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LOCAL IMMUNITY STATUS OF OROPHARYNX IN PATIENTS WITH SCLEROMA

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ABSTRACT

Introduction: Scleroma is a rare chronic granulomatous disease of the upper respiratory tract caused by Klebsiella pneumoniae subsp. rhinoscleromatis. To date its pathogenesis is as yet little understood. At the same time, scleroma is associated with a number of immune system disturbances.

The aim: To study local immunity status of oropharynx in patients with scleroma, and to compare its parameters in various clinical forms of the disease.

Materials and methods: 20 apparently healthy subjects and 92 patients with scleroma (33 males, 59 females) underwent clinical immunologic evaluation. There were 31 patients with dominating infiltrative form of scleroma, 30 – with dominating atrophic form, 31 – with dominating scarring form. Concentration of secretory and monomeric immunoglobulin A, immunoglobulin G, α-interferon, interleukin 1β in oropharyngeal secretion was determined by enzyme immunoassay.

Results: Patients with scleroma were found to have altered local immunity of oropharyngeal secretion. There was a strong tendency for decreased concentration of secretory immunoglobulin A - 1.3-2.0 times, and decreased immunoglobulin G level – 1.5-2.3 times ($p < 0.05$) as compared to the values in healthy subjects. Specific features of local immunity in oropharyngeal secretion in various forms of scleromatous inflammatory process in upper respiratory tract were found: the most significant decrease of α-interferon concentration in atrophic and scarring forms of the disease, and the largest increase of anti-inflammatory interleukin 1β and immune complex concentration in infiltrative form of scleroma.

Conclusions: The study revealed deficiency of local immunity factors in oropharynx, being indicative of immunopathogenetic role of diagnosed disturbances in development and persistence of chronic inflammation in scleroma, and emphasizing the necessity of immunocorrection in complex therapy of the disease.

KEY WORDS: scleroma, local immunity of oropharynx

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INTRODUCTION

Scleroma is a diagnostic and therapeutic challenge for clinicians due to its chronic course, need for prolonged treatment and relapses. It is characterized by a slowly progressive, specific (granulomatous) inflammation of the respiratory tract. According to the results of several studies, its pathogenesis is influenced by altered immune response because of cellular immunity disturbances [1, 2, 3]. Hence, there is an urgent need for the development of methods of targeted action on the immune status of patients with this pathology.

Disturbances in cellular immunity are of particular significance in scleroma development, as phagocytic reactions are unable to destroy pathogens (incomplete phagocytosis) resulting in persistence of K. rhinoscleromatis, accumulations of lymphoid cells and macrophages (granuloma). Sometimes this leads to nonspecific stimulation of macrophages causing increased resistance to other infections. Several studies have shown that scleroma may result from abnormal macrophage function [4]. Actually, Mikulicz cells are known to have specific features of their ultrastructure as well as enzyme histochemical properties of macrophages. Hence, it has been suggested that Mikulicz cells fail to develop into epithelioid cells, thereby inducing bacterial proliferation [4]. However, monocytes of patients infected with K. rhinoscleromatis demonstrate normal phagocytosis [5]. Thus, the probability of specific deficiency of destructive processes of K. rhinoscleromatis within macrophages cannot be excluded. Mutations affecting the genes, which encode the components of nicotinamide-adenine-dinucleotide-phosphate oxidase complex, proved to cause susceptibility to K. pneumoniae infection in mice [6].

M. Fusconi et al. (2018) hypothesized scleroma to be a type of immunodeficiency state with qualitative reduction in intervention of lymphocytes hyporeactive to K. rhinoscleromatis [7]. Decreased activity of CD4 T cells may result in insufficient activation of macrophages, which can be implicated in survival of Klebsiella spp. within the cells. The hypothesis on decreased CD4 activity, subsequently leading to Mikulicz cell formation, was confirmed in patients with HIV [8].

