

Recruitment, Follow-Up and Characteristics of HIV Infected Adults who Use Illicit Drugs in Southern Africa

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Abstract

Background: With one of the worst HIV prevalence rates in the world, Botswana has made great strides in addressing AIDS. Nevertheless, to fully contain the epidemic, outreach to marginalized groups, including illicit drug users, is critical.

Objective: To conduct targeted outreach within an intervention trial to recruit HIV-infected drug users and assess HIV disease and nutritional status.

Method: Recruitment strategies included safeguarding confidentiality, involving local health-care professionals, advertising, and participation incentives. Urine toxicology, CD4 cell count, HIV viral load, blood chemistry, plasma micronutrients, dietary history, drug use and morbidity were assessed for two years.

Results: Targeted outreach identified 138 HIV-infected persons who used marijuana; 18.1% had CD4 cell counts ≤ 350 cells/ μ L and 39.9% had low BMI. Eligible marijuana users (N=52) had significantly lower BMI (21.8 ± 3.7 vs. 24.3 ± 5.3 kg/m², $P=0.001$), higher HIV viral load (4.36 ± 0.89 vs. 4.09 ± 0.89 log₁₀, $P=0.018$), and higher kilocalorie intake (1924 ± 1055 vs. 1620 ± 926 Kcalories, $P=0.025$) than those who did not use marijuana (N=748) with similar CD4 cell count. Marijuana users ≥ 40 years old had more opportunistic diseases ($P=0.020$) than non-users of the same age. Benzodiazepine use was detected among 57 participants and they had higher BMI than marijuana users (24.4 ± 6.8 vs. 21.8 ± 3.7 kg/m², $P=0.017$).

Conclusion: A population stigmatized by illicit drug use and HIV-infection can be brought into a clinical research setting in Africa. HIV-infected marijuana users were at a risk for higher HIV viral load, lower BMI and more comorbidities than non-users. Outreach to this marginalized group is important for containing the HIV epidemic.

Keywords: Marijuana; Benzodiazepines; Botswana; HIV; Recruitment; Nutritional status, HIV disease progression

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Introduction

The majority of the world's current HIV infected cases occur in Sub-Saharan Africa, which is reporting hyperendemic proportions of the epidemic; in particular, Botswana has one of the worst HIV prevalence rates estimated at 25% [1, 2]. Great strides have been made in stemming the epidemic with the introduction of universal antiretroviral therapy (ART) [3], decreasing the death rates in some of the hardest-hit countries in Africa [4]. Various HIV prevention strategies have been used successfully, but

populations who use illicit drugs are difficult to reach, particularly in developing countries, due to stigma associated with both HIV positivity and illicit drug use. Therefore, these populations are of particular interest [1]. Drug use has not been adequately studied in Sub-Saharan Africa, especially in countries such as Botswana where the effect of drug use on HIV transmission has been considered minimal [5]. While the illegality of cannabis in most countries precludes an accurate estimation of its use, Sub-Saharan Africa has one of the lowest prevalence rates in the world [6, 7]. Use of cannabis however, contributes to HIV transmission [8, 9], disability, and is associated with higher mortality, and thus, needs to be considered [6]. Benzodiazepines, on the other hand, are the most commonly prescribed medication for a range of medical conditions [10], are also available over the counter in Southern Africa, and are frequently misused [11]. Benzodiazepines are frequently the primary drug of abuse, primarily in females aged older than 40 years [11], and their use is associated with psychological and physiological dependence [12].

