

Low-level genital HIV shedding in Thai HIV-infected women with suppressed plasma viral load after menopause: a longitudinal study

Nadia Kancheva Landolt^{1*}, Tanya Do¹, Naruporn Kasipong¹, Rosalin Kriengsinoyot², Sasiwimol Ubolyam¹, Apicha Mahanontharit¹, Tippawan Pankam², Tanakorn Apornpong¹, Anchalee Avihingsanon¹, Jintanat Ananworanich^{1,3,4,5}, Nittaya Phanuphak^{2,3} and Surasith Chaithongwongwatthana⁶

¹ HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok, Thailand

² Thai Red Cross AIDS Research Centre, Bangkok, Thailand

³ SEARCH, Bangkok, Thailand

⁴ University of Amsterdam, the Netherlands

⁵ US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA

⁶ Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract

Objectives: First, to evaluate the longitudinal changes of HIV RNA in genital secretions in HIV-positive women with plasma HIV RNA <50 copies/mL before and after the onset of menopause. Second, to assess inflammatory markers and prevalence of comorbidities after the onset of menopause.

Methods: This was a prospective observational study with two time points. HIV RNA in genital secretions (GVL) was measured in 15 HIV-positive menopausal women (second time point). Results were compared to earlier available data for GVL from the same participant before the onset of menopause (first time point).

Results: Median age at the first time point was 42 years, and 52 years at the second time point. Median time since the onset of menopause was 2 years and 33% of women were sexually active. Eighty per cent had at least one comorbidity. The GVL before menopause was >50 copies/mL in 27% of the participants, and in 40% after menopause. The GVL was <1000 copies/mL in all but one measurement. There was no significant difference between the two time points ($P=0.687$). Intermediate vaginal flora or bacterial vaginosis was found in 73% of participants during the second time point.

Conclusions: There was a high prevalence of low-level GVL shedding before and after menopause. This needs further investigation, especially in relation to the vaginal microbiome and the complex interactions between micro-organisms. HIV-infected women in menopause do not seem to present a major public health risk for HIV transmission. Nevertheless, safe sex should be discussed with all, regardless of age. The high prevalence of non-communicable diseases after menopause requires special attention and comprehensive care.

Keywords: HIV and women, menopause, genital viral shedding, metabolic syndrome

Introduction

Women represent approximately half of all HIV-positive individuals. With antiretroviral therapy (ART), more HIV-positive women are surviving and entering the menopause [1]. Furthermore, there is an increase in new HIV diagnoses among women >40 years [2]. HIV status does not seem generally to influence either the age at menopause onset nor menopausal symptoms [3,4], although data are still scarce in this field.

Menopause is a natural biological process. It is characterised with a decrease of hormone production, oestrogen and progesterone by the ovaries and cessation of menstruation. The protective role of oestrogens on the vaginal epithelium and the immune system is well described [5–7]. In a small study by Rollenhagen and Asin, enhanced HIV replication has been reported in all 16 samples of ectocervical tissue from menopausal women in comparison to only 60% out of 15 tissue samples from premenopausal women [8]. An increase in inflammatory markers, associated with disease progression and comorbidities, has also been noted [8]. In addition, healthy, HIV-uninfected, menopausal women might have increased CCR5 expression on endocervical CD4 T cells [9], as well as decreased innate anti-HIV-1 activity in the lower genital tract [10], which could facilitate HIV susceptibility.

In spite of these biological changes in women after the menopause, the few studies available have failed to find differences in clinical outcomes related to HIV disease progression and infectivity between pre- and postmenopausal women [3,11–13]. ART-naive postmenopausal women, respond to ART in the same way as premenopausal women [12]. Genital tract HIV RNA (GVL) shedding has been reported in women with undetectable plasma HIV RNA (PVL), regardless of age and menopausal status [11,14,15]. Suppression of PVL with ART appears to be the main predictor for reduced GVL shedding, and thus reduced risk for HIV transmission [16,17].

Better knowledge on GVL shedding in menopausal women will contribute to a better understanding of the pathogenesis of HIV infection in this target group, and of the risks of HIV sexual transmission from menopausal women. The objective of this study was to assess the longitudinal changes in GVL in HIV-positive women with undetectable PVL before and after the onset of menopause.