Evaluation of local immunity status of oropharynx appears to be of particular importance in patients with scleroma because of the necessity to administer proper therapy

Table 1. Immunoglobulin concentration in oropharyngeal secretion of control subjects and patients with various forms of scleroma (M ± σ)

Groups	n	sIgA, g/l	Ig G, g/l	mIg A, g/l
Control group	20	0.58 ± 0.2	0.06± 0.05	0.07 ± 0.06
Scleroma, infiltrative form	31	0.4 ± 0.1*	0.29 ± 0.15*	0.18 ± 0.14*
Scleroma, atrophic form	30	0.35 ± 0.32*	0.17 ± 0.08*#	0.17 ± 0.08*
Scleroma, scarring form	31	0.3 ± 0.18*#	0.24 ± 0.22*	0.24 ± 0.22*

Notes:
1. Arithmetical mean values of parameters studied (M) and standard deviations (σ) are presented in the table;
2. * – statistically significant difference ($p < 0.05$) compared to values in the control group;
3. # – statistically significant difference ($p < 0.05$) compared to values in the group of patients with dominating infiltrative form of scleroma.

and immune system correction. At the same time, the role of such immune regulatory factors as immunoglobulins of various classes, pro-and anti-inflammatory cytokines, regulatory peptides and other humoral immunity factors in genesis of the disease has rarely been addressed in the literature devoted to scleroma, and to date it is not clearly understood. This paper presents novel data on local immunity status of oropharynx in patients with various clinical forms of scleroma when compared to apparently healthy subjects.

THE AIM

To study local immunity status of oropharynx in patients with scleroma, and to compare its parameters between the groups with various clinical forms of the disease.

MATERIALS AND METHODS

92 patients with scleroma (33 males, 59 females) aged 23-79 years (mean age (M ± σ) – 53.4 ± 14.52 years) underwent clinical immunologic evaluation. The study was performed in compliance with the provisions of Declaration of Helsinki at Scleroma Center of Pirogov Regional Clinical Hospital, Vinnytsia.

Local immunity of oropharynx was studied in the following groups: 1) the control group - apparently healthy subjects (n=20); 2) patients with dominating infiltrative form (n=31); patients with dominating atrophic form (n=30); patients with dominating scarring form (n=31).

Sampling of oropharyngeal secretion was performed on the day of admission on an empty stomach before rinsing the oral cavity or brushing teeth and gums. After centrifugation of the whole oropharyngeal secretion in supernatant fluid the following local immunity parameters were determined by enzyme immunoassay:

- concentration of secretory and monomeric forms of A immunoglobulins, immunoglobulin G (chemical reagents Xema-Medica, Russian Federation);
- cytokine content - α-interferon, interleukin 1β (chemical reagents LLC Tsitokin, Russian Federation);
- level of immune complexes (precipitation method using 3.37% polyethylene glycol) [9].

Statistical processing of data was performed in statistical package "SPSS 23" (SPSS Inc.) using parametric and non-parametric methods of data evaluation.

RESULTS

Significantly decreased content of secretory immunoglobulin (sIg A) and significantly increased content of monomeric immunoglobulin (mIg A) and immunoglobulin G (IgG) in oropharyngeal secretion was found in all forms of scleroma (Table 1).

In healthy subjects median sIg A content in oropharyngeal secretion was 0.5 g/l, interquartile interval P_{25}-P_{75} ranged from 0.5 to 0.7 g/l. The most significant decrease in sIg A content was documented in atrophic and scarring forms, and less significant decrease - in infiltrative form of the disease. Thus, in infiltrative form of scleroma, sIg A content was 1.3 times less than the control value, $p = 0.001$ (the median concentration was 0.4 g/l, and P_{25}-P_{75} range - 0.3-0.5 g/l). In patients with atrophic and scarring forms of scleroma, the content of this immunoglobulin was 2 times lower than in the controls, $p < 0.001$ (median concentrations were 0.25 and 0.23 g/l, and P_{25}-P_{75} range - 0.2-0.35 and 0.15-0.4 g/l, respectively). No statistically significant difference in sIg A content in atrophic and scarring forms of scleromatous respiratory tract lesion was found.

In control subjects, median contents of Ig G and mIg A in oropharyngeal secretion were 0.1 and 0.07 g/l, and P_{25}-P_{75} range - 0-0.1 and 0.02-0.1 g/l, respectively. Depending on the form of scleroma, specific lesion of the respiratory tract was accompanied by various extent of increase in the content of those immunoglobulins in oropharyngeal secretion. The greatest increase in Ig G level (2.3 times, $p = 0.001$) was observed in infiltrative form of disease (median – 0.23 g/l, and P_{25}-P_{75} – 0.2-0.5 g/l), and less significant increase – in atrophic (1.8 times, $p < 0.001$) and scarring (1.5 times, $p < 0.001$) forms of scleroma (median Ig G content - 0.18 and 0.15 g/l, P_{25}-P_{75} – 0.1-0.2 and 0.15-0.4 g/l, respectively) (See Table 1).

