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Research Article

ROLE OF NUCLEIC ACID TESTING IN "DOUBLE" SCREENING OF BLOOD DONORS FOR A SAFE BLOOD TRANSFUSION

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Abstract-Background: A total of 30 million blood components are transfused each year in India. Blood safety thus becomes a top priority, especially with a population of around 1.23 billion and a high prevalence rate of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) in general population. Transfusion-transmitted viral infections (TTIs) continue to be a major threat to safe transfusion practices. Screening of all blood samples by highly sensitive and specific techniques will enhance the safety of blood transfusion by reducing the diagnostic window period as much as possible**. Aims:** The aim of this study is to show the value of NAT in blood screening. Settings and Design: Dhanavantari Blood Bank, Rajahmundry, Andhra Pradesh, India. **Methods:** Over a period of 2 years from January 2013 to December 2014, a total number of 26,148 blood donor samples were subjected to tests for HIV, HBV, and HCV by enzyme-linked immunosorbent assay (ELISA) method and 25,658 ELISA nonreactive samples were subjected for NAT using multiplex polymerase chain reaction technology. **Results:** Of the 26,148 donors tested, 490 were seroreactive. In 25,658 remaining ELISA negative blood samples subjected to NAT, 14 donor samples were reactive for HBV. The NAT yield was 1 in 1867. **Conclusion:** Based on Seroprevalence of TTI and NAT, we conclude that dual screening of all blood and blood components before transfusion by highly sensitive ELISA and NAT helps in detecting all potentially infectious blood units in all phases of infection that will increase safety in blood tranfusions and making near zero risk of post transfusion infections.

Keywords- Hepatitis B virus, Hepatitis C virus, Human immunodeficiency virus, Nucleic acid amplification testing, Transfusion-transmitted infection.

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Introduction

Globally Safe blood is an important aspect to prevent transfusion transmitted infections in recipients. With advancement of good component therapy millions lives are saved but unsafe transfusion practices leads to risk of TTI. Blood and blood components are important sources of HIV, HBV, HCV, Syphilis, Malaria and other infectious diseases in India. India has an estimated population of 1.23 billion. It has the world's largest population suffering of HIV/AIDS with adult HIV seroprevalence of 1% in 2014 [1]. Our country fills under category of HBV intermediate endemicity with HBsAg seroprevalence of 2-8% in general population [2].It is estimated that 40 million Chronic Hepatic B carriers exists in India out of 350 million carriers worldwide [3]. HCV is the leading cause of Hepatic Fibrosis, Cirrhosis of Liver and Hepato-cellular Carcinoma (HCC). India has a prevalence of 1.5% of HCV, which is an additional burden to Health Care Management System [4].

Even after the strict vigilance of screening of blood and blood components, occasional post transfusion infections are common. It might be due to prevalence of asymptomatic carriers in the society, blood donation during window period (WP) and escape of viral detection system by mutation of viruses [5].

The post transfusion infections can be minimised by banning professional donors and switch on to non-renumerated voluntary donors and screening of blood by highly sensitive serological techniques. The post TTI can be reduced further by strict curtailing of illegal blood bleedings, establishment of transfusion services, use of highly sensitive serological methods, strict supervision by health administrators and effective quality control systems. The present study was conducted in Dhanwantari Voluntary blood bank and blood components, Rajahmundry, Andhra Pradesh, India, to know the seroprevalence of TTI *viz.*, HIV, HBV, HCV, Malaria, Syphilis by dual screening by ELISA and Nucleic Acid Testing (NAT).

Subjects and Methods

A total number of 26,148 blood units were collected from voluntary and replacement donors from January 2013 to December 2014 in Dhanwantari Voluntary blood bank and blood components, Rajahmundry, Andhra Pradesh, India. Donor selection was made by criteria laid by Drugs and Cosmetic Act 1940. Strict clinical examination was done to eliminate professional donors. At the time of blood donations blood samples were collected for serology and NAT separately in EDTA Vacutainers. (3ml for serology, 6ml for NAT) serological screening for HIV, HCV, HBV infections.

All serum samples were screened for the presence of HIV 1, 2 antibodies and P_{24} antigen using fourth generation ELISA, HCV antibodies and HBsAg by ELISA, using fully automated ELISA processor, employing Biomedd test kits; and followed manufacturer's instructions. All samples that were borderline negative were tested again using same technique.

NAT Test

All reactive samples by serology were discarded and all nonreactive samples were subjected to NAT by fully automated Roche Cobas S201 system of MPX version. In NAT test, HIV1 0, HIV1 M, HIV 2 , HBV, HCV viral nucleic acids are detected by multiplex polymerase chain reactions. Testing involves 5 major steps: Initially, plasma is ultra-centrifuged to concentrate viral particles. Reverse transcriptase of

the target RNA is added to generate complementary DNA. Once that step is completed, amplification of target complementary DNA is achieved by PCR using bio specific complementary primers. The amplified products are then hybridized to oligonucleotide probes specific to the target, and the probe-bound amplified products are then detected by colorimetric measurement. The detection of amplified DNA is performed by using avidin-horseradish peroxidase. The entire test process takes approximately 6 hours. Briefly 6 serum samples are pooled to one automated Hamilton machine. Later samples are transferred to Roche Ampliprep machine where sample preparation takes place to release and purify target viral nucleic acids in K tubes. Later these K tubes are transferred to Roche Taq screen MPX machine where PCR reaction takes place by fully automated procedure employing fluorescent-based Taqrean Chemistry. The mini pool samples that showed pool reactive were retested individually by Resolution test to know the reactivity of individual sample. The reactive sample was subjected to Discrimination test which was out sourced by Roche Molecular Diagnostic of Asian Institute of Gastroenterology Hyderabad. Analyses of discriminatory test results were done.

