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IMMUNOHISTOCHEMICAL STUDIES OF THE AFFECTED SKIN OF PATIENTS WITH MICROBIAL AND TRUE ECZEMA

Abstract

The aim of the study was to investigate the immunophenotypic composition of inflammatory infiltrating cells in patients with microbial and true skin eczema.

37 biopsy specimens of patients for microbial and true eczema were examined by Immunohistochemistry method. The development of another form of eczema may be due to defects in the focus of inflammation itself. Consequently, it is unable to recruit distinct T-lymphocyte populations to the inflammatory process (CD4+ lymphocytes-for parenchyma and CD 8+ lymphocytes-for microbial eczema). Patients with eczema have also been shown to have decreased reactivity of some T-lymphocytes that circulate in the blood and pass through the focus of inflammation. It adequately responds to the signals of cell mediators of inflammation foci, cannot leave the vascular bed, and outside it participates in the formation of a qualitative effective inflammatory response.

Keywords: immunohistochemistry, CD4+ lymphocytes, CD8+ lymphocytes.

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ИММУНОГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ПОРАЖЁННОЙ КОЖИ У ПАЦИЕНТОВ С МИКРОБНОЙ И ИСТИННОЙ ЭКЗЕМОЙ

Аннотация

Целью исследования явилось изучение иммуннофенотипического состава клеток воспалительного инфильтрата у пациентов с микробной и истинной экземой кожи. Иммуногистохимическим методом были исследованы 37 биопсийных образцов пациентов с микробной и истинной экземой. Развитие той или иной формы экземы может быть обусловлено дефектами непосредственно в очаге воспаления. Вследствие этого очаг воспаления оказывается неспособным рекрутировать различные

популяции Т-лимфоцитов в воспалительный процесс (CD4+ лимфоциты — в паренхиме, CD8+ лимфоциты — при микробной экземе). Также установлено, что у пациентов с экземой снижена реактивность некоторых Т-лимфоцитов, циркулирующих в крови и проходящих через очаг воспаления. Они неадекватно реагируют на сигналы клеточных медиаторов воспаления, не могут покинуть сосудистое русло и за его пределами участвовать в формировании полноценного и эффективного воспалительного ответа.

Ключевые слова: иммуногистохимия, CD4+ лимфоциты, CD8+ лимфоциты.

Introduction

Considering the results of studies conducted on the immunological side of the pathogenesis of eczema, many researchers are inclined to believe that a malfunction of the immune system or its insufficiency is a central link in the pathogenesis of the disease [11], endogenous factors are assumed (disorders of the endocrine, nervous systems, genetic defects, etc.). d.), malfunction of the immune system, affects the development of eczema through the immune system [1].

Modern research leads us to the idea that the skin with its unique immunologic properties, manifested in its ability to locally process both internal and external antigenic signals, is the central point where imbalance or insufficiency of the local immune system is manifested by the development of pathologic processes with characteristic clinical manifestations [2]. At the same time, the ratio of endogenous and exogenous factors in the development of subsequent processes may be different [3].

The study of immunity in eczema by determining the degree of blood cells involvement in the immune process is not a sufficiently complete informative indicator, since the main immune processes are carried out directly

in the skin, and hematologic indices in this case reflect only the general mood of the body immune system [4].

The evaluation of the skin immune state, the most affected organ, should be of great importance in understanding the etiology of the eczema process, the results of which are interesting to compare with similar indicators in the peripheral blood [5]. This approach to the study of the etiology of eczema will allow not only to identify possible conditions for the development of its clinical form variants, but also to determine the direction of corrective therapy of the local immune system disturbed links [7].

The aim of the study was to investigate the immunophenotypic composition of inflammatory infiltrating cells in patients with microbial and true skin eczema.

Material and methods

Immunohistochemistry is a complex multi-step process, the result of which is influenced by a variety of factors, both outside the immunohistochemistry laboratory and within its walls. 37 biopsy specimens of patients for microbial and true eczema were examined by Immunohistochemistry method. Biopsy material was taken after the patient's written consent. The biopsy contained the patient's blood. Monoclonal antibodies CD3+, CD4+, CD8+, Cd1a+ and CD22+ were used for testing.

Quantitative ratios of reactive cells were determined in serial continuous sections. The number of epithelial cells and cells with characteristic expression of marker antigens in the epidermal layer were counted. The change in the quantitative distribution of reactive cells was judged by the change in percentage.

