

Research Article

Yeast-Like Fungal Colonization of the Oral Cavity in Patients with Sjögren's Syndrome

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Abstract

The frequency of yeast-like fungal colonization of the oral mucosa in patients with Sjögren's syndrome constitutes $40.0 \pm 1.63\%$. Fungi are detected on the mucous membrane of the tongue more often ($36.7 \pm 1.61\%$) than on the mucous membrane of the gums ($23.3 \pm 1.41\%$). The extent of tongue mucosa colonization is also higher in comparison with the gum colonization. The extent of *Candida* colonization of tongue mucosa is 2.5 times higher in women with Sjögren's syndrome as compared to men.

The objective of the research was to study the level of candida colonization of various oral mucosa areas in patients with Sjögren's syndrome.

26 fungal cultures were isolated from the examined patients. *Candida albicans* predominated among them constituting 14 strains ($53.8 \pm 1.92\%$). *C. albicans* colonization of the oral mucosa was detected in 10 patients with Sjögren's syndrome ($30.0 \pm 1.53\%$). The extent of tongue mucosa colonization was 3.95 ± 1.32 lg CFU/ml and the extent of gum colonization constituted 3.08 ± 0.60 lg CFU/ml.

Keywords

Sjögren's syndrome; oral mucosa; Candida as a yeast-like fungus

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Problem statement and analysis of the latest research

Yeast-like fungi are minor participants of microbiocenosis in different biotopes of the human body. *Candida* genus includes 186 species. Among them, *Candida albicans* is the most closely related to the human body from an ecological perspective. Different types of candida are found in the oral cavity (on the tongue, palatine, buccal mucosa) of healthy individuals. Additional contributing factors (diabetes mellitus, hormone imbalance, anemia, fasting, saliva flow disorder, prolonged use of antibiotics or corticosteroids, radiation therapy and chemotherapy for tumors in the areas of neck and head, leukemia, HIV infection, AIDS) provide the basis for oral thrush development. Its most common manifes-

tations include oral moniliasis, glossitis, cheilitis. Candida influence on the pathogenesis of prosthetic stomatitis, periodontitis is a serious problem for dental practice.

Sjögren's syndrome is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands, especially salivary and lacrimal glands, and leads to the development of mucous membrane dryness. Primary Sjögren's syndrome clinically manifests only in xerostomia and keratoconjunctivitis sicca. Secondary Sjögren's syndrome develops on the background of other systemic connective tissue diseases, in particular rheumatoid arthritis, systemic lupus erythematosus, primary biliary cirrhosis [7]. Oral manifestations of Sjögren's syndrome include xerostomy, oral mucosa (OM) pain and ulceration, salivary glands enlargement,

dysphagia, dysgeusia, increased liability to dental caries, higher frequency of tooth absence. At the same time, saliva flow disorder in case of Sjögren's syndrome is a contributing factor to the increased yeast-like fungal colonization of the mucosa and oral thrush development [2, 8, 9, 12, 14].

The objective of the research was to study the level of candida colonization of various OM areas in patients with Sjögren's syndrome.

1. Materials and Methods

Thirty patients with Sjögren's syndrome associated with rheumatoid arthritis underwent our observation. Comparison group included 15 apparently healthy individuals.

Microscopic method was applied to search for pseudomycelium and some candida cells in impression smears from different OM areas, namely gums, tongue, cheeks. Impression smears were stained according to the method by Romanovskyi.

Material for culture was selected with a sterile cotton ball from tongue and gum mucosa (in some cases from buccal surface, impression area and angular oral fissures) with an area of 1 cm² separated by a special stencil [1]. The cotton ball was placed in a test tube with 1.0 ml of a sterile normal saline solution where selected mucus was carefully resuspended. Fungal cultures were separated on chromogenic medium ChromID Candida (biomerieux, France) where 0.1 ml of the samples prepared by the above-described method was introduced. Cultures were grown in a thermostat at a temperature of 37°C for 24 hours followed by another 24 hours at room temperature. The number of colonies and their color were assessed on the chromogenic medium as well as the microscopic picture of smears made of them. The final identification of yeast-like fungal cultures was conducted on the basis of 40 biochemical tests with the use of VITEK 2 system using VITEK 2 YST ID card (biomerieux, France).

