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Screening for “window-period” acute HIV infection among pregnant women in rural South Africa

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Abstract

Objective—To evaluate HIV-1 RNA pooled nucleic acid amplification testing (NAAT) strategy to screen pregnant women in the “window period” of acute HIV infection (AHI) in rural South Africa.

Methods—In 2007 and 2008, 750 consecutive pregnant women at their first prenatal care visit to primary health care clinics were tested anonymously for HIV infection. HIV-1 RNA pooled NAAT was performed on HIV antibody negative samples. All positive pools were tested individually and positive samples were classified as incident cases to calculate HIV incidence.

Results—The overall HIV prevalence was 37.3% [95% confidence interval (CI) 34.3–41.3]. Of the 467 HIV antibody negative samples, four (0.9%) were HIV-1 RNA positive. The mean viral copies/ml in the four samples was 386260 (range 64 200 to 1228130). The HIV incidence was 11.2% per year (95% CI 0.3–22.1) and all women with AHI were ≤ 21 years of age.

Conclusions—Identifying AHI in pregnancy is important for health interventions to reduce perinatal and heterosexual transmission of HIV; and to estimate HIV incidence for epidemiological surveillance.

Keywords

pregnant women; screening; HIV-1 RNA; acute HIV infection; rural South Africa

Introduction

Epidemiological screening for HIV infection using standard antibody tests is crucial to understand and monitor the spread of HIV and to provide care and treatment for those infected [1]. In countries with generalized epidemics where heterosexual transmission is dominant, HIV seroprevalence surveys amongst pregnant women are frequently used. These surveys identify individuals with latent or advanced HIV disease and miss individuals with “window-period” acute HIV infections (AHI) who are more likely to transmit HIV due to high viral concentrations in the blood and genital tract [2,3]. Sensitive, validated and well

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Conflicts of interest: None

calibrated assays for HIV-1 RNA, p24 antigen and the fourth generation assays for the simultaneous detection of HIV antibodies and p24 antigen have been used with increasing frequency to diagnose AHI [4–8]. These tests have been used in cross sectional studies to estimate HIV incidence [5,6] and are useful to understand HIV transmission dynamics and assess impact of public health interventions [9]. The objective of this study was to evaluate the HIV-1 RNA pooled nucleic acid amplification testing (NAAT) strategy to screen pregnant women for “window-period” AHI and estimate HIV incidence.

Methods

Study setting and population

The study population comprised pregnant women attending seven public sector primary health care clinics in Vulindlela, a rural community about 150 kilometers west of Durban in the KwaZulu Natal Midlands. As part of the prevention of mother to child transmission of HIV infection all pregnant women at these clinics are offered HIV voluntary counseling and testing services and if infected have access to prevention of mother to child transmission of HIV programs and antiretroviral treatment if they meet the treatment initiation eligibility criteria.

Data and sample collection

This study was undertaken as part of the annual, cross sectional surveys conducted from 1 October to 30 November in 2007 and 2008. This survey coincided with the South African Department of Health’s National Antenatal Sentinel HIV and Syphilis Prevalence Surveys conducted annually among pregnant women and blood samples from this survey are tested using a single ELISA assay (Abbot Axysm System for HIV-1 / HIV-2) [10]. We included consecutive pregnant women who presented for their first prenatal care visit at one of the seven primary health care clinics, regardless of age. Screening for HIV infection was anonymous and in compliance with the World Health organization guidelines for using HIV testing technologies in surveillance [1]. Trained nurses collected two venous blood samples in pre-labeled EDTA and plain tubes. The age of the woman, her current partner’s age, if this was her first pregnancy and dates of prior pregnancies were recorded on a standardized case report form labeled with a unique participant identification number. Samples were transported to the central laboratory in Durban for testing.

HIV testing procedure

HIV antibody testing on serum samples was carried out using Enzygnost* Anti-HIV 1/2 Plus, an enzyme-linked immunosorbant assay (ELISA) for the detection of antibodies to HIV-1, HIV-2 and HIV-1 (subtype O) antigens (Dade Behring, Marburg, Germany). Plasma from all ELISA negative samples were batched and tested using the pooled NAAT strategy [5,6]. Each master pool was made up of 10 samples consisting of 100µl from each sample to a total volume of 1000µl and tested with qualitative HIV-1 RNA PCR assay (COBAS Amplicor™ system, Roche Molecular Systems). Master pools testing negative were considered HIV negative with no further testing. If any of the master pools tested positive for HIV-1 RNA, quantitative testing was performed on individual samples using the COBAS AmpliPrep/COBAS TaqMan (Roche) which has a detection level of ≥ 40 copies/ml. HIV antibody negative samples with detectable plasma HIV-1 RNA were retested using the third generation Abbott Determine HIV-1/2 rapid antibody test (Abbott Park, Illinois, USA). We calculated the cost of HIV-1 RNA testing by including the cost of consumables, test kits and technician time.

