

ORIGINAL RESEARCH ARTICLE

Human immunodeficiency virus in plasma and cervicovaginal secretions in Filipino women

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Summary: This study examined 30 HIV-infected women in Manila to assess the relationship between cervicovaginal and plasma HIV-1 viral load. An interview and gynaecologic examination was conducted and cervicovaginal lavage (CVL) and venous blood specimens were collected. HIV-1 RNA was detected in plasma samples of 24 patients (80%) and in CVL samples of 18 women (60%); 16 patients (53%) had detectable levels in both. CVL HIV-1 RNA was detectable in 75% of women (6/8) with plasma viral loads between 10,000 and 100,000 copies/mL and in 77% of women (10/13) with plasma viral loads higher than 100,000 copies/mL ($P=0.0086$). Among women with CD4 cell counts of less than 200, 200–500, and greater than 500/mm³, CVL HIV-1 RNA was detected in 73%, 69%, and 17% of women, respectively ($P=0.1428$). HIV-1 RNA shedding in the genital tract was significantly associated with plasma viral load.

Keywords: cervicovaginal, HIV-1 RNA, genital tract, shedding

Introduction

The World Health Organization (WHO) estimates that there are 5.6 million people infected with human immunodeficiency virus (HIV) in South and Southeast Asia¹. The prevalence of HIV is still very low in the Philippines (0.07%), but the number of HIV cases is increasing and may be underestimated². As of May 2001 the Philippine Department of Health HIV/AIDS Registry reports 1503 HIV-seropositive Filipinos, 503 (33%) of whom are living with AIDS³. Because the estimated reporting rate of HIV infection is low (5%), the WHO approximates that there are really 28,000 Filipinos living with HIV². The predominant mode of transmission in the Philippines remains heterosexual contact, accounting for 913 (61%) of reported cases³. Injection drug use accounts for less than 1% (6 of 1503 cases) of HIV infections, and perinatal transmission accounts for just over 1% (20 of 1503 cases)³. Approximately 40% (590 of 1503 reported cases) of HIV-infected individuals in the Philippines are women³.

Heterosexual exposure is the most common mode of HIV-1 transmission worldwide¹. Vertical transmission is the most common mode of HIV-1 transmission to children. The incidence of HIV-1 is

increasing rapidly among women⁴. HIV-1 shedding in the female genital tract influences both heterosexual and vertical (mother-to-child) transmission, but little is understood about the factors governing this shedding⁵. A better understanding of cervicovaginal HIV shedding may improve efforts to prevent both heterosexual and vertical transmission.

Polymerase chain reaction (PCR) technology has been used to document the presence of HIV-1 in the cervix and vagina^{6–12}. Major correlates associated with the presence of HIV-1 in genital secretions (HIV-1 shedding) include high plasma viral load, low CD4 cell count, and presence of concomitant genital tract infections^{13–20}. HIV shedding has also been associated with pregnancy, menses, use of oral contraceptives, vitamin A deficiency, cervical ectopy, and cervicitis^{11,12,21–26}. A negative association has been documented between highly active antiretroviral therapy (HAART) and detectable HIV-1 RNA in both genital secretions and plasma¹⁶.

To date there has been no research examining viral shedding in the genital tract of HIV-seropositive Filipino women. Because so few women in the Philippines have access to antiretroviral therapy, this population provides a unique opportunity to observe the natural state of cervical and vaginal HIV-1 shedding. This study examined 30 HIV-infected women in Manila to assess the relationship between cervicovaginal HIV-1 viral load, plasma HIV-1 RNA level, and CD4 cell count.

Methods

A cross-sectional study was conducted from June 1999 through June 2000 at San Lazaro Hospital in Manila, Philippines. All HIV-seropositive women currently seeking care during this time period were invited to participate in the study. The primary investigator (GU N-V) recruited, screened, and oriented all participants. Written informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Board (IRB) at Brown University and the Committee on Research Implementation and Development (CRID) at the University of the Philippines.

