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Characterization of oral candidiasis and the *Candida* species profile in patients with oral mucosal diseases

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ABSTRACT

Background: The oral mucosa is likely to be compromised by acquired systemic disease. There are no data available on the prevalence of oral candidiasis and the species distribution among patients with oral mucosal diseases. Therefore, it is necessary to conduct a study assessing the characterization of oral candidiasis and the species profiles in such patients.

Methods: Over a period of four consecutive years, patients with oral mucosal diseases were screened for oral candidiasis by a combination of clinical presentation and laboratory findings (smear test and *Candida* cultures). In addition, *Candida* species were isolated and identified for further analysis.

Results: In total, 9769 (6.09%) of the 160,357 patients screened were diagnosed with oral candidiasis on the basis of both clinical manifestations and laboratory testing. The ratio of females to males was 1:0.61, and females had higher overall infection rates than males in all age subgroups. Patients with HIV infection, anaemia-related stomatitis, Sjögren's syndrome/xerostomia, pemphigoid, and radiation-induced stomatitis were highly susceptible to oral candidiasis. Of the 11,161 isolated *Candida* strains, *C. albicans* remained the most common species (75.37%), followed by *C. tropicalis* (6.06%), *C. krusei* (2.79%), and *C. glabrata* (2.02%). Surprisingly, both the proportion and the number of *C. glabrata* isolates increased dramatically over the 4 consecutive years.

Conclusions: In this large-scale population-based study, the features of oral candidiasis prevalence and the species profile among patients with oral mucosal diseases were summarized. The information gleaned will enhance the understanding of and improve management strategies for oral candidiasis and the underlying systemic and oral conditions.

1. Introduction

The oral cavity is at the intersection of medicine and dentistry and is a window into general health [1]. In the oral cavity, the oral mucosa is perhaps the most likely tissue to be compromised by acquired systemic disease [2]. Thousands of diseases may initially present in the oral mucosa, and oral mucosal disorders may arise as a consequence of systemic diseases. Perhaps the most notable of these is oral candidiasis, the most common fungal infection of the oral cavity in humans [3].

Oral candidiasis typically reflects present or recent therapy with corticosteroids or other immunosuppressants, other immunodeficiencies (for example, HIV infection) or long-standing oral dryness (for example, that due to Sjögren's syndrome) [4]. To this end, dentists, physicians, and all primary care providers must increase their awareness of oral candidiasis and its link to general and oral health to minimize the impact of diseases and maximize the benefit to the patients. While *Candida albicans* is the most common species associated with oral infection, accounting for more than 80% of clinical isolates, several other *Candida* species, such as *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*, may produce clinical infections, some of which can be particularly insensitive or, indeed, resistant to antifungal therapy [5]. Furthermore, the presence of concomitant multiple *Candida* species in oral infections complicate the mycological features and enhance the clinical challenge [6].

To date, there are no data available on the prevalence of oral candidiasis and the species distribution among patients with oral mucosal diseases. Therefore, a large-scale population-based longitudinal study was conducted to characterize oral mucosal and clinical conditions and the prevalence profile of *Candida* species. The information gleaned will enhance the understanding of and improve management strategies for

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Table 1
Distribution of oral mucosal diseases among 9769 patients with oral candidiasis.

Primary diagnosis of oral disease	1st year	2nd year	3rd year	4th year	Total	%
Oral candidiasis (all types)	1056	883	764	767	3470	35.52
Oral lichen planus	549	485	401	441	1876	19.20
Sjögren's syndrome/Xerostomia	268	256	222	252	998	10.22
Recurrent oral ulceration	143	124	110	105	482	4.93
Burning mouth symptoms	100	57	63	62	282	2.89
Anaemia-related stomatitis	61	41	73	72	247	2.53
Fissured tongue	41	39	29	39	148	1.51
Chronic cheilitis	44	27	27	38	136	1.39
Discoid lupus erythaematosus	24	37	31	40	132	1.35
Geographic glossitis	40	33	23	30	126	1.29
Oral leukoplakia	31	24	22	31	108	1.11
Pemphigoid	32	22	16	26	96	0.98
Pemphigus	17	18	12	14	61	0.62
Radiation-induced stomatitis	13	12	7	15	47	0.48
Graft-versus-host disease	7	3	3	6	19	0.19
HIV infection	2	3	3	4	12	0.12
Others	280	418	415	416	1529	15.65
Total	2708	2482	2221	2358	9769	100

both oral candidiasis and the associated underlying conditions of the hosts.

