Original Article

NAT yield in blood donors: An observational study

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Abstract

Introduction: Individual donation nucleic acid testing (ID-NAT) is considered as highly sensitive technology for viral transfusion-transmissible infections (TTIs) in blood donors. The present study was aimed to analyze the results of ID-NAT with special reference to different types of donors, their age, gender, blood group ranges in a tertiary care center in north India. **Methodology:** The present study was done from 24th June 2019 to 31st December 2021 in Blood Center, Department of Immunohematology and Blood Transfusion, SMS Hospital, Jaipur. A total of 18313 apparently healthy adult donors were included in present study. **Result:** In 2019 Combined NAT yield was 1 in 754, in 2020 it was 1 in 2368 and in 2021 it was 1 in 741. With Total NAT yield was 1 in 1017 (0.09 %) over a period of study.NAT yield in HBV is 1 in 1077, in HCV 1 in 18313 and no NAT Yield in HIV. **Conclusion:** NAT testing for hepatitis B provides additional safety because ELISA does not pick up occult hepatitis. The non-seroconverting or delayed seroconverting disease is missed by ELISA alone and can be picked up by NAT.

Keywords: NAT yield, seroyield, transfusion transmitted infection

Introduction

Individual donation nucleic acid testing (ID-NAT) is considered a highly sensitive technology for viral transfusion–transmissible infections (TTIs) in blood donors. The present study aimed to analyze the results of ID-NAT with special reference to different types of donors, their age, gender, and blood group ranges in a tertiary care center in North India.

NAT provides an additional layer of safety for the blood supply. According to the Drugs and Cosmetics Act, in India, as per 1940 and the rules there in, NAT testing is not yet mandatory.

NAT techniques are based on the amplification of targeted regions of viral ribonucleic acid or deoxyribonucleic acid (DNA). It has reduced the window period of Hepatitis B virus (HBV) to 10.34 days, hepatitis C virus (HCV) to 1.34 days, and Human Immunodeficiency Virus (HIV) to 2.93 days.[1]

NAT also adds the benefit of resolving false reactive donations through serological methods.[2]

As this was the first study from Rajasthan, on NAT yield, seroprevalence of TTI marker varies according to geographical area. So, a study from our area based on NAT will be helpful here in future policymaking.

Materials and Methods

This study was conducted from June 24, 2019, to December 31, 2021, in Blood Center, Department of Immunohematology and Blood Transfusion, SMS Hospital, Jaipur. The study was conducted after taking ethical clearance [194/MC/EC/2020] from the Research Review Committee of the Institute.

A total of 18313 healthy adult donors were included in this study, on which additional NAT testing was performed for HIV,

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HBV, and HCV in association with Enzyme Linked Immuno Assay (ELISA) testing. Donors from both in-house and outdoor camps organized by SMS Hospital were included in the study. This study was a cross-sectional in nature. It was also a type of observational study.

Written informed consent was obtained for all participants. The sample size was calculated at a 95% confidence level as per the result of the seed article (Aramani SS *et al.*).[3] At 5% allowable error, a minimum of 1767 blood donors were required for this study, which were increased up to 18313. The inclusion criteria for participating in the study were a donor who was fit to donate blood according to Drug and Cosmetic Act and rules therein.

- For collecting samples, two pilot tubes labeled, one 3 ml for ELISA testing and one 6 ml for ID-NAT testing, were taken. The sample was taken from the sample pouch before starting blood collection. The fourth-generation ELISA test (Hepalisa Ultra) with a sensitivity of 0.050 ng/ml was used for testing HBsAg for all 11 subtypes, including mutant strains such as ad and ay. For HCV testing, the third-generation ELISA test (HCV Microlisa) was used, which has a sensitivity of 100% and a specificity of 97.4% based on the WHO evaluation report. For HIV testing, a fourth-generation test (Microlisa HIV Antigen (Ag) and Antibody (Ab)) was used, with an analytical sensitivity of 5 IU/ml. For NAT testing, ID-NAT Procleix Ultrio Elite assay kits were used in Grifols Procleix Panther System, which is based on transcription-mediated amplification (TMA) and has an analytical sensitivity of 18 IU/ml for HIV-1 Ribonucleic Acid (RNA), 10.4 IU/ml for HIV-2, 3.0 IU/ml for HCV RNA, and 4.3 IU/ml for HBV DNA.

