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ANALYSIS OF THE BACKGROUND LEVEL OF M1 AND M2 MACROPHAGE CYTOKINES IN PATIENTS WITH REVISION RHINOPLASTY

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The purpose of the study was to evaluate the background level of cytokines M1 and M2 of the macrophage phenotype in patients with revision rhinoplasty. The search for effective diagnostic methods will improve treatment and prevention of negative consequences of reconstructive rhinoplasty. 63 patients (18–45 years old) who underwent revision rhinoplasty using a rib graft were under supervision. Patients were divided into two groups based on fibrinogen levels for further studies. The levels of TNF-α, TGF-β1 cytokines (DRG Diagnostic Inc., Germany), IL-6, IL-10, IL-13 (IBL International, Germany), IL-12, IL-18 (ElabScience, USA) were evaluated in all patients. In patients after rhinoplasty who have postoperative complications in the form of nasal deformity, there was an increase in the synthesis of cytokines characteristic of macrophages of the M1 phenotype and a decrease in the level of cytokines of the M2 phenotype of macrophages. The identified differences require further research to study their pathogenetic role in developing complications in rhinoplasty patients.

Key words: revision rhinoplasty, rib implant, fibrosis, fibrinogen, cytokines.

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АНАЛІЗ ФОНОВОГО РІВНЯ ЦИТОКІНІВ М1 ТА М2 ФЕНОТИПУ МАКРОФАГІВ У ПАЦІЄНТІВ З РЕВІЗІЙНОЮ РИНОПЛАСТИКОЮ

Метою роботи було оцінити фоновий рівень цитокінів М1 та М2 фенотипу макрофагів у пацієнтів з ревізійною ринопластикою. Пошук ефективних способів діагностики дозволить покращити лікування та профілактики негативних наслідків реконструктивної ринопластики. Під наглядом знаходилося 63 пацієнти (18–45 років), яким було проведено ревізійну ринопластику з використанням реберного трансплантату. Для подальших досліджень пацієнти були розділені на дві групи за рівнем фібриногену. Усім пацієнтам проводили оцінку рівня цитокінів TNF-α, TGF-β1 (DRG Diagnostic Inc., Німеччина), IL-6, IL-10, IL-13 (IBL International, Німеччина), IL-12, IL-18 (ElabScience, США). У пацієнтів після ринопластики у яких виявлено післяопераційні ускладнення у вигляді деформації носу спостерігаються посилення синтезу цитокінів, які характерні для макрофагів М1 фенотипу та зниженням рівня цитокінів М2 фенотипу макрофагів. Виявлені відмінності потребують подальших досліджень для вивчення їх патогенетичної ролі в розвитку ускладнень у пацієнтів з ринопластикою.

Ключові слова: ревізійна ринопластика, реберний імплант, фіброз, фібриноген, цитокіни.

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Rhinoplasty is considered one of the most complex procedures in the field of facial plastic surgery. Various types of grafts are widely used in reconstructive rhinoplasty, but special attention is paid to grafts of human origin. Among them, transplants from the nasal septum, ear, and costal cartilage are the most popular. However, the main disadvantage of using autografts is their tendency to resorption. Despite the proven advantages of using costal cartilage compared to quadrangular cartilage of the nose and ear, graft resorption has a significant risk, which can lead to undesirable results in rhinoplasty [1, 2, 3, 4].

In recent years, according to the data of world scientific publications [3, 4], there has been an active discussion regarding the choice of transplants in reconstructive rhinoplasty. Researchers face challenges such as graft instability, cartilage resorption, and the lack of universally accepted solutions to overcome these challenges. Current research focuses on analyzing immunological aspects of the inflammatory response during transplantation. Special attention is paid to the study of innate immune reactions and the influence of the tissue microenvironment on the development of inflammation [5]. Scientific studies emphasize the importance of the inflammatory process as a necessary condition for initiating the healing process. Macrophages, the main cells that initiate the inflammatory response, are considered critical for regulating the healing process [6].