2. Materials and methods

2.1. Study design

The researchers conducted a retrospective study investigating the characterization of oral candidiasis and the profile of *Candida* species among patients with oral mucosal diseases over four consecutive years. This study was approved by the ethics committee of Peking University Health Science Center.

2.2. Participants

A total of 160,357 patients admitted to the Department of Oral Medicine, Peking University School and Hospital of Stomatology from January 1, 2010 to December 31, 2013 were included in the study.

2.3. Clinical data collection

All subjects underwent a thorough oral examination by experienced oral medicine specialists. When oral candidiasis was suspected clinically at the examination, further laboratory tests (smear test and saliva culture) were performed. The preliminary diagnosis of oral candidiasis was based primarily on the clinical symptoms (i.e., pain, burning, or loss of taste) and signs (i.e., loss of papillae on the dorsal tongue, erythema and fissuring of the tongue, erythema of other mucosal surfaces, or angular cheilitis). This diagnosis was confirmed by the mycological tests described below.

2.4. Sample collection

A smear test and culture for *Candida* were performed on samples from patients. Oral smears from the lesions were examined by microscopy by treatment with 10% potassium hydroxide. A saliva sample of 1ml was obtained for culture from patients with normal salivary flow. Oral rinse specimens were collected from patients with xerostomia or decreased salivary flow: patients were requested to rinse with 10 mL of normal sterile saline solution for 60 s and then to spit into a sterile container [7].

2.5. Candida species isolation and identification

Saliva samples were plated on Sabouraud agar (bioMérieux, Shanghai) and CHROMagar chromogenic medium (CHROMagar, Shanghai). Identification of *Candida spp.* was based on morphological criteria. The colony colour was recorded, and representative colonies of each morphological type and colour were subjected to Gram staining. An API 20C AUX kit (bioMérieux, France) was used as a supplementary identification method. Additionally, the total colony-forming units per millilitre (CFU/mL) was counted.

2.6. Statistical analysis

Continuous variables were compared using an independent Student's *t*-test. Multivariate analysis of variance (MANOVA) and least significant differences (LSD) post hoc analyses were used to evaluate differences by sex and age subgroups. A p-value of < 0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS software (ver. 19.0; IBM Corp., Armonk, NY, USA).

3. Results

3.1. Prevalence pattern

A total of 160,357 patients with a suspected clinical presentation of oral candidiasis were further screened by mycological testing. The diagnosis of OC was eventually confirmed in 9769 (6.09%) patients. OC could be concomitant with or secondary to other oral mucosal diseases or systemic conditions. By total number, the most common comorbidity was oral lichen planus, followed by Sjögren's syndrome/xerostomia, recurrent oral ulceration, burning mouth symptoms, anaemia-related stomatitis, fissured tongue, chronic cheilitis, discoid lupus erythaematosus, geographic glossitis, oral leukoplakia, pemphigoid, pemphigus, radiation-induced stomatitis, graft-versus-host disease, and HIV infection (Table 1). Oral manifestations of oral candidiasis are shown in Fig. 1.

3.2. OC susceptibility rate by disease

Patients with certain oral and systemic diseases are prone to oral *Candida* infection. Regarding the effect of different comorbidities on the OC susceptibility rate, 100% of the patients with HIV infection typically presented with pseudomembranous oral candidiasis as the initial sign, which was subsequently confirmed by screening. Patients with anaemia-related stomatitis (75.56%), Sjögren's syndrome/xerostomia (70.99%), pemphigoid (68.37%), and radiation-induced stomatitis (67.01%) were also highly susceptible to oral candidiasis (Table 2).