Results and discussion

Based on review of data, it was found that out of 37 cases, 21 (56.8%) patients were diagnosed with true eczema and 16 (43.2%) cases were diagnosed with microbial eczema [10] (Figure 1).

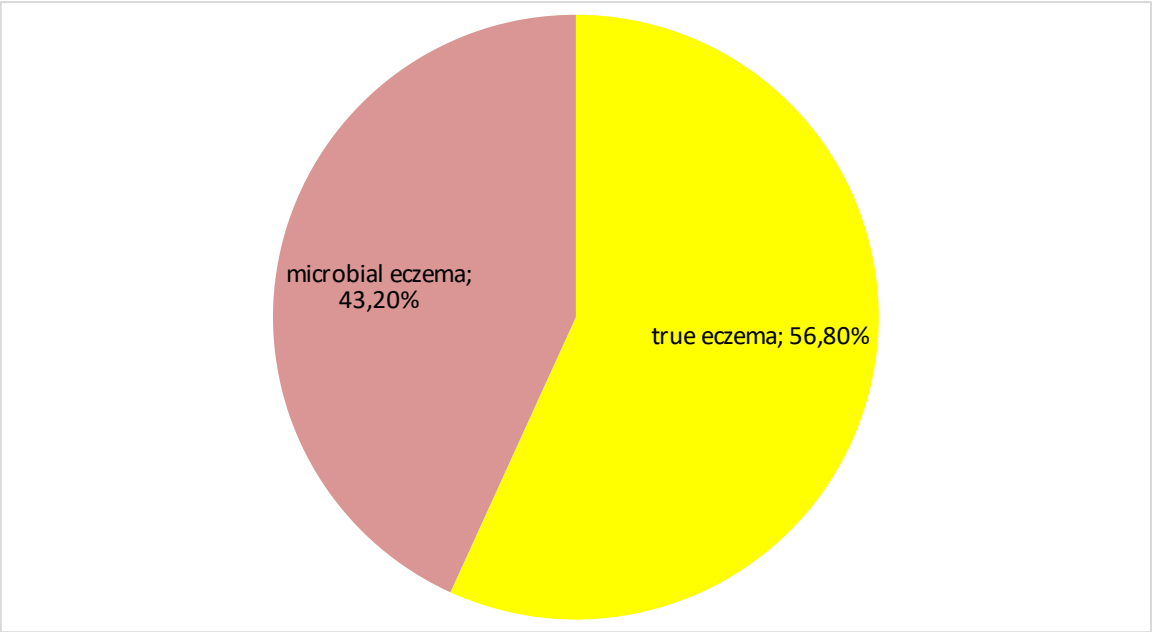

Fig. 1. Ratio of the skin eczema.

37 studied patients were divided by age as follows. In the group with true eczema, the age ratio was: young age (18-44 years) - n=11 (52.4%), middle age (45-59 years) - n=6 (28.6%), elderly age (60-74 years) - n=3 (14.3%), old age (75-90 years) - n=1 (4.8%), age of longevity (over 90 years) - n=0 (0 %). In the group with microbial eczema, these ratios were as follows: young age (18-44 years) - n=7 (43.7%), middle age (45-59 years) - n=4 (25%), old age (60-74 years) - n=2 (12.5%), old age (75-90 years) - n=1 (6.25%), age of longevity (over 90 years) - n=2 (12, 5 %) (Figure 2).