The acute inflammatory response after implantation is crucial for the initiation of angiogenesis. Monocytes activate after implantation. Activated monocytes secrete factors that contribute to the colonization of mononuclear macrophages. During the first week after implantation, macrophages attached to the implant, under the influence of pro-inflammatory cytokines (such as IL-6), transform into the M1 phenotype and, under the influence of various signals and TNF-α, participate in the recruitment of leukocytes. After a week, M1 macrophages transform into the M2 phenotype. M2 macrophages release cytokines such as IL-10 while producing extracellular stromal components such as type I and IV collagen, elastin, fibronectin, and aminoglucan, which contribute to remodeling the extracellular matrix required for late cells [6].

Although the inflammatory process is crucial for initiating bone healing, prolonged inflammation after the initial phase (about 4 days) leads to impaired tissue and bone healing [7].

The negative consequences of improper activation of inflammatory macrophages have been thoroughly studied in the context of aseptic loosening during total joint endoprostheses [8]. Fracture risk increases, and fracture healing is impaired in patients with chronic inflammatory diseases such as Crohn's disease [8].

M1 macrophages are known to secrete pro-inflammatory cytokines such as IL-β, IL-6, IL-12, IL-23, and TNF-α, which recruit and activate white blood cells during injury. In contrast, IL-4 and IL-10 stimulate the differentiation of macrophages into the M2 subtype, which exerts anti-inflammatory effects and secretes factors such as TGF-β and IL-13. M2 macrophages, including M2a and M2c subsets, promote angiogenesis and tissue regeneration, while M1 macrophages are anti-angiogenic [8, 9].

Thus, M1 macrophages are typically present in the early stages and initiate the angiogenesis process. M2 macrophages dominate in the later stages, contributing to the stabilization of blood vessels and the synthesis of extracellular matrix components. Suppose the transition of the M1 phenotype to the M2 phenotype is disturbed. In that case, this is reflected by a constantly increased number of macrophages of the M1 phenotype, and the damage occurs with chronic inflammation and disturbances in the healing and regeneration process [9].

The purpose of the study was to evaluate the background level of cytokines M1 and M2 of the macrophage phenotype in patients with revision rhinoplasty and to establish effective diagnostic methods that will improve treatment and prevention of negative consequences of reconstructive rhinoplasty.

Materials and methods. The study was performed on 63 patients who underwent revision rhinoplasty using a rib graft. All patients were examined based on the clinical laboratory U`Clinic (Kyiv), where surgical treatment was carried out after preliminary rehabilitation. The study was performed from 2021 to 2024. All patients complained about dissatisfaction with the shape of the external nose, pits, compaction at the tip of the nose, deformation of the nasal axis, difficulty in nasal breathing, and impaired nose sensitivity. The first surgery was performed no less than 1.5 years later.

For further studies, patients were divided into two groups based on the results of the fibrinogen level. The first group of patients with an elevated level of fibrinogen >350 mg/dL included 32 patients, and the second group had a mean level of fibrinogen <350 mg/dL, which accordingly included the other 31 patients. Among the examined, 23 (36.5%) were men and 40 (63.5%) women. The age of the patients ranged from 18 to 45 years. The mean age was 32.7±1.3 years [10].

Assessment of serum cytokine concentrations was determined using enzyme-linked immunosorbent assay kits – TNF-α, TGF-β1 using reagents DRG Diagnostic Inc. (Germany), IL-6, IL-10, IL-13" IBL International (Germany), IL-12, IL-18 ElabScience (USA). The results were evaluated using a "Sunrise" photometer (Austria).

The control group consisted of 20 healthy people aged 21–45 without rhinoplasty.

Statistical processing of the results was carried out using Microsoft Excel and Statistica for Windows 10.0 programs. Quantitative changes are presented as mean squared deviation (SD) and the arithmetic mean. A comparison of quantitative features and determination of the reliability of differences was evaluated using the Student's T-test. Differences were considered probable at $p < 0.05$.

Results of the study and their discussion. The results of the study of the cytokine profile in patients after revision rhinoplasty are shown in Fig. 1–2.

