Original Article

Impact Of Introduction Of Nucleic Acid Test And Its Role In Improving Blood Safety In A Tertiary Care Hospital

Mohd Zubair Qureshi, Shazia Handoo, Aruj bin Rashid, Sheikh Bilal

Abstract:

Background: Nucleic Acid Testing (NAT) is a molecular technique for screening blood donations to reduce the risk of Transfusion Transmitted Infections (TTIs) in the recipients, thus providing an additional layer of blood safety. It is a highly sensitive test which reduces the window period by detecting low levels of viral genomic materials that are present soon after infection but before the body starts producing antibodies in response to the virus.

Material and method: The study was conducted over a period of 10 months from September 2020 to June 2021.All samples were examined for HbsAg, HCV, HIV I & II by Enzyme Linked Immunosorbent Assay (ELISA). All seronegative cases by ELISA were subjected to Minipool-NAT in small pools of six to detect HBV DNA,HCV RNA and HIV 1 & 2 RNA.

Result: Out of all 15569 blood donations collected over study period, reactive samples by serological test (ELISA) were 40.

All the seronegative 15529 samples were tested by Minipool-NAT, out of which 22 were positive with total NAT yield of 1 in 706.

Conclusion: The routine use of NAT for detection of HBV, HCV & HIV should be mandatory for all seronegative donor blood to reduce the serological window period and hence reduce the incidence of transfusion transmission infections and increase the safety for the patients.

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Introduction

Nucleic Acid Testing (NAT) is a molecular technique for screening blood donations to reduce the risk of Transfusion Transmitted Infections (TTIs) in the recipients, thus providing an additional layer of blood safety. [1] The traditional method which is used for screening blood donations, known as immunoassay (or serology) testing, detect antibodies to viruses or viral antigens. With immunoassays, there is an interval between the donors' exposure to a virus until antibodies against the virus are produced, known as "window period". During this period there is risk of infection being missed in donated blood by immunoassay testing. These undetected window period infections are responsible for most of TTIs. Thus, NAT takes care of the dynamics of window period of viruses and provide safe blood for donation. [2]

Despite the current practice of screening blood with the newest generation serological tests of different sensitivities, a considerable residual risk of TTIs remains. Although these tests have shortened the pre-sero conversion window period, they still are not able to identify a number of newly infected blood donors. [3] NAT is highly sensitive test which detects the viral ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) by the amplification method. It reduces the window period by detecting low levels of viral genomic materials that are present soon after infection but before the body starts producing antibodies in response to the virus. This allows for earlier detection of infection and further decrease the possibility of transmission via transfusion and also detects mutants and occult cases. [4] Although NAT screening cannot completely eliminate the risk of TTIs, but it has reduced the risk of HBV, HCV and HIV-1, where it has been implemented. [5]

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Nucleic acid testing, Transfusion transmitted infections, window period. HBV, HCV and HIV

Implementation of NAT has introduced not only a new methodology and new logistic but when combined with sensitive serology it provides the most sensitive and specific screening platform for blood screening. [6] 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. [7] Various screening tests available for screening blood donors are Rapid tests, Enzyme Linked Immunosorbent Assay (ELISA), Chemiluminescence (CLIA) and Nucleic Acid Testing (NAT).There are two types of NAT, Individual donor (ID) NAT and Minipool (MP) NAT. Both are recognized by FDA as valid instruments for NAT testing. [8] NAT is a highly sensitive and advanced technique which has reduced the window period of HBV to 10.34 days, HCV to 1.34 days and HIV to 2.93 days. [9]

The AIM of this study is to assess the impact of the introduction of Minipool-NAT for HBV, HCV and HIV and its role in improving blood safety in a tertiary care hospital.

Materials and methods

The study was conducted at Blood Centre of Shri Maharaja Hari Singh (SMHS) Hospital / Government Medical College (GMC) Srinagar over a period of 10 months from September 2020 to June 2021. After Physical examination all medically fit donors were allowed to donate blood after obtaining informed consent for blood donation and screening for TTIs. Blood samples were collected in pilot test tubes at the time of bleeding. It was a non-interventional, retrospective, observational study.

All samples were examined for five TTIs namely HIV I & II, HBsAg, HCV, syphilis and malaria. Blood samples of six milliliters collected in a clean and dry test tube for the TTIs screening were centrifuged for serum and then tested by ELISA for HBsAg (HEPALISA by J Mitra& co Pvt Ltd) and anti-HCV antibody (OSCAR by Oscar Medicare PvtLtd.), anti-HIV 1+2 (MERILISA by Merilisa Diagnostic Pvt Ltd). Rapid kit tests were performed for Syphilis (RECKON by Reckon Diagnostic P Ltd) and Malaria antigen to Plasmodium Falciparum (RELIABLE By Reliable Pro-detect Biomedicals Pvt Ltd). All the data was stored for future reference.

All seronegative cases by ELISA were subjected to Minipool-NAT in small pools of six on Roche's CobasTaq 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 CobasTaq 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 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, HCV6.8 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.

The data were recorded on specially formed proforma, the recorded data were tabulated and analysed.

