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Multiple Oral *Candida* Infections in Patients with Sjögren's Syndrome — Prevalence and Clinical and Drug Susceptibility Profiles

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ABSTRACT. ***Objective.*** To determine the prevalence of oral candidiasis and multiple oral *Candida* infections in patients with primary Sjögren's syndrome (SS), and the clinical and drug susceptibility profile.

Methods. Thirty patients with primary SS were enrolled in our study. The diagnosis of oral candidiasis was based on the clinical manifestation, and confirmed by a concentrated rinse culture. *Candida* spp. assessment was accomplished using standard methods: Sabouraud dextrose agar with 50 mg/l chloramphenicol and CHROMagar were used for the rapid screening of clinical species, followed by the API 20C system for further species identification. *In vitro* antifungal drug susceptibility of *Candida* isolates was determined by the minimal inhibitory concentrations.

Results. In our study, 87% (26/30) of subjects had oral candidiasis, in which 42% (11/26) had multiple *Candida* spp. infection. Although *C. albicans* remains the predominant isolate, other rare species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* were present, alone or in combination. Chronic atrophic candidiasis is the most common clinical type of oral candidiasis in patients with SS. The susceptibilities of the 44 *Candida* isolates to 7 antifungal agents varied dramatically. The resistance to azoles was remarkable, and the phenomenon of cross-resistance between itraconazole and fluconazole was observed.

Conclusion. Patients with primary SS carry a high risk of oral candidiasis and a high frequency of multiple *Candida* infections. The azole resistance patterns of *Candida* spp. support the necessity for drug susceptibility testing as a routine procedure for patients with oral *Candida* infections. (First Release Aug 15 2011; J Rheumatol 2011;38:2428–31; doi:10.3899/jrheum.100819)

Key Indexing Terms:
SJÖGREN'S SYNDROME ORAL CANDIDIASIS DRUG RESISTANCE

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by dysfunction and destruction of the exocrine glands associated with lymphocytic infiltrates, and immunological hyperreactivity. Salivary and lacrimal glands are the most commonly affected glands, resulting in the typical clinical presentation of dryness of mouth (hyposalivation) and eyes (keratoconjunctivitis sicca)[1,2]. A study by Atkinson, *et al* found that 88% of the SS subjects had a reduced salivary flow rate (SFR)[3]. And xerostomia, the subjective complaint of dry mouth, has also been reported in high percentages (75% to 92%) of patients with SS[4].

Saliva has antibacterial and fungicidal properties, and plays a critical role in maintaining oral health. A continuous flow of saliva is important to prevent oral colonization by *Candida*. Several of the constituents of saliva are thought to inhibit fungal growth, including defensins, lysozymes, peroxidase, lactoferrin, secretory IgA, and histatin[5,6]. As a consequence of hyposalivation, patients with SS are prone to develop oral candidiasis. Various studies have reported a high prevalence of oral *Candida* carriage in patients with SS, ranging from 54.2% to 81.25%[7,8,9,10].

Although it is generally accepted that patients with SS have an increased risk for oral candidiasis, little is known about multiple *Candida* infection and *Candida* spp. distribution. In addition, patients with SS are likely to get recurrent oral *Candida* infections, and frequent antifungal treatments are given for symptom management. As a result, development of antifungal drug resistance is a growing problem, as with antibiotics.

The aim of our study was to assess the prevalence of oral candidiasis and multiple oral *Candida* infection in patients with primary SS, as well as the clinical and drug susceptibility profile. This knowledge would potentially lead to an

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improved strategy for managing oral candidiasis in patients with SS.

MATERIALS AND METHODS

Population. The subject population consisted of 30 patients with primary SS, who were randomly selected by computer-generated numbers from all the patients with SS who had followup visits at the Oral Medicine Clinic at the Stomatological Hospital of Peking University, China. All 30 patients were diagnosed using the revised European classification criteria[11]. Selection criteria included subjects who had not received corticosteroid, antibiotic, or antimycotic therapy over the previous 4 weeks. Thirty sex-matched and age-matched patients with oral candidiasis but without SS or other systemic diseases were studied in parallel for oral *Candida* distribution.

The Ethics Committee of Peking University Health Science Center approved the study.

Oral examinations. The diagnosis of oral candidiasis was based on the clinical presentation (such as loss of papilla of the dorsal tongue, erythema and fissuring of the tongue, erythema of other mucosal surfaces, or angular cheilitis), and then verified by positive potassium hydroxide slide and/or positive culture. Whole unstimulated saliva was collected in a sterilized plastic tube for 5 min. The SFR was recorded as ml/10 min. The oral hygiene, caries, and presence/absence of dentures were also recorded. All oral examinations were performed by the same examiner.

