SHORT COMMUNICATION

Acute HIV-1 infection among antigen/antibody seronegative blood donors in Dar es Salaam, Tanzania

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Abstract: Fourth generation human immunodeficiency virus (HIV) antigen (Ag)/antibody (Ab) enzyme-linked immunosorbent assay (ELISA) used in the current screening of blood donors at the National Blood Transfusion Service Centres in Tanzania has limited ability to detect HIV Ag/Ab during the first two weeks of the window period. The aim of this study was to determine the prevalence of acute HIV infection among HIV antigen/antibody negative blood donors. This cross-sectional study which was conducted based on a blood donation facility in Dar es Salaam from December 2009 to April 2010**.** Apparently healthy voluntary blood donors screened and accepted for blood donations were included. Blood donation screening questionnaires were used to obtain socio-demographic characteristics, history of past medical, sexual and blood transfusion of the study population. Blood specimen was collected for confirmation of the negative HIV Ag/Ab status by the Roche HIV-1 DNA polymerase chain reaction (PCR) test. A total of 552 blood donors (age=18-54 years) with negative HIV Ag/Ab donated blood were included in the study. About two thirds of the blood donors were in the age group of 18-27 years. Of 552 blood donors, 413 (75%) were males while 139 (25%) were females. Seventy two percent of blood donors were unmarried. About 71% were voluntary and the rest were replacement blood donors. The prevalence of acute HIV-1 infection by HIV-1 DNA PCR test was found to be 0.2% (1/552). It is concluded that many voluntary blood donors were found to be young, male and unmarried. Acute HIV-1 infection using HIV-1 DNA PCR test in the blood donors with negative HIV Ag/Ab donated blood was found to be very low. Further multi-centre study with larger sample size country wide is warranted to determine the magnitude of acute HIV infection in the blood donors with negative HIV Ag/Ab donated blood**.**

Keywords: HIV/AIDS, blood donors, ELISA, PCR, Tanzania

Human immunodeficiency virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) is a global burden, but predominantly prevalent in sub-Saharan Africa, where the pandemic is driven by multiple factors. The number of people living with HIV worldwide continued to grow in 2008 reaching an estimated 33.4 million (UNAIDS, 2009). In 2008, an estimated 2.7 million new HIV infections occurred. The latest epidemiological data indicate that globally the spread of HIV appears to have peaked in 1996, when 3.5 million new HIV infections

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occurred. In 2008, the estimated number of new HIV infections was approximately 30% lower than at the epidemic's peak 12 years earlier. Consistent with the long interval between HIV seroconversion and symptomatic disease, annual HIV-related mortality appears to have peaked in 2004, when 2.2 million deaths occurred. The estimated number of AIDS related deaths in 2008 was roughly 10% lower than in 2004 (UNAIDS, 2009).

Acute HIV infection is the period between infection with the virus and complete seroconversion defined by a positive Western blot test (Daar *et al.,* 2008). In the developed world, a few cases can be diagnosed during the acute phase of the disease. It is well established that HIV infection can be transmitted by infected blood and blood components including plasma and factor concentrates. The risk increases particularly in products prepared from donor pools (WHO, 1990). In 1982 the first case of transfusion-associated AIDS was described in an infant given transfusion for erythroblastosis fetalis (CDC, 1992) and more cases were reported thereafter. The risk for acquisition of HIV infection through blood transfusion is estimated to be 1 infection per 1000 units in sub-Saharan Africa (Jayaraman *et al.,* 2010). Blood transfusion has been the means of transmission accounting for about 15% of total patients infected with HIV in resource-limited country like India (Singh *et al.,* 2005). Guidelines formulated at the International Society for Blood Transfusion Workshop in October 1985 require that only blood and blood products found non reactive for HIV should be used for transfusions. In many countries national legislation also requires blood donations to be tested and be confirmed to be HIV-antibody-negative before being issued (Fleming, 1997). It should not be forgotten, however, that several months may elapse between HIV infection and appearance of circulating antibody. Blood for donation collected in the latency period may thus be infectious despite a negative antibody test (WHO, 1990).

