Original Research Paper

Volume - 12 | Issue - 09 | September - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

UTILITY OF MINI-POOL NAT STRATEGY FOR IMPROVING BLOOD SAFETY BY DETECTING OCCULT HEPATITIS B VIRUS INFECTION : A 5 YEAR STUDY

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ABSTRACT **Introduction:** The prevalence of transfusion transmitted infections (TTIs) is high in India. The aim of our study was to evaluate the sero-prevalence of TTIs using Nucleic acid technology (NAT) and detection of Occult Hepatitis B virus infection by Minipool NAT Strategy. **Materials and Methods:** A total of 89,639 units of blood donated at Dhanvantari Voluntary Blood Bank, Rajahmundry during the period from January 2012 to July 2016. The sero-prevalence for HIV, HBV and HCV was tested by 4th generation ELISA. Only sero-negative samples were tested on mini pool nucleic acid test (MP-NAT) to detect HIV1, HIV2, HCV and HBV viral nucleic acids. Combined NAT yield was calculated and compared the NAT yield with MPX test and MPX V2.0. We also evaluated the optical density (OD) values of ELISA for MP-NAT reactive donor samples to evaluate the efficacy of NAT in detecting window period cases. **Results:** The overall sero-prevalence of TTIs was 2.41% and HBV was predominantly present (66%). Total 29 ELISA negative blood donor samples were found reactive on NAT. All NAT reactive donors were of HBV only. Out of 29 cases, 12 were detected by MPX test from 26,008 donations and 17 by MPX V2.0 from 25,619 donations, providing NAT yield of 1:2169 and 1:1507 respectively. OD and Signal cut off values of ELISA for NAT reactive samples were found to be much below extended grey zone value (<0.3). **Conclusion:** Additional testing of nucleic acids by MP-NAT in combination of mandated primary serology by ELISA provides 99.99% of safe blood to needy patients; thus significantly reduces the risk of TTIs by identifying the window period cases.

KEYWORDS :

INTRODUCTION:

The risk of transfusion transmitted infections (TTIs) is one of the major problems identified in Transfusion Medicine. The prevalence of TTIs is directly proportional to prevalence of these infections in the blood donor population. The range observed from various studies is from 0.02% to 0.98% (Table 1). Hence, it is very critical to implement safety and quality systems in blood screening and transfusion processes to reduce the risk of TTIs in recipients. It has been noted that trends of TTIs were reported high in first time donors and replacement donors compared to voluntary donors [1-4].

NACO (National Aids Control Organization), division of Ministry of Health and Family Welfare which governs the HIV/AIDS control programme in India, has mandated blood testing for 5 TTIs; HIV (HIV1 and HIV 2 antibodies), HBV (HBsAg Antigen), HCV (HCV antibodies), Malaria and Syphilis [5]. NACO has not made Nucleic Acid Amplification testing (NAT) mandatory for blood screening but an additional test of safety in India. However, NACO in its recent guidelines on HIV testing mentioned that NAT for HIV diagnosis for early infant diagnosis and window period cases [6]. Multiple studies have shown that NAT is able to pick up the HIV HBV HCV infections in window period which may undetected leads to post transfusion TTIs in recipients with overt infection and misinterpreted as medico-legal negligence on blood banking services [7,8].

The aim of our study was to evaluate the sero-prevalence of TTIs in Dhanvantari Voluntary Blood Bank, Rajahmundry (Andhra Pradesh, India) since 2012 using Nucleic acid technology (NAT) and detection of Occult Hepatitis B virus infection by Minipool NAT Strategy over a 6 year period. We also compared latest NAT (**cobas®** TaqScreen MPX V2.0 Test) assay with the previous NAT (**cobas®** TaqScreen MPX Test) assay.

MATERIALS AND METHODS:

The study was conducted at Dhanwantari Voluntary Blood Bank (Rajahmundry, East Godavari district Andhra Pradesh, India), established in 2007, covers twin districts of Godavari (Andhra Pradesh, India). Dhanwantari blood bank has~ 15,000 blood donations and issues ~30,000 annually. It has all the required technology mandated by NACO and DCGI.

