Original Article

Absence of routine molecular testing and prevalence of HIV-2 infection in regions hardest-hit by HIV infection

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Abstract

Introduction**:** Investigating the incidence and dynamics of HIV-2 and false-negative HIV test results in a highly sexually active population where frequent opportunities exist for acquiring and transmitting infections provides additional understanding of the epidemiology of the virus in Africa.

Methodology**:** The HIV status of 900 active female sex workers (FSWs) was determined using two lateral flow rapid assays in series. The second rapid test device incorporates type-specific recombinant peptides that discriminate between HIV-1 and HIV-2 infection. HIV sero-negative samples were re-tested for HIV infection and their viral loads determined using the NucliSENS real-time nucleic acid sequence-based amplification (NASBA) platform.

Results: In total, 335 FSWs were determined to be HIV positive, the majority (227; 67.8%) of whom were between the ages of 20 and 30 years. Eighteen (5.4%) were found to have evidence of HIV-2 infection, 17 of whom were co-infected with HIV-1. Only one HIV-2 mono-infection was observed. Out of 565 HIV-negative individuals determined by serology, 11(1.9%; $p>0.05$) were found to be HIV-1 positive when tested via the NASBA platform.

Conclusion: False negative test results, HIV-2 infection, and complex transmission networks among FSWs may aid in fueling the HIV epidemic in the Nigerian population. These findings demonstrate the need to reevaluate the quality of HIV serological diagnostics, control services, and stress the need for widespread introduction of molecular testing among high-risk populations in the country.

Key words: HIV-2; false-negative diagnosis; FSWs; Nigeria

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Introduction

Nigeria has the second highest number of people living with HIV/AIDS in Africa after South Africa. National sentinel surveillance showed significant regional variation, from 1% in Ekiti State in the southwest zone to 10.6% in Benue State in the north-central zone [1]. The first case of HIV/AIDS in Nigeria was diagnosed in 1986; since then, confirmed cases of HIV-1 and HIV-2 have been reported [2, 3]. HIV-1 and HIV-2 are genetically similar but have very distinct epidemiologies [4]. HIV-1 is responsible for the vast majority of infections in the world [5]. HIV-2 is endemic in West Africa and has spread to a limited extent outside this region [6]. Compared with HIV-1, HIV-2 infection is characterized by a longer asymptomatic stage, lower plasma viral loads, slower decline in CD4 counts, and lower mortality rate due to AIDS, as well as lower rates of genital tract shedding and mother-to-child and sexual transmissions [7]. Treatment of HIV-2 infection is complicated by the intrinsic resistance of the virus to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the fusion inhibitor T-20 (enfuvirtide) [8].

Diagnosis of HIV infection in many laboratories in Nigeria is mostly based on serological assays and involves the detection of specific antibodies. These tests are usually inexpensive and do not require sophisticated equipment or high-skilled manpower. These assays have been shown to produce false-negative HIV results that may contribute to the large pool of infections in Nigeria; for instance, 1.2% of ready-to-be-transfused blood was found to be HIV-antigen positive [9]. We hypothesize that the rate of false-negative results will be even higher in populations with a high prevalence of HIV infections. False-negative results may occur because of the delay in the production of antibodies after acute infection and the loss of antibodies during late infection. [10].

Concern about false-negative HIV results has led to attempts to reduce the active window period by replacing viral lysates with recombinant antigens and the use of a double-antigen sandwich to enhance capture of IgM and IgG antibodies. The above studies were principally conducted in populations with low HIV prevalence.

To select a suitable sub-population where clinical trials of HIV products could be conducted, we evaluated HIV incidence in a population of sexually active female sex workers (FSWs) from north-central Nigeria where recent HIV infections are more likely to be detected [11]. Positive samples were retested using serological assays that distinguish between HIV-1 and HIV-2, and negative samples were subjected to nucleic acid amplification using Nucleic Acid Sequence Based Amplification (NASBA) technology. We investigated the frequency of HIV-2 infection and determined the rate of false-negative serology test results in FSWs, a high-risk population in which different variants of HIV could be found and transmitted at a faster pace.

Methodology

Study population and sample collection

This cross-sectional study was conducted from February to October 2006 in Nasarawa State in North-central Nigeria, West Africa [11]. Initial meetings were conducted with relevant people of the community to solicit support. These meetings allowed the study team to identify local brothels and key informants who were linked with brothels. As a result, 52 brothels were identified 900 FSWs living in brothels were recruited for the study. Participation was voluntary and written informed consent was obtained from all those who agreed to participate. Pre-test counseling for HIV testing was offered primarily in the rooms of the FSWs after which 5-10 mls of a single blood sample were collected at the time of enrolment. Data on demographics, clinical characteristics and behaviour was also obtained. Individuals were provided with a coded, preprinted card devoid of name or other personal identifiers to ensure anonymity; subjects used this card to obtain test results in an anonymous fashion. HIV results were disclosed after post-test counseling and positive FSWs were referred to an HIV/AIDS facility for care, support and antiretroviral therapy. The study protocol was reviewed and approved by the Nasarawa state Ministry of Health Research/Ethics Committee.