When analyzing the level of pro-inflammatory cytokines, it was found that patients in Group I showed a significant increase in the level of IL-6 and TNF-α. The level of IL-6 was 167.3±12.4 pg/mL (with a norm of 4.71±0.42 pg/mL), and the level of TNF-α was 75.7±13.2 pg/mL (with a norm of 2.44±0.09 pg/ml) (Fig.1).

When analyzing the level of IL-12 and IL-18 (Fig. 2), we also established that their level increased to 55.14±1.07 pg/ml and 341.3±14.7 pg/ml in comparison with the data of the control group 24.63±2.92 pg/ml and 110.4±24.4 pg/ml, respectively ($p<0.05$).

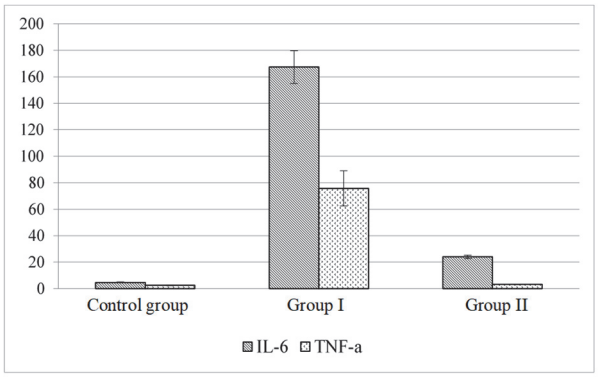

Fig. 1. IL-6 and TNF-α cytokine levels in patients with revision rhinoplasty.

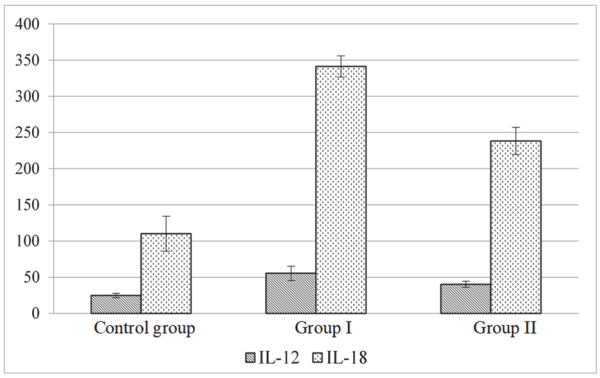

Fig. 2. Level of cytokines IL-12 and IL-18 in patients with revision rhinoplasty.

Analyzing the indices of pro-inflammatory cytokines in patients of Group II, a significant increase in IL-6 to 24.04±1.34 pg/mL was found. However, it was significantly lower compared to the indices of Group I ($p<0.05$). IL-6 and TNF-α levels probably did not differ from the indices in the group of healthy individuals. It was also established that the patients of this group had an increase in IL-12 and IL-18 levels to 40.12±4.43 pg/ml and 238.4±15.7 pg/ml, respectively. When comparing cytokine levels between patients of the I and II groups, probable differences in the levels of IL-6 and IL-18 were found ($p<0.05$).

The results of determining the level of anti-inflammatory cytokines in patients with revision rhinoplasty are presented in Figs. 3–4. As can be seen from the obtained data, IL-10 levels were slightly reduced compared to healthy individuals, but no probable changes in its level were detected. IL-13 and TGF-β1 concentrations were also reduced to 15.31±2.86 pg/mL and 152.3±34.7 compared to the group of healthy individuals ($p < 0.05$).

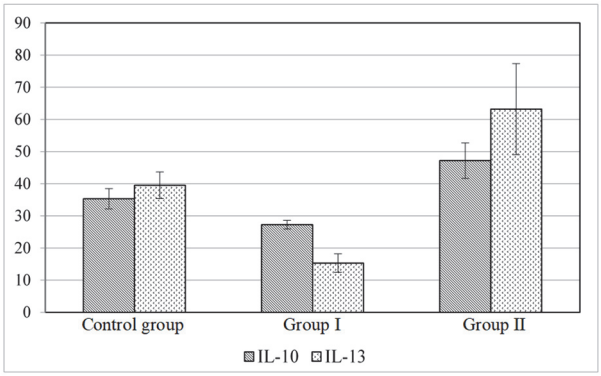

Fig. 3. Level of cytokines IL-10 and IL-13 in patients with revision rhinoplasty.

