

Seven Years Experience in NAT Testing of Blood Donors in a Tertiary Care Centre

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ABSTRACT

Introduction: Nucleic acid amplification testing (NAT) is a very sensitive and specific test for viral nucleic acids. It reduces the window period for detection of viruses. It is highly beneficial in countries like India which has a high incidence and prevalence of transfusion transmitted infections. NAT is expected to identify many NAT yield cases which are not detected by other serological tests.

Material and methods: A retrospective analysis of transfusion transmitted infection (TTI) reactive units in a tertiary care hospital was done over a period of seven years from Jan 2011 to Dec 2018. The blood units were tested by enhanced chemiluminescence technology and Individual donor nucleic acid testing (ID- NAT). NAT yield was calculated for Hepatitis B, C and HIV.

Results: Out of 23,378 collected blood units, 380 units (1.62%) were found to be reactive for one or more transfusion transmitted viruses by chemiluminescence and/or NAT. 371 units (1.58%) were found reactive by chemiluminescence and 190 units (0.81%) by NAT. All the NAT yield cases were for hepatitis B virus and it was 9 (1:2597).

Conclusion: NAT is more sensitive than chemiluminescence in detection of Hepatitis B. It detects both window period and occult infection. It has made a significant contribution towards ensuring safe blood transfusion by helping in reduction of window period transmission of Hepatitis B. It is important to implement NAT in developing countries like India to enhance transfusion safety.

Keywords: Chemiluminescence, NAT, Window Period, NAT Yield, Hepatitis B

INTRODUCTION

Ensuring blood safety in a developing country like India where the seroprevalence of transfusion transmitted infections (TTI) is high is a challenging task. TTI's pose a potential threat to safe blood transfusion practices.¹ The threat of TTI's was first observed in 1940's where HIV, Hepatitis B, Hepatitis C, malaria and syphilis were recognized as major diseases transmitted through blood.²

Donor prevalence of viruses in India stands at 0.24% for HIV, 1.18% for hepatitis B and 0.43% for Hepatitis C.³ The prevalence of TTI's among blood donors has been used as a surrogate marker for the population at large. In India as per the regulatory requirements of the drug and cosmetics act of 1940 (1st amendment 1992) it is mandatory to test each unit of blood for markers of HIV 1 and 2, Hepatitis B and C, malaria and syphilis.⁴ Various screening tests available for screening blood donors are rapid tests, Enzyme Linked Immunosorbent Assay (ELISA), Chemiluminescence (CLIA)

and Nucleic acid amplification testing (NAT).

NAT is a very sensitive and specific screening test for viral nucleic acids and is based on amplification of targeted regions of RNA and DNA. Currently approximately 33 countries have implemented NAT for HIV and 27 have implemented it for Hepatitis B.⁵ It was started in developing countries by the end of 1990's. There are two types of NAT, individual donor (ID) NAT and minipool NAT. Both are recognized by FDA as valid instruments for NAT testing.³ Various studies have reported ID NAT to be more sensitive as compared to minipool NAT.⁶ NAT takes care of the dynamics of window period of viruses. The estimated reduction in window period utilizing NAT for HIV is from 22 to 11 days, for hepatitis B is from 59 to 25-30 days and for hepatitis C is from 70 to 12 days.⁷ NAT is not yet a mandatory test for screening blood units in India.⁸ Only around 2% of blood banks in our country are doing NAT and approximately 7% of all collected blood units are NAT tested.³ The current mandatory screening strategy in India does not address the problem of critical window period detection.⁹ Even with the most sensitive, newest generation of serological tests a considerable residual risk of infection remains. Developing countries like India have a high prevalence and incidence of TTI and a high incidence of window period donations. NAT testing is highly beneficial in such a scenario and is expected to identify more NAT yield cases than developed countries. NAT also adds the benefit of resolving false positive reactions based on serological tests which is very important for donor notification and counselling.⁸

However NAT is highly technically demanding, involves high costs in infrastructure, equipment, consumables and requires technical expertise. Moreover it is not alone feasible in situations where the viral load is low and undetectable and antibodies can still be detected by ELISA or chemiluminescence. The feasibility of introducing NAT in India has been and is still a matter of debate. The aim of the study was to analyse the sensitivity of NAT in detection of transfusion transmitted infections in blood donors.

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MATERIAL AND METHODS

A retrospective analysis of TTI reactive units tested by chemiluminescence and NAT in a tertiary care hospital was done over a period of seven years from Jan 2011 to Dec 2018. The data was collected from the blood bank records. All the units were tested for HIV, HBV, HCV by enhanced chemiluminescence technology in Vitros ECI Immunodiagnostic System by Ortho Clinical Diagnostics. The test for syphilis was done by Rapid Plasma Reagin method using Toluidine Red Unheated Serum (TRUST) test kit by Tulip Diagnostics. Malaria was tested by rapid card method using SD Malaria Ag P.f/Pan by Standard Diagnostics INC. NAT was used as supplementary test along with routine serology. The units were tested by ID-NAT (Individual donor nucleic acid test) at the central NAT laboratory of our hospital group using Procleix Ultrio Elite assay kits in Procleix Panther System. 6 ml blood was collected in EDTA vacutainer tube from the sample pouch at the time of blood collection. The samples were transported in thermocol boxes to the central lab maintaining temperature below 25 degrees within 24 hours. The results were available the next day. The blood units were released from quarantine when results were available for both chemiluminescence and NAT. The study involves an analysis of retrospective data and does not involve any interventional procedures on animals or human participants.