Analysis

AHI was defined as HIV ELISA antibody negative, qualitative HIV-1 RNA positive with measurable HIV-1 RNA copies/ml. The proportion of women with AHI was calculated using the number of women who were HIV-1 RNA positive in the numerator and the total number of ELISA negative samples tested in the denominator. The annual HIV incidence was calculated using the formula ($I = [365/w] N_{inc}/[\text{number at risk}]$), where I is the incidence rate and w is the mean window of detection (28 days). The N_{inc} is the number of women found to be HIV-1 RNA positive. The denominator, number at risk, is the number of HIV ELISA seronegative women tested. The HIV incidence is reported as percent per year. The 95% confidence interval (CI) for the incidence estimate was calculated using $\pm 1.96 I / [\text{square root of } N_{inc}]$ [5,6].

Ethics approval

The Biomedical Research Ethics Committee of the University of KwaZulu Natal and the uMgungundlovu District KwaZulu-Natal Department of Health approved the study.

Results

A total of 750 consecutive samples were collected from pregnant women at their first prenatal care visit. The HIV prevalence at screening, patient demographics and HIV tests characteristics are shown in table 1. The overall HIV prevalence was 37.3% [95% confidence interval (CI) 34.3–41.3]. Of the 467 ELISA HIV antibody negative samples, four (0.9%) samples tested HIV-1 RNA positive and negative with the Abbott Determine rapid assay. The mean viral copies/ml was 386260 (range 64200 –1228130). Based on the HIV-1 RNA positive samples the point estimate of HIV incidence was 11.2 % per year (95% CI 0.3–22.1). All women diagnosed with AHI were ≤ 21 years of age. The ages of the current partner for two women was < 25 years and for the other two, the ages were > 25 years. Only one woman reported a history of a previous pregnancy. The mean age of women without AHI and their current partner was 22.3 (SD 6.8, range 12–45) years and 25.2 (SD 7.9; range 16–56) years respectively; 185/463 women reported having had at least one previous pregnancy.

Four of the 47 master pools testing positive with the qualitative HIV-1 RNA assay required 40 individual samples to be tested. A total of 87 tests were performed (47 master pools plus 40 individual tests) at a cost of R483.00 (~\$61 or £40) per test, totaled to R42021.00 (~\$5253 or £3502). The cost per individual HIV negative sample was R90.00 (~\$11 or £8), whilst the cost of identifying a single case of AHI was R10505.00 (~\$1313 or £876).

Discussion

In this study using the HIV-1 RNA pooled NAAT strategy we identified 0.9% of pregnant women with AHI in the absence of HIV antibodies. During the early years of the HIV epidemic, amongst mother-infant pairs attending immunization clinics in rural KwaZulu-Natal, 2% of women were diagnosed with acute incident HIV infections [4]. Our study reaffirms that a high proportion of pregnant women are likely to be missed being diagnosed with HIV infection and the potential for vertical and heterosexual transmission predicted by the magnitude of the viral load [2,3] during the acute stage of infection has important public health implications.

The HIV incidence of 11.2% per year in this study is similar to the 10.7/100 women years obtained following retesting of HIV negative pregnant women around the time of delivery from urban and rural facilities in South Africa [11]. Whilst measuring HIV incidence by the traditional follow-up of cohorts of HIV-uninfected individuals remains the gold standard,

these studies are usually time consuming, expensive and potentially biased by poor retention rates. From such studies, HIV incidence rates among 18 to 25 year old non-pregnant women in Hlabisa and Durban, South Africa was 8.9 and 8.5 /100 women years respectively [12], indicative of the unrelentingly high HIV incidence rates in young women in this region.

To estimate HIV incidence from cross sectional studies antibody based sensitive/less sensitive testing [13] and the BED-HIV-1 Capture enzyme immunoassay (BED-CEIA) [14] have been used. Using BED-CEIA, data from population-based household surveys in South Africa have shown the HIV incidence to be 5.6% among women aged 20–29 years compared to 0.9% in men of the same age group. Amongst women with a current pregnancy the HIV incidence was 5.2% (95% CI: 0.0–12.9) [14]. A key disadvantage of the BED-CEIA is that it is known to misclassify early or acute HIV infection with established long term infections and individuals on antiretroviral treatment [5]. In the absence of HIV antibodies, the measurement of HIV-1 RNA and p24 antigen are both highly sensitive and specific, with HIV-1 RNA having an added advantage of being detected much earlier compared to p24 antigen [5,6]. Further advancements in diagnosing AHI has been the development of fourth generation HIV-1 assays, detecting p24 antigen and HIV antibody simultaneously [8]. However, the detection levels of these assays differ as key viral and serological markers evolve in AHI.

