



# Discordant sero-positive HIV antigen/antibody assays among voluntary blood donors in North Central Nigeria

Damulak O. D.<sup>1\*</sup>, Bolorunduro S. A.<sup>2</sup>, Egesie J. O.<sup>1</sup>, Jatau E. D.<sup>1</sup>, Yakubu R. K.<sup>2</sup>, Godit P.<sup>2</sup>, Maan V. T.<sup>1</sup> and Ewuga O. J.<sup>1</sup>

<sup>1</sup>Department of Haematology and Blood Transfusion, Faculty of Medical Sciences, University of Jos, Jos, Nigeria.

<sup>2</sup>National Blood Transfusion Service, North Central Zonal Centre, Jos, Nigeria.

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## ABSTRACT

Haematogenous transmission of the human immunodeficiency virus is still a great risk to patients requiring blood transfusion particularly in areas with limited resources. The prevention of HIV transmission through this route requires tough donor selection criteria and meticulous screening of donated blood. This study aimed to determine the human immunodeficiency virus antigen and antibody status among voluntary blood donors using Determine and enzyme linked immune-sorbent assay (ELISA). Six hundred (600) units of blood were voluntarily donated to the North Central Zonal Centre of the National Blood Transfusion Service in Jos, for human immunodeficiency screening tests. Blood were tube grouped and haemoglobin electrophoretic patterns were determined. A total of 600 units of blood were collected randomly from voluntary blood donors with age range 18 to 59 years (mean 28.3). The percentage of males and females involved were 70.0% and 30.0%, respectively. The total blood units collected included 49.5% and 50.5% from repeat and first time donors, respectively. Overall, HIV sero-positivity was found in 18(3.0%) of all donor blood units studied; with 4(1.4%) among repeat donors and 14(4.6%) among the first time donors. Isolated Determine HIV sero-positivity occurred in 10(1.3%) of all donors while ELISA showed isolated positive reaction in 5(0.8%), P=0.2. The rate of HIV sero-positivity was significantly higher among blood group O donors (3.3%) when compared with blood group B donors (3.1%), P=0.01. Donor blood haemoglobin protein electrophoretic patterns were 22.2% AS and 78.8% AA, with equal prevalence of positive HIV reactions. It was concluded that HIV infection is still common among voluntary non-remunerated blood donors (VNRBD) with ELISA screening tests missing in some positive donors.

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## INTRODUCTION

Transmissions of the human immunodeficiency virus (HIV) through blood transfusion have been reported in literature. It has been a great risk to patients requiring

blood transfusion particularly in Africa where resources do not match the health demands of citizens.

The prevention of HIV transmission through this route requires tough donor selection criteria and meticulous screening of donated blood. The World Health Organization requires as minimum standard, antigen/antibody enzyme linked immuno-sorbent assay (ELISA) screening test for the determination of safe blood units for

\*Corresponding author. E-mail: ddamulak@yahoo.com. Tel: +2348035664635.

transfusion. This technology is not available or very few in most African countries with attendant under utilization due to low voluntary blood donor pool. The National Blood Transfusion Service (NBTS) relies solely on antigen/antibody ELISA screening for post donation HIV 1 and 2 blood testing.

Several publications have shown varying prevalence of HIV sero-positivity among blood donors and non-donors. A 1.2% transfusion related HIV transmission has been reported in Nigeria (Ubesie et al., 2010). A high rate of 8.7% HIV sero-positive reaction was reported among blood donors in North-Eastern Nigeria (Kagu et al., 2005). In Osogbo, South-Western Nigeria, poor donor selection strategy has been blamed for a 6.0% HIV sero-positivity among their blood donors (Busari et al., 2009). A prevalence of 2.8% HIV positivity has been reported among blood donors in Kaduna, North-Western Nigeria in 2008 (Hassan et al., 2008). A study among blood donors in Cameroon reported prevalence of 4.44% HIV sero-positivity ascribing it to the endemicity of the infection in the region (Ymele et al., 2012). Another work in the same region reported a 1.2% antigen and cDNA positivity in ELISA antibody screened negative blood units (Odaibo et al., 2008).

In South Africa where the prevalence of HIV in the general population is 11.4%, the introduction of measures to reduce transfusion related infection between 2001 and 2002 led to a fall in the prevalence of HIV sero-positivity from 0.17% to 0.08% among their blood donors (Connolly et al., 2004). Another study in South Africa also reported a reduction in sero-positivity to HIV among blood donors following the implementation of a structured blood safety policy in 2006 (Heyns et al., 2006). Andrade et al. (2002) reported a low prevalence of 0.149% HIV sero-positivity among blood donors donating at the Curitiba blood bank in Brazil.

A study done on the association between ABO, Rhesus blood group systems and haemoglobin genotypes among confirmed HIV/AIDS victims with tuberculosis co-infection in Enugu, Nigeria showed no relationship (Ukaejofe and Nubila, 2006). Jeremiah et al. (2007) reported that the low HIV prevalence among their premarital subjects was not related to the ABO, Rhesus blood group systems and haemoglobin genotypes of studied subjects.

Reports of studies done on discordant HIV test results confirmed inconsistency of reactivity between test methods. Bhole (2003) colleagues reported on HIV rapid test sero-positivity among pregnant women in India using Cadila, Determine and Oralquick test kits that were negative to conventional enzyme immune assay (EIA). Kannangai (2010) and others reported that only 61% of HIV antibody positive blood donors from India were confirmed by Western blot. Crucitti et al. (2011) documented a high percentage of reactive Determine HIV1/2 antibody testing in a multicentre phase 111

microbicide clinical trial in Kampala that were however Western blot negative.

Data is scanty on the discordant HIV sero-positivity to test methods and the association of ABO and haemoglobin protein electrophoretic pattern with HIV infection among voluntary blood donors in our setting.

The authors undertook this study to determine the discordant HIV sero-positivity of voluntary blood donors to rapid tests and antigen/antibody ELISA test to warrant recommendations that suit our setting.

## MATERIALS AND METHODS

This prospective study was carried out at the North Central Zonal Centre, Jos, of the National Blood Transfusion Service (NBTS) between October 2011 and June 2012. Six hundred (600) units of blood voluntarily donated, were subjected to human immunodeficiency virus screening by Determine HIV 1/2 rapid test kit and Antigen/Antibody HIV 1/2 ELISA screening kit. The 4th generation ELISA used detects both antigens and antibodies. Blood were tube-grouped using both forward (red blood cells against known sera) and reverse (donor serum against phenotyped red cells) methods and haemoglobin electrophoretic pattern was determined by alkaline cellulose acetate method using Shandon 60000001 electrophoretic tank. Each test was carried out according to the manufacturer's instructions. Results were presented in tables and analyzed using *Epi Info* statistical soft ware. Chi square was used to test for statistical significance.

## RESULTS

A total of 600 units of blood were collected randomly from voluntary blood donors within the age range of 18 to 59 years (mean 28.3), at the North Central Zonal Centre of the National Blood Transfusion Service in Jos, Nigeria, during the study period. 180(30.0%) units were collected from females and 420(70.0%) were from the male blood donors. 297(49.5%) and 303(50.5%) blood units were donated by repeat and first time blood donors, respectively. Overall, HIV sero-positivity was found in 18(3.0%) of all voluntary non-remunerated blood donors (VNRBD) studied, with 4(1.4%) among repeat donors and 14(4.6%) among the first time donors,  $P=0.03$  (Table 1).

Determine screening test showed sero-positivity in 13(2.2%) while ELISA showed positive in 10(1.7%);  $P=0.5$ . The sero-positive reactions affected 10(2.4%) males and 8(4.4%) females,  $P=0.2$ . Determine HIV screening was the isolated positive result in 8(1.3%) of all donors while ELISA was in 5(0.8%),  $P=0.2$ . Dual reactions with both Determine and ELISA were however

**Table 1.** Distribution of blood donors according to donor types, sex and HIV sero-positivity.

| Donor type | Male (%)  | Female (%) | Total (%) | HIV positive (%) | P-value |
|------------|-----------|------------|-----------|------------------|---------|
| FTBD       | 218(36.3) | 88(14.7)   | 306(51.0) | 14(4.6)          | 0.03    |
| RBD        | 202(33.7) | 92(15.3)   | 294(49.0) | 4(1.7)           |         |
| Total      | 420(70.0) | 180(30.0)  | 600(100)  | 18(3.0)          |         |

FTBD, first time blood donors; RBD, retained blood donors.

**Table 2.** Distribution of HIV sero-positivity according to the ABO blood groups of donors.

| Blood group | Number of donors (%) | Number of HIV positive (%) | P-value |
|-------------|----------------------|----------------------------|---------|
| O           | 302(50.3)            | 10(3.3)                    | 0.01    |
| B           | 163(27.2)            | 5(3.1)                     |         |
| A           | 106(17.7)            | 2(1.9)                     |         |
| O           | 302(50.3)            | 10(3.3)                    | 0.30    |
| B           | 163(27.2)            | 5(3.1)                     |         |
| A           | 106(17.7)            | 2(1.9)                     |         |
| AB          | 29(4.8)              | 1(3.5)                     | 0.09    |
| O           | 302(50.3)            | 10(3.3)                    |         |

observed in 5(0.8%).

There were 302(50.3%) blood group O, 163(27.2%) group B, 106(17.7%) group A and 29(4.8%) AB (Table 2). The rate of HIV sero-positivity was significantly higher among blood group O, (3.3%) than blood group B (3.1%),  $P=0.01$ . There was no significant difference in the sero-positivity rate between blood group A (1.9%) and O (3.3%),  $P=0.3$ . The difference in the rates of sero-positive reactions between group B and A, and between A and AB were also not significant,  $P>0.05$  (Table 2).

Donor blood haemoglobin protein electrophoretic patterns were 133(22.2%) AS and 467(78.8%) AA. 4(3.0%) AS were sero-positive while 14(3.0%) AA reacted to HIV screening,  $P=1.0$ .

## DISCUSSION

HIV is the causative agent of AIDS (Costello, 2001). This virus can be efficiently transmitted in cellular and plasma component of blood (Costello, 2001; McCullough, 2002). The Nigerian National Blood Transfusion Service (NBTS) ensures that blood units for transfusion are appropriately screened for transfusion transmissible infections (TTIs) including HIV by 4th generation ELISA which detects both antigens of and antibodies to the virus (The National Blood Transfusion Service, 2006). The 4th generation ELISA is the minimum test standard for blood screening for HIV in the blood service in Nigeria as well as in other

countries of the world practicing modern blood transfusion service.

In this study, an overall 3.1% HIV sero-positivity was found using both Determine rapid testing and 4th generation ELISA screening. This prevalence in our study is similar to the 3.2% reported by Olajubu et al. (2009) among blood donors in a Nigerian tertiary health centre. A research report from a centre in the same North Central Geopolitical Region of Nigeria with ours showed a high (12.0%) prevalence of HIV sero-positivity among male blood donors (Alao and Okwori, 2009). Another report from Ethiopia showed a high prevalence of 11.7% HIV sero-positivity with a higher crude TTIs positive reaction rate among blood donors (Azene et al., 2007). The lower prevalence in the donors' blood used in this study may be attributable to self deferral of prospective donors following self evaluation for behaviors with exposure to risk of contracting HIV, and hence, transmission. The prevalence of 3.1% HIV sero-positivity among the volunteer blood donors of this study is higher than 0.87% reported in Lagos, Southern Nigeria (Irene, 2002). The findings of this study is also higher than the 0.007% reported in Northern Pakistan among blood donors and 0.14% HIV sero-positivity in donated blood at a Curitiba Blood Bank (Khattak et al., 2002; Andrade et al., 2002). The prevalence of HIV in our study is also higher than the low rate documented by Shrestha and colleagues (2009), among blood donors in Kathmandu. This findings suggests the possibility of healthy persons

in our setting with risk exposure to contracting HIV, donating blood for the benefit of confidential testing. This calls for increased donor education on the implications of donating TTIs contaminated blood on the recipient. It also calls for diligent application of donor selection criteria to ultimately reduce donation from prospective donors with risk exposure to contracting HIV and other TTIs. Emphasis should be placed on persuasion of willing blood donors with undeclared positive history of multiple sexual partners, injection drug abuse, male to male sex, rape, sexual partner with multiple sexual partners; to permanently self defer from blood donation.

The rate of HIV sero-positivity was higher among female donors (4.4%) when compared with male donors (2.4%) although it was not statistically significant,  $P=0.19$ . This finding did not concur with earlier report by Fernandes and colleagues who found higher rate of TTIs including HIV among their male blood donors (Damulak et al., 2011).

The ABO blood group pattern of our donors in this study (Table 2) is similar to an earlier finding we reported, with blood group O dominating and AB the rarest (Damulak et al., 2013). The distribution of HIV sero-positivity among the various blood groups of our donors (Table 2) was only significantly higher among blood group O when compared with blood group B,  $P=0.01$ . A relationship between ABO blood group and the rate of HIV infection is not known as Ukaejofe and Nubila (2006) found no association between individuals with HIV 1 and 2 with TB co-infection and their ABO and Rhesus blood group systems and genotype. The findings of this study may however suggest the tendency of the immunodeficiency virus of humans to infect with relative ease, individuals with blood group O as this virus is known to frequently change its genetic and phenotypic characteristics. It may be because individuals who lack the A and B antigens have higher concentration of other antigens on their cell membranes. This finding may also be due to the small sample size of this study, hence the need to verify our report on a large scale study in other centres. Further studies are recommended to determine the association between ABO blood group and the densities of CD4 and Chemokine co-receptors on the CD4 positive lymphocytes of human in this setting. Higher density of these receptors on the lymphocytes of blood group O would suggest enhanced infectivity as both are required for HIV infection to occur. Whether blood group O provides the drive to HIV risk exposures such as multiple sexual partners and injection drug abuse is also not known to us.

The haemoglobin electrophoretic pattern of our donors were AA(77.8%) and AS(22.2%), similar to report of our earlier findings in Jos (Caspari et al., 1986) with equal HIV sero-positivity of 3.0%,  $P=1$ . This finding suggests a null relationship between HIV infectivity and haemoglobin

A or S types as concluded by report work (Ukaejofe and Nubila, 2006).

The prevalence of 1.4% and 4.6% HIV sero-positivity among our repeat and first time blood donors respectively are higher than 0.04% among the repeat and 0.11% in first time donors reported by Gosh (2002). This finding however confirms the superior safety of blood sourced from repeat donors and emphasizes the need to commit resources in blood donor retention.

We found discordant HIV sero-positive reaction of donor blood units to rapid and 4th generation ELISA screening tests. The outcome of our work collaborates with that of Bhore et al. (2003) who earlier reported negative conventional enzyme immunoassay (EIA) among Indian pregnant women who tested HIV positive by the rapid tests Codela, Abbott, Determine or Oralquick. It also agreed with the 2011 culticentre phase 111 microbicide clinical trial, where Crucitti et al. (2011) reported failure to detect HIV antigens and nucleic acid among a large percentage of Determine screened positive individuals. We agree with the high specificity and sensitivity of the 4th generation ELISA and the higher rate of false positivity due to background noise in the microbiological assay worse in developing countries (Healey and Bolton, 1993). This could mean that some of the positive reactions recorded in this study with both rapid and ELISA tests might be due to some naturally occurring antibodies such as anticarbohydrate antibodies or antibodies to tetanus vaccine (Alao and Okwori, 2009). It could be due to acquired antibody to rabies vaccination as reported by Pearlman and Ballas (1994). This calls for consideration of the history of vaccinations in permanently deferred blood donors who are HIV antibody positive but antigens and nucleic acid negative. There is need to determine true positive reactions and recall of donors permanently deferred for false positive reactions.

The application of Determine rapid test in screening donated blood in the studied centre identified sero-positive units which were sero-negative at ELISA test. While labeling of donors with HIV positive should be done with care in the face of false positive reactions. Such donors should be referred for counseling and confirmatory tests. For the purpose of safe blood transfusion, such units should be discarded while the donors contacted; and further evaluated performed using Western Blot and Nucleic Acid Testing (NAT). Western Blot and mini pool for NAT should be used to confirm true positive and negative HIV screening outcome. This may provide opportunity for scientific recall strategy of donors whose blood gave false positive reaction while all true positive reactors are permanently deferred. This would prevent donor loss to permanent deferral based on cross reacting antibodies to HIV tests (Pearlman and Ballas, 1994; Kleiman et al., 1987). NAT amplification testing of

antibody negative donors may save recipients of blood collected from donors in early phase of HIV infection. This is supported by the findings of Siesan et al. (2004), who reported positive nucleic acid to HIV-1 in 12 of their large antibody negative blood with P24 antigen detected in only two of the 12 (Karki et al., 2009). The finding of higher sero-positivity to HIV rapid than 4th generation ELISA, suggests the development of a staggered testing protocol in blood services to include rapid antibody screening and only units that are negative should be screened with ELISA. This could save energy consumption, wear and tear and prolong the life span of ELISA equipments as well as reduce the cost of testing. Donors persistently positive to rapid HIV test but negative to ELISA and western blot or NAT are suitable subjects for further study on cross reacting antibodies and their provoking antigens. Donors who remain NAT or Western blot negative should be re-evaluated for recall into blood givers if the cross reacting antibody is identified during follow up to prevent precious donor losses particularly in our setting that is not blood donation friendly. There is the need for the NBTS and its technical partner and other relevant bodies to review the testing protocol to improve blood safety in medical care.

Other steps to further reduce or eliminate transfusion acquired HIV infection includes detail discussions with first time prospective blood donors on life style with high risk of contracting and spreading blood borne viruses (Ogunkolo et al., 2006). This is supported by the report from a South African study where impressive reduction in HIV-1 from 0.17% to 0.08% was achieved following implementation of a structured blood safety policy anchored on enhanced donor education and selection which resulted in the reduction in high risk donation from 2.6 to 1.7% (Dutta, 2002). Reduction in the likelihood of HIV infected donors donating blood could be achieved with good donor education and stringent suitability determination process (Damulak et al., 2013).

## Conclusion

This study has shown that HIV infection is still common among first time donors, more frequent among blood group O, with frequent discordant sero-positivity to rapid Determine and 4th generation ELISA screening tests. Also, the 4th generation ELISA test alone may not identify all HIV contaminated donor blood.

## RECOMMENDATIONS

It is recommended that the development of a staggered HIV screening strategy to include rapid antibody screening and 4th generation ELISA in the blood service.

It is also recommended that the incorporation of Western blot and nucleic acid test in blood testing of all blood for transfusion employed and that donors with false positive antibody reactivity be recalled. Furthermore, there is great need to train and retrain personnel in blood service in the art of HIV testing.

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