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Risk indicators for oral candidiasis and oral hairy leukoplakia in HIV-infected adults

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Abstract – Objectives: Oral candidiasis (OC) and oral hairy leukoplakia (OHL) are the most common oral mucosal diseases associated with HIV infection. Independent risk indicators associated with these sentinel opportunistic diseases have not been established in mixed race and gender adult populations in the southeast USA. The purposes of this study were 1) to estimate prevalence of OC and OHL among an HIV-1 positive adult population, and 2) to develop explanatory multivariable models for each disease outcome. *Methods:* This cross-sectional study evaluated 631 adult dentate HIV-1 seropositive persons examined for HIV-associated oral mucosal diseases between 1995 and 2000 at University of North Carolina Hospitals in Chapel Hill, North Carolina using data collected from medical record review, interview questionnaire and clinical examination. We analyzed the data using *t*-tests, ANOVA, and unconditional logistic regression. *Results:* Prevalent OC was associated with low CD4+ cell count [<200 cells/μl, adj. OR = 12.7 (95%CI: 4.9–32.9)], antiretroviral combination therapy [OR = 0.6 (0.3–0.9)], and current smoking [OR = 2.5 (1.3–4.8)]. Prevalent OHL was associated with low CD4+ cell count [<200 cells/μl, OR = 7.2 (2.7–18.9)], antifungal medication use [OR = 1.8 (1.1–2.9)], current recreational drug use [OR = 2.5 (1.3–4.9)], and male gender [OR = 2.5 (1.3–4.8)]. *Conclusions:* While CD4+ cell count, and antiretroviral medication were important risk indicators for OC, and OHL, cigarette smoking appears to be an important risk indicator for OC in HIV-1-infected populations.

Key words: candidiasis; hairy leukoplakia; HIV; logistic regression; multivariable models; prevalence; smoking

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Oral candidiasis (OC) and oral hairy leukoplakia (OHL) are the two primary oral infections associated with human immunodeficiency virus (HIV) infection (1–28) that also serve as clinical markers of symptomatic HIV disease (29). A recent review has shown that current estimates suggest that between 20 and 50% of HIV-infected patients develop mucosal lesions in the oral cavity during the course of their disease (1).

One early study estimated that more than 90% of patients with AIDS would develop OC at some point (15). OC suggests immunological decline, and is an initial sign of HIV infection or progression to AIDS (22, 27). The most common *Candida* species in HIV-infected individuals is *C. albicans*, present in 63–93% of cases (30, 31). Other species include *C. glabrata* (14–21%), *C. krusei* (4–10%), and *C. tropicalis* (2–7%) (30, 31). *Candida non-albicans* spp. increase in prevalence with immune decline and previous antifungal drug exposure (1). OC responds to antifungal therapy, but eradication is rarely achieved unless the underlying immunocompromised state is resolved. OC has been found to be associated with low CD4+ T-lymphocyte count (CD4+ cell count) (<200/mm^3) and high viral loads (13, 19). MacPhail et al. (14) reported

statistically significant associations between OC and CD4+ cell count, viral load, recreational drug use and xerostomia in a women's HIV-1 positive cohort. The study also reported risk factors for different types of OC but did not include any effect estimates from their model, making it difficult to determine the relative strengths of the relationships with OC.

OHL is an opportunistic viral lesion caused by Epstein–Barr virus and is frequently detected among HIV-infected individuals (1, 10). OHL is a benign hyperplastic lesion usually of the lateral borders of the tongue, although it has also been reported on the ventral and dorsal tongue surfaces, and is frequently bilateral in presentation (1). Rare instances of OHL occurring on palate, buccal mucosa, floor of the mouth and oropharynx have been reported (32). Clinical presence of OHL has been shown to have 100% positive explanatory value for HIV infection/AIDS (10). OHL has been found to be associated with CD4+ counts below 200/mm^3, and the absence of anti-p24 antibodies in serum and saliva (13–19). OHL has been associated with more rapid progression to AIDS among HIV-infected individuals (11, 32, 33), and with HIV viral loads exceeding 20,000 copies/ml, independent of CD4+ cell count (19).

Some studies have identified potential risk indicators for HIV-associated oral diseases (3–28). However, definitions of many risk indicators have varied across studies. It has been reported that institution of highly active antiretroviral therapy (HAART) has resulted in a change in prevalence of HIV-associated oral diseases (2). However, the definition of HAART used in different studies has varied substantially. In conducting this cross-sectional study among 631 HIV-positive persons, our overall aim was to establish independent risk indicators for OC and OHL during a time period of rapid change in antiretroviral therapy approaches to the control of HIV disease.

Methods

Study data, variables, and power

Between 1995 and 2000, 631 HIV-positive adult volunteers were recruited from those examined and treated at the infectious diseases clinic of University of North Carolina (UNC) Hospitals in Chapel Hill, North Carolina to participate in this study approved by the UNC School of Medicine and UNC Hospitals Committee on the Protection of the Rights of Human Subjects. A trained social researcher administered a sociobehavioral questionnaire to each participant in a private room requiring about 45–60 min to complete. Afterwards, a single calibrated examiner trained in oral medicine conducted the clinical examination. Assessment of oral manifestations of HIV was based on the published standard presumptive clinical criteria (34). A medical record review was then conducted for each participant to ascertain laboratory and medication variables and AIDS case status, typically within a month. The following variables were included in the analyses as outcomes: OC, OHL; or as explanatory variables: age, race, sex, years of education, sexual orientation, smoking, drug use, CD4 cell count, plasma viral load, antiretroviral therapy, antifungal medication use, and antibiotic use. Immune suppression measured as the blood CD4+ cell count was considered as the main exposure in this set of studies.

