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Studying of biofilm formation by clinical strains of *Candida albicans* in interaction with Fusarium solani to predict the severity of atopic dermatitis

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Abstract

Aim. To assess the ability to form biofilms by clinical strains of the yeast *Candida albicans* isolated from patients with atopic dermatitis in exacerbation and remission stages under the effect of Fusarium solani micromycete and its absence.

Methods. The study included 70 strains of *C. albicans* and one strain of *F. solani*. Fungal biofilms formed according to the method of Ramage. The optical density of the biofilms measured using a micro plate reader at 620 nm. The effect of associated fungi on the biofilm-forming properties of *C. albicans* strains was studied by an extract from opportunistic *F. solani* fungi.

Results. The greatest biofilm formation was observed in strains isolated at the remission stage. The strains isolated in the acute period were inferior to them in the ability to form biofilms (average values of film formation were 0.143 and 0.087, respectively). Co-cultivation of *C. albicans* strains with *F. solani* fungus extract stimulated biofilm formation of *C. albicans* strains at a concentration of 1:10.

Conclusion. This study showed a possible synergism between *C. albicans* and *F. solani* in polymicrobial skin infections, because the products of the fungus *F. solani* increase one of the virulence factors of the fungus *C. albicans*; the possibility to assess of a stimulating effect of associated fungi on the virulence one of an agent of infectious disease process will allow predicting the disease severity.

Keywords: Candida albicans, Fusarium solani, biofilms, atopic dermatitis, symbiosis, microbial associations.

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Background

Recently, atopic dermatitis is a leading skin pathology, especially in industrialized countries. Among allergic diseases, the specific weight of atopic dermatitis reaches more than 60% [1]. This disease is accompanied by a long-term skin inflammatory process, and the development of which is associated with a complex process. Morphological skin changes at the same time provoke the colonization of bacterial and fungal microbiota on the skin, which can lead to the development of infectious complications. Thus, microscopic fungi, often found in immunocompromised patients, make worse the severity of atopic dermatitis because of allergen-specific immunoglobulin E induction and sensitization development [1,2].

Many patients with atopic dermatitis, especially with a long course of the disease, have fungi in microbiological crops, belonging to different groups. The results of a survey of patients who applied to the Mycology Laboratory of the Kazan Research Institute of Epidemiology and Microbiology (KRIEM) revealed that yeast-like fungi are one of the most frequently detected representatives of skin microbiocenosis. According to our research, the frequency of detection of *Candida albicans* fungi in the human body between 2017 and 2019 was approximately 64%. In 45% of cases, different types of mycelial fungi were isolated together with *C. albicans*. Thus, along with dermatomycetes, (*Trichophyton* spp.) mold fungi (*Aspergillus* spp., *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp.,

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Penicillium spp., *etc.*) were also sown. Moreover, this pattern was observed most often against the background of long-term skin lesions [3].

An increasing number of reports in the literature revealed that pathogenicity factors of fungi themselves play a huge role in the pathogenesis of mycotic complications. More than 65% of all infectious diseases are caused by microorganisms existing in the form of biofilms (highly ordered communities of microorganisms within the polysaccharide matrix formed by them) [4]. It is known that *C. albicans* fungi are the main fungal agents that can form biofilms [5–7]. This is a clinically significant ability since the resistance of microbial cells to traditional antifungal drugs increases. Biofilms can resist the host immune defense mechanisms.

The aim of the study. In this regard, our work aimed to determine the ability to form biofilms by clinical strains of *C. albicans* yeast fungi isolated from patients with chronic recurrent atopic dermatitis in the exacerbation and remission stages and the effect of the metabolites of *Fusarium solani* micromycetes on the biofilm formation process.

Material and methods

The objects of the study were strains of *C. albicans* ($n = 70$) and *F. solani* ($n = 1$) isolated from the skin surface of patients with clinical signs of superficial mycotic infection, who are on outpatient treatment, with a chronic relapsing course in the exacerbation and remission stages. The study included patients with chronic recurrent atopic dermatitis who underwent outpatient treatment with a dermatologist. The diagnosis of "atopic dermatitis" was made based on the criteria specified in the Federal Clinical Recommendations for Dermatovenerology in 2015 [8]. The study was approved by the local ethics committee of the Federal Budget Institution of Science of KRIEM of the Russian Agency for Health and Consumer Rights (Report No. 2 of 09-09-2019).