2. Results and Discussion

Yeast-like fungal cultures were isolated from 12 patients with Sjögren's syndrome (40.0±1.63%).

Mucous membrane colonization of both tongue and gums was not observed in 15 individuals of the comparison group who were apparently healthy and had no pathology of teeth and periodontal diseases. The obtained results indicated that yeast-like fungal colonization of tongue mucosa was detected more often (in 11 cases, 36.7 ± 1.61%) than gum colonization (in 7 patients, 23.3 ± 1.41%, p< 0.01) in the examined patients with Sjögren's syndrome. Moreover, fungal cultures were isolated from both tongue and gums mucous membranes in 6 patients (20.0 ± 1.33%), in 5 cases (16.7 ± 1.24%) only from tongue mucosa and in 1 patient (3.4 ± 0.60%) from gum mucosa. Growth of the fungal flora was not detected in the cultures of some patients isolated from the buccal surface, impression area and angular oral fissures. Determined frequency of candida colonization of tongue and gum mucous membranes in the patients with Sjögren's syndrome was much lower than the results presented in a number of literary sources, namely by 77-83% [3, 8, 13] and even by 92% [9]. Such difference had to be caused by different sensitivity of the applied research methods and the peculiarities of clinical material sampling.

According to our research, tongue mucosa of the patients with Sjögren's syndrome was characterized by more massive yeast-like fungal colonization in comparison with gum mucosa (Table 1). These data are consistent to some extent with the results of Almstahl A. et al. [5]. The scientists found that the maximum amount of yeast-like fungi in patients with Sjögren's syndrome was detected in supragingival calculus, much less amount was observed on the tongue mucosa and the minimum number was noted in the area of gingival sulcus. The extent of candida colonization of tongue mucosa was significantly higher in our research, and that of gum mucosa was comparable to their number in saliva, namely 1672±1455 CFU/ml [4]. At the same time, the determined extent of candida colonization of the tongue mucosa was comparable to its number in supragingival calculus, namely 1.8×10⁶ CFU/g in the patients with the primary Sjögren's syndrome and 0.4×10⁶ CFU/g in the patients with the secondary Sjögren's syndrome [14].

Thus, the differences in the extent of candida

Table 1. Frequency and level of yeast-like fungus colonization of the OM in patients with Sjögren's syndrome

Patients	Total number	Average level of colonization (CFU/ml)	The number of colonized patients	% of colonized patients	Average quantity of fungi in colonized patients
Tongue					
Men	5	20 100	2	40.0±9.80*	50 250
Women	25	45 820	9	36.0±1.92*	127 278
Total	30	41 533	11	36.7±1.61*	113 273
Gums					
Men	5	0	0	0	0
Women	25	720	7	28.0±1.80*	2 571
Total	30	600	7	20.0±1.33*	2 571
Total					
Men	5	20 100	2	40.0±9.80*	50 250
Women	25	46 540	10	40.0±1.96*	116 350
Total	30	42 133	12	40.0±1.63*	105 333

Note. * - the probability of difference from healthy individuals, $p < 0.05$.

colonization of the OM in the patients with Sjögren's syndrome depending on the sex (Table 1) were established. 5 men were examined constituting 17% (this was primarily connected with the peculiarities of rheumatoid arthritis spread). However, common patterns of the frequency of yeast-like fungal culture from the surface of the tongue and gums in men did not differ from female patients with Sjögren's syndrome associated with rheumatoid arthritis. Moreover, the extent of *Candida* colonization of the OM in women was 2.5 times higher ($p < 0.05$).