Both oral disease outcomes and the main exposure (CD4+ count) data were double entered and error checks were performed as quality assurance measures. Discrepancies and conflicts were resolved by crosschecking with the hardcopy questionnaire and medical record review. Overall, only 2% data points related to covariates used in multivariable models were missing. These data were crosschecked with and filled in from medical record review. Analyses were restricted to a complete data set. Because this study analyzed already collected, prospective data with fixed sample size, we calculated minimum detectable odds ratios (ORs) under different exposure (CD4+ cell count) prevalences. Assuming 5% disease prevalence among the unexposed (high CD4+ count), 10% prevalence among the exposed (low CD4+ count), and 80% power, we calculated the minimum detectable OR to be 1.85 (0.54 if OR <1.0).

Statistical analyses

Indicator variables with reference cell coding were used for all variables. Univariate distributions were evaluated, as were bivariable relationships between the covariates, CD4+ cell count, and each outcome variable (OC and OHL). Bivariable associations were evaluated with *t*-tests and ANOVA for continuous variables; and ORs and chi-square statistics for categorical variables. Correlations were evaluated between the covariates to help prevent co-linearity errors in the multivariable models. Statistical significance was inferred at 0.05 level using two-sided *P*-values. All analyses

were done in SAS (V8.2; SAS Institute Inc., Cary, NC, USA).

Multivariable analyses

For OC and OHL, separate unconditional logistic regression analyses were performed using the PROC LOGISTIC. The goals of these analyses were to control for potential confounders, and to find the best fitting, most parsimonious and biologically reasonable model to describe the relationship between the outcome variable and a set of independent explanatory variables in which CD4+ cell count was always included. For all multivariable analyses, hierarchically well-formulated models were used, implying that given any variable in the model, all lower order components of the variables must also be contained in the model (35). To arrive at a final model, a manual hierarchical backward elimination approach was employed, using the likelihood ratio test.

An initial model was defined as the full model that included all variables suggested in the literature, and those associated with the oral disease outcomes and CD4+ count in bivariable analyses. For proceeding with the manual backward selection strategy, the full model had to be significantly better than an intercept-only model. Starting with the full model, noncontributory variables were manually removed, through hierarchical sets of models using the likelihood ratio test. The guiding rules used for removal of variables were: 1) if the type-III analysis (variable-added last test) suggested that the variable was not significant in the model; and 2) removal of the variable did not change the OR of CD4+ with the outcome by more than 20% from that of the full model. Precision of ORs was examined using 95% confidence limit ratio (CLR) defined as the ratio of upper and lower confidence bounds of the 95% confidence intervals. Regression diagnostics were performed and utility of the models was evaluated by outputting predicted scores.

Results

The study sample included 25.2% women, 34.5% whites, and 62.3% below 40 years of age. About 56.5% were under combination antiretroviral therapy, 16.4% were under monotherapy and 27.1% were under no antiretroviral therapy at the study visit. Of the study sample, 59.0% were current smokers and 17.6% were current recreational drug users; 44.0% were men who had had male sexual partners. Prevalence of OC and OHL each was 17.4%, whereas 4.6% of subjects had both OC and OHL; and 69.7% had neither. Overall 6.2% of participants had erythematous candidiasis, 11.6% had pseudomembranous candidiasis, and 2% had both OC types. Other HIV-associated oral disease entities identified in this study population included salivary gland disease (4.8%), linear gingival erythema (3.2%), and minor aphthous ulcers (2.5%). Table 1 shows the distribution of prevalent OC and OHL cases by the study variables. Significantly more smokers (former as well as current) had OC compared with never smokers. No significant differences were seen in OC prevalence by age, sex, race, education, recreational drug use (henceforth called drug use), sexual orientation or antiretroviral medication use. OHL was significantly more prevalent among men, whites, bisexuals, and those taking antifungal medication.

Oral candidiasis

Table 2 summarizes the bivariable analyses comparing OC status with clinical and biological variables. Participants with low CD4+ cell count (crude OR = 10.7) and those with intermediate CD4+ cell count (OR = 2.9), smokers (ORs for current smokers: 2.6; former smokers: 1.4), and those under antifungal medication (OR = 2.0) were significantly more likely to have OC compared with their respective reference categories. Former drug users, older age groups, males, heterosexuals, whites, and those not using antiretroviral medication were slightly more likely to have OC, although the differences did not achieve statistical significance. Although not shown in the table, those with high viral load were three times as likely to have OC as those with low viral load (OR = 2.9; 95% CI: 1.6–5.7) and those with AIDS were 1.8 times as likely to have OC as those without AIDS (OR = 1.8; 95% CI: 1.1–2.7).

Table 2 also shows the logistic regression models for OC. In the full model, CD4+ cell count, antiretroviral medication and smoking were the significant factors. The ORs of some covariates with substantial crude effect came close to unity in the fully adjusted model. For example, use of antifungal medication showed a crude estimate of 2.0, which became 1.00 in the fully adjusted model. Similarly, effect estimates for age groups and drug users were diluted in the fully adjusted model. The ORs for sex, sexual orientation and race/ethnicity did not change appreciably from their crude values.

