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Variations in CD4 cell counts among HIV-uninfected and infected women in Uganda and Zimbabwe

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Summary

We conducted a cross-sectional study with 208 HIV-uninfected and 188 HIV-infected women in Uganda and Zimbabwe to investigate differences in median CD4 counts. Absolute CD4 counts were determined by flow cytometry. Multivariate analyses were used to examine the association of country and HIV-infection status on CD4 counts. Median CD4 counts were significantly lower in Zimbabwe than in Uganda overall (649 and 783 cells/mm^3, $P = 0.009$) and among HIV-infected women (470 and 614 cells/mm^3, $P = 0.003$). In separate multivariable models, CD4 counts were significantly lower in Zimbabwe in HIV-uninfected ($P = 0.014$) and infected ($P < 0.001$) women, controlling for age, contraceptive method, education and living with partner status. In a model combining HIV-uninfected and infected women, there was no significant interaction between country and HIV infection status ($P = 0.344$), suggesting that the relationship between country and CD4 count was not significantly modified by HIV infection status. This study reinforces the importance of establishing country-specific reference CD4 levels as CD4 count continues to be used as a key biomarker in clinical decision-making for HIV-infected individuals in sub-Saharan Africa.

Keywords

CD4 count; HIV infection; women; Africa

INTRODUCTION

The CD4+ T-lymphocyte cell count remains an important marker for monitoring human immunodeficiency virus (HIV)-1 disease progression. The use of the CD4 count in clinical

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decision-making for HIV-1-infected persons, however, is improved by knowledge of haematological reference ranges in the source populations.

While a CD4 reference range of 475–1616 cells/mm^3 has been established for healthy HIV-uninfected individuals in the United States,[1] this cannot be applied to other populations due to considerable variation in factors that impact CD4 levels.[2–5] Previous studies of CD4 in healthy HIV-uninfected African populations have established mean CD4 cell counts of 775 cells/mm^3 in Ethiopia,[6] 980 cells/mm^3 in Tanzania[7] and 1256 cells/mm^3 in Uganda.[8] In some HIV-uninfected African populations, background CD4 counts have varied by gender: at a national hospital in Tanzania, women had significantly higher mean CD4 levels than men.[5] A study of HIV counselling and testing clients in Uganda (38% women) had similar find-ings.[8] However, among 183 healthy HIV-uninfected miners (49% women) and 681 antenatal clinic attendees in Nigeria, CD4 counts were similar among the male and non-pregnant female miners (combined mean 828 cells/mm^3).[3]

In Ugandan and Zimbabwean women, who became HIV-infected while participating in the Hormonal Contraception and Risk of HIV Acquisition (HC–HIV) Study[9] and subsequently enrolled in an incident HIV infection cohort (Genital Shedding [GS] Study), CD4 counts were measured quarterly. Preliminary results demonstrated that median CD4 counts were lower in Zimbabwean women than in Ugandan women during early HIV-1 infection.[10] We conducted a sub-study in a sample of HIV-uninfected and infected women from these two cohorts to determine whether CD4 differences observed were associated with differences in (1) background levels in HIV-uninfected women; (2) the infection process (e.g. variation by HIV subtype); or (3) laboratory procedures.

METHODS

Between November 1999 and January 2004, women in Zimbabwe and Uganda were enrolled in the HC–HIV Study, a longitudinal study of hormonal contraception and HIV acquisition.[9] At enrolment, participants were aged 18–35 years, sexually active and seeking reproductive and general health-care services at three sites in Kampala (Uganda) and four sites in Harare and Chitungwiza (Zimbabwe). Participants had been using low-dose combined oral contraceptives (COCs), depot-medroxyprogesterone acetate or a non-hormonal contraceptive method for at least three months. If women became HIV infected, they were recruited into an incident HIV cohort, the GS Study, which started in March 2001.

Study population

Between July 2003 and January 2004, a sample of HIV-uninfected participants from the HC–HIV Study was recruited for the CD4 substudy. If they consented to participate, a CD4 T-lymphocyte count was conducted on routinely collected blood.

Between March 2001 and July 2007, GS Study participants were included in the CD4 substudy if they had a CD4 count available at six months (+1 month) following their estimated HIV infection date. CD4 counts measured approximately six months following the HIV infection were selected to capture early-stage HIV infection while allowing for recovery from the acute HIV infection CD4 nadir.

