EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Structural Peculiarities of Epidermal Barrier in Individuals with Syndrome of Undifferentiated Connective Tissue Dysplasia

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Histological, morphometric, and immunohistochemical analysis showed that the specific features of epidermal barrier structure in patients with undifferentiated connective tissue dysplasia syndrome are increases number of keratinocyte rows in the basal and prickly layer as a result of their higher mitotic activity, increased numerical density of Langerhans cells, and suppression of terminal keratinocyte differentiation.

Key Words: *epidermal barrier; keratinocytes; undifferentiated connective tissue dysplasia*

Normally, well-balanced stages of proliferation and subsequent terminal differentiation of keratinocytes maintain the development of the so-called epidermal barrier, which protects the internal environment of the body from external factors [13]. The epidermis and derma form an integral morphofunctional system, where dermal fibroblasts and epidermal antigen-presenting cells (Langerhans cells) control functional state of keratinocytes by secreting cytokines, primarily, growth factors, while keratinocytes modulate proliferative activity of connective tissue cells in the derma [5,9,12].

It was hypothesized that phenotypic manifestations of undifferentiated connective tissue dysplasia syndrome (CTD) are developed as a result of various defects of the main components of extracellular matrix, primarily collagen fibers, which are characterized by low stability and high degradation rate [4]. This leads to compensatory changes in fibroblast functional state, which can be associated with modulation of keratinocyte differentiation.

Here we studied structural peculiarities of the epidermal barrier in individuals with syndrome of undifferentiated CTD.

MATERIALS AND METHODS

Thu study included 28 female patients of cosmetic clinics (women of 28-40 years, mean age – 32.3±2.9 years), who passed blepharoplasty. Phenotypic signs of CTD (skin hyperelasticity, joint hypermobility, vertebral column and breast deformities, flatfoot, "thumb sign", "wrist sign", microvascular fragility, varicose veins, skin hemangiomas, and telangiectasias) were revealed during routine examination [2-4,11,14]. For evaluation of possible effects of CTD on epidermis structure, the subjects were divided into two age-matched groups: with phenotypic signs of CTD (12 patients, main group) and without these signs (16 patients, reference group). The main group included patients with medium or pronounced CTD with five or more phenotypic signs of the syndrome [6].

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The patients gave informed consent for participation in the study. Patomorphological analysis of eyelid skin removed during surgery was carried out. The material was prepared for light microscopy according to standard protocol [7]. Morphometry of the epidermis and derma was performed [1]: the width and depth of interpapillar processes (μ) were calculated, mitotic activity of keratinocytes was estimated (%), thickness of collagen fibres in the derma (μ) and thickness of the epidermis (μ) were determined; the number of cell rows in the basal prickly and granular layer of the epidermis in the region of interpapillar processes and beyond them was determined. To visualize and investigate Langerhans cells, immunohistochemistry on deparaffinized serial sections was performed with subsequent dehydratation in descending concentrations of ethanol and epitope retrieval using microwave oven. Molecular marker Lag^+ (Novocastra) was detected using Envision detection system (Dako). The sections were incubated with antibodies for 60 min at room temperature, after immunohistichemistry they were poststained with hematoxylin. Lag^+-positive reaction was seen as brown staining of Langerhans cells cytoplasm. Morphometric evaluation of numerical density of Langerhans cells per 1 mm^2 skin was performed [8].

The data were processed using analysis of variance. Significance of differences between the compared mean values was estimated using Student's *t* test. The difference was considered to be significant at $p<0.05$.

RESULTS

In CTD patients, the thickness of collagen fibers in the derma (the parameter reflecting degenerative processes) by 1.3 times surpassed the corresponding parameter in the control group (35.4±2.8 *vs*. 26.4±2.1 μ, respectively), fibres were arranged more regularly with sites of homogenization.

The most pronounced difference in epidermis structure between patients of the two groups were seen in the region of interpapillar processes: the number of keratinocyte rows in CTD patients was 1.5 times higher in the basal layer and 1.7 times higher in the prickly layer, which resulted in the increase of depth (1.2 times) and width (1.5 times) of epidermal interpapillar processes (Figs. 1 and 2) compared to the control group. On the contrary, the number of keratinocyte rows in the granular layer in the region of interpapillar processes in CTD patients was 1.2 times lower than in the control group (Fig. 1). These differences in the epidermis structure in patients of both groups were also noted beyond the regions of interpapillar processes, but they were less pronounced (Fig. 1). The increase in the number of keratinocyte rows in the epidermal basal and prickly layers in the CTD group was a result of a 1.4-fold increase in their mitotic activity (26.7±2.1 and 18.6±1.6% respectively). Signs of parakeratosis were also noted in the epidermis of patients with CTD: in some sites of the epidermis, the horny layer cells containing preserved nuclei, the thickness of the underlying stratum lucidum was significantly reduced, while keratinocyte rows in the prickly layer were violated. There were no signs of parakeratosis in subjects from the control group. Thickness of the epidermis in patients with CTD signs was 1.1 less than in the control group (Fig. 2).

