Prevalence and intraoral distribution of *Candida albicans* in Sjögren's syndrome

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SUMMARY An imprint culture technique has been employed to study the prevalence and intraoral distribution of *Candida albicans* in 16 patients with Sjögren's syndrome and in 16 healthy controls matched for age, sex, and dental status. The prevalence and intraoral density of *C. albicans* was found to be significantly higher at almost all sites in the Sjögren's patients than in the controls. The distribution of candida was also altered, being significantly higher in the floor of the mouth and anterior labial sulcus in the Sjögren's group.

There was an approximate inverse relationship between candida populations and rate of salivary flow. Mean candida densities were found to be significantly higher in those Sjögren's patients with detectable serum rheumatoid factor in the serum. However, patients with primary Sjögren's syndrome had significantly higher mean candida densities compared with patients with secondary Sjögren's syndrome.

Sjögren's (1933) syndrome is characterised by xerophthalmia, xerostomia, and, in most cases, an associated connective tissue disorder (Bloch *et al.*, 1965). Chisholm and Mason (1973) state, without supporting data, that oral candida infections are common in Sjögren's syndrome.

Using an imprint culture technique, a normal range for the oral density and distribution of *Candida albicans* has recently been established for healthy subjects (Arendorf and Walker, 1979a). In healthy carriers, there was an upper limit to the density of *C. albicans* found at any site, which was, however, regularly exceeded in candida infections.

In the present investigation, quantitative imprint cultures have been used to assess whether the prevalence or intraoral density and distribution of *C. albicans* is significantly altered in Sjögren's syndrome compared with that of normal subjects. The serum candida fluorescent antibody titres were also determined since Lehner (1966) suggested that this test could also discriminate between the healthy carrier state and oral candidosis.

Method

Sixteen patients with Sjögren's syndrome and 16 healthy controls matched for age, sex, and dental

status were studied, as detailed in Table 1. The diagnosis of Sjögren's syndrome was made when at least two out of three of the following components were present (Bloch *et al.*, 1965); keratoconjunctivitis sicca, xerostomia, or a connective tissue disease. All the patients studied complained of dry eyes and a dry mouth, and 12 out of 16 patients had a connective tissue disease.

Each patient was examined clinically with particular attention to any abnormal oral appearances. All patients underwent a routine haematological investigation (Challacombe *et al.*, 1977) together with a serum ferritin, liver function tests, rheumatoid factor (rheumatoid screening test), and ESR estimation. Ten patients consented to a labial gland biopsy.

The resting and stimulated whole salivary flow rate and stimulated parotid flow rates were measured in each patient. Salivary flow was stimulated by means of an acid drop sweet since most of the patients with Sjögren's syndrome were unable to tolerate application of citric acid. Parotid saliva was collected using a Carlson-Crittenden (1910) cup with an outer diameter of 20 mm and an inner diameter of 10 mm. Using an imprint culture, the prevalence and intraoral distribution of *C. albicans* were determined. Sterile 2.5 cm squares of plastic foam were dipped in Sabouraud's broth and placed for 60 seconds on the various oral mucosal or denture surfaces

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Table 1 Details of patients and normal subjects

Clinical diagnosis	No. of patients	Sex		Age range (yr)	Dentate	Denture wearers	Angular cheilitis	Atrophic glossitis
		M	F					
Sjögren's syndrome	16	2	14	28-81 (Mean 57)	5	11	3	6
Primary	5	0	5	51-81 (Mean 63)	2	3	1	2
Secondary								
Rheumatoid arthritis	10	2	8	28-70 (Mean 57.3)	2	8	2	4
Systemic lupus erythematosus	1	0	1	32	1	0	0	0
Healthy controls	16	2	14	29-79 (Mean 57)	5	11	0	0

listed in the key attached to Figure 1. For the lower denture the foam pad was halved, and the right and left halves of the fitting surfaces of the denture were sampled separately. Each foam pad was then pressed firmly on to a Sabouraud's agar plate containing 10 ml of Actidione¹ (0.4 mg per ml) and 10 ml of Crystamycin (0.15 mg per ml penicillin and 0.25 mg per ml streptomycin) per litre of agar, to

¹Upjohn, Kalamazoo, Michigan, USA.

Fig. 1 Mean candida density at each intraoral site, as determined by imprint culture technique.

prevent contamination. The foam pads were removed after the first 8 hours of the 48 hours of incubation of the plate at 37°C. The candida density at each site was determined using a Gallenkamp colony counter and expressed per unit area sampled. *C. albicans* was distinguished from other species of yeasts by means of serum germ tube and chlamydospore formation (Mackenzie, 1966) and sugar fermentation and assimilation tests (Dolan, 1971). Other yeasts were not identified.

At the initial examination, epithelial smears were taken from each site sampled by the imprint culture for comparison of the sensitivity of the two methods. The smears were stained with PAS and examined for the presence of spores and hyphae.

The fluorescent serum candida antibody titres were measured in the patients with Sjögren's syndrome and in a group of 11 healthy dentate dental hospital staff volunteers who were non-carriers of intraoral *C. albicans*.