Mycological evaluation. Saliva samples were collected and cultured using an oral rinse technique[12], in which patients swished 10 ml of sterile saline solution without preservative for 30 s, and then spat into a sterile container. Cultures were performed on Sabouraud agar and CHROMagar chromogenic medium (CHROMagar Microbiology, Paris, France). The total colony-forming units per milliliter (CFU/ml) were counted, and colony color was recorded. The *Candida* spp. identification was based on a combination of morphological and biochemical criteria: representative colonies of each morphologic type and color were isolated, routinely Gram-stained, then identified by API 20C kits (bioMérieux, Craponne, France), complemented with germ tube formation, growth at 37°C and 45°C. The results were subsequently interpreted according to the manufacturer's instructions.

Susceptibility testing. Seven antifungal agents (fluconazole, itraconazole, amphotericin B, flucytosine, ketoconazole, miconazole, and terbinafine) were obtained from their manufacturers. Stock solutions of each antifungal agent were prepared and stored at –20°C or lower.

The National Committee for Clinical Laboratory Standards-approved method (NCCLS M27-A)[13] for fungal drug susceptibility was employed, with a broth microdilution using RPMI-1640 medium buffered to pH 7.0 with 3-(N-morpholino) propanesulfonic acid (MOPS), and an inoculum of $0.5–2.4 \times 10^4$ cells/ml. Specifically, 5 colonies of each isolate were selected and placed in the medium with MOPS, and diluted to the desired concentration using spectrophotometric techniques. Serial dilutions of drugs were made from 0.03 to 128 μg/ml, to which the yeast cell inoculum was added and incubated at 35°C for 48 h. Two quality-control standard strains, *C. albicans* ATCC 90028 and *C. krusei* ATCC 6258, were tested in parallel.

RESULTS

Subjects. The ages of the 30 patients with primary SS ranged from 26 to 75 years (mean 48.6 yrs). All were women, all had a history of oral candidiasis, and all had received prior antifungal treatment. At the time of sample collection, they had not received antibiotic, corticosteroid, or antimycotic drugs for at least 4 weeks.

Prevalence of oral candidiasis. Of the 30 patients, 26 (87%) were diagnosed with oral candidiasis, based on the clinical presentation and a positive oral rinse culture. There was a significant inverse relationship between unstimulated salivary flow and candidal infection. In subjects (4/30) with an unstimulated SFR ≥ 1 ml/min, no clinical signs of oral candidiasis were detected, and subsequent oral rinse culture ruled out their infections. Conversely, all patients (26/30) with low SFR < 1 ml/min had detectable oral candidiasis.

Clinical profile. Oral manifestations (Table 1) included erythematous candidiasis of the tongue (17 patients), angular cheilitis (11 patients), denture-related candidiasis (6 patients), and median rhomboid glossitis (3 patients). Although median rhomboid glossitis is a type of erythematous candidiasis, it occurs uniquely in the posterior midline of the dorsum and was separated here from erythematous candidiasis.

Candida spp. distribution. A total of 44 *Candida* isolates were identified from 26 patients. Among the isolates, *C. albicans* was most common, accounting for 55% (24/44) of all isolated species. The next most frequent isolate was *C. tropicalis* (7 strains), followed by *C. glabrata* (5 strains), *C. gulliermodii* (4 strains), *C. krusei* (2 strains), and *C. parapsilosis* (2 strains).

There is a high diversity of *Candida* spp. distribution in this group of the population. In our study, 58% (15/26) of the cases were infected by single species. Co-infection with other *Candida* spp. was found in the rest of the cases (11/26). In the 15 single-species infections, 14 were by *C. albicans* and 1 by *C. gulliermodii*. The rest of the 11 cases were infected by 2 or 3 *Candida* spp. In 2 cases of multiple infections, *C. tropicalis* and *C. glabrata* were each "dominant" in 1 case, although *C. albicans* was "dominant" in unit counts in most multiple species infections. Table 2 shows the distribution and diversity of *Candida* spp. identified from those with single or multiple infections.

In the control group, composed of patients with oral candidiasis but without systemic disease, 83% (25/30) were mono-infected by *C. albicans,* and the remaining 5 cases were co-infected by 2 species (Table 3).