Screening for epidemiological purposes has typically described the prevalence of established infections, limiting the understanding of ongoing transmission dynamics. HIV prevalence from anonymous testing of pregnant women and from nationally representative population based household surveys remain the mainstay of HIV surveillance [10,15]. With increasing access to and uptake of antiretroviral therapy, survival time of those infected increases and the proportion with established infections increases over time, influencing the usefulness of HIV prevalence data for surveillance. Dissecting the relationship between prevalence and incidence becomes more complex as approaches to the epidemic become more advanced and widely available. Measuring HIV incidence provides a more sensitive way to monitor trends in HIV infection and behaviour. Enhancing current screening programs to include tests for HIV-1 RNA, p24 antigen or the newer fourth generation HIV-1 assays to monitor AHI and HIV incidence would provide a nuanced sophisticated understanding of the epidemic, allowing more focused prevention and treatment efforts to be implemented and evaluated [8].

Whilst the cost of identifying a single case of AHI may be excessive at the individual level, evidence for enhanced spread during this stage of infection and the importance for broader public health benefit at the population level compel detecting AHI to prevent secondary spread. Since this was an anonymous survey we were unable to refer women diagnosed with AHI for care and support. We also believe that the HIV-1 RNA pooled NAAT strategy rather than the BED-CEIA be incorporated into the annual anonymous Department of Health's National Antenatal Sentinel HIV and Syphilis Prevalence Surveys [10] to provide a parallel measure of incident HIV infections as antiretroviral therapy is scaled up [9].

There are several limitations to our study. It is difficult to extrapolate our data to the general population because of the small sample size; the survey population being pregnant women seeking antenatal care; and rates of new HIV infections are likely to be different during pregnancy [16]. However, the population represented is that of young sexually active women, most affected by the virus [14] as all acutely infected women were ≤ 21 years of age. The HIV-1 RNA pooled NAAT strategy is technically demanding requiring laboratory expertise, has cost implications, may fail to detect or under-amplify some non-B subtypes, lower specificity as detectable low viral copies are classified as positive and some loss of sensitivity due to testing pooled samples [6,8]. Since the ELISA was not repeated for all the

samples, HIV antibody negative samples could have been misclassified as false positive. Regardless of misclassification the viral loads in the AHI individuals were all higher than 3000 copies /ml and unlikely to represent false positive results [7,8]. Nevertheless, HIV-1 RNA pooled NAAT strategy has been used with great efficiency to diagnose AHI in pregnant women [17], high risk individuals from populations with low [18] and high HIV incidence [19,20].

Diagnosing pregnant women with AHI is critical in reducing perinatal and heterosexual transmission of HIV, underscoring the need for vigilant and rigorous testing for HIV infection at prenatal care visits. For epidemiological surveillance estimating HIV incidence is central to HIV prevention and understanding of transmission dynamics in generalized, hyperendemic HIV prevalence settings [9].

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References

1. UNAIDS/WHO. Guidelines for Second Generation HIV Surveillance: The next decade. Working group on Global HIV/AIDS and STI Surveillance. WHO/CDC/EDC/2000.5 UNAIDS/00.03E 2000.
2. Pilcher CD, Joaki G, Hoffman IF, Martinson FE, Mapanje C, Stewart PW, et al. Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection. *AIDS*. 2007; 21:1723–1730. [PubMed: 17690570]
3. Pilcher CD, Tien HC, Eron JJ Jr, Vernazza PL, Leu SY, Stewart PW, et al. Brief but efficient: acute HIV infection and the sexual transmission of HIV. *J Infect Dis*. 2004; 189:1785–1792. [PubMed: 15122514]
4. Rollins NC, Dedicoat M, Danaviah S, Page T, Bishop K, Kleinschmidt I, et al. Prevalence, incidence, and mother-to-child transmission of HIV-1 in rural South Africa. *Lancet*. 2002; 360:389. [PubMed: 12241784]
5. McDougal JS, Pilcher CD, Parekh BS, Gershy-Damet G, Branson BM, Marsh K, Wiktor SZ. Surveillance for HIV-1 incidence using tests for recent infection in resource constrained countries. *AIDS*. 2005; 19 Suppl 2:S25–S30. [PubMed: 15930838]
6. Quinn TC, Brookmeyer R, Kline R, Shepherd M, Paranjape R, Mehendale S, et al. Feasibility of pooling sera for HIV-1 viral RNA to diagnose acute primary HIV-1 infection and estimate HIV incidence. *AIDS*. 2000; 14:2751–2757. [PubMed: 11125894]
7. Patel P, Mackellar D, Simmons P, Uniyal A, Gallagher K, Bennett B, et al. Detecting acute human immunodeficiency virus infection using 3 different screening immunoassays and nucleic acid amplification testing for human immunodeficiency virus RNA, 2006–2008. *Arch Intern Med*. 2010; 170:66–74. [PubMed: 20065201]
8. Daar ES, Pilcher CD, Hecht FM. Clinical presentation and diagnosis of primary HIV-1 infection. *Curr Opin HIV AIDS*. 2008; 3:10–15. [PubMed: 19372938]