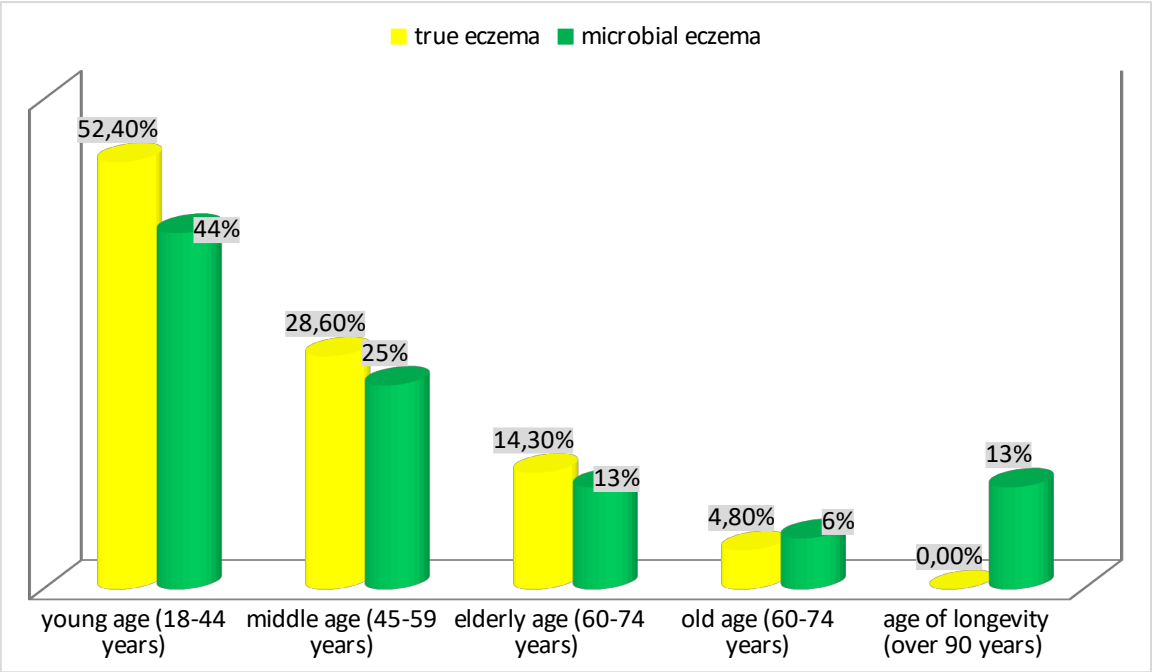

Fig. 2. Skin eczema ratio by age.

The study included 9 (42.9%) men and 12 (57.14%) women with true eczema; and 9 (56.25%) men and 7 (43.75%) women with microbial eczema (Figure 3).

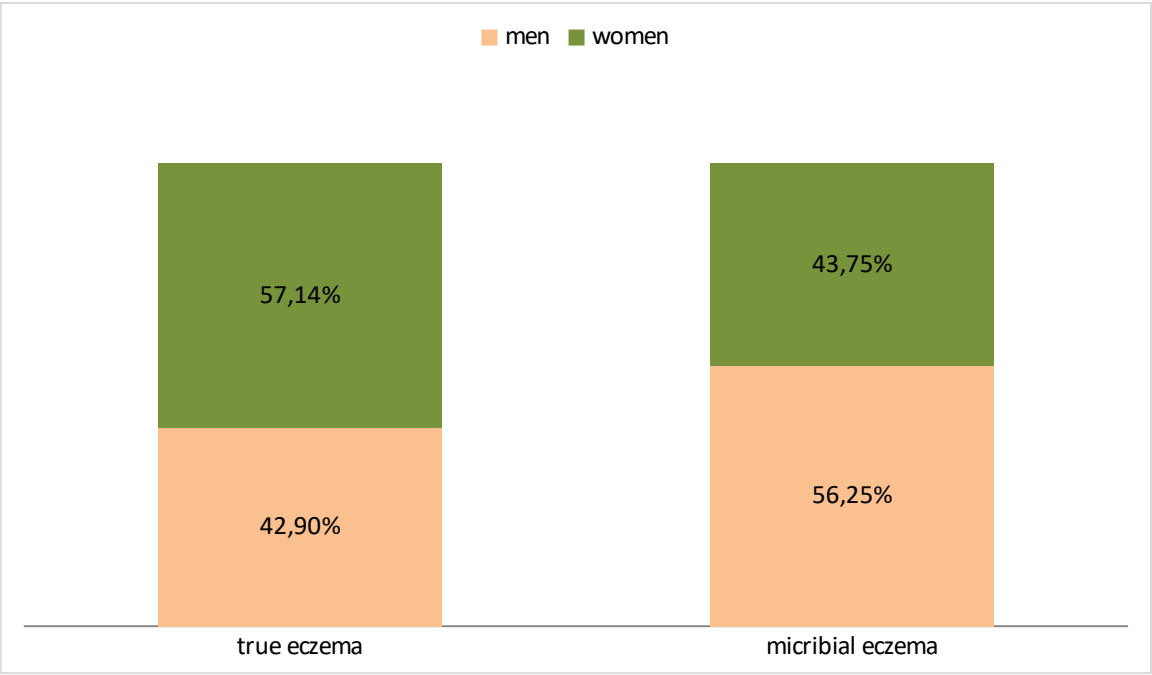

Fig. 3. Skin eczema ratio by gender.