Smears of a suspension of *C. albicans* LSH TM3153 containing 6.6×10^7 cells per ml were prepared and incubated with serial dilutions of the patient's serum. After washing, the antibody titre was determined by the indirect technique of Lehner (1966) using a 1 in 10 dilution of a polyvalent anti-human immunoglobulin serum (Wellcome Research Laboratories, Beckenham, Kent, BR3 3SB, UK). Preparations were viewed on the same day by incident ultraviolet light using an American Optical incident fluorescence microscope (Model 2071) with FITC filters. The highest dilution of serum giving a complete ring of green fluorescence around the cells and 'track lines' along the hyphae was taken as the titre of the serum. The antibody titres were expressed as reciprocals of the dilutions. The specificity of the fluorescence was studied by examining the autofluorescence of these cells and by applying saline and negative serum in the indirect technique. As a further control, attempts were made

specifically to inhibit binding of the fluorescent anti-human immunoglobulin serum by prior incubation of the yeast cells with a blocking unlabelled anti-human immunoglobulin serum.

The data were analysed using a Student's *t* test and chi squared test as appropriate.

Results

Table 1 shows the clinical findings in the various subject groups. Three patients had marked bilateral angular cheilitis and six had an atrophic glossitis. All but two patients had clinically dry mouths. Four patients gave a history of previous intraoral or vaginal candida infections. Three of the group had low serum iron concentrations associated with normal total iron binding capacity and serum ferritin levels, and had rheumatoid factor detectable in their serum, an association which has been described previously (Bloch *et al.*, 1965). Nine out of the 16 Sjögren's patients were seropositive for rheumatoid factor. The mean ESR of the patient group was 39 mm/h compared with 3 mm/h for the controls. No haematological abnormality occurred in the control group.

Table 2 shows the frequency of detection of *C. albicans* at various sites in patients with Sjögren's syndrome and in healthy subjects as determined by the imprint culture technique. All five dentate patients with Sjögren's syndrome had a positive imprint culture at one or more sites compared with only 20% of the control group. Sixty-four per cent of the 11 denture-wearing Sjögren's patients had at least one positive imprint culture compared with only 45% of the control group. For the Sjögren's group as a whole, the percentage of subjects with a positive culture at one or more sites was significantly greater (68%) than that of the controls (44%) ($P < 0.05$). The mean frequency of detection of candida at all sites was significantly higher at the

Table 2 Percentage detection of *C. albicans* in patients with Sjögren's syndrome and healthy controls at various sites by imprint culture technique

Site	Dentate		Denture wearers	
	SS (n = 5)	Controls (n = 5)	SS (n = 11)	Controls (n = 11)
Upper denture	—	—	64	36
Lower denture	—	—	55	45
Post tongue	100	0	45	36
Ant palate	100	0	45	36
L cheek	100	0	54	36
R cheek	100	0	45	27
Post palate	100	0	45	36
Ant palate	100	0	45	36
Floor of mouth	100	0	45	18
Ant labial sulcus	100	20	45	27
L commissure	100	20	45	27
R commissure	100	20	45	27

Fig. 2 Relationship between clinical findings and mean candida density in patients with Sjögren's syndrome.

1% level in both dentate and edentulous Sjögren's patients compared with the controls. The candida density was significantly higher ($P < 0.01$) at all sites except for the lower denture in the Sjögren's group compared with that of the normal subjects (Fig. 1). The difference in candida densities in the Sjögren's group, as compared with the controls, was greatest in the floor of the mouth and anterior labial sulcus ($P < 0.001$). Figure 2 shows that patients with atrophic glossitis had a significantly higher density of *C. albicans* on the tongue than other patients in the group ($P < 0.001$). Similarly, a significantly higher density of *C. albicans* was found at the commissures of patients with clinical angular cheilitis compared with the other patients in the group. Two out of the 16 patients with Sjögren's syndrome had negative imprint cultures on one occasion but positive cultures on subsequent examination. Sixty-three per cent of patients had colony counts suggestive of candida infection in at least one site on one or more occasions, that is, counts in excess of 30 colonies/cm² in non-denture wearers and 49 colonies/cm² in denture wearers (Arendorf and Walker, 1979a).

Figure 3 compares the mean candida density and whole stimulated salivary flow rates in Sjögren's patients and controls. The mean candida density appeared to be inversely related to the salivary flow rates, but this is not a straight line or logarithmic

Fig. 3 Relationship between mean candida density (all sites) and whole stimulated salivary flow rates in Sjögren's patients and controls.

relationship (regression coefficient = 0.3). A similar relationship was observed with whole, unstimulated and parotid-stimulated salivary flow rates.

The highest serum fluorescent candida antibody titres occurred in the Sjögren's group but the mean titre of patients with Sjögren's syndrome was not significantly higher than that of the control group (Table 3). There was no apparent relationship between the mean candida densities and the fluorescent serum antibody titres in the patients with Sjögren's syndrome. However, the variability factor was significantly increased in the Sjögren's group ($P < 0.005$).

