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Prevalence of HIV Sero-positivity in Blood Donors of Kolar District : 10 Years' Study

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Abstract

Background and Objectives: In India, about 7% of Human Immunodeficiency Virus (HIV) infection spreads through unsafe Blood Transfusion. Often blood donors are unaware till their blood unit tests reactive for HIV. This study finds out prevalence of HIV 1 & 2 amongst blood donors. **Material and Methods:** 23,624 blood units from voluntary and replacement donors screened by ELISA. Reactive units confirmed by Western Blot. **Results:** 1 hundred blood units, all from male replacement donors, tested reactive for HIV by ELISA, which were later confirmed by western blot. The disadvantaged population need education and motivation regarding voluntary blood donation. With no history of "risk behaviour", non-sexual transmission of HIV / AIDS needs exploration as a possible source.

Introduction

Transmission of HIV and other viral infections continues to be a threat to safe blood transfusion. Developing countries account for more than 90% of all new HIV cases. With the population exceeding 1 billion, HIV epidemic has remarkably impaired the economy of India and health of its people. Concealing the medical history by professional or replacement donor poses a great threat to safe blood supply. Problems are also due to prevalence of asymptomatic carries in the society, blood donations during window period, genetic variability in the viral strains and laboratory errors,

In India, safe blood transfusion still remains an issue of major concern. The first step towards blood safety is to encourage blood donations that are voluntary, non-remunerated and obtained from low-risk and regular donors (repeat donors). Despite careful donor selection, some donors may be sero-positive for HIV. Rigorous screening of all donated blood is, therefore, required to ensure the supply of safe blood. In a notification issued in July 1989 by the Government of India (Drugs and Cosmetics Act 194-IVth Amendment rules, 2001), it was made mandatory for every licensed blood bank to get every blood unit tested for freedom from HIV antibodies by ELISA, which now is an established approach in sero-diagnosis of HIV 1 & 2 infection. WHO recommends Testing Strategies for HIV screening, to maximize accuracy while minimizing the cost. For the objective of transfusion safety, Strategy - I is to be applied, where one combined HIV 1 & 2 ELISA assay needs to be done.

According to estimates from WHO and UNAIDS there were 40 million people around the world living with HIV at the end of 2001. During 2001 it has been estimated that there were 5 million new HIV infections and 3 million deaths due to HIV / AIDS. It has also been estimated that about 14 thousand new HIV infections occurred every day in the year 2001. More than 95 per cent of these were in developing countries, 2000 were in children under 15 years of age and about 12000 were in persons aged 15 to 49 years, of whom almost 50 per cent were women. The

epidemic is now spreading rapidly in Asia, where new infections are increasing faster than anywhere else in the world. AIDS first appeared in South East Asia in the 1980s. By 1997, over 65,000 cases had developed AIDS and an estimated 3.75 million HIV - infected persons were reported. By the end of 2001, WHO and UNAIDS estimated that approximately 6.1 million people living with HIV / AIDS and more than 135,000 cases have been reported in South East Asia.

India has the second highest number of estimated HIV infected people. The significant levels of HIV / AIDS in India suggest that the country is now at an advance stage epidemic. Epidemiological analysis at the end of year 2000 shows that:

1. Estimated number of HIV infected persons are 3.86 millions.
2. Predominant mode of transmission of infection in AIDS patients is through heterosexual contact (80.86%) followed by blood transfusion and blood product infusion (5.52%), IDUs (5.30%) and perinatal transmission 0.72 per cent and others 7.60 per cent.
3. Males account for 77 per cent of AIDS cases and females 23 percent (a ratio of 3:1).
4. Estimated aggregate costs of HIV / AIDS by the year 2000 were \$11 million (5% of India's GDP).