The material for the study was taken using a sterile cotton swab followed by flushing with distilled water along the perimeter of the most recent lesions. Then, the swab was placed in a sterile tube with 2 mL of distilled water. Seeding of the wash was performed on an agarized Saburo sphere in two Petri dishes, and ciprofloxacin was added to one cup in the amount of 50 U/mL of the sphere. We calculated the number of fungal cells in the flush from one swab in 1 mL of distilled water. Mushroom cultures were grown on Saburo sphere at a temperature of 30°C for 2–5 days (for yeast and mycelial forms, accordingly) [9].

Fungi were identified using conventional microscopic and biochemical methods. We used selective chromogenic spheres CandiSelect 4 (Bio-Rad) and commercial test systems based on the auxanogram study of Auxacolor 2 (Bio-Rad).

The formation of fungal biofilms was performed using the method of Ramage et al. [10]. The mushroom culture was sown in a liquid Saburo sphere and incubated in an orbital shaker (180 rpm) at 30°C for 1 day. Then, the culture was precipitated by centrifugation at 3000 rpm for 3 min, washed two times with a sterile phosphate buffer, and resuspended in a liquid Saburo sphere with a final density of 1.0×106 cells/mL. A suspension of cells in the amount of 100 μL was introduced into 96-well flat-bottomed polystyrene micropanels and incubated for 2 days at 37°C. After the formation of the biofilm, the plates were washed three times with a sterile phosphate buffer. The degree or quantity of biofilm formation was evaluated by colorimetric method. An aqueous solution (125 mL) of 1% crystalline violet was added to the wells with the formed biofilms and incubated for 20 min at 37°C. After removing dye excess and washing the wells, 95% ethanol was added in an amount of 125 μL, and the optical density was recorded on a reader with a vertical light beam using a 620 nm light filter [6].

To study the effect of associated fungi on the biofilm-forming properties of *C. albicans* strains, we used an extract from opportunistic fungi *F. solani*, developed by the Laboratory of Mycology of KRIEM in accordance with VFS 42-93-88 standards. The culture of fungi was grown on a liquid Saburo sphere for 5–7 days at a temperature of 30°C. Then, the mycelium was separated from the culture liquid and grounded in a mortar until the mull. After that, the crushed mycelium was mixed with culture liquid and filtered through asbestos filters. The inhibitory or stimulating effect was studied (evaluated) by the method of acute experience [11]. The culture of *C. albicans* cells was sown in a liquid Saburo culture sphere (150 μL) and poured into 96-hole flat-bottomed polystyrene micropanels, which were also filled with 15, 30, or 60 μL of the corresponding extract. The crops were cultivated for 48 h at a temperature of 37°C. Then, the biofilm formation level was determined as described above. As a control, a culture of *C. albicans* cells grown without the addition of extracts was studied.

Statistical analysis of the results processing was performed using the Biostat 4.03 program, and the arithmetic mean (M) and arithmetic mean error (m) were calculated. The reliability of the differences was determined using the Student's confidence index (t), and the differences were considered reliable at $p < 0.05$.

Table 1. Biofilm formation of clinical strains of *C. albicans* isolated for patients with atopic dermatitis with a chronic recurrent course in the exacerbation and remission stages

Localization of a strain	C. *albicans* strains isolated in the acute stage		*C. albicans* strains isolated in the remission stage	
	n	The amount of the formed biofilm (M ± m)	n	The amount of the formed biofilm (M ± m)
The surface of a glabrous skin	35	0.087 ± 0.01 (0.046–0.112)	35	0.143 ± 0.031 (0.1–0.326)

Note. The values of D620 (minus the background) of dye absorption in the wells of the micropanel are given. Differences in values between groups of *C. albicans* strains isolated in the acute and remission stages are significant, $p < 0.05$ ($p = 0.039$).

Results and discussion

The analysis of biofilm formation by *C. albicans* fungi was performed on the formed two groups of strains:

(1) isolated from patients in the acute period, where the number of fungi in microbiological seeding exceeded 10^3 CFU/mL[1];

(2) isolated from patients with chronic relapsing course in remission, and in the absence of expressed clinical signs of fungal damage, the number of fungi in microbiological seeding did not exceed 102 CFU/mL.

