Method for Evaluating the Effectiveness of Combined Treatment with Immunomodulators in Chronic Dermatologic Diseases

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The aim of the investigation was to determine the significance of the index of natural colonization of epithelial cells for evaluating the effectiveness of treatment and prevention using immunomodulators in patients with microbial eczema and yeast onychomycosis.

Materials and Methods. The level of natural bacterial colonization of buccal epithelial cells was determined in patients with microbial eczema and yeast onychomycosis before and after combined treatment with immunomodulators.

Results. The study showed that the level of natural colonization of epithelial cells increased in pathologic processes. The natural colonization level significantly decreased after combined therapy with Polyoxidonium (in microbial eczema) and Derinat (in onychomycosis), approximating the level in healthy people. Evaluation of changes in natural bacterial colonization on buccal epithelial cells makes it possible to estimate the efficiency of combined treatment with immunomodulators.

Key words: buccal epithelial cells; microbial eczema; onychomycosis; natural colonization; Polyoxidonium; Derinat; soluble adhesion molecules.

A substantial number of human pathologies occur in conditions of immunodeficiency, which determines the severity of clinical manifestations of chronic diseases as well as the development of complications and prognosis deterioration [1]. To achieve sufficient clinical effect in diseases torpid to the conducted standard therapy, immunomodulating medications are often introduced into combined treatment regimen [2–4]. Dermatologic diseases develop due to changes in responsiveness of an organism, they are accompanied by immune factor work impairment and tend to have chronic relapsing course. Therefore, the use of immunomodulators in their management is an important part of combined therapy. However, there is still no algorithm providing the possibility to make an appropriate choice and evaluate the effectiveness of immunomodulators used in combined therapy of various pathological states [5–7].

A simple and noninvasive method for revealing pathological changes in a human body is assessing the level of natural bacterial colonization of buccal epithelial cells [8, 9]. The capacity for adhesive interactions is the most important functional characteristic of epithelial cells and this may serve as a source of important diagnostic and prognostic information [10].

In this work, we have made an attempt to estimate the informative value of the index of natural colonization of epithelial cells while evaluating the effectiveness of combined therapy for chronic pathologies: microbial eczema and yeast onychomycosis using immunomodulatory agents, Polyoxidonium and Derinat. These preparations were chosen due to their wide use in various fields of medicine (pediatrics, gynecology, cardiology, oncology, etc.) [2].

The aim of the investigation was to determine the significance of the index of natural colonization of epithelial cells for evaluating the effectiveness of treatment and prevention using immunomodulators

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in patients with microbial eczema and yeast onychomycosis.

Materials and Methods. The natural colonization of buccal epithelial cells was studied in 52 patients suffering from microbial eczema. Some patients with microbial eczema (n=28) received therapy according to the standard of treatment for this disease. The patients in the group with combined therapy (n=24) additionally received immunomodulator Polyoxidonium (azoximer bromide) (Petrovax Pharm, Russia) 24 mg 2 times a day during 10 days.

The work also involved analysis of buccal epithelial cells obtained from patients with yeast onychomycosis, collected before and after treatment course: after classical treatment with antimycotic agents (n=26) and combined treatment with preparation Derinat (sodium deoxyribonucleate, Technomedservice, Russia) (n=27) intramuscularly, 5 ml of 1.5% solution with the interval of 24–72 h, the course consisting of 5–10 injections. The control group comprised healthy men and women (n=28) aged 21–38 years.

The study complies with the Declaration of Helsinki (adopted in June 1964, Helsinki, Finland and revised in October 2000, Edinburgh, Scotland) and was approved by the Ethics Committee of Nizhny Novgorod State Medical Academy. All the patients gave informed consent to participate in the study.

Buccal epithelial cells were collected from the internal surface of the cheek in the morning while fasting, washed twice (40 g, 5 min) with buffered saline solution, suspension in concentration of 10^6 cells/ml was prepared. Assessment of natural colonization in patients was carried out by counting the total number of bacterial cells adhered onto epithelial cells. Smears were prepared from the suspension of buccal epithelial cells, fixed in methanol (10 min), stained with 0.25% water solution of Azure A (Sigma-Aldrich, USA). The index of natural colonization was measured by the number of bacterial cells per one epithelial cell (bact./ep.). After counting 100 epithelial cells the mean value was taken into consideration (Figure 1).