The theme of the 20th International AIDS conference was "Stepping up the Pace" reflecting the principle of inclusiveness of key affected populations in "hotspots," which were defined as geographic areas of high HIV incidence and prevalence [13]. Botswana is one of the HIV "hotspots" and the first resource-limited country in Africa that was involved in a large-scale effort to provide universal ART [2]. Nevertheless, to conquer the HIV epidemic in Botswana, it is critical to reach all groups who are at high risk for HIV infection. HIV infected persons in the general population have largely been identified and have been receiving ART [14, 15]. One of the factors that has impeded greater coverage has been the difficulty in reaching and engaging persons who use illicit drugs [16-19], due to criminalization and a dual stigma associated with HIV positive status and the use of illegal drugs [20, 21]. Illicit drug use is related to unsafe sexual practices and an increased risk for HIV infection [8]. In addition, the stigma associated with drug use is a powerful barrier to accessing healthcare, and for recruitment into scientific studies which are designed to address health and welfare issues of this population [22].

Marijuana contains a psychoactive ingredient, tetrahydrocannabinol (THC), which has been shown to suppress immune function [23] and may affect HIV disease progression, directly or indirectly [24, 25]. The use of marijuana has also been reported to increase the risk for contracting HIV-infection [9]. A derivative of the drug has been recommended as an appetite stimulant in HIV infection, but, its effects on nutritional status and HIV disease progression are still controversial [25]. Over the counter use of benzodiazepines is of interest as its misuse was the primary problem for which patients sought substance abuse treatment service in South Africa [11]. The purpose of this study was to assess the recruitment, follow-up, HIV disease progression, nutritional status and retention of HIV-infected persons who use illicit drugs and were enrolled in a trial of micronutrient supplementation among HIV positive treatment-naïve patients in Botswana.

Method

Recruitment strategies

Several strategies were used to enhance outreach of the hard-to-reach HIV-infected population that used illicit drugs. We obtained written assurances from the Botswana Ministry of Health to safeguard the confidentiality of the drug use reported by the study participants. Other strategies to identify HIV-infected persons who use illicit drugs included defining behavioral and geographic characteristics of the population, conducting focus-groups with health-care professionals from areas known for illicit drug use to evaluate the recruitment materials, utilizing mass media advertising for recruitment, and referrals from detoxification programs. Incentives for participation and adherence to study visits included free laboratory testing, clinical follow-up, and reimbursement for transportation.

The research team conducted a rapid assessment strategy [26] to target HIV-infected drug users in which we partnered with health-care workers serving a population known to include HIV-infected persons who used marijuana in Botswana, including a rural area of Mochudi and the capital of Botswana, Gaborone. These health care workers shared their local knowledge about the populations of interest and helped to develop and evaluate recruitment materials. Separately, HIV-infected persons who used illicit drugs also evaluated the recruitment strategy devised by the research team. The formulation of the strategy involved developing a profile of the HIV-infected drug-using population, including their locations, means of contacting them, and determining appropriate incentives for their participation. Mass media campaigns advertised the study recruitment within identified marijuana user clusters many of whom were likely to be HIV-infected, emphasizing confidentiality and benefits of HIV testing. Liaisons within the communities who used marijuana were identified to facilitate recruitment, and incentives for participation were provided. The identified HIV-infected persons who used illicit drugs were evaluated for eligibility to participate in a 2-year follow-up clinical trial of micronutrient supplementation described elsewhere [27].

Participants who did not use marijuana were recruited from the clinics where HIV testing and treatment occurs. All participants were eligible for the study if they were HIV seropositive, their CD4 cell count was >350 cells/ μ L and they were ART-naïve. Other criteria were a body mass index (BMI) >18 Kg/ m^2 for women and 18.5 Kg/ m^2 for men, age 18 years or older, and no current AIDS-defining conditions or history of AIDS-defining conditions. Women were excluded if pregnant or becoming pregnant during the study. When the participants became eligible for ART according to the standards in Botswana at the time, their study participation was discontinued and they were referred for antiretroviral treatment.

The study was conducted by the Florida International University in collaboration with the Harvard School of Public Health and the Botswana-Harvard Partnership in Gaborone, Botswana, from December 2004 until July 2009. The investigation was approved by the Institutional Review Boards of all three institutions, and monitored by a Data Safety Monitoring Board [27].

