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Undernourishment in patients with connective tissue dysplasia: the role of proinflammatory cytokines and adipokines, and genetic factors

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Summary

Objective

Evaluate the levels of specific and non-specific mediators of inflammation (interleukins 1 and 6 (IL1 and IL6, respectively), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), adipokines (leptin, soluble leptin receptor, adiponectin, resistin) and the frequency of mutations of soluble leptin receptors (Arg223Gln) in undernourished patients with connective tissue dysplasia

Materials and methods

A cross-section study involved 94 patients (50% males, 50% females). Average age of patients was 30,5±0,8 years. The I group included 34 patients with connective tissue dysplasia and signs of undernourishment, the II group consisted of 30 patients with connective tissue dysplasia and no signs of undernourishment, the control group included 30 patients without connective tissue dysplasia. The groups were similar with respect to age and gender. We estimated the levels of IL1, IL6, TNF-α, CRP, leptin, soluble leptin receptors, adiponectin, resistin, and the frequency of mutations of soluble leptin receptors (Arg223Gln).

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Results

Undernourishment was associated with changes of immune status in patients with connective tissue dysplasia. These changes consisted of leucopenia, lymphocytopenia, decreased CRP concentration, higher levels of proinflammatory IL1 and IL6. IL6 changes correlated with the severity of undernourishment in connective tissue dysplasia (moderate negative correlation), the severity of leucopenia correlated with the degree of TNF-α levels decrease (significant direct correlation). We registered the change of adipokines' concentrations that was expressed as low leptin and resistin levels, higher concentration of adiponectin and soluble leptin receptors. In 73,44% of patients these changes were associated with soluble leptin receptor gene polymorphisms: Arg223Gln A/G was present in 50,0% of patients, and Arg223Gln G/G was found in 23,44% of patients.

Conclusion

Change of the levels of adipokines in patients with connective tissue dysplasia may be used not only as diagnostic criteria of severity of undernourishment, but also as factors determining different risks of associated pathologies. Increased IL-6 levels combined with low CRP concentration can be a sign of latent inflammatory process, autoimmune and allergic diseases, and decreased concentration of TNF-α associated with leucopenia can be an evidence of the risk of infectious or oncologic diseases.

Key words

Connective tissue dysplasia, leptin, adiponectin, resistin, c-reactive protein, tumor necrosis factor-α

Introduction

Malnutrition is an important phenomenon for internal medicine due to bad prognosis associated with this syndrome, high risk of chronic diseases development and high letality.

It is known that a part of metabolic disorders are genetically determined and that mutations of receptors of adipokines, hormones produced by adipose tissue, can lead to metabolic abnormalities [1].Although the frequency and the intensity of impaired trophologic status in patients with connective tissue dysplasia (CTD), malnutrition remains poorly studied [2, 3].

The objective of this study was to evaluate the levels of specific and non-specific mediators of inflammation (interleukins 1 and 6 (IL1 and IL6, respectively), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), adipokines (leptin, soluble leptin receptor, adiponectin, resistin) and the frequency of mutations of soluble leptin receptors (Arg223Gln) in undernourished patients with connective tissue dysplasia

Materials and methods

A cross-section study involved 94 patients (50% males, 50% females). Average age of patients was 30,5±0,8 years. The I group included 34 patients with connective tissue dysplasia and signs of undernourishment, the II group consisted of 30 patients with connective tissue dysplasia and no signs of undernourishment, the control group included 30 patients without connective tissue dysplasia. The groups were similar with respect to age and gender. Diagnosis of genetically determined (non-syndromic) CTD was defined according to the National guidelines [4]. Estimation of trophological status was performed according to the Russian guidelines (2012) [5]. Hormone levels were evaluated using enzyme-linked immune sorbent assay (ELISA) and the following kits:l Leptin- ELISA «DBC», Canada; human leptin receptor - ELISA«BioVendor», Czech Republic; resistin- ELISA «Mediagnost», Germany; adiponectin - ELISA «Mediagnost», Germany. Genetic mutations were estimated using polymerase chain reaction and electrophoretic detection of reaction products with amplificator of scientific production company "Liatech". Evaluation of proinflammatory cytokines levels was done using appropriate test-systems for ELISA (ELISA-BEST) and multiwall spectrophotometer iMark (BIORAD). Statistical analysis of the results was performed using Statistica 6.0 software.

