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SHORT COMMUNICATION

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

ABM Kharsany,[1] N Hancock,[2] JA Frohlich,[1] HR Humphries,[1] SS Abdool Karim[1,3] and Q Abdool Karim[1,3]

[1]*Centre for the AIDS Programme of Research in South Africa (CAPRISA), Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa,* [2]*Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, USA and* [3]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA*

Objectives

The aim of this study was to evaluate the 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 on their first antenatal care visit to a primary health care clinic 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 load in the four samples was 386 260 HIV-1 RNA copies/mL (range 64 200–1 228 130). 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: acute HIV infection, HIV-1 RNA, pregnant women, rural South Africa, screening

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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 who are infected [1]. In countries with generalized epidemics where heterosexual transmission is dominant, HIV seroprevalence surveys among pregnant women are frequently used. These surveys identify individuals with latent or advanced HIV disease and miss individuals with 'window-period' acute HIV infection (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-calibrated assays for HIV-1 RNA and 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 the 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 km west of

Correspondence: Dr Ayesha BM Kharsany, Centre for the AIDS Programme of Research in South Africa (CAPRISA), Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa.
Tel: + 27 31 260 4558; fax: + 27 31 260 4566; e-mail: kharsany@ukzn.ac.za

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 voluntary HIV counselling and testing services and, if infected, have access to programmes designed to prevent mother-to-child transmission of HIV and antiretroviral therapy (ART) if they meet the eligibility criteria for initiation of treatment.

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 are tested using a single enzyme-linked immunosorbent assay (ELISA) (Abbott Axsym System for HIV-1/HIV-2; Abbott Laboratories, Chicago, IL, USA) [10]. We included consecutive pregnant women who presented for their first antenatal 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 prelabelled ethylenediaminetetraacetic acid (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 labelled 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 (Dade Behring, Marburg, Germany), an ELISA for the detection of antibodies to HIV-1, HIV-2 and HIV-1 (subtype O) antigens. Plasma from all ELISA-negative samples were batched and tested using the pooled NAAT strategy [5,6]. Each master pool comprised 10 samples, consisting of 100 μL from each sample to a total volume of 1000 μL, and tested with qualitative HIV-1 RNA polymerase chain reaction (PCR) assay (COBAS Amplicor™ System, Roche Molecular Systems; Systems, Inc., Branchburg, New Jersey, USA). 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 Molecular Systems) which has a detection level of ≥ 40 HIV-1 RNA 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 Laboratories). We calculated the cost of HIV-1 RNA testing by including the cost of consumables, test kits and technicians' 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 by dividing the number of women who were HIV-1 RNA-positive by the total number of ELISA-negative samples tested. The annual HIV incidence was calculated using the formula $I = (365/w)N_{\text{inc}}/(\text{number at risk})$, where I is the incidence rate and w is the mean window of detection (28 days). 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 a percentage per year. The 95% confidence interval (CI) for the incidence estimate was calculated using $\pm\ 1.96\ I/(\sqrt{N_{\text{inc}}})$ [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 during their first antenatal care visit. The HIV prevalence at screening, patient demographics and HIV test characteristics are shown in Table 1. The overall HIV prevalence was 37.3% (95% CI 34.3–41.3]. Of the 467 ELISA HIV antibody-negative samples, four (0.9%) tested HIV-1 RNA-positive and antibody-negative with the Abbott Determine rapid assay. The mean viral load was 386 260 copies/mL (range 64 200–1 228 130). 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 were < 25 years and, for the other two, > 25 years. Only one woman reported a history of a previous pregnancy. The mean ages of women without AHI and their current partner were 22.3 years (SD 6.8, range 12–45) and 25.2 years (SD 7.9, range 16–56), respectively; 185 of 463 women reported having had at least one previous pregnancy.

Table 1 HIV prevalence, incidence and test characteristics among pregnant women with acute HIV infection (patients 1–4)

Characteristics	Overall sample
Sample size	$n = 750$
HIV prevalence	37.3% (95% CI 34.3–41.3)
HIV antibody-negative samples tested	$n = 467$
HIV incidence	11.2% per year (95% CI 0.3–22.1)

	Patient 1	Patient 2	Patient 3	Patient 4
Patient demographics				
Age (years)	18	21	19	18
Partner's age (years)	23	25	21	28
Number of prior pregnancies	0	1	0	0
Year of prior pregnancy	NA	2004	NA	NA
HIV test characteristics				
ELISA*	Negative	Negative	Negative	Negative
Abbott Determine HIV-1/2 rapid test	Negative	Negative	Negative	Negative
HIV-1 RNA	Positive	Positive	Positive	Positive
Viral load (HIV-1 RNA copies/mL)	64 200	1 228 130	94 258	158 453

Including repeat ELISA with Enzygnost Anti-HIV-1/2 Plus.
CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; NA, not applicable.

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 and 40 individual tests) at a cost of 483 South African rand (R483; approximately US$61, £40) per test, making, in total, R42 021.00 (US$5253, £3502). The cost per individual HIV-negative sample was R90.00 (US$11, £8), while the cost of identifying a single case of AHI was R10 505.00 (US$1313, £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, among 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 with HIV infection are unlikely to be diagnosed, 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 per 100 person-years obtained following retesting of HIV-negative pregnant women around the time of delivery from urban and rural facilities in South Africa [11]. While 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 18–25-year-old nonpregnant women in Hlabisa and Durban, South Africa, were 8.9 and 8.5 per 100 person-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 HIV-1 subtypes B, E, and D immunoglobulin G 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 with 0.9% in men of the same age group. Among 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 AHI with established long-term infections and individuals on ART [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 than p24 antigen [5,6]. A further advance 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 remains the mainstay of HIV surveillance [10,15].

With increasing access to and uptake of ART, 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 of monitoring trends in HIV infection and behaviour. Enhancing current screening programmes to include tests for HIV-1 RNA and 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].

While 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 support the need to detect AHI to prevent secondary spread. As 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, should be incorporated into the Department of Health's annual anonymous National Antenatal Sentinel HIV and Syphilis Prevalence Surveys [10] to provide a parallel measure of incident HIV infections as ART 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; because the survey population comprised pregnant women seeking antenatal care; and because 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]. 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; has lower specificity, as detectable low viral load is classified as positive; and has some loss of sensitivity due to the testing of 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, the HIV-1 RNA pooled NAAT strategy has been used with great efficiency to diagnose AHI in pregnant women [17] and in high-risk individuals from populations with low [18] and high HIV incidence [19,20].

Diagnosing pregnant women with AHI is critical to reducing perinatal and heterosexual transmission of HIV, underscoring the need for vigilant and rigorous testing for HIV infection at antenatal 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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Conflicts of interest: None

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