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Role of Nutritional Status and Weight Loss in HIV Seroconversion Among Rwandan Women

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Summary: To investigate nutritional status and heterosexual human immunodeficiency virus (HIV) transmission, we performed a nested case-control study of sexually active, adult women in Kigali, Rwanda. Forty-five women who seroconverted during the 24-month study period were compared to 74 women who remained seronegative throughout the study. Seroconvertors and nonseroconvertors did not differ in preseroconversion serum levels of vitamin A, carotenoids, vitamin E, selenium, albumin, ferritin, or cholesterol. Weight loss, however, was a significant predictor of eventual HIV seroconversion. Subsequent seroconvertors lost an average of 1.5 kg during the first 6 months of the study compared with a 1.0-kg gain ($p = 0.001$) for nonconvertors. Nine of 27 (33%) seroconvertors, compared with one of 44 (2%) controls, lost at least 5 kg in the 6-month period beginning 1 year before their seroconversion (odds ratio, 21.5, 95% confidence interval 4.1–112). The association between weight loss and seroconversion was independent of other potential risk factors such as socioeconomic status, pregnancy, and genital ulcer disease. In addition to these findings for measured weight loss during follow-up, reported weight loss before enrollment was also a risk factor for subsequent seroconversion. Additional studies of heterosexual HIV transmission are needed to determine whether or not weight loss is causally related to susceptibility for HIV infection. **Key Words:** Human immunodeficiency virus—AIDS—Heterosexual—Nutrition—Vitamin A—Vitamin E—Carotenoids—Selenium—Ferritin—Albumin—Weight—Africa.

Compared with industrialized countries, heterosexual human immunodeficiency virus (HIV) transmission in East Africa is remarkably high. Up to 30% of the sexually active adult population of some East African cities are infected with HIV (1), and seroconversion rates of 2–5% per year in urban adults are common.

HIV transmission in Africa occurs primarily from heterosexual contact (2). Heterosexual contact with multiple partners (3,4) and conditions affecting genital mucosal integrity (5), including genital ulcerative diseases (6–8), are risk factors for HIV sero-

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conversion in Africa. These factors, however, are not unique to Africa and do not fully explain the high rates of heterosexual HIV transmission.

One factor that is common in impoverished populations and may contribute to HIV seroconversion is malnutrition. Some micronutrients, such as vitamin A, are important in maintaining both mucosal integrity (9,10) and immune competence (11). Vitamin A deficiency has also been associated with increased risk of infection for a variety of viral and bacterial pathogens (12). To investigate the possibility that poor nutritional status may contribute to high rates of HIV seroconversion, we examined preseroconversion nutritional status in a nested case-control study of sexually active Rwandan women.

METHODS

Nested Case-Control Study

A nested case-control study was performed on 1,458 women enrolled in an ongoing cohort study in Kigali, Rwanda (1,13). Members of this cohort were sexually active women, aged 18 to 40 years, selected by stratified random sampling from consecutive prenatal care and pediatric clinic visitors in Kigali, Rwanda in 1988. This cohort's demographic characteristics and HIV seroprevalence rates are similar to the age- and sex-adjusted characteristics for the general urban female population of Kigali.

Women enrolled in our study were examined at baseline and at each 6-month interval for 2 years. At each clinic visit, serum was drawn for HIV testing and a brief questionnaire was completed. Participants were weighed at each examination by staff unaware of the participant's serostatus and always using the same single-beam balance weight scale.

Of the 1,458 women enrolled in the cohort study, 460 (32%) were HIV seropositive at the baseline examination in 1988 and were excluded from our case-control study of seroconversion. Forty-nine (5%) of the 998 remaining HIV-seronegative women seroconverted during the first 18–24 months of follow-up and were considered cases. A control group of 100 women was selected using a random number generator from among the participants who did not seroconvert during the study. Among the cases, 4 of 49 seroconvertors missed one or more visits before confirmed seroconversion and 26 of 100 controls missed one or more of the five examination visits. These women were excluded from the analysis because weight changes between visits and dates of seroconversion for these women could not be precisely determined (a higher proportion of controls were excluded because cases were followed until seroconversion and controls were followed until the end of the study). The remaining 45 cases and 74 controls had been weighed and tested for HIV at each of the five examination dates (baseline, 6, 12, 18, and 24 months).