This substudy was approved by the institutional review boards of collaborating institutions in the United States, Zimbabwe and Uganda, and all women provided informed consent prior to study participation.

Laboratory procedures

CD4 T-lymphocyte counts were determined on site by standard flow cytometry using FACSCount or FACSCalibur (Becton Dickinson, Sparks, MD, USA) analysis within 4–6 hours of specimen collection. Both countries upgraded technology from FACSCount to FACSCalibur in 2003. Manufacturer's instructions were followed for all analytical procedures. Herpes simplex virus (HSV)-2 infection was diagnosed by ensyme linked immunosorbent assay (ELISA) (Focus Diagnostics, Cypress, CA, USA).

Every effort was made to ensure that the laboratories in Uganda and Zimbabwe followed the same procedures for flow cytometry and for quality assurance procedures. Both study laboratories participated in the CD4 proficiency testing programme administered by the UK National External Quality Assessment Service, as required by the US National Institutes of Health, Division of AIDS. Additionally, a subset of GS Study samples tested by each study laboratory was sent to a local reference laboratory for reliability testing.

Statistical analysis

The outcome was defined as differences in CD4 cells/mm^3 by group. The main effects of interest were country and HIV infection status. Age, contraceptive method, education level, living with partner and pregnancy status (HIV-infected women only) were examined as covariates. Sexual risk behaviour in the participant and their primary partner was also examined.

Differences in population characteristics were examined using Fisher's exact test. The Mann–Whitney–Wilcoxon test was used to test the hypothesis that median CD4 counts differed significantly by country and by HIV serostatus. In multivariate analyses, generalized estimating equations were used to evaluate the impact of the main effects on CD4 cell counts among HIV-uninfected and infected women separately and then in a single model with an interaction term for HIV infection status and country, controlling for covariates. The Wald method was used to compute confidence intervals for estimated CD4 counts.

RESULTS

Characteristics of the study population

The study population comprised 188 HIV-infected and 208 (53%) uninfected women. Among HIV-infected women, 100 (53%) were Zimbabwean and 88 (47%) were Ugandan; among HIV-uninfected women, 103 were from Zimbabwe and 105 from Uganda.

In both the HIV-infected and uninfected cohorts, women in Zimbabwe and Uganda were similar in age, and Zimbabwean women had more education than those in Uganda (Table 1). More Zimbabwean women were living with a partner compared with their Ugandan counterparts. In the HIV-infected cohort only, more Zimbabwean than Ugandan women

reported using hormonal contraceptive methods. No significant difference in HSV-2 infection status by country was observed between either cohort.

Variation in median CD4 cell counts

The two sites each received 25 identical proficiency panels as part of the UK-based external proficiency testing programme. Comparison of the results of the panels tested by each laboratory showed no difference in absolute CD4 counts (sign test: $P = 0.69$). In addition, GS Study samples tested in parallel at local reference laboratories in Uganda and Zimbabwe demonstrated a high correlation with the original values from the study laboratories ($r_S = 0.86$, $P < 0.0001$).

Overall, median CD4 cell counts were significantly lower in Zimbabwe (649, interquartile range [IQR]: 453–881) than in Uganda (783, IQR: 586–999) ($P = 0.009$) (Table 2). This difference was statistically significant in the HIV-infected women, where median CD4 cell counts were 470 (IQR: 357–579) in Zimbabwe and 614 (IQR: 463–783) in Uganda ($P = 0.003$). Among the HIV-uninfected women, we did not find a statistically significant difference in median CD4 counts between the two countries, although a similar trend was noted (Zimbabwe: 838 [IQR: 679–1041], Uganda: 912 [IQR: 757–1156]; $P = 0.244$).

Predictors of CD4 levels in HIV-uninfected and HIV-infected women

In separate multivariable models for HIV-uninfected and infected women, the CD4 count was significantly lower in Zimbabwe than in Uganda, controlling for age, education level, living with partner and contraceptive method, in both HIV-uninfected ($P = 0.014$) and infected ($P < 0.001$) women (Table 3). Covariates including current age, education level and living with partner were not associated with CD4 levels in either model; COC use was associated with higher CD4 levels among HIV-infected women ($P = 0.019$) (Table 3). Pregnancy status was significantly associated with decreased CD4 counts in HIV-infected women ($P = 0.031$). In a model combining all women, there was no significant interaction between country and HIV infection status ($P = 0.344$), suggesting that the relationship between country and CD4 count was not significantly modified by HIV infection status.