In Tanzania, the demand for blood transfusion services is high due to endemicity of infections causing anaemia, malnutrition, surgical and obstetrical emergencies which are associated with blood loss (Gumodoka *et al.,* 1993). Limited information exists regarding the magnitude of acute HIV infection among blood donors. In a pilot study that was conducted at Muhimbili National Hospital in Dar es Salaam in 1999, among 300 blood donors, the overall frequency of anti-HIV antibody was 8.7% (Matee *et al.,* 1999). Blood safety remains an issue of major concern in transfusion medicine in Tanzania where national blood transfusion services and policies, appropriate infrastructure, trained personnel and financial resources are inadequate. Between April 2004 and May 2005, seroprevalence of HIV infection was found to be 3.8% among 1599 consecutive donors (Matee et *al.,* 2006). The strategy of blood donation is focused on low risk of HIV, voluntary non-enumerated blood donors and discouraged gradually replacement/family blood donors due to high-risk of transfusion transmissible in replacement/family donors (MoHSW, 2006).

Fourth generation HIV screening (Ab) enzyme-linked immunosorbent assay (ELISA) that make combined HIV antigen (Ag) and antibody (Ab) detection possible simultaneously in a single test offers the advantage of early detection of HIV infection via p24 antigen detection (Aboud *et al.,* 2006). The Ag/Ab ELISAs (Vironostika® HIV Uni-Form II and Abbott Murex) have a sensitivity of 100% and specificity of 98.9% and the diagnostic window is reduced by 4-5 days compared to that for antibody detection alone with third generation ELISAs (Aboud *et al.,* 2006). Since the establishment of National Blood Transfusion Services (NBTS) in 2004, no study has been done to determine the magnitude of HIV infection that could potentially be transmitted through blood transfusion after screening by the fourth generation Ag/Ab ELISA. The aim of the current study was to determine the prevalence of acute HIV infection in HIV Ag/Ab negative blood donors.

This was a cross-sectional study which was conducted based on a blood donation facility at the Eastern Zone Blood Transfusion Services Center in Dar es Salaam, Tanzania, from December 2009 to April 2010. Apparently healthy voluntary blood donors with negative HIV Ag/Ab donated blood after screened by the Vironostika® HIV Uni-Form II Ag/Ab ELISA (Biomerieux, the Netherlands) were included in the study while blood donors with positive HIV Ag/Ab donated blood were excluded from the study.

A systematic random sampling method was used in which every second blood sample was selected when met the requirement. Blood donation screening questionnaires were used to obtain socio-demographic, history of past medical, sexual and blood transfusion characteristics of the study population.

Blood specimens were collected for confirmation of the negative HIV Ag/Ab status by the HIV-1 DNA PCR test. Peripheral blood mononuclear cells (PBMC) separation and HIV-1 DNA PCR testing were performed in the PCR laboratory, Department of Microbiology and Immunology at Muhimbili University of Health and Allied Sciences (MUHAS). Amplicor HIV-1 DNA assay version 1.5 (Roche Molecular Systems, Inc., Branchburg, NJ) was used for testing. The PBMC were stored at -80ºC freezer until the time for DNA extraction. Each DNA extract was amplified and tested by Roche Amplicor HIV-1 DNA assay version 1.5 (Roche Molecular Systems, Branchburg, NJ) as used previously (Lyamuya *et al.*, 2000). Three negative and one positive control were included in each HIV DNA PCR assay run as per manufacturer's instructions. In addition, known Virology Quality Assurance (VQA) proficiency testing panel pellets from USA were included in every assay run. All assay runs were validated to ensure accuracy and reliability of results.

Ethical clearance to conduct the study was obtained from the Senate Research and Publication Committee of the Muhimbili University of Health and Allied Sciences, in Dar es Salaam, Tanzania. Permission to conduct the study was obtained from EZBTS centre. Written informed consents were obtained from all participants prior to HIV testing and inclusion into the study. Individuals found to be HIV-infected were referred to HIV care and treatment centre at Amana Municipal Hospital and managed according to the prevailing national treatment guidelines.

Data were analyzed using the Epi Info version 3.5.1. Demographic characteristics such as age and sex, history of past medical history, sexual and blood transfusion were summarized using frequency distribution. Proportions of different variables were determined.

A total of 552 blood donors with negative HIV Ag/Ab donated blood were included in the study. The overall mean age of blood donors was 26 years (18-54 years). About two thirds of the blood donors were in the age group of 18 - 27 years (Table 1).