Yeast-like fungi were separated on chromogenic medium ChromID *Candida* (biomerieux, France) [10, 11]. Details of the chromogenic component of the medium providing staining of the colonies of different *Candida* species into blue, green, pink, purple, gray and white are not disclosed by the manufacturer (CHROMagar Company, France). However, *C. albicans* differentiation is known to be based on the ability of this species fungi to produce β -N-acetylgalactosaminidase which determines chro-

mogenic or fluorogenic changes of hexosaminidase substrates added to the medium. This provides an opportunity to perform preliminary identification of *C. albicans* directly in the primary cultures of the clinical material.

Identification based on 40 biochemical tests using VITEK 2 system with the application of VITEK 2 YST ID card (biomerieux, France) was additionally used for some *Candida* cultures that provided rather high indices of mucous membrane colonization. According to final identification, the color of colonies on ChromID *Candida* agar did not always accurately show the species belonging of cultures (Table 2).

In particular, additional biochemical identification established that not all cultures with blue type colonies belonged to *C. albicans* species. Some cultures were identified as *C. lusitaniae* and *Cryptococcus laurentii*. On the other hand, 3 cultures with white-gray type colonies were identified as *C. albicans*. Therefore, the results of our research

Table 2. Dependence of color of yeast-like fungal colonies from the OM in patients with Sjögren's syndrome on their species belonging (n=20)

	Blue			White-grey			Pink		
	Number of strains	Percentage by species	Percentage by color	Number of strains	Percentage by species	Percentage by color	Number of strains	Percentage by species	Percentage by color
<i>C. albicans</i> (n=10)	7	77.8*	70.0*	3	37.5*	30.0*	0	0	0
<i>C. lusitaniae</i> (n=4)	1	11.1*	25.0*	2	25.0*	50.0*	1	33.3*	25.0*
<i>C. kefyr</i> (n=2)	0	0	0	0	0	0	2	66.7*	100*
<i>C. lipolytica</i> (n=2)	0	0	0	2	25.0*	100*	0	0	0
<i>C. tropicalis</i> (n=1)	0	0	0	1	12.5*	100*	0	0	0
<i>Crypt. laurentii</i> (n=1)	1	11.1*	100*	0	0	0	0	0	0

Note. * - the probability of difference from healthy individuals, $p < 0.05$.

as well as literature data on the possible coloring of colonies of 22 yeast-like fungal species on ChromID Candida chromogenic medium [11] indicated a definite need for a profound biochemical identification of all cultures isolated from clinical material in this medium. *C. albicans* was significantly more prevalent in terms of quantity in the examined patients with Sjögren's syndrome constituting 14 strains ($56.0 \pm 1.98\%$) with an average colonization rate of 3.95 ± 1.32 lg CFU/ml on the tongue mucosa and 3.08 ± 0.60 lg CFU/ml. *C. albicans* colonization of OM was detected in 10 patients of the examined group ($30.0 \pm 1.53\%$ of all examined individuals). This species definitely dominated both on the gums and tongue mucous membranes (Table 3). *C. albicans* is the main and most common development of candidiasis. The majority of yeast-like fungal species (63-92% of strains) detected on the OM of the patients with Sjögren's syndrome by virtually all other groups of researchers pertained to *C. albicans* [3, 8, 13, 15]. N. Ding et al. [6] argued that *C. albicans* predominated in patients with both primary and secondary Sjögren's syndrome.

In addition, on the OM of the patients with Sjögren's syndrome we identified 4 *C. lusitaniae* strains isolated from 3 patients ($16.0 \pm 1.47\%$), 3 *C. kefyr* (*Kluyveromyces marxianus*) strains isolated from 2 patients ($12.0 \pm 1.30\%$), 2 *C. lipolytica* (*Yarrowia lipolytica*) strains isolated from 2 pa-

tients ($8.0 \pm 1.09\%$), 1 *C. tropicalis* ($4.0 \pm 0.78\%$) strain and 2 *Cryptococcus laurentii* strains isolated from different biotopes of 1 patient ($8.0 \pm 1.09\%$).