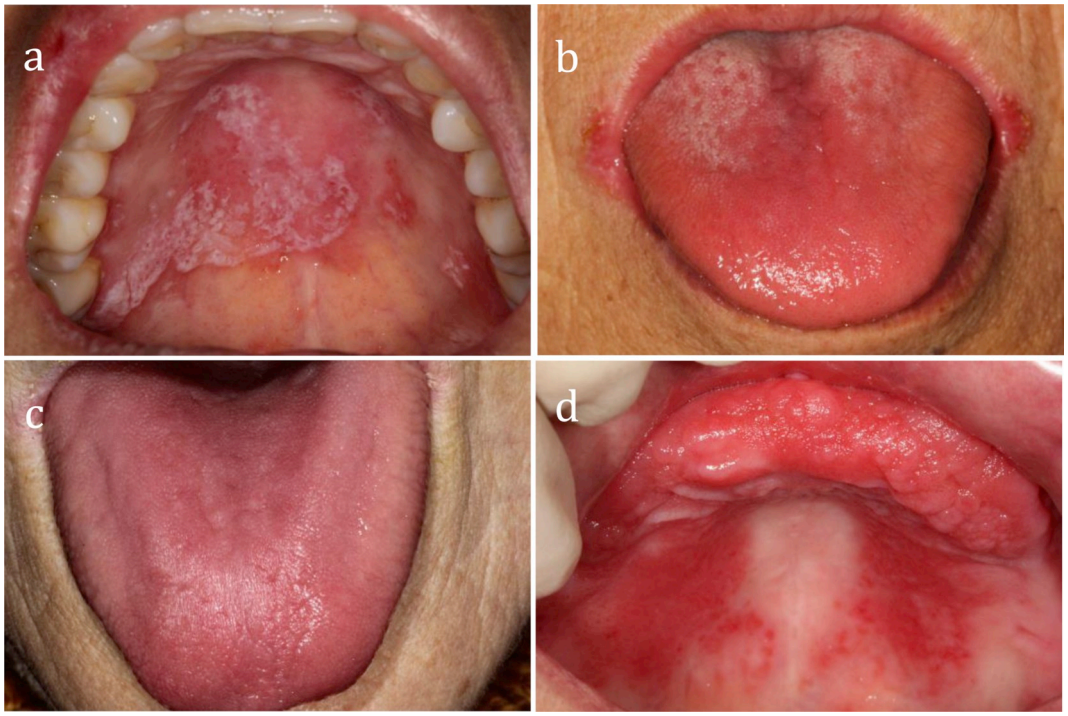

Fig. 1. Variations in clinical manifestation of oral candidiasis in our patients. a. Thrush (pseudomembranous candidiasis) in HIV infection. b. Chronic erythematous candidiasis and candidial cheilitis in anemia-related stomatitis. c. Chronic erythematous candidiasis in Sjögren's syndrome. d. *Candida*-associated denture stomatitis (Newton III type).

Table 2
Oral candidiasis prevalence (%) by disease.

Primary diagnosis of oral disease	1st year	2nd year	3rd year	4th year	Average
HIV infection	100	100	100	100	100
Anaemia-related stomatitis	78.21	67.21	80.22	76.6	75.56
Sjögren's syndrome/ Xerostomia	78.33	67.55	66.67	71.39	70.99
Pemphigoid	76.19	57.9	72.73	66.67	68.37
Radiation-induced stomatitis	69.23	75	57.14	66.67	67.01
Discoid lupus erythaematous	51.06	68.52	73.81	68.97	65.59
Recurrent oral ulceration	61.9	52.32	56.12	52.5	55.71
Oral lichen planus	57.07	51.87	51.61	50.34	52.72
Pemphigus	58.62	48.65	44.44	45.16	49.22
Graft-versus-host disease	50	30	33.33	50.01	40.84
Oral leukoplakia	50	35.82	37.29	39.74	40.71

3.3. Demographic features

The ages of the OC patients ranged from 1 to 98 years (mean, 51.7 ± 21.5), and the oral *Candida* infection rate increased with age. The ratio of females to males was 1:0.61, and the females had significantly higher overall infection rates than the males in all age subgroups ($P = 0.008$). However, the gap in the oral *Candida* infection rate between females and males narrowed with increasing age.

3.4. Candida spp. Distribution

A total of 11,161 *Candida* strains were isolated from 9769 OC patients: *C. albicans* was the most common (8412, 75.37%), followed by *C. tropicalis* (676, 6.06%), *C. krusei* (311, 2.79%), and *C. glabrata* (226, 2.02%). The number of *C. glabrata* isolates increased more than twofold from 2010 to 2013, which is a dramatic change. In addition, the rate of mixed infection (with 2 or more *Candida spp*.) progressively increased yearly, accounting for 261 (9.64%), 300 (12.09%), 284 (12.79%) and 403 (17.09%) cases in years 1, 2, 3, and 4, respectively (Table 3).

4. Discussion

Oral candidiasis is the most common opportunistic infection affecting the human oral cavity. In addition to *Candida* species, local and systemic co-factors, such as a reduced salivary flow rate, oral mucosal erosion, vitamin deficiency, and generalized immune suppression [8], play contributory roles. Hence, oral candidiasis may reflect immunological changes and micro-environmental variations.

In this large-scale study, we found that certain population groups are prone to develop oral candidiasis: patients with immunodeficiency (HIV infection), under treatment with systemic immunosuppressants (pemphigus, pemphigoid, lupus, or graft-versus-host disease), with dry mouth (Sjögren's syndrome/xerostomia or head and neck radiation) and under frequent topical steroid treatment (lichen planus and recurrent oral ulcers). Notably, patients with anaemia showed a rather high prevalence rate of oral *Candida* infection, and the possibility of *Candida* infection may be due to impaired cellular immunity and epithelial abnormalities. It has been reported that some cases of stomatitis-related oral candidiasis could be cured by supplemental therapy alone [9].

