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Original Research Article

Seroprevalence among Blood Donors Using Comparison of ELISA and Rapid Screening Methods at a Tertiary Care Hospital

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Abstract:

Introduction: Transfusion of blood and blood components, a special modality of patient management is known to save millions of lives worldwide to reduce morbidity and mortality. Transfusion department plays an important role to screen, monitor and control infections transmitted by blood transfusion. The objective of blood screening is to detect markers of infection in order to provide safe blood and blood components for clinical use.

Materials and Methods: 3000 donations were screened for viral markers namely HIV 1and 2, HBV, HCV by ELISA and Rapid testing methods. Results: 35 out of 3000 donors are HBV positive (1.16%), 7 cases of HIV positive (0.23%), 2 cases positive for HCV among 3000 donors (0.06%) are noted by ELISA method. 5 out of 3000 donors (0.16%) are reactive for HIV, 29 (0.96%) are reactive for HBV, 2 (0.06%) reactive for HCV by rapid method. 5 out of 7 donors (71.4%) are reactive for HIV by rapid screening method.

Conclusion: Screening of donated blood with higher generation sensitive ELISA kits and avoiding Rapid screening methods can help to identify reactive donors accurately. The higher number of false negative results by Rapid tests is of concern to blood safety, hence should not be used routinely in Blood Centre for screening of blood donors.

Keywords: ELISA, Rapid test, Blood Donors, TTI.

Introduction

Transfusion of blood and blood components, a special modality of patient management is known to save millions of lives worldwide to reduce morbidity and mortality [1]. Every year more than 90 million units of blood are collected worldwide [2]. Amongst the complications Transfusion transmitted infections (TTI) are major issue in blood transfusion to the recipients of blood or blood products [3].

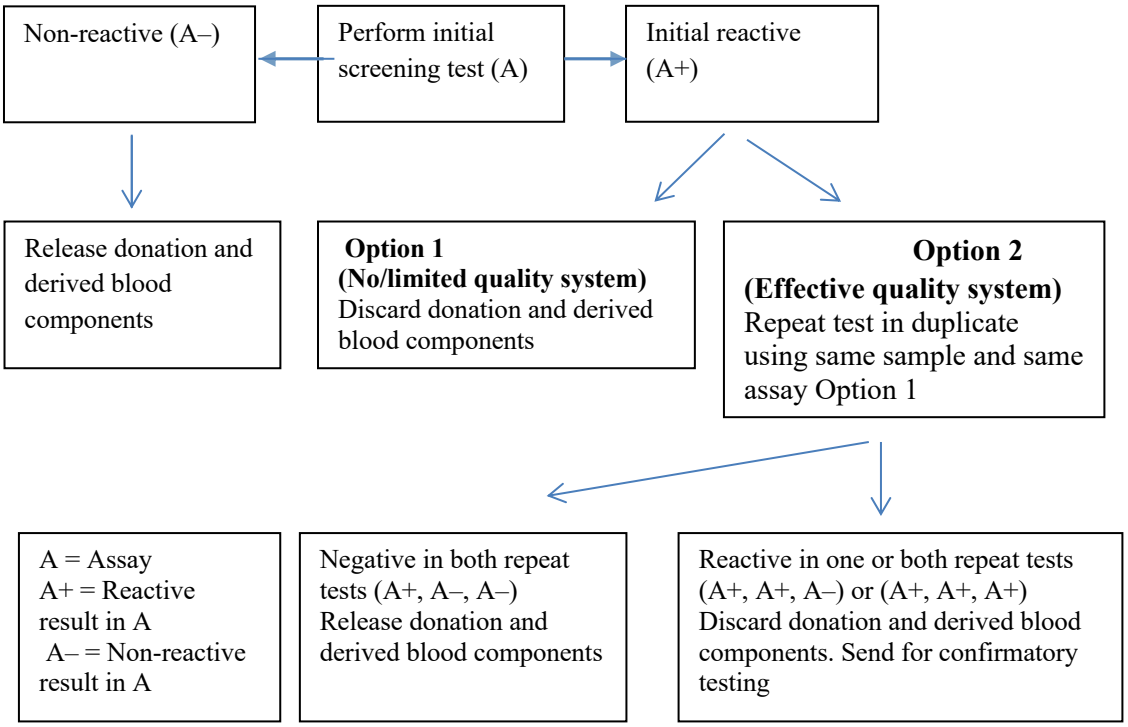
Regardless of testing modality, a nonzero risk of disease transmission still exists [4]. Transfusion medicine is the field that has developed in the second half of the century to reduce lethal effects of transfusion [5]. The priority objective of blood transfusion services is to ensure safety, adequacy, accessibility and efficiency of blood supply at all levels [2]. Transfusion Medicine department plays a pivotal role to screen, monitor and control infections which are transmitted by blood transfusion [1]. Blood Centres and Plasma Manufacturing industries have adopted strategies to reduce the risks of Transfusion transmitted infections which include Donor evaluation, laboratory screening tests and pathogen inactivation procedures as crucial tools [5]. The objective of blood screening is to detect markers of infection in order to provide safe blood and blood components for clinical use [2].

