Original research article

HIV Testing Among Blood Donors -Acomparative Study Between HIV Nucleic Acid Amplification Testing and HIV Antibodies Enzyme Linked Immunosorbent Assay

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Abstract

Background: 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.

Objectives: To observe any discordant results between HIV Nucleic acid Amplification Testing and HIV antibodies ELISA screening and Note relative sensitivity , specificity and diagnostic accuracy.

Materials and Methods: This is a comparative study of two different HIV diagnostic methods for sensitivity, specificity and diagnostic accuracy .It involves voluntary blood donors during period of two years. Blood Bank, IGIMS, Patna.

Conclusion: NAT could detect HIV cases in blood donor samples which were undetected by ELISA. It's widespread use in blood banks would ensure additional layer of safetyin blood transfusion.

Keywords: HIV, ID-NAT,ELISA,TTIs.

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 prevalence rate of HIV among blood donors in India is around 0.32% and decreasing over the years.[3, 4,5,6] In India National AIDS Control Program (NACP) has been established under

Department of AIDS, Ministry of Health and Family Welfare, Government of India. Ministry of Health and Family Welfare has released a report on HIV Estimations 2012:According to the report, HIV prevalence rate in the country is 0.27%. The overall adultHIV prevalence has declined from 0.41% in 2001 to 0.27% in 2011 and in blood donors is 0.32% .[2,5,6]. 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.[8]Overall, India's HIV epidemic is slowing down with a 32% decline innew HIV infections.[7] 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.[9,10]In India as per the regulatory requirement of the Drug andCosmetics act of 1940, (1st Amendment rules 1992) it is mandatory to test each donatedunit of blood for markers of HIV- I and II, HBV, HCV, Malaria and Syphilis [11]. A single unit of blood or its components may be transfused into as many as 1-4recipients and thereby spreading HIV infection quite rapidly.[12] Due to increased demand of blood transfusion especially in patients receiving multiple blood transfusions such as in Thalassemia, Hemophilia, Road traffic accidentswith major bleeds, Malignancies and Gynaec/Post partum cases with heavy bleeds thereis more chance of transmission of HIV infection to the recipients.[13]. Efficient blood screening for HIV can help save lives by reducing transmissionof HIV infection from donors to recipients.[14]

Individual Donor-Nucleic Acid Testing (ID-NAT) looks for the genetic materialof disease causing organisms and can detect infections in donated blood earlier than routine serology tests, which can either detect an antigen or antibodies to the organismin response to the body's immune system.NAT (Nucleic acid Amplification Testing)is an advanced technology directly detects small amount of a virus before antibodies or viral proteins are detected. NAT assays are highly sensitive and specific as it targets against specific viral nucleic acid sequences. It also detects mutants,occult cases and false negatives from serology.[12]Bloodsafety is a major concern all over the world and NAT (Nucleicacid Amplification Testing) has been adopted in many countries of Europe, USA, CANADA & JAPAN as per blood screening protocols.[15].

Objectives

To observe any discordant results between HIV Nucleic acid Amplification Testing and HIV antibodies ELISA screening and Note relative sensitivity, specificity and diagnostic accuracy**.**

Review of Literature

HIV, the causative agent of AIDS, belongs to the lentivirus subgroup of the[15,16]family Retroviridae. Retroviruses possess a unique enzyme called reverse transcriptase that directs the synthesis of DNA from the viral RNA. The family Retroviridae includes three subfamilies and seven genera, out of which two genera contain viruses that are pathogenic to humans. Genus Lentivirus: Contains human immunodeficiency virus (HIV)-1 and 2. Genus Deltaretrovirus: Contains human T cell lymphotropic virus-1(HTLV-1) HIV is 90-120 nm icosahedral, enveloped RNA virus, which comprises of an outer envelope consisting of glycoprotein gp120 and gp 41,The nucleocapsid has an outer icosahedral shell and an inner cone shaped core,enclosing the ribonucleoproteins. The genome is diploid, composed of two identical single stranded, positive sense RNA copies and the enzyme reverse transcriptase(RT).The core also contains viral enzymes integrase and protease .

MODES OF HIV TRANSMISSION

Efficiency of different routes of HIV transmission and their contribution to totalnumber of

cases.

