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Individual Donor Nucleic Acid Testing for HIV, HBV and HCV among Blood Donors in a Tertiary Care Hospital

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A B S T R A C T

Transfusion-transmitted infections (TTIs) continue to be a threat to safe transfusion practices. 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) in general population in India. Nucleic acid testing (NAT) in blood donor screening has been implemented in many developed countries to reduce the risk of transfusion-transmitted viral infections(TTIs). The objective was to assess the role of individual donor-NAT (ID-NAT) for human immunodeficiency virus-1 (HIV-1) its role in blood safety. It involves voluntary blood donors during period of one year from 1st June 2016 to May 2017 attending Chigateri Blood Bank, Davangere. Over a period of 1 year from 1st June 2016 to 31st May 2017, a total number of 9423 blood donor samples were subjected to test for HIV by Individual Donor Nucleic acid testing (ID-NAT), 9402 donors (99.8%) were negative by ID-NAT. 21 donors (0.2%) were positive by ID-NAT. Most of donors were in age group of 20-29 years & sex ratio of male: female was 70:1.Maximum donors belonged to O blood group. NAT could detect HIV cases in blood donor samples. Its widespread use in blood banks would ensure additional layer of safety in blood transfusion.

Introduction

Infection with human immunodeficiency virus (HIV), which causes AIDS has become a worldwide epidemic and is one of the major public health concerns for all countries.[1,2]

The HIV prevalence at national level has

continued its steady decline from an estimated peak of 0.38% in 2001-03 through 0.34% in 2007 and 0.28% in 2012 to 0.26% in 2015.[3] Overall, India's HIV epidemic is slowing down with a 32% decline in new HIV infections.[4]

Blood safety status in India is challenging task with a population of more than 134 crores & with at least 2.1 million HIV infected individuals (0.24%-0.3%) among general population. India is the home for third largest HIV epidemic in the world after South Africa & Nigeria.[5,6] In India as per the regulatory requirement of the Drug and Cosmetics act of 1940, (1st Amendment rules 1992) it is mandatory to test each donated unit of blood for markers of HIV- I and II, HBV, HCV, Malaria and Syphilis[7].

A single unit of blood or its components may be transfused into as many as 1-4 recipients and thereby spreading HIV infection quite rapidly.[8]

Currently, in India all the blood donations are screened for various infectious markers using ELISA or rapid methods. The NAT tests of high sensitivity rely on amplification of intended regions of viral nucleic acid for detection. The purpose of this study is to provide better understanding of the role of NAT in reduction of the risk of acquiring TTIs. NAT testing is not yet mandatory for screening blood units in India but has been started in a few centers to enhance blood safety[9].

NAT detects infection before serological tests 9.4 days earlier for HIV-1.[10].

The objective was to assess the role of individual donor-NAT (ID-NAT) for human immunodeficiency virus-1 (HIV-1) its role in blood safety.

Materials and Methods

Study Design and settings

Around 9423 voluntary blood donors during period of one year from 1st June 2016 to 31st May 2017 were included. Permission from Hospital authorities and ethical clearance from institutional ethical committee were obtained before starting the study.

All samples were subjected to ELISA for HIV reactivity, irrespective of its results were subjected to the reference center for ID-NAT, where multiplex PCR company employed by Karnataka Government under "TOWARDS ZERO RISK BLOOD" Program at Bowring &Lady Curzon Hospitals attached to Bangalore Medical College & Research Institute.

Specimen Collection

With strict aseptic precautions, 10 ml of venous blood sample was collected by vein puncture.

From that, 7 ml of blood was directly collected in EDTA vacutainer for obtaining plasma and was processed for ID-NAT (Individual Nucleicacid Amplification Testing) using Procleix Ultrio Plus Assay by qualitative in-vitro Nucleicacid Amplification Test as per standard protocol supplied by manufacturer (*i.e* Novartis Diagnostics, Emeryville, California) Year 2012-07, 502432 Rev.A.).

Remaining 3 ml of blood was collected in plain vacutainer and was centrifuged to get serum and was tested/processed for HIV antibodies by using SD HIV1/2 ELISA 3.0 KIT (Third Generation of Anti-HIV1/2 ELISA) according to standard protocol supplied by manufacturer (i.e, Sd Bio Standard Diagnostics Pvt. Ltd, Haryana, India) Year 2014, SD/02/IB/96/0100.)

Sample subjected to two tests

ID-NAT (Individual Nucleicacid Amplification Test)

HIV 1 & 2ELISA

The test was run in batches once in 3-4 days.
In our study we followed two-step procedure, transcription of the RNA to cDNA is performed first. Transcription occurs between 40^{o}C and 50^{o}C, depending on the properties of the reverse transcriptase enzyme utilized. Products of that reaction are then amplified in a separate reaction.

