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Original Research Paper

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DIAGNOSTIC ACCURACY OF RAPID CARD TEST AGAINST NUCLEIC ACID AMPLIFICATION TEST FOR SCREENING OF HIV INFECTION AMONG BLOOD DONORS

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ABSTRACT

Background- Methods- The present study was conducted at the Blood bank of SRG Hospital in the Department of Pathology, Jhalawar Medical College, Jhalawar, Rajasthan, India. The present study was conducted from 01 August 2020 to 31 July 2021. The total of 1331 blood samples were taken from blood donors aged >18years old, presenting in the Blood bank of SRG Hospitalfor the study. The study was carried out after obtaining approval from the Institutional Research Ethical Committee. An informed and written consent was obtained from all the blood donors. **Results-** Out of total 3 reactive cases diagnosed with NAT in which 2 were also found reactive (True positive) with RCT while one was found non-reactive (False negative). Out of total 1328 non-reactive cases diagnosed with NAT, no false positive was reported by RCT, all cases were true negative. Sensitivity and specificity of RCT against NAT was 66.67%, and 100% respectively. Positive and negative predictive values were assessed to be 100% and 99.2% respectively. Thus accuracy of RCT was found 99.92%. **Conclusion-** The study concluded that RCT is a highly sensitive and specific test in identifying HIV infection in blood donors. So, RCT can be used in place of NAT in resource limited settings with high accuracy. The anotherimportance of our study for a physician is to have an idea about accessibility of a particular blood group, so that a patient can be managed in time during the state of emergencies like trauma, postoperative careand Rh incompatibility. It is required to have knowledge about blood group profile of the society where the patients are to be managed by general physicians. The blood groupavailability depends on donor of that particular blood group in that area.

KEYWORDS : NAT, RCT, HIV

INTRODUCTION

Rapid diagnostic tests (RDTs) are so called as they produce a test result quickly, usually in less than 30 minutes. The broad class of HIV-1/2 RDTs includes lateral-flow (immunochromatographic) and vertical-flow (immunofiltration) assay formats, which detect the presence of HIV-1/2 antibodies and/or HIV p24 antigen. RDTs are not only quick but also are easier to perform than assays that require a laboratory. With training, both health-care professionals and lay providers can perform HIV testing with high accuracy and reliability using RDTs. Most RDTs and their accompanying reagents can be stored between 2 and 30 °C. They do not require any additional equipment and, thus, do not need to necessarily be performed in a laboratory. This means that RDTs are suited for use in both community- and facility-based settings, including sites with limited infrastructure that process low numbers of specimens daily.[1,2]

Nucleic acid testing (NAT) utilizes molecular techniques that may be used qualitatively to assist the diagnosis of HIV infection and quantitatively to monitor the progression of HIV infection and the response to ART. They include NAT technologies that detect the presence of HIV viral nucleic acid (RNA, DNA) via techniques based on amplification of viral nucleic acids, such as polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA), or on amplification of the bound probe signal, as in branched-DNA (bDNA) assays. NAT has the ability to detect very low quantities of viral nucleic acid (high analytical sensitivity); a typical limit of detection for most NAT technologies is at about 50 copies/ml.[3]

Qualitative nucleic acid testing is commonly used for early infant diagnosis (under 18 months of age), given the interference of passively transferred maternal antibody with serological methods. It may also be used to assist in the qualitative diagnosis of adults, including acute infection, when it is validated by the manufacturer as part of the intended use. Quantitative NAT is typically not recommended for diagnosis; as these assays are usually only validated for monitoring the level of virus in HIV-positive individuals, not as a diagnostic assay.[4]

Laboratory-based technologies for NAT require sophisticated equipment, rigorous laboratory conditions and specimen collection, and highly trained staff who can perform precision steps and avoid contamination. Not all NAT technologies detect all HIV-1 subtypes equally well, and certain NAT technologies may not detect HIV-2 well unless they are optimised to do so. Newly developed NAT technologies that are simpler and more robust are intended for use at the point of care and may avoid some of the logistical and technical disadvantages of laboratory-based NAT technologies.[5]

MATERIAL AND METHODS

The study was carried outat the Blood bank of SRG Hospital in Department of Pathology, Jhalawar Medical College, Jhalawar, Rajasthan, India to estimate the proportion of HIV infection among blood donors by Rapid card test and Nucleic acid Amplification Test (NAT); to find the sensitivity and specificity of Rapid card test against NAT; to determine the distribution pattern of ABO and Rhesus (Rh) blood groups among blood donors.