The study established the ability of all strains of *C. albicans* fungi to form biofilms. The effectiveness of biofilm formation of clinical strains of *C. albicans* fungi was evaluated by optical density, and the range of values of which varied from 0.046 to 0.326 units. The greatest biofilm formation was observed in strains isolated in the chronic phase of the disease, whereas the strains isolated in the acute period were significantly (1.5 times) inferior. The average values of film formation were 0.143 and 0.087 units, respectively (Table 1). Meanwhile, the maximum values of the biofilm formation in the strains isolated in the chronic and acute phases of the disease reached optical densities of 0.326 and 0.112, respectively.

Low values of biofilm formation of strains isolated in the acute period are probably due to the influence of external conditions, which has a possible effect on the induction of several other pathogenicity factors: a complex of extracellular proteolytic enzymes that can destroy the skin proteins, provide tissue penetration of the macroorganism, and form hyphae [12]. However, it should be noted that in the group of strains isolated in the chronic phase of the disease, five strains have low values of biofilm formation, which may indicate possible morphological changes of cells from yeast-like forms to hyphae, which provides invasion (spread) of the pathogen in the tissue and, as a result, exacerbation of the infectious process.

Joint cultivation of *C. albicans* strains with *F. solani* mushroom extract revealed a stimulating effect on the biofilm-forming properties of *C. albicans* strains. The effect was achieved only when strains were cultivated with a minimum extract volume of 15 μL, and the density of the biofilm increased by 1.5 times compared with that of the biofilm formed in the absence of the extract (Fig. 1). The addition of 60 μL of the extract had a pronounced inhibitory effect on the growth of *C. albicans* strains. However, the introduction of 30 mL of the extract during the cultivation of fungi led to activation of the formation of *C. albicans* tube germination (hyphae formation). Thus, the stimulating effect of *F. solani* mushroom extract on the biofilm-forming characteristics of *C. albicans* strains was observed with a coculturing in a ratio of 1:10.

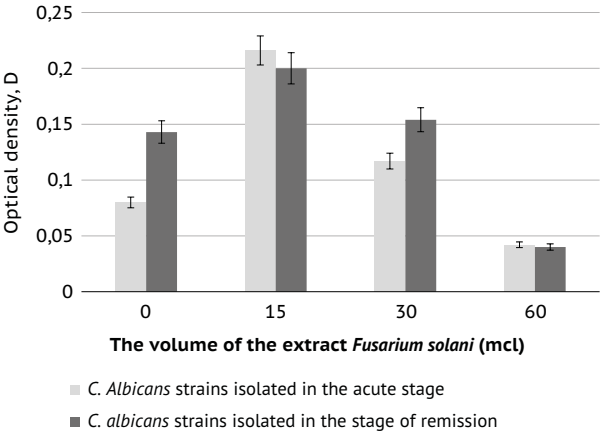

Fig. 1. Optical density of biofilms formed by *Candida albicans* fungi coculturing with Fusarium solani mushroom extract in various volumes

In clinical practice, it should be remembered that a low contamination level of the organism with fungi in chronic forms of the disease may indicate the development of a biofilm structure—one of the main strategies for the survival of a microorganism in the human body. The study of the formation of microbial associations, as well as the mutual influence of microorganisms in associations, will allow us to predict the development of the infectious process.

[1]CFU — colony-forming units.

Conclusions

1. There is a correlation between biofilm formation of strains and the course of cutaneous mycotic disease.

2. A high degree of film formation is typically for the strains isolated from patients with a chronic relapsing course at the remission stage of the disease.

3. It is possible to synergize *C. albicans* and *F. solani* in fungal skin infections. The possibility of the increase of one of the pathogenicity factors of the fungus *C. albicans* under the influence of the life products of the fungus *F. solani* is shown.

Contribution of authors. S.A.L. and R.I.V. conducted the research. A.A.SH., E.B.Ф., and I.M.H. selected the groups of patients and analyzed the results. E.V.H. and G.SH.I. collected and analyzed the results. S.A.L. managed the project.

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Conflict of interest. The authors declare no conflicts of interest.

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