Material and Method

Blood Bank at Sri Devaraj Urs Medical College, and R.L. Jalappa Hospital and Research Centre, Tamaka, Kolar was started at January 1999. All blood donations, received between January 1999 to December 2006, from voluntary and replacement donors of either sexes, with ages ranging from 18 to 60 years, were included in this study. As per the Drugs & Cosmetics Act of India, guidelines were stringently followed for careful donor selection and mandatory screening of every blood unit for HIV - 1 & 2, Hepatitis B & Hepatitis C by ELISA, in addition to VDRL and Malaria. All the donated blood units were screened for HIV - 1 & 2 by Enzyme-linked Immunosorbent Assay (ELISA) using Vironostika HIV Uni-Form II Ag/Ab ELISA kit. It is a 4th generation HIV screening test based on a 3-step 'sandwich' method, that detects anti-HIV-1, anti-HIV-2 and anti-Hiv-1 group 0 antibodies as well as HIV-1

antigen in human serum/plasma. Test methodology was strictly followed as per kit insert. To confirm the presence of antibodies against HIV-1 & 2, HIV western blot (J.Mitra Co., India) was used. Test methodology was strictly followed as per kit insert.

Results

Table 1 From January 1999 to December 2006, 23,624 blood units were collected. Of these 98% were 1st time

Table 1 Shows the annual distribution of blood donors at R.L.J.H.		
Sl. No	Annual Distribution	No. of Donors
01	1997	500
02	1998	1500
03	1999	2003
04	2000	2100
05	2001	2565
06	2002	2887
07	2003	3150
08	2004	3353
09	2005	3560
10	2006	2400
Total		25,624

donors, **Table 2** shows the sex distribution amongst the blood donors of R.L. Jalappa Hospital and Research Centre with only 24 adult females. This calls for more motivation

Table 2 Shows the Gender distribution amongst the blood donors of RLJH		
Sl. No	Gender	No. of Donors
01	Male	25,601
02	Female	23
Total		25,624

among the female population to donate blood. **Table 3** shows number of sero positive cases according to voluntary and replacement donors. HIV sero positivity was predominantly seen in male replacement donors but in

Table 3

Shows the total number of HIV reactive blood donors

Sl. No.	Year	Total HIV Donors	Replacement HIV Donors	Voluntary HIV donors
01	1997	0	0	0
02	1998	3	3	0
03	1999	5	3	0
04	2000	10	9	1
05	2001	11	8	3
06	2002	11	9	3
07	2003	11	7	2
08	2004	14	14	1
09	2005	16	17	1
10	2006	19	18	1
		100	88	12

Table 4

Shows the age distribution amongst HIV reactive donors at RLJH

Sl. No.	Age group	Number of donors
01	18-22 years	10
02	23-27 years	18
03	28-32 years	30
04	33-37 years	40
05	38-42 years	02
06	43-47 years	00

contrast to other studies, a significant proportion of voluntary donors show HIV sero positivity. This highlights 2 important facts: 1) The so called voluntary donors could be actually professional donors who were paid by the patient's relatives. 2) Wide prevalence of HIV sero positivity as this area falls within the high risk zone for HIV / AIDS disease. Hence more concerted efforts must be made to detect such cases. **Table 4** shows maximum sero-prevalence of HIV in 33-37 age group which is economically and sexually an active age group. This finding has an alarming consequence to the local socio-economic scenario of Kolar district. **Table 5** shows that around 50% of the HIV positive blood donors in our study had a body weight

Table 5

Shows the Weight distribution (kgs) amongst HIV reactive donors at RLJH

Sl. No.	Weight (kgs)	Number of donors
01	45-50	20
02	51-55	30
03	56-60	40
04	61-65	10
05	66-70	00

Table 6

Shows the vocational profile amongst HIV reactive donors at RLJH

Sl. No	Vocational profile	Number of donors
01	Agriculture	37
02	Students	15
03	Transport (Driver, Mechanics)	48

Table 7

Shows the mode of Transmission amongst HIV reactive donors at RLJH

Sl. No.	Mode of Transmission	Number of donors
01	Sexual	45
02	Blood transfusion, Surgical & Dental procedures etc.	15
03	Unknown/ unaware	40

ranging from 45-50 kg. Though, within the permissible limit, the weight of these donors were apparently towards the lower limit. Studies by Batterham *et al*¹ have shown that viral load had a significant association with weight loss in HIV reactive cases. As the facility to determine viral load is not available with us, further correlation of this observation could not be done. **Table 6** shows the vocational profile of HIV sero positive blood donors in our study. Although 48% of the patient population came from transport and transport related services such as mechanics and attenders. A significant 15% were college students showing the high risk behaviour including unsafe sexual practices and drug abuse. **Table 7** shows the mode of transmission of HIV amongst the blood donors. Although sexual mode of