All participants submitted informed consent before enrolment. A hospital based cross-sectional study conducted in Blood bank of SRG Hospital, Department of Pathology, Jhalawar medical college, Jhalawar and R.N.T. Medical College, Udaipur.

Rapid card test was done at Blood bank of SRG Hospital, Jhalawar NAT testing was done at R.N.T. Medical College, Udaipur.

01 August 2020 onwards for one year (duration includes time required for data collection, analysis and report writing).

SUBJECTS

Blood donors who donated blood on Monday and Tuesday of every week during study period.

INCLUSION CRITERIA

All blood bag samples which were obtained from blood donors and sent for NAT testing at R.N.T. Medical College, Udaipur.

EXCLUSION CRITERIA

Haemolysed blood bag samples.

The study was started after approval from Ethical Committee of Jhalawar Medical College, Jhalawar.

METHODOLOGY

After obtaining approval and clearance from the institutional ethical committee, only those blood donors meeting the inclusion and exclusion criteria were enrolled for the study. Informed consent was obtained from each participant.

In this cross-sectional study, a total of 1331 blood samples collected at bloodbank of SRG Hospital, Jhalawar and sent for NAT testing at R.N.T. Medical College, Udaipurwere assessed in the study at our Institute. The samples were collected to estimate the proportion of HIV infection among blood donors by Rapid card test and Nucleic acid Amplification Test (NAT); to find the sensitivity and specificity of Rapid card test against NAT; to determine the distribution pattern of ABO and Rhesus (Rh) blood groups among blood donors.

After enrollment the following parameters were considered and/or measured in all patients: name, age, gender, occupation, address, general physical examination.

STATISTICAL ANALYSIS

The data was collected and complied on M.S.Excel 2020 and data was analyzed using SPSS 20.0 version. Data were analyzed and statistically evaluated using Statistical Package for Social sciences (SPSS)-PC-20 software (version 20, SPSS, Inc, Chicago, IL, USA). Data were presented as mean and standard deviation (SD) for normally distributed continuous variables and as frequencies for categorical variables. Sensitivity, Specificity and Accuracy of Rapid Card Test was assessed against NAT Test for screening of HIV Infection among blood donors.

RESULTS

TABLE NO. 1: DISTRIBUTION OF BLOOD SAMPLES ACCORDING TO RAPID CARD TEST RESULT

RAPID CARD TEST RESULT	NO. OF SAMPLES	PERCENTAGE
NON REACTIVE	1329	99.85
REACTIVE	02	0.15
TOTAL	1331	100.0

Out of total 1331 blood sample, 99.85% samples were non-reactive, whereas 0.15% were reactive in nature.

TABLE NO. 2: DISTRIBUTION OF BLOOD SAMPLES ACCORDING TO NAT RESULT

NAT RESULT	NO. OF SAMPLES	PERCENTAGE
NON REACTIVE	1328	99.77
REACTIVE	03	0.23
TOTAL	1331	100.0

Out of total 1331 blood sample, 99.77% samples were non-reactive, whereas 0.23% were reactive in nature.

TABLE NO. 3: COMPARISON OF RAPID CARD TEST AND NAT FOR HIV INFECTION

TEST RESULT	RAPID CARD TEST RESULT	NAT RESULT
NON REACTIVE	1329	1328
REACTIVE	02	03
TOTAL	1331	1331

Out of total 1331 blood sample, 99.85% samples were non-reactive, whereas 0.15% were reactive in nature by Rapid card test.Out of total 1331 blood sample, 99.77% samples were non-reactive, whereas 0.23% were reactive in nature by NAT test.

TABLE NO. 4: ACCURACY, SENSITIVITY AND SPECIFICITY OF RAPID CARD TEST (RCT) AGAINST NAT FOR HIV INFECTION

RCT	NAT		TOTAL
	REACTIVE	NON REACTIVE	
REACTIVE	02 (TP)	0 (FP)	02
NON REACTIVE	01 (FN)	1328 (TN)	1329
TOTAL	03	1328	1331

TRUE POSITIVE	TRUE NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE
02	1328	0	01

Accuracy = (TP+TN) / (TP+TN+FP+FN) X 100 = 1330/1331 X 100 = 99.92%

Sensitivity = TP / (TP+FN) X 100 = 2/3 X 100 = 66.67%
Specificity = TN / (TN+FP) X 100 = 1328/1328 X 100 = 100%

Positive Predictive Value (PPV) = TP / (TP+FP) X 100 = 2/2 X 100 = 100%

Negative Predictive Value (NPV) = TN / (TN+FN) X 100 = 1328/1329 X 100 = 99.92%

Out of total 3 reactive cases diagnosed with NAT in which 2 were also found reactive (True positive) with RCT while one was found non-reactive (False negative). Out of total 1328 non-reactive cases diagnosed with NAT, no false positive was reported by RCT, all cases were true negative. Sensitivity and specificity of RCT against NAT was 66.67%, and 100% respectively. Positive and negative predictive values were assessed to be 100% and 99.2% respectively. Thus accuracy of RCT was found 99.92%.

