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Oral status and *Candida* colonization in patients with Sjögren's Syndrome

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Abstract

Objective: To determine the oral status, salivary flow rate, *Candida* carriage in saliva, and prevalence of *Candida albicans* colonization in several areas of the mouth in patients with primary and secondary Sjögren's syndrome as opposed to those of healthy subjects.

Study design: Thirty-seven patients with Sjögren's syndrome (SS), [14 patients with primary SS (SS-1) and 23 patients with secondary SS (SS-2)], along with 37 healthy controls were examined in regard to number of teeth, pro-bing pocket depth (PPD), approximal plaque index (API), bleeding on probing (BOP), presence of prosthetic appliances and smoking habits. Salivary flow rate (SFR), *Candida* carriage in saliva, presence of *Candida albicans* colonization on buccal, angular, palatal and sulcular areas, on dentures and on the tongue's dorsal surface were determined. Statistical analyses were performed using the 2-tailed Fisher exact and Kruskal-Wallis test.

Results: No statistically significant difference was found between SS-1 and SS-2 groups based on the parameters analysed. Statistically significant differences were observed between patients with SS and healthy subjects in terms of SFR, oral signs and symptoms, API, BOP, *C. albicans* colonization on tongue and buccal area, and *Candida* carriage in saliva. In the gingival crevicular fluid positive *C. albicans* colonization was found in only one subject of SS subgroup.

Conclusions: SS patients carry a higher risk of having periodontitis and are more predisposed to develop candidiasis. *C. albicans* is scarcely detected in gingival crevicular fluid despite high scores on *C. albicans* colonization in different areas of the oral cavity in SS patients.

Key words: *Sjögren's syndrome, candida colonization, oral and periodontal status.*

Introduction

Sjögren's syndrome (SS) is a systemic chronic autoimmune disease characterized by severe dryness of the mouth (xerostomia) and eyes (keratoconjunctivitis sicca), along with major salivary gland enlargement caused by lymphoid infiltration (1). SS is classified as secondary (SS-2) when it is associated with other autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and as primary (SS-1) when there is no other connective tissue disease (1, 2). Several epidemiologic studies on SS have shown that the disease affects primarily females (nine fold higher than men) during the fourth and fifth decades of life (3).

Subjective xerostomia has been reported in higher percentages (75.18% to 91.84%) in patients with SS (4). As a consequence, these patients are prone to develop a variety of signs and symtoms such as dental caries, angular chelitis, redness of the tongue, atrophy of filiform papilae, halitosis, difficulties and pain on swallowing and burning syndrome (4). Studies have demonstrated a higher gingival bleeding and plaque index in subjects with hyposalivation but without showing a correlation between salivary flow rate and gingival bleeding index or plaque index resulting from higher risk of having periodontitis (5, 6).

A continuous flow of saliva is important to prevent oral colonization by *Candida*. Various investigators have reported a high prevalence of oral *Candida* species in patients with SS as compared with those of healthy controls (5, 7-11), while others have found that there is no significant difference between patients with SS and healthy controls in terms of presence of candidiasis (8, 12). Most reports indicate that *C. albicans* is the predominant yeast isolated in gingival crevicular fluid and in periodontal pockets of the periodontal patients as well as in healthy subjects, although *Candida glabrata* and *Candida tropicalis* have also been found, albeit infrequently (9, 11, 13).

The aim of this study was to compare the oral status, oral hygiene, smoking habits, salivary flow rate, presence of prosthetic appliances, number of teeth, bleeding on probing, approximal plaque index, probing pocket depth, *Candida* carriage in saliva, and prevalence of *C. albicans* colonization in gingival crevicular fluid, on dentures, on buccal, angular and palatal areas, and on the tongue's dorsal surface of SS-1 and SS-2 patients as opposed to those of healthy subjects.

Study design

Subject population consisted of 37 patients with SS, of whom 14 patients were diagnosed as having primary SS (SS-1) and 23 patients with secondary SS (SS-2), and 37 healthy individuals. SS patients were diagnosed at Istanbul University, Faculty of Medicine, Department of Rheumatology, based on the recently modified internationally agreed-on criteria for Sjögren's syndrome. Oral and periodontal examination and mycological analyses were performed in all groups. Selection criteria included only subjects who did not receive periodontal, antibiotic or antimycotic therapy over the last three months. The study was approved of the Ethic Committee of Istanbul University, Faculty of Medicine.