Assessments

To identify active drug users, we used two main strategies: (1) urine toxicology and history of drug and alcohol use with validated questionnaires [28, 29] were performed at baseline and every 6 months in all the participants, and (2) use of the active outreach strategies described above to access the drug-user clusters. Illicit drugs were those that were detected in the urine of the participants but were not prescribed by physicians as confirmed by the medical charts.

At baseline and every three months, physical examination and medical history were performed by a trained nurse or physician, blood was drawn for assessment of CD4⁺ cell count, and dietary intake was determined using 24-hour food recalls conducted by trained interviewers. Quantitative estimates, using several 24-hour recalls, provided an estimate of a person's diet. At baseline and every six months, blood was also drawn to evaluate HIV viral load, blood chemistry and plasma micronutrients. Medical history included inter-current health events and currently prescribed medications; a review of medical charts was used to verify prescriptions and determine changes in health status. Morbidity information was collected by questionnaires at screening, and at every monthly visit, and confirmed by documentation in the medical chart.

To take in account the difference in required caloric intake due to gender and age, we used a formula that estimates basal metabolic rate (BMR) [30] from bioimpedance measures, which takes into consideration gender, age, weight and height. Individual caloric requirement was estimated by multiplying BMR x Activity factor of 1.55 for asymptomatic HIV people [31, 32]. We calculated the ratio of actual intake to individual requirement to create a variable that shows adequacy (≥ 1), or inadequacy (<1) and compared men who used THC with men who did not use drugs, and for women who used benzodiazepine compared to women who did not use drugs to investigate whether the differences between groups were due to gender, individual caloric requirements or types of drugs.

Biochemical assays

Urine was collected under observation at baseline and every six months. The American Biomedica Rapid Drug kits were used to determine the presence of amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, methamphetamines, opiates, morphine, and tricyclic antidepressants.

Lymphocyte phenotype was determined with a four-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Sysmex XE-2100 hematology instrument and corroborated with cytocentrifuge smears.

HIV-viral load was determined using an in-vitro nucleic acid amplification test (Amplicor reagents and protocol, Roche-Diagnostics, Branchburg, NJ). For quality assurance we used standard reference materials, and 5% of the samples underwent duplicate testing blinded to the laboratory personnel.

Plasma zinc (Zn) and selenium (Se) levels were determined by flame atomic absorption spectrophotometry [33, 34]. Plasma vitamins A and E were determined by high performance liquid chromatography (HPLC) [35]. Folate levels were determined by microbiological assay using standardized procedures employing *Lactobacillus casei* [36].

Statistical analysis

Statistical analyses were performed with the use of SAS software (SAS 9.3, SAS Institute, Cary, NC). At baseline, the participants who used drugs were compared to those who did not use drugs for continuous variables with the Student t-test, non-parametric ANOVA and Wilcoxon rank-sum test, and for categorical variables with the Chi-Square and logistic regressions. Over time analyses used the Mixed Linear Models procedure controlling for age, gender, baseline CD4 cell count, baseline HIV viral load (log₁₀ scale), and baseline BMI. All P-values reported were two-sided; statistical significance was defined as $P < 0.05$. The data was censored on July 24, 2009, when the last scheduled 24-month visit was completed.

Results

This is a descriptive study of targeted outreach for HIV-infected persons who use illegal drugs from the Micronutrient Supplementation Study in Botswana, Africa [27]. Using the community outreach strategies for HIV-infected persons who use drugs, 138 HIV-infected marijuana users were identified who were willing to be pre-screened for eligibility for the study (Table 1). From this group, only 52 qualified for the study. The main two reasons for ineligibility among the HIV-infected persons who used marijuana were a CD4 cell count ≤ 350 cells/ μ L (18.1%), and BMI < 18 Kg/m² for women and 18.5 Kg/m² for men (39.9%) as the goal of the study was to supplement micronutrients to those who were early in the HIV disease progression and were not wasting. Those who did not qualify for the study or were not willing to participate (4.3%) were referred for follow-up and treatment according to the Botswana's guidelines at the time of the study. The great majority (92%) of the marijuana users admitted readily to using drugs and the reported frequency of weekly use was median 5.00 (IQR=2.0-12.0).

