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THE IMPORTANCE OF PRO-INFLAMMATORY CYTOKINES IN THE IMMUNOPATHOGENESIS OF EPIDERMOLYSIS BULLOSA

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ABSTRACT

Aim: to study the role of some pro-inflammatory immunological markers and the possibility of carrying out specific immunotherapy for EB to improve some clinical manifestations of EB, such as wound healing, reduction of blistering and alleviation of itching. **Materials and Methods of research.** We collected serum samples from 42 patients with EB (19 men and 23 women), consecutively examined and hospitalized in the center, where patients were diagnosed using clinical criteria and basic laboratory signs of the disease and by electron microscopy of skin tissue. In accordance with the cross-sectional study design, serum samples were collected at one time during routine follow-up visits. **Results.** Levels of the cytokines IL-1β, IL-6, IL-2 and TNF-α were analyzed in serum samples from patients with EB. In patients with EB, peripheral serum levels of IL-1β were significantly increased compared to normative values ($p < 0.01$), while IL-6 levels were also significantly increased in the entire group of patients with EB than in the normative group values. An interesting fact was that IL-2 levels were significantly increased in patients with EB compared to normative values, indicating activation of adaptive cellular immunity. The level of TNF-α was significantly reduced in patients with EB than in the group of control normative values. We attempted to look at the correlation between clinical manifestations and proinflammatory cytokine values. A positive correlation of clinical manifestations with the level of IL-6 (r=0.6941) and TNF-alpha (r=0.5503) in the blood serum was revealed. **Conclusions:** Thus, based on the data presented above, it should be noted that the altered levels of cytokines noted in the present study may be a consequence of genetic defects and the formation of blisters on the skin based on these immunopathogenetic mechanisms. Of course, further studies are needed on a larger group of patients to select the most specific research markers, and which will be associated with the characteristics of the clinical course, which will suggest their immunopathogenetic role in the deregulation of the adaptive immune response. And it should be said that looking to the future, our results will support the use of biological agents that target specific cytokine networks. This alternative therapeutic approach may be useful in counteracting both cutaneous manifestations and systemic long-term complications, thereby avoiding life-threatening consequences and improving quality of life.

KEYWORDS: pro-inflammatory cytokines, epidermolysis bullosa.

Relevance. Epidermolysis bullosa (EB) is a rare group of severe inherited defects known as genodermatoses and is characterized by abnormalities of skin adhesion.[1,5,10,12] In particular, the clinical phenotype is characterized by abnormal fragility of the skin and mucous membranes due to disruption of the epidermal junction in response to mechanical trauma, resulting in disfiguring scars, local and systemic infections, syndactyly and esophageal strictures.[2,3,11,12]

Specifically, BE types are identified according to the level of blistering development and also include the simple form with mechanical fragility and blistering limited to the epidermis; nodular BE, consisting of all subtypes with blisters arising within the clear lamina of the dermal epidermal basement membrane; and dystrophic form, including subtypes with blisters arising in the uppermost part of the dermis.[4,5,7,9]

It is known that the simple form occurs mainly due to mutations in the K5 and K14 genes, encoding intermediate keratin filaments of types I and II, while DEB occurs due to mutations in the COL7A1 gene, encoding type VII collagen, the main component of the anchoring fibrils on the dermis. epidermal junction.[6,8,13,14]

Immunological tolerance to self-antigens is the result of central and peripheral tolerance mechanisms. Central tolerance in the thymus results in either negative selection of autoreactive T cells or the development of autospecific suppressor regulatory T cells, both of which require the expression and presentation of autoantigens to developing thymocytes.[11,12,15] In addition, various peripheral tolerance mechanisms protect the body from harmful reactions to its own tissues. These include anatomical sequestration of autoantigens, deletion of peripheral autoreactive lymphocytes, development of functional lymphocyte unresponsiveness (anergy), and the action of regulatory T cells. Over the past decade, there has been a huge number of clinical trials registered to evaluate treatments for primary and secondary pathologies associated with various forms of EB and its clinical manifestations.[5,8,9,13] But many drugs are based on immunopathogenesis, most likely on the cytokine regulation of the immunopathogenesis of EB. In this regard, it is important for us to study the role of some cytokines in the immunopathogenesis of EB.