In the dermal component of the affected skin of patients with microbial eczema, histopathological changes are observed in most cases:

- smoothing of the papillae;

- upper dermal edema;

- blood vessel dilation and vessel rupture, emphasized by the formation of a strong perivascular infiltrate of mononuclear cells.

In the epidermis, uneven thickness of the epithelial layer was associated with localized pronounced spongiosis, where infiltration and accumulation of mononuclear cells were observed.

Scattered CD3+ lymphocytes were observed in the papillary and reticular layers of the dermis and were localized mainly at the border with the epidermis; CD3+ lymphocytes were rarely observed in the epidermis, except in spongy lesions, where they tended to infiltrate loose epithelial layers (Tab. 1).

Table 1

Number of inflammatory infiltrate in the dermis distributed in the perivascular area

	(%)
CD3+- lymphocytes	66.1±1.3%
CD4+- lymphocytes	47.1±1.9%
CD8+- lymphocytes	13.1±0.9%
CD4/ CD8	1.4%
CD1a+- lymphocytes	13.5±1,4 %
CD22+- lymphocytes	11.3±0.9%
HLA-DR	51.4±2.9 %

CD4+ lymphocytes were observed as single cells in the reticular layer of the dermis and at the border with the epidermis, CD4+ cells tended to infiltrate

the basal part of the epithelium, especially where there were pronounced trabecular processes. Very rarely, CD4+ lymphocytes were observed in the thickness of the epidermis between epithelial cells.

CD8+ lymphocytes were localized predominantly in the perivascular infiltrate; Like CD4+ lymphocytes, CD8+ lymphocytes tended to migrate to the subepithelial region and invaded the epithelium at the site of trabecular formation. CD8+ lymphocytes were rarely found in the deeper epithelial layers.

Outside the infiltrate, CD1a+ cells tended to accumulate in subepithelial areas, mainly adjacent to trabeculae. This type of CD1a+ cells was characterized by a direct reaction in the cytoplasm rather than in the periphery, with an accentuated oval cell nucleus and a well-defined contour. Dendritic structures were observed in these cells, branching in different directions along the intercellular spaces.

51.4±2.9% of all skin cells from patients with true eczema expressed HLA-DR antigens on the cell surface or in the cytoplasm. Both lymphoid and endothelial cells showed HLA-DR+ expression activity, highlighting the dermal vasculature. Individual HLA-DR+ cells were scattered in the edematous friable stroma and were irregularly shaped. They had a dendritic structure and were characterized by relatively large nuclei.

In the epidermis, CD22+ cells accounted for 17.4±2.4% of cells in the entire epithelial layer. These cells were distributed throughout the epithelial layer, with a tendency to predominate in the basal sections. CD22+ cells (B-lymphocytes) were found in a single number both in the deep dermis and in its reticular section, constituting such a small proportion in the investigated inflammatory infiltrates that they cannot be taken into account.

The formation of inflammatory infiltrates distributed around vessels and vascular slits was observed in the dermal part of the affected skin of patients with microbial eczema.

The following were observed in the epidermis:

- foci of spongiosis;

- expansion of intercellular gaps;

- formation of bubble elements.

CD3+ lymphocytes made up the bulk of the inflammatory infiltrates (73.4±1.2%), which were predominantly distributed around the vessels. Focal accumulations were also observed in the upper dermis, in areas of pronounced spongiosis, into which CD3+ cells tended to invade. In the epidermis, CD3+ cells were found mainly as single cells embedded between keratinocytes. However, in areas of spongiosis and vesicular elements, CD3+ cells were observed in small groups.

CD4+ lymphocytes made up for 46.2±1.8% of inflammatory infiltrates cells. CD4+-lymphocytes were distributed as single cells in the upper dermis, mainly along the border with the epidermis, between the cells of the basal section of the epithelial layer. Cell infiltration was also noted in places of dystrophic changes of the epithelium.

CD8+ lymphocytes constituted a rather small proportion of inflammatory infiltrates (9.03±0.81%.) CD8+ cells were consistently observed in the basal parts of the epidermis. However, they were not noted in its upper parts even under conditions of spongiosis or the formation of vesicular elements. The CD4+/ CD8+- lymphocyte ratio index was noted to be 3.7.