Methods

This was a prospective observational study with two time points. Part of the data for the first time point was gathered through a secondary analysis. There were two study sites: Anonymous Clinic, Thai Red Cross AIDS Research Centre and HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT). No formal sample size calculation was performed. We screened all female participants who had taken part between 2002 and 2009 in three studies conducted at the two study sites (HN 058 STACCATO; HN

*Corresponding author: Nadia Kancheva Landolt, HIV-NAT, Thai Red Cross AIDS Research Centre, 104 Rajdamri Road, Pathumwan, Bangkok, Thailand 10330
Email: nadia.kl@hivnat.org

079 HIV-STAR; HPV study, Anonymous Clinic), and who had genital samples collected and either stored or tested for GVL. All eligible participants were enrolled. Inclusion criteria included women in natural menopause (second time point), with at least one result of GVL (available result/stored sample) of vaginal secretions from earlier studies (first time point). At the first time point, participants would have had regular natural menstrual periods and would not have been pregnant (eligibility criteria for the three earlier studies from which participants were enrolled). All participants should have had an undetectable PVL (<50 HIV-1 copies/mL), at the time of GVL testing (first and second time points). Menopause is defined as not having had any menstrual bleeding for a period of at least 12 months, owing to natural age-related changes in hormones. At the second time point, demographic and reproductive health data were gathered, along with vaginal swabs for GVL testing and Gram's stain, as well as endocervical swabs for testing *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in case of detectable GVL (GVL >50 HIV-1 copies/mL, GVL shedding). Additionally, we collected blood during the second time point for measuring high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6). Blood samples were centrifuged and stored at -80°C . Participants gave informed consent. The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University.

Laboratory tests for GVL, PVL and inflammatory markers were performed at the HIV-NAT Research Laboratory, Thai Red Cross AIDS Research Centre, Bangkok, Thailand. The GVL was tested in dry vaginal swabs at both time points, using COBAS Ampliprep/COBAS TaqMan HIV-1 Test, Version 2.0. Samples had been stored at -80°C . CT and NG were tested with a qualitative polymerase chain reaction (qPCR) test using COBAS 4800 CT/NG. Vaginal flora was assessed on Gram's stain by Nugent score at the Department of Microbiology, Chulalongkorn University.

Heterosexual HIV transmission is rare among persons with PVL <1500 HIV-1 copies/mL [18] and almost absent when the PVL is <400 HIV-1 copies/mL [19]. There are no such thresholds determined for GVL. All the study participants were found to have a PVL <50 copies/mL at both time points. Therefore, for the purpose of this study, we have defined low-level GVL shedding, as GVL <1000 HIV-1 copies/mL.

Data were analysed by calculating the median and interquartile ranges (IQR) for quantitative characteristics, and the number and percentages for categorical characteristics, using STATA version 11.2 (Statacorp LP College station, Texas, USA). McNemar's test was applied for assessing the significance in the change of GVL shedding prevalence before and after menopause. Logistic regression was applied to assess factors associated with the prevalence of GVL shedding during menopausal years.

Results

Baseline characteristics

We preselected 106 women, out of 441, aged 45–65 years at the second time point screening. These women could possibly then be in menopause (second time point), while being at reproductive age with regular natural menstrual periods during the earlier study (first time point). Of these, 17 women corresponded to the inclusion/exclusion criteria and were enrolled in this study between January 2016 and June 2016. Non-eligibility reasons were loss to follow-up/referred to another hospital ($n=35$); regular menstrual period ($n=38$); menopause during the first time point/hysterectomy ($n=8$); not willing to participate in the study/death ($n=8$).

Fifteen women contributed to the analysis. Two women were excluded due to detectable plasma viraemia at the second time point and incomplete data from the first time point. All participants were of Thai ethnicity. Median age at the first time point was 42 years (IQR 40–45), and 52 years (IQR 51–54) at the second time point. Participants had been in menopause for a median of 2 years (IQR 1–9) at the second time point. Median body mass index during the second time point was 22.5 kg/m² (IQR 21.8–25.3). At the second time point all participants were on ART for a median of 14 years with 73% on a non-nucleoside reverse transcriptase inhibitor-based regimen and 17% on a protease inhibitor-based regimen. No participant had missed a dose in the last month prior to the second time point visit; however, 20% reported a delay of more than 1 hour in taking medication.

