciparum (HRP-2, LDH My Test by Bio-footprints Health Care Pvt Ltd). All the data was stored for future reference.

All seronegative cases were subjected to MP-NAT in small pools of six on Roche's Cobas Taq Screen MPX assay v2.0 on Cobas System s 201(Roche Diagnostics Gmbh, Mannheim) to detect HIV-1 (groups M and O RNA), HIV-2 RNA, HCV RNA and HBV DNA.

The Cobas Taq Screen MPX assay comprises of four automated steps which include (i)pooling of samples,(ii) sample preparation,(iii) real time Polymerase Chain Reaction(PCR) amplification ,detection, (iv)data management and reporting.This is also involves quality control by processing one replicate of the Negative Control (MPX (–) C, v2.0) and one replicate of each of the three Positive Controls (MPX M(+)C, v2.0, MPX O(+)C, v2.0 and MPX 2(+)C, v2.0) in each batch. Reactive (created) pools were retested individually to confirm and to know the infection in donor sample. Limits of detection (with 95% probability) for various analytes on Taqscreen MPX v 2.0 are : HIV -1 Group M- 46.2 IU/mL, HIV -1 Group O - 18.3 Copies /ml, HIV-2- 56.2 copies /ml, HCV-6.8 IU /mL, HBV- 2.3 IU /mL. HIV-1 Group M, HCV and HBV are calibrated against WHO International Standards while HIV-1 Group O and HIV-2 are calibrated against FDA Reference reagents.

If the results of either serology and/or NAT were found to be positive, blood unit was discarded as per hospital SOPs and donor was notified of his/her status either by telephone or by letter. The case was closed only if the donor did not respond to any of the three telephone calls/letters and the case was labeled as nonresponder. Donors who responded to the call/letters and came back to transfusion facility were counseled and retested by ELISA with fresh blood sample. Donors whose results from fresh sample were concordant with earlier tests were referred to

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concerned clinical specialty and donors who tested nonreactive were asked to remain in follow-up.

RESULTS:

A total of 58,191 donations were received in the period between July 2016 to October 2018 consisting of 55,336 donations by males and 2855 by female donors. 31,519 units were collected as voluntary and 26,672 as replacement donations and there was wastage of 273 blood units due to low collection from the outdoor voluntary camp sites. Out of these, total 57,918 units of blood were tested consisting of 31,453 number of units donated voluntarily and 26,465 units donated through replacement respectively. Total number of male donations tested was 55,106 and female donation tested was 2812 respectively. Reactive samples through serological tests came out to be 629(1.086%) consisting of 299 (0.95%) of voluntary sample and 330(1.24%) of replacement samples. (Fig 1) Total 11(0.39%) of females and 618(1.12%)of male donors were seroreactive. The 629 number of seroreactive units consisted of 444(0.76%) of HBV followed by 100(0.17%) of HCV cases, 59 cases(0.1%) of HIV,25 cases (0.042%) of syphilis and 1 case(0.0017%) of malaria.(Fig 2) A total of 57,918 number of seronegative units consisting of 31,154 number of voluntary and 26,135 number of replacement units subjected to the NAT testing showed positive results in 53 (0.092%) cases. Out of 53 NAT positive units 25 cases (0.094%) were from replacement donors and 28 cases (0.089%) from voluntary donors respectively and consisted of 49 number of males and 4 number of females. NAT yield was 1 in 1080 cases and showed 46 cases (0.080%) of HBV, 4 cases (0.0069%) of HIV and 3 cases(0.005%) of HCV.(Fig 3)

Of all the 682 donors who were notified of their reactive status only 534 donors could be contacted & reported back to transfusion facility. These reported cases consisted of 47 number of HIV, 375 cases of HBV, 87 cases of HCV, 25 cases of syphilis and one case of malaria.(Table 1) Donors residing in the urban nearby areas responded better than those who lived in rural or far-off areas. Donor notification using telephone was more beneficial as more donors turned up to transfusion facility.