A total number of 89,639 blood donors from 2010-2016 were screened for HIV (P24antigen and GP41 antibody), HBV (HBsAg) and HCV (antibody). All positives and grey zone units are discarded. All negative samples are subjected for MP-NAT test.

We implemented mini pool NAT (MP-NAT) in 2012 with cobas®TaqScreen MPX test (Roche Molecular Systems, Branchburg, NJ) on cobas® s201 platform (Roche Instrument Center, Rotkreuz, Switzerland). We upgraded NAT to cobas® TaqScreen MPX Test, version 2.0 (MPX V2.0) in September 2014.

The MPX and MPX V2.0 tests are intended as an advanced donor screening test to detect HIV-1Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in human plasma [22, 23]. MPX V2.0 test is more advanced over MPX where the target viral infection is known in resolution test itself and no separate discrimination test is needed. Moreover the lower level of detection of viral pathogens is better in MPX V2.0 All blood donations/units were screened by 4th generation ELISA of BIORAD employing fully automated ELISA processor - EVOLVIS. Only Seronegative samples were tested on NAT using MPX or MPX V2.0.

For MPX test - NAT was performed in pools of 6 and the reactive pools were resolved by further testing of the individual blood donations/samples comprising the pools. Individual reactive specimens were further tested using the cobas Taqman monitor test (for HBV followed by HCV and then followed by HIV) for viral discrimination. All reactive samples were outsourced and discriminated at Asian Institute of Gastroenterology (AIG), Hyderabad, using the cobas TaqMan HBV, HCV and HIV viral load assay.

For MPX V2.0 – The same procedure was employed as for MPX. The reactive pool was subjected for individual testing for NAT by resolution. No viral discriminatory test was required for MPX V2.0. We also evaluated the optical density values of ELISA for NAT reactive donor samples, to know the efficacy of NAT in detecting window period cases.

RESULTS:

The details of number of samples tested on ELISA and sero-positivity for HIV, HBV and HCV is given in figure 2. Total sero-prevalence TTIs was 2.41%. Sero-prevalence rate found to be decreased over the period years (2010 - 3.38%, 2011 – 3.01%, 2012 – 3.03%, 2013 – 1.91%, 2014 – 1.84%, 2015 – 1.75% and 2016 – 1.36%). HBV was predominantly present, ~66% of all positive cases. The sero-reactivity of HIV in blood

donors is decreased over a period of years due to compulsory donor screening by qualified staff, use of donor deferral software and counselling of reactive donors with follow up for treatment in VCTC centres (sero prevalence of HIV in 2010- 0.60%; 2011- 0.61%; 2012- 0.53%; 2013- 0.33%; 2014-0.19%; 2015- 0.23%; and 2016- 0.22%). Similarly the sero-prevalence of HCV is also decreased over a period of years from 0.79% in 2010 to 0.14% in 2016.

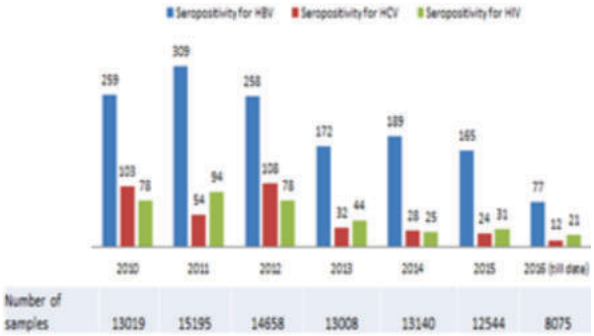

Figure 1: Seropositive cases by ELISA test

A Total of 51,627 donations were tested on Mini pool NAT of Roche Cobas. Of which 26,008 samples tested on MPX test and 25,619 donations were on MPX V2.0 test. Total 29 donors were detected on NAT screening which were negative on ELISA test. All NAT reactive donors were of Hepatitis B virus infection. No NAT reactive samples found for HIV and HCV. Out of 29 cases, 12 were detected by MPX test from 26,008 donations and 17 by MPX test V2.0 from 25,619 donations, providing NAT yield of 1:2169 and 1:1507 respectively [Table 2].

