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RESEARCH ARTICLE

SEROPREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS IN BLOOD DONORS OF WESTERN PART OF INDIA USING THIRD AND FOURTH GENERATION ENZYME LINKED IMMUNOSORBENT ASSAY

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ABSTRACT

Background: The percentage of HIV cases due to blood transfusion has decreased significantly in the last 2 decade. The newer 4th generation enzyme linked immunosorbent assay (ELISA) has been shown to have increased detection rate compared to 3rd generation ELISA.
Objectives: To estimate the seroprevalence of HIV among blood donors using 4th generation ELISA assay and to compare it with the 3rd generation ELISA.
Materials and Methods: This retrospective and prospective study involved 32,360 blood donors- 25,420 were voluntary donors and 6,940 were replacement donors. All blood units were tested with 3rd as well as 4th generation ELISA. All samples found reactive or in grey zone with either 3rd or 4th generation ELISA were retested by re-ELISA & Rapid HIV Card Test.
Results: The seroprevalence of HIV was estimated to be 0.55/1000 donations (0.05%) with 3rd generation ELISA compared to 0.89/1000 donations (0.09%) with 4th generation ELISA. The seroprevalence of HIV among voluntary donors was estimated to be 0.47/1000 donations (0.04%) with 3rd generation ELISA and 0.74/1000 donations (0.07%) with 4th generation ELISA. The prevalence of HIV among replacement donors was 0.86/1000 donations (0.08%) with 3rd generation ELISA and 1.44/1000 donations (0.14%) with 4th generation ELISA.
Conclusion: 4th generation HIV ELISA detects a higher number of seroreactive donors compared to 3rd generation ELISA. However, larger studies are required with confirmatory tests for both 3rd and 4th generation ELISA for making any policy changes.

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INTRODUCTION

Transfusion associated HIV/AIDS is defined as "AIDS" occurring in a person who has received transfusion after 1977, but has no other risk factors for HIV infection (Mollison and Engelfriet, 2005). Transmission of HIV through blood and blood products can be reduced to a great extent by efficient and reliable screening of the blood to be transfused by confirmatory diagnostic ELISA testing. The 4th generation ELISA assays simultaneously detect antibodies against HIV-1 and 2 and the presence of p24 antigen and thus shorten the window period to about 14 days, as compared to about 22 days with 3rd generation Enzyme linked immunosorbent assay (ELISA) assay (Weber, 2006).

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This study was undertaken to estimate the seroprevalence of HIV among blood donors at a tertiary care institute in India using a 4th generation ELISA (antigen + antibody) assay and to compare it with the 3rd generation ELISA (antibody) assay.

MATERIALS AND METHODS

The Department of Pathology, P.D.U. Medical College & Hospital- Rajkot collects approximately 15,000 units of blood annually. Of these, approximately 11,000 are voluntary donors and 4,000 are replacement donors. Among these we selected blood donors from January 2013 to June 2015 time period for taking incidence of HIV seroprevalence among blood donors. The donors were divided into two groups - voluntary donors and replacement donors. The test was performed on serum and it was separated from the clot as soon as possible to avoid any hemolysis. Specimens with observable particulate matter were

centrifuged prior to testing as suspended fibrin particles or aggregates may yield false reactive results.

MATERIALS AND METHODS

As per Drugs and Cosmetics Act (3rd amendment 2001) (Mallik, 2003), Govt. of India, all blood units were tested for HIV antibodies using 3rd generation ELISA (Enzaids – HIV-1+2 microwell ELISA kits manufactured by Span Diagnostic Pvt. Ltd.). In addition all donors were screened with 4th generation HIV Ag-Ab ELISA (Merilisa - HIV advance 4th generation microwell ELISA kits manufactured by Meril Diagnostic Pvt. Ltd). All samples found reactive or in grey zone with either 3rd or 4th generation ELISA were retested by Rapid HIV Card Test (Immunochromatographic Assay for HIV-1 & HIV-2 manufactured by Reckon Diagnostic Pvt. Ltd).