Among the HIV-infected women with HIV subtype data, CD4 count was significantly lower in Zimbabwe (196 fewer cells), where all HIV infections were subtype C, compared with women in Uganda with subtype A infections. However, when CD4 counts in Ugandan women with subtypes A and D were compared, there was no statistical difference in the CD4 count. COC use and pregnancy remained statistically significant in the model with HIV subtype included.

DISCUSSION

We found that CD4 levels were lower in Zimbabwe than in Uganda in both HIV-uninfected and infected women. HIV infection status did not modify the relationship between country and CD4 levels, suggesting the difference in CD4 levels among HIV-infected women is due to background CD4 levels rather than to the HIV infection process itself. We also found high levels of agreement between the two study laboratories during proficiency testing.

The specific reason behind the differences in background CD4 levels between Ugandan and Zimbabwean women remains unclear. We attempted to control for the potential effects of other underlying factors where data were available. For the most part, contraceptive method was not associated with the CD4 count, although we did observe significantly higher CD4 count in HIV-infected women who used COCs compared with the non--hormonal referent group. A similar effect was noted among COC users in a study of HIV-infected women in the USA.[11]

There was an association between pregnancy and lower CD4 count among HIV-infected women. While Zimbabwean women were significantly more educated and likely to be living with their regular partner, neither these factors nor age was independently associated with CD4 levels.

Although we found no difference in the CD4 count among HIV-infected women in Uganda with subtype D compared with those with subtype A, two independent cohort studies in Kenya and Uganda showed faster CD4 decline among women with subtype D HIV infection compared with women with other HIV subtypes.[12,13] Those studies, however, followed women over many years. Such a difference may not have been detectable in our study since all women had early HIV infection. Subtype C is predominant in Zimbabwe and subtype A in Uganda, but we cannot gauge whether the difference in CD4 counts is due to HIV subtype or to other factors.

Compared with a previous study by Tugume *et al.*[8] that reported a mean CD4 count of 1425 cells/mm^3 in a group of HIV-uninfected women in Uganda, our study found a much lower mean of 840 (and median of 783) among HIV-uninfected Ugandan women. Tugume *et al.*[8] reported the mean count, which may have been skewed due to outliers. Although both studies used the same laboratory, variations in laboratory procedures may explain the difference, because the previous study was conducted approximately 10 years earlier than the present study. Additionally, Tugume *et al.* utilized FACScan rather than FACSCount or FACSCalibur and calculated the CD4 count using the absolute lymphocyte count and CD4 percentage rather than measuring the absolute CD4 count directly. We are not aware of any comparable published studies of CD4 counts among HIV-uninfected Zimbabweans.

We lacked information on other factors that may have contributed to lower CD4 counts in Zimbabwe such as systemic infectious diseases including malaria, diarrhoeal or respiratory diseases and tuberculosis. Differential treatment and care of such co-morbidities across the two countries may also have contributed to the lower median CD4 counts in HIV-infected Zimbabwean women.

Despite the above limitations, our study contributes to the understanding of differential CD4 counts in HIV-uninfected and infected women in two sub-Saharan African countries. Ongoing and future studies should include examination of CD4 levels in non-pregnant women, particularly those who are known to be HIV-uninfected, to create a knowledge base on background levels of CD4 count in healthy individuals. Further, factors that may explain observed differences in background CD4 count should be examined to better understand the ‘natural history’ of CD4, particularly before and after HIV infection. Establishing reference

CD4 levels specific to sub-Saharan African countries is necessary for refining the interpretation of this key biomarker that continues to play a major role in clinical decision-making for HIV-infected individuals.