Table 1:

EXPOSURE ROUTE	PERCENT EFFICIENCY	PERCENTAGEOFTOTAL	
		WORLD	INDIA
Blood transfusion	90-95	5	7.05
Perinatal	20-40	10	15
Sexual intercourse	0.1 to 1	75	74.15(heterosexual) 0.58 (homosexual)
Injecting drugs use	0.5-1.0	10	7.3
Needle stick exposure	<0.5	0.1	0
Others (Tattooing, Organ/ Tissue transplants)	0.2-0.4	0.2-0.4	0.4

Viral replication occurs predominantly in the peripheral lymphoid organs, especially the spleen, lymph nodes and gut-associated lymphoid tissue. Target cells include mature CD4+ T-cells, developing T-cells in the thymus, and the ubiquitous tissue macrophages Binding and Fusion: HIV uses CD4 molecules on the surface of the lymphocytes as a primary receptor. Viral gp120 binds the CD4 molecule on the surface of CD4 T- lymphocyte leading to the conformational changes exposing binding site for co- receptors (chemokine receptors) present on the surface of a CD4 T-lymphocyte. The virus envelope fuses with host cell membrane leading to the release of viral RNA copies in the protoplasm of host cell. Barreto et al., from Brazil during period from 1995-2001, estimated that HIV NAT assays would detect 10.8 unitsin window period among 1 million first time donations .Soldan K et al., in 2003 in United Kingdom, had observed that NAT has reduced the risk of HIV infection by 10%Pragati Chigurupati et al., in blood bank, Rajamundry in Andhra Pradesh in 2012, observed that among 8000 ELISA non-reactive samples from blood donors, 4 were reactive by NAT. In 2012 in New Delhi, Nitin Agarwal et al., in their study tested 73,898 samples for. HIV by both ELISA and NAT & obtained NAT yield of 1 in 610 donations. Chandrashekar Shivaram et al., in their study in 2010 at private blood transfusion centre in documented NAT yield of 1:53260 for HIV In France and Spain in 2005, the HIV NAT yield rates decreased from 0.59 and 2.48 respectively to 0.3/million donations after implementation of NAT among blood(17)donors In a study done in 2013 by Nitin et al., there was estimated risk reduction of the Window period utilizing NATfor HIV by 85%. The overall prevalence of HIV using third generation ELISA was 3.1% in a study by Damulak et al.,in the year 2013 in Nigeria. Another study by Olajubu et al.,(18) in 2009 documented prevalence of HIV as 3.2% among blood donors. In a study by Anuradha et al.,(19) in 2014 in Perambulur, Tamilnadu, among 2964 donors2 (0.07%) were infected with HIV virus. ELISA was used to screen HIV, HBV and HCV. Ghosh K and Mishra K(20) in 2017 calculated the risk of HIV NAT negative but infected blood units slipping away HIV screening process because of infectious window and stressed the importance of donor pre-assessment for selection of an ideal donor.

Material and methods

This study involved voluntary blood donors attending Blood Bank Indira Gandhi institute of

medical sciences, Patna, and Tertiary Centre, Bihar. Study duration of Two years.
Permission from Hospital authorities and ethical clearance from the institutional ethical committee were obtained before starting the study.
All samples were subjected to ELISA for HIV reactivity, irrespective of its results (reactive/non-reactive) were subjected to the reference center for ID-NAT.

Statistical Analysis:
Qualitative data was represented in the form of frequencies and percentages.
Quantitative data was represented using mean & Sd. Validity of the Test in comparison to gold standard was done with Specificity,Sensitivity & Accuracy of the diagnosis.

Inclusion criteria
Donors selected based on selection criteria for healthy donor.
Any donor who is fit and not suffering from any transmittable diseases can donate the blood. Negativity for Transfusion Transmitted Infections(TTIs) being one of them.
Donor must be 18-60 years of age and having a minimum weight of 50Kg.
Donor's hemoglobin level must be minimum 12.5%.A donor can again donate blood after 3 months of last donation of blood. Donor pulse rate must be between 50 to 100/min without any irregularities.

Exclusion criteria
All donors who do not fulfill the criteria for an ideal donor were excluded. (Consumption of alcohol in last 24 hours, HIV and other TTI reactive individuals, Cardiac diseases, Hypertension, Kidney diseases, Epilepsy, Diabetics, woman with bad Obstetric history for first 6 months, under treatment for Malaria for first 3 months, Immunization in last one month period, has recently undergone major surgery or Dental procedure).

Samples with pre-analytical errors like Hemolysis, improper blood – anticoagulant ratio, use of wrong vacutainer/tube and anticoagulant ,partially clotted sample, contaminated sample, improperly preserved sample were excluded.

