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Chemokine Expression in Neutrophils and Subcutaneous Adipose Tissue Cells Obtained during Abdominoplasty from Patients with Obesity and Normal Body Weight

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The study was aimed at assessing the role of chemokines in inflammatory changes in tissue following abdominoplasty. The levels of neutrophil-coupled chemokines and their receptors in the serum and blood cells, as well as in cells isolated from the subcutaneous adipose tissue sampled during abdominoplasty were compared in patients with obesity and normal body weight. The levels of chemokines CCL3, CCL3, and CCL5 in blood serum and expression of chemokine receptor CXCR2 and CXCR6 on blood neutrophils were significantly higher (p<0.05) in obese patients in comparison with patients with normal body weight. Elevated expression of chemokines CCL2, CCL3, CCL4, CCL5, CCL18, and CCL20 (p<0.05) was detected in subcutaneous adipose tissue cells isolated obese patients in comparisons with persons with normal body weight. These findings attest to favorable conditions for enhanced neutrophil migration to the adipose tissue in patients with obesity, which can promote leukocyte infiltration of the suture site after abdominoplasty and serves as additional risk factor for the development of postoperative complications associated with activity of neutrophil-derived proteolytic enzymes.

Key Words: *chemokines; obesity; abdominoplasty; neutrophils*

Chemokines is a superfamily of proteins with a molecular weight ~8-10 kDa acting as chemotactic molecules for cells of different types [5]. Chemokines are divided into 4 subfamilies based on sequential arrangement of their first two cysteine residues in the N-terminal part of the peptide chain. The two largest families are the CXC family, in which these two cysteines are separated by any single amino acid, and the CC family, in which the first two cysteines are adjacent. Chemokines of a minor XC subfamily have only one N-terminal cysteine. In chemokines of the CX3C subfamily, N-terminal cysteine residues are separated by three amino acids [12].

Most chemokine receptors are G-protein-coupled receptors with 7 transmembrane domains acting as monomers, homodimers, and heterodimers. The expression of chemokine receptors is organ- and cell-specific [13]. Activation of CXCR1/2 chemokine receptors is related to the development of postsurgical pain syndrome [11], and their binding to CXCL8 ligand leads to neutrophil migration to the inflammation site.

G-protein-coupled receptors FPR1 and FPR2 expressed on the surface of neutrophils recognize chemotactic bacterial peptides, in particular, fMLP (N-formyl-L-methionyl-L-leucyl-L-phenylalanine), that initiate neutrophil migration to the dermis in the wound area in inflammation and tissue damage; they are considered the key regulators of events in the inflammation focus [7,9,10]. Neutrophils migrate through the endothelium to the infection focus and phagocytize pathogens or destroy them by releasing proteolytic enzymes from primary and secondary granules.

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Elevated expression of chemokines and their receptors in obesity leads to leukocyte infiltration of the adipose tissue and accumulation of tissue macrophages. This leads to a number of diseases associated with obesity. CCL20 chemokine and its receptor CCR6 are considered as the markers indicating the risk of these complications [8]. Proinflammatory cytokine IL-1β induces the expression of *CCL20* gene, while TNFα and IFNγ increase the expression of CCR6 receptor on neutrophils [6].

Our aim was to assess the role of chemokines in the inflammatory changes in tissues after abdominoplasty. To this End, we compared the levels of chemokines in blood cells and subcutaneous adipose tissue, as well as their receptors, in patients with normal body weight and obesity.

MATERIALS AND METHODS

The study included 320 patients (262 women and 58 men) aged 18-65, who underwent abdominoplasty surgery. We compared patients with metabolic alimentary obesity grades I-III (160 women, 36 men) and patients with normal body weight (102 women, 22 men).

The blood was collected from the medial cubital vein on an empty stomach into vacutainers with EDTA. The blood cells were counted on a Pentra Nexus HORIBA hematology analyzer (Horiba ABX SAS). Chemokines in the blood serum were assayed using commercial kits: MILLIPLEX kit Human Cytokine/Chemokine Panel I (Cat.# HCYTMAG-60K) and MILLIPLEX kit Human Cytokine/Chemokine Panel III (Cat.# HCYP3MAG-63K) (Merck-Millipore). Measurements were performed on a Luminex 200 Immunoassay Multiplex Analyzer (Luminex Corporation). The procedures of cell isolation from the surgical material and staining of receptors and intracellular proteins with antibodies were described earlier [1-4]. The fluorescence intensity was measured on a FACSCalibur flow cytometer (Becton Dickinson) using SimulSet program. The protein concentration was measured by Western blotting (Bio-Rad Protein Assay).

The obtained data were processed statistically by ANOVA and presented as $M \pm m$. Two groups were compared using the Student's t test; nonparametric Newman—Keuls multiple comparison test was used for statistical processing of several groups; the differences were significant at $p \leq 0.05$.

RESULTS

The levels of chemokines CCL2, CCL3, and CCL5 in the blood were measured on the next day after abdominoplasty. The chemokine levels in women were slightly lower than in men; at the same time, chemokine levels in both women and men with obesity were significantly higher ($p<0.05$) than in subjects with normal body weight (Table 1).