Oropharyngeal secretion showed similar changes in mIg A content in various forms of scleroma (Table 1). In patients with infiltrative and atrophic forms of disease mIg A level was 2.4 times higher than in the control group, $p < 0.001$ (median content - 0.18 and 0.17 g/l, P_{25}-P_{75} – 0.1-0.25 and 0.1-0.2 g/l, respectively), and it was 2.9 times higher in those with scarring form, $p = 0.002$ (median concentration – 0.2 g/l, and P_{25}-P_{75} – 0.15-0.4 g/l). No statistically significant difference between monomeric immunoglobulin content in atrophic and scarring forms of scleromatous respiratory tract lesion was found.

Thus, significant decrease in the content of secretory immunoglobulin (sIg A) and significantly increased content of monomeric immunoglobulin (mIg A) and immunoglobulin G (IgG) was found in the patients with various forms of scleroma. Such changes in immunoglobulin content are consistent with the studies of local immunity factors in other infectious and inflammatory diseases of the upper respiratory tract.

Concentration of interleukin 1β (IL-1β) – one of the key factors in regulation of inflammatory reaction and induction of immune response – was assessed in oropharyngeal secretion as well (Fig.1). In the control group its content appeared to be Me - 21.5 pg/ml, P_{25}-P_{75}-12.0-24.0. In all forms of scleromatous lesions, oppositely directed alterations in interleukin 1β concentration were revealed in oropharyngeal secretion. In infiltrative form its level was Me -54.0 pg/ml, P_{25}-P_{75} - 28.0-62.0, i.e. 2.5 times higher than in the control group ($p < 0.001$). High IL-1β concentration in infiltrative form of scleroma can suggest that on this stage of disease exudative reactions as well as activation of helper lymphocytes Th1 *и* Th17, leading to activation of specific humoral immune response, are most pronounced, and this can be confirmed by the measurement of immune complex level in oropharyngeal secretion (Fig. 1).

In atrophic form of scleroma, IL-1β level in oropharyngeal secretion was found to be 1.7 times lower than in the control group, $p < 0.001$ (median concentration - 13.0 pg/ml, P_{25}-P_{75} – 10.0-16.0 pg/ml), while in dominating scarring form no significant changes in its level was observed. The data received corroborate the suggestion that forms of scleroma represent various stages of the disease. IL-1β level in oropharyngeal secretion proved to be significantly higher in infiltrative form as compared to that in the other forms of scleroma ($p < 0.05$) (Fig. 1).

Study of α-interferon concentration in oropharyngeal secretion showed its level to be Me - 13.5 pg/ml, P_{25}-P_{75} - 10.0-20.0 in the control group. Specific respiratory tract lesion occurring in scleroma was accompanied by decreased α-interferon level, most evident in atrophic and scarring forms and less significant in infiltrative form. In atrophic and scarring forms, α-interferon content was 2.25 (2.0-3.0) and 2.75 (2.0-4.5) pg/ml, respectively, being 6 and 4.9 times lower as compared to the control ($p < 0.001$), while in infiltrative form its content was 11.0 (10.0-18.0) pg/ml being 1.2 times lower as compared to the control. α-interferon content was significantly higher in dominating infiltrative form as compared to that in other forms of scleroma. No statistically significant differences in α-interferon level were found between atrophic and scarring forms of the disease (Fig.2).

Inflammatory process activity was evaluated by the content of immune complexes in oropharyngeal secretion (Fig.3). The level of immune complexes in normal individuals is known to range from 0 to 23 ODU (median – 9.0 ODU, P_{25}-P_{75} – 0-12.0 ODU). Patients with scleroma showed significant continuous increase in the content of immune complexes, being most evident in dominating infiltrative form (4.3 times, $p < 0.001$) (median – 38.5 ODU; P_{25}-P_{75} – 37.0-42.0 ODU). Atrophic and scarring forms of the disease were associated with less significant increase in immune complexes content. Increased level of immune complexes in oropharyngeal secretion in infiltrative form of scleroma were due to intensive processes of pathogen elimination by antibodies of various classes, entering the focus of inflammation from different sources including blood vessels. In atrophic scleroma the content of immune complexes exceeded the control values 1.2 times ($p > 0.05$) (median – 10.75 ODU, P_{25}-P_{75} – 10.0-12.0 ODU). Scarring form of scleroma was associated with 1.7 time increase ($p < 0.001$) in the content of immune complexes as compared to the controls (median – 15.25 ODU, P_{25}-P_{75} – 12.0-20.0 ODU). The content of immune complexes was significantly higher in dominating infiltrative form as compared to other forms of scleroma ($p<0.05$).