HIV Testing

Sera drawn at each visit were screened for HIV antibodies in Rwanda using HIV-1 enzyme-linked immunosorbent assay (ELISA) and confirmatory Western blot or immunofluorescent assay at the Rwanda National AIDS Control Program Reference Laboratory. Sera repeatedly positive by ELISA and Western blot or immunofluorescence were considered positive for HIV infection (1,9). Sera were stored at −20°C in Rwanda until shipped to the United States for additional testing. During transit, these serum specimens thawed for approximately 24 h, but were promptly refrozen at −70°C on arrival. There was no evidence of deterioration of the specimens by turbidity or subsequent micronutrient analyses.

To ensure that seroconvertors were not seropositive at entry into the study, baseline specimens from women who seroconverted were retested for HIV-1 and HIV-2 at the Centers for Disease Control, Atlanta, GA, U.S.A. by both ELISA and Western blot. All baseline sera from cases were negative for HIV-1 or HIV-2 antibodies.

Micronutrient Assays of Baseline Sera

Micronutrient levels were measured in baseline, nonfasting sera (200 μl) by high-performance liquid chromatography using the method of Sowell et al. (14) with the following changes: In addition to nonapreno-β-carotene, retinyl butyrate was used as an internal standard and a Waters Model 490 detector was used for detection of vitamin A at 325 nm, vitamin E at 300 nm, and carotenoids at 450 nm. Cholesterol was measured using the Dupont ACA methods (DuPont ACA Manual, Wilmington DE, 1982). Serum albumin was measured using the Dupont ACA methods (DuPont ACA Manual 1984). Serum ferritin was measured using the Bio-Rad Quantimune ferritin IRMA kit (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Selenium levels in baseline sera were determined by the methods of Lewis et al. (15) and Paschel and Kimberly (16) using a Perkin-Elmer Model 3030 atomic absorption spectrometer with Zeeman background correction at 196 nm.

Statistical Analysis

Continuous variables were compared using a Student's t test and dichotomous variables were compared using a χ^2 test (Statistical Analysis Systems, Cary, NC, U.S.A.). If an expected cell had less than five members, a Fisher's exact test was calculated using EPIINFO version 5.0 (USD Inc., Stone Mountain, GA, U.S.A.). Potential confounding between weight loss and other exposure variables was evaluated by logistic regression analysis.

RESULTS

Of the 45 women with HIV seroconversion during the 24-month follow-up ("cases"), 18 were first seropositive at the 6-month examination, 12 at the 12-month examination, 8 at the 18-month examination and 7 at the 24-month examination. Cases and unmatched controls were similar in age, height, body weight, body mass index, religion, income, and number of lifetime sex partners at enrollment (Table 1).

TABLE 1. *Demographic and physical characteristics of seroconvertors (cases) and controls at baseline examination*

	Sero-convertors, (n = 45)	Controls, (n = 74)	p value
Age, yr (mean ± SD)	28.1 ± 4.4	29.4 ± 4.7	0.16[a]
Height, cm (mean ± SD)	160 ± 6	162 ± 7	0.15[a]
Weight, kg (mean ± SD)	58.1 ± 9.4	60.0 ± 13.6	0.37[a]
Body mass index, kg/m^2 (mean ± SD)	22.6 ± 3.4	22.8 ± 4.6	0.81[a]
Subject's income, Rwandan francs/month			
0–999	34 (76%)	54 (73%)	0.49[b]
1,000–9,999	7 (16%)	10 (14%)	
>10,000	4 (9%)	10 (14%)	
Primary partner's income, Rwandan francs/month			
0–2,999	19 (42%)	22 (30%)	
3,000–9,999	9 (20%)	18 (24%)	
>10,000	17 (38%)	34 (46%)	0.21[b]
Religion			
Catholic	30 (67%)	42 (57%)	
Other Christian	6 (13%)	19 (26%)	0.52[b]
Moslem	9 (20%)	11 (15%)	
Other	0	2 (3%)	
Number of sexual partners in year before enrollment (mean ± SD)	1.2 ± 0.8	1.0 ± 0.2	0.19[c]

[a] Student's t test.
[b] Cochran-Mantel-Haenszel statistic.
[c] Wilcoxon rank-sum test.