CD1a+ cells made up 7.3±0.4% of dermal cells. The cells had a diverse configuration, characterized by a large nucleus, reactogenic cytoplasm. In the loose dermis, out of connection with the vessel, the cells manifested themselves as cytoplasmic outgrowths, and near the vessels they had a rounded shape.

In the epidermis, CD1a+ cells accounted for 19.3±2.6%. HLA-DR+ antigen was expressed in 65.3±2.6%. HLA-DR+ cells had different morphological properties:

- cells of the lymphocytic type, represented by a nucleus and a scanty rim of cytoplasm;

- cells with signs of macrophages;

- cells that form the lining of blood vessels - endothelial cells.

In the epidermis of patients with microbial eczema, HLA-DR+ cells accounted for 2.7±1.3% and had clear dendritic structures typical for Langerhans cells.

The distribution of cells in the epidermis was uneven. At the sites of spongiosis, simplification of cellular structures was observed in HLA-DR+ cells: the cells had shortened outgrowths and acquired a more rounded shape with a simplified surface. Cells with features of keratinocytes, also exhibiting HLA-DR+ positivity, were clustered around individual HLA-DR+ cells. B-lymphocytes (CD22+) were observed in sporadic numbers in both the upper and lower dermis.

If we compare the number of CD1a+ cells in the dermis in microbial and true eczema, the lower number of CD1a+ cells in microbial eczema is noteworthy. On the contrary, a high level of CD1a-positive cells in the dermal part of the skin may indicate an intensive transit of Langerhans cells from the epidermis to the regional lymph node.

Comparing the data for the different forms of eczema, it should be noted that the number of cells expressing HLA-DR was higher in microbial eczema than in true eczema. The noted changes in the expression of CD1a+ and HLA-DR indicate the intensity of the inflammatory process and reflect the features of each form of eczema.

Analysis of changes in the lymphocytic component of immunity in various forms of eczema shows that each form is characterized by its own individual immunophenotypic profile. First of all, it should be noted that B-lymphocytes practically did not participate in the eczematous process. Their content in inflammatory infiltrates of the dermis was so low that this type of lymphocytes could not be taken into account. This observation confirms the data

of studies, which also indicate the predominant participation of only T-lymphocytes in the formation of inflammatory focus in contact dermatitis.

In this study, it is noteworthy that the accumulation of CD3+-lymphocytes dominated the formation of the inflammatory process. CD3+-lymphocytes were the main cellular component in inflammatory infiltrates of the dermis, and their proportion in the formation of infiltrates was approximately the same in all forms of eczema. The results of this study coincide with the data of studies, which found that in conditions of contact dermatitis 80% of the cellular composition of dermal infiltrates have been formed at the expense of CD3+-lymphocytes. The data according to which 97% of cells isolated from eczematous inflammatory infiltrates were CD3+-lymphocytes are close in significance.

When comparing the groups of patients with microbial and true eczema, it turned out that microbial eczema had its own individual characteristics, which were clearly manifested when comparing the percentages of CD4+ and CD8+ cells. It follows from the analysis that the development of the process of microbial eczema was associated with a decrease in the number of CD8+-lymphocytes, and the formation and development of true eczema with a decrease in the number of CD4+-lymphocytes [9].

Conclusion

The above specificity of each form of eczema can be related to two findings:

It can be assumed that there is a decrease in the reactogenic properties of a certain part of T-lymphocytes circulating in the blood and transiting the site of inflammation and their inability to adequately respond to the signal of cellular mediators of the site of inflammation [8], to leave the vascular bed, beyond which to participate in the formation of qualitatively efficient inflammatory reaction. This defectiveness of a certain subpopulation of T-lymphocytes should manifest itself in a violation of the quality of the inflammatory process, which

acquires the specific features of microbial eczema (defect of CD8+ lymphocytes) or true eczema (defectiveness of CD4+ lymphocytes).

The development of a certain form of eczema may be associated with a defective focus of inflammation itself, which is unable to attract a certain population of T-lymphocytes (CD4+ lymphocytes for true eczema and CD8+ lymphocytes for microbial eczema) to participate in the inflammatory process. In terms of the assumptions made, it is of interest to compare changes in each of the forms of eczema in the ratios of the T-lymphocyte population observed in the skin with changes in the ratios of T-lymphocytes in the blood.

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The article is published for the first time and is part of a scientific work.

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