DISCUSSION

Scleroma is a chronic granulomatous infectious disease that affects the nose and other parts of the respiratory tract down to the trachea [10]. A delay in the diagnosis can lead to complications such as physical deformity, upper airway obstruction and, rarely, sepsis [11, 12]. The disease has classic histopathologic features consisting of Mikulicz cells, Russell bodies, and gram-negative bacilli. In many cases, the causative agent is identified as Klebsiella rhinoscleromatis [13].

The exact pathogenesis in which Klebsiella spp result in Mikulicz cell formation and eventual rhinoscleroma is unclear. Klebsiella rhinoscleromatis, like K pneumoniae, is characterized by a mucopolysaccharide capsule that contributes to inhibition of phagocytosis, thus facilitating intracellular survival [12, 14]. Clearly, capsule polysaccharide plays an important role in the interplay between K. pneumoniae and the innate immune system [12].

There are many immune system abnormalities found in patients affected by scleroma [7]. According to the first hypothesis, Klebsiella rhinoscleromatis could induce an alteration of the immune system, thus allowing the evolution of the infection into a chronic form. The second is that the immune system, already altered by other unknown mechanisms, allows KR attacking the body.

It has been postulated that cellular immunity is impaired with a decreased CD4 to CD8 ratio but with humoral immunity remains intact. Lessened activity by CD4 T cells may result in improper activation of macrophages, further promoting intracellular survival of Klebsiella. Cytokines are key mediators of immune responses and the anti-inflammatory cytokine IL-10 has been shown to be highly produced after K. rhinoscleromatis infection and to play a crucial role in the establishment of a proper environment leading to Mikulicz cells maturation [12].

At the same time, the role of such immune regulatory factors as immunoglobulins of various classes, pro-and anti-inflammatory cytokines, regulatory peptides and other humoral immunity factors in genesis of the disease has rarely been addressed in the literature devoted to scleroma, and to date it is not clearly understood. This paper presents novel data on local immunity status of oropharynx

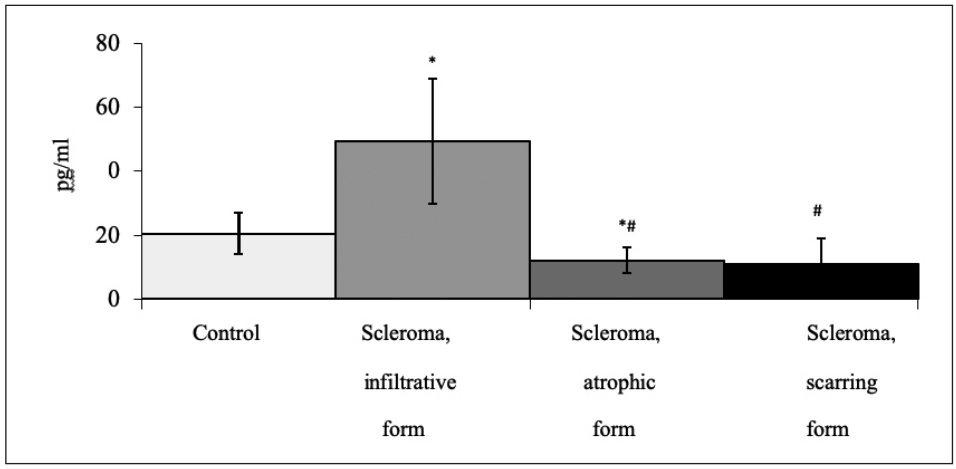

Fig. 1. Interleukin 1β concentration in oropharyngeal secretion of control subjects and patients with various forms of scleroma (M ± σ)

Notes: * – statistically significant difference ($p < 0.05$) as compared to the control group; # – statistically significant difference ($p < 0.05$) as compared to the patients with atrophic form of scleroma.