RESULTS

A total of 23,378 blood units were collected over a period of seven years from Jan 2011 to Dec 2018. Out of 23378 units, 380 units (1.62%) were found to be reactive for one or more transfusion transmitted viruses by chemiluminescence and/or NAT. A total of 371 units (1.58%) were found reactive by chemiluminescence [Table 1, Fig 1]. Out of this, 158 units (42.5%) were for HBsAg [Fig 2], 162 units (43.6%) were for HCV [Fig 3], 51 units (13.7%) were for HIV [Fig 4]. A total of 190 units (0.81%) were found reactive by NAT [Table 1,

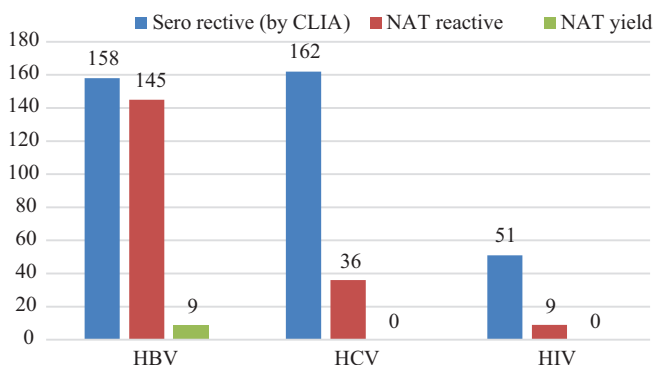


Figure-1: Diagrammatic representation of Hep B, Hep C and HIV reactive units by CLIA and NAT.

Reactive units	Hepatitis B	HIV	Hepatitis C	Total
Reactive by CLIA	158 (42.5%)	51 (13.7%)	162 (43.6%)	371
Reactive by NAT	145 (76.3%)	9 (4.7%)	36 (18.9%)	190
NAT yield	9 (6.2%)	0	0	9

Table-1: TTI reactive data from 2011 to 2018.

Fig 1]. Out of this, 145 units (76.3%) were for HbsAg [Fig 2], 36 units (18.9%) for HCV [Fig 3], 9 units (4.7%) were for HIV [Fig 4]. Out of 145 reactive units for HbsAg, 136 units (93.7%) were reactive by both the methods while 9 units (6.2%) were reactive only by NAT. Based on this, the NAT yield detected was 9 (1:2597) for HbsAg. No NAT yield cases were detected for HCV and HIV.

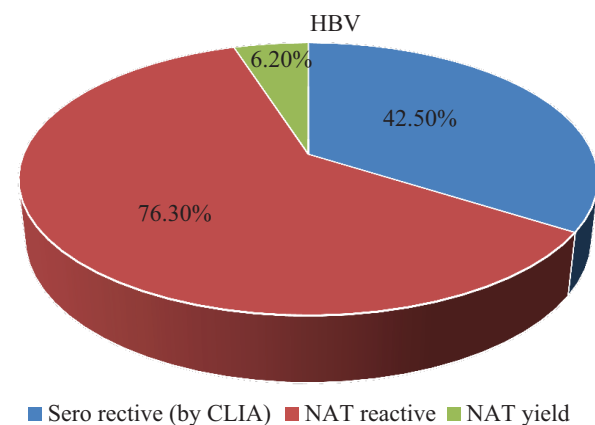


Figure-2: Percentage of Hepatitis B reactive units.

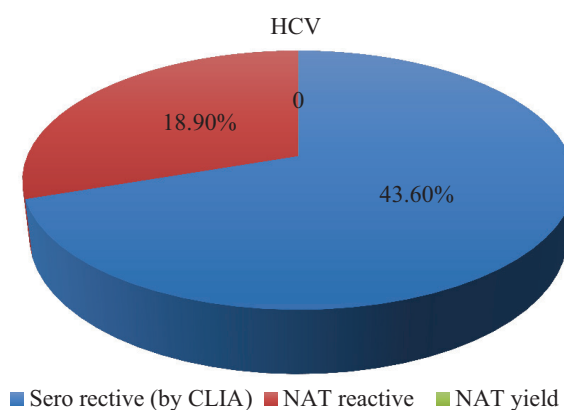


Figure-3: Percentage of HCV reactive units.

