Oral Diseases (2006) 12, 41–44. doi:10.1111/j.1601-0825.2005.01155.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Association of asymptomatic oral candidal carriage, oral candidiasis and CD4$^+$ lymphocyte count in HIV-positive patients in China

X Liu[1], H Liu[1], Z Guo[2], W Luan[1,2]

[1]*School of Stomatology, Peking University, Beijing, China;* [2]*Department of Dentistry, Beijing Hospital, Beijing, China*

OBJECTIVES: To compare the prevalence of asymptomatic oral candidal carriage in healthy volunteers with human immunodeficiency virus (HIV)-positive patients in China, as well as to investigate the relationship between CD4$^+$ lymphocyte count and oral candidal colonization or oral candidiasis.

METHODS: Oral candidal carriage and oral candidiasis were investigated in 101 patients with HIV-infection seen at Youan Hospital, Beijing, China. Two hundred and seventeen healthy volunteers were involved as a control. Culture from saliva was used to test for the presence of oral *Candida*. CD4$^+$ lymphocyte count was measured by flow cytometry. All data were analyzed statistically by SAS.

RESULTS: Asymptomatic oral candidal carriage rate (28.6%) in HIV-positive group was similar to that in the healthy group (18.0%; $P = 0.07$). No significant difference in CD4$^+$ lymphocyte count was found between oral *Candida* carriers and non-carriers among HIV-positive subjects ($P = 0.89$). However, the frequency of oral candidiasis increased with the decrease in CD4$^+$ lymphocyte count ($P < 0.0001$), and pseudomembranous candidiasis was predominant in HIV-positive patients with CD4$^+$ <200 cells μl^{-1} (66.7%).

CONCLUSIONS: In HIV-positive subjects, asymptomatic oral candidal colonization is not related to CD4$^+$ lymphocyte count of blood, and the carriage rate is similar to that in the healthy population. Oral candidiasis is more likely to be observed in HIV-positive patients who have a low CD4$^+$ lymphocyte count.

Oral Diseases (2006) 12, 41–44

Keywords: HIV; oral candidiasis; asymptomatic oral candidal carriage; CD4$^+$ lymphocyte

Correspondence: Prof. Wenmin Luan, Department of Dentistry, Beijing Hospital, Beijing 100730, China. Tel: +86 10 6513 2266 ext 7245, Fax: +86 10 6512 7933, E-mail: wenmin5@yahoo.com.cn
Received 12 October 2004; revised 15 March 2005; accepted 15 April 2005

Introduction

Oral candidiasis is most common in HIV-related oral lesions. About 90% of patients were found to suffer from oropharyneal or esophageal candidiasis in various stages of AIDS (Darouiche, 1998). AIDS is a chronic progressing disease characterized by immunodeficiency that is the result of human immunodeficiency virus (HIV) attacking the host's CD4$^+$ lymphocytes. It is generally thought that immunodeficiency is the main cause in HIV-infected and AIDS patients of their susceptibility to various opportunistic infections. CD4$^+$ lymphocyte count in peripheral blood is important when evaluating the immune status of the host. In this study, we investigated the prevalence of oral *Candida* in HIV-positive individuals in China. The relationship between CD4$^+$ lymphocyte count and asymptomatic oral carriage or oral candidiasis was also investigated.

Subjects and methods

Subjects

During 2002–2003, 101 individuals seen at the outpatient clinic of Youan Hospital, Beijing, China were enrolled in this study. All of them were diagnosed with HIV-infection by means of enzyme-linked immunosorbant assay (ELISA) and Western blot assay. Inclusion criteria for these subjects were as following: (i) no antiretroviral therapy; (ii) no antifungal, antibiotic and immune suppression agents such as steroid treatment during the previous 3 months; (iii) without non-candidal oral lesions. Criterion (i) was to eliminate the potential effects of proteinase inhibitors used in antiretroviral therapy and criterion (iii) was to eliminate the effects of non-candidal lesions on candidal carriage. Patients with diabetes, xerostomia or salivary gland disease, patients undergoing chemotherapy or radiotherapy in the head or neck and pregnant or nursing women were excluded from this study. Among this population, 77 subjects with normal oral mucosa were compared for asymptomatic candidal carriage with the healthy group. The

remaining 24 subjects diagnosed with oral candidiasis were used to investigate the relationship between oral candidiasis and $CD4^+$ lymphocyte count in peripheral blood.