Results

Clinical characteristic of patients is present in the Table 1. Patients with CTD and undernourishment had lower body weight, body mass index (BMI), brachial muscles circumference (BMC), triceps skin-fold thickness (TSFT), and number of lymphocytes. These parameters are standard for diagnostics of undernourishment and they are included in the "scale of organism's nutritional status" [5].

Apart from the change of "standard parameters", patients with CTD and undernourishment had statistically significant reduction of leukocyte levels in

Table 1. **Clinical characteristic of patients**

Parameter	I group (n=34)	II group (n=30)	Control group (n=30)	p
	M±m	M±m	M±m	
Height, cm	169,5± 0,96	172,0±0,70	166,5±1,97	>0,05
Body weight, kg	48,5±0,74	65,0±1,04	59,0±2,08	<0,016
BMI, kg/m^2	17,76±0,16	22,5±0,21	22,85±0,28	<0,017
BMC, cm	23,0±0,31	24,0±0,50	24,5±0,35	>0,053
TSFT, cm	7,8±0,06	10,6±0,04	10,65±0,12	<0,012
Total protein, g/L	58,0±0,49	60,5±0,57	65,0±0,47	>0,052
Albumin, g/L	34,0±0,23	33,5±0,49	33,5±0,49	>0,056
Lymphocytes, %	14,0±0,88	27,0± 0,77	25,5±0,69	<0,005
Leukocytes	5,75±0,21	6,75±0,12	6,8±0,10	<0,036

Comment: BMI – body mass index, BSA – body surface area, BMC – brachial muscles circumference, TSFT – triceps skin-fold thickness.

Table 2. **Proinflammatory cytokines' levels in studied groups**

Characteristic	I group (n=34)	II group(n=30)	Control group (n=30)	P
CRP	1,86±1,19	3,52±0,69	4,21±1,92	<0,01
IL -1	8,01±0,83	7,28±0,40	6,79±0,67	<0,02
IL -6	1,59±0,18	1,368±0,15	1,16±0,37	<0,01
TNF- α	11,76±1,41	13,03±1,79	9,71±1,25	<0,01

Comment: C-reactive protein, IL-1 – interleukin 11, IL-6 – interleukin-6, tumor necrosis factor α – TNF-α

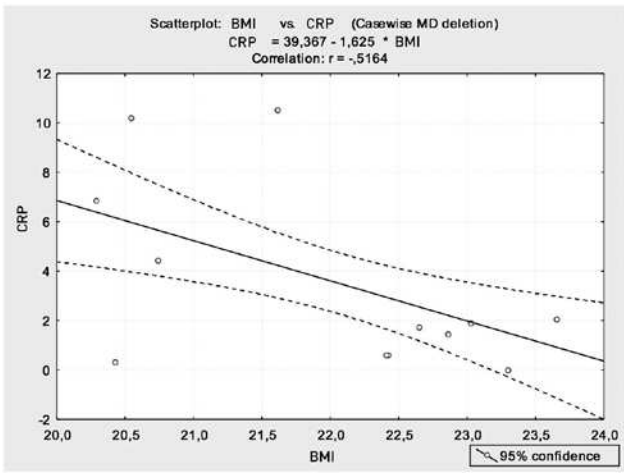
peripheral blood (5,75±0,21 × 10^9/L, comparing with the II group - 6,75±0,12 × 10^9/L and the control group - 6,8±0,11 × 10^9/L, (p=0,036; p= 0,043 respectively).

Complex evaluation of immunological status demonstrated that patients with CTD and undernourishment had higher levels of interleukin-1 (IL-1) and interleukin-6 (IL-6), whereas the concentrations of C-reactive protein (CRP) and tumor necrosis factor α (TNF-α) were lower than in patients of the II group and the control group (Table 2).