HIV-1&2 infections

Evidence of HIV infection was assessed using two rapid HIV test assays [11], Smart Check (GlobaleMed, World Diagnostics, Alexandria, VA, USA; sensitivity = 99.2%, specificity = 99.8%) and Tri-line 1&2 lateral flow rapid test (Shantha Biotechnics Ltd., Hyderabad, India: sensitivity = 100%, specificity = 99.9%) used in series [11]. Samples that were reactive in the first rapid test kit were retested (confirmed) using a second test kit that distinguishes HIV-1 from HIV-2 infection. The results of both assays were interpreted according to the manufacturers' instructions. Samples were considered HIV sero-positive if there was a positive reaction in both HIV rapid test kits [11].

Nucleic acid determination

To determine whether the two rapid antibody assays detected all positive cases, HIV-negative serological samples were subjected to DNA amplification. The NucliSENS miniMAG platform version 1.0 (revision 1) for nucleic acid extraction and NucliSENS EasyQ HIV type 1 (HIV-1) analyzer version 2.0 (revision 0) (BioMérieux , Boxtel, The Netherlands) were used for real time quantification of viral load in plasma samples by combining NASBA amplification with molecular beacon (MB) detection technology. NASBA amplification primers and MB detection probes were directed towards a conserved region of the HIV-1 genome. RNA extraction was performed with a silica-based procedure using the NucliSENS extractor. NASBA amplification and real-time detection of HIV-1 RNA and internal calibrator RNA was performed in a single tube using a temperature-controlled fluorescence reader. Quantitative results were calculated using a curve fitting of signal curves for both the HIV-1 RNA and an internal calibrator. In a single run, test results were obtained in 90 minutes and involved less than 30 minutes of hands-on time [12]. All assays were conducted according to the manufacturer's instructions and good laboratory practice standards. Further details of the procedure are described by Weusten *et al.* [12] and Stevens *et al*. [13]. All values were reported according to the limits (linear range: 25 IU/ml to 3,000,000 IU/ml) set by the EasyQ assay. Data were analyzed using the manufacturer's NucliSENS EasyQ director software on the NucliSENS EasyQ analyzer. Testing of negative samples was performed initially in pools of sixties; negative pools were regarded as negatives while positive pools were further analyzed in pools of thirties, fifteens and sevens. At this stage

pools still testing positive were analyzed individually to determine the positive sample(s) in the pool [14].

All statistical analysis was performed using the PASW statistics 18 software (IBM, SPSS Inc., Chicago, IL, USA). A *P*-value of < 0.05 was considered to be significant.

Results

Three hundred and thirty-five (37.2%) HIV sero-positivite cases were detected. The distribution of HIV infection by age group shows that individuals between 20-30 years of age (67.8%, n = 227) had significantly higher ($P < 0.05$) rates of infection compared with other age groups in this study. Risk of acquiring HIV infection was 3.5 times higher in subjects younger than 30 years old compared with those above 30 years. Seventeen participants had HIV-1&2 co-infections (94.4%); only one (n = 1) had HIV-2 mono-infection; 5.1% had dual HIV-1&2 infection; and 0.3% had HIV-2 mono-infection. HIV-2 infections were found in 18 (5.4%) individuals. Considering that about 3 million individuals in Nigeria are infected with HIV and the prevalence of HIV-2 is 5.4%, this implies that the total number of individuals infected with HIV-2 in the country can be assumed to be ~160,000 persons. The mean age of the 18 individuals with evidence of HIV-2 infection was 25.6 years. The distribution of HIV-2 infection by location was 10, 3, 3, and 2 for Lafia, Keffi, Karu and Masaka respectively. The chance of being infected with HIV-2 was over three times higher among FSWs in Lafia (Nasarawa state capital), suggesting that HIV-2 was either first introduced there or may be a reflection of increased promiscuity in Lafia.

A total of 565 FSWs were found to be HIV-negative by serology testing. Results based on NucliSENS nucleic acid amplification assay revealed that 11 out of the 565 HIV-negative samples were positive and had viral loads ranging from 130 to 140,000 IU/ml. Analysis of their viral load did not reveal any definite infection pattern. This observation stresses the importance of using a more sensitive diagnostic test and reveals that rapid assays alone may not be sufficient for HIV diagnosis. All individuals with false-negative results were below 30 years of age.