Table 8

Shows the level of education amongst HIV reactive donors at RLJH

Sl. No	Education	Number of donors
01	Un-educated	15
02	Primary education	55
03	Higher Secondary / Beyond	30

Table 9

Shows the Marital status amongst HIV reactive donors at RLJH

Sl. No.	Marital status	No. of donors
01	Married	55
02	Un-married	45

Table 10

Shows the comparative data of HIV reactive donors across India

resent study	Delhi	Mumbai	Kolkata	Vellore
0.39%	1.35%	1.04%	0.04%	0.44%

Transmission is the predominant one, a significant 40% of the patient population could not provide any information regarding their likely mode of transmission for acquiring this HIV infection. This could be due to: (1) social taboo in discussing sex and sex related issues openly. (2) Conversely, some of the patients might genuinely be unaware of any occupational exposure to HIV, for example through contaminated sharps. **Table 8** shows that only 30% of HIV reactive donors have received higher education and the greater majority are either un-educated or have received just primary education including a higher proportion of school dropouts. This calls for greater mass education regarding the risk and prevention of HIV / AIDS. **Table 9** shows that 55% of the HIV reactive donors are married and hence have higher chance to transmit HIV infection to their wives / sexual partners. Such cases have been referred to our Voluntary Center for Disease Control (VCDC). **Table 10** shows the comparative data of HIV reactive blood donors amongst the various cities of India. Our study shows Kolar district of Karnataka state has 0.39% prevalence of HIV reactive blood donors.

Discussion

The first AIDS cases were detected in homosexual men in 1981. Transmission through blood products was reported in 1982 when 3 hemophiliacs, who had no other risk factor except for transfusion of factor VIII concentrate, developed AIDS¹. The first reported case of blood transfusion associated AIDS was in an 18-month-old infant who had been transfused repeatedly since birth². In 1983, the agent was identified as a retrovirus, which was later named as HIV. With increasing knowledge about HIV it was realized that the symptomatic carrier stage of HIV infection could exist in the population with a considerable lag period between the initial exposure and onset of symptoms, allowing these people to donate blood.

The special features of HIV infection that allow a high index of transmission through blood are: 1) Long asymptomatic phase by which the potential donor can go unsuspected and undetected on history and examination. 2) Window period of the infection. This is defined as the time between infection / exposure and appearance of markers indicative of infectivity, which allows the potentially infectious donors to go undetected even after doing tests like anti-HIV ELISA (3-4 weeks), p24 antigen testing (2 weeks), nucleic acid amplification for HIV RNA (1 week). 3) Both cell associated and cell free stage of virus capable of surviving at 4°C and frozen state implicating the range of blood components and plasma fractions. 4) Isolation of virus from infected lymphocytes of "donor-recipient" pair, both of whom developed AIDS continued that the virus causing AIDS was transmitted through blood transfusion and commercial products fractionated from source and recovered plasma³. The donor selection criteria were then modified to exclude high-risk donors and this strategy was shown to be effective⁴. Transfusion-associated HIV infection is the most efficient way of HIV transmission. Even a small amount of transfusion of infected blood can result in seroconversion in up to 95% cases⁵. The transmission rate correlates with the number of transfusions, the viral load and the immunosuppressive effect of transfusion. The progress of HIV infection to AIDS is much faster in transfusion related infection. A study suggests that 50% of persons infected by transfusion develop AIDS within 7 years⁵ of infections, in comparison only 33% of non-transfusion-related HIV infections progress to AIDS in the same period⁵. Development of AIDS in the recipient is also related to the clinical course in the donor. A

higher number of AIDS cases with shorter incubation periods were observed if the donor also developed AIDS shortly after blood donation⁶.