Baseline Micronutrient Levels

Nonfasting levels of vitamin A, carotenoids, vitamin E, ferritin, selenium, albumin, and cholesterol in baseline sera did not differ significantly between cases and controls (Table 2). The lack of association between these nutritional factors and seroconversion was not affected by adjustment for potential confounding factors such as age, income level, oral contraceptive use and number of sex partners (data not shown). To determine whether or not short-term micronutrient fluctuations might increase the risk of seroconversion, 6-month seroconvertors alone were also compared with the control group. No significant differences in micronutrient levels between these two groups were found (Table 2).

Seroconversion and Weight Loss

At entry into the study, cases were more likely than controls to report "noticeable weight loss except from pregnancy or delivery" during the previous year [22 of 45 cases vs. 16 of 74 controls; odds ratio 3.5; 95% confidence intervals (C.I.) 1.6–7.6]. A similar association was noted for direct measurements of body weight changes between enrollment and the first 6-month examination. Although the baseline body weights of cases and controls were similar (Table 1), subsequent seroconvertors lost 1.5 kg on average between enrollment and the first 6-month visit, while control patients gained 1.0 kg during the same period (p = 0.001, Student's t test). During the first six months of the study, the risk of subsequent seroconversion was highest for women losing at least 5 kgs (Table 3). Overall, 10 (22%) of 45 cases and 1 (1%) of 74 controls lost 5 kg or more during the first 6-month period (odds ratio 20.9; 95% C.I. 2.7–917). This did not represent protein-calorie malnutrition since the mean weight among seroconvertors who lost 5 kgs or more was still 57 kg after the weight loss.

TABLE 2. *Comparison of baseline serum micronutrient levels between cases and controls*

	All seroconvertors (n = 45) mean ± SD	Six-month seroconvertors only (n = 18) mean ± SD	Controls (n = 74) mean ± SD	p value[a]	
				All seroconvertors vs. controls	Six-month seroconvertors vs. controls
Vitamin A (μg/dl)	51.7 ± 18.3	51.1 ± 23.5	50.1 ± 15.4	0.63	0.86
Carotenoids (μg/dl)					
α-Carotene	22.7 ± 12.0	24.4 ± 14.8	23.0 ± 15.9	0.92	0.67
β-Carotene	22.6 ± 13.1	24.5 ± 16.6	23.7 ± 18.2	0.68	0.86
Lutein/zeaxanthin	27.4 ± 15.7	28.5 ± 14.2	28.2 ± 13.9	0.78	0.95
Cryptoxanthin	2.6 ± 1.5	2.8 ± 1.8	2.8 ± 2.0	0.58	0.96
Lycopene	5.2 ± 3.2	5.0 ± 3.6	5.3 ± 4.4	0.94	0.81
Vitamin E (μg/dl)	708 ± 192	691 ± 171	721 ± 187	0.71	0.54
Selenium (ng/ml)	103 ± 21	98 ± 18	101 ± 21	0.71	0.55
Ferritin (ng/ml)	61 ± 39	61 ± 38	61 ± 49	0.97	0.99
Cholesterol (mg/dl)	111 ± 33	114 ± 33	113 ± 25	0.66	0.90
Albumin (g/dl)	3.9 ± 0.6	3.7 ± 0.8	3.9 ± 0.6	0.66	0.26

[a] Student's t test.