Table 1. Characteristics of the study sample and mean CD4+ counts and viral loads

Characteristic	Level	Total number	OC (% total)	OHL (% total)	CD4+ (cells/μl)		Viral load (cp/ml)	
					Mean	SD	Mean ($\times 10^4$)	SD ($\times 10^4$)
Blood CD4+ count (cells/μl)	<200	270	30*	26*	75*	59	9.83*	17.1
	200–499	221	10	14	335	81	3.67	10.5
	500 or more	132	4	4	736	228	7.61	1.83
Plasma viral load (HIV RNA copies/ml)	Below 20,000	219	13*	9	399	299	0.30	0.43
	20,000 – 50,000	35	9	14	304	184	2.95	0.73
	More than 50,000	72	31	17	151*	170	21.8*	20.3
Sex	Male	472	18	21*	284*	262	5.5	13.4
	Female	159	17	8	378	312	4.91	11.9
Race	Whites	218	18	23*	325	279	4.14	12.7
	Blacks	413	17	15	298	278	5.85	13.1
Age (years)	18–29	81	14	15*	315	242	5.63	12.4
	30–39	312	18	21	309	283	5.20	12.6
	40+	238	18	13	303	284	5.43	13.7
Education	12 years or less	386	19	18	311	280	6.13	14.1
	12+ years	245	16	17	301	277	3.74	10.3
Smoking	Never	135	10*	13	314	274	3.69	9.2
	Former	124	13	15	279	263	6.26	15.1
	Current	372	22	20	314	285	5.59	13.3
Drug use	Never	169	15	14	285	245	4.29	8.8
	Former	351	19	17	311	294	5.41	13.9
	Current	111	15	25*	332	276	6.83	14.8
Sexual orientation	Heterosexual	353	19	15*	308	251	5.48	14.6
	MSM	182	15	18	306	291	5.35	14.2
	Bisexual	96	18	28	308	282	4.58	11.6
Antiretroviral medication	Combination therapy	354	16	16	301	260	4.30	11.8
	Monotherapy	103	16	21	233	220	2.47	1.99
	None	170	22	19	366*	270	9.23*	16.2
Antifungal	Yes	145	26*	30*	119*	150	10.4*	16.9
	No	482	15	14	365	283	4.26	11.7

*Significantly different (*t*-test/Scheffe's test).

Table 2. Logistic regression model for oral candidiasis (presence of candidiasis)

Variable	Level	Full model		Bivariable (crude)		Final model	
		OR (95% CI)	CLR	OR (95% CI)	CLR	OR (95% CI)	CLR
CD4+ (cells/μl) (Ref: ≥500)	<200	13.6 (5.1–36.2)	7.2	10.7 (4.2–27.1)	6.4	12.7 (4.9–32.9)	6.7
	200–499	3.3 (1.2–9.1)	7.6	2.9 (1.1–7.9)	7.3	3.3 (1.2–8.9)	7.4
Antiretrovirals (Ref: None)	Combination	0.4 (0.2–0.8)	3.3	0.7 (0.4–1.1)	2.8	0.6 (0.3–0.9)	3.0
	Monotherapy	0.7 (0.4–1.1)	2.9	0.7 (0.4–1.3)	3.3	0.5 (0.2–1.0)	5.0
Smoking (Ref: Never)	Current	2.5 (1.2–4.9)	3.9	2.6 (1.4–4.9)	3.5	2.5 (1.3–4.8)	3.7
	Former	1.2 (0.5–2.7)	5.4	1.4 (0.6–3.0)	4.7	1.2 (0.5–2.7)	5.1
Antifungal (Ref: No)	Yes	1.0 (0.6–1.7)	2.8	2.0 (1.3–3.2)	2.5		
Age (years) (Ref: 18–29)	40+	1.2 (0.6–2.6)	4.8	1.4 (0.7–2.8)	4.2		
	30–39	1.2 (0.6–2.5)	4.6	1.4 (0.7–2.9)	4.0		
Sex (Ref: women)	Men	0.8 (0.4–1.5)	3.4	1.0 (0.7–1.7)	2.6		
Sexual orientation (Ref: Heterosexual)	MSM	0.7 (0.4–1.4)	3.7	0.7 (0.5–1.2)	2.6		
	Bisexual	0.9 (0.4–1.8)	4.2	0.9 (0.5–1.7)	3.2		
Race/ethnicity (Ref: Whites)	Blacks	0.7 (0.4–1.1)	2.8	0.9 (0.6–1.4)	2.4		
Drug use (Ref: Never)	Current	0.9 (0.4–1.9)	4.3	1.0 (0.5–1.9)	3.8		
	Former	1.1 (0.6–1.9)	3.1	1.3 (0.8–2.2)	2.7		

MSM, men who have sex with men.

The final model for OC included CD4+ cell count, antiretroviral medication and smoking. After adjusting for antiretroviral medication and smoking, those with low CD4+ counts were 13 times as likely as those with high CD4+ cell counts to have OC (adj. OR = 12.7). A dose–response

effect was noted with higher odds of OC for lower CD4+ cell count. After adjusting for CD4+ cell count and antiretroviral medication; current smokers had 2.5 times the odds of OC compared with those who had never smoked (OR = 2.5). This risk was lesser for former smokers (OR = 1.2). Antiretroviral medication showed a significant protective effect (OR = 0.6 for combination therapy). The crude OR (10.7) for the low CD4+ count–OC relationship was confounded 16% downwards compared with the final model OR (12.7). Estimate precisions were similar in both models.