Initial reactive NAT (IR) samples were further processed for discriminatory testing. Serology-negative and NAT-reactive sample up to discriminatory were considered true NAT yields. NAT nonreactive and serology-positive samples were considered seroyield.

Statistical analysis

The data obtained in this study were processed in Microsoft Excel 2007. Appropriate statistical tests of significance were applied for the analysis of the data collected using IBM Statistical Package for the social Sciences (SPSS) Statistics version 22.

The categorical data were presented as numbers (percent) and were compared among groups using the Chi-square test. The quantitative data were summarized as mean and standard deviation and further analyzed by Student's *t*-test. The probability was considered significant if less than 0.05.

Result

Of the total 18313 study population, 17898 (97.73%) were male donors and 415 (2.27%) were female donors. Among the total study population, 13548 (73.98%) donors were replacement donors and 4765 (26.02%) donors belonged to the voluntary blood donor category. Blood donors belonged to the age group ranging from 18 to 60 years. The mean age of donors was 28.73 ± 7.52 years. Maximum donors were in the 18–30 years age group with a percentage prevalence of 61.55% followed by 31–40 yr. (30.4%), 41–50 yr. (7.03%), and 51–60 yr. (0.98%).

Of a total of 18313 blood donors, 12824 (70.02%) blood donors were residing in urban areas at the time of donation, while 5489 (29.98%) donors had a residence in rural areas. Here, urban area means an area situated in a tehsil and municipality or its equivalent level and below that level was considered a rural area.

Mostly donors were in blood group B (33.96%) followed by O group (29.01%), A group (21.89%), and AB group (7.76%). The prevalence of the blood group in our study population was B > O > A > AB. On evaluation of Rh D status, 92.63% of donors were Rh D positive and 7.37% of donors were Rh D negative. The NAT yield and seroyield of these donor according to demographic characteristics are presented in Table 1.

Of the total study population of 18313, 194 donors were ELISA reactive for Hepatitis B virus surface antigen (HBsAg), 59 donors were found ELISA reactive for HCV, and 15 donors were ELISA reactive for HIV, with percentage prevalence of HBV, HCV, and HIV 1.05%, 0.32%, and 0.08%, respectively. The details are mentioned in Table 2.

Of the total study population of 18313, 234 blood donors were found reactive with Procleix Ultrio Elite NAT technology, 207 of them were reactive for HBV, 12 for HCV, and 15 for HIV, with a percentage prevalence of HBV, HCV, and HIV, 1.13%, 0.06%, and 0.08%, respectively.

In this study, 18 blood donors were reactive by ID-NAT, but nonreactive by ELISA (NAT yield), resulting in a NAT yield of 1 in 1017 (0.09%). Among these 18 donors, 17 were reactive for hepatitis B and one were reactive for HCV. Not a single case of HIV was found to be reactive. The details are mentioned in Table 3. Thus, HBV NAT yield was 1 in 1077 (0.09%) and for HCV virus 1 in 18313. Thus, maximum contribution to NAT yield is due to HBV in our study. The details are mentioned in Table 4.

In the study, 52 (0.02%) blood donors were ELISA reactive, but NAT nonreactive. Among these, 48 were anti-HCV reactive, and four were HBsAg reactive. Thus, seroyield for HCV was 1 in 381 (0.26%) and for HBV seroyield was 1 in 4578 (0.02%).

NAT yield in voluntary donors was 1 in 1588 (0.06%), and in replacement donors, it was 1 in 903 (0.11%).

In 2019, the combined NAT yield was 1 in 754, in 2020 it was 1 in 2368, and in 2021 it was 1 in 741. Total NAT yield was 1 in 1017 (0.09%) over a period of study. The details are mentioned in Table 4.

