

Immunobiochemical characteristics of ulcerative skin lesions

Mavlyanova Sh.Z., Obidov S.Z., Khakimov D.R., Zokirov B.X., Sadiev Sh.F.,
Khonkhodjaev Sh. Sh., Parpiev B.R.

Republican Specialized Scientific and Practical Medical Center of Dermatovenerology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan, Tashkent.

KEYWORDS

ulcerative skin lesions, cytokines, procalcitonin, staphylococcus spp.

ABSTRACT

The article presents immuno-biochemical aspects of the pathogenesis of ulcerative skin lesions. 44 patients with UC aged 23 to 64 years were examined. All patients underwent clinical, immunological, biochemical, microbiological and statistical studies. The results of the study showed the failure of the cytokine status in the direction of hyperproduction of pro- (TNF-alpha, IL-6) and hypoproduction of anti-inflammatory cytokines (IL-4, IL-10), an increase in the level of procalcitonin by 2.9 times against the background of increased contamination of opportunistic flora staphylococcus spp. in the lesions.

The pathogenesis of trophic ulcers (TU), regardless of their origin, is based on similar mechanisms caused by disorders of microcirculation, cellular activity, extracellular matrix synthesis (EMS), release of growth factors and neovascularization [1,4,7-11,13]. At the same time, the leading factor in the development of ulcerative lesions is the deterioration of the microcirculatory system (MCS) caused by venous hypertension, which determines the earliest symptoms of chronic venous insufficiency (CVI), including macro and microcirculatory disorders in the venous bed, biophysical, biochemical and immunological processes, as well as impaired lymphatic drainage, affecting the course of reparative processes in the tissues of the legs. [2,3, 5].

However, the presence of immune shifts and the presence of a constant factor that causes and supports inflammation leads to the formation of a continuous cascade of perivascular cellular reactions with a pathological transformation of the substances released during this process [8,9].

In this aspect, it is important to evaluate the immunobiochemical processes in the mechanism of development of trophic ulcers.

The aim of our research was to study the cytokine status in patients with trophic skin ulcers, taking into account the assessment of procalcitonin and the skin microbiome in patients with ulcerative skin lesions.

The material is research methods. We examined 44 patients with ulcerative skin lesions. The age of the patients ranged from 18 to 75 years. Among them, 27 were female and 17 were male. Clinical, functional, histological, immunological, and microbiological studies were performed in all patients. To assess the cytokine link, the state of pro-TNF-alpha, IL-6, and anti-inflammatory IL-4 and IL-6 cytokines in blood serum was studied by ELISA using standard sets of Vector-Best test systems (Novosibirsk). The state of procalcitonin in blood serum by immunofluorescence analysis using a Finecare FIA Meter PluspFS-113 fluorescence analyzer.

Microbiological studies were characterized by back-seeding of skin scales on nutrient media of Saburo, 5% blood agar, endo, Levine to determine myco- and microflora. The control group consisted of 39 healthy individuals of the appropriate age without skin pathology.

Statistical processing of the obtained material was carried out by the method of variation statistics. For statistical calculations, Pentium – 4 PCs, standard (MS Excel – 2000, Statistica 6.0) and specially designed programs were used to ensure the effective use of statistical analysis

methods. The results were considered reliable at $P < 0.05$. Correlation analysis was performed using the Pearson method.

The results of the study. According to the clinical form, among 44 patients with IAP, 13 were diagnosed with trophic ulcer, 15 with ulcerative necrotic vasculitis, and 16 with chronic ulcerative pyoderma. The analysis of the prescription of the disease showed that 7 patients suffered for up to 1 year, 23 for 1-5 years, and 14 patients for more than 5 years.

