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STUDY OF THE ADHESIVE PROPERTIES OF CANDIDA STRAINS IN AN IN VITRO TEST USING ERYTHROCYTES AS TARGET CELLS

Abstract: The appearance of endogenous toxins in the blood leads to disturbances in the biotransport system of toxins-a decrease in the sorption capacity of erythrocytes, albumin concentration, as well as a decrease in the concentration of total protein and fibrinogen, redistribution of protein and phospholipid fractions.

Keywords: candidiasis, mucous membrane of an oral cavity, erythrocyte, adhesive.

Currently, there is an increase in fungal opportunistic infections, among which a significant part is candidiasis. The wide prevalence and diversity of Candida environment, their resistance, high susceptibility of the population, the possibility of developing persistence and carrier, the polymorphism of clinical manifestations makes candidal infection a very urgent problem of our days. The development of infection depends on the virulence of the candida, the condition of colonized tissue, and the severity of immune responses [1; 3]. At the same time, the question remains about the triggers of candidiasis and the mechanisms of interaction of Sandida with the macroorganism.

The factors of pathogenicity factors that provide adhesion and invasion of C.albicans are fairly well characterized [2; 8; 9].

Research in the system "microorganism – a macroorganism" can help to identify mechanisms of adaptation of pathogens to changing conditions of existence, to solve problems of chronicization and exacerbation of the disease.

Objective of research: To assess the adhesion of Candida fungi to erythrocytes of patients with different clinical course of the disease.

Materials and methods. The venous blood of patients with oral candidiasis served as a material for obtaining red blood cells, as a control, the blood of patients without signs of oral pathology was used.

As an anticoagulant, a 3.8% solution of sodium citrate (1 : 10) or heparin (3.0 U/ml blood) was used. Not later than 24 hours after taking blood, red blood cells were washed three times with a tenfold volume of sterile 0.9% sodium chloride solution (pH 7.2) by centrifugation at 300 g for 10 min, after which it was suspended in the same solution. Tanified erythrocytes were prepared according to the method described by Bondarenko and co-authors (Bondarenko et al., 1987), formalized erythrocytes – according to the method of Kolganova (Kolganova, 2003).

When the possibility of registration of adhesion was revealed, the proposed method used the Candida culture, isolated in patients with candidiasis of mucous membrane of an oral cavity (MMOC). As a comparison, Candida strains isolated from donors were used. The microbial cells were suspended in a sterile 0.9% solution of sodium chloride (pH 7.2). The final concentration of bacteria in the suspension corresponded to 1.0 unit optical density (OD) at a wavelength of transmitted light of 540 nm and the optical path length of the cuvette of 5 mm. To comply with standard conditions at this stage of the experiment, the erythrocytes of only one 0 (I) Rh + blood donor were used. 2.5 ml of a suspension of microbial cells and 1.0 ml of a suspension of erythrocytes in a concentration of 0.1 × 109 /ml were introduced into the tubes. Controls were samples

containing; 2.5 ml of a suspension of microbial cells and 1.0 ml of a 0.9% solution of sodium chloride (pH 7.2) (control sample No. 1); 1.0 ml of erythrocyte suspension and 2.5 ml of 0.9% sodium chloride solution (pH 7.2) (control sample No. 2). Experimental and control samples were incubated at a temperature of 37 ± 1 °C on a rotating platform for 30 minutes, and then centrifuged at 80 g for 1.5 min to precipitate the red blood cells. After this, a supernatant of 2.0 ml was taken from the samples and its OP was measured.

The level of adhesion was calculated using the formula for determining the hydrophobic properties of microbial cells:

$$AI = (D_{к1} + D_{к2} - D_{оп})/D_{к1} \times 100\%,$$

where AI is the adhesion index, Dk1 is the supernatant of the supernatant in control sample No. 1, Dk2 is the supernatant supernatant in control sample No. 2, Dop is the supernatant of the supernatant in the test sample.

The study of the adhesive properties of Candida strains in an in vitro test using erythrocytes as target cells was carried out in two series of experiments:

I. Studying the adhesion of Candida strains to donor erythrocytes.

1.1. Adhesion of Candida strains to donor erythrocytes.

1.2. Adhesion of Candida strains to donor erythrocytes in the presence of blood plasma in patients with candidal stomatitis.