Calculation and Interpretation of the Results

The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/or HIV-2 was determined by comparing the absorbance measured for each sample to the calculated cut-off value. Samples with absorbance values less than the cut-off value were considered to be ELISA non-reactive. Sample with absorbance values equal to greater than the cut-off value were initially considered to be ELISA reactive. Sample with absorbance values within 10% of cut off value were considered in grey zone. Samples initially found in grey zone were retested by re-ELISA test and if again found in grey zone were termed as possibly reactive and included in analysis.

All samples found reactive or possibly reactive with either 3rd or 4th generation ELISA were further tested by Rapid HIV Card Test (Immunochromatographic Assay for HIV-1 & HIV-2 manufactured by Reckon Diagnostic Pvt. Ltd). The results of Rapid Card Test were interpreted as reactive or non-reactive individually as a HIV-1 and HIV-2. All the details regarding demographic profile of the donors (age, sex, number of donations), whether voluntary or replacement donors and the results of HIV seroreactivity with 3rd or 4th generation ELISA was recorded. HIV seroprevalence among blood donors was estimated by both the 3rd and 4th generation ELISA as percentages with confidence limits of 95%. Performance of 4th generation ELISA was compared against 3rd generation ELISA using chi square test.

RESULTS

Out of the 32,360 samples tested, 18 were found to be seroreactive for HIV using 3rdgeneration ELISA and result of 3 samples were in grey zone. On repeat testing, these 3 samples were negative, thus giving a prevalence of 18/32360 i.e; 0.55 per 1000 donations (0.05%) with 3rd generation ELISA. Combining the results of the subgroups showed the seroprevalence of HIV among voluntary donors to be 0.47/ 1000 donations (12/25420 donations or 0.04%) and the prevalence of HIV among replacement donors was 0.86/ 1000 donations (6/6940 donations or 0.08%). There was statistically significant difference in HIV seroprevalence between replacement and voluntary donors, there were approximately double prevalence among replacement donors. Of the 32,360 samples, 25 were found to be seroreactive for HIV using 4th generation ELISA and result of 4 samples were in grey zone.

Table 1. 3rd Generation ELISA and retested by Rapid HIV Card Test of reactive samples by 3rd Generation ELISA (*Source*: P. D. U. Medical College & Hospital, Blood Bank – Rajkot 2015)

Blood Donors	Total Number of Tested Donors	HIV Reactive by 3rd Generation ELISA	HIV Reactive by Rapid HIV Card Test	Prevalence (per 1000 Blood Donors) by 3rd Generation ELISA	% of Seroreactivity by 3rd Generation ELISA
Total Donors	32360	18	16	0.55	0.05
Voluntary Donors	25420	12	10	0.47	0.04
Replacements Donors	6940	6	6	0.86	0.08

Table 2. 4th Generation ELISA and retested by Rapid HIV Card Test of reactive samples by 4th Generation ELISA (*Source*: P. D. U. Medical College & Hospital, Blood Bank – Rajkot 2015)

Blood Donors	Total Number of Tested Donors	HIV Reactive by 4th Generation ELISA	HIV Reactive by Rapid HIV Card Test	Prevalence (per 1000 Blood Donors) by 4th Generation ELISA	% of Seroreactivity by 4th Generation ELISA
Total Donors	32360	29	21	0.89	0.09
Voluntary Donors	25420	19	13	0.74	0.07
Replacements Donors	6940	10	8	1.44	0.14