Approach to blood screening is recommended for blood safety, depending on whether or not an effective quality system has been established in the laboratory in which the testing is carried out in terms of testing the duplicate sample [6]. The present study was conducted to compare the efficacy of ELISA test kits and Rapid test kits for screening of blood donors.

Materials and Methods

This prospective study was conducted on all the donors in the Department of Transfusion Medicine at Kamineni Institute of Medical Sciences, Narketpally from August 2018 to November 2018. During this period blood samples from 3000 blood donors were collected and tested for HIV 1& 2 (Microlisa), HCV (Microlisa), Hepatitis B surface Antigen (Hepalisa kits) by third generation ELISA kits (J.Mitra& Co. Pvt. Ltd., New Delhi, India.).

Algorithm for Blood Screening:[6]



The samples which were reactive by ELISA method were tested by rapid test kits HBsAg "Hepacard" (J. Mitra & Co. Ltd), HIV Tridot (J. Mitra & Co. Ltd), and HCV Tridot (J. Mitra & Co. Ltd).

The criteria for selecting the donors for the study included various parameters such as age, blood group, fulfilling the inclusion and exclusion criteria and by evaluating the total number of cases reactive for the viral markers and by comparing the reactive cases with ELISA and Rapid screening method for transfusion transmitted infections.

Inclusion Criteria:

1. Age 18-65yrs
2. Donors of both sex included
3. Weight >45kg
4. Haemoglobin>12.5gm/dl
5. Blood pressure-120/80mmHg

Exclusion Criteria: Blood donors who are unfit to donate blood according to standard blood donors selection criteria (as per NACO guidelines) [7].

Statistical Analysis:

Data was analyzed using SPSS version 19 for calculating Chi-square test for comparison of screening tests. A chi-squared test, also written as x^2 test, is any statistically hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true.

Without other qualification, chi-squared test' often is used as short for Pearson's chi-squared test. Chi–squared tests are often constructed from a sum of squared errors, or through the sample variance. Tests statistics that follow a chi-squared distribution arise from an assumption of independent normally distributed data, which is valid in many cases due to the central limit theorem. A chi-squared test can be used to attempt rejection of the null hypothesis that the data are independent.

Performance of kits used for screening tests was evaluated in terms of Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value defined as follows:

1) Sensitivity is the ability of an assay to detect truly infected individuals and very small amounts of analyte. It can be calculated by following formula.

Sensitivity= [True Positives/ (True Positives + False Negatives)] X 100

2) Specificity is the ability of an assay to correctly identify all the uninfected individuals and there should be no false positives. It can be calculated by following formula.

Specificity= [True Negatives/ (True Negatives + False Positives)] X 100

3) **Positive Predictive Value (PPV) =** It is the ability of a test to identify actually infected individuals

among all the persons giving a positive result with the kit being used. It can be calculated by following formula.

