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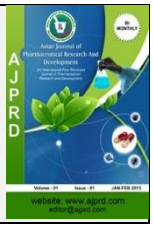

Open Access Research Article

The Risk of Transfusion of Transmissible HIV Infections in Sokoto, the North West of Nigeria

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A B S T R A C T

The risk of transfusion of transmissible HIV infection was investigated amongst potential blood donors from three health care institutions in Sokoto, North West Nigeria. Three hundred and fifty (350) blood samples from study subjects were screened for HIV antibody using Determine kit for HIV 1&2 (by Alere) and Uni-gold kit (biotech) for second antibody screening. All blood samples negative for the HIV antibodies using RDT were further screened for thep24 antigen using Enzyme Linked Immunosorbent Assay (ELISA). The results obtained showed that blood samples from 3(0.9%) study subjects were sero-positive for HIV antibody using the serial algorithm. There was no statistically significant difference (p=1.000) between the two RDT screening kits used. Nine blood donors 9(2.6%) were positive for HIV-1 p24-antigen using ELISA test. Overall results showed that the prevalence rate of HIV was 3.4% amongst all study subjects. However, sensitivity and specificity of RDT was 3.4% and 96% while the positive and negative predictive value was 100% and 97% respectively. The low incidence of 0.9% obtained using RDT clearly indicates that the antibody tests had failed to detect the newly infected blood donors. This is confirmed by the more sensitive and specific p24 antigen test.Thus, the risk of transmitting undetected HIV antigens from such sero-negative blood samples is expectedly high. Even though transmission of blood and blood products is a routine in our health care settings, it should only be done when benefit clearly outweighs the risk.

Key words: Transfusion, HIV, Blood donors, p24

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INTRODUCTION

Transmission of HIV through blood transfusion is a well-established associated risk as evidenced by many patients such as hemophiliacs and others who received blood components prior to the discovery of the virus who subsequently developed the infection[1,2]. By 1992 transfusion transmitted AIDS accounted for ten percent (10%) of all cases of AIDS in Africa at which time many countries do not screen, though this risk exists even in countries where all blood products are fully screened[3].

All blood components can transmit HIV infection and this is due to collection of blood from infectious donors during the “window period” which is said to be about one in every 60,000 or laboratory errors in one out of 2. 6 million donations in the US[4]. Thus, diagnosis of acute HIV infection remains a challenge especially in the early stages of infection. Acute retroviral syndrome symptoms may not be present, and standard detection methods such as antibody tests will fail to detect the new infection[5, 6].

Blood transfusion is one of the known therapeutic interventions that cut across a number of clinical disciplines but the practice is not without risks, as 10%-15% of HIV transmission in Africa has been attributed to transfused blood[7, 8]. To date, the internationally accepted method for screening and diagnosis of HIV carriers relies on the detection of HIV-specific antibodies. Enzyme-linked

immunosorbent assay (ELISA) and other antibody detection methods have been improved to detect significantly low levels of early IgM antibodies and display excellent specificity and sensitivity[9]. However, during the early stages of the "window period," when viruses lay latent without prompting immune responses, serum antibody screening has proven ineffective in identifying the majority of low-level HIV carriers[6, 10]. Therefore, any method that facilitates the detection of HIV carriers during this window period is critical both as a measure of the incidence and risk level of a given population, and as a tool for reducing that risk[11] .

The current testing strategy in some Nigerian hospitals supported by US funding agencies includes the use of three (3) antibody detection kits and p24 antigen testing. A donor that tests antibody negative with a rapid test (RDT) is accepted for donation, further testing screens for the p24 antigen for all antibody negative units[12]. This translates to a residual risk of transmitting HIV infected blood to about 33,000 units per million compared to a residual risk of one in 1.6 to 2 million units in developed nations[13, 14]. In the study area, transfusion of blood/blood products is a routine but screening for the HIV p24 antigen before transfusion has never been the practice in all the health care center's selected except the University Teaching Hospital. Thus, donors within the window period (who are seronegative for the virus antibody) if allowed to donate to patients, invariably increases the chances of transmission of the virus to the recipients.

Even though RDT based seroprevalence studies amongst blood donors has been regularly investigated in Nigeria[15-20], the risk associated with transmission of undetected HIV antigens in blood samples of RDT sero-negative individuals in the study area has not been much documented. It is in view of the forgoing that the present study aims at carrying out seroprevalence studies of HIV P24 antigen in the study subjects and documenting the risk associated with undetected HIV antigens in blood samples of sero-negative blood donors in three selected health care institutions in Sokoto, north-west Nigeria.

MATERIALS AND METHODS

The Study Area

The study was conducted in Sokoto, the capital of Sokoto State, Nigeria. Sokoto is one of the 36 States in Nigeria situated between longitude 4° and 6° East and latitudes 11° 30' and 13° 50' North. The state has a total population of 3.696,999 and 0.3% growth rate according to 2006 population census[21]. Sokoto metropolis has a population of 427,760[21], one major tertiary health care center, three secondary health care centers and a number of primary health care centers all distributed within the State. Study setting is the infectious disease laboratory, Usmanu Danfodiyo University, Sokoto. The laboratory receives and process blood samples from the other health care facility laboratories for diagnosis of major infectious diseases.

Ethical Approval

Ethical approval was obtained from the Ethical Committee of the Ministry of Health of the state.

Study Subjects

The study was conducted at the University Teaching Hospital (UDUTH). A total of three hundred and fifty (350) participants were recruited for the study. The target populations were adults aged 18 to 50 years of age who satisfy the criteria for blood donation; such as individuals having adequate blood, are apparently healthy and have not donated blood in the previous month. Informed consent was obtained from each donor prior to commencement of the research.

Inclusion Criteria

All potential blood donors who meet the criteria for blood donation and are willing to participate in the study.

Exclusion Criteria

All potential blood donors below 18 years and above 50 years were excluded.

All potential blood donors who did not meet the criteria for blood donation were excluded.

Sample Size

Sample size for the study was determined using the standard formula for the calculation of minimum sample size.

$$n = \frac{(Z1\text{-}a)^2 (p)(1-p)}{d^2}$$

Where n= minimum sample size desired;

Z1-a = the value of standard normal deviate which at 95% confidence interval has been found to be 1.96;

p= the best of the proportion prevalence obtained from the literature review;

d= the difference between the true population rate and the sample that can be tolerated (in percentage point) on either side of the population.

The prevalence rate of 10 % was obtained in a previous study therefore, at an established prevalence of 10 %, using 5 % precision at 95 % confidence level, the minimum sample size (n) for seronegative patients is as follows:

$$n = \frac{(1.96)^2 (0.1)(1-0.1)}{(0.05)^2}$$

$$= \frac{3.84 \times 0.1 \times 0.9}{0.0025}$$

$$= 138.24$$

$$= 138.$$

As calculated, the minimum sample size is 138. However, 212 donors will be added to improve precision. Therefore, three hundred and fifty (350) blood donors were consecutively recruited for the study.

Blood Sampling Technique

Three ml (3 ml) of blood were collected in ethylene diamine tetra acetic acid (EDTA) tube. This was centrifuged at 3000 rpm for 5 minutes. Plasma was

extracted from the blood samples and transferred to a cryovial, and these were transferred to the site of analysis in a cold box and stored at -20°C until tested.

Reagents and Test Kit

The reagents and test kits used for the research are as follows:

a. Determine kit for HIV 1&2 by Alere: for initial antibody screening.
b. Uni-gold kit (biotech): for second antibody screening.
c. Gene screen UltraHIV Ag-Ab ELISA kit: for p24 Antigen Detection.

Analysis of Blood Samples

i. HIV Screening

Antibody screening was done using HIV 1&2 Determine kit as follows**:**

HIV 1 and 2 Determine kit was used and procedures were based strictly on manufacturer's instruction as follows: The protective foil cover was removed from each test strip and 50 µl of plasma was applied to the sample pad. It was allowed to stand for a minimum of 15 minutes. Result was observed for the appearance of bars both on the test and control regions.

i. Interpretation of Test Result

a. **Positive (two bars):** Red bars appearing in the control and patient window of the strip was interpreted as a positive test.

b. **Negative (one bar):** One red bar appearing in the control window (labeled "control"), and no red bar appearing in the patient window of the strip was interpreted as a negative test.

Confirmation of Positive Antibody Test Using Unigold

i. Procedure:

The protective foil cover was removed from each test strip and 50 µl of plasma was applied to the sample pad and allowed to stand for a minimum of 15 minutes. Result was observed for the appearance of bars at both the test and control regions**.**

ii. Interpretation of Test Result

a. **Positive:** A positive reaction was visualized by pink/red band in the test and control regions of the device.

b. **Negative:** A negative reaction is indicated by the absence of pink/red bands.

P24-Antigen Screening

All HIV negative serum samples using both RDT tests were then screened for the p24 antigen using the Gene screen ultra HIV Ag-Ab qualitative enzyme immunoassay kit (www.biorad.com).

Procedure:

Each serum sample was analyzed based on the manufacturer's instructions: Exactly 75 µl of Conjugate 1 (biotinylated polyclonalantibody to p24 HIV-1 Ag) was added into the microplate wells followed by the addition of 75 µl plasma samples and controls into the appropriate wells.

After incubation at 37°C the plate was washed and 75 µl conjugate 2 was added and re-incubated at 18-30°C.The unbound conjugate 2 fraction was removed by washing. After incubation in the presence of 75 µl of substrate solution at room temperature (18-30°C), 75 µl of stop solution was added and the absorbance read at 450 nm. The absorbance measured on a sample determined the presence or absence of HIV Ag or HIV-1 and/or HIV-2 antibodies using the cutoff value. Samples with absorbance values less than the cut-off value were considered negative. Each cutoff value was calculated as follows:

$$\text{Cut-off value} = \frac{NC_1 + NC_2}{2} + K$$

Where: NC_1= optical density of negative control 1

NC_2= optical density of negative control 2

K= constant (0.200).

Statistical Analysis

All data generated from this study was recorded on excel spread sheet and statistically analyzed using the Statistical Package for Social Science (SPSS) software package version 10 (SPSS Inc. Chicago, USA).

RESULTS

Seropositivity of HIV-1 antibodies among donors using the conventional (RDT) was 3 out of the 350 blood samples analyzed 3(0.9%) (Table 1). All the 3(0.9%) positive samples were positive for HIV antibodies using determine and unigold (Table 2). No discordant result was obtained. Table 3 shows the result of the HIV-1 p24-antigen test as (confirmatory). The HIV-1 p24 antigen was detected in 9(2.6%) of the 347(97.4%) sero-negative individuals tested. Table 4 compares the HIV-1 antibody screening using the two RDT screening tests and p24-antigen ELISA test. The result showed that sero positivity was obtained in 3(0.9%) while that of the p24-antigen was 9(2.6%). The overall seroprevalence of HIV-1 among the study subjects using antibody screening and p24 antigen ELISA test is presented in Table 5.

Table1: Incidence of HIV Antibody Amongst Potential Blood Donors Using RDT

Total Number of Sample	**Number of Reactive (%)**	**Number of Non-Reactive (%)** 350
350	3(0.9)	347(99.1)
p-value :1.0209^N		
(x^2=4.9, df=1, p=1.0209)		

Table 2: Results of Serial Algorithm of Sero-Positive Blood Samples

Test Kit Number	**Tested Number**	**Positive (%)**
Determine	350	3(0.9)
Unigold	350	3(0.9)
p-value :1.000^N		
(X^2=0, df=1, p=1.000)		

Table 3: Detection of p24-Antigen in Sero-Negative Blood Samples.

Number Tested (%)	Number Positive (%)	Number Negative (%)
347	9(2.6)	338(97.4)

Table 4: Correlation Between Rapid Screening (RDT) and p24-Antigen Tests

Method	Number of Tested	Number of Positive (%)
RDT	350	3(0.9)
P24- antigen	347	9(2.6)
p.value : 0.0013[S]		
$X^{2=}$10.3, df=1, p=0.0013)		

Table 5: Overall seroprevalence of HIV Among study subjects (RDT and p24 ELISA)

Number Tested	Number of Positive (%)	Number of Negative (%)
350	12(3.4%)	338(96.5)
P24- antigen	347	9(2.6)
p.value :0.0208[S]		

DISCUSSION

In this study, both rapid testing (RDT) screening methods (Determine and Uni-gold)gave the same 0.9% HIV sero-positivity in the first phase of the study. No discordant result was obtained as the two antibody screening kits gave the same test values (which indicates that the two testing kits have the same sensitivity). Many clinical laboratories in the study state use both rapid HIV antibody tests to detect infection before blood donation or in order to institute therapy at the earliest time following exposure. However, any newly infected donor who has not yet developed HIV antibodies, will be missed. Thus, the risk of transmitting the virus via transfusion of blood from such sero-negative donor is expectedly high[21, 22]. Blood transfusion should only be done when benefit clearly outweighs the risk.

In the second phase of this study, HIV p24 antigen was detected in 9 (2.6%) out of the 347 sero-negative individuals tested. Meaning these nine are in the first few weeks after exposure (window period) and are yet to build up antibodies to the virus. Transmission of HIV by blood transfusion occurs almost exclusively during acute infection when the donor is sero-negative[4, 23]. Since the mid-1990s antigen testing was instituted in the United States to supplement antibody screening of donated blood. By instituting p24 antigen screening of blood, an estimated 4 to 6 cases of transfusion-associated HIV infections can be prevented per year[24, 25]. The risk varies according to geographic regions, ranging from zero in low incidence areas to 1 in 70,000 in high incidence areas[24, 26]. Even though the current testing strategy in major Nigerian hospitals includes the use of the three (RDT) antibody detection kits and p24 antigen testing, many health care institutions especially in resource poor settings rely on the rapid (RDT) test only before donation. This translates to a residual risk of transmitting HIV infected blood to about 33,000 units per million compared to a residual risk of one in 1.6 to 2 million units in developed nations[13, 18, 27].

CONCLUSION

The results of the present study show a HIV incidence rate of 0.9% using RDT, 2.6% using p24 antigen test and an overall 3.4% for both methods. This study shows that conventional screening method can only detect antibody to HIV in the serum of a very few intending blood donors. Thus, such RDT seronegative individuals, if allowed to donate blood, invariably increase the chances of transmission of the virus to the recipients.

Recommendations:

We recommend the use of ELISA for routine screening of the HIV p24-antigen among sero negative donors before donation of blood in all health care centres.

The establishment of the National Blood Transfusion Services (NBTS) centres in Nigeria has gone an extra mile in reducing the scourge by the establishment of zonal centers where blood/blood product from donors are screened for HIV antibodies and p24 antigen protein. Overall program-wide benefit can be optimized if NBTS centres be established in all the states of the federation.

Conflict of Interest: None.

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