Quality Control

All pooled samples and individual samples are tested with internal Quality control target along with Positive, Negative controls of all Viral Nucleic Acids. If any deviations occurred, the cause is analysed and corrective action was taken.

Results

Of the 26,148 blood donors screened for HIV, HBV, and HCV, 490 were seroreactive (1.9%). Of these 361 (1.38%) were HBsAg reactive, 60 (0.22%) were anti-HCV reactive, and 69 (0.69%) were anti-HIV reactive. Year wise distribution of the data of reactivity is shown in [Table-1].

The 490 reactive samples were discarded and the remaining 25,658 samples that were subjected to NAT yielded 14 reactive cases. All the samples were found positive for HBV nucleic acids. The viral quantification results were analyzed [Table-2]. The NAT yield result was 1 in 1867.

Table-1 ?????????

S. No	Year	No. of Donors	HIV(%)	HBV(%)	HCV(%)	VDRL	MP
1.	2013	13008	44 (0.33%)	172(1.32%)	32 (0.24)	-	-
2.	2014	13140	25(0.19)	189(1.43)	28(0.21)	-	-
	Total	26148	69(0.26%)	361(1.38%)	60(0.22%)	-	-

Table-2 ?????????

S. No	Blood Bag	HBV Viral Load
1.	3615/2013	20 IU/ml
2.	4928/2013	<6 IU/ml
3.	6984/2013	14 IU/ml
4.	8538/2013	<6 IU/ml
5.	9729/2013	<6 IU/ml
6.	12939/2013	<6 IU/ml
7.	8737/2013	6 IU/ml
8.	61/2014	61 IU/ml
9.	86/2014	<6 IU/ml
10.	1866/2014	7 IU/ml
11.	2248/2014	100 IU/ml
12.	9541/2014	20 IU/ml
13.	10002/2014	29 IU/ml
14.	120941/2014	32 IU/ml

Discussion

Blood is a life saving fluid connective tissue and there is no natural substitute for it. One unit of blood can save the lives of 3 or 4, when given at right timing or correct blood component. Advancement of medical technology by the usage of 4th generation ELISA leads to best available screening tests, which could detect low concentration of antigen and antibody with enhanced sensitivity and speciality of test results. In spite of all the precautionary measures used by blood-collecting agencies to avoid inoculating infectious agents into transfusion recipients, it is possible to transmit disease when blood from a recently infected donor fails to be identified by routine screening tests [6]. This is because of the so-called WP after a donor is infected, but before the condition is detectable by routine methods. This technological limitation puts blood recipients at a definite though infrequent risk for transmissible diseases. Since viremia precedes serocon-version by several days to weeks, tests that detect viral nucleic acids are considered a significant technological advancement and an additional step in our quest to achieve the goal of zero risk for blood transfusion recipients.

Nucleic acid amplification test (NAT) technologies have the potential to detect viremia earlier than current screening methods, which are based on serocon-version. NAT is a molecular technique to detect viral nucleic acids of HIV 1–2, HBV, and HCV at a very low concentration in donor blood by Nucleic acid amplification technology [7]. The primary benefit of NAT is the ability to reduce residual risk of infectious WP donations. The estimated reduction of the WP utilizing NAT for HCV is 70–12 days, HIV from 22 to 11 days, and HBV from 59 to 25–30 days [8].

In the present study, the overall TTI reactivity is 1.87%. HIV reactivity is 0.26 %, HBV reactivity is 1.38% and HCV reactivity is 0.22%.

Since 14 samples are reactive for nucleic acids and all are HBV only, imagining the bag is issued without NAT, 14 patients might be affected and in a component-oriented blood bank, 42 patients might be affected by HBV infections, which are very high, a yield of 1 in 610. Similar studies in other countries have also demonstrated high yields [9-14].One study done in India have noted a NAT yield of 1 in 650 [15]. We published a similar result earlier wherein NAT yield of 1 in 666 was noted [16].

In the present study the viral load of HBV indicates less than 6 IU/ml to 100 IU/ml. (Normal detection level is 6 IU/ml to 1.10 million /ml). It indicates donors are in window period where in viral nucleic acids positive with absence of significant detectable concentrations of HBsAg. Detection of low concentrations of HBV to the tune of 6 IU/ml will definitely enhances the sensitivity of the test, safe to recipient.

Studies on the feasibility of NAT implementation in developing countries like India will help extend the message to blood centers that NAT can be an effective method for safeguarding the blood supply. The potential for NAT yield in India is staggering when compared to other countries that have already implemented the technology, especially when one takes notice of the high number of carriers of HIV (2.5 million), HBV (43 million), and HCV (15 million) as per the statistics provided by the NACO (national AIDS control organization) India [17-19].

Conclusion

In conclusion, near zero risk of blood transfusions cannot be achieved by routine screening of all blood bags by only ELISA as primary screening .If it is followed by NAT to all ELISA negative bags to detect window period donations, the same can be achieved. Hence double or dual screening of all blood bags by two different techniques will provide 99.99% safe blood globally.

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