DISCUSSION:

Blood safety is a challenge in India because of the high prevalence of HIV, HCV, and HBV, the relatively low percentage of voluntary donors and the lack of standardization of screening procedures of the transfusion transmitted infections. Prevalence of TTI in India is 1.8–4%, 0.4–1.09%, 0.2–1%, and 0.05–0.9% for HBV, HCV, HIV, and syphilis, respectively.[6-11] Prevalence of TTI in the present study was 0.76% of HBV followed by 0.17% of HCV cases, 0.1% of HIV,0.042% of syphilis and 0.0017% of malaria in agreement with other seroprevalence studies of TTI carried out in various parts of India. NAT yield in our study was 1 in 1080 donations which is comparable to a previous study performed by Sadhana et al who find NAT yield to be one in 974 cases.[12] The prevalence of HIV, HBV, and HCV in India is respectively about 1%, 4%, and 1.5% [13]compared to 0.0097%, 0.3%, and 0.07% in the US blood donors, respectively.[14] In spite of all the precautionary measures taken by blood banks to avoid transmission of infectious agents through transfusion to the recipients, it is possible to transmit disease when blood from a recently infected donor fails to be identified by routine screening tests.[15]This is because of the window period after a donor is infected, but before the condition is detectable by routine screening methods. This technological limitation puts blood recipients at a risk for transmissible diseases. Since viremia precedes seroconversion by several days to weeks, tests that detect viral nucleic acids are considered a significant technological advancement and an additional step in our quest to improve the blood transfusion safety.

Nucleic acid amplification test (NAT) technology has the potential to detect viremia earlier than current serological screening methods. NAT is a molecular technique to detect viral nucleic acids of HIV 1-2, HBV, and HCV at a very low concentration in donor blood by Nucleic acid amplification technology.[16]The primary benefit of NAT is the ability to detect the risk of infections in donations in a lesser window period. The estimated reduction of the window period utilizing NAT for HCV is from 70 to 12 days, HIV from 22 to 11 days, and HBV from 59 to 25-30 days.[17] After NAT was adopted, the residual risk for HCV transmission prior to NAT in France and Spain reduced from 0.64/million and 3.94/million to 0.1/million and 2.33/million, respectively. HIV NAT yield rates were estimated at 0.3/million donations in

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France and Spain as opposed to 0.59 and 2.48, respectively preceding NAT.[18] The NAT yield rate from other blood Centers in India is 1 in 753,[19] 1 in 610,[20] 1 in 1528,[21] 1 in 2622,[22] 1 in 2000,[23] and 1 in 2972.[24] The higher NAT yield compared to other studies [21,22,23,24] is probably on account of higher prevalence in the population reaching to this tertiary care medical college & hospital.

Analytical sensitivity is the key factor for the performance of the NAT assay. The analytical sensitivity is generally determined by testing dilutions of standardized materials such as WHO International Standards and subsequent calculations of 95% limit of detection by probit analysis. The analytical sensitivity for HBV NAT is long doubling rate of 2.6 days during which the viral count is generally low. In comparison to HBV, the doubling rate of HCV and HIV-1 are 14.9 h and 20.5 h, respectively which are shorter. The WHO international standards analytical sensitivity for HIV-1, HCV, and HBV is 44, 10.7, and 3.7 IU/ml.

Amongst these 17 cases of NAT yield (1 in 974), HBV NAT yield was 1 in 1379 donations which was almost similar to the yield shown by other studies i.e. 1 in 1012[17] and 1 in 1221.[16] Our HBV NAT yield was higher than those found by Chigurupati et al (1 in 2000)[20] and Chandrashekhar (1 in 26630)[21] which might be due to higher prevalence of HBV in our population.

