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J Infect Dis. Author manuscript; available in PMC 2009 May 1.

Published in final edited form as:
J Infect Dis. 2002 December 1; 186(11): 1669–1672. doi:10.1086/345364.

Cervical Shedding of Human T Cell Lymphotropic Virus Type I Is Associated with Cervicitis

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Abstract

Human T cell lymphotropic virus type I (HTLV-I) is sexually transmitted. The purpose of this study was to determine the prevalence and risk factors for cervical shedding of HTLV-I DNA among Peruvian sex workers. HTLV *tax* DNA was detected in cervical specimens from 43 (68%) of 63 HTLV-I–infected sex workers and in samples obtained during 113 (52%) of 216 clinic visits between 1993 and 1997. Detection of HTLV DNA was associated with the presence of ≥30 polymorphonuclear cells (PMNs) within cervical mucus per 100×microscopic field (odds ratio [OR], 4.3, 95% confidence interval [CI], 1.8–10.1) and with the presence of cervical secretions (OR, 2.0; 95% CI 1.2–3.4). Hormonal contraceptive use (OR 1.7; 95% CI, 0.8–3.6) and concomitant cervical infection by *Chlamydia trachomatis* (OR, 1.5; 95% CI, 0.3–4.3) or *Neisseria gonorrhoeae* (OR, 1.1; 95% CI, 0.6–3.7) were not significantly associated with HTLV-I shedding. Our results suggest that cervicitis may increase cervical HTLV-I shedding and the sexual transmission of this virus.

Human T cell lymphotropic virus type I (HTLV-I) causes HTLV-I–associated myelopathy/tropical spastic paraparesis and adult T cell leukemia [1]. Like human immunodeficiency virus

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Presented in part: 9th International Conference on Human Retrovirology, April 1999, Kagoshima, Japan (abstract SP31).

Informed consent was obtained from all patients. Approval was obtained from the Human Subjects Review Committee of the University of Washington, the Office for Protection from Research Risks of the National Institutes of Health, and the Institutional Review Board of the Universidad Nacional Mayor de San Marcos in Lima, Peru.

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(HIV), HTLV-I is transmitted sexually, from mother-to-infant, or by blood transfusion or injection drug use [1]. The prevalence of HTLV-I infection in female sex workers (FSWs) has ranged from 3.2% in Kinshasa, Zaire, to 5.7% in Fukuoka, Japan, and to 21.8% in Callao, Perú [2].

In Latin America, the Caribbean, and the United States, HTLV-I infection has been associated with the number of sexual partners and the duration of commercial sex work or homo-sexuality [3]. Serologic evidence of HTLV-I infection has been associated with ulcerative (syphilis, herpes simplex virus [HSV] type 2, and chancroid) and nonulcerative (gonorrhea and chlamydia) sexually transmitted diseases (STDs) [4].

Male-to-female transmission of HTLV-I infection has been found to occur more frequently than female-to-male transmission [5]. Higher rates of male-to-female transmission were associated with older male partners, length of relationship, high antibody titer against *env* or whole virus proteins, and high virus titer in lysed peripheral blood mononuclear cells (PBMC) [5]. Syphilis and genital ulcer disease in men have been associated with higher rates of female-to-male HTLV-I transmission, whereas a history of STD was associated with HTLV-I seropositivity in men and women [3,6]. Shedding in the genital tract has been examined only by Belec et al. [7], who detected HTLV-I DNA in 3 (20%) of 15 cervicovaginal secretions from HTLV-I–infected women tested, but they did not examine potential risk factors for shedding. The present study was undertaken to identify the prevalence of and risk factors for HTLV-I shedding in cervical secretions in a large cohort of asymptomatic HTLV-I–infected Peruvian FSWs.

Materials and Methods

Study design

All registered FSWs in Lima and Callao, Peru, were eligible to participate and underwent gynecological examination at a public health clinic every 2 weeks. A study social worker recruited FSWs and administered a standard questionnaire to each participant. The gynecological examination included collection of a vaginal specimen for direct microscopic evaluation and 2 endocervical specimens: one was used for Gram's stain, and the other was placed in either 2SP medium (1993–1995) or a cryovial (1996–1997), which then was frozen at −70°C. Specimens were subsequently used for polymerase chain reaction (PCR) assays for HTLV DNA, *Neisseria gonorrhoeae*, or *Chlamydia trachomatis*. All study subjects provided written informed consent; each received 25 condoms in return for participation.