Antifungal susceptibility testing. Although almost all the isolates (98%) were sensitive to flucytosine, this medication is rarely used in our clinic because of its unfavorable adverse effect profile and rapid development of resistance. For the triazoles, fluconazole and itraconazole, which are

Table 1. Signs of Candidiasis on oral examination of subjects with primary Sjögren's syndrome.

Oral Manifestations	No. Subjects
Erythematous candidiasis of the tongue	10
Angular cheilitis	2
Median rhomboid glossitis	2
Denture-related candidiasis	1
Erythematous candidiasis of the tongue + angular cheilitis	5
Denture-related candidiasis + angular cheilitis	3
Erythematous candidiasis of the tongue + denture-related candidiasis	2
Median rhomboid glossitis + angular cheilitis	1

Table 2. *Candida* spp. isolated from 26 patients with Sjögren's syndrome.

Candida spp.	Frequency
C. albicans	14
C. gulliermodii	1
C. albicans + *C. tropicalis*	1
C. albicans + *C. glabrata*	1
C. albicans + *C. gulliermodii*	1
C. tropicalis + *C. glabrata**	1
C. albicans + *C. tropicalis** + *C. glabrata*	1
C. albicans + *C. tropicalis* + *C. glabrata*	1
C. albicans + *C. tropicalis* + *C. gulliermodii*	1
C. albicans + *C. tropicalis* + *C. krusei*	1
C. albicans + *C. gulliermodii* + *C. krusei*	1
C. albicans + *C. glabrata* + *C. parapsilosis*	1
C. albicans + *C. parapsilosis* + *C. tropicalis*	1

* Predominant in mixed *Candida* spp.

Table 3. *Candida* spp. distribution among 30 patients with oral candidiasis who did not have Sjögren's syndrome.

Candida spp.	Frequency
C. albicans	25/30
C. albicans + *C. tropicalis*	1/30
C. albicans + *C. parapsilosis*	1/30
C. albicans + *C. glabrata*	1/30
C. albicans + *G. penicillatum*	1/30
C. albicans + *Saccharomyces cerevisiae*	1/30

Table 4. Susceptibility test of 44 isolated *Candida* spp.

Antifungal Agents	Sensitivity, % (n)	SDD, % (n)	Resistance, % (n)
Flucytosine	97.73 (43)	—	2.37 (1)
Ketoconazole	52.27 (23)	47.73 (21)	0 (0)
Amphotericin B	45.45 (20)	—	54.55 (24)
Miconazole	22.73 (10)	22.73 (10)	54.55 (24)
Fluconazole	47.73 (21)	11.36 (5)	40.91 (18)
Itraconazole	56.82 (25)	15.91 (7)	27.27 (12)
Terbinafine	9.09 (4)	0 (0)	90.91 (40)

SDD: susceptible dose dependent.

Table 5. Sensitivity of 6 *Candida* spp. to azole drugs. Data are frequency/number.

Candida spp.	Fluconazole			Itraconazole		
	S	SDD	R	S	SDD	R
C. albicans	11/24	3/24	10/24	13/24	1/24	7/24
C. tropicalis	4/7	0/7	3/7	4/7	1/7	2/7
C. glabrata	1/5	1/5	3/5	1/5	1/5	3/5
C. gulliermodii	3/4	1/4	0/4	2/4	2/4	0/4
C. krusei	0/2	0/2	2/2	0/2	2/2	0/2
C. parapsilosis	2/2	0/2	0/2	2/2	0/2	0/2

S: sensitivity; SDD: susceptible dose dependent; R: resistant.

first-line medications in the management of oral candidiasis, susceptibility was 48% and 57%, respectively (Table 4). Cross-resistance between itraconazole and fluconazole was rather high, at 67% of all *Candida* isolates. This phenomenon was found in all isolated *Candida* spp. (Table 5).

The minimal inhibitory concentration range for the quality control strains *C. albicans* ATCC 90028 and *C. krusei* ATCC 6258 were within the recommended range at every test occasion.

DISCUSSION

Oral candidiasis is recognized as one of the common, painful, and often chronic complications of SS. It is important that practitioners have a thorough knowledge about the clinical and mycological profile of these infections.

In our study, most of the patients had severe dry mouth symptoms and low SFR. Patients with low SFR were at a higher risk for developing oral candidiasis. Interestingly, of the 4 subjects in our study with flow rates ≥ 1 ml/min, none had clinical signs of oral candidiasis or infections detectable by *Candida* culture. This finding is consistent with other studies that reported an inverse relationship between salivary flow and *Candida* carriage[7], which highlights the critical role that saliva plays as a protective mechanism.