Women were interviewed to collect information about demographic characteristics, sexual behaviour, and reproductive and medical history. Following the interview a gynaecologic examination was conducted and cervicovaginal lavage (CVL) and venous blood specimens were collected. Two endocervical swabs were collected for chlamydia and gonorrhoea cultures. Additional swabs of the posterior fornix were taken and samples were placed on two slides for microscopic examination. One slide was buffered with normal saline to screen for trichomoniasis; a second was buffered with 10% potassium hydroxide (KOH) solution to screen for fungal elements. Patients received appropriate treatment upon detection of any of these infections. A CVL specimen was collected by instilling 10 mL of phosphate-buffered saline (PBS) solution directed at the cervical os and aspirating back as much fluid as possible. Ecto- and endocervical swabs were obtained for Papanicolaou smear. Phlebotomy for measurement of CD4 count and plasma viraemia, as well as serology testing for syphilis, was also conducted at this time.

Quantification of HIV-1 RNA in plasma and CVL specimens was performed using the nucleic-acid sequence-based amplification assay (Organon Teknika Corporation, Durham, NC, USA), according to manufacturer's protocol. Results were expressed as copies of HIV-1 RNA per mL of plasma or CVL fluid. The lower limit of detection for the assay was 400 copies/mL.

Univariate and multiple regression analyses were applied to identify associations among CVL HIV-1 RNA, plasma viral load, CD4 count, and genital tract infections. The Spearman rank correlation test was used to assess the relation between CVL viral HIV-1 RNA levels and plasma viral load levels. Differences in the levels of plasma and CVL HIV-1 RNA among different levels of CD4 count were assessed with the Kruskal-Wallis rank test.

Results

The mean age of the women was 30 years. Twenty-six (86.7%) of the participants were infected with HIV through the heterosexual route. The CD4 cell

counts ranged from 21 to 1,222 cells/mm³ and 11 of 30 women (37%) had CD4 counts lower than 200/mm³. Only one patient was on combination antiretroviral therapy.

Samples adequate to measure viral load were obtained from 27 women. Plasma HIV-1 RNA was detected in 24 patients (80%). HIV-1 copy numbers in plasma ranged from 110 to 570,000 copies/mL. HIV-1 RNA was detected in CVL samples of 18 women (60%). HIV-1 copy numbers in CVL ranged from 500 to 48,000 copies/mL. Sixteen patients had detectable levels of HIV-1 RNA in both plasma and CVL (53%). All women who had positive HIV-1 RNA in both plasma and CVL had higher levels in plasma. HIV-1 RNA was detected in plasma samples but not in CVL specimens of 8 women out of 30 (27%). Three women out of 30 (10%) were negative for HIV-1 RNA in both plasma and CVL. There were no women with detectable CVL and undetectable plasma viral load.

None of the women with plasma viral load less than 10,000 copies/mL had detectable cervicovaginal viral load. Cervicovaginal HIV-1 RNA was detectable in 75% of women (6/8) with plasma viral loads between 10,000 and 100,000 copies/mL and in 77% of women (10/13) with plasma viral loads higher than 100,000 copies/mL ($P=0.0086$). Among women with CD4 cell counts of less than 200, 200-500, and greater than 500/mm³, CVL HIV-1 RNA was detected in 73%, 69%, and 17% of women, respectively, but this relationship was not statistically significant ($P=0.1428$). None of the women with detectable CVL HIV-1 RNA were receiving antiretroviral therapy. There were no significant differences in age or risk factors between those with detectable and undetectable cervicovaginal HIV-1 RNA (Table 1).

A Spearman rank correlation test showed a significant relation between the levels of HIV-1 RNA in CVL and the levels of HIV-1 RNA in plasma ($P=0.003$). Kruskal-Wallis testing showed a significant negative association between the level of HIV-1 RNA in plasma and CD4 count ($P=0.004$) among those with detectable plasma viral load. The association between the level of HIV-1 RNA in CVL samples and CD4 count among those with detectable CVL viral load approached but did not achieve statistical significance ($P=0.065$) (Table 2).

Age, infection route, CD4 cell count, plasma viral load, trichomoniasis infection, and presence of vulvar warts were selected for inclusion in multivariate analysis. Multivariate logistic regression analysis did not reveal any statistically significant associations between CVL HIV-1 RNA and these factors.