In recent years, much interest has focused on the possible association of oral candidiasis with potentially malignant disorders of the oral mucosa. We found that oral candidiasis can present in potentially malignant oral disorders such as oral lichen planus, discoid lupus erythaematosus, and oral leukoplakia. Accumulated evidence suggests that oral candidiasis is a predisposing factor that increases the likelihood of malignant transformation in potentially malignant disorders [10]. Although the exact pathologic significance of *Candida* infection remains unknown, it is generally accepted that there is a positive association between oral *Candida* infection and epithelial dysplasia [11,12]. Therefore, accurate diagnosis and timely use of antifungal therapy are essential to manage these lesions.

While the sex and age of the host have been suggested as

Table 3
Oral *Candida* species distribution.

Candida species	Number of patients (N, %)							
	1st year		2nd year		3rd year		4th year	
Single species isolated								
Candida albicans (CA)	1994	(73.63)	1919	(77.32)	1689	(76.05)	1735	(73.58)
Candida tropicalis (CT)	120	(4.43)	53	(2.14)	55	(2.48)	47	(1.99)
Candida krusei (CK)	31	(1.14)	17	(0.68)	10	(0.45)	8	(0.34)
Candida glabrata (CG)	8	(0.3)	9	(0.36)	12	(0.54)	16	(0.68)
Other *Candida spp.* (OC)	294	(10.86)	184	(7.41)	171	(7.7)	149	(6.32)
Dual species isolated								
CA + OC	91	(3.36)	146	(5.88)	90	(4.05)	160	(6.79)
CA + CT	75	(2.77)	56	(2.26)	58	(2.61)	56	(2.37)
CA + CK	22	(0.81)	20	(0.81)	28	(1.26)	36	(1.53)
CA + CG	7	(0.26)	10	(0.4)	44	(1.98)	56	(2.37)
CT + OC	20	(0.74)	13	(0.52)	11	(0.5)	22	(0.93)
CT + CK	3	(0.11)	2	(0.08)	3	(0.14)	6	(0.25)
CT + CG	1	(0.04)	0	(0)	0	(0)	3	(0.13)
CK + OC	12	(0.44)	9	(0.36)	13	(0.59)	19	(0.81)
CK + CG	0	(0)	2	(0.08)	0	(0)	1	(0.04)
CG + OC	2	(0.07)	2	(0.08)	2	(0.09)	3	(0.13)
Three or more *Candida* spp. isolated	28	(1.03)	40	(1.61)	35	(1.58)	41	(1.74)
Total	2708	(100)	2482	(100)	2221	(100)	2358	(100)

determining factors in *Candida* infection [13,14], few studies have evaluated sex differences in a large population. In this study, both the female and male subgroups demonstrated the same tendency towards increased oral *Candida* infection rates with increasing age. In addition, females showed significantly higher overall infection rates than males in all age subgroups, but the gap between the sexes decreased with increasing age. In summary, female patients are more susceptible to OC than males, and old age may be a predisposing factor for oral candidiasis, possibly due to complex systemic conditions, decreased salivary flow, increased medication intake, and denture wearing.

Previous mycological studies [15] have shown that *C. albicans* accounts for over 80% of isolates and that the number of infections due to non-*albicans Candida* (NCAC) species, especially *Candida glabrata*, has increased significantly in the last decade, which is similar to the results of our study. In particular, *C. glabrata* is an increasing concern as a cause of mucosal infections and is related to approximately 15% of all *Candida*-related systemic bloodstream infections [16]. More importantly, *C. glabrata* exhibits reduced susceptibility to commonly used antifungal drugs, and it is proposed that the increase in *C. glabrata* infections is due to its intrinsically low susceptibility to azoles. Moreover, we found that the rate of mixed infection (with 2 or more *Candida spp.*) progressively increased. This phenomenon is of great clinical importance, since susceptibility to antifungals differs dramatically among *Candida spp*. [17].

5. Conclusions

In this large-scale population-based study, the characterization of oral candidiasis and the profile of *Candida* species in patients with oral mucosal diseases were assessed for the first time. Awareness of the prevalence and clinical significance of oral candidiasis by healthcare providers may promote more intensive clinical and laboratory monitoring and possibly the initiation of prophylaxis against and reasonable treatment of the systemic condition as well as this opportunistic oral fungal infection.

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Conflicts of interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Declarations of interest

None.

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