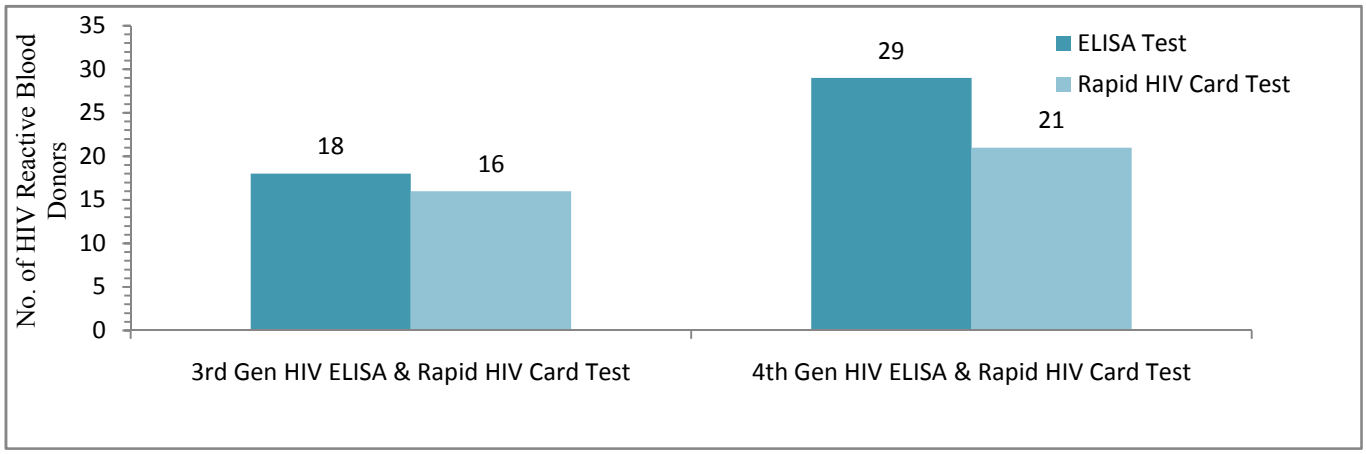

Table 3. Results of various studies from India estimating HIV seroprevalence among Blood Donors

Authors	Years	Donors (n)	Seroreactivity	Test used	Province
Present study	2013-15	32,360	0.89/1000	ELISA 4th Gen	Rajkot
Sudha, *et al.*	2003-2004	23,609	47/1000 HIV1 2/1000 HIV2	ELISA and RT-PCR	Hyderabad
Thakral, *et al.*	2003-05	39,764	1.6/1000	ELISA and WB	Chandigarth
Choudhury, *et al.*	1993-98	65,288	0.2/1000	ELISA and WB	UP
Bhushn, *et al.*	1988-93	14,084	1.9/1000	ELISA and WB	Tamilnadu
Khurana, *et al.*	1988-97	79,553	0.8/1000	ELISA and WB	Punjab
Kapoor, *et al.*	1989-97	3,33,054	0.72/1000	ELISA and WB	Delhi
Sharma, *et al.*	1996-2002	2,35,461	3.0/1000	ELISA	Chandigarth
Nanu, *et al.*	1989-96	1,32,093	5.5/1000	ELISA	Delhi
Makroo, *et al.*	1989-93	5,66,928	3.0/1000	ELISA	Delhi

On repeat testing, these samples were again seen in grey zone (possibly reactive), thus giving a prevalence of 29/32360 i.e.; 0.89 per 1000 donations (0.09%). Although 4th generation ELISA could detect significantly higher number of seroreactive samples against 3rd generation ELIZA (29 vs 18 per 32360 donations), yet the difference in seroprevalence expressed per 1000 donations was statistically not significant (0.55/1000 Vs 0.89/1000 donations). Shows the number of donor samples reactive for HIV in individual groups using both 3rd and 4th generation also retested by Rapid HIV Card Test. Comparing 3rd generation ELISA with Rapid Card Test, it was seen that of the 18 samples found reactive with 3rd generation ELISA, 16 were confirmed to be reactive and 2 were non-reactive with Rapid HIV Card Test. A similar comparison between 4th generation ELISA and Rapid Card Test observed that of the 29 samples found reactive or possibly reactive with 4th generation ELISA, among them 21 were Rapid HIV Card Test reactive, 8 were non-reactive. Shows the comparison of ELISA reactive samples using 3rd generation ELISA and 4th generation ELISA with Rapid HIV Card Test.