Oral hairy leukoplakia

Table 3 shows that participants with low CD4+ count (crude OR = 9.1), intermediate CD4+ cell count (OR = 4.1); men (OR = 3.2); bisexuals (OR = 2.3); current drug users (OR = 2.2); and those taking antifungal medication (OR = 2.7) were significantly more likely to have OHL compared with their respective reference categories. Being black (OR = 0.6) was significantly protective, compared with being white, against development of OHL. Smokers, older age groups, those with no antiretroviral medication, men who have sex with men (MSM) and bisexuals were slightly more likely to have OHL, although the differences did not achieve statistical significance. Although not shown in the table, those with high viral load were twice as likely to have OHL as those with low viral load (OR = 2.1; 95% CI: 1.0–4.6) and participants with AIDS were 1.6 times as likely to have OC as those without AIDS (crude OR = 1.6; 95% CI: 1.1–2.5).

Table 3 shows the logistic regression models for OHL. In the full model, CD4+ cell count, sex, race/ethnicity, and drug use were the significant factors. Compared with crude ORs, estimates for smoking, sexual orientation and antifungal medication were diluted in the fully adjusted model, each moving substantially closer to unity. In the full model, the ORs for drug use, age and antiretroviral medication did not change substantially from their crude values. Compared with crude estimates, each fully adjusted estimate was slightly less precise (larger CLR).

The final model included CD4+ cell count, sex, race/ethnicity, antifungal medication, and drug use as significant independent variables associated with OHL. Use of antifungal medication was statistically not significant in the full model, but became significant in the final model. Compared with the full model, all ORs in the final model gained in precision as seen in slightly lesser CLR values.

After adjusting for sex, race, antifungal medication, and drug use, those with low CD4+ counts were seven times as likely as those with high CD4+ cell counts to have OHL (adj. OR = 7.2). Those with intermediate CD4+ cell count were 3.9 times as likely as those with high CD4+ cell count to have OHL. A dose–response effect was noted with higher odds of OHL with lower CD4+ cell count. Similarly, after adjusting for CD4+ cell count, race/ethnicity, and antifungal medication, men were 2.5 times as likely to have OHL compared with females. Blacks had 35% lesser risk compared with whites, while those using antifungal medications

Table 3. Logistic regression model for oral hairy leukoplakia (presence of OHL)

		Full model		Bivariable (crude)		Final model	
Variable	Level	OR (95% CI)	CLR	OR (95% CI)	CLR	OR (95% CI)	CLR
CD4+ (cells/μl) (Ref: ≥500)	<200	7.7 (2.9–20.6)	7.1	9.1 (3.6–23.1)	6.5	7.2 (2.7–18.9)	7.0
	200–499	3.4 (1.3–9.3)	7.3	4.1 (1.6–10.9)	6.9	3.9 (1.4–10.4)	7.2
Sex (Ref: Women)	Men	2.5 (1.2–5.2)	4.3	3.2 (1.7–5.9)	3.5	2.5 (1.3–4.8)	3.7
Antifungal (Ref: No)	Yes	1.6 (0.9–2.6)	2.7	2.7 (1.7–4.1)	2.4	1.8 (1.1–2.9)	2.7
Drug use (Ref: Never)	Current	2.2 (1.1–4.3)	3.9	2.2 (1.1–4.1)	3.8	2.5 (1.3–4.9)	3.7
	Former	1.1 (0.6–2.0)	3.2	1.3 (0.8–2.1)	1.7	1.2 (0.7–2.1)	3.0
Race/ethnicity (Ref: Whites)	Blacks	0.6 (0.4–0.9)	2.7	0.6 (0.4–0.9)	2.3		
Age (years) (Ref: 18–29)	40+	0.7 (0.3–1.5)	4. 8	0.8 (0.4–1.8)	4.2		
	30–39	1.2 (0.6–2.4)	4.3	1.5 (0.7–3.0)	4.1		
Antiretrovirals (Ref: None)	Combination	0.8 (0.5–1.5)	3.2	0.8 (0.5–1.3)	2.6		
	Monotherapy	0.8 (0.4–1.4)	3.1	1.2 (0.7–2.2)	3.1		
Smoking (Ref: Never)	Current	1.3 (0.7–2.5)	3.5	1.7 (0.9–2.9)	3.1		
	Former	0.9 (0.4–2.0)	4.7	1.3 (0.6–2.6)	4.1		
Sexual orientation (Ref: Heterosexual)	MSM	0.6 (0.3–1.2)	3.5	1.2 (0.8–1.9)	2.7		
	Bisexual	1.3 (0.7–2.4)	3.6	2.3 (1.4–3.9)	2.9		

MSM, men who have sex with men.

had a 73% higher risk for OHL. After adjusting for the covariates, current drug users were 2.4 times as likely as never users to have OHL. The risk for former users was lower (OR = 1.3). The crude OR for low CD4+ count–OHL relationship (OR = 9.1) was confounded 26% upwards compared with the final adjusted OR (7.2).