Results and discussion. The study of the adhesive properties of Candida against donated erythrocytes showed a significant increase in the adhesive potential of fungal strains isolated from patients with candidal stomatitis. Adhesive activity of the strains isolated in the control group ranged from 6.52 + 0.22% to 3.02 + 0.12%; while in patients with candidal stomatitis, there was an increase in adhesive activity that was about 8–10 times higher relative to the control group ($P \leq 0.01$). Thus, the values of AI S. albicans. fluctuated within the limits of 45.11 ± 2.01% – 66.60 ± 2.72%; C.tropicalis 35.00 ± ± 1.65% –55.31 ± 2.65%; C.globrata 26.81 ± 1.07% – 45.92 ± ± 2.02%, C.crusei 26.85 ± 1.16% – 43.11 ± 1.59% and C. Gulermonde-27.81 ± 1.21% – 50.82 ± .2.03%. The obtained results, which testify to the expressed ability of Candida to attach to erythrocytes, suggested the presence of the phenomenon of Candida adhesion to red blood cells under macroorganism conditions. It is known that Candida fungi are able to use Hb as a universal source of iron and porphyrins, necessary for the normal life of a microbial cell and for the synthesis of DNA (Kutyreva, MP, et al. 2012). In our opinion, adhesion can be the initial stage of such intercellular interaction, preceding the invasion. Analysis of these experiments showed a sharp increase in the adhesion of the strains studied to the erythrocytes of donors incubated with plasma patients. At the same time, in the control group, on the contrary, a decrease in adhesion was observed. It is obvious that the plasma of patients without candidal lesions does not have inflammatory effectors and other factors determining adhesive candida reactions, so the incubation of donor erythrocytes with plasma of healthy individuals (control group) led to the stabilization of erythrocyte membranes and a decrease in adhesion. Thus, in the AI control group, S. albicans decreased by 50.61%; C.tropicalis at 52.15%; C.globrata – by 48.36%, C.crusei 33.11%% and C. Gulermonde- 52.59%.

In contrast, in patients with candidiasis, MMOC AI increased depending on the nosological form and stage of candidiasis for S. albicans by 30.07% – 44.14%; C.tropicalis at 28.02% – 62.56%; C.globrata – by 28.78% – 90.02%, C.crusei 28.12% – 62.86% and C. Gulermonde – 36.81% – 75.98%. Thus, the blood plasma of patients with candidal stomatitis is able to significantly increase AI candida, realized in systems with erythrocytes. It is shown that the efficacy of Candida adhesion to erythrocytes depends on the pathogenic potential of the fungal cell and the state of the systemic metabolic processes that accompany the development of candidiasis lesions. An increase in the adhesive potential of Candida is the first and mandatory stage in the development of candidiasis. At the same time, many factors can influence the realization of the adhesive potential in the "candida-erythrocyte" system, both from the candidiasis and from the host organism. The latter, in our opinion, can include toxic products of tissue decay caused by the presence of a lesion lesion on the MMOC, which cause a decrease in the structural and functional characteristics of red blood cells.

It is obvious that the accumulation of endogenous toxic substances in the body during the development of candidiasis of CRS can have a damaging effect on cellular structures and their metabolism [3; 7; 9]. A model system for the study of structural and functional characteristics of cells is erythrocytes. Today it is reliably known that red blood cells are involved in the pathological process and undergo serious changes in structure and function in diseases of different genesis. It has been proved that the revealed regularities of the structure and function of the erythrocyte membrane can be extrapolated to other membrane systems [4; 5]. The researchers' interest in erythrocyte in diseases of different genesis is caused by its participation in the processes associated with the maintenance of homeostasis, including immune ones [6]. Existing data leave no doubt that erythrocytes are an important link in the mechanism of immunoregulation in pathological conditions and under stress conditions [3–6].

In this connection, the next stage of the research was the study of the adhesive properties of Candida strains to their own erythrocytes in patients with candidal stomatitis, as well as the influence of donor blood plasma on the adhesive properties of Candida strains of patients with candidal stomatitis in an in vitro experiment.