Direct solid phase antiglobulin ELISA is the method most commonly used.The antigen is obtained from HIV grown in continuous T lymphocyte cell line or by recombinant techniques and should represent all groups and subtypes of HIV 1 and HIV 2.The antigen is coated on microtitre wells or other suitable solid surface. Thetest serum is added, and if the antibody is present, it binds to the antigen .After washing away the unbound serum, antihuman immunoglobulin linked to a suitable enzyme is added, followed by a colour-forming substrate.If the test serum contains anti-HIV antibody, a photometrically detectable colour is formed, which can be read by special ELISA reader.
Test validation:
The individual values of the absortances for the control sera were used tocalculate the mean value if-

$$-0.010 < A\ (neg) < 0.200$$

$$A\ (Pos.) > 1.000$$

Calculation of Cut-off value:
Calculate the mean absorbance of the negative controls, then calculate the cut-offvalue by

adding 0.300

Results

The present study was carried out on 9423 voluntary blood donors attending IGIMS, Patna and tertiary care centre, In Bihar. to document HIV ID-NAT & ELISA test results.
The observations made from the study are shown in the following tables.

Table 2:

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

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,15donors(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.

Table 3:

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

Sex wise distribution of voluntary blood donors sex wise distribution of voluntary blood donorsFrom the above table, males were 9290 (98.6%) and females were 133 (1.4%).

Table 4:

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

Blood groups distribution among voluntary blood donors

Table 5:

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

Distribution of Rh Blood Groups among blood donors

Table 6:

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

Results of HIV ID-NAT among voluntary blood donors

Table 7:

Elisa test	Frequency	Percentage
Positive	20	0.3
Negative	9403	99.7
Total	9423	100.0

Results of HIV ELISA among voluntary blood donors

When compared to ID-NAT, ELISA shows a sensitivity of 90% andspecificity of 99%. Positive predictive value (PPV) of 95% and negative predictive value of 99%. Accuracy of ELISA results was 99%. The ROC curve depicts 97.5% accuracy (Area covered is 97.5%) by ELISA in comparison with ID-NAT. ROC= Receiver Operating characteristics curve, a statistical tool.

Discussion

With over 93 million donations made every year worldwide, blood transfusion continues to save millions of lives each year and improve the life expectancy and quality of life of patients. An attempt has been made to compare two diagnostic methods to detect HIV among blood donors.

Comparison of age group (%) with other studies:

Table 8:

AUTHORS	AGE GROUP (%)
Chatterjee et al	14-30 yrs (62%)
Bareeto et al	18-29yrs (56%)
Sharma et al	15-35 yrs (52%)
Present study	20-29 yrs (64.1%)

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. The least donors were in age group of 60 years & above because many of the people in this group have health issues like hypertension, diabetes, ischemic heart diseases and considered unfit for blood donation.[21]

Comparison of sex ratio (M:F) in other studies:

Table 9:

AUTHORS	SEX RATIO(M:F)
Chatterjee et al	62:1
Sharma et al	40:1
Rao & Annapurna et al	70:1

Rose et al	70:1
Present study	70:1

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.,[12], Rao and Annapurna et al.,[22] and Rose et al.,[23] Decreased female blood donations could be due to physiological issues like menstruation, pregnancy, lactation and high prevalence of anaemia deferrals, though females happen to be the major beneficiaries of blood transfusion(60%).The distribution of Rh groups varies markedly in different races, ethnic groups and socio-economic groups in different parts of the world. From the above table it is quite evident that the percentages of Rh blood groups in Chandra et al., [24], Kaur et al.,[25], Piyush et al.,[26] & Giri et al.,[27] studies varies in different regions of country. The Rh+ve blood group in our study (96.6%) correlates well with the studyby Chandra et. In a study by Mohammadali et al., there was a significant association between 'A' blood group and HIV infection but there was no significant association between HIV infection and Rh status.. The prevalence of ABO blood group in general & Rh typing varies amongdifferent countries & population within the nation. While in countries like Japan &China Rh-ve blood groups are almost non-existant (Rh-ve groups <0.2%) to 15% inCaucasian population. In India, Punjabis have highest prevalence of Rh-ve bloodgroups and it is 5% in Karnataka. The results in our group reflects the sameprevalence. Rh-ve grouping can cause additional stress in blood requirement,prevention of Rh incompatibility by anti-D prophylaxis among women of reproductive age group. The sensitivity, specificity, Negative Predictive Value and Positive Predictive Value in our study were comparable in Sheetal et al.,[28] study where third generation ELISA was employed as in our study. The accuracy of ELISA for testing HIV among blood donors in our study fromROC curve was 99% and was comparable to study by Sheetal et al.[28].

Conclusion

Based on the prevalence study of HIV screening among blood donors by dual testing strategy using ELISA test and ID-NAT test , our study reveals the decreasing trend of prevalence of HIV among blood donors due to increased donor awareness about TTIs. ID-NAT is a reliable method to ensure near cent percent blood safety which looks for the genetic material of disease causing organism and can detect infections in donated blood earlier than routine serological testing. Our results reveal that it could pick up only 2 more extra cases than ELISA. Of more concern are false negativeresults which cannot be picked up & are capable of spreading the infection.

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