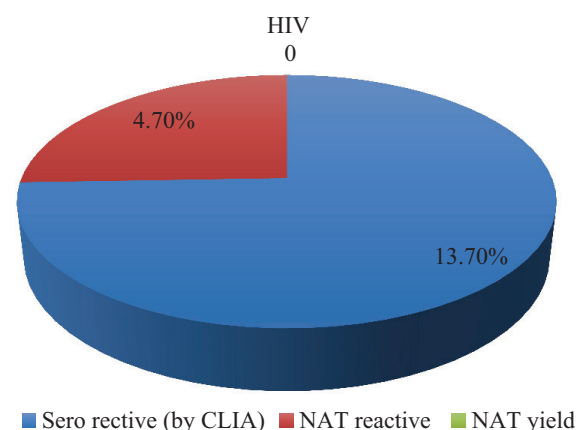


Figure-4: Percentage of HIV reactive units.

NAT yield is defined as units which are reactive by NAT and Non- reactive by serology. 51.2% units found reactive by chemiluminisense were found nonreactive by NAT. This could be false positive reactions due to high sensitivity of chemiluminisense technology and would require follow up and further testing for confirmation.

DISCUSSION

A total of 30 million blood components are transfused each year in India.¹⁰ Out of 9.3 million units of blood collected every year, replacement donors even now contribute 50% of the total blood collection.¹¹ Basic quality assured blood transfusion services includes voluntary, non remunerated donors, donor notification and quality assured sensitive serological methods.¹¹ Blood safety involves effective donor education, motivation and recruitment strategy. Need for safe blood is particularly important for chronically transfused patients of thalassemia, haemophilia, sickle cell disease.¹² NAT testing for TTI's is an important recent advancement to ensure blood safety by reducing the window period for detection of viruses. According to a large study conducted by Makroo et al NAT could interdict 3272 infectious donations a year among our approximately 5 million annual donations.¹³ NAT yield for various viruses varied from 1:476 to 1:440 in different studies. 70- 80% of NAT yields are related to hepatitis B. HIV and HCV accounts for 10%- 20%. Across the globe, Hepatitis B is the most common cause of NAT yield.³

The NAT yield in our study was 9 (1:2597). All the NAT yields were due to Hepatitis B. The yield in our study was comparable to yields obtained in previous Indian studies like 1:2622 in AIIMS in¹⁴,1:2972 in Jaipur¹⁵ and 1:2000 in Andhra Pradesh.¹⁰ Our yield was less compared to an yield of 1:686 in Apollo, New Delhi¹⁶, 1: 476 in another study in AIIMS¹⁷ and 1:1125 in RML hospital in Delhi¹⁸ and much higher compared to an yield of 1:4403 in Medanta,¹⁹ 1:5000 in Rotary TTK²⁰ 1:17753 in Manipal Hospital.²¹

Reasons for variability in yield is due to several factors like wide variation in the pattern of infections among donors, type of test employed, type of kit, sensitivity of the test and accuracy of methods.²⁰ The performance of NAT assay is essentially dependent on analytical sensitivity. Our yield may be lower than some studies due to stringent donor screening criteria followed by us while the yield we obtained is higher than some previous studies may be due to greater sensitivity of the test method employed.

Yield obtained in developed countries is much lower compared to India. A study conducted in USA found a NAT yield of 1 : 2 million for HIV and 1: 270,00 for HCV for 66 million donations.²² A European study found a yield of 1: 600,000 for HCV and 1: 1.8 million for HIV after screening 3.6 million donations.²³

This is due to the higher prevalence and incidence of infections in developing nations. Developing countries like S. Africa, Thailand, Kuwait, Malaysia have Hepatitis B NAT yield of 1:52303, 1:4868, 1:24275 and 1:3616 respectively. NAT yield in high prevalence developing countries are 1:232

in Ghana,1:2609 in Egypt, 1 :501 in Lebanon, 1 :125 in Iran, 1:193 in Pakistan, 1:865 in Mexico,1:81 in Mongolia, 1:1430 in China.²⁰

In developing countries and across the globe Hepatitis B is the most common cause of NAT yield.³ Not all NAT positive samples transmit infection. Studies have shown that upto 19% to 83% of NAT positive but seronegative donors may transmit infection with higher risk associated with window period donation rather than occult hepatitis B infection.²⁴ Strict donor selection criteria and immunization of adults for hepatitis B is very important to ensure blood safety in India. NAT is a very sensitive test for early detection and prevention of transfusion transmitted infections. But it has its limitations and is not the only answer for safe blood. NAT may be false negative in situations where viral load is low. Initial NAT yields may not be true NAT yields. Supplementary tests and donor follow up is important.¹⁹ Moreover it is technically demanding and requires advanced resources and trained manpower. To ensure blood safety, in addition to NAT testing it is very important for a quality assured blood transfusion system to be in place. This includes proper donor screening and counselling, donor notification, scope of self deferral, encouragement of voluntary blood donation and quality assured sensitive serological methods for TTI testing.

CONCLUSION

NAT yield in our study for hepatitis B is 1:2597 which is comparable to yields in previous Indian studies. Introduction of NAT has helped in reducing window period donation thus making a significant contribution towards ensuring safe blood transfusion and restoring confidence in blood safety. It is important to implement NAT in developing countries like India as an additional layer of blood safety. Despite certain limitations and cost constraints the importance of NAT testing in blood banking cannot be overlooked.

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