Discussion

OC and OHL are the two most common oral diseases associated with HIV/AIDS (1–22). Most studies have reported higher prevalence of OC than OHL (36, 37), in contrast to our findings that prevalence of OC and OHL were both around 17%. Among US HIV-infected women under medical care, the prevalence of OHL (6.1%) has been reported to be substantially less than that of OC (13.7%) (38), suggesting a decreased risk of OHL among women, as confirmed in our multivariable model. Although many published studies evaluating risk indicators for HIV-associated oral diseases have methodological weaknesses such as inadequate sample size, biased/convenience samples, misclassification of disease, different categorization of variables, poor control of potential confounders, and lack of multivariable analyses (36, 37), recent well-designed studies (38) have shown sounder analytic approaches to determine risk indicators beyond the well-established lower CD4 counts and somewhat less well-established higher viral loads. The current study attempted to address many of these issues in accordance with guidelines for future research (36, 37). However, this cross-sectional data could not be analyzed to address issues related to changes in observations in a time-dependent manner. Additional aspects of risk for these HIV-associated oral lesions may not have been either conceptualized or assessed in our study design.

We used CD4+ cell count as our main explanatory variable. While modeling to ascertain potential etiological mechanisms, we followed the suggestions by Rothman and Greenland (39) that variables falling in the direct causal pathway should not be used as covariates in the same model. Because CD4+ cell count was our main exposure variable, we did not use plasma viral load as a covariate in the multivariable models. Greenspan et al. (38) used viral load and CD4+ cell count in the same model for each of the outcomes, possibly resulting in an attenuated effect of CD4+ cell counts in their study.

We used antiretroviral therapy as a potential confounder in the models. Antiretroviral therapies have a variable effect on plasma viral load owing to differences in bioactivity, tissue penetration, half-life, ease of developing resistant strains, tolerance, toxicity and regimen complexity that influence adherence (40). Thus, HIV therapies are often not equally successful in reducing viral load for substantial periods. Their effect on CD4+ cell count therefore would be variable and indirect. In addition, some antiretroviral medications are secreted in the saliva, suggesting that systemic anti-HIV medications may alter the oral milieu through either direct antimicrobial effects on oral pathogens or a common side effect such as reduced saliva production. In light of these issues, we used antiretroviral therapy as a covariate in the most logical form within the constraints of our data to demonstrate the role of these therapies compared with more ecological evaluations or intervention with protease inhibitors alone (2, 5, 6).

It is intuitive to expect a reduction in disease odds following antiretroviral therapy. In a study described in 1994, Lamster et al. (41) found a significantly higher risk of OC among those receiving antiviral therapy. In a study reported 6 years later, Hilton et al. (42) described a similar finding although the effect was not statistically significant in their study. In contrast, we found significant protective effects of antiretrovirals for OC, but no effect on OHL alone. There are several possible explanations for these seemingly contradictory results. First, the antivirals used before 1994 were typically administered in monotherapy or two-drug combinations and did not include potent protease inhibitors. Because these early therapies were not very effective, patients could have experienced inadequate suppression of viremia with CD4+ cell loss continuing despite treatment. Second, antiretrovirals were typically prescribed at late, rather than early, stages in the HIV/AIDS disease spectrum and may account for the higher risk of OC described in the Lamster et al.'s study (41). Third, because of the variety of medication regimens available from 1991 (Lamster et al.'s study) to 2000 (current study), the various studies may have used different definitions of antiretroviral therapy (e.g. combination therapies including multiple reverse transcriptase inhibitors versus regimens that contained both reverse transcriptase and protease inhibitors).

While various studies have discussed the changing prevalence of HIV/AIDS and diseases before

and after the availability of HAART, a clear definition of what constituted HAART in these studies was not uniformly available until the US Public Health Service guidelines were published in 1998 (40). Our study was initiated before these guidelines, and data were collected before, during, and after the phase of initiation and establishment of HAART. Our data are not suitable for a HAART/non-HAART dichotomy. Instead of using HAART as a definition, we evaluated antiretroviral therapy as a paradigm by categorizing antiretroviral therapy into a three level variable – no antiretroviral medications, monotherapy, and combination therapy. Monotherapy is no longer recommended, as numerous combinations of antiretroviral agents have shown superior efficacy in reducing viral load and restoring immune function in individuals who have not experienced development of antiretroviral drug resistance.

Among studies reporting multivariable analyses for OC and OHL, the effect of cigarette smoking has varied between no (16), mild (43), and strong associations (44). Palmer et al. (43) used the stepwise regression analyses, a technique that has been criticized as a modeling strategy (39). In those models (43), age and tobacco use were the significant variables associated with OC, with tobacco use imparting a 50% higher risk. The results from a study conducted by Nittayananta et al. (16) had some intriguing features. First, in the multivariable model presented, current smoking exhibited a protective effect for any oral lesion. Second, current smoking was significant when any HIV-associated oral disease was the outcome. However, smoking was not reported in the models for OC and OHL analyzed independently. Our results are contradictory in that not only was current smoking associated with significantly higher risk of OC, there was also a suggestion of a dose–response relationship if former smokers are considered as low-dose exposed. In contrast, smoking did not contribute significantly to OHL. Construction of the variables in the various studies may explain some of these differences. Additionally, smoking was a common social behavior among the study population, with 59% being current smokers and 20% being former smokers.