Figure 4 compares the sensitivity of the imprint culture with the epithelial smear techniques for detecting candida at various sites in Sjögren's

Table 3 Comparison of fluorescent candida antibody titres in serum of 15 Sjögren's patients and 11 healthy controls

Group	Total No.	No. with antibody titres (reciprocal)									
		1	2	4	8	16	32	64	128	256	512
Sjögren's patients	15	4	0	4	1	1	1	0	1	2	1
Healthy subjects	11	4	1	2	1	1	2	0	0	0	0

Fig. 4 Incidence of positive imprint culture and positive epithelial smears for candida at various sites.

patients. In all sites positive findings with imprint cultures were significantly more frequent compared with those in epithelial smears at the 0.1% level. Four of the 16 patients had positive epithelial smears. Hyphae were present in all positive smears but spores were found in only three of the four positive smears.

In Fig. 5, the mean candida density in Sjögren's

Fig. 5 Relationship between the presence of serum rheumatoid factor and mean candida density in Sjögren's patients and healthy controls.

patients with rheumatoid factor detected in their serum is compared with that in seronegative Sjögren's patients. The mean candida density in seropositive patients was significantly higher at the 5% level than in seronegative patients. No correlation was found between the presence of rheumatoid factor in the blood and salivary flow rates. In patients with primary Sjögren's syndrome, the mean candida density was 29.3 ± 31.5 colonies cultured per cm² surface sampled, compared with 14.0 ± 16.2 in patients with secondary Sjögren's syndrome ($P < 0.005$).

Discussion

The increase in carrier rate and in prevalence and density of *C. albicans* at all but one of the oral sites sampled confirms and extends the report of MacFarlane and Mason (1974). Those authors estimated the number of candida organisms in swabs from four intraoral sites in 20 subjects, 10 with Sjögren's syndrome and 10 healthy controls. However, their methods did not permit an estimation of the density of organisms per unit area sampled. Swabs are a less sensitive method of detection of *C. albicans* in the mouth than other methods (Bartels and Blechman, 1962).

The findings that the greatest increase in candida density and prevalence occurred in the floor of the mouth and anterior labial sulcus, where saliva normally pools, is further evidence that oral candida populations are normally limited by an adequate salivary flow. The inverse relationship observed between the mean density of candida colonisation and salivary flow rates is consistent with this hypothesis. As in normal subjects (Arendorf and Walker, 1979b), the tongue, cheeks, and palate were the most frequently and densely colonised oral surfaces in Sjögren's syndrome.

In health, the tongue appears to be the primary oral reservoir for *C. albicans* in that this was the only positive oral site in one-third of normal dentate carriers (Arendorf and Walker, 1979b). Presumably the secondary colonisation of sites such as the floor of the mouth and anterior labial sulcus by candida shed from the tongue is limited in health by the mechanical flushing effect of saliva. Moreover, secretory immunoglobulin IgA, the major antibody of saliva, has the property of inhibiting adherence of microorganisms to oral epithelium (Williams and Gibbons, 1972). Other constituents in saliva may be involved. The histological abnormalities reported in oral epithelium in Sjögren's syndrome by Adams (1973) may be accompanied by a surface which is more susceptible to colonisation by yeasts.

Little information is available on the qualitative

and quantitative changes in microorganisms in the oral flora in Sjögren's syndrome. However, the reciprocal relationship existing between *C. albicans* and lactobacillus in the mouth (Young *et al.*, 1956), to name but one other organism, does suggest that the increase in candida populations in Sjögren's syndrome could equally result from an altered bacterial flora.

Using different sampling methods, Arendorf and Walker (1979b) and Williamson (1972) have found that the carrier or non-carrier state in healthy subjects was a constant one. In this study, two of the patients were found to have candida only intermittently. A normal flow of saliva may therefore be necessary to maintain the carrier or non-carrier state.

The reason why Sjögren's patients with a positive rheumatoid factor appeared to be more susceptible to candida infection is unknown. It may be speculated that rheumatoid factor could interfere with normal cellular or humoral defence mechanisms for eliminating candida from the oral cavity.

Candida infection in Sjögren's patients may have been unrecognised in the past owing to the use of diagnostic techniques such as epithelial smears which we have shown to be particularly unreliable in this condition.

Previously, no more than 30 or 49 colonies of *C. albicans* per cm² have been detected at any time in healthy dentate and denture-wearing subjects, respectively, with a tendency for these limits to be exceeded in oral candidosis. The significant number of patients with Sjögren's syndrome without clinically overt candidosis, in which the density limits have been exceeded, may be interpreted in several ways. Saliva may dilute and reduce the number of organisms removed during sampling of normal oral mucosa. The usefulness of the imprint culture technique in distinguishing between a carrier state and a frank infection may thus be limited in Sjögren's syndrome unless allowance for reduced salivary flow can be made. Alternatively, the large candida populations detected on the oral mucosa in Sjögren's syndrome may amount to a subclinical infection by the yeast. That the discomfort, atrophy, and fissuring of the mucosa and angular cheilitis in Sjögren's syndrome may result from this candida overgrowth is supported by the higher candida densities associated with these mucosal changes in the present series.

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