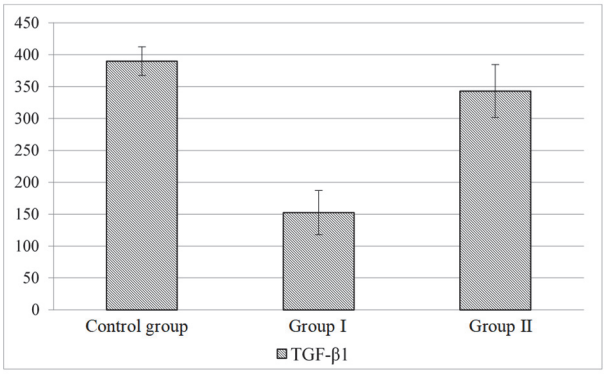

Fig. 4. TGF-β1 level in revision rhinoplasty patients.

In contrast to the patients of group I, the level of cytokines in patients of group II had a slightly different profile. Thus, there was an increase in the level of IL-13 to 63.23±14.18 pg/ml compared to the group of healthy individuals, while the levels of IL-10 and TGF-β1 did not significantly differ from the norm, and their levels were 47.17±5.48 pg/ml 343.1±41.6 pg/ml. When comparing cytokine levels in the two study groups, a significant difference in IL-4, IL-13, and TGF-β1 levels was found ($p< 0.05$).

Thus, our study allowed us to establish that in patients with revision rhinoplasty, who were found to have an increase in the level of fibrinogen, there is an increase in the serum concentration of almost all cytokines of the M1 macrophage phenotype (IL-6, IL-12, IL-18, and TNF-α). Changes in IL-6 and TNF-α levels were the most pronounced among all cytokines. It is known that IL-6 is a central regulator of

inflammatory reactions and the most powerful cytokine that stimulates the release of vasoactive substances, inducing the secretion of fibrinogen and the production of C-reactive protein [9, 11] and promoting the development of inflammation. Another powerful cytokine produced by activated macrophages is TNF-α. It was established that TNF-α is one of the critical cytokines temporarily expressed by polarized macrophages at the early regeneration stage. TNF-α promotes the recruitment of macrophages, which may be an enhanced cycle of macrophages accumulating in the wound area. It is known that this cytokine also belongs to the regulators of immune inflammation. It is synthesized by many cells but primarily by activated macrophages.

IL-12, also known as natural killer cell-stimulating factor, is mainly produced by dendritic cells, macrophages, and B-lymphocytes and is a growth-stimulating factor of immune cells with various biological effects. IL-12 can regulate the balance between Th1/Th2 cells and promote the proliferation of Th1-type cells [8, 10, 12]. However, as a pro-inflammatory factor, continuous IL-12 secretion is not beneficial for tissue repair and delays bone healing and formation.

IL-18, being a pleiotropic proinflammatory cytokine, stimulates the production of IFNγ, TNF-α, IL-1, IL-2, adhesion molecules, and apoptosis factors, increases the proliferative activity of T lymphocytes, and increases the lytic activity of NK cells. IL-18 forms cellular and humoral immunity and innate and acquired immune responses [12]. In experimental conditions on mice, it was shown that the synergistic effect of IL-18 and IL-12 leads to a decrease in the production of IgE and IL-13 by basophils and mast cells. Still, the administration of IL-18 alone in these mice stimulates the secretion of IgE, IL-4, and IL-13 by basophils, mast cells, and CD4+ by T lymphocytes [4, 12].