The healthy group included 217 HIV-negative volunteers who were postgraduate students from a university in Beijing. They were physically healthy with normal oral mucosa, and had not received treatment during the previous 3 months with any antifungals, antibiotics or immune suppression agents such as steroids.

All individuals in this study were examined by trained dentists according to the diagnosis criteria of the EC-Clearinghouse on Oral Problems Related to HIV Infection (EC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Center on Oral Manifestations of the Immunodeficiency Virus, 1993). $CD4^+$ lymphocyte count in peripheral blood was measured by flow cytometry within 48 h of oral examination. Informed consent was obtained from all participants.

Microbiologic procedures

The subjects fasted and had no drinking or any oral-cleaning procedure for 1 h before sampling. 1 ml of unstimulated whole saliva from each subject was collected and incubated aerobically on Sabouraud Dextrose Agar plate (Jinzhang Medical New Technology Institute, Tianjin, China) at 37°C for 48 h. Subsequently, for each sample, colonies of different morphology were streaked onto CHROMagar plates (Biocell Institute, Zhengzhou, China) and incubated at 37°C for 48 h. Presumptive identification of yeast isolations was determined to the species level according to the colors of the colonies growing on CHROMagar plates and confirmed using the API 20C AUX system (bioMérieux, France), germ tube formation assay and growth assay at 45°C.

Statistical anaylsis

SAS, version 9.0 (SAS Institute Inc, Cary, NC, USA) was used to manage and analyze the data. Quantitative variables and percentages were compared by Student's *t*-test and chi-squared test, respectively. Fisher's exact test was used when the sample size was quite small. Wilcox–Mann–Whitney rank sum test was used to analyze the relationship between $CD4^+$ lymphocyte count and asymptomatic oral carriage. The relationship between the oral candidiasis rate and the $CD4^+$ lymphocyte count was analyzed by chi-squared test for linear trends. Statistical significance for all analysis was $P < 0.05$.

Results

Among the 217 HIV-negative healthy volunteers, 68 (31.3%) were men and 149 (68.7%) were women. The mean age was 30 years (range, 25–44 years). *Candida* was isolated from the oral cavities of 39 individuals. Therefore, the asymptomatic oral candidal carriage rate was 18.0% (39 of 217) in this control group.

Among the 77 HIV-positive individuals with normal oral mucosa, 29 (37.7%) were men and 48 (62.3%) were women. The mean age was 41.9 years (range, 28–58 years). *Candida* was isolated from 22 patients. The asymptomatic candidal carriage rate was 28.6% (22 of 77) in the study group, higher than that of the control group (18.0%). However, there was no statistically significant difference (chi-square test, $P = 0.07$) as shown in Table 1. The 22 candidal carriers were compared with the remaining 55 non-candidal carriers as shown in Table 2. No statistically significant differences were found in age and gender between the candidal carriers (mean age, 40.1 ± 7.8 years; male, 45.5%) and the non-candidal carriers (mean age, 42.6 ± 6.4; male, 34.5%; *t*-test, $P = 0.16$; chi-square test, $P = 0.53$). There were 21 smokers (five candidal carriers and 16 non-candidal carriers) and four people with dentures (all non-candidal carriers) in these subjects. Similarly, no significant differences were found in smokers and denture wearers (chi-square test, $P = 0.78$; Fisher's exact test, $P = 0.25$). Mean $CD4^+$ lymphocyte counts in candidal carriers and non-candidal carriers were 426.05 ± 276.39 cells μl^{-1} (range, 18–1163 cells μl^{-1}) and 466.18 ± 315.07 cells μl^{-1} (range, 68–1520 cells μl^{-1}), respectively. No statistically significant difference was observed (Wilcox–Mann–Whitney rank sum test, $P = 0.89$).