In comparison, participants who did not use marijuana were selected from HIV screening and treatment clinics in Botswana

Table 1. Characteristics of HIV-infected marijuana (THC) users engaged through targeted outreach strategies in Botswana.

Characteristics	HIV-Infected THC Users N=138
CD4 cell count < 350 cells/ μ L (N, %)	25 (18.1%)
HIV viral load (log ₁₀ copies/mL)	4.26 \pm 0.95
BMI < 18 kg/m ² for men, < 18.5 kg/m ² for women	55 (39.9%)
Not Willing to participate in the study	6 (4.3%)

There were 138 individuals recruited for this study through targeted outreach strategies in Botswana who were HIV-infected and used marijuana. Of these, 62.3% were not eligible for the study due to low CD4 cell count (18.1%), low BMI (39.9%) and lack of willingness to participate (4.3%).

and at the time of screening for this study their HIV status and CD4 cell count were known; thus all of the participants who did not use drugs were HIV positive with CD4 cell count >350 cells/ μ L, and BMI >18 Kg/m² for women and 18.5 Kg/m² for men. None of the participants considered drug non-users admitted using marijuana and were negative by urine screens for drugs.

Several substances were identified during the follow-up of the study participants recruited from the HIV screening clinics. The urine drug screens identified use of benzodiazepines among 57 participants, 13 participants used opiates, 12 used tricyclic antidepressants, 7 used methamphetamine, 4 used amphetamines, and one participant used methadone. Review of the medical charts indicated that none of these substances were prescribed by physicians. None of the participants who were not specially recruited for their drug use however, used marijuana. Only two participants were positive for three types of drugs and one participant was positive for two illicit drugs, but neither one of these individuals were positive for marijuana. In the group that used benzodiazepines, three participants were excluded from the analyses because they used other drugs, mainly opiates.

As shown in Table 2, multivariate analyses indicate that participants who used marijuana were predominantly men (90.3% vs. 23.5% among drug non-users, $P < 0.001$), and compared to those who did not use illicit drugs, had significantly lower BMI (21.8 kg/m² \pm 3.7 vs. 24.3 kg/m² \pm 5.3, $P = 0.004$), higher HIV viral load (4.36 log₁₀ \pm 0.89 vs. 4.09 log₁₀ \pm 0.89, $P = 0.018$), higher

kilocalorie intake (1924 Kcalories \pm 1055 vs. 1620 Kcalories \pm 926, $P = 0.025$), consumed fewer number of meals/day (2.5 \pm 0.5 vs. 2.8 \pm 0.5, $P = 0.005$, not shown in the table), tended to have higher protein intake (85.6 gm \pm 59.1 vs. 72.9 gm \pm 60.7, $P = 0.15$), had higher plasma levels of selenium (0.068 mg/dL \pm 0.03 vs. 0.048 mg/dL \pm 0.01, $P = 0.032$), and folate (14.5 ng/mL \pm 5 vs. 10.6 ng/mL \pm 9.8, $P = 0.009$), and tended to have higher plasma zinc levels (0.85 mg/dL \pm 0.47 vs. 0.60 mg/dL \pm 1.14, $P = 0.092$). Lower percentage of those who used marijuana were engaged in exercise or other physical activity than drug non-users (65.38% vs. 79.5%, $P = 0.016$). Marijuana users were similar to drug non-users in age (33.5y \pm 7.8 vs. 33.2y \pm 7.9, $P = 0.45$), serum albumin levels (41.3mg/dL \pm 4.9 vs. 41.19 mg/dL \pm 4.7, $P = 0.842$), CD4 cell count (477cell/ μ L \pm 153 vs. 461 cell/ μ L \pm 180, $P = 0.595$), and plasma vitamins A (41.5 μ g/dL \pm 13 vs. 45.32 μ g/dL \pm 16.0, $P = 0.262$) and E (8.1 μ g/mL \pm 1.9 vs. 9.8 μ g/mL \pm 5.1, $P = 0.075$).