The active components of drugs usually consist of small molecules or biologics. While small molecules with low molecular weight can be produced chemically and have clear advantages in terms of delivery and route of administration, biologics, which are much larger, often target certain immunopathomechanisms very specifically and generally exhibit less toxicity.[10,13][14,15] Functionally, small molecules are often developed as inhibitors of, for example, enzymes, while biologics usually have a specific active function (eg, antibodies, enzymes, nucleic acids). Based on the above, it follows that work in this direction has not been carried out previously, and the search for drugs in the study of the immunopathogenesis of many clinical manifestations of EB has also not been carried out previously.

This topic corresponds to the priority areas of scientific research carried out in the Republic of Uzbekistan on the topic of orphan diseases, adopted in 2019. on diagnosis, treatment, prevention and rehabilitation of these diseases.

Based on the above, the purpose of the study was to investigate the role of some pro-inflammatory immunological markers and the possibility of carrying out specific immunotherapy for EB to improve some clinical manifestations of EB, such as wound healing, reduction of blistering and alleviation of itching.

Based on the purpose of the study, we set the following tasks, such as conducting research on the values of pro-inflammatory cytokines in EB in order to understand the basic immunological mechanisms that assess the severity and prognosis of the disease, and also based on the identified values, conducting and monitoring the use of the drug Diacerein, which represents is a small molecule that interferes with the expression and signaling of the proinflammatory cytokine IL-1β at various levels.

Materials and methods of research. We collected serum samples from 42 patients with EB (19 men and 23 women), consecutively examined and hospitalized in the center, where patients were diagnosed using clinical criteria and basic laboratory signs of the disease and by electron microscopy of skin tissue. In accordance with the cross-sectional study design, serum samples were collected at one time during routine follow-up visits.

The main objective of the study was to compare the cytokine profiles of patients between differences in clinical presentations in order to be able to identify the role of proinflammatory cytokines involved in the immunopathogenesis of the disease. The secondary aim of our study was to investigate any potential correlation between cytokine levels and clinical features.

The study protocol was reviewed and approved by the Ethics Committee of the Medical University of Bari.[8,10,12,15]

Levels of serum cytokines IL-1β, IL-6, IL-2, and TNF-α were measured by ELISA assay according to the manufacturer's instructions. Patient serum and HCs were added to each microplate well. The sera were incubated for 3 hours at room temperature. After washing to remove any unbound proteins, the substrate tetramethylbenzidine (TMB) was added and incubated for 10 minutes at room temperature. An acid solution was then added to each well to stop the enzymatic reaction and stabilize color development. The value in each sample was obtained by comparing the optical density (OD) of the sample with the OD of the calibrator. For the research, we used a set of test systems produced by Vector-Best, Russia.

Statistical analysis was performed using GraphPad Prism 5 software. Continuous variables are expressed as mean ± standard deviation for normally distributed data. P values <0.05 were considered statistically significant.

Results obtained and discussion. Levels of the cytokines IL-1β, IL-6, IL-2 and TNF-α were analyzed in serum samples from patients with EB. In patients with EB, peripheral serum levels of IL-1β were significantly increased compared to normative values ($p < 0.01$), while IL-6 levels were also significantly increased in the entire group of patients with EB than in the normative group values. An interesting fact was that IL-2 levels were significantly increased in patients with EB compared to normative values, indicating activation of adaptive cellular immunity. The level of TNF-α was significantly reduced in patients with EB than in the group of control normative values. We attempted to look at the correlation between clinical manifestations and proinflammatory cytokine values. A positive correlation of clinical manifestations with the level of IL-6 (r=0.6941) and TNF-alpha (r=0.5503) in the blood serum was revealed.

What was interesting for us was the decrease in serum TNF-α levels in patients with EB compared to normative values. According to previously published literature, reduced levels of TNF-α in patients with EB may play a role in the reduction or impairment of autoimmune activity in EB.

