

Phenotypic change in neutrophils in patients with Acne Vulgaris

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Abstract

Background: The widespread prevalence, material and moral damage, a tendency to chronicity and frequent recurrence, lack of effectiveness of therapy determine the relevance of the studied problem.

Aims: to evaluate the phenotype of neutrophils in patients with severe Acne.

Methods: The study included 77 people aged 18 to 35 years, 3-4 stage of severity of acne vulgaris was diagnosed in 49 of them and the remaining 28 were practically healthy individuals, without pathology at the time of the investigation. All participants underwent a study of the phenotyping of neutrophilic granulocytes (NG) in peripheral venous blood. CD 16+, CD 45+, and CD 95+ receptors on their surface identified subpopulations of neutrophils.

Results: In the group of patients with vulgar acne compared to control group, there was a significant increase in the number of (CD16 +) receptors in neutrophils by 1.4 times ($26.5 \pm 1.1\%$ / $18.8 \pm 0.9\%$). Expression of CD45 + receptors decreased on average to $28.4 \pm 1.2\%$ (in control group - $37.5 \pm 2.1\%$). Expression of CD95 + receptors exceeded in control group ($27.4 \pm 0.8\%$ / 22.1 ± 1.3) ($p < 0.05$). With pustular skin lesions, neutrophils are activated and migrate to the site of local inflammation. Wherein there is marked changes in the membrane expression of superficial NG receptors, such as an increase in the number of cells with (CD16 +), responsible for cytostatic function and ability to immune phagocytosis, an increase in the number of cells carrying apoptosis receptor (CD95 +), while the number of neutrophils with activated (CD45 +) significantly decreases relatively to the control data groups. An increase in the effector system (CD16 + and CD95 +) receptors amid declining of phagocytic activity contributes to the transition of the inflammatory process into a chronic form.

Conclusions: In patients with acne vulgaris, a reorganization of the expression of surface neutrophil receptors is observed. The obtained results show the necessity for correction of disorders in the phagocytic link in patients with pustular skin lesions.

Keywords: acne vulgaris, neutrophilic granulocytes, phenotype, surface receptors.

Background

Acne vulgaris (acne) is an extremely widespread disease. 70-80% of the population have experienced some form of acne during their lifetime. Acne occurs not only in adolescence, but also in adulthood. Quite wide distribution, material and moral damage, a tendency to chronicity and frequent recurrence, as well as insufficient effectiveness of therapy determine the urgency of the problem [1; 2]. The incidence is steadily increasing and the number of patients with severe course and resistance to therapy is increasing. Numerous studies show the complex multifactorial nature of the pathogenesis of acne. The anatomical localization of the pathological process is the sebaceous glands and the orifice of the hair follicles. Sebocytes express more than 40 receptors, including receptors for beta-endorphins, interleukins 1-alpha and 1-beta, interleukins-6 and -8, TLR-2 (Toll-like receptor-2), TLR-4 (Toll-like receptor -4), TLR-6 (Toll-like receptor-6), TNF-alpha (tumor necrosis factor alpha). This diversity of receptors indicates that the sebaceous gland can respond to various stimuli and changes in the body. The microbial environment in the funnel of hair follicle promotes the proliferation of *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus epidermidis*. Both types of bacteria are part of the normal facial skin microbiota, but their actively multiply contribute to the development of acne.

According to the latest data there is no difference in the amount of *P. acnes* in individuals with and without acne vulgaris and microbiome disruption plays an important role in the development of acne. A significant (up to 19%) increase in the population of the bacterium *Staphylococcus* leads to the activation of toll-like keratinocyte receptors (receptors that recognize the structures of microorganisms and activate the cellular immune response), which in turn causes excessive production of antimicrobial peptides and increased inflammation [3]. The leading etiological agents in pustular forms of acne are *Staphylococcus aureus* and *Staphylococcus haemolyticus*, the latter is considered the predominant causative agent of dermatosis with a persistent course resistant to treatment [4]. In the works of modern authors, these diseases are not considered only from the point of view of local damage to the integrity of the skin, since their emergence and further progression is based on a multiplex complex of still insufficiently studied etiopathogenetic factors. In the development of acne the importance of the pathology of the gastrointestinal tract, nervous, endocrine and urinary systems has been proved. Great importance is attached to the immunity system, the imbalance of factors of innate and adaptive immunity [4–7]. Chronic bacterial skin lesions can be manifestations of secondary immunodeficiency conditions. In most patients, a violation of the phagocytic link of immunity is revealed, leading to a decrease in the functional activity of neutrophils. Neutrophilic granulocytes realize their broad functional capabilities thanks to a powerful receptor apparatus that provides interconnection with cells of the immune system and various tissues [1; 8; 9; 10].