Laboratory testing

We tested serum samples for HTLV-I anti-body by use of ELISA (Cambridge Bioscience) and confirmed ELISA-positive results by use of an rp21e-enhanced Western blot assay (Cambridge Bioscience). ELISA-positive specimens were considered to be HTLV-I seropositive if the Western blot revealed bands at p24, as well as at gp46 or rp21env. If only other virus-specific bands were present, such as p53 or p19, the individual's results were considered to be indeterminate. Only women who were positive for HTLV by means of Western blot on all serum samples tested were included in the analyses.

Detection of HTLV DNA in genital specimens was accomplished by means of nested PCR amplifying for the *tax* gene, as described by Tuke et al. [8]. In brief, genital specimens were lysed using a volume of lysis buffer (10 m*M* Tris-HCl [pH 8.3], 50 m*M* KCl, 0.01% gelatin, 0.45% NP-40, 0.45% Tween 20, and 0.6 mg/mL proteinase K) equal to sample volume and were incubated at 56°C for 1 h and then heat-inactivated at 90°C for 15 min. For the primary HTLV *tax* PCR, 10 μL of lysate was used for first-round PCR amplification. For second-round

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HTLV *tax* PCR, 5 μL of the primary amplification was added to the second-round PCR cocktail and amplified. The secondary PCR amplification products (128 bp) were visualized on a 2% agarose gel in 1× Tris Borate EDTA (pH 8) (TBE). The sensitivity of the PCR was 1 HTL-infected cell/100,000 cells. To ensure that each sample contained amplifiable material, β-globin was amplified by use of 25 μL of lysate added to the PCR cocktail (1× PCR buffer II, 1.5 m*M* $MgCl_2$, 40 pmol of each primer, 200 μ*M* each dNTP, 1 U AmpliTaq (Applied Biosystems), and sterile dH_2O to a total volume of 80 μL). Amplification conditions consisted of a hold at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. The primers (PC03, ACAAACTGTGTTCACTAGC; PC04, CAACTTCATCCACGTTCACC) produced a 110-bp amplicon, as visualized on a 2% agarose gel in 1× TBE. The sensitivity for the β-globin primers was determined to be 10 cell equivalents.

PCR for N. gonorrhoeae *and* C. trachomatis

DNA was purified from genital samples by use of Masterpure DNA purification kits (Epicentre Technologies). *N. gonorrhoeae* and *C. trachomatis* were detected by use of the Roche Amplicor multiplex assay system.

Definition of mucopurulent cervicitis (MPC)

The number of polymorphonuclear cells (PMNs) present within cervical mucus per 100× microscopic field were counted, and MPC was defined as ≥30 PMNs, as described by Brunham et al. [9].

Diagnosis of vaginal infection

Bacterial vaginosis was diagnosed by Gram's stain or Amsel's criteria [10]. Wet-mount examination of vaginal fluid was used to diagnose *Trichomonas vaginalis* infection (presence of motile trichomonads) and *Candida* vulvovaginitis (presence of yeast cells or mycelia).

Statistical analysis

Using generalized estimating equation models to account for multiple visits per woman over time, we were able to examine predictors of cervical shedding of HTLV-I, comparing visits where a participant shed with visits where no shedding occurred. These models correctly adjusted standard errors (and hence, *P* values) for repeated observations per participant, and model coefficients were interpreted in a manner similar to those derived through logistic regression. The correlation between visits was assumed to be autoregressive (i.e., declining as days between observations increased), as described by Heagerty and Zeger [11].

Potential predictors considered were as follows: age, race, place of birth, age of sexual debut, age at which individual entered prostituion, duration of sex work, number of clients in previous week, place of prostitution, sex with a foreigner, marital status, education, abortion history, parity, use of hormonal contraceptives, vaginal pH, vaginal cleansing in last 36 h, and presence of *N. gonorrhoeae, C. trachomatis*, trichomoniasis, bacterial vaginosis, grossly visible cervical secretions, cervical lesions, or ≥30 PMNs. Variables statistically significant at $\alpha = 0.2$ in univariate models or previously associated with genital shedding of HIV (presence of *N. gonorrhoeae* or *C. trachomatis* or hormonal contraceptive use) were included in the adjusted model. Statistical analyses were completed using S-plus (Insightful).