TABLE 3. *Weight loss during the first 6 months of the study among seroconvertors (cases) and controls*

Weight change, baseline to 6 months	Sero-convertors (n = 45)	Controls (n = 74)	Odds ratio	95% confidence interval
>0 kg gain	15	37	1.0	
0–4.9 kg loss	20	36	1.4	0.6–3.4
≥5 kg loss	10	1	24.7	2.9–1,098

Since weight loss among the women who seroconverted could be due to intercurrent acute infection with the HIV virus, this analysis was repeated after excluding those who became HIV positive at the 6-month examination. After excluding the 18 women seropositive at the 6-month visit, 7 (26%) of the remaining seroconvertors lost 5 kg or more between 0 and 6 months compared with 1 (1%) of 74 controls (odds ratio 25.6; 95% C.I. 2.9–1166). All 27 of the seroconvertors had at least one negative HIV test between the end of the weight measurement period and their first positive HIV serology.

Delivery after pregnancy could result in weight loss and might be associated with HIV seroconversion. This possibility was controlled for by examining only cases and controls who were not pregnant at baseline. When pregnant women were excluded from the analysis, 6 (16%) of the remaining 37 nonpregnant seroconvertors and 1 (2%) of the remaining 61 nonpregnant controls lost 5 kg or more between 0 and 6 months (odds ratio 11.6; 95% C.I. 1.3–542). After excluding both women who were pregnant and women who seroconverted at the 6-month visit, 5 (22%) of the remaining 23 nonpregnant seroconvertors and 1 (2%) of the remaining 61 nonpregnant controls lost 5 kg or more during the first 6 months of the study (odds ratio 16.7; 95% C.I. 1.6–801).

The association between weight loss and seroconversion also remained significant when the cases were compared with an independent control group. A second control group of 74 seronegative women with complete follow-up was identified by random selection without replacement from the seronegative cohort and compared with the cases. Cases were still significantly more likely to lose 5 kg or more during the first 6 months of the study compared with this second, independent group of controls [10 (22%) of 45 cases vs. 4 (5%) of 74 controls; odds ratio 5.0; 95% C.I. 1.6–15.7].

Weight loss was also examined relative to the seroconversion date of the case patients. Control patients were randomly matched to cases and weight changes were compared in cases and controls beginning 1 year before the case's seroconversion (weight changes were measured between 0 and 6 months for 12-month seroconvertors and controls, between 6 and 12 months for 18-month seroconvertors and controls, and between 12 and 18 months for 24-month seroconvertors and controls). Six-month seroconvertors and their matching controls were excluded from the analysis. This ensured that all of the seroconvertors had one negative HIV test between the period of weight loss and their first positive HIV test. Overall, 9 (33%) of 27 seroconvertors lost 5 kg or more compared with 1 (2%) of 44 controls during the 6-month period beginning 1 year before seroconversion (odds ratio 21.5; 95% C.I. 4.1–112).

Interaction Between Weight Loss and Other Risk Factors

To determine whether or not weight loss was confounded by other risk factors, the risk of seroconversion associated with 0- to 6-month weight loss was adjusted for a variety of potential confounding factors by logistic regression (Table 4). We did not

TABLE 4. *Seroconversion and ≥5-kg weight loss at 0 to 6 months, adjusted for potential confounding by nested logistic regression*

Risk factor	Odds ratio for HIV seroconversion[a]	95% confidence interval
≥5-kg weight loss, unadjusted	20.7	7.2–85.3
≥5-kg weight loss adjusted for		
Pregnancy	22.6	7.7–66.4
Breast-feeding	18.5	6.2–55.0
Income ≥5,000 Rwandan francs/month	21.1	7.24–61.6
Primary partner's income ≥5,000 Rwandan francs/month	29.7	10.0–88.2
Genital inflammation, ulceration, or discharge	21.1	7.2–62.2
History of tuberculosis in year before enrollment[b]	—	—
History of ≥1 month diarrhea in year before enrollment	22.9	7.6–69.7

All exposures measured at baseline unless otherwise noted.
[a] Maximum likelihood estimate.
[b] One case and no controls had a history of tuberculosis in the year before enrollment.

find that the association between weight loss and seroconversion was due to any of the potential confounding factors shown in Table 4, nor to other factors measured at baseline, including a history of transfusion, injections, oral or injected hormonal contraceptive use, white cell count, eosinophil count, Westergren erythrocyte sedimentation rate, hematocrit, baseline vitamin A, vitamin E, and selenium levels. None of the participants admitted to having a sex partner with AIDS. This suggests that weight loss in seroconvertors was not due to the psychological or economic stress of having a sex partner disabled by symptomatic HIV disease.