All participants had at least one child. Five participants (33%) were sexually active with one partner at the second time point with a median frequency of sexual intercourse four times a month. Eighty percent (four out of five) of sexually active women were using condoms consistently after menopause versus 53% (8 out of 15) during reproductive years. All participants had normal-for-age gynaecological examination findings and normal Pap smear results. On Gram stain, four participants out of 15 (27%) had normal vaginal flora, seven (46%) intermediate vaginal flora and four (27%) bacterial vaginosis. Out of 15 participants, 12 (80%) had at least one non-communicable comorbidity, which in 83% was a metabolic syndrome.

Genital viral shedding and inflammatory markers

Low-level GVL shedding was common before menopause: four participants (27%), and after menopause: six participants (40%) (Table 1). GVL was <1000 HIV-1 copies/mL in all but one measurement (first time point). There was no significant difference between the two time points, ($P=0.687$). Two participants (13%) were shedding virus at both time points, six (40%) at only one of the two time points and seven (47%) had undetectable GVL. QPCR for CT and NG were negative in the six participants with GVL shedding at the second time point. GVL shedding during menopause in our cohort was not associated with any of the factors under study.

Inflammatory markers during the menopausal years were within the laboratory norms: hs-CRP at a median of 0.83 mg/L and IL-6 of 2.71 pg/mL.

Discussion

We found a comparable prevalence of GVL shedding in a small, longitudinal cohort of HIV-infected women with undetectable PVL before and after the onset of menopause. Levels of GVL were low. These results are similar to other reports in the literature. Kovacs *et al.* found a 33% prevalence of detectable GVL with PVL <500 HIV-1 copies/mL in a cross-sectional study, in reproductive-aged women [15]. Melo *et al.*, in a cross-sectional study, compared GVL in pre- and postmenopausal women visiting a gynaecological clinic with predominantly sexually transmitted infection (STI) symptoms and no significant differences, potentially influenced by menopause, were found [11]. However, the cross-sectional study design and higher prevalence of STI in younger women were acknowledged to be important shortcomings of the study, which could have confounded the interpretation of results [20]. Cu-Uvin *et al.*, in a longitudinal analysis testing GVL every four weeks for a period of one year, found 37% prevalence of detectable GVL with undetectable PVL [14]. In addition, the authors have reported that some women had detectable GVL over several visits (7%), some had intermittently detectable GVL (31%) and some had undetectable GVL (46%). When considering our study, 13% of

Table 1. Genital viral shedding and inflammatory markers before and after menopause

Variable	Years before menopause, median (IQR) 6(5–10) years n=15 median (IQR)/n (%)	Years after menopause, median (IQR) 2(1–9) years n=15 median (IQR)/n (%)	P-value
PVL <50 (copies/mL)	15 (100)	15 (100)	
GVL >50 (copies/mL)	4 (27)	6 (40)	0.687
Median GVL, (copies/mL)	149 (82–956)	148 (90–183)	
Maximum GVL, (copies/mL)	1755	660	
NG among women with GVL shedding	—	0	
CT among women with GVL shedding	—	0	
Bacterial vaginosis	—	4 (27)	
hs-CRP (mg/L)	—	0.83 (0.40–2.97)	
IL-6 (pg/mL)	—	2.71 (2.23–5.41)	

CT: *Chlamydia trachomatis*; GVL: genital HIV-RNA viral load; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; NG: *Neisseria gonorrhoeae*; PVL: plasma HIV-RNA viral load.

the participants had detectable GVL before and after menopause, 40% at only one of the two time points and 47% had undetectable GVL.

The somewhat ‘sporadic’ nature of GVL shedding has also been described by others [16]. Some authors have found an association between GVL shedding and factors such as STIs, vaginal pH (a proxy for vaginal dysbiosis) and the type of ART taken [11,16,21]. However, such associations were not always confirmed when an undetectable PVL was present [16]. Therefore, the PVL seems to be the main predictor of GVL [16,17,22]. As all our study participants had an undetectable PVL, it is not surprising that we have not found any association between GVL shedding and these variables.

