Section: Transfusion Medicine

Original Research Article

EVALUATION OF NUCLEIC ACID AMPLIFICATION TESTING(NAT) YIELD AMONG THE DONATED BLOOD- A THREE YEARS RETROSPECTIVE STUDY

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Abstract

Background: The Drugs and Cosmetics Act, 1940 of India, currently mandates tests for screening of blood donation in India which include: anti HIV 1 and 2 antibodies, anti HCV antibodies, hepatitis B surface antigen, serological tests for syphilis and malarial parasite. However, the screened seronegative donations, tested by conventional methods like enzyme linked immunosorbent assay(ELISA), chemiluminescence immunoassay(CLIA) and rapid tests, are still at risk since the donation could have taken place in the window period. Nucleic acid amplification testing(NAT) has a prominent place in transfusion medicine as a complementary screening test to serologic testing as it detects early infection before seroconversion as well as occult infections thereby improving blood safety. However, NAT is not made mandatory in India yet. **Aims and object:** This study was taken up to estimate the NAT yield, which is the number of seronegative samples found to be positive in NAT testing. **Materials and Methods:** The study is a retrospective crossectional one conducted for a period of 3 years. All the blood samples are tested for HIV, HBV, HCV, syphilis and malaria using ELISA, ECLIA or rapid tests. Any sample found to be positive in any of the above tests is marked as seropositive and discarded. The rest are known as seronegative and these seronegative samples are put for NAT. NAT yield and NAT yield rate (total number of NAT positive divided by total number of seronegative samples) are calculated. **Results:** Thirty-one samples were found to be NAT positive (NAT yield). Among the 31 NAT yield, 20 samples were positive for HBV, 11 were positive for HCV. No sample was found to be positive for HIV by NAT. Overall NAT yield rate in our study is 1 in 1120(0.089%). NAT yield rates in HBV and HCV are 1 in 1737 and 1 in 3158(0.031%) respectively. **Conclusion:** Since there is 100% component separation in our centre, we can conclude that by NAT we additionally stopped the transmission of infections to 93(31x3) patients. This is a huge achievement in improving blood safety. Thus, we conclude that NAT adds an additional layer of safety in blood transfusion recipient.

INTRODUCTION

Safe and quality supply of blood and blood products have become a priority issue in health care system. Transfusion transmissible infections (TTIs) can be transmitted through several factors like inability of the test to detect the disease in the pre-seroconversion or "window" phase of their infection, immunologically variant viruses, non-sero converting chronic or immunosilent carriers and laboratory testing errors.[1] Conventional methods like enzyme linked immunosorbent assays(ELISA), electrochemiluminescence (ECLIA), and rapid tests are generally used for screening for the presence of antigens/antibodies, however many cases could be missed if such tests are done during the window period subjecting the recipient susceptible for infection.[2]

To overcome these shortcomings and to provide an additional layer of protection, techniques with better sensitivity and specificity like nucleic acid amplification testing (NAT) has emerged. In this technology, a specific RNA/DNA segment of the virus is targeted and amplified in vitro. The amplification step enables the detection of low levels of virus in the original sample by increasing the amount of specific target present to a level that is easily detectable. NAT is able to detect viruses during the "window period" and thus allowing for earlier detection of the presence of TTIs and avoiding the possibility of transmission via transfusion. NAT testing has not only increased blood safety, but has also provided insights into the epidemiology, natural history, and pathogenesis of viral infection.[3] It was introduced in the developed countries in the late 1990s and currently many countries in the world have implemented NAT for detection of TTIs.[4]

In India as per the regulatory requirement of the Drug and cosmetics Act, 1940 and Rules, 1945, NAT is not a mandatory screening test for screening of blood for TTIs. Earlier studies with high yield of NAT suggest higher prevalence of TTIs in India and thus the need for NAT in blood banks for screening the donations.

The present study is taken up to determine the efficacy of NAT techniques by estimating the NAT yield, which is the number of seronegative samples found to be positive in NAT testing. This type of study has never been taken up in this part of the country. The observation from the study will help to understand the prevalence of TTIs and other occult infections and their timely detection during the window period.

MATERIALS AND METHODS

The study is a retrospective crossectional one conducted in a Tertiary care teaching hospital, Imphal, Manipur, India for a period of three years, from January 2021 to December 2023. After getting approval from the Hospital ethical committee, both replacement and voluntary blood donors who came to donate blood during the study period were enrolled for the study. Donors who pass the pre-donation criteria as per the regulatory guidelines laid down in the Drugs and Cosmetics Act 1940 and Rules 1945 were included for the study. The excluded donors were those who do not fulfill the pre donation criteria as per the regulatory guidelines laid down in the Drugs and Cosmetics Act 1940 and Rules 1945.