Almost 5% to 10% of all AIDS cases globally are contributed by transmission through transfusion. By mid 1990s transfusion associated AIDS constituted about 2.3% of all AIDS cases in US⁶. Till March 1998, of the 5,204 AIDS cases reported in India, the probable source of infection was blood transfusion in 7.05% and intravenous drug abuse in 7.3% cases. By 31st March 2001, of the 20,304 cases of AIDS reported to National AIDS Control Organization (NACO), 4% and 4.2% were transmitted by infected blood use and intravenous drug abuse respectively⁷. In USA approximately 70% of patients with hemophilia A and 35% of patients with hemophilia B developed HIV antibodies before the introduction of methods for viral inactivation of blood derivatives⁷. The difference may be partly due to an uneven partitioning of HIV in infected plasma during fractionation, HIV going to the cryoprecipitate fraction. As many as 1% of thalassaemics and 4.4% of hemophiliacs in India have been reported to be infected with HIV⁸. The demographic characteristics of TAA (Transfusion-Associated-Aids) demonstrate several differences from those of the syndrome in general. A disproportionately high number of cases of TAA occur in pediatrics (3 to 12 years old) age group, where sexual transmission and intravenous drug use are not the common routes of exposure⁹. TAA is also more common in people older than 59 years, as this population is most likely to have received transfusion¹⁰. The prevalence of anti-HIV seroreactivity in blood donors in India by one time ELISA positivity, as reported to NACO¹¹, varies from 0.1 to 1.5%. Studies conducted at Lucknow and other centres to determine the true prevalence of HIV positivity by repeat testing in duplicate and confirmation with other kits have shown much lower prevalence (0.3% to 0.5%). Confirmation by western blot has even lesser positivity of 0.022% to 0.08%¹².

The laboratory diagnosis of HIV infection¹² depends upon the detection of various markers of infection in the blood. Detection of specific antibodies for screening is done by tests like ELISA, rapid test, simple test or supplemental tests including immunoblot (IB) / western blot (WB), indirect immunofluorescence assay (IFA) and radio-immuno precipitation assay (RIPA). Detection of specific antigens is based on p24 antigen detection by dissociation or enzyme

immunoassay (EIA) or a reverse transcriptase assay while detection of viral nucleic acid is done by *in situ* hybridization or polymerase chain reaction (PCR).

Blood transfusion in advanced countries has become very safe due to strict donor procedures, awareness, self-deferral, and total dependency on the voluntary blood donation programme. With the use of 3rd generation ELISA kits and nucleic acid amplification techniques NAT, the risk of TAA in USA presently is only 1 in 917,000¹³, whereas the risk remains very high in developing countries. There is a report of residual risk of HIV transmission from donors in the window period, and supports the implementation of nucleic acid testing in blood banks. No transmission of HIV attributable to coagulation factors has been documented in USA since mid 1980s with the introduction of donor screening and virus inactivation techniques¹⁴. It is mandatory to screen all blood and blood products for anti-HIV 1 and 2 (including subtype O) antibodies using screening tests, which include ELISA and other rapid tests like the dot blot tests, particle agglutination (gelatin, RBC, latex micro beads), HIV Spot and Comb test, of fluorometric micro particulate technologies.

There are several reports that persons with transfusion transmitted HIV and injection drug users have passed on the infection to their sexual partners. Women are at higher risk of getting infected with HIV, because of gender inequality, cultural and social customs, poor access to knowledge, information and education and for biological reasons. HIV infection among women, who are not sex workers, is increasing in India and worldwide and the most likely mode of transmission is through unprotected sex with their husbands. The high infection rate among the non-sex workers has been confirmed in studies from Indian National AIDS Research Institute, Johns Hopkins University and National Institute of Allergy and Infectious disease². As per joint report from UNAIDS / WHO Dec 2001, there have been 12000 persons with new infections a day in the year 2001 and out of these 50 per cent were women⁶. The increased prevalence of HIV infection among women has far reaching implications as the neonate can get HIV infection through perinatal transmission⁶. Surveillance studies show that in the high prevalence states of Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Manipur and Nagaland, HIV prevalence in antenatal women is more than 1 percent⁶. The reported risk of mother to child transmission is between 13-60 per cent. According