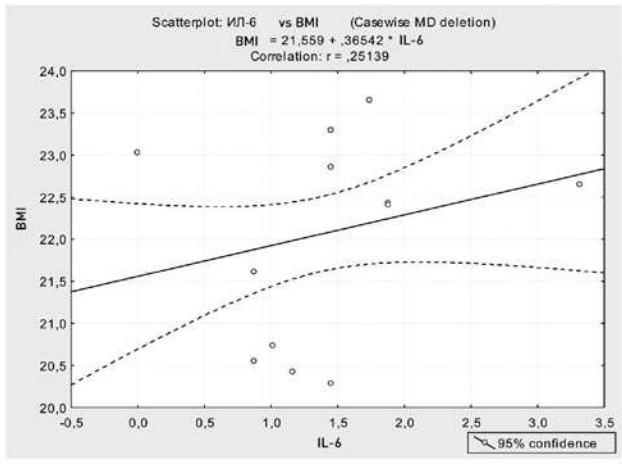
Correlation analysis revealed significant reverse correlation of moderate power between BMI dynamics and CRP concentration in patients with CTD and undernourishment (r=-0,35, p=0,438, Figure 1A) or normal body weight (r=-0,52, p=0,0327, Figure 2A), at the same time there were no significant correlations in the control group (Figure 3A). Undernourished patients with CTD demonstrated significant reverse correlation of BMI and IL-6 concentration (r=-0.39, p=0,0041, Figure 1B), and this correlation was not observed in the II group and in the control group (Figures 2B and 3B, respectively).

Patients with CTD and undernourishment demonstrated significant direct correlation between TNF-α levels and leukocyte concentration in peripheral blood (r=0,41 p=0,0381) (Figure 4A), at the same time there was no correlation between this parameter and lymphocyte concentration (one of standard parameters characterizing undernourishment) (Figure 4B).

ELISA essay with the use of allele-specific polymerase chain reaction revealed significant differences in serum concentration of several fat tissue me-



A

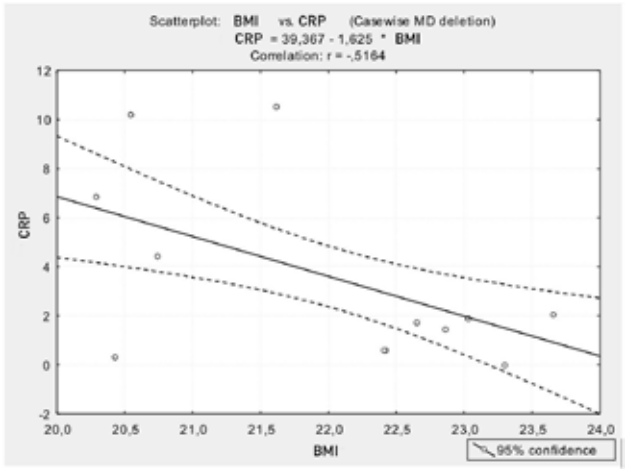


B

Figure 1

A. Correlation between BMI and CRP in patients with CTD and undernourishment

B. Correlation between BMI and IL-6 in patients with CTD and undernourishment



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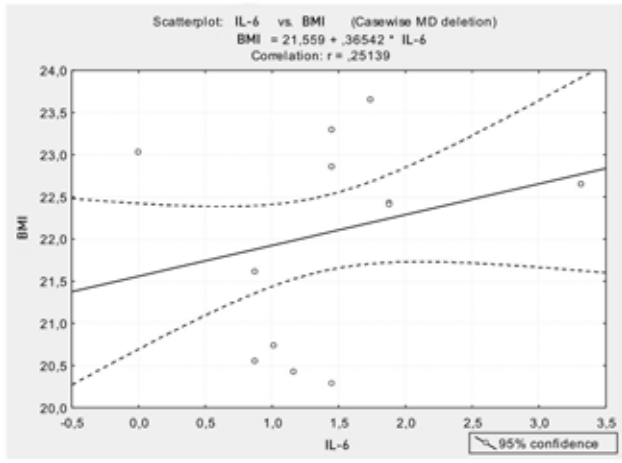
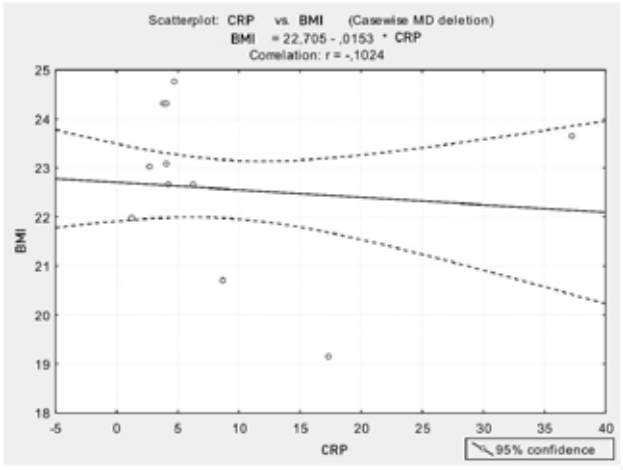