Sample size is calculated as below by using the formula:N=4PQ/L2 Where , N=sample size, P=prevalence of seroreactive and NAT reactive, Q=100-P, L=relative error(20% of P) and as per the study conducted by Mahapatra et al5 the overall prevalence of seroreactive and NAT reactive is 1.1%. Therefore, N=4x1.1(100-1.1)/0.0484=8990=9000(approximately) donors need to be recruited during the study period. Sampling will be nonprobability convenience sampling.

Procedure and data collection. At the end of phlebotomy, 2ml of blood from donor was collected in plain vial for serological test (ELISA or ECLIA) and 6 ml of blood was collected in EDTA vacutainer for NAT testing. All samples found negative in serological tests (ELISA or ECLIA) were subjected to NAT testing.

A total of 35497 blood donor samples were run for serological tests by using ELISA or ECLIA. Tests for anti HIV antibodies and p24 antigens were done using fourth generation ELISA (Erba Lisa from Transasia bio-medicals Ltd/ Merilisa from Meril Diagnostics private Ltd) or ECLIA (Roche cobas e411). Tests for HbsAg were done using third generation ELISA (Erba Lisa from Transasia bio-medicals Ltd /Merilisa from Meril Diagnostics private Ltd) or ECLIA (Roche Cobas e411). Tests for antibodies against HCV were done using third generation ELISA (Erba Lisa from Transasia biomedicals Ltd/merilisa from Meril Diagnostics private Ltd) or ECLIA (Roche Cobas e411). Rapid tests were done for syphilis and malaria. All samples found to be serologically negative were put up for NAT testing for HIV, HCV, HBV by using Cobas s 201 system's MPX v2.0 test. The Cobas s 201 system is an automated system. Sample pooling is performed on the Hamilton MICROLAB STAR IVD/STARlet IVD pipettor. Extraction is performed on the COBAS Ampliprep instrument. Amplification and detection are performed on the COBAS TaqMan analyzer. Results analysis and reporting are performed using PDM software. Minipools (6 samples in one pool) are subjected to nucleic acid amplification testing. If any pool is found to be positive then the six corresponding bags are held in quarantine and resolution is done by running the six samples individually.

NAT yield All samples that were negative for anti HIV antibody, p24 antigen, anti HCV antibody and

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HBsAg by ELISA or ECLIA but positive in the MPX NAT were earmarked as NAT yields.
Independent study variables which include gender and donor types(replacement/voluntary) were recorded. The study involves an analysis of data and does not involve any interventional procedures on animals or human participants.

RESULTS

During the study period of 3 years 35497 blood units were collected of which 7401 were from voluntary donors and 28096 were from replacement blood donors (shown in Table 4). All the 35497 blood samples were subjected to serological testing for HIV, HCV, HBV, syphilis and malaria. A total of 754 blood samples were found to be serologically positive, out of which 28 were from female donors and 726 were from male donors. 34743 samples were found to be seronegative and were put to NAT testing. Among the seronegative samples 2828 were from female donors and 31915 were from male donors. 7179 seronegative samples were from voluntary donors and 27564 seronegative samples were from replacement donors.
31 samples were found to be NAT positive (NAT yield), as shown in Table 1. Among the 31 NAT yield, 20 samples were positive for HBV, 11 were positive for HCV. No sample was found to be positive for HIV by NAT. Overall NAT yield rate in our study is 1 in 1120(0.089%). NAT yield rates in HBV and HCV are 1 in 1737 and 1 in 3158(0.031%) respectively.
Among the NAT yield 5 samples were from voluntary donors and 26 were from replacement donors (Table 3). NAT yield rate in voluntary and replacement donors are 1 in 1435(0.069%) and 1 in 1060(0.094%) respectively. Only 1 sample was found to be NAT positive from female donor. NAT yield rates in male and female donors are 1 in 1063(0.09%) and 1 in 2828 (0.035%) respectively. [Table 2]

Table 1: Showing year wise NAT yield distribution and total NAT yield and NAT yield rate

	NAT yield HIV	*NAT yield HBV*	*NAT yield HCV*	*Total*	*Chi square value of 3.04 & P value of 0.08 (NS)*
2021	0	12	3	15	
2022	0	6	6	12	
2023	0	2	2	4	
Total NAT positive	0	20	11	31	
***NAT** yield rate*	0	1 in 1737(0.057%)	1 in 3158(0.031%)	1 in 1120(0.089%)	

Table 2: showing the distribution of gender wise NAT yield distribution

Seronegative	***NAT yield in HIV***	***NAT yield in HBV***	***NAT yield in HCV***	***Total NAT yield (Rate)***	*Chi square value of 0.57 & P value of 0.45 (NS)*
Male (31,915)	0	19	11	1 in 1063(0.09%)	
Female (2828)	0	1	0	1 in 2828((0.035%)	