It is worth noting that 73% of our study participants had laboratory findings consistent with vaginal dysbiosis, including reduced *Lactobacillus* spp. *Lactobacillus* spp and their associated lactic acid production is understood to contribute to vaginal health [23]. This suggests that we may get a better understanding of GVL shedding with enhanced understanding of the vaginal microbiome and the complex interactions between micro-organisms [23–25].

All women in the study were sexually active during their reproductive years, in comparison to 33% after the onset of menopause. Most sexually active menopausal women were consistently using condoms during sexual intercourse. This finding corroborates another report on declining sexual activity and declining unprotected sexual intercourse in HIV-positive women after the onset of menopause [26]. All our study participants had an undetectable PVL and GVL levels were low, in spite of a 40% prevalence. Therefore, HIV-infected women in menopause with undetectable PVL do not seem to pose a major risk for HIV transmission.

Among our participants, 80% had at least one comorbidity, a metabolic syndrome being the most common condition. HIV infection is often associated with dyslipidaemia, insulin resistance and ART – the standard of care for HIV-infection – increases the incidence of metabolic risk factors [27], as does being menopausal [28,29]. In spite of HIV viral suppression, ageing HIV-infected women have chronic immune activation and are, therefore, at an increased risk of age-associated end-organ diseases compared to uninfected age-matched controls [30]. Inflammatory markers in our study were within the laboratory limits. Metabolic conditions have been described in younger HIV-infected women as well, highlighting the necessity to integrate non-communicable diseases

(NCD) within HIV care programmes [31]. We believe that interventions should start as early as HIV diagnosis [31].

The modest study sample size, as well as the PVL <50 HIV-1 copies/mL as an inclusion criterion, do not allow us to generalise our findings. However, the prevalence of low-level GVL shedding in women needs further investigation and could improve our understanding of the vaginal microbiome and the complex interactions between micro-organisms. The low sexual activity of HIV-infected women in our cohort, high percentage of condom use and well-suppressed PVL by ART, does not seem to present a major public health risk of HIV transmission. Nevertheless, as some women were sexually active, safe sex should be discussed with all, regardless of age. In addition, the high prevalence of NCD requires special attention and comprehensive care.

Acknowledgements

We are grateful to the research and clinical staff and clients of the Thai Red Cross Anonymous Clinic and the HIV-NAT Clinic, who contributed to this study, as well as to the research, clinical staff and clients of the earlier studies, including STACCATO (HN058), HIV-STAR (HN079) and the HPV study.

Conflict of interests

The views expressed are those of the authors and should not be construed to represent any of the institutions mentioned above. The authors have no conflict of interest.

Funding

The study was funded by the National Research Council of Thailand (NRCT).

References

- Hogg R, Lima V, Sterne JA *et al.* Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008; **372**: 293–299.
- Espinoza L, Hall HI, Hardnett F *et al.* Characteristics of persons with heterosexually acquired HIV infection, United States 1999–2004. *Am J Public Health* 2007; **97**: 144–149.
- Cejtin HE, Kim S, Taylor RN *et al.* Menopause in women in the Women’s Interagency HIV Study (WIHS). XV International AIDS Conference, July 2004, Bangkok, Thailand. Abstract WePeD6504s.
- Lui-Filho JF, Valadares AL, Gomes Dde C *et al.* Menopausal symptoms and associated factors in HIV-positive women. *Maturitas* 2013; **76**: 172–178.
- Hel Z, Stringer E, Mestecky J. Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. *Endocr Rev* 2010; **31**: 79–97.
- Mingjia L, Short R. How oestrogen or progesterone might change a woman’s susceptibility to HIV-1 infection. *Aust N Z J Obstet Gynaecol* 2002; **42**: 472–475.