PPV= [True Positives/ (True Positives + False Positives)] X 100

4) **Negative Predictive Value (NPV) =** It is the ability of a test to identify correctly the real non-infected individuals among all the persons giving a negative result with the kit being used. It can be calculated by following formula.

NPV= [True Negatives / (True Negatives + False Negatives)] X 100

Results

A total of 3000 samples were tested for HIV 1 & 2, HBsAg and HCV over a period of August 2018 to November 2018. 99% of the donors are males in the age group of 18-25 years (50.2%). Maximum numbers of donors are O Rh Positive (39.3%) with male predominance (38.9%). 35 out of 3000 donors are HBV positive (1.16%), 7 cases of HIV positive (0.23%), 2 cases positive for HCV among 3000 donors (0.06%) are noted by ELISA method. 5 out of 3000 donors (0.16%) are reactive for HIV, 29 (0.96%) are reactive for HBV, 2 (0.06%) reactive for HCV by rapid method. 5 out of 7 donors (71.4%) are reactive for HIV by rapid screening method. The Chi square statistic for HIV is 1734. The p value is <0.0000001. The result is significant at p<0.05, Sensitivity of rapid test is 71.4% while the Specificity of rapid test is 100%, Positive predictive value is 100% and the Negative predictive value is 99.9%,False negativity by rapid test is 0.06%. 29 out of 35 donors(82.8%) are reactive for HBV by rapid screening method The Chi-square statistic is 2480. The p value is <0.00000001.

The result is significant at p<0.05, Sensitivity of rapid test is 82.8%, while the Specificity of rapid test is 100%, Positive predictive value of the test is 100% and the Negative predictive value is 99.7%, False negativity by rapid test is 0.2%. 2 cases(100%) of HCV reactive by both rapid and ELISA The Chi-square statistic p-value is 1687. The p value is<0.0000001.The test is significant at p<0.05, Sensitivity of the rapid test is 100% while the Specificity of the rapid test is 100%, Positive predictive value is 100% and the Negative predictive value is 100%, False negativity by rapid test is 0%.

Table 1: Age wise and Sex wise distribution of blood donors (N=3000)

Age	18-25 Years (%)	26-40 Years (%)	>40 Years (%)	Total (%)
Males (%)	1481 (49.4%)	1374 (45.8%)	115 (3.8%)	2970 (99%)
Females (%)	25 (0.83%)	4 (0.14%)	1 (0.03%)	30 (1%)
Total (%)	1506 (50.3%)	1378 (45.9%)	116 (3.80%)	3000 (100%)

Table 2: Distribution of blood groups among donors (N=3000)

Blood group	A+ve (%)	A-ve (%)	B+ve (%)	B-ve (%)	AB+ve (%)	AB-ve (%)	O+ve (%)	O-ve (%)	Total (%)
Males (%)	522 (17.4)	43 (1.4)	929 (31)	69 (2.3)	159 (5.3)	8 (0.3)	1166 (38.9)	74 (2.4)	2970 (99)
Females (%)	4 (0.11)	0	8 (0.3)	0	4 (0.13)	0	12 (0.4)	2 (0.06)	30 (1)
Total (%)	526 (17.5)	43 (1.4)	937 (31.2)	69 (2.3)	163 (5.4)	8 (0.3)	1178 (39.3)	76 (2.6)	3000 (100)

Table 3: TTI screening showing positivity with ELISA method:

Elisa	Reactive (%)	Nonreactive (%)	Total
HIV	7(0.23%)	2993(99.7%)	3000
HBV	35(1.16%)	2965(98.8%)	3000
HCV	2(0.6%)	2998(99.9%)	3000

Table 4: TTI screening showing positivity with Rapid method

Rapid Method	Reactive (%)	Nonreactive (%)	Total
HIV	5(0.16%)	2995(99.8%)	3000
HBV	29(0.96%)	2971(99.0%)	3000
HCV	2(0.06%)	2998(99.9%0	3000