Table 1 Comparison of asymptomatic oral candidal carriage rates in HIV^+ and HIV^- groups

	Salivary culture for Candida [no. (%)]		
	Positive	*Negative*	*Total number*
HIV-positive	22 (28.6)	55 (71.4)	77 (100)
HIV-negative	39 (18.0)	178 (82.0)	217 (100)
Total	61	233	294

Table 2 Comparison of HIV^+ asymptomatic oral candidal carriers and HIV^+ non-candidal carriers

	HIV^+ candidal carriers	*HIV^+ non-candidal carriers*	
Number (%)	22 (28.6)	55 (71.4)	
Age[a]			
Mean ± s.d.	40.1 ± 7.8	42.6 ± 6.4	0.16
Range	28–56	31–58	
Sex [no. (%)][b]			
Male	10 (45.5)	19 (34.5)	0.53
Female	12 (54.5)	36 (65.5)	
Smoking [no. (%)][b]			
Yes	5 (22.7)	16 (29.1)	0.78
No	17 (77.3)	39 (70.9)	
Denture [no. (%)][c]			
Yes	0	4 (7.8)	0.25
No	22 (100)	51 (92.2)	
$CD4^+$ cell count (cells μl^{-1})[d]			
Mean ± s.d.	426.05 ± 276.39	466.18 ± 315.07	0.89

[a]Student's *t*-test.
[b]chi-square test.
[c]Fisher exact test.
[d]Wilcox–Mann–Whitney rank sum test.

Table 3 Relationship of CD4$^+$ cell count and oral candidiasis in HIV-infection

	Oral candidiasis/total (n)	*Frequency (%)*	*P*
CD4$^+$ cell count (cells μl^{-1})			
≥500	0/25	0	0.0001
200–499	9/48	18.8	
< 200	15/28	53.6	

The relationship between CD4$^+$ lymphocyte count and oral candidiasis in HIV-positive patients is shown in Table 3. Among all 101 HIV-positive patients (with and without oral candidiasis), 28 patients were at the status of CD4$^+$ < 200 cells μl^{-1}, of whom 53.6% (15 of 28) suffered from oral candidiasis and 17.9% (five of 28) were asymptomatic *Candida* carriers. Twenty-five subjects were at the status of CD4$^+$ ≥500 cells μl^{-1}, of whom eight patients were asymptomatic *Candida* carriers status, none with oral candidiasis. In addition, 48 individuals were at the status of CD4$^+$ = 200–499 cells μl^{-1}, of which 18.8% (nine of 48) suffered from oral candidiasis, the percentage was the same as asymptomatic *Candida* carriers. The data were analyzed by chi-squared test for linear trends, $P < 0.01$. This means that the frequency of oral candidiasis increased significantly with the decrease of CD4$^+$ lymphocyte count in HIV-positive patients.

According to the classification and diagnostic criteria for HIV-related oral candidiasis by WHO: among the 24 patients who suffered from oral candidiasis, 11 cases had pseudomembranous candidiasis, 10 cases had erythematous candidiasis and one case had angular candidiasis. In addition, two cases were found with more than one form of oral candidiasis, one of whom had pseudomembranous and erythematous candidiasis and the other with pseudomembranous and angular candidiasis. Fifteen patients were at the status of CD4$^+$ cell count < 200 cells μl^{-1}, of whom 66.7% (10 of 15) presented pseudomembranous candidiasis and the rest with erythematous candidiasis. Moreover, nine patients were at the status of CD4$^+$ = 200–499 cells μl^{-1}, and erythematous candidiasis was the main form in these subjects (55.6%). The relationship of oral candidiasis form with CD4$^+$ lymphocyte count is shown in Table 4.