The results of the ELISA study of cytokine status revealed a failure in the production of pro- and anti-inflammatory cytokines, characterized by an increase in TNF-alpha and IL-6 by 2.3 and 2.4 times compared with the indicators of healthy individuals ($P < 0.05$). (Table 1)

Table 1. Indicators of pro – TNF-alpha, IL-4 and anti-inflammatory cytokines IL-4 and IL-10 in blood serum in patients with ulcerative skin lesions. (pg/ml)

Group	TNF -alpha	IL-6	IL-4	IL-10
Healthy n=39	5,2 $\pm$ 0,03	4,6 $\pm$ 0,8	4,2 $\pm$ 0,08	5,5 $\pm$ 0,13
Patients with trophic ulcer n=44	12,1 $\pm$ 0,3*	11,4 $\pm$ 0,2*	2,01 $\pm$ 0,03*	3,1 $\pm$ 0,2*

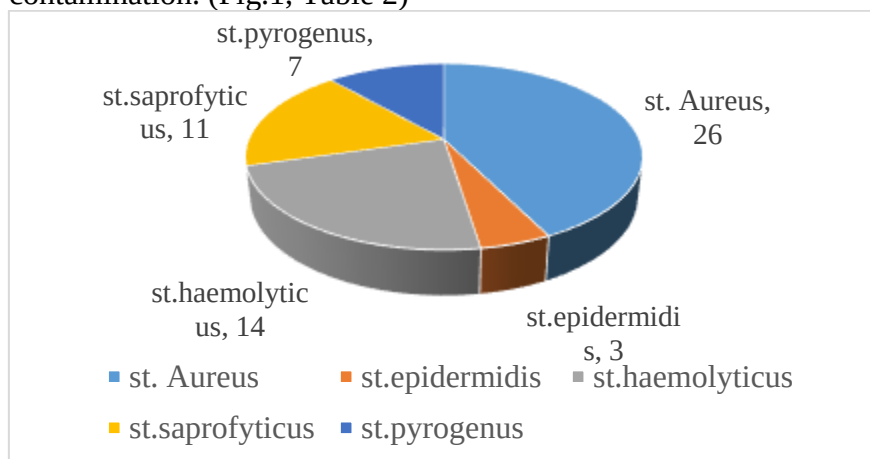
Note: * reliability in relation to the indicators of healthy individuals. ($P < 0.05$)

As follows from the table, the levels of anti-inflammatory cytokines IL-4 and IL-10 were 2.1 and 1.7 times lower than in healthy individuals and averaged 2.01+0.03 pg/ml and 3.1 $\pm$ 0.2 pg/ml, respectively.

The results obtained indicate an imbalance in cytokine status, characterized by uncontrolled hyperproduction of proinflammatory cytokines TNF-alpha and IL-6 and a decrease in the functional activity of anti-inflammatory cytokines IL-4 and IL-10.

In our opinion, an increase in the pro-inflammatory cytokine TNF-alpha and IL-6 is associated with a response to microbial components released from microorganisms - endotoxin or lipopolysaccharide (LPS). By affecting the endothelium of blood vessels, they participate in the migration of leukocytes into tissues, which is accompanied by infiltrative damage to the tissue surface.

Thus, the results of microbiological studies of skin lesions in patients revealed an increased detectability of pathogenic forms of opportunistic microorganisms staphylococcus spp. with high contamination. (Fig.1, Table 2)



As follows from the figure, among the pathogenic forms, St.aureus was most often isolated in 26 (59.1%), St.haemolyticus in 14 (31.8%), and St.saprophyticus in 11 (25%). Whereas among 36 healthy controls, only 4 were isolated from St.epidermidis, which accounted for 11.1% of cases. Moreover, the colonization rate of staphylococcus spp. the average was 76.9+1.3 CFU/g.