9. Wilson D, Halperin DT. "Know your epidemic, know your response" a useful approach, if we get it right. *Lancet*. 2008; 372:423–426. [PubMed: 18687462]
10. Department of Health. 2008 National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa. 2009.
11. Moodley D, Esterhuizen TM, Pather T, Chetty V, Ngaleka L. High HIV incidence during pregnancy: compelling reason for repeat HIV testing. *AIDS*. 2009; 23:1255–1259. [PubMed: 19455017]
12. Ramjee G, Kapiga S, Weiss S, Peterson L, Leburg C, Kelly C, Masse B. The value of site preparedness studies for future implementation of phase 2/Ib/III HIV prevention trials: experience from the HPTN 055 study. *J Acquir Immune Defic Syndr*. 2008; 47:93–100. [PubMed: 17984760]
13. Novitsky V, Woldegabriel E, Wester C, McDonald E, Rossenkhan R, Ketunuti M, et al. Identification of primary HIV-1C infection in Botswana. *AIDS Care*. 2008; 20:806–811. [PubMed: 18608056]
14. Rehle T, Shisana O, Pillay V, Zuma K, Puren A, Parker W. National HIV incidence measures--new insights into the South African epidemic. *S Afr Med J*. 2007; 97:194–199. [PubMed: 17440667]
15. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Pillay-van-Wyk V, et al. South African national HIV prevalence, incidence, behaviour and communication survey 2008: A turning tide among teenagers? Cape Town. HSRC Press. 2009
16. Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, et al. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. *Lancet*. 2005; 366:1182–1188. [PubMed: 16198767]
17. Patterson KB, Leone PA, Fiscus SA, Kuruc J, McCoy SI, Wolf L, et al. Frequent detection of acute HIV infection in pregnant women. *AIDS*. 2007; 21:2303–2308. [PubMed: 18090278]
18. Patel P, Klausner JD, Bacon OM, Liska S, Taylor M, Gonzalez A, et al. Detection of acute HIV infections in high-risk patients in California. *J Acquir Immune Defic Syndr*. 2006; 42:75–79. [PubMed: 16763493]
19. Fiscus SA, Pilcher CD, Miller WC, Powers KA, Hoffman IF, Price M, et al. Rapid, real-time detection of acute HIV infection in patients in Africa. *J Infect Dis*. 2007; 195:416–424. [PubMed: 17205481]
20. Pilcher CD, McPherson JT, Leone PA, Smurzynski M, Owen-O'Dowd J, Peace-Brewer AL, et al. Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population. *JAMA*. 2002; 288:216–221. [PubMed: 12095386]

Table 1

HIV prevalence, incidence and test characteristics among pregnant women with acute HIV infection

Characteristics				
<i>Sample population</i>				
Sample size	n=750			
HIV Prevalence	37.3% (95% confidence interval 34.3–41.3)			
HIV antibody negative samples tested				
	n=467			
HIV Incidence	11.2 % per year (95% confidence interval 0.3–22.1)			
<i>Patient demographics</i>				
Age in years	18	21	19	18
Partners age in years	23	25	21	28
Number of prior pregnancies	0	1	0	0
Year of prior pregnancy	NA	2004	NA	NA
<i>HIV test characteristics</i>				
ELISA *	Neg	Neg	Neg	Neg
Abbott Determine HIV-1/2 rapid test	Neg	Neg	Neg	Neg
HIV-1 RNA	Pos	Pos	Pos	Pos
Viral load copies/ml	64 200	1228130	94258	158453

* including repeat Enzyme linked immunosorbent assay (ELISA) with Enzygnost* Anti-HIV 1/2 Plus NA= not applicable