Table 5: Comparison of reactive samples with ELISA and rapid test method

	Reactive by Elisa	Percentage Reactivity	Reactive by rapid test	Percentage Reactivity	False negative by rapid test
HIV	7	0.23%	5	0.16%	0.06%
HBV	35	1.16%	29	0.97%	0.2%
HCV	2	0.06%	2	0.06%	0

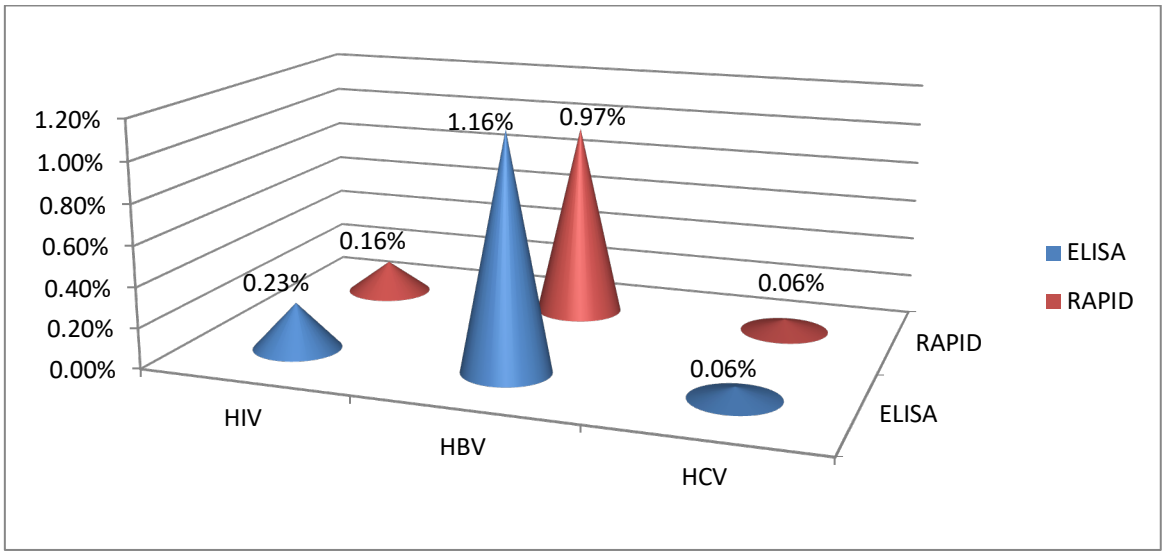


Figure 1: Bar diagram showing comparison of both Screening tests

Table 6: Comparison of HIV positive results

HIV	Test Positive by Elisa	Test Negative by Elisa	Total
Test Positive by Rapid	5(a)	0(b)	5
Test Negative by Rapid	2(c)	2993(d)	2995
Total	7	2993	3000

Table 7: Comparison of HBV positive results

HBV	Test Positive by Elisa	Test Negative by Elisa	Total
Test Positive by Rapid	29(a)	0(b)	29
Test Negative by Rapid	6(c)	2965(d)	2971
Total	35	2965	3000

Table 8: Comparison of HCV positive results

HCV	Test Positive by Elisa	Test Negative by Elisa	Total
Test Positive by Rapid	2(a)	0(b)	2
Test Negative by Rapid	0(c)	2998(d)	2998
Total	2	2998	3000

Table 9: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value

Viral markers	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	False negatives (%)
HIV	71.4	100	**100**	**99.9**	**0.06**
HBV	**82.8**	**100**	**100**	**99.7**	**0.2**
HCV	**100**	**100**	**100**	**100**	**0**

Discussion

With every unit of blood, the risk of transfusion associated problems is 1% including Transfusion transmitted infections. Risk of TTI has reduced in high income nations over the years, because of extraordinary success in preventing HIV and other transfusion transmitted viruses from entering the blood supply [1]. Globally quantitative immunoassays (EIA, ELISA, PCR etc) are considered to be more sensitive tests, widely used at almost all central blood banks. Rapid tests are intended for qualitative detection of the viral markers [8].