Discussion

Generally, *Candida* exists in normal oral cavities without harm. Due to the various collection methods and different backgrounds of subjects, rates of asymptomatic oral candidal carriage in healthy cohorts published in previous studies are different. For instance, the carriage rates were 12.5% among healthy children in Hong Kong (Sedgley *et al*, 1997), 53% among adults seen at dental outpatients (Masipa *et al*, 1992) and 29.3% among healthy adults (Campisi *et al*, 2002). In the present study, subjects were postgraduate students from a university in Beijing. The carriage rate was 18.0% by means of salivary cultures. Various methods are often used for sample collection, such as epithelial smears, mucosal swabs, salivary cultures, oral rinses and imprint cultures. Although the salivary culture method is less susceptible than the last two methods (Samaranayake and MacFarlane, 1982; Arendorf and Walker, 1979), it is easy to conduct, especially for large sized samples. This is why the rate in our study was lower than that in previous papers.

Although no significant difference was found in the present study, oral *Candida* carriage rate in HIV-positive patients (28.6%) was a little higher than that in the healthy group (18.0%). This may be related to the worse oral-hygiene status of HIV-positive subjects. Most of the subjects were farmers and thus *Candida* species were more likely to be isolated from them. As reported by Hauman *et al* (1993), our findings suggest that HIV-infection could not change the asymptomatic oral candidal carriage rate. In contrast, Campisi *et al* (2002) and Fong *et al* (1997) thought that *Candida* were more frequently isolated from HIV-positive individuals.

It is generally agreed that cellular immunodeficiency is the main cause for oral candidiasis in subjects with HIV-infection. Kolokotronis *et al* (1994) proved this view by their investigation, in which oral candidiasis was observed to be associated with CD4$^+$ < 200 cells μl^{-1}. Furthermore, in other studies, pseudomembranous candidiasis was shown to be most closely related to immune suppression (CD4$^+$ < 200 cells μl^{-1}), and considered to be a useful clinical marker of progression of disease from HIV-infection to AIDS. However, erythematous candidiasis and angular candidiasis were not found to possess that property (Feigal *et al*, 1991; Schuman *et al*, 1998; Patton, 2000; Campo *et al*, 2002). Similarly, our study revealed that the frequency of oral candidiasis increased significantly with the decrease in CD4$^+$ lymphocyte count, and pseudomembranous candidiasis was predominant among HIV-positive patients with CD4$^+$ < 200 cells μl^{-1} (66.7%), while erythematous candidiasis was principal among patients with CD4$^+$ = 200–499 cells μl^{-1}. Our findings suggest that systematic oral examination for HIV-positive patients should be emphasized, especially in economically less developed regions where laboratory means could not be used periodically to survey the patients' immune status.

Table 4 Relationship of oral candidiasis form and CD4$^+$ lymphocyte count

	Pseudomembranous form (n = 11)	*Erythematous form (n = 10)*	*Angular form (n = 1)*	*Hybride form (n = 2)*
CD4$^+$ cell count (cells μl^{-1})				
≥500 [no. (%)]	0	0	0	0
200–499 [no. (%)]	1 (11.1)	5 (55.6)	1 (11.1)	2 (22.2)
< 200 [no. (%)]	10 (66.7)	5 (33.3)	0	0

Yeast colonization on oral mucosa is the precondition of oral candidiasis. Regarding the relationship between candidal carriage and the circulating $CD4^+$ lymphocyte count, Fetter *et al* (1993) and Schoofs *et al* (1998) reported that higher frequency of asymptomatic oral candidial carriage was closely associated with $CD4^+$ <400 and <200 cells μl^{-1}, respectively. In contrast, no significant association was found in the present study, which partly supports the results from Gottfredsson *et al* (1999) and Campisi *et al* (2002). In addition, Gottfredsson *et al* revealed that oropharyngeal candidial carriage was strongly associated with plasma HIV-1 load, and explained that high levels of HIV-1 RNA replication led to local defenses being compromised, and the increase of yeast proliferation, while Campisi *et al* found only smoking was associated with candidial colonization.