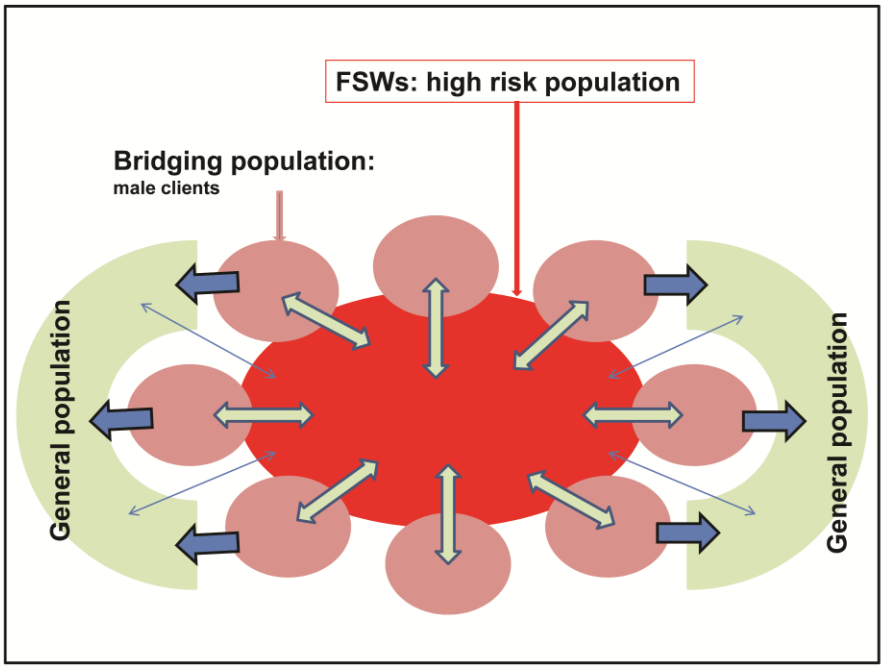
Discussion

The prevalence of HIV infection reported in this group of FSWs (38.4%) is dramatically high, especially compared with the HIV prevalence among adults aged 15 to 49 years in Nigeria (3.9%) [1]. As expected, for every FSW there are hundreds of male partners. These males are the plausible conduit that spreads the HIV/AIDS infection from the high-risk FSW community to the general population (Figure 1). These male partners usually have wives and girlfriends with whom they insist on engaging in sexual activities without the use of condoms for the purpose of childbearing, perceived safety or other reasons. A few of the FSWs have stable relationships with male partners in their hometown of origin and this constitutes another important outlet of HIV into the general population. Interestingly, only approximately 42% of the FSWs in this study said that they were never married at any point during their lives. FSWs in Nigeria typically do not know their HIV status and some who do know do not care about transmitting the infection to others. In fact, some may want to get revenge by intentionally transmitting the virus to others. The spread of HIV infection in Nigeria involves a complex network of sexual transmission (Figure 1). It is possible that controlling commercial sex activity in Nigeria could be predictive of the HIV/AIDS prevalence in the general population.

The incidence of HIV found in this study (38.4%) was approximately four-fold higher than the HIV incidence in the general population in Nasarawa state (10%) where this study was conducted [1]. It appears that the actual HIV prevalence in the general population in each state of the federation can be estimated by multiplying the HIV rates among FSWs by a factor of 0.25. This estimation works only in the regions of sub-Sahara hardest hit by HIV, where prostitution is not actively controlled by legislators. The high prevalence of infection found among FSWs between the ages of 20 and 30 years demonstrates that sexual promiscuity and youthfulness may play an important role in the evolution of HIV in Nigeria. The high HIV prevalence reported in this study may indicate that current preventive measures are ineffective. Offering HIV antibody testing to FSWs without offering preventive and supportive interventions may not be effective in curbing the spread of the virus. This situation calls for more continuous HIV interventions with measurable goals tailored for this group of people.

In this study, 5.4% of the FSWs had evidence of HIV-2 infection. These cases were mostly concentrated in Lafia, the Nasarawa state capital. HIV-2 may have been introduced into Lafia through FSWs or their clients (who typically patronize more than one brothel) and then spread to other regions of the state. The presence of HIV-2 in Nigeria has previously been confirmed serologically and genetically in low-