Additional yield with 4th generation HIV ELISA

Of the 11 samples which were tested non-reactive with 3rdgeneration ELISA, 8 were tested reactive and 3 samples were found to be possibly reactive with 4th generation ELISA.

DISCUSSION

HIV can be easily transmitted by blood transfusion. Study shows that up to 95% of persons receiving HIV seroreactive blood become infected (hp://www.nacoonIine.org). Recent findings demonstrate that in primary HIV infection, random blips of low level viremia occur which can last up to 25 days with HIV concentration in plasma between 1 and 10 copies/ml (Weber, 2006). Plasma donations during this stage can be infectious though HIV transmission through sexual contact is relatively improbable, since the threshold for heterosexual transmission of HIV is 1500 copies/ml. It is thus important for us to reduce the transfusion transmitted HIV to minimum possible limits and to stop further addition to already growing population of PLHA (people living with HIV/AIDS). The most critical component of blood safety is the screening of blood for infectious markers. Testing blood donors for HIV was introduced in 1985 (Barbara and Dow, 2009) and has been mandated for blood screening ever since. HIV kits have undergone a considerable range of performance improvements over this time with the aim of shortening the window period between infection and the detection of HIV. In the late 1990s, fourth generation or combined antigen/antibody ELISA assays were introduced, which incorporate in a single assay the advantages of sensitive anti-HIV detection as well as p24 antigen detection (Perry *et al*., 2008). The p24 antigen is detectable in blood several days before anti-HIV appears. This window period can be shortened to about 2 weeks using p24 antigen assays. In the present study, HIV seroprevalence was estimated to be 0.55 per 1000 donations using 3rd generation ELISA. Alvarez *et al*. (2002) estimated the HIV incidence rate to be 3.23 per 100,000 donor-years in Spain using 3rd generation kits. The HIV incidence among blood donors is much lower in our study probably because of better donor education and increased donor awareness. In our study, although seroreactivity was less in voluntary as compared to replacement donors, the results were not statistically significant.

Using the 4th generation ELISA, HIV seroprevalence was estimated to be 0.89 per 1000 donations. Sudha, *et al*., evaluated the TRI-DOT Rapid HIV test for the early detection of human immunodeficiency virus (HIV) infection in comparison with a 4th generation ELISA (Vironostika HIV Uniform II) in 23,609 samples between January 2003 and April 2004. In the case of discordance, sera were retested by Western Blot, and qualitative RT-PCR. The seroprevalence is high as compared to our study i.e. 0.09% because of the author′s study is from a region of high HIV prevalence (prevalence of HIV in Andhra Pradesh is 0.9% vs 0.09% in blood donors at Rajkot) (NACO, 2012). In our study, of the 16 samples which were ELISA reactive and Rapid HIV Card Test reactive using 3rd generation ELISA, all were found to be reactive with 4th generation ELISA.

It is estimated that use of combined antigen-antibody assay would give a better result as for retesting of those which are tested reactive by the current 3 rd generation assays. Of the 29 samples reactive or possibly reactive with fourth generation ELISA in the present study, 21 were also reactive with Rapid HIV Card Test. However, true yield cannot be estimated without repeat fourth generation ELISA testing. Limitations of our study include NAT or Western blot was not applied to confirm the results of 4th generation ELISA. Another limitation of the 4th generation ELISA is the relatively high false reactive rate (Ly *et al*., 2001). This may be of special concern in low HIV seroprevalence regions and paradoxically may have a negative impact on the blood donation services (Sickinger *et al*., 2008).

Conclusion

To conclude, this study includes comparing the HIV seroprevalence among blood donors with 3rd and 4th generation ELISA and Rapid HIV Card Test on the same donor population. Results of our study show better performance of 4th generation ELISA compared to 3rd generation ELISA in terms of HIV seroreactivity. Although both 4th generation ELISA and NAT are suitable for testing sizeable number of samples, can be easily adapted to automated platforms and have high stability, the former offers an advantage over NAT in that it is relatively less expensive and simple to perform.

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