DISCUSSION

As there is no cure for AIDS, but the etiological factors by which the infection happens are quite recognized, thus the main stress has been placed to prevent the transmission of HIV. AIDS can be transferred from one person to other by unprotected sex (either homosexual or heterosexual relationships), transfusions of blood and vertically from an infected mother with HIV to the child during her pregnancy, at the time of delivery or breast feeding.

In various health care settings, like blood banks, or hospitals, the main initiative given is to fight against the transmission of HIV that mainly contain the thorough organization of blood products that are used for transfusions.

Various different methods are now available for detecting HIV infection that identifies the anti-HIV antibody or the HIV antigen or both presence. Nucleic acid based tests have been introduced for blood donor screening, that are either transcription mediated amplification or PCR are used for identification of the viral nucleic acid [7]. The two commonly used assays are specialized rapid assays and the Western blot that discriminate between HIV-1 and HIV -2 types.[6] The procedure of screening of the blood supply is a cost-efficient modality that helps in reduction of transmission of HIV, and still the implementation of this technique is not consistent in various developing countries.

In resource-limited settings, rapid diagnostic tests (RDTs) are commonly being used for point-of-care identification of HIV infection.[23] Various easy instrument-free assays that can be done by people who have even received very limited training sessions, and where no lab facilities are needed.

Thus the present study was conducted to estimate the proportion of HIV infection among blood donors by Rapid card test and Nucleic acid Amplification Test (NAT); to find the sensitivity and specificity of Rapid card test against NAT; to determine the distribution pattern of ABO and Rhesus (Rh) blood groups among blood donors.

In present study, we found that according to Rapid card test result for HIV infection, out of total 1331 blood sample, 99.85% samples were non-reactive, whereas 0.15% were reactive in nature. According to NAT result for HIV infection, Out of total 1331 blood sample, 99.77% samples were non-reactive, whereas 0.23% were reactive in nature.

Rapid diagnostic Test (RDT) kits are the commonly used assays for Human immunodeficiency virus (HIV) testing in different countries across the world. In a study by Owusu-ofori et al.[6], advised complement serologic rapid test with Nucleic Acid Test (NAT) in screening of blood donors before blood donation. They mentioned that NAT has various technical and economic limitations. Due to this reason, various studies have been conducted on much affordable HIV RDT kits. The studies have been carried to estimate the sensitivity and specificity of these test kits with different test specimens.

In present study, we also assessed the Accuracy, sensitivity and specificity of Rapid card test (RCT) against NAT for HIV infection. Out of total 3 reactive cases diagnosed with NAT in which 2 were also found reactive (True positive) with RCT while one was found non-reactive (False negative). Out of total 1328 non-reactive cases diagnosed with NAT, no false positive was reported by RCT, all cases were true negative. Sensitivity and specificity of RCT against NAT was 66.67%, and 100% respectively. Positive and negative predictive values were assessed to be 100% and 99.2% respectively. Thus accuracy of RCT was found 99.92%.

Similar to our study, Boadu R et al.[7] tested First Response HIV-1-2 kits using Electro-chemi-luminescence assay (ECLIA) as reference assay. They found that RDT kit showed 100 % sensitivity and 100 % specificity with whole blood specimen and 100 % sensitivity and 82.86 % specificity with serum specimen for the detection of HIV-1. The positive and negative predictive values were found to be 100, 100 and 85.35, 82.86 % for whole blood and serum respectively.

The results were also in accordance with study by Anzala et al.[8] who evaluated Rapid Diagnostic Test (RDT) kits for their sensitivity and specificity, being used for counselling and testing in Kenya and Uganda. They found that RT sensitivity was high for all assays at different sites (97.63-100%).