Aim of the study: to assess the phenotype of neutrophils in patients with severe acne.

Methods of the study

Study design

A cross-sectional observational single-center study was carried out.

Compliance criteria

Inclusion criteria:

- patients with acne vulgaris of 3-4 degrees of severity (according to the classification of the severity of acne vulgaris AAD (American Academy of Dermatology), which includes the criteria: the presence of a large number of papulopustular and nodular elements, cysts, one or more nodes, severe inflammatory reaction, the presence of cicatricial changes);
- disease in the acute stage;
- Prolonged absence of systemic therapy (6 months or more).

Exclusion criteria: age under 18 and over 35; the presence of immunodeficiency conditions (HIV, pregnancy, renal failure, liver failure, condition after splenectomy), autoimmune diseases.

Criteria for inclusion in the control group: absence of any pathologies at the time of treatment and blood test; similar age range.

Conditions of conducting

Patients were admitted at the Republican Dermatovenerologic Clinical Hospital of the Ministry of Health of the Republic of Uzbekistan (Tashkent). Laboratory studies were carried out at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan (Tashkent).

Study duration

The collection of samples for research was carried out over 2 years (from March 2018 to December 2019).

Study outcomes

The study evaluated the relationship between changes in membrane expression of surface neutrophil receptors (CD 16+, CD 45+, CD 95+) in severe acne.

Outcome registration methods

The material for the study was peripheral venous blood taken in the morning on an empty stomach in vacuum tubes vacutainers containing the anticoagulant K3 EDTA (ethylenediaminetetraacetic acid), purple marker. The study of the blood NG phenotype was carried out by the method of indirect rosette formation. NG was isolated from the obtained venous blood in a double density gradient of ficoll-verographin (1.093 / 1.077 g /

ml). The cells were washed; their number was counted in the Goryaev chamber and brought to a concentration of 2 million per 1 ml for neutrophils. Subpopulations of neutrophils were studied by identifying CD 16+, CD 45+, CD 95+ receptors on their surface in an immunofluorescence test using monoclonal antibodies against human CD receptors (Sorbent, RF) and labeled FITC (fluorescein isothiocyanate) F (ab) 2 - fragments of antibodies to mouse Ig G. Fluorescence was recorded using a Lumam R-8 microscope.

Ethical review

The study was carried out as part of scientific research and with the informed consent of patients. Approved by the ethical commission at the Tashkent Medical Academy at the department meeting; protocol No. 2 dated 02/12/2018.

Statistical analysis

The sample size was not pre-calculated. Statistical processing of the research results was carried out using the Statistics v. 6, MS Excel spreadsheets. The significance of the differences between the samples was established using the Mann – Whitney U test and Pirson x2 test. A p-value <0.05 was considered statistically significant. Data are presented as arithmetic means (M) and their corresponding standard errors of the mean (m).

Results

Objects (participants) of the study

The object of the study was 77 people, including 49 patients with acne vulgaris of 3-4 degrees of severity; and 28 practically healthy people. The average age of patients with acne was 23.1 ± 0.6 years with a range of 18-34 years, in the control group - 24.4 ± 1.0 years with a range of 19-35. The sex and age characteristics of the groups of patients with acne and persons of the control group are shown in Table. 1.

Table 1. Distribution of patients with acne vulgaris and persons of the control group by sex and age.

Index	Acne patients n = 49	Control group (healthy persons), n = 28
Age range	18–34	19–35
Average age	23,1±0,6	24,4±1,0
Sex, m / f,%	63,3/36,7	57,1/42,9

Key research findings

Clinically, there was a papulopustular rash (ranging in size from 0.5 to 1.5 cm in diameter), a pinkish-red hue with a pronounced inflammatory reaction. Against the background of the rash, multiple comedones, small atrophic scars, and secondary hyperpigmentation were also observed. The patients had a severe chronic recurrent course of the disease with frequent relapses, 37% had the formation of hypertrophic scars. Acne conglobata occurred in the sample in 24.5% (12 patients). Patients with phlegmonous acne applied to the clinic in isolated cases (2 patients).

Almost 70% (34) of patients with acne were diagnosed with concomitant diseases of the gastrointestinal tract (Table 2).