Table 1: NAT and seroyield distribution in accordance with demographic characteristics of population

Variables	Total no.	NAT positive ELISA negative (18)			NAT negative ELISA positive (52)			Combined NAT yield	Combined seroyield
Total donations	18313	HBV	HCV	HIV	HBV	HCV	HIV		
Male	17898	17 (1 in 1053)	1 (1 in 17898)	0	4 (1 in 4475)	48 (1 in 373)	0	1 in 994	1 in 344
Female	415	0	0	0	0	0	0	0	0
Donor type									
Voluntary	4765	3 (1 in 1588)	0	0	2 (1 in 2383)	5 (1 in 953)	0	1 in 1588	1 in 680
Replacement	13548	14 (1 in 968)	1 (1 in 13548)	0	2 (1 in 6774)	43 (1 in 315)	0	1 in 903	1 in 301
Age group									
18–30 yr	11273	10 (1 in 1127)	1 (1 in 11273)	0	2 (1 in 5636)	21 (1 in 536)	0	1 in 1024	1 in 490
31–40 yr	5573	5 (1 in 1115)	1 (1 in 5573)	0	1 (1 in 5573)	18 (1 in 310)	0	1 in 929	1 in 293
41–50 yr	1288	2 (1 in 644)	0	0	1 (1 in 1288)	9 (1 in 143)	0	1 in 644	1 in 129
51–60 yr	179	0	0	0	0	0	0	0	0
Residence									
Urban	12824	15 (1 in 855)	0	0	0	38 (1 in 337)	0	1 in 855	1 in 337
Rural	5489	3 (1 in 1829)	0	0	0	14 (1 in 392)	0	1 in 1829	1 in 392
Blood group									
A+	4009	4 (1 in 1002)	0	0	1 (1 in 4009)	12 (1 in 334)	0	1 in 1002	1 in 308
A-	307	0	0	0	0	0	0		0
B+	6219	5 (1 in 1244)	0	0	2 (1 in 3109)	16 (1 in 389)	0	1 in 1244	1 in 346
B-	490	1	0	0	0	1 (1 in 490)	0	1 in 490	1 in 490
O+	5314	5 (1 in 1062)	1 (1 in 5314)	0	1 (1 in 5314)	15 (1 in 354)	0	1 in 886	1 in 332
O-	459	0	0	0	0	1 (1 in 459)	0	0	1 in 459
AB+	1422	1 (1 in 1422)	0	0	0	3 (1 in 474)	0	1 in 1422	1 in 474
AB-	93	1 (1 in 93)	0	0	0	0	0	1 in 93	0

Table 2: Seroprevalence of viral marker in donor population

Seroprevalence	Total	ELISA positive NAT positive (prevalence)	NAT positive ELISA negative	ELISA positive NAT negative
HIV	15	15 (0.08%)	0	0
HBV	211	190 (1.03%)	17	4
HCV	60	11 (0.06%)	1	48
Total	286	216 (1.17%)	18	52

Table 3: Year-wise NAT reactive donor

Year	HBV	HCV	HIV
2019	7	0	0
2020	2	1	0
2021	8	0	0

Table 4: Year-wise NAT yield distribution

Year	NAT yield (combined)	NAT yield (HBV)	NAT yield (HCV)	NAT yield (HIV)
2019	1 in 754 (0.001)	1 in 754	0	0
2020	1 in 2368 (0.0004)	1 in 3553	1 in 7106	0
2021	1 in 741 (0.001)	1 in 741	0	0
Total	1 in 1017 (0.0009)	1 in 1077	1 in 18313	0

Discussion

The NAT yield obtained in this study is 1 in 1017, which is similar to that observed in previous studies conducted by Mahapatra S *et al.*,[4] Mangwana *et al.*,[5] and Hans R *et al.*,[6] which was 1 in 1078, 1 in 974, and 1 in 1031, respectively.

The NAT yield in this study was lower than that obtained by Kumar R *et al.*,[7] Agarwal *et al.*,[8] and Kabita C *et al.*,[9] which was 1 in 753, 1 in 610, and 1 in 847, respectively.

The NAT yields obtained in this study are higher than those obtained by Chatterjee K *et al.*,[10] Jain R *et al.*,[11] and Chigurupati P *et al.*,[12] which were 1 in 2622, 1 in 2972, and 1 in 2000, respectively.

