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.

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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, IgЕ. 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.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adhesion index (bact./ep.)</th>
<th>Serum factors</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>sICAM-1 (ng/ml)</td>
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<tr>
<td>Healthy volunteers (n=28)</td>
<td>14.31±6.91</td>
<td>377.60±92.78</td>
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<tr>
<td>Patients with eczema before treatment (n=52)</td>
<td>36.79±15.52*</td>
<td>514.73±110.57↑</td>
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<tr>
<td>Patients with eczema after standard treatment (n=28)</td>
<td>35.12±15.21</td>
<td>510.54±110.26</td>
</tr>
<tr>
<td>Patients with eczema after combined treatment* (n=24)</td>
<td>26.47±12.03↓</td>
<td>505.61±114.29</td>
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</table>

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