7. Rodriguez-Garcia M, Biswas N, Patel MV *et al*. Estradiol reduces susceptibility of CD4+ T cells and macrophages to HIV-infection. *PLoS One* 2013; **8**: e62069.
8. Rollenhagen C, Asin SN. Enhanced HIV-1 replication in *ex vivo* ectocervical tissues from post-menopausal women correlates with increased inflammatory responses. *Mucosal Immunol* 2011; **4**: 671–681.
9. Meditz AL, Moreau KL, MaWhinney S *et al*. CCR5 expression is elevated on endocervical CD4+ T cells in healthy postmenopausal women. *J Acquir Immune Defic Syndr* 2012; **59**: 221–228.
10. Chappell CA, Isaacs CE, Xu W *et al*. The effect of menopause on the innate antiviral activity of cervicovaginal lavage. *Am J Obstet Gynecol* 2015; **213**: 204 e201–206.
11. Melo KC, Melo MR, Ricci BV, Segurado AC. Correlates of human immunodeficiency virus cervicovaginal shedding among postmenopausal and fertile-aged women. *Menopause* 2012; **19**: 150–156.
12. Patterson KB, Cohn SE, Uyanik J *et al*. Treatment responses in antiretroviral treatment-naive premenopausal and postmenopausal HIV-1-infected women: an analysis from AIDS Clinical Trials Group Studies. *Clin Infect Dis* 2009; **49**: 473–476.
13. van Benthem BH, Vernazza P, Coutinho RA *et al*. The impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women. *AIDS* 2002; **16**: 919–924.
14. Cu-Uvin S, DeLong AK, Venkatesh KK *et al*. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. *AIDS* 2010; **24**: 2489–2497.
15. Kovacs A, Wasserman SS, Burns D *et al*. Determinants of HIV-1 shedding in the genital tract of women. *Lancet* 2001; **358**: 1593–1601.
16. Homans J, Christensen S, Stiller T *et al*. Permissive and protective factors associated with presence, level, and longitudinal pattern of cervicovaginal HIV shedding. *J Acquir Immune Defic Syndr* 2012; **60**: 99–110.
17. Spencer LY, Christiansen S, Wang CH *et al*. Systemic immune activation and HIV shedding in the female genital tract. *J Acquir Immune Defic Syndr* 2016; **71**: 155–162.
18. Quinn TC, Wawer MJ, Sewankambo N *et al*. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med* 2000; **342**: 921–929.
19. Attia S, Egger M, Muller M *et al*. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *AIDS* 2009; **23**: 1397–1404.
20. Tsibris AM. Human immunodeficiency virus and the postmenopausal woman: genital shedding may remain the same. *Menopause* 2012; **19**: 124–125.
21. Low AJ, Konate I, Nagot N *et al*. Cervicovaginal HIV-1 shedding in women taking antiretroviral therapy in Burkina Faso: a longitudinal study. *J Acquir Immune Defic Syndr* 2014; **65**: 237–245.
22. Natividad-Villanueva GU, Santiago E, Manalastas RM, Jr *et al*. Human immunodeficiency virus in plasma and cervicovaginal secretions in Filipino women. *Int J STD AIDS* 2003; **14**: 826–829.
23. Ravel J, Gajer P, Abdo Z *et al*. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011; **108** Suppl 1: 4680–4687.
24. Muhleisen AL, Herbst-Kralovetz MM. Menopause and the vaginal microbiome. *Maturitas* 2016; **91**: 42–50.
25. Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. *J Physiol* 2017; **595**: 451–463.
26. Taylor TN, Weedon J, Golub ET *et al*. Longitudinal trends in sexual behaviors with advancing age and menopause among women with and without HIV-1 infection. *AIDS Behav* 2015; **19**: 931–940.
27. Husain NE, Ahmed MH. Managing dyslipidemia in HIV/AIDS patients: challenges and solutions. *HIV AIDS (Auckl)* 2015; **7**: 1–10.
28. Kanapathipillai R, Hickey M, Giles M. Human immunodeficiency virus and menopause. *Menopause* 2013; **20**: 983–990.
29. Kojic EM, Wang CC, Cu-Uvin S. HIV and menopause: a review. *J Womens Health (Larchmt)* 2007; **16**: 1402–1411.
30. Alcaide ML, Parmigiani A, Pallikkuth S *et al*. Immune activation in HIV-infected aging women on antiretrovirals—implications for age-associated comorbidities: a cross-sectional pilot study. *PLoS One* 2013; **8**: e63804.
31. Sobieszczyk ME, Werner L, Mlisana K *et al*. Metabolic syndrome after HIV acquisition in South African women. *J Acquir Immune Defic Syndr* 2016; **73**: 438–445.