Results

Between February 1993 and July 1997, 1119 FSWs were enrolled. At initial testing (all but 3 conducted at the baseline visit), 97 women (8.7%) were HTLV-I seropositive by ELISA and

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Western blot, 1009 (90.2%) were seronegative, and 13 (1.2%) had indeterminate results. Of the 97 HTLV-I–seropositive women, 63 had β-globin detected in at least 1 cervical specimen and were consistently seropositive. Of these 63 women, cervical specimens collected that were β-globin positive were included in further analyses. Forty-three (68%) had HTLV *tax* DNA detected by PCR assay in at least 1 cervical specimen, and, of 216 cervical specimens collected longitudinally, 113 (52%) had detectable HTLV DNA. The mean age of these 63 women was 36 years (range, 20–72 years), the mean duration of sex work was 8 years (range, <1–45 years), and the mean number of clients in the prior week was 19 (range, 0–119 clients) (table 1).

Cervical shedding of HTLV DNA was observed at 29 (81%) of 36 visits for women with ≥30 PMNs on Gram's stain of the endocervical swab and at 81 (49%) of 166 visits for women with <30 PMNs (odds ratio [OR], 4.3; 95% confidence interval [CI], 1.8–10.1; table 2). Shedding also was associated with grossly visible cervical secretions (OR, 2.0; 95% CI, 1.2–3.4).

Six women had gonoccoccal infection and 11 had chlamydial infection; of these, 1 had both infections. The association of HTLV shedding with cervical infection by *N. gonorrhoeae* or *C. trachomatis* (adjusted OR, 1.4; 95% CI, 0.6–3.2) was not statistically significant. One or more vaginal infections were diagnosed in 30 women (24 women with bacterial vaginosis, 12 with candidiasis, and 2 with trichomoniasis), but none were associated with HTLV-I shedding.

Cervical HTLV DNA shedding was more common among women using hormonal contraceptives and among women aged <30 years, but these observations did not reach statistical significance in either unadjusted or adjusted analysis (table 2). Other characteristics examined, as detailed in Materials and Methods, were not associated with shedding.

Discussion

We detected cervical shedding of HTLV DNA in two-thirds of HTLV-I–infected women and found significant associations of cervical shedding of HTLV with the presence of ≥30 PMNs in endocervical mucus and of grossly visible cervical secretions, but not with concomitant STD or hormonal contraception. Mucopurulent cervicitis has been defined by various criteria, including concentration of PMNs in endocervical mucus, visible yellow endocervical discharge, and easily induced cervical bleeding [12]. The presence of ≥30 PMNs in cervical mucus has been associated with cervical infection due to *N. gonorrhoeae*, *C. trachomatis*, or HSV [12]. Infection with *N. gonorrhoeae* or *C. trachomatis* in our study was uncommon and was not significantly associated with detection of HTLV DNA in the specimen; a larger study with a higher incidence of these cervical infections would be required to test associations with shedding of HTLV DNA. Although we did not classify inflammatory cytologic patterns in cervical secretions in this study, our group previously defined inflammatory cytologic smears as containing PMNs, lymphocytes, transformed lymphocytes, plasma cells, and histiocytes—each of which we found to be associated with the presence of one or more cervical infections [13]. The association of HTLV shedding with inflammatory patterns remains a potential area for future research.

Whether HTLV-I is a cause or an effect of cervicitis remains to be clarified. It is plausible that cervicitis, which is associated with cervical microulcerations and increased lymphocytes in cervical secretions, results in shedding of cell-associated HTLV-I. It is also possible that cervicitis among the FSWs in this study was caused by other coinfections or conditions that were not detected. For example, we did not test for HSV, *Treponema pallidum*, or *Mycoplasma genitalium*, which would cause ulcerations or nongonococcal cervicitis. Furthermore, we did not perform colposcopy to determine whether these women had cervical ectopy. Future studies could assess the influence of other cervical infections and cervical ectopy or other colposcopic abnormalities on cervical HTLV-I shedding.