Langerhans cells in the skin of patients of both groups were located between keratinocytes mainly in the lower rows of the epidermal prickly layer and did not form intercellular contacts with them. Cyto-

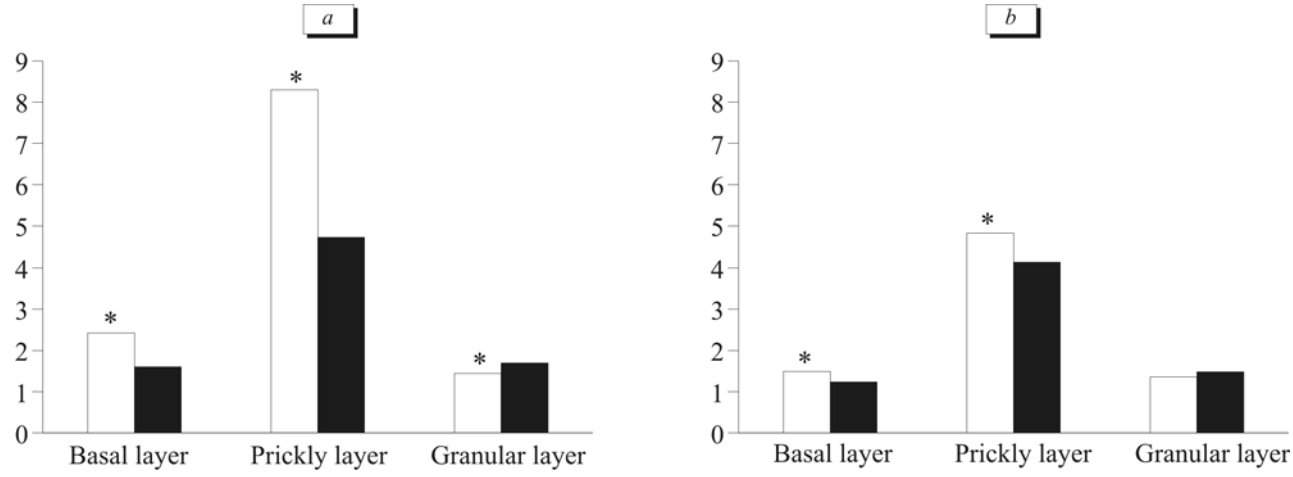

Fig. 1. Number of keratinocyte rows in different epidermal layers in patients with signs of non-differentiated CTD syndrome (light bars) and without these signs (dark bars). *a*) in the region of interpapillar processes, *b*) beyond this region. Here and on Fig. 2: **p*<0.05 compared to patients without CTD manifestations.

plasmic processes of Langerhans cells were mainly laterally directed, some of then ascended vertically up to the granular layer and ended with button-like nodes, single processes in some sections were directed towards the epithelial basal membrane. However, the mean number of Langerhans cells in the CTD group was 1.2 times higher than in the control group (395.57±21.40 and 321.6±18.5 cells per 1 mm^2 of skin, respectively). Moreover, the number of cytoplasmic processes of Langerhans cells was higher in patients with CTD signs than in the control group: 3.10±0.18 and 2.60±0.11 processes per cell, respectively. Higher numerical density of Langerhans cells in patients with CTD signs along with lower epidermis thickness (Fig. 2) can be considered as a compensatory response. On the other hand, it is well known that Langerhans cells can enhance proliferative activity of malpighian layer keratinocytes [5,9,12], and the increase in their density along with other factors can promote the increase in mitotic activity of keratinocytes observed in the study.

Thus, CTD is characterized by enhanced proliferative activity of malpighian layer keratinocytes and suppression of their terminal differentiation, which leads to impairment of keratinization and formation of the epidermal corneal layer and impairs the efficacy of the epidermal barrier. The increase in the number of epitheliocyte rows in gingival stratified epithelium along with the decrease in the size and density of desmosome contacts between cells was previously shown in CTD patients with chronic gingivitis [10]. The observed specific features of surface epithelia in people with CTD seem to be a compensatory adaptive response with unknown mechanisms.

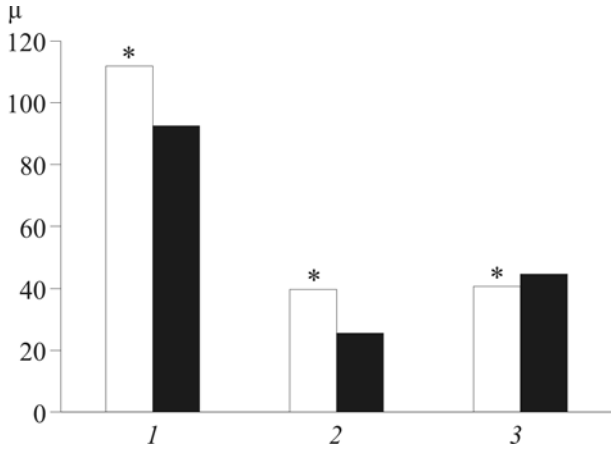

Fig. 2. Morphometry of epidermis in patients with signs of non-differentiated CTD syndrome (light bars) and without these signs (dark bars). *1*) depth of interpapillar processes, *2*) width of interpapillar processes, *3*) thickness of epidermis beyond the region of interpapillar processes.

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