In our study, we found that patients in this group also showed an increase in IL-12 and IL-18, which in turn can lead to inhibition of the synthesis of anti-inflammatory cytokines and the formation of chronic inflammation, leading to cartilage damage. Indices of the background level of cytokines of the M1 macrophage phenotype in patients with normal fibrinogen levels differed from those with increased fibrinogen levels. IL-6 levels were slightly elevated and lower than in patients in Group I, but IL-12 and IL-18 levels were significantly different compared to those in Group I.

Analyzing the concentration of cytokines produced by M2 macrophages (IL-10, IL-13, TGF-β1) revealed that in revision rhinoplasty patients with an increased level of fibrinogen, the levels of anti-inflammatory cytokines were reduced. Among them, IL-13 and TGF-β1 probably differed from the indices of the control group of healthy individuals, and the level of IL-10 tended to decrease.

IL-10 is a critical molecule that antagonizes Th1 cells with a direct inhibitory effect. Therefore, IL-10 is defined as an inhibitor of cytokine synthesis [11]. They experimentally established that IL-10 can affect the recruitment of macrophages to the focus of inflammation and can play a role in the control of early infiltration, and IL-10 deficiency leads to a change in the phenotype of macrophages towards a more pro-inflammatory phenotype [11].

TGF-β is also a cytokine involved in skeletal muscle repair and regeneration. TGF-β is produced in skeletal muscle during the response to injury and plays a role in various stages of injury healing [11]. During the inflammatory response phase, TGF-β can recruit more inflammatory cells and enhance the inflammatory response. During the proliferative phase, TGF-β stimulates extracellular matrix production, angiogenesis, and epithelialization, and during maturation, TGF-β induces fibroblast formation and promotes wound healing by contraction. Accordingly, a deficiency of this cytokine leads to a violation of the immune response during healing and contributes to the development of chronic inflammation [11].

In contrast, in the group of patients with normal fibrinogen levels, IL-13 levels were slightly elevated, while IL-10 and TGF-β1 levels were probably not different from normal values. Given that an increase in IL-18 was observed in patients of this group, it can be assumed that the increase in the synthesis of IL-18 in the conditions of a high level of sIL-2R production determines the switching of the synthesis to the M2 type of cytokines, such as IL-13. It can be confirmed by the fact that in our study, an increase in the serum concentration of IL-13 was found in patients from group II. However, the detected differences between the M1 and M2 phenotype cytokine levels in patients with revision rhinoplasty require further research to study their pathogenetic role in developing complications after rhinoplasty.

Thus, our results allow us to state that patients after rhinoplasty in whom postoperative complications in the form of nasal deformity are detected have an increased synthesis of cytokines, which are characteristic of macrophages of the M1 phenotype. This is reflected by an increase in their level in the peripheral blood and a decrease in the level of cytokines of the M2 phenotype of macrophages, which

may indicate a violation in the switching of the M1 phenotype of macrophages to the M2 phenotype, which is accompanied by chronic inflammation and impaired healing and engraftment of cartilage. The revealed differences between M1 and M2 phenotypes cytokine levels in revision rhinoplasty patients require further studies to study their pathogenetic role in developing complications in rhinoplasty patients.

Conclusions

1. In patients with revision rhinoplasty in whom an increased level of fibrinogen was detected, we observed an increase in the concentration of cytokines of the M1 phenotype of macrophages (IL-6, IL-12, IL-18, and TNF-α) and a decrease in the concentration of cytokines of the M2 phenotype of macrophages (IL-10, IL-13, TGF-β1).

2. The level of cytokines in patients with revision rhinoplasty, in whom the level of fibrinogen is normal, is characterized by the absence of an increase in the serum concentration of M1 cytokines of the macrophage phenotype (IL-6, IL-12, IL-18) and an increase in the level of cytokines of macrophages of the M2 phenotype (IL-10, IL-13).

3. An increase in the synthesis of IL-18 detected in patients with rhinoplasty can serve as a criterion for switching the synthesis of cytokines characteristic of the M1 phenotype of macrophages to cytokines of the M2 phenotype of macrophages, such as IL-10 and especially TGF-β1.

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