Among marijuana users, HIV viral load was significantly and inversely correlated with plasma albumin ($r = -0.33$, $P = 0.0047$), and those with low plasma zinc (Zn < 0.75 mg/L), and selenium levels (Se < 0.065 mg/L) had significantly higher HIV viral load ($P = 0.004$ and, $P = 0.036$, respectively). When marijuana users were compared to non-users at different age ranges using non-parametric t-test, marijuana users who were 40 years or older had significantly more AIDS defining conditions (7/10) 71% than non-users (126/434) 29%, $P = 0.020$ in the same age range. Alcohol use did not significantly affect these analyses.

Table 2. Characteristics of HIV-infected marijuana and benzodiazepine users, compared to HIV-infected drug non-users in the micronutrient supplementation trial in Botswana [1].

Characteristics	THC users Group 1 N=52	BDZ Users Group 2 N=54	Drug Non-Users** Group 3 N=748	P-values		
				Between Groups 1 & 2	Between Groups 1 & 3	Between Groups 2 & 3
Age (years)	33.5 \pm 7.8	37.3 \pm 9.5	33.2 \pm 7.9	0.029*	0.758	<0.001*
Male Gender	90.3%	19.2%	23.5%	<0.001*	<0.001*	0.479
Income <1000 Pulas/month	30.6%	22%	23.4%	0.335	0.252	0.820
BMI (kg/m ²)	21.8 \pm 3.7	24.4 \pm 6.8	24.3 \pm 5.3	0.017*	<0.001*	0.932
CD4 count (cells/ μ L)	477 \pm 153	477 \pm 211	461 \pm 180	0.408	0.595	0.530
HIV Viral load (log ₁₀)	4.36 \pm 0.89	4.20 \pm 0.94	4.09 \pm 0.89	0.865	0.018*	0.413
Albumin (mg/dL)	41.3 \pm 4.9	40.0 \pm 3.7	41.19 \pm 4.7	0.134	0.842	0.040*
Plasma Zinc (mg/L)	0.85 \pm 0.47	1.22 \pm 0.85	0.60 \pm 1.14	0.246	0.092	0.120
Plasma Se (mg/L)	0.068 \pm 0.03	0.067 \pm 0.01	0.048 \pm 0.01	0.936	0.032*	0.322
Folate (ng/mL)	14.5 \pm 5.8	10.0 \pm 5.2	10.6 \pm 9.8	0.042*	0.009*	0.864
Vitamin A (μ g/dL)	41.5 \pm 13	46.7 \pm 13.3	45.32 \pm 16.0	0.339	0.262	0.828
Vitamin E (μ g/mL)	8.1 \pm 1.9	9.1 \pm 3.33	9.8 \pm 5.1	0.432	0.075	0.782
Calorie Intake (kcal)	1924 \pm 1055	1799 \pm 1013	1620 \pm 926	0.547	0.025*	0.192
Protein Intake (grams)	85.6 \pm 59.1	84.9 \pm 61.5	72.9 \pm 60.7	0.957	0.150	0.181
Engaged in Sports Activity	65.38%	67.35%	79.5%	0.837	0.016*	0.043*

*Statistically significant, $P < 0.05$

**This group of participants did not use marijuana, benzodiazepines, amphetamines, barbiturates, methadone, methamphetamines, opiates, morphine, or tricyclic antidepressants.

Multivariate analyses using ANOVA and controlling for baseline age, gender, baseline CD4 cell count, baseline viral load (log₁₀ scale), and baseline BMI. Marijuana and benzodiazepine are the two main drugs of abuse in Botswana. Marijuana users differ from Benzodiazepine users by being mostly male, younger; have lower BMI, and higher plasma folate levels. Marijuana users differ from those who do not use drugs by being mostly male, having lower BMI, higher HIV viral load, higher plasma folate and selenium levels, higher caloric intake and less physical activity. Those participants who used benzodiazepines were more similar in characteristics to drug non-users, but were significantly older, more sedentary, with lower levels of serum albumin. Alcohol use did not significantly affect any of these analyses.