In conclusion, asymptomatic oral candidal colonization is not related to $CD4^+$ lymphocyte count of blood in individuals with HIV-infection, and the candidal carriage rate is similar to that in the healthy group. The frequency of oral candidiasis increases with the decrease in $CD4^+$ lymphocyte count and pseudomembranous candidiasis could predict the immune suppression status of the host.

References

Arendorf TM, Walker DM (1979). Oral candidal populations in health and disease. *Br Dent J* **147:** 267–272.

Campisi G, Pizzo G, Milici ME *et al* (2002). Candidal carriage in the oral cavity of human immunodeficiency virus-infected subjects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **93:** 281–286.

Campo J, Del Romero J, Castilla J *et al* (2002). Oral candidiasis as a clinical marker related to viral load, CD4 lymphocyte count and CD4 lymphocyte percentage in HIV-infected patients. *J Oral Pathol Med* **31:** 5–10.

Darouiche RO (1998). Oropharyngeal and esophageal candidiasis in immunocompromised patients: treatment issues. *Clin Infect Dis* **26:** 259–274.

EC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Center on Oral Manifestations of the Immunodeficiency Virus (1993). Classification and diagnostic criteria for oral lesions in HIV infection. *J Oral Pathol Med* **22:** 289–291.

Feigal DW, Katz MH, Greenspan D *et al* (1991). The prevalence of oral lesions in HIV-infected homosexual and bisexual men: three San Francisco epidemiological cohorts. *AIDS* **5:** 519–525.

Fetter A, Partisani M, Koenig H *et al* (1993). Asymptomatic oral candida albicans carriage in HIV-infection: frequency and predisposing factors. *J Oral Pathol Med* **22:** 57–59.

Fong IW, Laurel M, Burford-Mason A (1997). Asymptomatic oral carriage of Candida albicans in patients with HIV infection. *Clin Invest Med* **20:** 85–93.

Gottfredsson M, Cox GM, Indridason OS *et al* (1999). Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal Candida colonization. *J Infect Dis* **180:** 534–537.

Hauman CHJ, Thompson IOC, Theunissen F *et al* (1993). Oral carriage of Candida in healthy and HIV-seropositive persons. *Oral Surg Oral Med Oral Pathol* **76:** 570–572.

Kolokotronis A, Kioses V, Antoniades D *et al* (1994). Immunologic status in patients infected with HIV with oral candidiasis and hairy leukoplakia. *Oral Surg Oral Med Oral Pathol* **78:** 41–46.

Masipa JN, Hauman CH, Raubenheimer EJ (1992). Oral carriage of *Candida* species in patients visiting the Medunsa Dental Clinic. *J Dent Assoc S Afr* **47:** 407–409.

Patton LL (2000). Sensitivity, specificity, and positive predictive 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* **90:** 182–188.

Samaranayake LP, MacFarlane TW (1982). Factors affecting the *in vitro* adherence of the fungal oral pathogen *Candida albicans* to epithelial cells of human origin. *Arch Oral Biol* **27:** 869–873.

Schoofs A, Odds FC, Colebunders R *et al* (1998). Cross-sectional study of oral *Candida* carriage in a human immunodeficiency virus (HIV)-seropositive population: predisposing factors, epidemiology and antifungal susceptibility. *Mycoses* **41:** 203–211.

Schuman P, Ohmit SE, Sobel JD *et al* (1998). Oral lesions among women living with or at risk for HIV infection. *Am J Med* **104:** 559–564.

Sedgley CM, Samaranayake LP, Chan JC *et al* (1997). A 4-year longitudinal study of the oral prevalence of enteric gram-negative rods and yeasts in Chinese children. *Oral Microbiol Immunol* **12:** 183–188.