Table 10: Prevalence of HIV, HBV, and HCV by ELISA in comparison to other studies

	Sample size (N)	HIV (%)	HBV (%)	HCV (%)
Sangeeta Pahuja et al[9] (2006)	28956	0.56%	2.23%	0.66%
Leena MS et al[10] (2012)	6939	0.27%	0.71%	0.14%
NiraliS et al[11] (2013)	92778	0.162%	0.977%	0.108%
Tulika C et al[12] (2014)	180371	0.08%	0.24%	0.001%
Pragnesh J. Patel[13](2014)	15368	0.14%	0.38%	0.06%
Bhawna Sethi et al [14](2014)	7884	0.19%	0.63%	0.20%
Yadav BS et al [15](2015)	4007	0.14%	1.77%	0.09%
Neetukukar et al[16] (2017)	11879	0.36%	1.06%	2.7%
Davendra Swarup et al[17] (2018)	34342	0.11%	1.74%	1.50%
Present study	3000	0.23%	1.16%	0.6%

Yadav BS et al [15], Sangeeta Pahuja et al [9], Davendra Swarup et al [17] studies showed a similar observation where HBV is the commonest transfusion transmitted infection. Pragneshj Patel et al [13] study observed HBV (0.38%) to be more prevalent followed by HIV (0.16%). Nirali et al [11] study also observed that HBV (0.977%) has more prevalence than that of HIV (0.162%) and HCV (0.108%). Tulika et al [12] study observed that HBV (0.24%) has more prevalence than compared to HIV (0.08%) and HCV (0.001%). Bhawna Sethi et al [14], Leena MSet al [10] studies also observed the same with more prevalence towards HBV.

The present study is similar to the all the studies mentioned above in comparison to the prevalence of HBV (1.16%) being more followed by HIV (0.23%). India has been placed in the intermediate zone of prevalence of Hepatitis B by World Health Organization (2-7% prevalence rates) and has been estimated to be home for over 40 million HBsAg carriers [1]. The prevalence of HBV infection is lower in United States and Western Europe (0.1-0.5%) and is reported to be higher 5-15%, in Southeast Asia and China [1].

Despite the fact that a safe and effective vaccine has been available since 1982, the HBsAg prevalence in India remains high. This is mainly because hepatitis B vaccination is not a part of our national immunization program. Gupta et al [1] study observed more anti HBC positivity than HBsAg suggesting the ability to detect HBV infection in window period. For HIV, India is second to South Africa with respect to number of people living with HIV. In India, NACO reported overall prevalence of 0.91% in 2005 with 0.25% in Delhi [16]. Globally, the highest prevalence of HIV has been reported in Sub-Saharan Africa at 7.4% 9. The present study showed an HIV seroprevalence of 0.23%. WHO report states that viral dose in HIV transmission through blood is very large that positive transfusion leads to death, after 2 years in children and 3 to 5 years in adults on an average [9].

HIV detection by ELISA method has window period of 2-8 weeks. During this time, person remains falsely negative. Nucleic acid test helps to identify reactive samples in window period but not cost effective. The most effective way to minimize TTI is to reduce the blood usage by rationale use of blood and taking donations from safer donor groups such as safe transfusion practices by avoiding single donors and practicing autologous blood transfusions should be encouraged [13].

The wide variations of HCV seroprevalence in India are due to the use of different generation of ELISA kits with different sensitivities and specificities. Garg et al [1] study reported lower prevalence of HCV with being similar to other studies in the donors of western India. Prevalence of TTI's in blood donors varies from place to place due to difference in environmental conditions and also due to poor hygienic and health conditions. [17]

Table 11: Comparison of ELISA with Rapid test for detection of HIV, HBsAg, and HCV among blood donors with respect to other studies