Nittayananta et al. (16) used 'symptomatic' disease as a covariate, along with AIDS status and CD4+ cell count to model OC status. In their model, being symptomatic for OC showed a 42 times higher risk of OC compared with not being symptomatic. The use of the symptom of a disease as an explanatory variable for the disease outcome seems questionable. AIDS staging has been reported as a predictor for OC (16). OC and OHL have been shown to be highly predictive of AIDS (25, 33, 45). Therefore, conceptually, AIDS status should not be used as an explanatory variable. At the same time, it is likely that AIDS status is correlated with CD4+ cell count. Covariates that are causally linked should not be used together (39). It is likely that the high odds ratios associated with AIDS symptoms (OR = 42.1) and status (OR = 24.2) as predictors of candidiasis in the Nittayananta models (16) may have arisen as a function of their model specification.

One repeat-measure analysis using transitional models (42) found history of candidiasis to be a good predictor for OC. This is generally true for most diseases in that past occurrences of a disease predict a future event of the same or associated disease very well. Unless evaluating multiple occurrences of a disease, conceptually, when the goal is to look for potential etiological models for a disease, history of the same disease should not be specified as an explanatory variable because it will be strongly correlated with all strong predictors for the disease outcome that are used as explanatory variables (39). For example, the Spearman correlation statistic for history of candidiasis and CD4+ cell count in our data was 0.56, which suggests moderate correlation. We analyzed history of OC and AIDS staging separately for consistency with literature. We tested both full and final models by introducing these factors after model selections were complete. While history of candidiasis predicted OC very strongly, it did not influence the significance or direction of effect estimates of any other factors, but it attenuated the strength of CD4+ cell count and OC association by about 50%. For the OHL model, results were similar to the OC model. AIDS staging showed strong bivariable association with OC and OHL, but the effect disappeared after adjustments in multivariable models.

As reported in comprehensive reviews of other studies (37), we found men to be at greater risk for OHL. This higher risk persisted after adjustment for all the covariates in the full model, and in the final model. Change in precision of the estimate between the crude and adjusted OR was not appreciable (similar CLR). Several authors have discussed the higher risk of OHL for men (3, 8, 10–12, 36, 37). Although MSMs have been generally associated with greater risk for OHL (8, 37), we did

not find MSMs to be at higher risk. In an effort to explore the potential issues that may explain the differences, we found that among men, proportion with OHL was higher among never smokers (15%), former smokers (18%) and current smokers (24%) compared with women (10, 6 and 7%, respectively), although not statistically significantly different. A similar trend was also found among drug users. Furthermore, 84% of the men with OHL had multiple risk factors including cigarette smoking and the use of cocaine, marijuana and crack cocaine. None of these differences achieved statistical significance. Carefully designed studies evaluating these interactions may be able to report an advance. Campisi et al. (46) reported a higher proportion of women with OC and attributed it to a purportedly different genetic makeup of Mediterranean women. Based on our results and other studies (37), it seems more likely that smoking, drug use and other such factors might explain the observed differences between men and women.

We found that drug use was a significant factor for risk of OHL but not for OC. Current drug users were 2.5 times as likely to have OHL compared with never users. The risk was lesser and non-significant (OR = 1.24) for former drug users. Because most men with OHL had multiple potential risk factors such as drug use and smoking, it is possible that a multiway interaction could be at play.

An intriguing result was that use of antifungal medications was a strong risk indicator for OHL. Although the adjusted final risk estimate (OR = 1.82) was 32% lower than the crude estimate (OR = 2.66), it remained significant in the final model. Several plausible explanations exist: (a) those with OHL also had OC and were treated with antifungals, and at examination, OC had resolved but OHL had not; (b) antifungals were prescribed because OHL was either misdiagnosed as OC or persons with OHL were deemed to be at risk for OC; or (c) antifungals were prescribed as secondary prophylaxis against chronic mucosal candidiasis or other systemic fungal disease among some with severe immune suppression, who were likely to also be susceptible to EBV infection presenting as OHL.

It is important to address the limitations of our study and the quality assurance steps that we undertook to enhance the study design. As mentioned earlier, this cross-sectional study could not address the issue of temporality etiological mechanisms. Because data documenting the time since HIV-1 seroconversion were not available, inference regarding events related to time since exposure was not incorporated in the analyses. In addition, diagnoses in this study were based on clinical grounds without biopsy or cultures, which could raise concerns for potential misclassification. However, OC and OHL have distinct clinical presentation, and as suggested by the presumptive diagnostic criteria (34, 35), clinical diagnoses are valid. The quality of the data described here was enhanced through multiple data entry, multiple record review and crosschecking missing and out of range values. The study population is representative of the North Carolina HIV-infected population in age, sex and racial/ethnic groups, and has slightly fewer injecting drug users and more heterosexuals than the North Carolina AIDS Surveillance Report (47). However, it is not a statistical representation of all HIV-infected persons of the nation, which could only be achieved through a national probability sample of HIV-infected persons. At the same time, it must be recalled that in a study addressing potential etiological relationships, limited generalizability may not be viewed as a serious limitation. Epidemiologic study designs are usually stronger if subject selection is guided by the need to make a valid comparison, which may require severe restriction of admissible subjects to a narrow range of characteristics, rather than by attempts, in a survey sampling sense, to make the subjects representative of the potential target populations (39). Without an assessment of adherence to antiretroviral therapy regimens or access to plasma levels of antiretroviral drugs, we area unable to verify that we have not misclassified individuals according to use of antiretroviral medications. Additionally, the cross-sectional nature of the study design limits our ability to determine cause and effect in the antifungal–OHL relationship; however, it is unlikely that OHL is a side effect of antifungal drugs.