Oral examination comprised the evaluation the clinical aspects of the lips, the tongue, the floor of the mouth, the gingiva, the cheeks, and the palate, focusing mainly on the signs and symptoms related to SS. Presence of any systemic disease as well as smoking habits were recorded. All oral clinical examinations were performed by the same examiner from the Department of Oral Medicine and Surgery.

Periodontal examination included number of teeth (NT), bleeding on probing (BOP) (expressed as the percentage of sites that bled upon gentle probing), approximal plaque index (API) (expressed as the percentage of sites which presented plaque), probing pocket depth (PPD) (the distance from the crest of the gingival margin to the base of the pocket). BOP, API and PPD were measured at 4 sites of each tooth: buccal, mesial, lingual and distal. All the measurements were performed by the same examiner attendant at the Department of Periodontology using a 0.5 mm-diameter Hu-Friedy periodontal probe with Williams's markings.

Mycological examinations included *Candida* carriage in saliva and prevalence of *C. albicans* colonization on dentures, in gingival crevicular fluid, on the tongue, and on buccal, angular and palatal areas. Samples of buccal, angular, palatal areas, tongue and denture surfaces were taken by a sterile cotton swab, and subgingival plaque samples were taken by sterile paper points. All samples were transferred into a vessel with 1 ml saline solution and mixed 20 seconds for homogenization by a Vortex mixer. Aliquots of 0.01 ml of swab, subgingival plaque and saliva samples were plated onto Sabouroud Agar (Merck, Darmstad, Germany) and incubated at 37°C and 45°C for 48 h. The numbers of the typical colonies of the saliva samples were enumerated and calculated as CFU/ml. *C. albicans* isolates were identified by the germ tube test.

Whole unstimulated saliva was collected in a sterilized plastic tube for 5 minutes. The salivary flow rate (SFR) was recorded as mL/min. Subjects with SS were also divided into two groups: one with a non-pathological level of resting saliva (normal saliva group > 0.1 mL/min) and the other with pathological level (low saliva group: < 0.1 mL/min). Statistical analyses were performed by using the Chi-Square and Kruskall Wallis one-way ANOVA tests. Statistical significance was set at $p < 0.05$.

Results

The age of the patients with SS ranged from 26 to 78 years (mean, 53.27), while the age of the healthy sub-

jects ranged from 25 to 94 years (mean, 54.27). There was no statistical significant difference between the groups in terms of age, gender and smoking habits ($p > 0.05$). Rheumatoid arthritis was the most commonly seen autoimmune disease in secondary SS group (SS-2) observed in 21 (91.30%) patients. Systemic lupus erythmatosus was found in only two patients (8.70%).

Oral examination of the patients with SS revealed no statistically significant difference between SS-1 and SS-2 patients in regard to the oral signs evaluated ($p > 0.05$). Significant differences were observed between patients with SS and healthy subjects in terms of the clinical oral findings associated with SS (Table 1).

There were 10 edentulous patients with SS (three with SS-1 and seven with SS-2) and they all were using total prosthesis, while 12 healthy subjects were using total prosthesis ($p > 0.05$). Dentate patients with SS (11 with SS-1 and 16 with SS-2) had 12.70 ± 1.75 teeth, while dentate healthy subjects had 12.73 ± 1.89 teeth ($p > 0.05$). There was no statistically significant difference between patients with SS and healthy controls regarding to their tooth brushing habits ($p > 0.05$). There was no statistically significant difference between patients with SS-1 and SS-2 regarding their periodontal status ($p > 0.05$); however, as shown in the table 2, a statistically significant difference was observed between patients with SS and healthy subjects in regard to BOP ($p = 0.025$) and API ($p = 0.001$).

The difference between the SS patients and the healthy subjects in terms of SFR was statistically significant ($p = 0.001$). No statistical significant difference was found between the two subgroups of SS patients regarding their mean ustimulated whole salivary flow rate ($p > 0.05$).