Table 3: Showing the distribution of NAT yield in voluntary and replacement donors

Seronegative	***NAT yield in HIV***	***NAT yield in HBV(rate)***	***NAT yield in HCV(rate)***	***Total NAT yield(rate)***	*Chi square value of 1.56 & P value of 0.21 (NS)*
VBD (7179)	0	2(1 in 3589 or 0.027%)	3(1 in 2393 or 0.041%)	5(1 in 1435 or 0.069%)	
RBD (27564)	0	18(1 in 1531 or 0.065%)	8(1 in 3445 or 0.029%)	26(1 in 1060 or 0.094%)	

Table 4: Showing the distribution of total voluntary and replacement donors

	2021	**2022**	**2023**	**Total**	*Chi square value of 294.1 & P value of 0.000 (HS)*
Voluntary donations	1627	2346	3428	7401	
Replacement donations	8378	9553	10165	28096	
Total	10005	11899	13593	35497	

DISCUSSION

The study recorded highest NAT yield in HBV followed by HCV. There was no NAT yield for HIV. In most other studies also, NAT yield has been reported to be highest for HBV. We found a total NAT yield of 31 and NAT yield rate of 1 in 1119(0.089%). We identified 31 infections from 34714 seronegative samples.
With a seroprevalence of 2-8% among the general population and 0.75-2.61% among blood donors, HBV is the most frequent TTI in India.6 The doubling time for HBV is longer (2.6 days) compared to that of HIV (20.5 hours) and HCV(14.9 hours). The diagnostic window period for serological assays is longer for HBV, compared with HIV and HCV. Occult HBV infections may also contribute to higher NAT yield of HBV as compared to HCV and HIV. In India, due to the high prevalence of HBV, the proportions of occult infections may be higher than window period

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infections.[7] All these factors may contribute to higher NAT yield in HBV virus.

Various studies have described NAT yield rates in India. A 2017 systematic review pooled the results of various studies reporting NAT yields from across 12 blood banks in India.[8] According to this review, 3,89,387 underwent NAT testing. NAT yield was 286 and NAT yield rate was 1 :1361. The highest NAT yield and NAT yield rates were for HBV(221,1:1761) followed by HCV(71,1:5484) and HIV(6,1:66000).

Pathak et al 6 in 2021 identified 97 infections from 155,211 seronegative samples. As compared to our study they observed a lower NAT yield rate of 1:1600. In their study the NAT yield rates were 1:1784 for HBV, 1:17,246 for HCV and 1:155,211 for HIV. The NAT yield in this study was lower than that obtained by Sharma et al7, Mahapatra S et al,[5] Mangwana et al,[10] Hans R et al,[11] Makroo et al,[9] which was 1 in 1017, 1 in 1078, 1 in 974, 1 in 1031 and 1 in 687 respectively. Other studies which obtained higher NAT yield than our study are Kumar R et al,[12] Agarwal et al,[13] Kabita C et al 14and they found NAT yield of 1 in 753,1 in 610 and 1 in 847 respectively.

However, Chatterjee K et al,[15] Jain R et al,[16] and Chigurupati P et al,[17] found lower NAT yields than our study which were 1 in 2622,1 in 2972 and 1 in 2000 respectively.

There may be various reasons for the variability in NAT results. Contributing factors may be pattern of infections among donors, type of kit, the sensitivity of the test and accuracy of methods.[4]

We did not get NAT yield in HIV. This may be because prevalence of HIV is lower as compared to HCV and HBV in this region. A prospective study conducted in our centre for seven years (from 2012 to 2018) found the prevalence of HBV, HCV and HIV among blood donors to be 0.67%,0.79% and 0.19% respectively.[18]

NAT is still not made mandatory in the blood centres in India due to high cost of the equipments. However, if we look at the cost of treatment of HBV and HCV (high cost medications including interferon, investigations, development of hepatocellular carcinoma and overall loss of productive years) we can safely conclude that NAT will help in reducing the burden of infections on the economy of the country in the long run.

CONCLUSION

The study recorded a total of 34743 seronegative blood samples with NAT yield of 31 and NAT yield rate of 1 in 1119(0.089%) , 1 in 1735(0.057%) for HBV and 1 in 3155(0.031%) for HCV. We didn't find any NAT yield for HIV. Since there is 100% component separation in our centre, so we can conclude that by NAT we additionally stopped the transmission of infections to 93(31x3) patients. This is a huge achievement in improving blood safety. Thus, we conclude that NAT adds an additional layer of safety in blood transfusion recipients.

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