In summary, comparing both models, a picture emerged that confirms low CD4+ cell count as an important risk factor for OC and OHL and that antiretroviral medications are particularly protective for OC. Among our population with a high smoking prevalence, cigarette smoking appears to increase the risk for OC and was identified as a significant OC risk indicator, while smoking remains a relatively unimportant factor for OHL. Our knowledge of HIV-associated oral disease pathogenesis will be advanced by addressing 1) the impact of continually evolving antiretroviral drug regimens, including new drug classes such as

the fusion inhibitors that prevent entry of HIV into the target cell, on the prevalence of OC, OHL, and other HIV-associated oral diseases, and 2) the potential influence of oral mucosal disease on immune reconstitution and systemic viremia following effective therapy as measured by improvements in plasma markers of HIV disease.

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References

1. Patton LL, van der Horst C. Oral infections and other manifestations of HIV disease. Infect Dis Clin North Am 1997;13:879–900.
2. Patton LL, McKaig RG, Strauss RP, Rogers D, Eron JJ Jr. Changing prevalence of oral manifestations of HIV in the era of protease inhibitor therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:299–304.
3. Bendick C, Scheifele C, Reichart PA. Oral manifestations in 101 Cambodian patients with HIV infection and AIDS. J Oral Pathol Med 2002;31:1–4.
4. Campo J, del Romero J, Castilla J, Garcia S, Rodriguez C, Bascones A. Oral candidiasis as a clinical marker related to viral load, CD4 lymphocyte percentage in HIV infected patients. J Oral Pathol Med 2002;31:5–10.
5. Ceballos-Salobrena A, Gaitan-Cepeda LA, Ceballos-Garcia L, Lezama-Del Valle D. Oral lesions in HIV/AIDS patients undergoing highly active antiretroviral treatment including protease inhibitors: a new face of oral AIDS? AIDS Patient Care STDs 2000;14:627–35.
6. Diz Dios P, Ocampo A, Miralles C, Otero I, Iglesias I, Rayo N. Frequency of oropharyngeal candidiasis in HIV infected patients on protease inhibitor therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87:437–41.
7. Eyeson JD, Tenant-Flowers M, Cooper DJ, Johnson NW, Warnakulasuriya KAAS. Oral manifestations of an HIV positive cohort in the era of highly active anti-retroviral therapy (HAART) in South London. J Oral Pathol Med 2002;31:169–74.
8. Feigal DW, Katz MH, Greenspan D, et al. The prevalence of oral lesions in HIV infected homosexual and bisexual men: three San Francisco epidemiological cohorts. AIDS 1991;5:519–25.
9. Gottfredsson M, Indridason OS, de Almeida GMD, Heald AE, Perfect JR. Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal *Candida* colonization. J Infect Dis 1999;180:534–7.
10. Greenspan JS, Greenspan D, Palefsky J. Oral hairy leukoplakia after a decade. Epstein–Barr Virus Rep 1995;2:123–8.
11. Greenspan JS. Sentinels and signposts: the epidemiology and significance of the oral manifestations of HIV disease. Oral Dis 1997;3:S13–7.
12. Khongkunthian P, Grote M, Isaratanan W, Piyaworawong S, Reichart PA. Oral manifestations in HIV-positive adults from Northern Thailand. J Oral Pathol Med 2001;30:220–3.
13. Koloktronis A, Kioses A, Antoniades D, Mandraveli K, Doutos I, Papanayotou P. Immunologic status in individuals infected with HIV oral candidiasis and hairy leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;78:41–6.
14. MacPhail LA, Komaroff E, Alves EAF, Navazesh M, Phelan JA, Redford M. Differences in risk factors among clinical types of oral candidiasis in the Women's Interagency HIV study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:45–55.
15. McCarthy GM, Mackie ID, Koval J, Sandhu HS, Daley TD. Factors associated with increased frequency of HIV-related oral candidiasis. J Oral Pathol Med 1991;20:332–6.
16. Nittayananta W, Chanowanna N, Sripatanakul S, Winn T. Risk factors associated with oral lesions in HIV infected heterosexual people and intravenous drug users in Thailand. J Oral Pathol Med 2001;30:224–30.
17. Nittayananta W, Chanowanna N, Winn T, et al. Co-existence between oral lesions and opportunistic systemic diseases among HIV infected subjects in Thailand. J Oral Pathol Med 2002;31:163–8.
18. Patton LL, McKaig RG, Strauss RP, Eron JJ Jr. Oral manifestations of HIV in a southeast USA population. Oral Dis 1998;4:164–9.
19. Patton LL, McKaig RG, Eron JJ Jr., Lawrence HP, Strauss RP. Oral hairy leukoplakia and oral candidiasis as predictors of HIV viral load. AIDS 1999;13:2174–6.
20. Ravina A, Ficarra G, Chiodo M, Mazzetti M, Romagnani S. Relationship of circulating CD4+ T-lymphocytes and p24 antigenemia to the risk of developing AIDS in HIV infected subjects with oral hairy leukoplakia. J Oral Pathol Med 1996;25:108–11.
21. Shiboski CH. Epidemiology of HIV-related oral manifestations in women: a review. Oral Dis 1997;3:S18–27.
22. Samaranayake LP, Holmstrup P. Oral candidiasis and human immunodeficiency virus infection. J Oral Pathol Med 1989;18:554–64.
23. Barr CE, Lopez MR, Rua-Dobles A, Miller LK, Mathur-Wagh U, Turgeon LR. HIV-associated oral lesions; immunologic, virologic and salivary parameters. J Oral Pathol Med 1992;21:295–8.
24. Greenwood I, Zakrzewska JM, Robinson PG. Changes in the prevalence of HIV-associated mucosal disease at a dedicated clinic over 7 years. Oral Dis 2002;8:90–4.
25. Patton LL. Sensitivity, specificity, and positive explanatory value of oral opportunistic infections in adults with HIV/AIDS as markers of immune suppression and viral burden. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:182–8.