Anorexia in the year before enrollment was a frequent complaint among both subsequent seroconvertors and nonseroconvertors. Among seroconvertors who reported weight loss, 55% reported anorexia in the same interval, compared with only 26% of seroconvertors without weight loss ($p < 0.05$).

DISCUSSION

Although a number of risk factors for heterosexual HIV transmission have been identified, the reasons for its rapid spread in Africa and other developing regions of the world remain unclear. In the United States and Europe, heterosexual HIV transmission is relatively inefficient, even between HIV-discordant couples with a high degree of exposure (17,18). In contrast, HIV seroprevalence in the general populations of several African cities is high, suggesting that heterosexual seroconversion may be more efficient in these settings. Genetic susceptibility is unlikely to account for this rapid spread of HIV in Africa since high heterosexual seroconversion rates have been found in widely scattered populations throughout the developing world (19).

To evaluate possible reasons for the high HIV transmission rate in sub-Saharan Africa, we investigated nutritional status in a nested case-control study. By examining stored serum specimens drawn at enrollment, we were able to investigate micronutrient levels prior to HIV seroconversion. Several nutrients known to affect mucosal integrity (vitamin A, carotenoids, vitamin E, and selenium) were evaluated and no correlation was found between serum levels of these micronutrients and subsequent HIV seroconversion. With the exception of cholesterol and carotenoids, these serum levels were similar to normal levels found during nutritional surveys of American women (20). We cannot exclude the possibility that other micronutrients, which we were unable to measure, play a role in HIV susceptibility. Zinc, vitamin C, and B-complex vitamins are other examples of micronutrients that are also important for maintaining skin integrity (9).

In contrast to micronutrient levels, weight loss was significantly associated with HIV seroconversion. Symptomatic HIV disease causes weight loss, raising the possibility that occult HIV infection prior to seroconversion is an explanation for our results (21). Acute retroviral infection may also be accompanied by flu-like symptoms and could cause weight loss. Baseline sera from women who subsequently seroconverted, however, were negative for HIV-1 antibodies tested by two independent laboratories, and the association between weight loss and seroconversion persisted even when weight loss preceded seroconversion by more than 6 months. Recent studies of high-risk homosexual men (22–24) and female partners of HIV-infected hemophiliacs (25) suggest that prolonged infection without seroconversion is extremely unusual. Nonetheless, the duration between HIV infection and antibody production in African populations has not been well studied and this remains a possible explanation for our findings.

It is also unlikely that this association was due to chance alone, since weight loss was significantly correlated with seroconversion in a second control group and at different time periods during the study. Cases who subsequently seroconverted were not only more likely to have a measured weight loss during the first 6-month period, but were also more likely to report weight loss before enrollment in the study.

Weight loss in seroconvertors may be merely a marker for an underlying illness or a high-risk behavior which directly places women at risk for HIV seroconversion. Cases in our study may have contracted an illness, such as genital ulcer disease, which could lead to both weight loss and HIV susceptibility. In our study, the association between weight loss and seroconversion was independent of genital infection or other laboratory evidence of systemic infection. We cannot exclude the possibility, however, that other illnesses or behaviors placed these women at increased risk and simultaneously caused them to lose weight.

Since our study was not designed to examine nutritional risk factors, we were unable to determine directly whether weight loss was due to decreased food intake, decreased calorie absorption, or in-

creased metabolism. Of interest, however, was the finding that half of the women who reported weight loss before enrollment also reported anorexia during the same interval. This suggests that a decrease in food intake might be responsible for the weight loss in some cases.

Regardless of the mechanism, weight loss was clearly an important predictor of seroconversion: One third of seroconvertors lost at least 5 kg in the 6-month interval beginning 1 year before seroconversion. If this finding is confirmed in other settings, direct investigations into the causes and consequences of weight loss in adults at risk for exposure to HIV are warranted.

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