Reasons for variability in yield are due to several factors such as the wide variation in the pattern of infections among donors, type of kit, the sensitivity of the test, and accuracy of methods.[13]

NAT yield is higher in HBV, whereas seroyield was higher in HCV in our study. NAT yield was mainly due to hepatitis B in our study. High NAT yield in hepatitis B might be because of non-seroconverting or delayed seroconverting disease unable to be picked by ELISA testing. Besides this, NAT testing is also helpful in detecting an occult hepatitis infection. In India, due to the high prevalence of HBV, the proportions of occult

infections may be higher than window period infections. Such occult HBV infections are generally associated with low levels of circulating HBV DNA.

In developing countries and across the globe, hepatitis B is also the most common cause of NAT yield.[14] Chronic occult HBV infections, which are not detected by HBsAg testing, are a major transfusion risk. The high sensitivity of NAT is required for the detection of occult HBV infection.[15]

In this study, a total of 59 cases were found ELISA reactive for HCV, and on further testing by ID-NAT, only 11 (18.6%) cases were found NAT reactive. A similar type of reactivity pattern was also found in another study.[16]

The number of ELISA-positive/NAT-negative cases may vary due to reasons such as resolved infections in donors, failure to develop detectable viral RNA/DNA, low viral load, and intermittent viral RNA/DNA,[17] and it may be due to the biological sero-false positivity of donors.

The circulating HCV RNA titer may vary considerably. So, a single-negative test does not exclude viremia and may reflect only a viral load below the detection limit of the assay.[18] United States (US) and Centers for Disease Control and Prevention (CDC) also mentioned the need for further medical evaluation in single HCV RNA-negative individuals.[19]

Follow-up of seroreactive and NAT-negative donor was not done, so we cannot comment whether it was true seroyield or not.

High NAT and seroyield were seen in male donor of 18–30 yr. of age, and donor belongs to urban population and replacement in type of donation and blood group B positive. Studies such as Makroo RN *et al.*[20] have shown high seroreactivity of TTI in replacement donors compared with voluntary donors.

The seroyield for HBsAg was 0.02% (1 in 4578) in our study. This may be due to remarkable difference between the release of viral structural proteins in circulation and the appearance of full viral particle. There is a tendency of non-encapsidated viral DNA to undergo rapid destruction. While surface antigen produced by either infected cells or integrated viral genome may remain detectable in circulation for prolonged periods of time, leading to HBsAg seroyield.

We did not get an HIV NAT yield over our study duration. This may be due to the low seroprevalence of HIV in our study population. Thus, NAT yield in blood donors varies region-wise based on the prevalence of infections in donor populations. If we talk about economic benefit, a single safe transfusion saves three lives. The cost of therapy of one case of HBV/HCV (including drug therapy or interferon, investigations, hospital administrations, hepatocellular carcinoma, and liver transplant) is approximately 15 lakh, which is multiplied to three times the mean of 4.5 million rupees. Thus, the combined saving of HBV and HCV is approximately 9 million rupees/donation.[21]

Key points

1. Maximum contribution to NAT yield is due to HBV in our study 1 in 1077 (0.09%).
2. There was no NAT yield in HIV.
3. Seroyield for HCV was more than NAT yield (0.26 v/s 0.005).
4. Seroyield in HBV was 0.02% as compared to NAT yield 0.09%.

Thus, NAT is not a replacement of serology method. It adds value only to HBV detection as per our study.

Conclusion

Thus, we find that NAT yield in HBV is 1 in 1077, in HCV 1 is 18313, and no NAT yield in HIV. Thus, NAT adds an additional layer of safety in TTVI. NAT yield strengthens support for the use of NAT despite its cost factors.

Hence, NAT testing for hepatitis B provides additional safety because ELISA does not pick up occult hepatitis. The non-seroconverting or delayed seroconverting disease is missed by ELISA alone and can be picked up by NAT. A prevention strategy regarding HBV should be implicated. Besides this, the goal should be zero risk from transfusion transmission viral infection. For this, additional methods such as universal pathogen reduction in conjugation with NAT testing should be implicated.

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Conflicts of interest

There are no conflicts of interest.

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