Table 2. Concomitant diseases of the gastrointestinal tract in patients with acne

Diseases	female	male	Total
Gastritis	6	9	15
Stomach and / or duodenal ulcer	3	6	9

Chronic cholecystitis	9	11	20
Chronic hepatitis	1	2	3
Intestinal motor dysfunctions	7	5	12

Among 34 patients, 15 (44%) were diagnosed with chronic gastritis, gastroduodenitis, 9 (26.5%) patients had stomach and duodenal ulcers, and some patients had a combination of these pathologies. Chronic cholecystitis was observed in more than half of the patients - 20 patients (58.8%). There is also a combination of cholecystitis with gastritis and peptic ulcer disease. In 12 patients (35.3%), there is a violation of the motor intestinal function. Our data coincide with the literature, which noted the pathology of the digestive system in patients with pyoderma in 48 - 91.7% of cases [2; 3]. It is known that gastrointestinal diseases are accompanied by an immunodeficiency state of both cellular and humoral immunity [2; 3]. A special place in inflammation is occupied by factors of innate immunity, among which the leading role belongs to phagocytes.

We have conducted a study of surface neutrophil receptors in patients with severe acne. The degree of interaction of immunocompetent cells is provided by the intensity of expression of their surface receptors [2; 3]. According to our data, the expression of the low-affinity Fc receptor for IgG (CD16 +) on neutrophils averaged $18.8 \pm 0.9\%$, CD45 + receptors that enhance the signal from TCR and BCR were detected on average in $37.5 \pm 2.1\%$ neutrophils of healthy individuals. ... The level of neutrophils with readiness for apoptosis (CD95 +) was $22.1 \pm 1.3\%$ (Table 3).

Table 3. Expression of surface receptors of neutrophilic granulocytes

	Neutrophil phenotype		
	CD16 ⁺	CD45 ⁺	CD95 ⁺
Acne patients, n = 49	26,5±1,1	28,4±1,2	27,4±0,8
Control group (healthy individuals), n = 28	18,8±0,9	37,5±2,1	22,1±1,3

In inflammatory processes of the skin, neutrophils are activated and migrate to the site of inflammation. Depending on the severity of the clinical course, the expression of surface receptors changes significantly. There was an increase in the number of (CD16 +) neutrophils ($26.5 \pm 1.1\%$) with a range of individual values from 21% to 33%. Expression of CD45 + receptors decreased on average to $28.4 \pm 1.2\%$ ($p < 0.05$ relative to control). Expression of CD95 + receptors significantly exceeded the data of the control group ($p < 0.05$). A higher level of neutrophils carrying a proapoptotic receptor indicates the readiness of neutrophils for apoptosis.

Discussion

Summary of the main research finding

In patients with acne vulgaris, in contrast to the control group, there is a reorganization of the expression of surface neutrophil receptors. There was a significant increase in the number of CD16 + neutrophils by 1.4 times compared to the control, a decrease in the expression of CD45 + by 1.3 times, an increase in the number of CD95 + NG receptors by 1.24 times ($p < 0.05$).

Discussion of the main research result

Thus, in inflammatory skin lesions, activation and migration of neutrophils to the site of local inflammation occurs. Wherein there is marked changes in the membrane expression of superficial NG receptors, such as an increase in the number of cells with (CD16 +), responsible for cytostatic function and ability to immune phagocytosis, an increase in the number of cells carrying apoptosis receptor (CD95 +), while the number of neutrophils with activated (CD45 +) significantly decreases relatively to the control data groups. An increase in the effector system (CD16 + and CD95 + receptors) amid declining of phagocytic activity contributes to the transition of the inflammatory process into a chronic form.

Research limitations

The limitations of this study are the relatively small sample size of patients, which is due to the criteria for

enrolling patients in the study. The study was conducted in one medical institution, was not a randomized multicenter, but is representative for the analysis of the activity of the neutrophilic link of immunity in patients with severe forms of acne.

Conclusion

The results obtained show the necessity to correction disorders in the phagocytic link of immune system in patients with pustular skin lesions, cause despite all the achievements of modern medicine, acne remains an urgent problem. The effectiveness of therapy remains low, there is an increase in treatment-resistant severe forms of acne. It is necessary to search for new effective methods of correction aimed at improving the clinical and pathological mechanisms of various forms of acne.

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Contribution of authors

A.Sh. Aliev, M.V. Zalalieva, U.A. Tashkenbaeva – study design; A.Sh. Aliyev – a set of samples and a clinical study; M.V. Zalalieva, Sh.R. Aliev – laboratory research; A.Sh. Aliyev – data analysis; W.A. Tashkenbaeva, F.Kh. Abboskhonova – work with literary sources, their translation into Russian; A.Sh. Aliev, M.V. Zalalieva, Sh.R. Aliev – the text of the article. All authors made significant contributions to the research and preparation of the article, read and approved the final version before publication.

Conflict of interests

The authors of this article have confirmed that they have no conflicts of interest to report.

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