Discussion

In this study, cervicovaginal HIV-1 RNA shedding was present in a majority of women with no

Table 1. Subject characteristics and presence of human immunodeficiency virus-1 RNA in cervicovaginal lavage (CVL) samples

Characteristic	Total No. (n=30)	CVL (+) (n=18)	Odds ratio	P-value
Age (years)			1.67	0.8224
Under 35	20	11 (55%)		
35 or older	10	7 (70%)		
Risk factor				
Occupational exposure	1	0 (0%)	0.61	0.7586
Injection drug use	1	0 (0%)	0.61	0.7586
Sex	26	17 (65%)	5.95	0.2779
Blood transfusion	2	1 (50%)	0.60	1.0000
CD4 cell count (mm ³)			0.38	0.1428
<200	11	8 (73%)		
200–500	13	9 (69%)		
>500	6	1 (17%)		
Plasma viral load			4.9	0.0086
<400 (undetectable)	4	0 (0%)		
400–9999	3	0 (0%)		
10,000–100,000	8	6 (75%)		
>100,000	13	10 (77%)		
Genital tract infections				
Chlamydia	1	0 (0%)	0.61	0.7586
Gonorrhoea	1	0 (0%)	0.61	0.7586
Trichomoniasis	2	0 (0%)	4.91	0.2700

Table 2. Human immunodeficiency virus-1 RNA in plasma and cervicovaginal lavage (CVL) with different CD4 cell counts

CD4 (mm ³)	Plasma (+)*			CVL (+)**		
	No.	Range (copies/mL)	Median	No.	Range (copies/mL)	Median
<200	8/11	110,000–570,000	355,000	8/11	2800–37,000	6600
200–500	12/13	22,000–390,000	77,500	9/13	500–48,000	4000
>500	4/6	1100–37,000	4950	1/6	1100	1100

*P=0.004

**P=0.065

antiretroviral therapy. Detectable CVL HIV-1 viral load was significantly associated with plasma viraemia. Although the relationship did not achieve statistical significance, CVL HIV-1 shedding was more common in women with lower CD4 cell counts. Because of the small sample size and low prevalence of genital tract infections, there was not statistical power to detect significant associations between CVL HIV-1 shedding and the presence of genital tract infections.

The role of female genital HIV-1 shedding in heterosexual and vertical transmission remains unclear. It has been shown that HIV-seropositive women with more advanced disease transmit HIV-1 to their male partners more frequently²⁷. Plasma viral load is the strongest predictor of risk of HIV-1 heterosexual transmission²⁸. Likewise, vertical transmission is more likely to occur in women with higher levels of HIV-1 RNA in plasma^{29–31}. It is plausible that women with more advanced disease and higher levels of viral load in plasma also have higher levels of CVL viral load. Thus women with higher levels of CVL HIV-1 RNA shedding may be more likely to transmit HIV-1 to their male sexual partners and/or to their babies. In agreement with

other current literature on this topic, the findings of this study strongly support a correlation between plasma and CVL HIV-1 viral loads.

In this study, CVL RNA shedding occurred in 67% (16/24) of women with detectable plasma viral RNA. In a 2001 study of 311 HIV-infected women, genital tract HIV-1 shedding occurred in 80% (130/163) of women with detectable plasma RNA¹⁹. In keeping with our study findings, Kovacs *et al* concluded that plasma RNA concentration was the most important predictor of genital HIV shedding¹⁹. In a 1997 study of 72 HIV-infected women, CVL RNA shedding occurred in 44% (27/61) of women with detectable plasma RNA; this study also documented a dose-dependent increase in genital HIV-1 shedding with increasing plasma viral load⁵. Among 205 HIV-infected women in a 2000 study, 34.9% (51/146) with detectable plasma viral load exhibited genital HIV-1 shedding¹⁶.

Other recent studies have documented significant associations between genital HIV-1 shedding and vaginal infections and low CD4 cell count. A 2001 study of 37 women found genital HIV shedding to be significantly associated with plasma viral load and vaginal infections¹⁷. In a 2001

Senegalese study, HIV-1 shedding was associated with advanced disease stage and immunosuppression, as well as basic vaginal pH²⁰. Neither presence of genital tract infections nor low CD4 cell count attained statistical significance as predictors of genital HIV-shedding in our analyses. However, it is likely that these associations do in fact exist, but are masked by our small sample size and the low prevalence of sexually transmitted diseases in our population.

Recent data suggest that HAART is associated with below-detectable levels of both plasma and genital tract HIV-1 RNA¹⁶. This study indicates that HIV-1 RNA is detectable in both plasma and genital tract of the majority of women not receiving antiretroviral therapy. In the Philippines where most HIV-infected women have no access to antiretroviral therapy, the promotion of safe sexual practices in HIV-1 infected women has an important role in the prevention of HIV transmission. Access to antiretroviral therapy should decrease plasma as well as genital tract viral load.

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