The Positive Predictive Value (PPV) is the value determined by the proportion of patients with a positive test who have the disease actually, whereas the Negative Predictive Value (NPV) is the value determined by the proportion of patients with a negative test who are without any disease. The performance of rapid diagnostic testing for HIV, in a given population is generally affected by Positive and Negative Predictive values of the RDT kits being used. Thus sensitivity and specificity values achieved from test kit evaluation (as mentioned in manufacturer's instruction manuals) before licensing and marketing of the kit, is not necessarily be achieved in practice.
In present study, Positive and negative predictive values were assessed to be 100% and 99.2% respectively, showing a good reliability of RCT in relation to NAT for diagnosing HIV in blood donors.

In a study by Parekh BS et al.,[9] a PPV of 85.37 % was noted with serum specimen that defines the high False Positives and the low negative prediction values of the test kit as compared to ELISA. This indicates that around 15% of people who were tested with serum specimen on First Response HIV-1-2 RDT havereceivedFalse Positive results. Mehra B et al.[10] sensitivity, specificity, and negative and positive predictive values of the first test were 77.5%, 99.3%, and 98.8% and 86.1%, respectively, taking ELISA as the standard test.

CONCLUSION

The study concluded that RCT is a highly sensitive and specific test in identifying HIV infection in blood donors. So, RCT can be used in place of NAT in resource limited settings with high accuracy. The anotherimportance of our study for a physician is to have an idea about accessibility of a particular blood group, so that a patient can be managed in time during the state of emergencies like trauma, postoperative careand Rh incompatibility. It is required to have knowledge about blood group profile of the society where the patients are to be managed by general physicians. The blood groupavailability depends on donor of that particular blood group in that area.

REFERENCES

1. BhanuMehra, SonaliBhattar, PreenaBhalla, DeeptiRawat, "Rapid Tests versus ELISA for Screening of HIV Infection: Our Experience from a Voluntary Counselling and Testing Facility of a Tertiary Care Centre in North India", International Scholarly Research Notices, vol. 2014, Article ID 296840, 5 pages, 2014.
2. Wei Huang, Wei Wei, Xiao Tian Shi, Tianlun Jiang. The analysis of the detection performance of nucleic acid testing and ELISA for HIV, HBV and HCB. Frontiers in Laboratory Medicine. 2017;4(1):200-202.
3. Patidar GK et al. Distribution of ABO and Rh (D) Blood groups in India: A systematic review. ISBT Sci Series. 2021;16(1):37-48.
4. Legese B et al. Distribution of ABO and Rhesus Blood Group Phenotypes Among Blood Donors at Bahir Dar Blood Bank, Amhara, Northwest Ethiopia: A Retrospective Cross-Sectional Study. 2021 Sep 16;12:849-854.
5. Rana R, Ranjan V and Kumar N (2021) Association of ABO and Rh Blood Group in Susceptibility, Severity, and Mortality of Coronavirus Disease 2019: A Hospital-Based Study From Delhi, India. Front. Cell. Infect. Microbiol. 11:767771.
6. Owusu-Ofori S, Temple J, Sarkodie F, Anokwa M, Candotti D, Allain JP. Predonation screening of blood donors with rapid tests: implementation and efficacy of a novel approach to blood safety in resource-poor settings. Transfusion. 2005 Feb; 45(2):133-40.
7. Boadu R, Darko G, Nortey P, Akweongo P, Sarfo B. Assessing the sensitivity and specificity of First Response HIV-1-2 test kit with whole blood and serum samples: a cross-sectional study. AIDS Res Ther. 2016 Feb 16;13:9. doi: 10.1186/s12981-016-0092-0. PMID: 26889203; PMCID: PMC4756447.
8. Anzala O, Sanders EJ, Kamali A, Katende M, Mutua GN, Ruzagira E, Stevens G, Simek M, Price M. Sensitivity and specificity of HIV rapid tests used for research and voluntary counselling and testing. East Afr Med J. 2008 Oct; 85(10):500-4.
9. Parekh BS, Kalou MB, Alemnji G, Ou CY, Gershy-Damet GM, Nkengasong JN. Scaling up HIV rapid testing in developing countries: comprehensive approach for implementing quality assurance. Am J ClinPathol. 2010 Oct; 134(4):573-84.
10. Mehra B, SonaliBhattar, PreenaBhalla, DeeptiRawat, "Rapid Tests versus ELISA for Screening of HIV Infection: Our Experience from a Voluntary Counselling and Testing Facility of a Tertiary Care Centre in North India", International Scholarly Research Notices, vol. 2014, Article ID 296840, 5 pages, 2014.