The *Candida* counts in saliva was lower than 10^3 CFU/mL in five SS-1 (35.71%), nine SS-2 (39.13%) and 13 healthy subjects (86.66%), between 10^3 and 10^4 CFU/mL in four SS-1 (28.6%), five SS-2 (21.74%) and two healthy subjects (13.33%) and higher than 10^4 CFU/mL in five SS-1 (35.71%) and nine SS-2 (39.13%) subjects. Statistically significant difference was observed between patients with SS and healthy controls in terms of *Candida* carriage in saliva ($p= 0.001$). Despite the considerable differences in the median CFU/ml for each subgroup of SS, these differences were not statistically significant ($p > 0.05$). *C. albicans* colonization in the gingival crevicular fluid was detected in only one tooth of one SS-1 subject. There was no statistically significant difference between SS-1 and SS-2 subjects on the prevalence of *C. albicans* colonization on dentures, in gingival crevicular fluid, on buccal, angular, palatal areas and the tongue ($p > 0.05$). Statistically significant differences were observed between the patients with SS and healthy controls in terms of *C. albican*s colonization on tongue and buccal area (Table 2).

Table 1. Positive objective and subjective signs on oral clinical examination of the patients with SS.

	SS (n=37) N, %,	HS (n=37) N, %,	p
Angular Chelitis	8 (21.62%)	0 (0%)	0.005 (S)
Oral Ulcerations	13 (35.13%)	0 (0%)	0.0001 (S)
Atrophic Mucosa	28 (75.67%)	3 (8.10%)	0.0001 (S)
Dry Mucosa	23 (62.16%)	1 (2.70%)	0.0001 (S)
Reddened Mucosa	23 (62.16%)	5 (13.51%)	0.0001 (S)
Atrophy of Filiform Papilla	18 (48.65%)	4 (10.81%)	0.001 (S)
Xerostomia	32 (86.49%)	5 (13.51%)	0.0001 (S)
Burning Sensation	29 (78.38%)	5 (13.51%)	0.0001 (S)
Pain on Swallowing	23 (62.16%)	4 (10.81%)	0.0001 (S)
Dysgeusia	30 (81.08%)	3 (8.10%)	0.0001 (S)
Hypersensitivity	22 (59.46%)	0 (0%)	0.0001 (S)

SS: Sjögren's syndrome
HS: healthy subjects; N: number; %: percentage; S: statistical significant; NS: no statistical significant

Table 2. The periodontal status and positive *Candida albicans* colonization (PCAC) in different areas of the oral cavity of the patients with SS.

	SS mean ± SEM / N, %,	HS mean ± SEM / N, %,	P
Number of Teeth (NT)	17.73±8.42/n=27	24.26±3.82/n=25	0.07 (NS)
Bleeding on Probing (BOP) (%)	62.29±30.35/n=27	42.96±29.70/n=25	0.025 (S)
Approximal Plaque Index (API) (%)	76.84±26.22/n=27	48.94±27.54/n=25	0.001 (S)
Probing Pocket Depth (PPD) (mm)	1.88±0.41/n=27	1.95±0.63/n=25	0.643 (NS)
(PCAC) on tongue	28 / n=37 75.68%	19 / n=37 51.35%	0.030 (S)
(PCAC) on the buccal area	21 / n=37 56.76%	11 / n=37 29.73%	0.019 (S)
(PCAC) on the palatal mucosa	12 / n=37 32.43%	13 / n=37 35.14%	0.806 (NS)
(PCAC) on the angular area	10 / n=37 27.03%	4 / n=37 10.81%	0.075 (NS)
(PCAC) in gingival crevicular fluid	1/ n=27 3.70%	1 / n=24 4.16%	0.750 (NS)
(PCAC) on dentures	16 / n=20 80.00%	12 /n=22 154.54%	0.195 (NS)

SS: Sjögren's syndrome HS: healthy subjects; N: number; %: percentage; S: statistical significant; NS: no statistical significant

Discussion

For a preventive measure in the oral cavity of subjects with SS, it is important that practitioners have a thorough knowledge about the effect of decreased salivary flow on the oral status. In this study, oral status and *C. albicans* colonization of the patients with SS-1 and SS-2 were compared with those of control healthy subjects.