B

Figure 2

A. Correlation between BMI and CRP in patients with CTD and normal body weight

B. Correlation between BMI and CRP in patients with CTD and normal body weight



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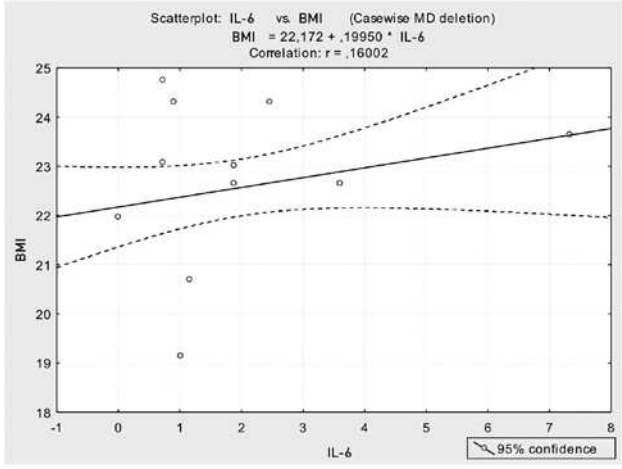
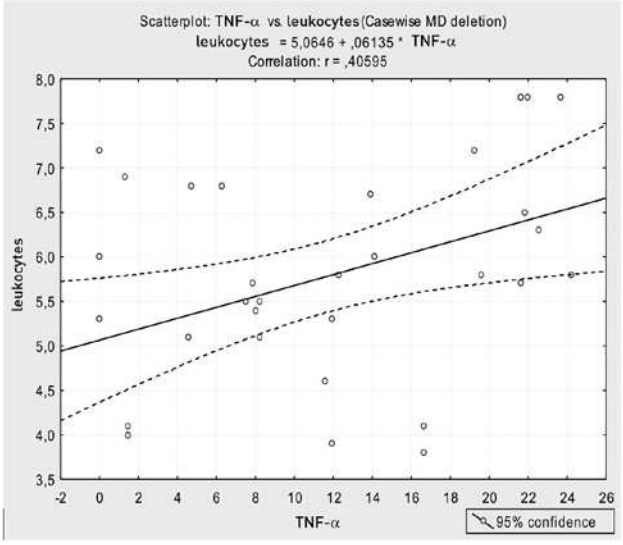


B

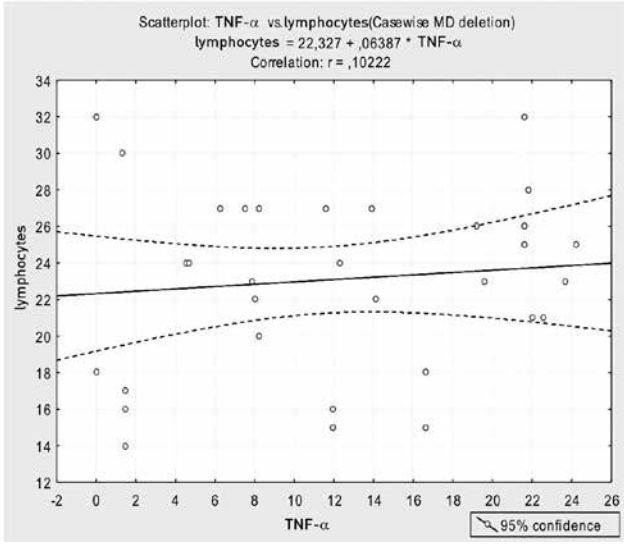
Figure 3

A. