Original Article

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Nucleic Acid Amplification Testing Detects HIV Transmission Risk in Serologically-Tested Blood Donor Units

Microbiology Section

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ABSTRACT

Introduction: Blood transfusion is an essential life-saving intervention in the healthcare delivery. Mandatory screening of donor units helps prevent transfusion-transmissible infections, such as Human Immunodeficiency Virus (HIV).

Aim: The purpose of this study was to use Nucleic Acid-Amplification Testing (NAAT) to screen for the presence of HIV-1 in blood-banked samples labelled as "safe for transfusion" per serological testing algorithm.

Materials and Methods: This hospital-based cross-sectional diagnostic study was conducted in May 2016 on serologically tested donor blood units in the Koforidua Regional Hospital blood bank. One hundred (100) donor samples were analysed using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qual Test for the detection of HIV-1 RNA and proviral DNA.

Results: Of the 100 donor samples that tested sero-negative for HIV-1 and 2 using the antibody screening kit, four (4) samples (4%) were reactive by NAAT. Blood donors who came for donation were only males and majority (90%) were between the ages of 17-27 years. Additionally, all the NAAT HIV-1 positive samples were from participants in the 17-27 year group.

Conclusion: NAAT demonstrated that a significant number of HIV-infected individuals are misdiagnosed at Ghanaian points-of-care. This finding has necessitated the need for inclusion of NAAT in donor blood screening in areas prevalent for HIV-1 in Ghana, considering the risk involved in using the licensed antibody test provided by the health authorities. In cases where NAAT screening may not be feasible, newer tests that have greater sensitivity compared to the FDA-licensed 3rd generation EIA which only detects HIV antibodies can be adopted.

Keywords: Blood safety, HIV diagnosis, Transfusion transmissible infections

INTRODUCTION

Blood transfusion is a life-saving intervention that has an essential role in patient management within health care systems. Over the past few decades, there has been a major increase in the safety of blood supply [1]. The main aim of HIV testing in donors is to eliminate the risk of HIV transmission through blood transfusion. The clinically significant time interval between infection and detection of antibodies (window period) is however vital. This period is characterized by a sero-negative result from an antibody-based test, detectable antigenemia (p24) and viraemia (as measured by RNA). A reason why majority of donors in Africa may be in the window phase of infection is that a significant proportion of these donors are commercial remunerated donors [2]. Studies among healthcare workers who acquired HIV through nosocomial exposure estimate that the median length of the entire window period is 40 days although, the viraemic phase of the HIV window period is approximately 22 days [3,4].

Despite Enzyme Immunoassays (EIA) having high sensitivity and specificity, they can only detect an infected blood after the window period. The p24 antigen becomes detectable approximately 2-3 weeks after initial infection and, can be detected approximately 6 days before antibodies are measured. However, NAAT allows the detection of viral RNA from 5 to 10 days before p24 antigen detection [5]. NAAT was originally introduced in some European countries in 1995 as a method to reduce viral loads for plasma-derived products, but was implemented for all donations in the United States, Canada, Australia, Japan, and much of Europe in 1998 to 2000 to reduce the residual risk of HIV and HCV transmission by all components [6].

The need for blood is universal however, there is still a major imbalance between developing and advanced countries in the level of access to safe blood [7]. Poor screening practices and contaminated blood supply in Ghana have been reported by Adjei AA et al., [8]. The commonly used Rapid/simple single-use assays (rapid tests) in Ghana detect antibodies produced against HIV after a period of 12 weeks (3 months) compared to line immunoassay which reduces the window period to 5 weeks; thus, people who have contracted the virus before these periods of detection would falsely test negative. Hence, the aim of this study was to determine whether blood-banked samples labelled as "safe for transfusion" test positive for HIV-1 using NAAT and to evaluate the effectiveness of including NAAT to the algorithm of tests done during screening for blood donation in prevalent areas in the country.

MATERIALS AND METHODS

Study Site: This was a hospital-based cross-sectional diagnostic study carried out in the Koforidua Regional Hospital Blood Bank located in the Eastern Region, Ghana. Eastern Region has recorded the highest prevalence for HIV since 2010. However, the region ranked third in the 2016 HIV sentinel survey in the country according to the National Aids Control Programme [9].

Sampling Technique: Hundred (100) plasma EDTA-anticoagulated samples which had passed the preliminary donor screening tests (HBV, HCV, Syphilis, and HIV-1 & 2) were used for the study. These samples were labelled using a number coding system thus, ensuring privacy and participant confidentiality.

Ethical Approval: The study was approved by the Institutional Review Board, University of Cape Coast (IRB/UCC), National AIDS Control Programme, and authorities of the Koforidua Regional Hospital.

Sample Testing: Plasma EDTA-anticoagulated samples (1.5ml) which had passed the preliminary donor screening tests were collected into Eppendorf tubes and stored per the manufacturers' instructions. The tubes were incubated at 56°C and then shaken continuously at 1,000 rpm for 10 minutes. One ml of the sample was tested for HIV-1 DNA using the COBAS AmpliPrep/COBAS TaqMan System (Roche Diagnostics, Indianapolis, IN) [10]. Samples

which were confirmed to be positive using NAAT was re-tested using the antibody kit thus ensuring the validity of the initial antibody screening.

Data Analyses: Data were entered and analysed using Microsoft Excel and presented as percentages.