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Table 1

Characteristics of HIV-uninfected and infected women in Uganda and Zimbabwe (n = 396)

	HIV uninfected (n = 208)			HIV infected (n = 188)		
Characteristic	**Uganda (n = 105) n (%)**	**Zimbabwe (n = 103) n (%)**	***P* value***	**Uganda (n = 88) n (%)**	**Zimbabwe (n = 100) n (%)**	***P* value***
Age (years)†						
18–24	23 (22)	20 (19)	0.904	28 (32)	19 (19)	0.128
25–29	38 (36)	39 (38)		33 (38)	42 (42)	
≥30	44 (42)	44 (43)		27 (31)	39 (39)	
≤9 years of education‡	81 (78)	34 (33)	<0.001	60 (68)	39 (39)	<0.001
Living with partner§	87 (83)	96 (93)	0.032	43 (51)	72 (72)	0.004
Majority contraceptive use†**						
COC	33 (31)	37 (36)	0.752	13 (15)	24 (24)	<0.001
DMPA	35 (33)	30 (29)		32 (38)	55 (55)	
NH	37 (35)	36 (35)		40 (47)	21 (21)	
Currently pregnant†	0	0	NA	4 (5)	4 (4)	1.000
HSV-2 positive†	68 (67)	56 (54)	0.087	69 (81)	87 (87)	0.314

*Based on Fisher's exact test

†At CD4 study visit, which occurred between July 2003 and January 2004 for the HIV-uninfected group and between March 2001 and July 2007 for the HIV-infected group

‡At Hormonal Contraception and Risk of HIV Acquisition (HC–HIV) screening visit (13–45 months prior to the CD4 visit)

§At HC–HIV screening visit (13–45 months prior to the CD4 visit) for the HIV-uninfected group; at GS enrolment visit (0–6 months prior to the CD4 visit) for the HIV-infected group

**Combined oral contraceptive (COC), depot-medroxyprogesterone acetate (DMPA) and non-hormonal (NH) contraceptive methods

HSV-2 = herpes simplex virus-2; NA = not applicable

Table 2

Median and interquartile ranges (IQR) of CD4 T-cell counts among Ugandan and Zimbabwean women by HIV infection status ($n = 396$)

	Zimbabwe		Uganda		
	n	**Median CD4 (IQR)**	***n***	**Median CD4 (IQR)**	***P* value***
Total	203	649 (453–881)	193	783 (586–999)	0.009
HIV infected	100	470 (357–579)	88	614 (463–783)	0.003
HIV uninfected	103	838 (679–1041)	105	912 (757–1156)	0.244

*Based on Mann–Whitney–Wilcoxon test for H_O: Median CD4 (Uganda) − Median CD4 (Zimbabwe) ≤60 cells

Table 3

Predictors of CD4 counts in separate multivariable models for HIV-uninfected and infected women in Uganda and Zimbabwe

	HIV uninfected (*n* = 208)			HIV infected (*n* = 180[*])		
Variable	**Estimated CD4**	**95% CI**	***P* value[†]**	**Estimated CD4**	**95% CI**	***P* value[†]**
Intercept	927	774, 1080	<0.001	658	547, 770	<0.001
Country						
Zimbabwe	−114	−206, −23	0.014	−202	−287, −117	<0.001
Uganda	Reference			Reference		
Majority contraceptive use[‡§]						
COC	−27	−126, 71	0.590	131	21, 240	0.019
DMPA	−38	−138, 62	0.454	20	−65, 105	0.649
NH	Reference			Reference		
Age (years)[§]						
18–24	27	−82, 135	0.629	34	−64, 133	0.496
25–29	50	−42, 141	0.287	−1	−79, 77	0.980
30 and up	Reference			Reference		
≤9 years of education[**]	39	−51, 129	0.395	−19	−93, 54	0.608
Living with partner[††]	39	−87, 165	0.544	9	−69, 87	0.817
Currently pregnant[§]	–	–	–	−154	−294, −14	0.031

[*] Eight women were excluded in the HIV-infected model due to missing data

[†] Based on chi-square test by the generalized estimating equation model with Gamma distribution

[‡] Combined oral contraceptive (COC), depot-medroxyprogesterone acetate (DMPA) and non-hormonal (NH) contraceptive methods

[§] At CD4 study visit, which occurred between July 2003 and January 2004 for the HIV-uninfected group and between March 2001 and July 2007 for the HIV-infected group

[**] At Hormonal Contraception and Risk of HIV Acquisition (HC–HIV) screening visit (13–45 months prior to the CD4 visit)

[††] At HC–HIV screening visit (13–45 months prior to the CD4 visit) for the HIV-uninfected group; at GS enrolment visit (0–6 months prior to the CD4 visit) for the HIV-infected group