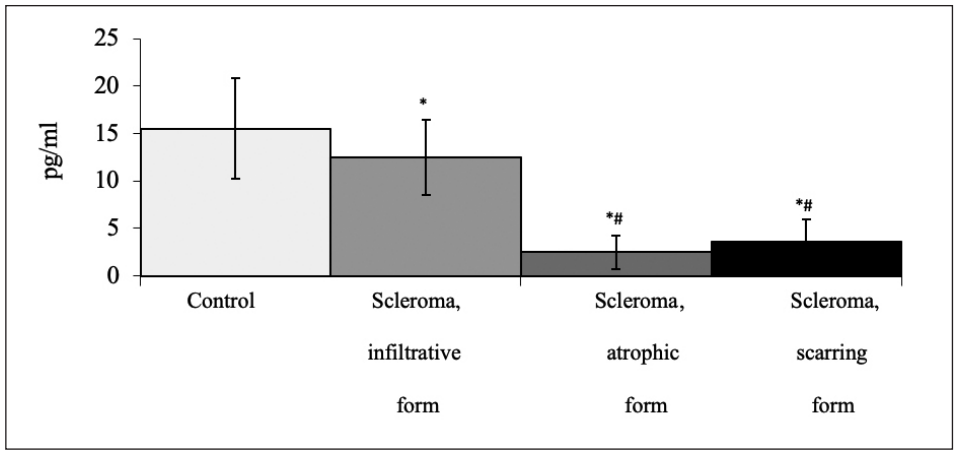

Fig. 2. Concentration of α-interferon in oropharyngeal secretion of control subjects and patients with various forms of scleroma (M ± σ)

Notes: * – statistically significant difference ($p < 0.05$) as compared to the control group; # – statistically significant difference ($p < 0.05$) as compared to the patients with atrophic form.

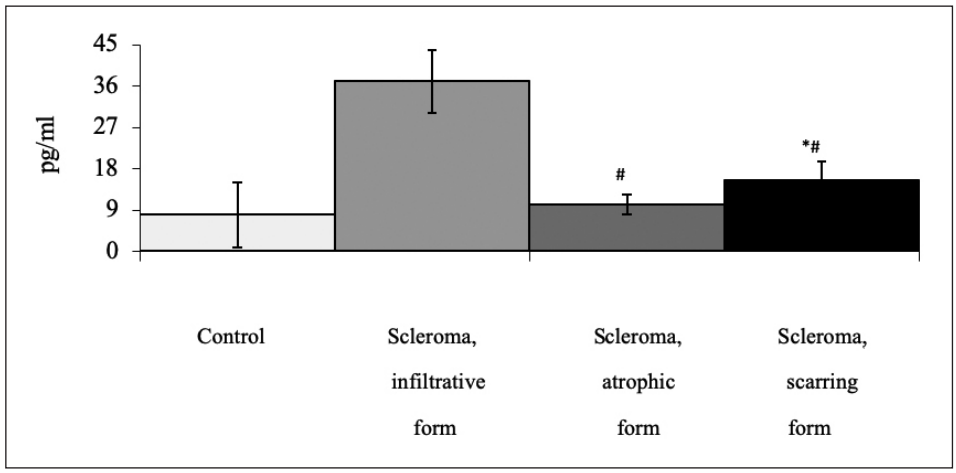

Fig. 3. Concentration of immune complexes in oropharyngeal secretion of control subjects and patients with various forms of scleroma (M ± σ)

Notes: * – statistically significant difference ($p < 0.05$) as compared to the control group; # – statistically significant difference ($p < 0.05$) as compared to the patients with atrophic form of scleroma.

in patients with various clinical forms of scleroma when compared to apparently healthy subjects.

The study demonstrated scleromatous lesion of the upper respiratory tract to be associated with deficiency of local immunity in oropharynx (decreased concentration of secretory immunoglobulin A and immunoglobulin G). The data obtained are indicative of immunopathogenetic role of the diagnosed disturbances in development and persistence of chronic inflammation in scleroma, emphasizing the necessity of immunocorrection in complex therapy of the disease.

Concentration of α-interferon was decreased in scarring and atrophic forms of scleroma, while the content of anti-inflammatory iterleukin-1β and immune complexes were the highest in infiltrative form – these findings are likely to be used as additional criteria for verification of disease form in scleroma.

CONCLUSIONS

1. In patients with scleroma, deficiency of local immunity in oropharynx was found: concentration of secretory im-

munoglobulin A and immunoglobulin G demonstrated strong tendency to decrease – 1.3-2.0 and 1.5-2.3 times, respectively, ($p<0.05$) as compared to healthy subjects.
2. Specific features of local immunity in oropharengeal secretion were detected in various forms of scleromatous inflammatory process in upper respiratory tract: the greatest decrease of α-interferon concentration in atrophic and scarring forms, and the highest concentration of anti-inflammatory iterleukin-1β and immune complexes in infiltrative form of the disease.

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