Table 2: NAT Yield on cobas TaqScreen MPX test and MPX test V2.0

	NAT Tested Samples	NAT Reactive	NAT Yield
MPX	26008	12	1:2167
MPX V2.0	25619	17	1:1507

Optical Density (OD) values of ELISA for NAT reactive samples were found to be much below from the signal cut off ratio [Table 4]. The viral load of the samples ranged from <6 IU/ml to 1002 IU/ml indicating the presence of Occult Hepatitis B virus infection.

DISCUSSION:

In our retrospective analysis, we found that NAT yield of 1 in 2,167 for MPX and 1 in 1,507 for MPX V2.0 and all were of HBV. Total 29 donors found reactive on NAT who were negative on serology testing. No NAT reactive donors found for HIV and HCV. With consideration of three blood components from one blood donation, total 87 lives saved from getting infected from HBV.

No NAT reactive donor for HIV could be due to implementation of best screening practices at Dhanwantari Blood Bank, such as donor database, repeat non remunerative donors, thorough screening, health questionnaires and counseling for HIV positive donors to exclude such high risk donors from donor list. Similar findings were observed for HBV and HCV with reduction in detected cases over the period of 5 years. The findings are in aligned with the prevalence data published by Khan MI et al on HBV (0.96%) and HCV (0.01%) in blood donors (13). Our study results are similar to results published by Jain R *et al*. As compared to sero-prevalnce of 2.41%, Jain R *et al* published the sero-prevalence rate of 2.62%. Combined NAT yield of their study was 0.034% (0.057% in our study) and all 8 cases NAT reactive were of HBV DNA (1: 2972 donations) [19].

Other publication from India reported NAT yield of 156 cases from 35,722 donations in northern India, with high NAT reactivity (69.2%) for HBV. NAT yield for HBV was 1:627 in this study which was much higher to our results i.e. 1:1507 [20]. However, another study has shown much lower NAT yield for HBV (1 in 26,630) on in house MP-NAT probably due to lower sero-positive donor or low sensitivity of assay [21].

The NAT yield was improved after up gradation from MPX to MPX V2.0, detected higher number of HBV cases per number of donations. MPX V2.0 detects (LOD) as low as 2.3 IU/ml (95% limit of detection) viral load for HBV compared to MPX 3.8 IU/ml [22, 23], which is best in class available test to detect HBV at such low concentration. Improved yield was due to improved sensitivity to HBV. Our study showed that MP -NAT screening detects the low viral load for HBV, could be found in window period infections or inactive chronic hepatitis B infections or chronic carriers.

OD values of ELISA tests for NAT reactive samples was much below the signal cutoff ratio, indicating the window period infection with low viral load which were undetected by the fourth generation ELISA. NAT due to high sensitivity could able to detect these donors at low viral load. With 4th generation ELISA or chemiluminiscence assay, the sensitivity would be expected to improve in detecting such cases [24,25].

In case of MP-NAT by MPX, the discrimination test was outsourced at Asian Institute of Gastroenterology, Hyderabad to know the viral pathogen and load by Real time PCR technology. Analysis of results shows all are HBV positive and viral loads are less than 200 IU/ml except one which is 1002 IU/ml. All these donors were in low viral load probably they may be in window period phase, or Occult hepatitis B infection. Transfusion of such blood or blood components would definitely produce overt hepatitis B infection in recipients due to high infective dose. In one case of viral load of 1002 IU/ml could be due to false occult hepatitis B infection. The ELISA test could not pick up the positive result of surface antigen probably due to surface antigen mutation leading to viral mutants and NAT is able to give positive reaction in this case.

CONCLUSIONS:

In countries with high population like India, the risk of TTIs is high due to high TTIs prevalence in blood donors and window period donations. The single ELISA test for TTI prevention is not 100% safe for transfusion. The addition of MP-NAT test is able to detect TTIs in window period, chronic carriers, chronic hepatitis cases, viral mutants and occult hepatitis B infections. Hence, NAT should be made mandatory in blood bank screening to eliminate the burden of TTIs in India.

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