To evaluate the significance of the index of natural colonization of buccal epithelium in determining the effectiveness of combined therapy, we compared its changes with the changes in soluble intercellular adhesion molecules sICAM-1 and immunoglobulins IgG, IgE. Their levels were analyzed by enzyme immunoassay using spectrophotometer Bio-Rad 480 (Bio-Rad Laboratories, USA). Blood from the median cubital vein was drawn in the morning fasting state in amount of 5 ml, the serum was obtained by centrifugation (1,000 g, 20 min, 4°C). Commercial test systems (Vector-Best, Russia and Bender MedSystems, Austria) were used to determine the levels of immunoglobulins and sICAM-1.

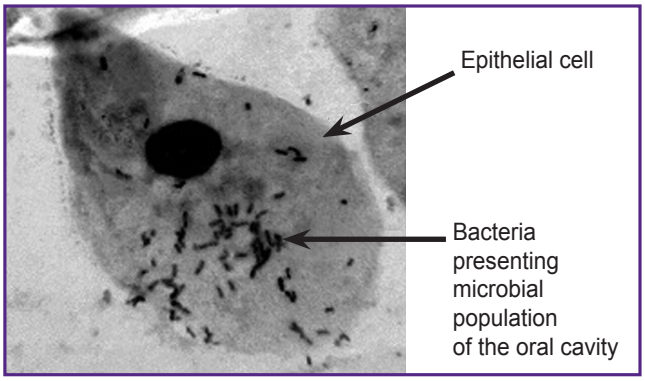

Figure 1. Natural colonization of a buccal epithelial cell; ×900; Azure A staining

Statistical data was processed using standard methods. Mean and standard error of mean (M±m) were calculated. Significance of differences was evaluated using Wilcoxon–Mann–Whitney test. The differences were regarded as statistically significant at $p<0.05$.

Results and Discussion. The study showed that mean values of all selected indices increased in patients with microbial eczema (See the Table). The standard treatment regimen resulted in insignificant reduction of natural colonization index, from 36.79±15.52 to 35.12±15.21 bact./ep. After combined therapy with Polyoxidonium preparation, natural colonization index

Natural colonization of buccal epithelium and serum indices in patients with microbial eczema in different treatment regimens (M±m)

Groups	Adhesion index (bact./ep.)	Serum factors		
		sICAM-1 (ng/ml)	IgG (mg/ml)	IgG (mg/ml)
Healthy volunteers (n=28)	14.31±6.91	377.60±92.78	10.45±0.68	25.53±5.51
Patients with eczema before treatment (n=52)	36.79±15.52↑	514.73±110.57↑	12.92±0.93	42.38±15.74↑
Patients with eczema after standard treatment (n=28)	35.12±15.21	510.54±110.26	12.92±0.89	42.85±15.25
Patients with eczema after combined treatment* (n=24)	26.47±12.03↓	505.61±114.29	12.87±0.56	43.81±16.87

N o t e: * combined treatment with the use of Polyoxidonium preparation; ↑ increase in the index, compared to the healthy group; ↓ decrease in the index during the treatment.

decreased more significantly (p>0.05) and equaled 26.47±12.03 bact./ep. approximating the level in the control group — 14.31±6.91 bact./ep. (Figure 2).

The concentration of serum factors sICAM-1, IgG and IgE in blood serum increased in case of pathology, however, in contrast to natural colonization index, it did not reflect the dynamics of positive changes in the course of disease treatment (See the Table).

The next stage of the study was to evaluate the changes in the level of natural colonization of buccal epithelium against the background of combined treatment for onychomycosis caused by fungi of *Candida* genus, with the use of Derinat preparation.

It was established that the index of natural colonization of buccal epithelial cells in patients with onychomycosis was also significantly higher than in the healthy: 45.32±22.14 bact./ep. vs 14.30±7.89 bact./ep. (Figure 3).

After the course of standard treatment for yeast onychomycosis, natural colonization index was lower and equaled 34.19±17.21 bact./ep. (p>0.05). In case of combined treatment with Derinat preparation, natural colonization index decreased more significantly, up to 23.71±11.0 bact./ep. (p>0.05), approximating that in the control group.