It should be noted that we detected certain species of yeast-like fungi (in particular, *C. lusitaniae*, *C. lipolytica*, *Cryptococcus laurentii*) on the OM of the patients with Sjögren's syndrome for the first time. Different researchers isolated *C. parapsilosis* (from oral fluid and supragingival calculus) [2, 6, 14], *C. tropicalis* [6, 8, 15] and *C. glabrata* [2, 8, 14, 15] more often (from 10 to 13% of patients) among other candida species (not *C. albicans*). The analysis of various literary sources indicated that only single fungal cultures from patients with Sjögren's syndrome are identified as *C. krusei* [8, 14, 15], *C. guilliermondii* [2, 14, 15], *C. kefyr* (*C. pseudotropicalis*) [2], *C. pelliculosa* [14], *C. dubliniensis* [14], *C. zeylanoides* [9]. However, only *C. glabrata* and *C. kefyr* were associated with symptoms of oral candidiasis [2].

We consider a number of little-known species of yeast-like fungi isolated from the patients with Sjögren's syndrome to deserve more detailed description.

***C. lusitaniae* (*Clavispora lusitaniae* – fertile form).** An increase in the incidence of candidemia induced by this species (in case of leukemia, in newborns) has been observed for the last 20 years. It may also cause septic arthritis, osteomyelitis. The

Table 3. Species variety of yeast-like fungi isolated from patients with Sjögren's syndrome (n=30)

Species	Tongue	Gums	Total	Average level of colonization (CFU/ml)
<i>C. albicans</i>	8 (20.0%†)	6 (20.0%)	14 (30.0%)	3.65±0.17
<i>C. lusitaniae</i>	4 (10.0%)	0	4 (10.0%)	4.06±1.18
<i>C. kefyri</i>	2 (6.7%)	1 (3.3%)	2 (6.7%)	3.51±0.72
<i>C. lipolytica</i>	2 (6.7%)	0	2 (6.7%)	2.70±0.10
<i>C. tropicalis</i>	1 (3.3%)	0	1 (3.3%)	3.00±0.10
<i>Cryptococcus laurentii</i>	1 (3.3%)	0	1 (3.3%)	4.00±0.10
Total number of isolates	18	7		
Number of not colonized patients	19 (63.3%)	23 (76.7%)	18 (60.0%)	
Isolated 1 species	8 (26.7%)	6 (20.0%)	8 (26.7%)	
Isolated associations of species	3 (10.0%)	1 (3.3%)	4 (13.3%)	

Note. † - the percentage of colonized patients.

risk factors for infection are bone marrow transplantation, anticancer chemotherapy. It is usually characterized by high resistance to antimycotic agents, including amphotericin B, imidazoles and triazoles.

The presence of *C. lusitaniae* in the oral fluid of the patient with secondary Sjögren's syndrome was described [14]. During our research, 4 strains of *C. lusitaniae* with a colonization extent of 4.03 ± 0.94 lg CFU/ml were isolated from the tongue mucosa of 3 patients with secondary Sjögren's syndrome ($16.0 \pm 1.47\%$).

***C. kefyri* (*C. Pseudotropicalis* – its synonym; *Kluyveromyces marxianus* – fertile form).** It is usually used in biotechnology as a producer of feed protein and enzymes (ribonuclease in the pharmaceutical industry and lactase). It is often detected in cheese and dairy products.