26. Ramirez-Amador V, Esquivel-Pedraza L, Sierra-Madero J, Anaya-Saavedra G, Gonzalez-Ramirez J, Ponce-de-Leon S. The changing spectrum of human immunodeficiency virus (HIV)-related oral lesions in 1,000 consecutive patients: a 12-year study in a referral center in Mexico. Medicine 2003;82:39–50.
27. Lifson AR, Hilton JF, Westenhouse JL, et al. Time from HIV-seroconversion to oral candidiasis or hairy leukoplakia among homosexual and bisexual men enrolled in three prospective cohorts. AIDS 1994;8:73–9.
28. Hilton JF. Functions of oral candidiasis episodes that are highly prognostic for AIDS. Statist Med 2000;19:989–1004.
29. Centers for Disease Control. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 1992;41:1–9.
30. Hunter KD, Gibson J, Lockhart P, Pithie A, Bagg J. Fluconazole-resistant *Candida* species in the oral flora of fluconazole-exposed HIV-positive individuals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:558–64.
31. Tumbarello M, Tacconelli E, Caldarola G, Morace G, Cauda R, Ortona L. Fluconazole resistant oral candidiasis in HIV infected individuals. Oral Dis 1997;3:S110–2.
32. Triantos D, Porter SR, Scully C, Teo CG. Oral hairy leukoplakia: clinico-pathologic features, pathogenesis, diagnosis, and clinical significance. Clin Infect Dis 1997;25:1392–6.
33. Katz MH, Greenspan D, Westenhouse J, et al. Progression to AIDS in HIV infected homosexual and bisexual men with hairy leukoplakia and oral candidiasis. AIDS 1992;6:95–100.
34. EEC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Center on Oral Manifestations of the Immunodeficiency Virus. Classification and diagnostic criteria for oral lesions in HIV infection. J Oral Pathol Med 1993;22:289–91.
35. Klienbaum DJ. Logistic regression – a self learning text. New York: Springer-Verlag; 1994.
36. Eyeson JD, Warnakulasuriya KAAS, Johnson NW. Prevalence and incidence of oral lesions – the changing scene. Oral Dis 2000;6:267–73.
37. Patton LL, Phelan JA, Ramos-Gomez FJ, Nittayananta W, Shiboski CH, Mbuguye TL. Prevalence and classification of HIV-associated oral lesions. Oral Dis 2002;8:98–109.
38. Greenspan D, Komaroff E, Redford M, et al. Oral mucosal lesions and HIV viral load in the Women's Interagency HIV Study (WIHS). J Acquir Immune Defic Syndr 2000;25:44–50.
39. Rothman KJ, Greenland S. Modern epidemiology. 2nd edn. Philadelphia: Lippincott Williams & Wilkins; 1998.
40. Centers for Disease Control and Prevention. Report of the NIH panel to define principles of therapy of HIV infection and guidelines for the use of antiretroviral agents in HIV infected adults and adolescents. MMWR CDC Surveill Summ 1998;47(RR-5): 1–82.
41. Lamster IB, Begg MD, Mitchell-Lewis D, et al. Oral manifestations of HIV infection in homosexual men and intravenous drug users; study design and relationship of epidemiologic, clinical, and immunologic parameters to oral lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;78:163–74.
42. Hilton JF, Donegan E, Katz MH, et al. Development of oral lesions in human immunodeficiency virus-infected transfusion recipients and hemophiliacs. Am J Epidemiol 1997;145:164–74.
43. Palmer GD, Robinson PG, Challacombe SJ, et al. Aetiological factors for oral manifestations of HIV. Oral Dis 1996;2:193–7.
44. Palacio H, Hilton JF, Canchola AJ, Greenspan D. Effect of cigarette smoking on HIV-related oral lesions. J Acquir Immune Def Syndr Hum Retrovir 1997;14:338–42.
45. Glick M, Muzyka BC, Lurie D, Salkin LM. Oral manifestations associated with HIV-related disease as markers for immune suppression and AIDS. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;77:344–9.
46. Campisi G, Pizzo G, Milici ME, Mancuso S, Margiotta V. Candidal carriage in the oral cavity of human immunodeficiency virus-infected subjects. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:281–6.
47. McKaig RG, Thomas JC, Patton LL, Strauss RP, Slade GD, Beck JD. Prevalence of HIV-associated periodontitis and chronic periodontitis in a southeastern US study group. J Public Health Dent 1998;58: 294–300.