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Sexual transmission of HTLV-I infection has occurred more frequently from man to woman than from woman to man [5]. Transmission from female to male may depend on several factors, including the presence of an STD. It remains to be determined whether sexual transmission is associated with higher proviral copy number in blood or genital fluids, and we have not yet examined the relationship of cervicitis to HTLV-I concentration in cervical specimens. As with HIV-1, higher HTLV-I copy numbers may increase transmission of infection to a sexual partner, and STDs may increase susceptibility to acquiring HTLV-I. Consistent condom use with clients increased from 10% in 1990 to 50% in 1991–1992 among registered FSWs working in brothels in Lima [14]. In a subsequent study of Peruvian FSWs in Lima and Callao by our group, 71% reported always using condoms with clients (authors' unpublished data). Increased condom use by Peruvian FSWs during the 1990s was temporally associated with the decrease in HTLV-I seroprevalence, from 21.8% in 1987–1988 to 8.7% in this study, and could help explain this decrease. Seroprevalence within the general population was much lower: of 2492 pregnant women tested for HTLV-I in Lima in 1998–1999, 42 (1.7%) were determined to be seropositive (J.O.V.A. and K.K.H., unpublished data). Injection drug use is known to be very uncommon in Peru and would not explain the high seroprevalence of HTLV among FSWs. Of 400 FSWs in Lima and Callao who were questioned, none acknowledged ever using injection drugs [15]. The lack of evidence of parenteral transmission and the much higher level of HTLV-I seropositivity among FSWs than in the general population in Lima and Callao support the premise that sexual transmission was responsible for the infection of most of the FSWs in our study. If cervicitis and cervical shedding of HTLV are associated with infectivity, then treatment of cervicitis could reduce sexual transmission of HTLV-I from woman to man. The influence of genital ulceration and urethritis on HTLV-I shedding remains to be studied.

Acknowledgements

Financial support: National Institutes of Health (NIH; grants TW00679 and AI0714P); Fogarty International (grant T22-TW-00001); University of Washington Center for AIDS Research (grant AI-27757); New Investigator Award from the NIH-funded University of Washington Sexually Transmitted Diseases–Cooperative Research Center (grant AI31448 to J.R.Z.).

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Table 1

Baseline characteristics of 63 human T cell lymphotropic virus type I (HTLV-I)–seropositive female sex workers (FSWs).

Characteristic	Variable
Age, mean years ± SD (range)	36 ± 10 (20–72)
Race	
Mixed	59 (98)
Asian	1 (2)
Born in Andes mountains	20 (33)
Attended secondary school	30 (49)
Married or cohabitating	9 (15)
Age sexual debut, mean years ± SD (range)	17 ± 3 (8–25)
Age began FSW, mean years ± SD (range)	26 ± 6 (14–40)
Duration as FSW, mean years ± SD (range)	8 ± 8 (0–45)
Clients last week, mean no. ± SD (range)	19 ± 23 (0–119)
Worked in brothel	53 (87)
HTLV-I DNA in cervical specimens	43 (68)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. HTLV-I seropositive defined as seropositive for all visits that serologic analysis was performed. Questionnaire data available for 61 of 63 women.

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Table 2

Characteristics of human T cell lymphotropic virus type I (HTLV-I)–seropositive women at clinic visits with and without cervical shedding of HTLV-I–infected cells.

Variable	Total no. of visits (n = 216)	No. (%) of visits		OR (95% CI)	Adjusted OR (95% CI)
		Without shedding	With shedding		
Cervicitis, ≥30 PMNs				4.3 (1.8–10.1)	4.1 (1.7–9.9)
Yes	36	7 (19)	29 (81)		
No	166	85 (51)	81 (49)		
Cervical secretions (grossly visible)					
Any	107	42 (39)	65 (61)	2.0 (1.2–3.4)	1.9 (1.1–3.3)
None	93	50 (54)	43 (46)		
Hormonal contraceptive use				1.7 (0.8–3.6)	1.9 (0.8–4.2)
Yes	143	62 (43)	81 (57)		
No	68	39 (57)	29 (43)		
Age <30 years				2.1 (0.9–4.9)	1.3 (0.5–3.6)
Yes	70	21 (30)	49 (70)		
No	141	80 (57)	61 (43)		
STD *(Neisseria gonorrhoeae* or *Chlamydia trachomatis)*					
Yes	26	9 (35)	17 (65)	1.4 (0.6–3.1)	1.4 (0.6–3.2)
No	182	89 (49)	93 (51)		
N. gonorrhoeae				1.1 (0.3–4.3)	1.1 (0.2–5.2)
Yes	7	2 (29)	5 (71)		
No	201	96 (48)	105 (52)		
C. trachomatis				1.5 (0.6–3.7)	1.5 (0.6–3.8)
Yes	20	7 (35)	13 (65)		
No	187	90 (48)	97 (52)		

NOTE. Generalized estimating equations used to adjust for observations correlated within person. Odds ratios (ORs) were adjusted for hormonal contraceptive use, age, cervicitis, and cervical secretions, except sexually transmitted diseases (STDs), which were adjusted for hormonal contraceptive use and age but not for cervicitis, because ORs for these STDs may cause cervicitis. CI, confidence interval; PMNs, polymorpho-nuclear cells.