Thus, increase in the level of natural colonization of buccal epithelial cells was observed in both pathologies (See Figures 2, 3). At the same time, the level of microbial colonization of buccal epithelial cells decreased rather fast (within 10–14 days) in case of combined therapy, approximating the values characteristic of healthy people. Besides, when

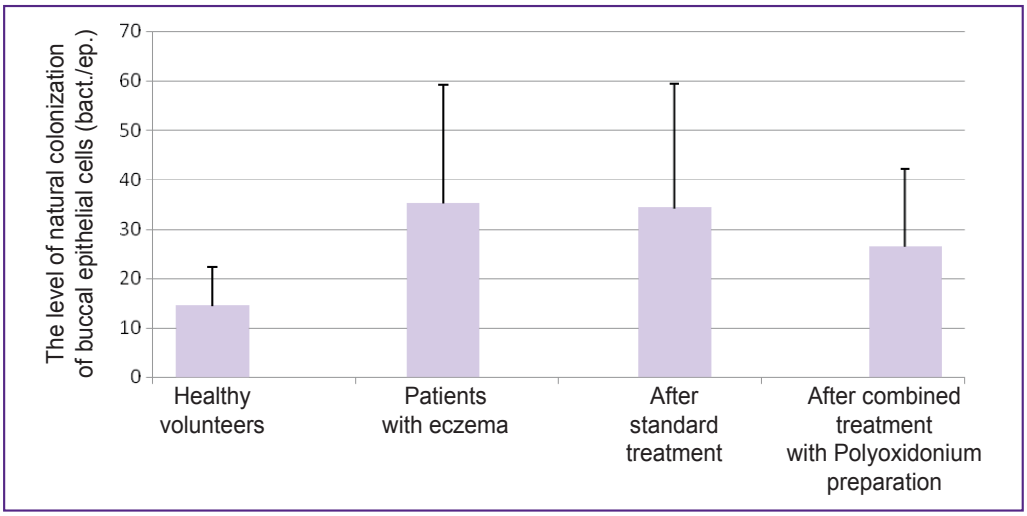

Figure 2. The level of natural colonization of buccal epithelial cells in patients with microbial eczema after treatment with immunomodulator Polyoxidonium

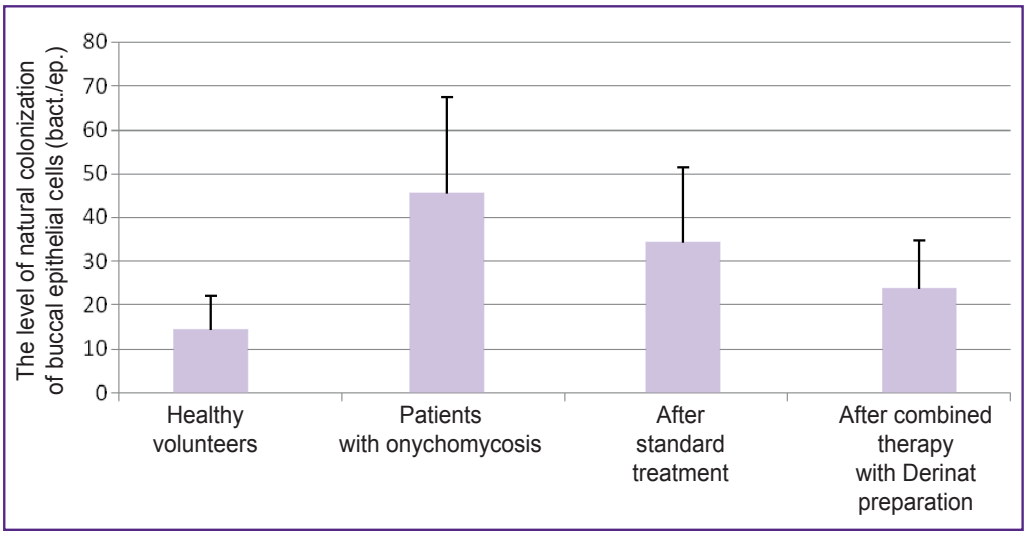

Figure 3. The level of natural colonization of buccal epithelial cells in patients with onychomycosis after treatment with Derinat immunomodulator

conducting combined therapy for microbial eczema, the changes in the level of natural colonization were more pronounced than those of serum indices (sICAM, IgE).