Kumar *et al*² the vertical transmission rate in India is 48 per cent, whereas in a study conducted by Arcon in Mumbai, Sangaonkar *et al*² reported mother to child transmission rate of 36 per cent. Health education and behavioural modification are the only ways of interrupting transmission⁸. Cultural taboos that surround sexual behaviour, general talks about sex, negotiating safe sexual practices etc. pose a significant challenge. It is, therefore, important to have formation and education programmes for all men and women, including adolescents. It is very important that sex education be given to adolescents before they become sexually active. It implies that education must begin in early teenage years and studies demonstrate that sex education is the most effective way of postponing sexual activity⁵. We are confronted today with a very serious public health problem where multiple partners such as medical fraternity, paramedical people, school and college teachers, NGOs, religious leaders, media, scientists and politicians need to play their respective role for prevention and control of HIV infection². A strong will on the part of the government is required to create policies and to exercise leadership for campaigns and development of resources. There have to be policies formulated to control and check transfusion transmitted diseases.

Since the positive predictive value is low in a population with low HIV prevalence, WHO has evolved strategies to detect HIV infection in different population groups to fulfill different objectives. For screening blood for transfusion purposes, strategy-I is used. According to this strategy, a random sample of the donor is subjected to ELISA / simple rapid test once and, if positive, it is taken as HIV positive and the unit is discarded. For negative it is considered free from HIV and used for transfusion⁴. The presently available, 3rd generation ELISA for the detection of anti-HIV antibodies uses recombinant HIV antigens and is very specific. The antibody negative window period has been reduced to 22 to 25 days. Within this period the donor can be infectious but negative for anti-HIV antibody. International regulatory authorities like the American Association of Blood Banks (AABB) and the Food and Drug Administration (FDA) have also mandated p24 antigen testing and screening for HIV RNA by PCR¹⁵. The p24 antigen testing was introduced by FDA in 1996 and made mandatory by AABB and FDA. It shortens the window period to 16 to 19 days¹⁶. Studies have shown that p24 antigen screening has contributed very less to blood transfusion safety. In fact, only four antigen positive,

antibody negative donations were observed among 24 million collections tested¹⁷. Nucleic acid amplification technology (NAT) could reduce the window period for HIV detection further from 16 to 10 days, reducing the current risk of HIV transfusion to 1:1000,000 per unit¹⁸. Updating their review of 2 years of HIV NAT, testing in US blood donors, Stramer *et al* showed that only 8 out of 26,339,192 antibody negative donors were NAT positive, giving a NAT yield of 1:3,292,400¹⁹. P24 antigen testing and NAT testing is expected to give higher yield in countries with greater first time donor volume, higher incidence rate of HIV infection and higher window period donations.

In the United States, two prospective studies conducted between 1985 and 1991 estimated the risk of HIV transmission from antibody-screened donations at approximately 1 case in 60,000 units⁴. These studies were discontinued in 1992 because of high cost, and the realization that prospective monitoring of donors and recipients represents an insensitive and inadequate approach for assessing the small residual risk of HIV transmission from screened transfusions. Since then an alternative approach has been developed for estimating the risk of HIV infection from transfusions, now known as the incidence-window period model⁷. It is based on the premise that the risk of virus transmission by means of blood transfusion in a given geographic area is primarily a function of the incidence (number of newly infected blood donors per person-time of observation) and the length of the window period (time infectiousness to seroconversion according to results of screening tests). The risk of HIV-1 transmission from repeated donors and, with some adjustments, from first-time donors, can be calculated. Window period estimates for HIV-1 infection in the United States decreased from a median of 45 days (96% confidence interval, 34-55 days) for the overall period from 1985 to 1990 to approximately 22 to 25 days with routine introduction in 1992 of new format for anti HIV-1 -HIV-2 combination tests to detect HIV specific immunoglobulin M antibody 10 to 15 days earlier than with previously available assays⁸. By combining the 25-day window period estimate with data on the frequency of HIV seroconversion in large U.S. donor populations, two independent studies derived point estimates for the risk of HIV transmission during the 1992 through 1995 period of 1:450,000 and 1:495,000⁵ antibody-negative units were intercepted since the test was introduced, it is thought that its contribution to risk reduction has been minimal. On the other hand,