Table 2. Colonization rate of staphylococcus spp. on the skin of lesions in patients with TU (CFU/g)

	staphylococcus spp. (CFU/g)
Patients with trophic ulcer n=44	76,9 $\pm$ 1,3
Healthy controls n= 36	up to 8

Note: * is a confidence indicator in relation to the control group of healthy individuals (P <0.05)

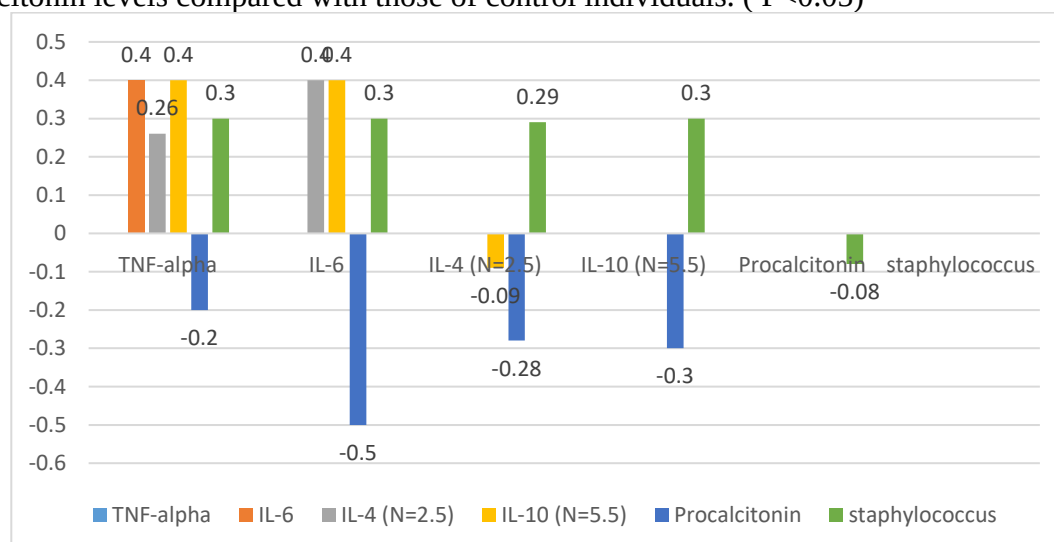
To assess the degree of bacterial infection in patients with trophic ulcer, we conducted studies of the level of procalcitonin in the blood. (Table 2)

Table 1. Comparative characteristics of procalcitonin levels in patients with ulcerative skin lesions with indicators of healthy individuals. (M+m) ng/ml

Group of patients	Patients with trophic ulcer N=44	A healthy group N=23
Procalcitonin	0,3 $\pm$ 0,01*	0,1006 $\pm$ 0,0002

Note: * confidence score in relation to healthy individuals (P <0.05)

The results of the Elisa study showed that patients with trophic ulcer have an increase in procalcitonin levels compared with those of control individuals. (P<0.05)



2. Correlation analysis of cytokine status and proliferation of bacteria and staphylococcus spp. (r)

As follows from the figure, correlation analysis of the study results showed that the level of procalcitonin had a significant inverse correlation with IL-6 – r=-0.5, IL-4 – r=- 0.28, IL-10 – r=- 0.3 (P<0.05), increased colonization of staphylococcus spp. It had a direct correlation with pro - and anti-inflammatory cytokines - r =+0.3 (P <0.05), respectively.

The analysis of the obtained results indicates the severity of the inflammatory process, characterized by a violation of the production of pro- and anti-inflammatory cytokines, which are directly involved in increasing the permeability of the vascular wall, the adhesion of shaped blood elements with the formation of leukocyte and platelet plugs, the release of lysosomal enzymes, active radicals, toxic oxygen metabolites. These processes are a favorable condition for the reproduction of pathogenic forms of bacterial flora and an increase in procalcitonin levels, contributing to the development of chronic inflammation and cytokinesis. , reducing the activity of skin fibroblast production.

Conclusions:

1. In patients with ulcerative skin lesions, cytokine status dysfunction is noted, expressed by 2.1 and 1.7-fold decreases in the production of anti-inflammatory cytokines IL-4 and IL-10 and by 2.1 and 2.6-fold increases in pro-inflammatory cytokines TNF-alpha and IL-6 compared to the healthy control group. ($P < 0.05$)
2. In patients with trophic ulcer, the development of an invasive form of bacterial infection is noted against the background of an increase in procalcitonin levels by 2.9 times compared with the healthy control group and averaged $0.3 \pm 0.01 \text{ ng/ml}$ and was significant.

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