Figure. Dynamics of HIV transmission in Nigeria. Double edge arrows indicate possible transmission routes



prevalence areas. Between July 1987 and December 1988, blood samples collected from a population of blood donors were screened for HIV-2 and 0.33% were confirmed to be HIV-2-positive [15]. During the early stage of the spread of HIV in Nigeria (1989-90) 2.8% of men and women seen at a Special Treatment Clinic in Ibadan were found to be HIV-2-positive [16]. In 1990/1991 the HIV-2 sero-prevalence among prostitutes in 11 local government areas in Lagos state was 2.1% [17]. The prevalence of HIV-2 in samples collected in 18 rural locations between 1992 and 1994 was reported to be 0.8% [2]. In 1996 an HIV-2-specific antibody was detected in 4.5% of samples collected in six regions of the country (Lagos, Cross River, Borno, Kano, and Jigawa states) [18]. The prevalence of HIV-2 in blood samples collected from 35 out of 36 states in Nigeria was found to be 4.3% in 1999 [3]. These studies show that HIV-2 is more prevalent in sexually active populations. However, studies conducted after 1994 [3,18] suggest that HIV-2 viral strains are widespread in Nigeria and are not restricted to specific sub-populations. As such, patients whose HIV-1 test results are inconsistent with clinical findings should be considered for the possibility of HIV-2 seropositivity. Furthermore, HIV tests that do not incorporate both HIV-1 and HIV-2 in their algorithms should not be approved for use in this region because they would have negative implications for diagnosis, prognosis and treatment of HIV [8]. The average HIV-2 prevalence was approximately 1.5% between 1987 and 1994 [19] and increased threefold between 1994 and 1996, after which it remained stable. The factors that led to elevated rates of HIV-2 infection between 1995 and 1996 remain unclear; however, the elevated rates of HIV-2 during this period coincide with increased inflation and unemployment. It could also be a reflection of the increased prevalence of HIV in Nigeria at that time [1]. Although HIV-2 transmission appears to be limited in this country, an appreciable pool of people is probably infected with HIV-2 (~160,000 persons) considering the large number of people infected with HIV. Although the chances of being infected with HIV-2 are limited, this may play a role in the evolution of the HIV epidemic in Nigeria. This study confirms the results of other studies that show that HIV-2 homotypic infections in Nigeria are extremely rare. HIV-2 is usually found together with HIV-1 [3], which suggests that HIV-2 may have evolved as a "cheater" virus that may not be suitably adapted to infect the Nigerian population and exists more often in unison with HIV-1 for survival purposes [20]. Also, the FSWs have multiple HIV exposures that may result in superinfection with different HIV types or subtypes, which would lead to a greater chance of acquiring HIV-1 and HIV-2 co-infections and a

smaller chance of acquiring HIV-2 mono-infection. A novel finding of this study is that HIV-1&2 co-infection is not a rare event in West Africa as previously thought.

Using molecular testing, our study revealed that 1.9% of samples classified as negative were indeed positive. This figure is similar to that found in previous studies that assessed the safety of blood transfusion in two large blood banks in Ibadan, Nigeria, where 1.2% of screened blood that passed as negative for HIV-1&2 actually contained HIV antigen [9]. Therefore, missed diagnosis is not limited to FSWs and occurs irrespective of risk levels and frequency of HIV infection in the population. To reduce the incidence of false-negative results, some laboratories in Nigeria have included HIV p24 antigen detection together with HIV antibody detection; this method decreases the window between infection and diagnosis but does not eliminate it [21]. As can be seen from this study the absence of molecular testing can lead to misclassified HIV-positive individuals. The percentage of false-negative results may actually be higher than the 1.9% reported since the NASBA assay is less reliable for accurate viral load measurements across HIV subtypes [22]. In Nigeria the HIV epidemic is dominated by HIV-1 clade CRF02_AG and G [23], for which NASBA has less specificity [22]. The majority of molecular tests have been optimized for detection of the HIV-1 subtype B that is prevalent in North America and Europe; as such their use in regions whose epidemic is driven by non-B subtypes should be investigated. The incorporation of HIV-1 clade CRF02_AG and G into the NASBA technology might render it a powerful tool for the routine diagnosis of HIV infection and might be useful for large-scale epidemiological studies and screening programs in Nigeria and other regions whose epidemic is driven by these subtypes.

Serological assays remain the primary method of HIV testing in Nigeria. Expanding access to state-of-the-art molecular testing would lead to earlier diagnosis, reduced mortality and morbidity related to HIV, and reduced transmission. Zonal, state or local government laboratories could be empowered to perform molecular testing to reduce the risk of transfusion-associated HIV infection and false-negative results; such a strategy was implemented in Kenya with success [24].This molecular testing should be adopted in high-risk populations. Although the incidence of misclassified infection remains low, FSWs with undetectable HIV infection have the potential to rapidly spread HIV to the general population. The findings from this study emphasize the importance of a close collaboration between the clinic and the laboratory, and illustrate the need to integrate molecular testing with the HIV screening process to develop strategies to improve HIV diagnosis in the country.

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