The level of natural colonization of buccal epithelial cells is known to be largely dependent on individual peculiarities of a person, his/her mucosal immunity as well as the presence of associated pathologies, particularly, diseases of the oral cavity (caries etc.) [11]. This explains the fact that changes in the index of natural colonization in a group of patients both during the disease and after treatment manifested themselves as a tendency, with such dynamics being absent in a number of patients. However, return to normal levels of natural colonization of epithelial cells was observed in most patients after treatment with immunomodulators. For example, statistically significant ($p<0.05$) lowering and normalization of natural colonization index were noted in 62.6% of patients with onychomycosis after combined therapy with Derinat preparation. Effectiveness of combined therapy with immunomodulator Polyoxidonium in microbial eczema was even higher: pronounced positive dynamics was observed in 72.3% of patients ($p<0.05$). In a smaller group of patients, the level of natural colonization of buccal epithelial cells did not change significantly after the conducted treatment, which may suggest either the absence of therapeutic effect in the given group of patients or the presence of additional (unidentified) pathology in them. All in all, it can be stated that introduction of immunomodulators into combined therapy for microbial eczema and yeast onychomycosis brings positive results, though it is not a cure-all solution.

Conclusion. Comparing the levels of natural colonization of buccal epithelial cells in patients with chronic dermatologic diseases before and after the conducted course of treatment is an available method providing the possibility to evaluate within a short time the effectiveness of therapeutic intervention, including treatment with immunomodulatory preparations.

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References

1. Sepiashvili R.I. From immunotherapy to personalized targeted immunomodulating therapy and immunorehabilitation. *Allergologiya i immunologiya* 2015; 16(4): 323–328.

2. Khaitov R.M., Pinegin B.V. The mechanism of action and clinical application of immunomodulators. *Allergiya, astma i klinicheskaya immunologiya* 2003; 8: 43–49.

3. Allakhverdieva L.I., Eyubova A.A., Akhmedova G.P. The influence of immunomodifying therapy on the immune characteristics and apoptosis in children suffering atopic bronchial asthma. *Immunologiya* 2011; 32(3): 160–163.

4. Karaulov A.V. *Polioksidoniy v klinicheskoy praktike* [Polyoxidonium in clinical practice]. Moscow: GEOTAR-Media; 2008; 136 p.

5. Bulgakova V.A. The practice of clinical use of azoximer bromide for therapy and prevention of respiratory infections. *Rossiyskiy allergologicheskiy zhurnal* 2014; 3: 73–81.

6. Márquez-González H., López-Gallegos D., González-Espinosa A.M., Zamudio-López J.O., Yáñez-Gutiérrez L. Effect of immune therapy in the prognosis of viral myocarditis in pediatric patients. *Rev Med Inst Mex Seguro Soc* 2016; 54(Suppl 3): S296–S301.

7. Abdrakhimova N.A., Khismatullina Z.R., Mustafina G.R., Nadyrchenko R.M., Gareev E.M. Rol' immunomoduliruyushchey terapii v lechenii mikrobnoy ekzemy. V kn.: *Problemy meditsiny v sovremennykh usloviyakh* [The role of immunomodulating therapy in management of microbial eczema. In: Problems of medicine in the modern context]. Kazan; 2015; p. 31–33.

8. Mayansky A.N., Abadjidi M.A., Mayanskaya I.V., Zaslavskaya M.I., Makhrova T.V. Reactivity of buccal epitheliocytes: indication of local and general impairment in hemostasis (literature survey). *Klinicheskaya laboratornaya diagnostika* 2004; 8: 31–34.

9. Makhrova T.V. The influence of staphylococcal metabolites on adhesive reactions in the system "Candida albicans — buccal epithelial cells". *Zhurnal mikrobiologii, epidemiologii i immunobiologii* 2004; 5: 4–7.

10. Polyakova V.O., Pal'tseva E.M., Krulevskiy V.A. *Bukkal'nyy epiteliy. Novye podkhody k molekulyarnoy diagnostike sotsial'no-znachimoy patologii* [Buccal epithelium. New approaches to molecular diagnosis of socially significant pathology]. Saint Petersburg: N–L; 2015; 128 p.

11. Ratsyuk M.M., Lukinykh L.M., Zelenova E.G. An index of the buccal epithelium natural colonization in a therapeutic stomatology clinic. *Nizhegorodskiy meditsinskiy zhurnal. Stomatologiya* 2003; Suppl: 65–66.