Considering the importance of chemokines for inflammation, the obtained results suggest that the level of signals attracting leukocytes to the adipose tissue is significantly higher in patients with obesity. During the post-operative period, this can promote the development of complications such as inflammation of the suture site, delayed healing of the surgical wound, and seroma development.

In patients with obesity, the expression of chemokine receptors CXCR2 and CXCR6 in blood neutrophils after abdominoplasty was significantly higher than in subjects with normal body weight. The values in men and women did not differ significantly, so they are pooled (Table 2).

Chemokine receptor CXCR2 activates chemotaxis signals in neutrophils and can work in synergy with receptors FPR1 and FPR2 for fMLP, which controls the chemotactic response to bacteria. After abdominoplasty, the patients with obesity demonstrate significantly higher expression of receptors FPR1 and FPR2 on blood neutrophils (in comparison with patients with normal body weight). Binding of CXCR6 receptor with CCL20 chemokine activates signal pathways of proinflammatory cytokines (IL-1β) that are regulated by nuclear transcription factor NF-κB. As a result, the synthesis of proinflammatory cytokines is enhanced, the inflammatory signals are

TABLE 1. Serum Concentration of Chemokines (pg/ml) in Patients after Abdominoplasty ($M \pm m$)

Chemokine	Men		Women	
	BMI 19.8±1.7 kg/m^2 (N=22)	BMI 38.3±4.1 kg/m^2 (N=36)	BMI 19.8±1.7 kg/m^2 (N=102)	BMI 38.3±4.1 kg/m^2 (N=160)
CCL2	161.42±9.11	217.38±10.08*	124.51±8.76	182.73±7.24*
CCL3	8.32±1.16	27.54±2.35*	11.73±1.89	24.97±2.13*
CCL5	205.21±11.84	284.53±12.22*	182.18±8.24	225.93±9.22*

Note. BMI: body mass index; *$p<0.05$.

TABLE 2. Comparison of the Expression of for Chemokine and fMLP Receptors Responsible for Neutrophil Chemotaxis in Patients with Normal Body weight and Obesity after Abdominoplasty (*M*±*m*)

Receptors		BMI 19.8±1.7 kg/m² (*N*=124)	BMI 38.3±4.1 kg/m² (*N*=196)
CXCR2	expression, %	21±4	32±3*
	fluorescence intensity, arb. units	104±8	186±11*
CXCR6	expression, %	44±3	62±5*
	fluorescence intensity, arb. units	121±5	206±7*
FPR1	expression, %	33±6	48±7*
	fluorescence intensity, arb. units	244±32	315±21*
FPR2	expression, %	29±4	41±5*
	fluorescence intensity, arb. units	244±32	315±21*

Note. BMI: body mass index; *p<0.05.

amplified, and inflammation in tissues is maintained at the constant level.

In patients with normal body weight, the expression of CCL20 chemokine was identified in 39.2±3.8% cells; in patients with obesity grades I, II, and III, CCL20 chemokine was expressed in 49.8±2.6, 57.1±2.1, and 71.5±3.1%, respectively (p<0.05). In persons with normal body weight, CCL20 fluorescence intensity (reflects the density of molecules on the cell) in blood neutrophils was 320±8 arb. units; in patients with obesity grades I, II, and III, this parameter increased to 339±5, 356±5, and 413±7 arb. units, respectively (p<0.05 for obesity grade II and grade III in comparison with the normal). This indicates high risk of inflammation after abdominoplasty in patients with obesity.

The expression of some chemokines of the CCL subfamily was significantly higher (p<0.05) in cells isolated from the subcutaneous adipose tissue of obese patients (Table 3).

In patients with grade I obesity, the expression of chemokines CCL2, CCL18, and CCL20 on adipose tissue cells was significantly higher (p<0.05). In grade II and III obesity, the level of all assessed chemokines was significantly higher in comparison with the corresponding parameters in patients with normal body weight.

Our findings suggest that expression of chemokines in adipose tissue cells and expression of chemokine receptors on neutrophils were enhanced in patients with obesity compared to persons with normal body weight. This creates prerequisites for elevated persistence of neutrophils at the site of post-operation scar, development of inflammatory alterations in this area, and can serve as an additional risk factor for the development of postoperative complications.

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TABLE 3. Expression of CCL Family Chemokines in Cells isolated from Specimens Obtained during Abdominoplsty (arb. units; *M*±*m*)

Chemokine	BMI<24 (normal) (*N*=25)	BMI>25 (*N*=18)	BMI>30 (grade I obesity) (*N*=22)	BMI>35 (grade II obesity) (*N*=24)	BMI>40 (grade III obesity) (*N*=21)
CCL2	82±3	86±4	141±6*	201±13*	232±8*
CCL3	114±6	119±7	131±5	189±7*	198±11*
CCL4	67±8	66±5	84±7	133±6*	181±5*
CCL5	93±4	98±4	112±9	148±8*	163±5*
CCL18	54±2	61±3	91±3*	124±4*	122±6*
CCL20	109±2	116±3	144±2*	171±5*	236±8*

Note. BMI: body mass index; *p<0.05.

relationship with the state of adipose tissue cells. Annaly Plast. Rekonstrukt. Estet. Khir. 2017;(1):100-101. Russian.
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