HBV NAT yield in the present study is 1 in 1245 and 1 in 1379 in Indian scenario, which is much higher than studies done in Europe and USA who reported the prevalence to be 1:600,000 to 1:350,000.[25] Similarly, higher prevalence of HIV-1 and HCV in India and also in this study (1 in 14,322 and 1 in 19,096 respectively) as compared to western Europe and USA (1:300,000 to 1:3,000,000)[25,26] are leading to increased HCV and HIV-1 yield cases. The high prevalence of HBV and HCV in India is considered to be in the intermediate level of HBV endemicity with over 40 million HBV carriers [26] and lesser number of voluntary donations. Developed countries are spending resources on NAT screening to detect only one window period donation in 300,000 to 3,000,000 donations while in developing countries with high NAT yield of 1 in 1080 (as in this study), NAT screening is 300 to 3000 times more beneficial. There is clear advantage of saving three lives at a time and cost effectiveness of NAT screening in developing over developed countries.

Transfusion safety begins with donation of blood from healthy donors. The important part of preventing TTI is to notify and counsel reactive donors. Donor notification and counseling protect the health of the donor, prevent secondary transmission of infectious diseases to sexual partners, reduces risk of vertical transmission and provide feedback about the effectiveness of donor selection procedures such as predonation education and medical history [27]. We attempted to contact all 682 reactive donors about their TTI status either telephonically or by letter. About 534(78%) reactive donors which constituted 75% of HIV, 76% of HBV , 84% of HCV , 96% of syphilis and 100% of malaria cases responded to the notification and reported back to transfusion facility. The response was better because of the nearby urban population and continuous pursuance of our counselor. The donors who did not turn up to transfusion facility (nonresponders) may continue to donate blood at other centers especially those centers which do not use biometric donor identification, hence posing serious threat to safety of blood supply.

Till date ELISA is the recommended and preferred screening technique. Many blood centers still do not have this facility and rely on “rapid kits” which may have high false-negative rate. Donors who are ELISA/NAT reactive elsewhere may escape TTI screening. These donors usually show an angry behavior in blood bank and question the accuracy of screening performed in blood bank. Despite best effort, the meaning of sensitivity and specificity of testing methods can’t be explained to the donors. We need to follow up such cases over 6 months as 95% of infected persons will seroconvert in this timeframe.

Our study has two limitations. First, we did not perform confirmatory testing of TTIs prior to notification. Second, repeat NAT was not done on the returning reactive donors. Studies on the feasibility of NAT implementation in developing countries like India will help extend the message to blood centers that NAT can be an effective method for safeguarding the blood supply. Transfusion safety rests heavily on the health of blood donors. Donors should undergo optimal predonation counseling so as to educate them about the risk of infections and the

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window period. It is the collective duty of transfusion community to inform these donors and do as much as possible to allay their anxiety about reactive result and to advise them about available treatment options. There is an urgent need to implement latest technology like NAT and formulate the nationally acceptable guidelines for notification of all reactive donors for availability of safe blood supply.

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Figure 1:

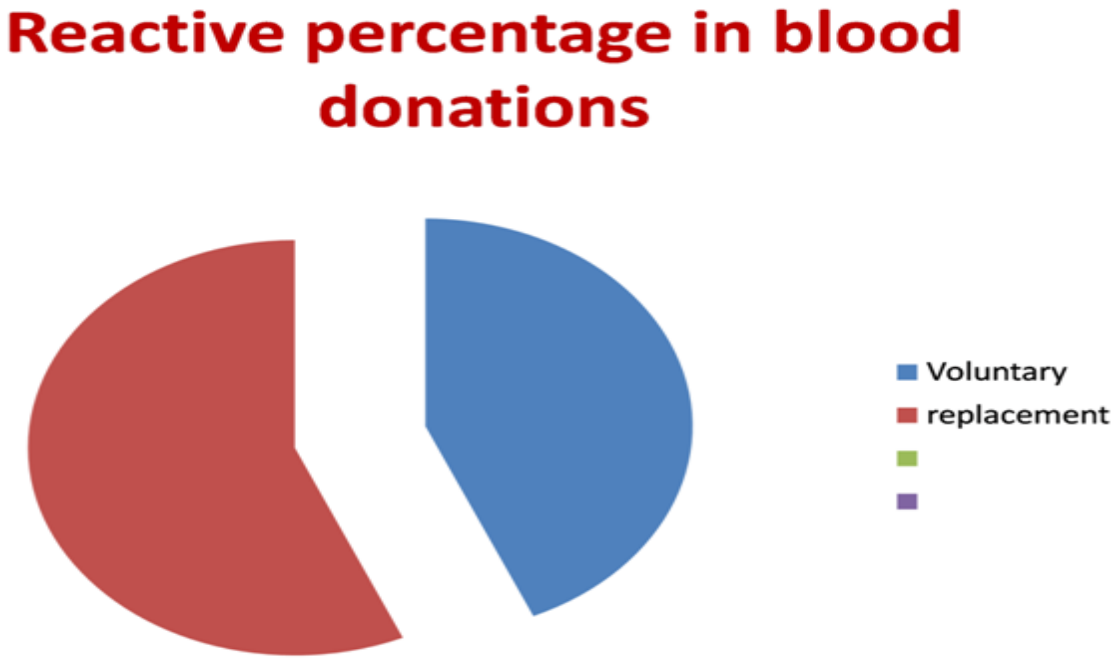

Figure 2:

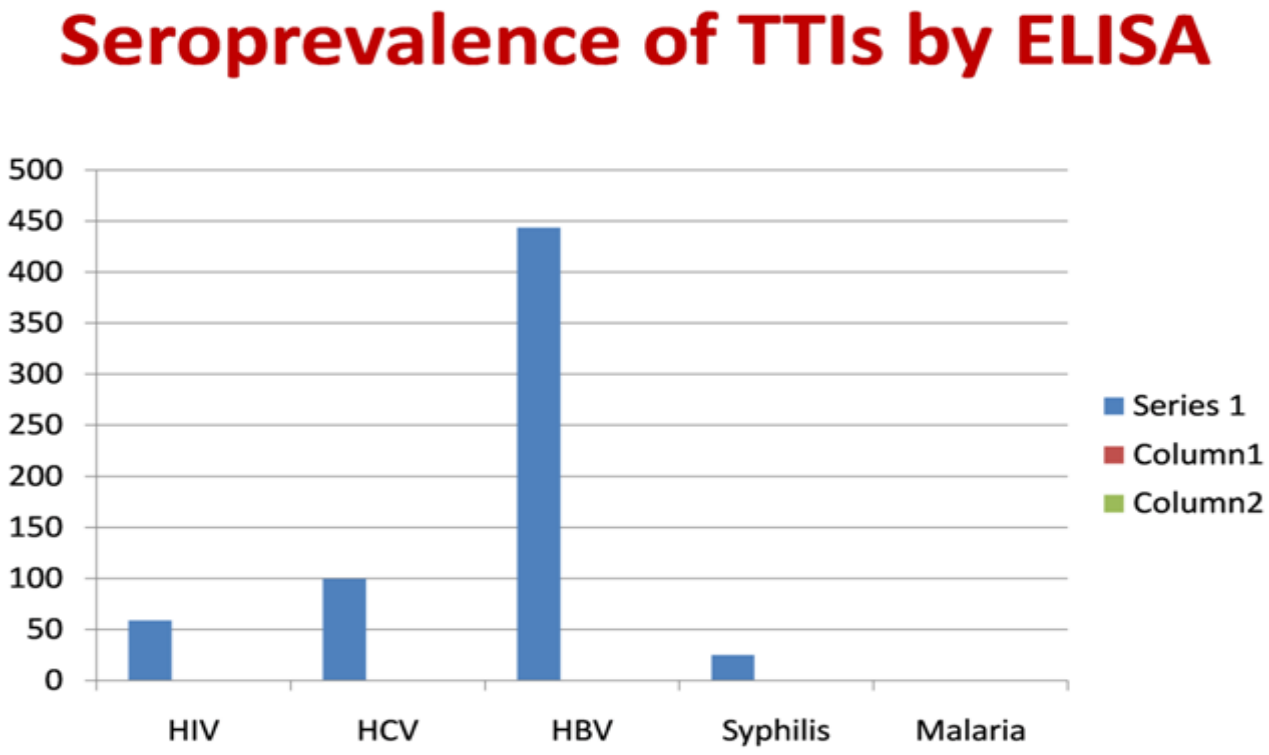

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Figure 3:

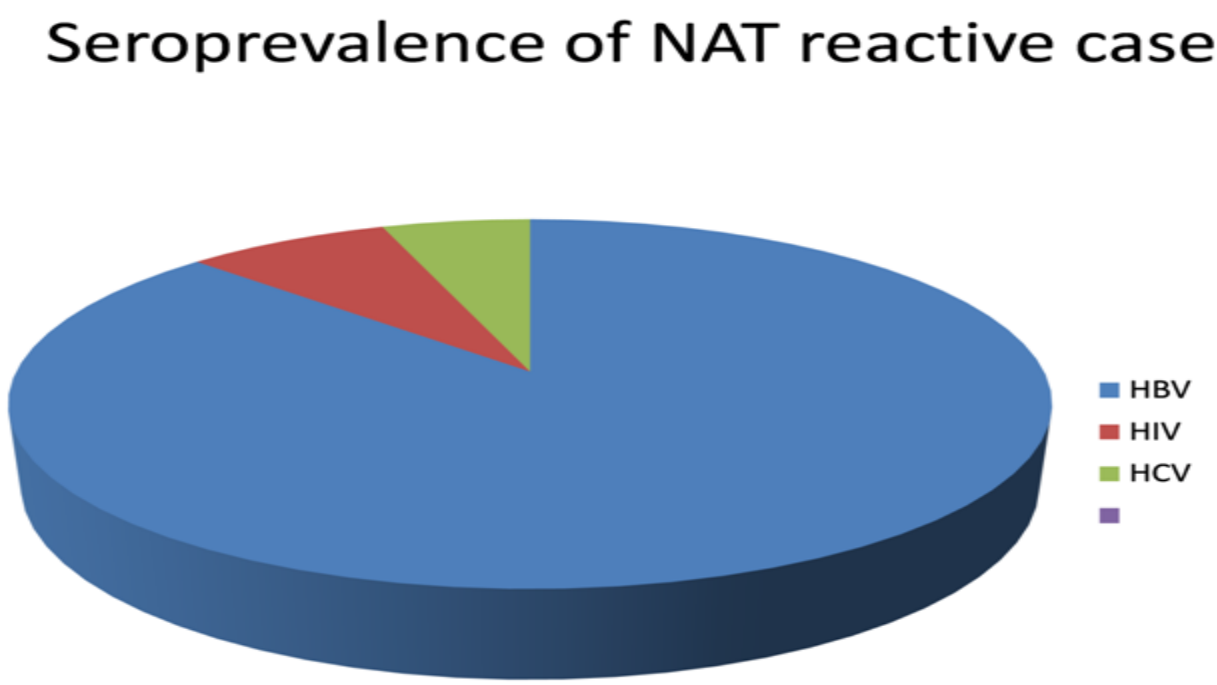

Table 1

Post donation notification

Cases	No of cases	Contacted	Not reachable	Not responded	Called back	% of contacted	% of noncontacted
HIV	63	47	10	6	Neg conf 2	75%	25%
HBV	490	375	112	3	One conf pos	76%	24%
HCV	103	87	6	10		84%	16%
Syphilis	25	24		1		96%	4%
Malaria	1	1				100%	

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