Table 1: Socio-demographic and blood transfusion characteristics of the study population

Characteristics	Response	No.	%
Age group (years)	18 – 27	359	65.0
	28 - 37	104	18.8
	38 – 47	63	11.4
	48 – 57	26	4.7
Sex	Male	413	75
	Female	139	25
Marital status	Married	147	26.6
	Unmarried	399	72.3
	Divorced/separated	4	0.7
	Widowed	2	0.2
Type of blood donors	Voluntary	391	70.8
	Replacement	161	29.2

(N=552)

Of the 413 male and 139 female blood donors, none of them had past medical history of any long term illness and injection outside the hospital settings. None had a history of being a victim of sexual assault (rape, sodomy or engaged in anal sex), sexually transmitted disease and new sexual partner in the past 6 months. There was no history of prior transfusion of blood or blood products. The prevalence of acute HIV-1 infection using HIV-1 DNA PCR test in blood donors with negative HIV Ag/Ab donated blood was found to be 0.2% (1/552). The HIV-infected individual was a male voluntary blood donor, 38 years old with no prior medical history of any injections or any long term illness. He had neither a history of being a victim of sexual assault, sexually transmitted disease and a new sexual partner in the past 6 months nor history of prior blood transfusion or blood products.

Our study findings showed that most of the blood donors come from the age group of 18-27 years and there is preponderance of male over female blood donors at a male to female ratio of 3:1. Our study findings are in line with those reported previously in Kenya where more younger and male donors were readily available to donate blood than female donors (Charles & Nam, 1999). The current study population included non-remunerated voluntary blood donors and replacement blood donors. In Tanzania, the strategy of blood donation mostly focus on voluntary blood donors and discourage replacement blood donors from families due to high-risk of transfusion transmissible diseases in replacement/family donors (MoHSW, 2006).

The proportion (0.2%) of acute HIV-1 infection found in the current study is lower compared to 1.8% found in a previous study in Lilongwe, Malawi among a group of 1360 men in an STD clinic. This difference could be explained by the difference in the study population in which the Malawi study involved men who were more at risk of HIV. In the Lilongwe study, analysis of HIV antibody negative specimens revealed that 24 men (1.8%) had unrecognized acute HIV infection, which represented 5.0% of all HIV infections detected (Pilcher *et al.*, 2004). This could be possibly explained by the fact that donated blood at EZBTS is screened using Vironostika Ag/Ab ELISA which has been shown to detect early HIV infection in the first 2 weeks and to reduce the window period (Aboud *et al.*, 2006) as well as careful selection of blood donors. Recent data from the Ministry of Health and Social Welfare showed that while the number of blood donors increased steadily from 2,294 in

2006 to 12,640 in 2008, the overall prevalence of HIV among the voluntary donors decreased from 4.0% in 2006 to 2.8% in 2007 and remained at 2.7% in 2008. Among women, the HIV prevalence remains at a relatively high level (3.2%) compared with 2.5% among male donors (MoHSW, 2006). Our study findings compared to the Ministry of Health and Social Welfare findings suggest that occurrence of acute HIV-1 infection among blood donors with negative HIV Ag/Ab donated blood could be a rare occasion possibly because of overall decline of HIV prevalence and coordinated and strengthened screening of HIV, selection of low HIV blood donors and other potentially infectious agents to improve blood safety country wide.

The current study is limited by the fact that the sample size included was relatively small and thus cannot be generalisable. It would have been preferred if larger sample size would be used. Parallel testing of all donated blood by HIV Ag/Ab ELISA together with HIV-1 DNA PCR and/or viral load would have been preferred to compare the ability to detect acute HIV infection in blood donors. This was not possible due to limited budget allocated.

In conclusion, many voluntary blood donors were found to be young unmarried males. Acute HIV-1 infection using HIV-1 DNA PCR test in the blood donors with negative HIV Ag/Ab donated blood was found to be very low. It is recommended that further multi-centre study with larger sample size country wide is warranted to determine the magnitude of acute HIV infection in the blood donors with negative HIV Ag/Ab donated blood. The NBTS programme should build capacity of selection, recruitment and retention of safe blood donors.

Acknowledgements

We would like to thank the study participants for consenting to participate in the study, staff at the EZBTS Centre for assisting with recruitment of participants and specimen collection, technical staff in the Department of Microbiology and Immunology, MUHAS for assisting with processing of blood specimens and HIV-1 DNA PCR testing. The study was supported by Field Epidemiology and Laboratory Training Programme of Ministry of Health and Social Welfare, Tanzania.

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