It should be said that our study was aimed at searching for cytokine markers in patients with EB and exploring potential associations between the profiles of major proinflammatory cytokines in peripheral blood serum and disease activity. Thus, we found increased levels of IL-1β and IL-2 in the serum of patients with EB. As well as increased values of IL-6 in patients with EB, and against the background of active clinical manifestations, a significant increase in the value of these cytokines was revealed. TNF-alpha levels were reduced. All this confirms the presence of a systemic lesion with a pronounced inflammatory potential associated with mucocutaneous manifestations in patients with EB, and furthermore, extracutaneous signs suggest a potential multisystem involvement. It should be noted from the literature that IL-1 beta is considered responsible for abnormal fat metabolism, cardiovascular complications, renal sclerosis, weight loss and systemic amyloidosis in the most severe cases of EB. Moreover, from the literature, constitutive activation of IL-1β signaling in keratinocytes of patients with EB is known, which leads to activation of the stress pathway of kinases and overexpression of IL-1β. All this confirms the role of IL-1β in inducing systemic damage in patients with EB.

Regarding IL-6, high levels of IL-6 were found in the blood serum of patients with EB. This cytokine, mainly produced by activated macrophages, monocytes, antigen-presenting cells and lymphocytes, is capable of stimulating the differentiation of Th17 cells and monocytes, as well as inducing the production of acute phase proteins, thereby playing a key role in the switch from innate to adaptive immunity. It is important that the activation of IL-6 was previously identified in patients with EB who complain of more severe clinical manifestations of EB, which confirms our results showing the correlation of IL-6. In this regard, high values of IL-6 in patients with EB may be of a diagnostic and prognostic nature in individuals with severe disease.[7,9,13,14] Thus, we also identified a possible connection with disease activity in patients with severe BE.

Regarding IL-2, it is noteworthy that the present study found that IL-2 was significantly elevated in severe EB patients than in milder EB patients, necessitating further research to better understand the role of IL-2 in such patients. In any case, the different expression of IL-2 in patients with different course of the disease, as well as the higher levels of IL-6 found in also with severe EB, probably reflect different immunopathogenetic mechanisms supported by different genetic and immunophenotypic defects.

It is known from the literature that a significant increase in IL-2 levels was found in patients with EB. In this context, IL-2 has been shown to potently activate the keratin K15 promoter in human epidermal keratinocytes, while IL-2 blockers have been successfully proposed as a possible pharmacological approach for the treatment of BE patients. It is also noteworthy that in patients with EB, IL-2 levels were positively correlated with anti-BP, anti-BP and anti-collagen VII autoantibody titers, indicating its role in immunomodulation and regulation of the adaptive immune response.[4,10,13,15] In addition, titers of autoantibodies to collagen VII and BP were negatively correlated with IL-5, indicating the promoter of CD4 + CD25 + T-regulatory cells that are involved in the suppression of autoimmunity.[6,13]

CONCLUSIONS

Thus, based on the data presented above, it should be noted that the altered levels of cytokines noted in the present study may be a consequence of genetic defects and the formation of blisters on the skin based on these immunopathogenetic mechanisms. Of course, further studies are needed on a larger group of patients to select the most specific research markers, and which will be associated with the characteristics of the clinical course, which will suggest their immunopathogenetic role in the deregulation of the adaptive immune response. And it should be said that looking to the future, our results will support the use of biological agents that target specific cytokine networks. This alternative therapeutic approach may be useful in counteracting both cutaneous manifestations and systemic long-term complications, thereby avoiding life-threatening consequences and improving quality of life. That is, therapeutic approaches using monoclonal antibodies to IL-1 beta may represent the optimal opportunity for low infectious risk and wound healing potential. In conclusion, the imbalance of several pro-inflammatory cytokines highlighted in the present study confirms that EB is a systemic inflammatory disease and not a limited skin disease. Moreover, cytokine studies, especially those cytokines that play an important role in the immune mechanisms of development and relapse of EB, represent a very useful and non-invasive laboratory tool to complement the currently used severity scores aimed at understanding disease activity. Therefore, these cytokines may become potential targets for new biotherapeutics, which requires further research, which may open new avenues for the treatment of moderate to severe forms of EB disease.

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