The maximum adhesive activity was detected when the Candida strains were adhered to their own erythrocytes in patients with candidal stomatitis. Thus, in patients with candidal stomatitis AI S. albicans ranged from 86.00 ± 4.11% to 92.11 ± ± 4.11% increasing to 100.0% with exacerbation of chronic forms of the disease; C. tropicalis, respectively, at 78.41 ± ± 3.14% – 87.81 ± 4.09% and 95.00 ± 3.25%; C. globrata – at 65.30 ± 3.02% – 70.28 ± 3.33% and 90.31 ± 4.25%; C. crusei at 63.42 ± 2.66% – 71.41 ± 3.02% and 88.23 ± 3.81% and C. Gulermonde – 59.42 ± 2.31% – 69.81 ± 2.81% and 92.31 ± ± 4.00% (Table 1).

Table 1. – The indices of adhesion (AI in%) of Candida fungi to donor erythrocytes

№	Type Candida	Control	Antilactoferrin activity ng / ml				
			acute		chronic		
			in 37.00	in 37.01	in 37.02	in 37.03	exacerbation
adhesion to red blood cells of donors							
1.	C.albicans	6.52 ± 0.22*	52.33 ± 2.61·	54.21 ± 2.66·	41.42 ± 2.23·	45.17 ± 2.01·	66.60 ± 2.18·
2.	C.tropicalis	4.41 ± 0.16*	36.92 ± 1.55··	40.32 ± 2.01··	39.42 ± 1.80··	35.07 ± 1.65··	55.31 ± 2.65··
3.	C.globrata	3.22 ± 0.11*	37.52 ± 1.62··	37.11 ± 1.52··	30.82 ± 1.37··	26.81 ± 1.07··	48.92 ± 2.07··
4.	C.crusei	3.02 ± 0.12*	41.61 ± 1.77··	30.25 ± 1.44··	26.85 ± 1.16··	31.22 ± 1.32··	43.11 ± 2.01··
5.	C.gulermonde	4.05 ± 0.18*	38.25 ± 1.61··	93.10 ± 1.37··	30.32 ± 1.32··	27.81 ± 1.21··	50.82 ± 2.43··
adhesion, after the incubation of red blood cells of donors with blood plasma of patients with candidal stomatitis							
1.	C.albicans	3.22 ± 0.12*	68.32 ± 3.21·*	70.51 ± 3.42·*	65.32 ± 3.02·	65.02 ± 3.11·*	88.32 ± 3.65·*
2.	C.tropicalis	2.11 ± 0.009*·	59.25 ± 2.62·*·	60.02 ± 2.59·*·	61.02 ± 2.52·*·	68.11 ± 3.12·*·	70.81 ± 3.44·*·
3.	C.globrata	1.62 ± 0.07*·	48.32 ± 2.11·*·	58.76 ± 2.74·*·	49.03 ± 2.17·*·	51.0 ± 2.51·*·	66.25 ± 3.14·*·
4.	C.crusei	2.02 ± 0.08*·	60.01 ± 2.58·*·	44.21 ± 2.01·*·	40.21 ± 1.68·*·	40.0 ± 1.48·*·	70.21 ± 3.33·*·
5.	C.gulermonde	1.92 ± 0.09*·	52.33 ± 2.60·*·	58.75 ± 2.81·*·	48.26 ± 2.33·*·	40.37 ± 1.51·*·	73.45 ± 3.59·*·

Note: · – P <0.05 in relation to the control

** – P <0,05 with respect to adhesion to donor erythrocytes*

· – P <0,05 in relation to C.albicans

At present, one of the most promising areas in biomedical research is the study of the nature of changes in the surface charge of red blood cells.

Conclusion. An analysis of the adhesiveness of Candida strains to erythrocyte membranes of patients with candidal stomatitis revealed ambiguous relationships between the pathogenicity of strains and the level of their adhesion to host erythrocytes. It is obvious that the accumulation in the organism's environment of diseased products of altered metabolism of exogenous and endogenous origin leads to a disruption in the structure and function of cell membranes, inevitably accompanied by an increase in the adhesion of Candida cells to them and an increase in pathogenic potential. It should be noted that membrane damage determines such functions of red blood cells as the binding and transportation of various compounds, including drugs that enter the body. This may be one of the causes of torpidity of the disease in patients with candidiasis of the oral cavity. The obtained data reveal the pathogenetic basis of the development of endogenous infections in patients with candidal stomatitis of the oral cavity.

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