Table 2 also shows that the participants who used benzodiazepines, as evidenced by a routine screening of urine for drugs during the study visits, were mainly females (80.8%), they were older than marijuana users (37.3 years \pm 9.5 vs. 33.5 years \pm 7.8, $P < 0.029$), and were also older than drug non-users (37.3 years \pm 9.5 vs. 33.2 years \pm 7.9, $P < 0.001$). Benzodiazepine users had higher BMI than marijuana users (24.4 kg/m² \pm 6.8 vs. 21.8 \pm 3.7 kg/m², $P = 0.017$), but the BMI of the benzodiazepine users was not different from the participants who did not use illicit drugs (24.4 kg/m² \pm 6.8 vs. 24.3 kg/m² \pm 5.3, $P = 0.923$). Plasma levels of folate were significantly lower among the benzodiazepine users than among the marijuana users (10.0 ng/mL \pm 5.2 vs. 14.5 ng/mL \pm 5.8, $P = 0.042$) but similar to those of drug non-users (10.0 ng/mL \pm 5.2 vs. 10.6 ng/mL \pm 9.8, $P = 0.864$). The benzodiazepine users were also less engaged in sports activities than the drug non-users (67.35% vs. 79.5%, $P = 0.043$). The participants who used non-prescription benzodiazepines did not perceive themselves as drug users.

Table 3 shows longitudinal analyses, using the Mixed Linear Models. Marijuana users had consistently lower BMI over time compared to participants who did not use drugs ($\beta = -0.75$, $P = 0.04$), indicating that every unit increase in marijuana use was associated with 0.75 units decrease in BMI. The energy intake of those who used marijuana remained higher over time than that among drug non-users ($\beta = 278$, $P = 0.04$, not shown), indicating that every unit increase in marijuana use was associated with 278 Kcalorie increase in energy intake. Marijuana users also had higher HIV viral load over time ($\beta = 0.29$, $P = 0.013$) than drug non-users, indicating that every unit increase in marijuana use was

associated with 0.29 log₁₀ increase in viral load after controlling for micronutrient supplementation, baseline CD4 cell count, and baseline HIV viral load. Marijuana users aged 40 years or older had significantly higher morbidity than participants who did not use any illicit drugs of the same age range ($P = 0.02$). Marijuana users had the same rate of CD4 cell count decline as non-users. After 2 years of follow-up, marijuana users were not significantly different from non-users in adherence to study supplement, but showed a trend towards non-adherence at the end of the study ($P = 0.075$) (Table 4). Comparing adherence rates between men who used marijuana and men who did not use drugs, no significant differences were found between those who used drugs and those who did not use drugs in adherence. These results suggest that the possible lack of adherence among men who are marijuana users appears to be due to gender rather than due to marijuana use.

When we compared women who used benzodiazepine, with women who did not use drugs, those using benzodiazepine were significantly older (36.5 vs. 32.3 years, $P = 0.008$), and the ratio of caloric intake to requirement was higher (0.911 ± 0.503 vs. 0.761 ± 0.459 , $P = 0.053$), but did not reach significance. However, the two female groups did not have significantly different BMI (24.3 ± 7.2 vs. 24.8 ± 5.5 , $P = 0.618$) or levels of reported physical activity by Chi-Square test ($P = 0.128$).

When we compared men who used marijuana with men who did not use drugs, however, those who used marijuana had significantly lower BMI ($21.5 \text{ kg/m}^2 \pm 3.8$ vs. $22.8 \text{ kg/m}^2 \pm 3.7$, $P = 0.044$) but their age, caloric intake, or the ratio of caloric intake

Table 3. Comparison of HIV-Infected marijuana users and non-users: longitudinal changes among marijuana users over two years using mixed linear models, N=52. The analyses controlled for baseline CD4 cell count and baseline HIV viral load.