It was reported that oral *Candida* carriage in patients with primary SS, secondary SS, and xerostomia was 81%, 67%, and 71%, respectively[7,8,9,10]. The high prevalence of oral candidiasis in patients with primary SS in our study may be due to the detection method and the population studied. An oral rinse technique (> 300 CFU/ml confirmed the oral candidiasis) was applied in our study, and it is reported to provide a better *Candida* detection sensitivity compared to the traditional culture method, especially for subjects with a low SFR.

As with previous observations[7,8], *C. albicans* was the predominant species, and was isolated from 25/26 patients diagnosed with oral candidiasis. However, only 56% of these patients (14/25) had a mono-*C. albicans* infection. In the remaining cases, multiinfections with either 1 or 2 *Candida* spp. were identified (Table 2).

For patients with SS who had candidiasis, our findings differed from previous observations — a remarkably high proportion (11 out of 26) had multispecies oral *Candida* infections. Although oral candidiasis has been reported with great frequency, reports of multiple *Candida* spp. isolation and distribution in patients with SS are rare[7,8,9,10]. This might be due to conventional isolation and methods used to identify infections, which detect mostly single causative species instead of multiple infections. There are some methods, such as polymerase chain reaction methods, or those that use a differential isolation medium containing chromogenic substrate[14], that are able to detect multiple *Candida* spp. We used CHROMagar *Candida* chromogenic medium because of its ease of use. We found that in Beijing there was a rather high frequency of mixed colonization, with 2 or 3 *Candida* spp. simultaneously. On the other hand,

non-*C. albicans* spp. (*C. tropicalis, C. glabrata, C. parapsilosis, C. krusei,* and *C. gulliermodii*) accounted for slightly under half (20/44) of the isolates.

This phenomenon is clinically relevant, since susceptibility to antifungal medication differs significantly among *Candida* spp. For example, *C. krusei* has a natural resistance to fluconazole, a standard antifungal agent commonly used in the clinic. Another example is *C. glabrata*, which possesses a low-level intrinsic resistance to azoles. These treatments are not effective in 15%–20% of *C. glabrata* infection cases. Therefore, *Candida* spp. other than *C. albicans* warrant more research. Undoubtedly, multiple-species infections will make antifungal treatment far more difficult, requiring increased diagnostic testing. It also provides a possible answer to the frustrating clinical question of why many patients with SS are so resistant to antifungal treatment. Antifungal susceptibility testing prior to prescribing medication is therefore highly recommended. It has been universally accepted that *in vitro* standardized susceptibility testing can provide predictive utility for clinical outcome and a reliable reference for decision-making[15].

In susceptibility testing, 43% of *Candida* isolates were not responsive to fluconazole, a firstline prescription antifungal drug. While some have said that the organism possesses an innate immunity to the drugs, it is more likely that the organism possesses an evolved resistance to the drugs. More importantly, we found a significant cross-resistance between fluconazole and itraconazole. This means that clinically, when 1 triazole antifungal medication has failed in treating *Candida* infection, a switch to another azole agent may also be ineffective. In addition to azoles, polyene antimycotics such as amphotericin B and nystatin are alternatives, because some strains are still highly vulnerable to amphotericin B. When mixed *Candida* infection and diverse susceptible strains are present, a combination of medications might be required to achieve the best effect. We also advise considering non-azoles and topical antifungal agents as part of the therapy, especially when species such as *C. krusei* and *C. glabrata* are involved.

Another finding of our study is a high rate of resistance to triazole agents in *C. albicans*, which is typically susceptible to fluconazole and other azoles. This could be primary or, more likely, secondary resistance. The population group in our study had a recurrent candidal infection history, which is common in patients with SS. Accordingly, the common resistance to fluconazole more likely developed through repeated treatment. There are reports about *C. albicans* resistance to azoles, especially in hosts with the human immunodeficiency virus (HIV) who have undergone repeated courses of antifungal therapy[16,17]. Such repeated treatments make it important clinically to apply drug susceptibility testing (especially to azoles) to patients with SS who have oral candidiasis. We also advise considering non-azole and topical antifungal agents in patients with extensive prior azole exposure, as well as medication to enhance salivation.

Our study supports the requirement for regular stomatologic surveillance of patients with SS, and underlines the clinical importance of identifying mixed *Candida* spp. and their antifungal susceptibility. The detection of multiple *Candida* spp. will lead to early and better antifungal management of oral candidiasis in SS.

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