incorporation of NAT of pooled samples into routine blood donor screening, which began on a research basis in 1999, increased the risk to an estimated 1 per 725,000 to 835,000 screened donations¹⁶. By multiplying the per-unit risk estimates by 18 million transfused components per year in the United States, it can be expected that a maximum of 2 to 25 recipients per year receive transfusions with blood infected with HIV-1. Fewer than 5 of these infected recipients would be expected to contract AIDS-related diseases before dying of other causes. The risk of transmission of HIV-2 has been estimated at less than 1 in 5 million, other rare subtypes (e.g., HIV-1 subtype) being even a lesser concern¹⁷. It is impressive to recognize that the combined risks are nearly 7,000-fold lower than the risk that existed at the peak of the transfusion AIDS epidemic between 1982 and 1984¹⁸.

Current Indian regulations require that the unit of blood be autoclaved and discarded if the screening test is positive. The blood bank will then direct the donor to linked voluntary counselling testing centre (VCTC) for confirmation on a fresh sample. Opinion is divided with some rationale on whether or not to inform the donor of the positive result, based on the fear that informing and counselling the donors would only draw high-risk people to donate blood to know their status in a confidential manner without having to go to a screening centre. The scientific community is coming to the conclusion that ELISA-positive cases should be confirmed with a more specific test and the donor informed with proper back-up for counselling and care¹⁶. This strategy has recently been implemented in Indian blood banks. American Association of Blood Banks and US FDA have made it mandatory to inform the donor and provide counselling after confirmation by a more specific test.

Repeated voluntary donors are found to be the safest²⁰. Thus, recruitment and retention of repeat donors should be encouraged by the centres. Cumming *et al*¹ have demonstrated that blood from repeat female donors is 9 times as safe as blood from a male donor. Professional blood sellers are banned now in India (since 1997 by a Supreme Court verdict) as they have a higher prevalence of transfusion transmitted disease markers and a tendency to hide high-risk status, because the motivation for giving blood is monetary gain²¹. A health history interview is taken to exclude donors belonging to high-risk groups, which include men who have had sex with men since 1977, intravenous drug abusers, people living in tropical Africa,

commercial sex workers, hemophiliacs who have received clotting factor concentrates, and the sexual partners of people in all these groups. Donors previously having a high-risk lifestyle, but currently enjoying good health might feel that they do not belong to the high-risk category and continue to donate. US FDA has thus recommended direct questioning of donors about high-risk activities. Many studies also emphasize the importance of pre-donation counselling to exclude the donors with high-risk behaviours²¹. Finally, even after the donation each donor is given an option to confidentially indicate whether this unit should be discarded through a process called confidential unit exclusion.

Conclusion

The ability to decrease the infectious window period through implementation of new molecular assays has resulted in dramatically enhanced safety, but at a very high cost. Whereas serologic screening of donors for HBV, HIV, and HCV was essentially cost-neutral (i.e. the cost of testing was offset by the savings in prevented infection or disease), the cost for NAT testing exceeds a million dollars per infection prevented or per quality-adjusted life-year saved. Moreover, every discovery of a new infectious agent in humans leads to consideration of potential blood safety implications, often resulting in expanded deferral (as with vCJD and severe acute respiratory syndrome (SARS) or screening recommendations (as for NEW infections). Pathogen reduction methods currently under development promise protection from TTV and other pathogenic organisms but could nearly double the cost of platelet and red blood cell components. There is a growing pressure to control the escalating costs of medical care in general, and of blood transfusions in particular. Although blood safety has had a relatively high level of political and financial support over the past decade, limits on additional funding for blood safety initiatives are likely. This reinforces the need to accurately define the value of new safety initiatives, and to reassess the utility of old procedures as new measures are introduced. Decision-making regarding blood screening policies must be based on accurate estimates of the incremental safety benefit balanced against the need to maintain an adequate and affordable blood supply.

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