	Sample size	ELISA reactivity (%)			Rapid test reactivity (%)			False negativity (%)		
		HIV	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV
Torane VP et al[18] (2008)	60	50.0	50.0	50.0	21.6	21.6	--	28.3	28.3	50.0
Khan JK et al[19] (2010)	68	--	--	77.9	--	--	36.8	--	--	41.1
Khan JK et al[19] (2010)	57	--	66.6	--		35.1		--	31.6	
Bhanu Mehra et al[20](2014)	787	5.08	--	--	4.58	--	--	0.50	--	--
NeetuKukar et al[16] (2017)	11879	0.36	1.06	2.7	0.15	0.8	1.9	0.21	0.26	0.8
Present study	3000	0.23	1.16	0.06	0.16	0.97	0.06	0.06	0.2	--

Present study deals with evaluation of performance of both ELISA and rapid assay for detection of three major blood borne pathogens namely HIV, HBV, and HCV using separate panel sera for each. Torane et al [18] study observed that rapid test results are inferior compared to ELISA for all the three viral markers tested with a false negativity by rapid test being 50%, where both modalities were used to screen healthy blood donors for all markers and RDT missed 17 out of 30 samples confirmed reactive by ELISA. Khan JK et al [19] study on 68 donors for HCV evaluation showed that ELISA (77.9%) is superior to rapid test (36.8%) with a high false negative value by rapid test (41.1%). Khan JK et al [16] study on 57 donors for HBV evaluation showed that ELISA (66.6%) is superior to rapid test (35.1%) with a high false negative value by rapid test (31.6%). In a study by Bhanu mehra et al [20] on 787 donors showed 5.08% positivity by ELISA and 4.58% reactivity by rapid test, false negativity being 0.5%. Neetu kukar et al15 study conducted in the year 2017 showed that ELISA is more reliable than rapid tests for screening of infections HIV, HBV, HCV. H Kaur et al [16] study also concluded low sensitivity of rapid tests, in concordance to B Mehra et al20 study done on HIV 1 and 2 with sensitivity and specificity of 77.5% and 99.3%. Certain studies showed that rapid tests are easy to use and more convenient. A Pakistani study showed 100% sensitivity of latex agglutination and immunochromatographic technique with a specificity of 91.7% and 99.2% for HBsAg [8]. In a study from India the rapid kits of HBsAg were found to be 100% specific and 93.4% sensitive [21]. In another study from Seoul, using rapid technique showed 97% sensitivity and 100% specificity for detecting HBsAg [21]. Both ELISA and rapid tests are widely employed immunological assays for serodiagnosis of TTI's. Discrepancy between results obtained by the two techniques is common [20].

An ideal rapid test should have high degree of positive predictive value (PPV) and low degree of false negative results [20]. Discordance between ELISA and rapid test could be due to low antibody titres especially in recent infections where the levels may well be below the detection limit of rapid test but are picked up by the more sensitive enzyme immunoassay and its spectrophotometric format of result analysis [22].

Conventional enzyme linked immunosorbent assay is most referred screening technique and possibly an accuracy of 99.9% with improved sensitivity but some of the kits have reported to have lower specificity. Additionally this method is laborious, time taking and needs proficient skill to perform and also not available in many blood banks. Comparatively rapid test are easier, quicker and require less skill to perform and no requirement of instruments [23]. The present study is similar to all the studies mentioned above in respect to that ELISA is more promising than rapid which has a higher false negative value.

Conclusion

HBV is the most common TTI among apparently healthy donors, followed by HIV and HCV. Strict and proper implementation of donor selection criteria and thorough history and examination should be followed. Screening with higher generation sensitive ELISA kits and avoiding rapid screening methods can help to identify reactive donors accurately. This may help to avoid transfusion of infectious whole blood and blood products, especially in patients requiring repeated transfusions as a part of therapy.

This study also indicates that ELISA is more sensitive than rapid tests for screening of infections like HIV, HBV, and HCV. The higher number of false negative results by Rapid tests is of concern to blood banks, hence should not be encouraged in Blood centre for routine screening blood donors.

In blood centres of India, ELISA still remains the appropriate assay for screening. Improving the sensitivity of Rapid kit will help resource at a basic setting with limited resources. With proper evaluation of the mechanism of the kits will help ensuring availability of quality commercial kits thus decreasing the infections transmitted by Blood Transfusion.

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