Concerning the periodontal status, a statistically significant difference was found between patients with SS and healthy controls, which is in agreement with findings reported in other similar studies (5, 6, 14), although there is no consensus in this matter, since other studies showed that SS patients did not present a higher risk for periodontitis compared with that of the general population (4, 7, 14-16).

Xerostomia and objective oral signs were highly prevalent in SS patients compared with those of the control subjects. But both groups, SS and controls, did not present significant difference in terms of tooth-brushing efficiency. Thus, the higher prevalance of peridontitis in SS patients is very likely to be due to their low SFR.

Most of the studies did not find a statistically significant difference between primary and secondary SS patients in terms of their periodontal status (4, 7, 14, 17). Our results are in agreement with these findings. Accordingly, the periodontal status seems not to be affected by the type of SS.

Clinically, *C. albicans* can be cultured from swabs of the buccal mucosa, tongue, teeth, denture surfaces, and dental plaque samples. The flushing effect of saliva and

anti-candidal salivary components such as lysozyme, histatins, lactoferrin, and calprotectin are the innate host defenses which act to remove or kill invading yeasts (8). The decreased salivary flow means the decreased host defense. In our study, candidal colonization on the buccal epithelial and the dorsal tongue was found to be in higher in SS patients than in healthy controls. In colonized individuals with no clinical symptoms of candidiasis, *C. albicans* is most frequently found on the dorsum of the tongue. Although Almståhl & Wikström (9) did not find an increase of frequency of *Candida* in subjects with hyposalivation, those authors did not analyse *Candida* colonization on the tongue's dorsal surface, which is the main ecological niche for *Candida* in the oral cavity.

Denture wearing is one of the major predisposing factors in humans for oral candidiasis. In denture wearers, the fitting surface of the denture is the main reservoir of the yeasts (10). Angular chelitis is commonly associated with denture-induced stomatitis. In our study, no statistically significant difference was found between SS and healthy subjects on the prevalence of *C. albicans* colonization on dentures, palatal and angular areas who use dentures with similar cleaning habits.

As there are limited findings in healthy subjects, yeasts especially *C. albicans* have been recovered from periodontal pockets of patients with chronic periodontitis in different rates (7.1 to19.6%). The gingival crevicular fluid (GCF) is considered a transudate, a passage of fluid from bloodstream (18,19). But it is also known that amount of GCF increases with periodontal disease, which leads us to think that GCF renews itself continuously (18). In our study, SS patients and control subjects showed slight to moderate signs of inflammation. Despite high scores of PCAC in different areas of the mouth in SS-1 and SS-2 patients, PCAC in the GCF was found in only one SS subject (2.7%) of SS-1 subgroup. The very low prevalence of *C. albicans* in the GCF should be related to the continuous flow rate of this area, making the enviroment difficult for fungi colonization. Rhodus and Michalowicz (14) found almost the same result in their pilot study, in which they compared the periodontal status and prevalence of sulcular *C. albicans* between subjects with SS-1 and healthy control subjects.

There were direct correlations between PCAC on buccal area and dry mucosa, hypersensitivity and pain on swallowing with no specific reason. Additionally, a weak correlation between *Candida* carriage in saliva and pain on swallowing was detected. Logemann et al. reported that xerestomia affects the sensory process of swallowing (20). It is well known that positive *Candida* carriage in saliva is mostly the result of the lower levels of salivary flow rate. From this available evidence, it can be assumed that difficulties and pain on swallowing might occur due to positive *Candida* carriage in saliva. But more studies with higher number of patients with SS are needed to confirm or refuse this association.

The limitation of the present study were the reduced number of the patients with SS, which was mostly due to the newly diagnosed SS patients according to the recently modified internationally agreed-on criteria for Sjögren's Syndrome since 2004 and the lack of determining of the number of decayed, missing and filled teeth which is known as DMF-T index.

Conclusion

Patients suffering from SS may experience great impairment in their general health. Regarding oral involvement xerostomia is a predominant finding and causes many oral discomforts, including infection by *Candida albicans* and periodontitis. Oral hygiene should be encouraged for all these patients in addition to palliative measures to alleviate dry mouth.

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