Results

A total of 15569 blood donations were collected over the period of 10 months from Sep 2020 to June 2021. Of these, the majority of the donors 15190 (97.57 %) were males and 379 (2.43%) were females. There were 11852 (76.13%) replacement donors and 3717 (23.87%) voluntary donors. Out of a total 3717 voluntary donors 3499 (94.14%) were first time voluntary donors and only 218 (5.86%) were repeat voluntary donors (Table1). Out of all 15569 blood donations, reactive samples by serological test (ELISA) were 40, consisting of 17 (0.11%) of HBV, 23 (0.14%) of HCV and 0 for HIV (Table 2). All the Sero-negative 15529 samples were tested by Minipool-NAT out of which 22 were positive, 16 were positive for HBV, 6 were positive for HCV & 0 for HIV. NAT yield i.e., Units reactive by NAT and Non-reactive by Serology was 1 in 970 for HBV, 1 in 2588 for HCV, with total NAT yield of 1 in 706 (Table 3).

Discussion

The purpose of introduction of NAT in Blood Centres is to provide an additional layer to blood safety. NAT is highly sensitive and specific for viral nucleic acids and is based on amplification of targeted regions of RNA and DNA and thus is the technique of choice. By early detection than serology, the window period of HBV, HCV and HIV infections narrows. In India, mandatory blood screening for HBV, HCV and HIV is done by serological tests. The screened seronegative donations are still at risk for TTIs and thus, need for a sensitive screening test arises. The residual risk has been significantly reduced over the last two to three decades in western countries by implementation of NAT. In order to mitigate the residual risk, NAT has been started in few centers in India, but it is not a mandatory screening test for TTIs as per Drug and Cosmetics Act, 1940. [10]

However because of continued hemovigilance it is now being considered that NAT screening may prove to be more beneficial keeping in view the burden of

Table1: Demographic details of donation

	Donor Demographics (n=15569)	
	(Number)	**(%)**
Gender		
Male	15190	97.57%
Female	379	2.43%
Donation Type		
Replacement	11852	76.13%
Voluntary	3717	23.87%
Donor Repeatability		
First time Voluntary Donors	3499	94.14%
Repeat Voluntary Donors	218	5.86%

Table 2: Seroreactivity of HBsAg, HCV and HIV by ELISA

Screening (By Elisa)	Screen Reactive (n=15569)
HBsAg (3^{rd} Generation)	17 (0.11%)
HCV (3^{rd} Generation)	23 (0.14%)
HIV (4^{th} Generation)	0
HbsAg + HCV + HIV	40 (0.25%)

Table 3: NAT Yield per donation tested.

Virus detected	Total no of Seronegative donation (N=15529)	
	No. of NAT yield donation	NAT yield
HBV	16	1 : 970
HCV	6	1 : 2588
HIV	0	0
Total NAT yield donations	22	1 : 706

the transfusion transmissible infectious and the endemicity of Hepatitis B and C with high seroprevalence of transfusion transmissible infectious agents.

The studies done so far are also in favour of introduction of NAT on a wider basis to enhance the safety of blood and blood products in India. [11-15]

In the present study 15569 blood donor samples were tested, by serology tests (ELISA), out of which 40 were seropositive with seroprevalence of 0.11% for HBV, 0.14% for HCV & 0% for HIV. Among all the seronegative 15529 samples tested by Minipool-NAT 22 were positive, 16 for HBV, 6 for HCV and none for HIV. The combined NAT yield for blood donors of all three viruses was 1 in 706 samples tested, which was comparable with study from Kumar R etal. [16] The NAT yield rate from other Blood Centres in India is 1 in 3182, [17] 1 in 2972, [12] 1 in 2622, [18] and 1 in 1528, [11] which is lower than our NAT yield rate.

NAT Yield obtained from 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. [19] Another study from Europe found a NAT yield of 1: 600,000 for HCV and 1: 1.8 million for HIV after screening 3.6 million donations. [20] One of the reasons for this lower NAT yield is that these countries mostly collect blood through voluntary blood donations and much lower prevalence of these viral infections in the population. NAT screening may thus prove to be more beneficial where the seroprevalence of transfusion transmissible infectious agents is high, as is the case in most developing countries.

In our study, there were 23.87% voluntary blood donors (which included only 5.86% repeat voluntary blood donors) and the remaining 76.13% were replacement donors. The majority of voluntary donors being first-time voluntary donors may not be safer than replacement donors and it could explain the higher NAT yields in our study as compared to some other centers. [21]

The implementation of NAT as an add on test for blood safety has been reported in various studies in India. The cost of implementation of NAT as a quality and safety measure is much lower than the cost of treating infected patients after receiving blood from window period donations. The cost of disease burden and treatment of HBV and HCV is very high and cannot be overlooked in view of millions of carriers already in the country and the lack of facilities and resources for treatment including hepatocellular carcinoma or liver transplantation. The benefits of NAT are especially important in patients who receive multiple blood transfusions for diseases such as thalassemia, chronic kidney disease, malignancies etc. Such patients need regular, repeated and life-long blood transfusions and are at higher risk of being infected with serious TTIs.

There are certain limitations of this study. First the sample size was relatively small and secondly we have used Minipool-NAT in our study as compared to ID-NAT in most of the other studies. But it should be kept in mind that ID-NAT marginally reduces the window period of the three infections compared to Minipool NAT by 2 days only, moreover several developed countries continue to use Minipool-NAT even today. [22]

Conclusion

By implementing Minipool-NAT we detected TTIs in 22 samples of donated blood which were missed by serological tests with an overall NAT yield of 1 in 706. The routine use of NAT for detection of HBV, HCV & HIV should be mandatory for all seronegative donor blood to reduce the serological window period and hence reduce the incidence of TTIs and increase the safety for the patients. The issue of higher cost in the developing countries accounts for the limitation of ID-NAT, hence if finance is the problem then Minipool-NAT also could be an acceptable beginning in the road to transfusion safety.

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