RESULTS

All the 100 blood donors were males. This study found four (4) out of the 100 donor blood samples reactive for HIV-1 using NAAT [Table/Fig-1]. Majority of the blood donors (90%) were between the ages of 17-27 years, with a mean age of 21 years [Table/Fig-2]. Additionally, all the NAAT HIV-1 positive samples belonged to the 17-27 year group. NAAT positive samples that were retested with the antibody based test kit were once again negative.

Variables	Serology (Antibody Testing) (N=100)	
NAAT	Negative	Positive
Reactive	4 (4%)	nil
Non-reactive	96 (96%)	nil

[Table/Fig-1]: Nucleic acid amplification testing on negative HIV-1 serology tested donor samples.

Age group	NAAT HIV status		Total (N = 100)
	Negative	Positive	
17-27	86	4	90
28-37	7	0	7
38-47	3	0	3

[Table/Fig-2]: Age ranges of blood donors stratified by their NAAT HIV status.

DISCUSSION

The safety of blood products is a major issue in the field of transfusion medicine. Screening of blood donors for transmissible agents, play a major role in decreasing the risk of transfusion of infected units. The limitation of serological techniques including window period between infection time and detection time, and antigenic variability has allowed for the use of NAAT in the detection of infectious organisms. We report that 4% of serologically non-reactive donor blood samples were reactive for HIV-1 using NAAT. More so, all the NAAT HIV-1 reactive samples were from donors in the 17-27 year age group.

The finding of 4% newly diagnosed HIV infections missed by rapid serological-based tests on donor blood samples shows that a substantial number of HIV-infected individuals are misdiagnosed at the points-of-care in Ghana. This observation is consistent with other studies. A study in South Africa showed that substantial number of individuals in the acute and early stage of HIV infection are misdiagnosed as negative upon initial screening with rapid antibody based tests [11]. A similar study by Stramer SL et al., concluded that NAAT has helped prevent the transmission of approximately 5 HIV-1 infections annually and has reduced the residual risk of transfusion-transmitted HIV-1 to approximately 1 in 2 million blood units [12]. There is therefore, the need for an improvement in the HIV testing guidelines in HIV hyper-endemic setting like Ghana to facilitate earlier detection of HIV-infected individuals who are misdiagnosed by rapid HIV tests at the points-of-care.

Interestingly, based on serological testing, the Eastern regional HIV-1 prevalence was reported to be 2.6% in 2016 [13]. The prevalence reported herein is suggestive that the prevalence is likely to be higher had a more sensitive testing algorithm like NAAT been used. NAAT is considered the most sensitive of the methods used in the diagnosis of HIV infection [14], making it the ideal test for the detection of HIV especially, infection in the early or the acute phase. However, this method is not practical in most developing countries, as it is expensive and generally requires complex laboratory equipment and skilled technicians to perform. The consequence is that most infections in the early and acute stages are missed and this has a great implication in the spread of the virus [15,16].

Donors were all males and majority was between the ages of 17-27 years. This age group represents the sexually active group and is at increased risk of contracting Sexually Transmitted Infections (STIs). This is a problem worth addressing because majority of the transfusion blood are donated by this age group. However, a previous HIV sentinel survey in 2016 reported a prevalence rate of 1.1% for participants between the ages of 15 and 24 [13] which is at variance with the major findings reported herein. That survey rather reported that 45-49 years' group represented the highest risk group with HIV-1 prevalence of 5.6%. We believe these apparent disagreements may be due to the fact that whereas the 2016 HIV sentinel recruited all participants, our study had one layer of serological screening test that eliminated some individuals before the participants were subjected to NAAT testing.

Different generations of HIV immunoassays have been developed differing in performance, source, nature of antigen used and the immunoglobulin target [17-19]. Each new generation achieved an increased test performance and shortened the time from infection to a positive result. It has been shown that the highest rates of infectivity occur during the acute stage of HIV infection [15] and as such, tests with shorter window periods are more likely to detect donors in the acute stage of HIV infection when HIV viral loads are high and there is a greater chance of transmission of HIV to recipients. Rapid HIV tests are commonly used for the diagnosis and screening of HIV infection in resource constraint settings. The sensitivities of these rapid tests vary, ranging between 86-99% in one study [20]. Although these tests are less effective for detection of early HIV infection, they continue to be used in most low resource settings because it is usually the only available test. On the contrary, careful donor screening and implementation of more cutting-edge technologies such as NAAT are used to eliminate the vast majority of transfusion-transmitted viral infections in the advanced countries. With viral specific antigen and antibody testing, and possible addition of NAAT to the algorithm of tests done for blood screening in prevalent areas like Koforidua in the Eastern Region of Ghana, the risk of viral-transmitted infections (in this case HIV-1) would be reduced significantly.

The First Response HIV rapid test strip which was used for the initial testing of the donor blood has sensitivity of 100%, implying that the test is capable of ruling out all blood samples that have antibodies to HIV 1 & 2. A probable reason accounting for those samples missed by first response could be attributed to the fact that those individuals were in the window period, where antibodies to HIV were either absent or below detectable limit. A blood donation during the window period constitutes the predominant risk for each of the major viral agents. For example, in HIV and Hepatitis B Virus (HBV) infection, at least 90 percent of the risk is attributable to donations of window-period units [21]. Thus, even in situations in which serological testing algorithm are the mainstay, selection should be based on test kits that are able to detect the lowest possible antibody titre to protect the prospective blood recipients.

LIMITATION

This research focused only on HIV-1 infection, thus the PCR could not detect HIV-2 infections which were also recorded in the hospital.

CONCLUSION

The over reliance on serological-based screening algorithms is exposing prospective blood recipients to 4% chance of HIV-1 transmission in the Eastern region, Ghana.

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