Variables	Parameter Estimate	SE	P-value
BMI (kg/m ²)	-0.75	0.37	0.039*
HIV Viral Load (log ₁₀)	0.29	0.12	0.013*
Lack of adherence (missing >1 pill in 30 days)	-0.06	0.037	0.075
CD4 cell count <250 cells/ μ L	-10.41	24.35	0.669
AIDS-defining conditions	0.01	0.03	0.855

*Statistically significant, $P < 0.05$

Over two years, marijuana users had significantly lower BMI and higher HIV viral load. There were no significant differences between marijuana users and non-users in reaching CD4 <250 cells/mm³ and in occurrence of AIDS defining conditions. Alcohol use did not significantly affect these analyses.

Table 4. Adherence through time: Percent at each assessment visit of those who missed more than one day of the nutritional supplement in the last month [1].

Lack of Adherence with supplement for more than one day in the last month at assessment visits.	THC users Group 1 N=52	BDZ Users Group 2 N=54	Non-drug Users Group 3 N=748	P-values		
				Between Groups 1 & 2	Between Groups 1 & 3	Between Groups 2 & 3
6 months	26.7%	29.8%	21.9%	0.743	0.462	0.418
12 months	29.2%	21.4%	19.2%	0.418	0.116	0.717
18 months	18.9%	13.2%	17.0%	0.502	0.769	0.538
24 months	30.3%	10.5%	19.5%	0.043*	0.075	0.174

*Statistically significant, $P < 0.05$

Only at the last assessment visit, marijuana users had significantly less adherence than the benzodiazepine users, but the adherence was not significantly different from the drug non-users. Benzodiazepine users did not have significantly different adherence than drug non-users at any point.

to caloric requirement were not different. We also examined if age was an independent factor from gender and drug use in predicting BMI, and found that both, men and women older than 40 had significantly higher BMI ($23.7 \text{ kg/m}^2 \pm 4.2$ vs. $22.1 \text{ kg/m}^2 \pm 3.6$, $P=0.01$ for men, and $26.6 \text{ kg/m}^2 \pm 7.0$ vs. $24.4 \text{ kg/m}^2 \pm 5.3$, $P<0.002$ for women) regardless of drug use. Women more than 40 years of age also had higher plasma folate (14.3 vs. 8.7 ng/mL , $P=0.045$) than younger women.

Discussion

Marijuana users in Botswana did not attend the HIV testing clinics, most likely due to stigma and discrimination associated with HIV-positivity and with use of illicit drugs [2]. However, to conquer the HIV epidemic, it is critical to reach all groups who are at high risk for HIV infection, including persons who use illicit drugs who may be at a higher risk for HIV transmission [2, 37]. To identify HIV-infected participants who use marijuana and bring them for HIV testing, it was necessary to develop and implement specific targeted outreach strategies, address perceived barriers to testing, establish trust, and conduct network-based recruitment and retention activities. Frequent (monthly) follow-up was critical to ensure a significant retention of this hard-to-reach population in the research study. Although there was no significant difference in adherence to the study supplement between the marijuana users and non-users in this two-year study, adherence decreased among the marijuana users towards the end of the study, and it was necessary to reinforce adherence counselling. This is an important factor to consider when HIV-positive persons who use marijuana are initiated on ART, which requires more than 95% adherence [38]. Comparing adherence rates between men who used marijuana and men who did not use drugs, and between women who used benzodiazepine and women who did not use drugs, no significant differences were found between the stratified gender groups in adherence. This suggests that the differences between the two groups of drug users in adherence are indeed related to gender and not to drug use.