Results and Discussion

The observations made from the study are shown in the following tables.

Among 9423 blood donors, highest no. of donors were in the age group of 20-29 years, 6038 donors (64.1%) and least in the >60 years age group,15 donors(0.16%). Other groups were 433(4.6%) in the age group <20 yrs, 2375(25.2%) in the age group 30-39 yrs, 515(5.5%) in the age group 40-49 yrs and 47(0.5%) in the age group 50-59 yrs.

Out of 9423 donors, maximum (3175) were O +ve group comprising 33.7% and least were AB-ve (30) comprising 0.3%. From the table.3, majority of donors were Rh+ve 9104 (96.6%) & Rh –ve were 319(3.4%).

Among 9423 donors, 9402 (99.8%) were negative and 21(0.2%) were positive by only ID-NAT. In the present ID-NAT study which is first ever in Davangere, 9423 blood donors samples were tested for a one year period from 1st June 2016 to 31st May 2017 attending Chigateri Blood Bank.

Maximum no of donors were in age group of 20-29 yrs (64.1%). Though the age criteria for donor selection was followed in Bareeto *et al.*, study, studies by Chatterjee *et al.*, & Sharma *et al.*, had adolescent blood donors. It could be due to inclusion of adolescents with enthusiasm for voluntary blood donation or due to social/peer pressure.

Most of the donors were males 98.6% with sex ratio of 70:1 similar to the observations in all other studies like Chatterjee K *et al.*,[(11)], Rao and Annapurna *et al.*,[(12)] and Rose *et al.*,[(13)]

Most of the donors in our study belong to O+ve blood group (33.7%), and it correlates well with studies P K Das *et al.*,[(14)] Apecu *et al.*,[(15)] & Das *et al.*,[(16)].O is the most common blood group among general population and is commonly regarded as universal donor & most needed group for patients.

In present study, the HIV ID-NAT positive percentage was (0.2%) which was high when compared with study by Rajesh *et al.*,[(17)] 43 (0.06%) out of 32,978 donor samples.

The reason for high percentage of HIV ID-NAT in present study could be that our blood bank is attached to Government Tertiary Care Hospital & many patients most of times comes from economical weaker sections & HIV is more common in people from lower socioeconomic status.[(18)] & also NAT can pick up at 4.7(4-5) days of entry of HIV virus in blood.

The percentage of HIV positive cases in our study (0.2%) correlates well with study by Makroo *et al.*,[(19)] but their sample size was large & it was undertaken for duration of 11 years. Another reason for low percentage of HIV positive donors may be availability of 4th Generation ELISA and reduction of false positive cases due to increased specificity of the test.

Table.1 Age wise distribution of voluntary blood donors

Age In years	Frequency	Percentage
< 20	433	**4.6**
20-29	6038	**64.1**
30-39	2375	**25.2**
40-49	515	**5.5**
50-59	47	**0.50**
≥ 60	15	**0.16**
Total	**9423**	**100.0**

Table.2 Sex wise distribution of voluntary blood donors

Gender	Frequency	Percentage	Ratio
Male	9290	98.6	**70 : 1**
Female	133	1.4	
Total	**9423**	**100.0**	

Table.3 Blood groups distribution among voluntary blood donors

Blood Group	Frequency	Percentage
A+ve	2265	**24.0**
B+ve	3033	**32.2**
O+ve	3175	**33.7**
AB+ve	631	**6.7**
AB-ve	30	**0.3**
A-ve	89	**0.9**
B-ve	86	**0.9**
O-ve	114	**1.2**
Total	**9423**	**100.0**

Table.4 Distribution of Rh Blood Groups among blood donors

Rh Positive Blood Groups	Rh Negative Blood Groups
9104(96.6%)	**319(3.4%)**

Table.5 Results of HIV ID-NAT among voluntary blood donors

ID-NAT	No. of Cases	Percentage
Positive	21	**0.2**
Negative	9402	**99.8**
Total	**9423**	**100.0**

The reason for higher yield in our study could be that our Blood Bank is attached to Government Tertiary Care Center and many patients most of times comes from economical weaker section where as study by Stramer *et al.,*[20] was carried out in non-Government organizations.

Since viremia precedes sero conversion by several days in cases of HIV infection, so routine implementation of ELISA & NAT in blood banks can be significant step to achieve the goal of "Towards Zero Risk Blood" a programme by Karnataka Government.

NAT helps to detect potentially infectious blood units in all phases of infection which in turn helps in enchancing the safety of the blood and blood components for transfusion.

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