C. kefyri is a rare causative agent of candidiasis, mainly with skin and nails surface lesions. It occurs in case of newborns' candidiasis. It rarely causes systemic candidiasis (< 1% of all cases of fungemia). However, the research in France (2000-2005) indicated a rather high level of this species colonization of patients in oncohematology units. Attention is drawn to this fungus liability to colonize the intestine. *C. kefyri* is usually characterized by good antimycotics sensitivity. Single *C. kefyri* cultures were isolated from patients

with primary and secondary Sjögren's syndrome. Moreover, this species relation to the symptoms of oral candidiasis was indicated [2]. We isolated 3 *C. kefyri* strains from 2 patients ($12.0 \pm 1.30\%$) with secondary Sjögren's syndrome: namely, from the tongue mucosa with the extent of colonization of 3.50 ± 0.41 lg CFU/ml in 2 patients and additionally from the gums (the extent of colonization of 2.7 lg CFU/ml) in one of those patients.

***C. lipolytica* (*Yarrowia lipolytica* – its fertile form).** It is usually of some interest to biotechnology and the microbiological industry as a producer of lipids, proteo- and lipolytic enzymes. *C. lipolytica* is a very rare causative agent of candidiasis. A hypothesis about the connection of *C. lipolytica* with seborrheic dermatitis development has been made.

We isolated 2 *C. lipolytica* strains from tongue mucosa in 2 patients ($12.0 \pm 1.30\%$) with secondary Sjögren's syndrome with a colonization extent of 2.7 lg CFU/ml. There is no literature data on the presence of this candida species on the OM of the patients with Sjögren's syndrome.

***Cryptococcus laurentii*.** Literary sources described cases of this yeast fungus colonization. It belongs to opportunistic microorganisms of the human ENT organs. The most important risk factors for the development of appropriate opportunistic infections are the weakening of T-cellular component

of the immune system and the presence of invasive devices (venous catheters primarily). Generally, it causes fungemia (associated to leukemia, cancer in premature infants). It may be a causative agent of meningitis in HIV-infected patients, peritonitis in patients undergoing peritoneal dialysis, diarrhea in oncologic patients. Cases of skin infections in children with immune deficiency and after kidney transplantation have been described.

In our research, *Cryptococcus laurentii* was detected on the tongue mucosa of one patient ($8.0 \pm 1.09\%$) with a colonization extent of $4.0 \lg \text{ CFU/ml}$. This species of yeast-like fungus on the OM in patients with Sjögren's syndrome has been described for the first time.

3. Conclusions

1. The frequency of yeast-like fungal colonization of the OM in patients with Sjögren's syndrome constitutes $40.0 \pm 1.63\%$. Fungi are detected on the mucous membrane of the tongue more often ($36.7 \pm 1.61\%$) than on the mucous membrane of the gums ($23.3 \pm 1.41\%$). The extent of tongue mucosa colonization is also higher in comparison with the gum colonization.
2. The extent of *Candida* colonization of tongue mucosa is 2.5 times higher ($p < 0.05$) in women with Sjögren's syndrome as compared to men.
3. *C. albicans* predominated among yeast-like fungal cultures isolated from the examined patients with Sjögren's syndrome and constituted 14 strains ($56.0 \pm 1.98\%$). The frequency of *C. albicans* colonization constituted $30.0 \pm 1.53\%$, the extent of tongue mucosa colonization was $3.95 \pm 1.32 \lg \text{ CFU/ml}$ and the extent of gum colonization constituted $3.08 \pm 0.60 \lg \text{ CFU/ml}$.
4. Some species of yeast-like fungi (*C. lusitanae*, *C. lipolytica*, *Cryptococcus laurentii*) were detected on the OM of the patients with Sjögren's syndrome for the first time.
5. Isolation of yeast-like fungal cultures from the clinical material with the use of ChromID Candida chromogenic medium should

be followed by their further biochemical identification.

Prospects for further research

Further research will focus on the study of yeast-like fungi isolated from the patients with Sjögren's syndrome, the determination of their sensitivity to modern antimycotics, and the development of methods for dental prophylaxis.

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Received: 2018-11-19

Revised: 2019-02-27

Accepted: 2019-03-18