The targeted outreach was successful in identifying HIV-infected participants who used marijuana. Once identified, a large proportion (58%) of the HIV-infected marijuana users was not eligible for the study due to either low CD4 cell count or low BMI at the time of recruitment. The study-eligible participants with CD4 cell count $>350 \text{ cells}/\mu\text{L}$ at recruitment, had significantly lower BMI despite higher calorie intake throughout the study, and better plasma levels of some micronutrients than drug non-users, most likely due to the higher food intake. Lower BMI in marijuana users is consistent with previous findings [39], and may be related to the consistently higher HIV viral load compared to marijuana non-users. Higher HIV viral load was particularly pronounced in those with overly low levels of plasma zinc and selenium. This is consistent with our previous findings with zinc supplementation preventing immune failure [40], and selenium supplementation reducing HIV viral load in HIV infected drug users [41]. Moreover, an animal study showed that exposure to THC significantly enhanced HIV replication [42], and an epidemiological study indicated that THC may be associated

with faster HIV disease progression in men who have sex with men [43]. In contrast, a 21-day placebo-controlled cannabinoid trial in patients on stable ART did not show increase in HIV viral load [44]. In a simian model however, cannabinoids slowed SIV disease progression in males, but not in females [45, 46]. Further research is needed to establish whether long-term regular use of marijuana impacts HIV disease progression.

The older marijuana users (age 40 or older) also had significantly more opportunistic diseases than non-users in the same age ranges. There were no differences in CD4 cell count between the drug using and non-using groups. When we compared men who used marijuana with men who did not use drugs, however, those who used marijuana had significantly lower BMI but their age, caloric intake, or the ratio of caloric intake to caloric requirement were not different. However, the lower BMI, higher HIV viral load, more opportunistic diseases among the older marijuana users, and the late presentation into the healthcare system, potentially places them at a higher risk for faster HIV disease progression and for HIV transmission.

In contrast, benzodiazepine users were among the participants recruited from the HIV testing clinics. However, review of their medical charts indicated that benzodiazepines identified in urine were not prescribed by their physicians. Their characteristics were very similar to those of the participants who did not use drugs, and by self-report, did not consider themselves drug users. This perception has been observed previously with benzodiazepine use as anxiolytics both with and without medical prescription [47]. These diverse attitudes between the marijuana and benzodiazepine users may be derived from the fact that marijuana production, trade and possession are criminalized in Botswana, while benzodiazepines may be prescribed by physicians or obtained over the counter in pharmacies [48, 49]. Due to lack of self-admission, it was difficult to assess if the number of cases of positive non-prescribed benzodiazepines was due to illegal use of Mandrax, an anxiolytic manufactured in South Africa and widely available in this region that contains both benzodiazepines and methaqualone [50], or to benzodiazepines dispensed in pharmacies without prescription. Marijuana use was an activity usually done by males while the use of benzodiazepines, was mostly found among females. The use of marijuana in the rural areas of Botswana is known, but the use of benzodiazepines was surprising, and the finding of opioid use was also unexpected.

Conclusion

This study demonstrates that a population stigmatized by drug use and HIV-infection can be brought into a clinical and research setting using targeted recruitment strategies in Southern Africa. HIV-infected participants who used marijuana in Botswana were at risk for higher HIV viral load, and very low BMI at diagnosis compared to those who did not use marijuana. HIV-infected marijuana users, despite significantly higher energy intake than non-users over time, had lower BMI and among older users, more opportunistic diseases than HIV-infected marijuana non-users. Adherence to the nutritional supplement was not

different between marijuana users and non-users, although a sustained trend towards decreased adherence towards the end of the follow-up was observed, suggesting a need to reinforce adherence at each visit. Benzodiazepine use was detected among 57 participants, mostly females, and they had higher BMI than marijuana users ($24.4 \text{ kg/m}^2 \pm 6.8$ vs. $21.8 \pm 3.7 \text{ kg/m}^2$, $P=0.017$). To contain the HIV epidemic, it is critical to develop programs that can outreach to this difficult-to-reach group, and provide medical treatment and nutritional interventions.

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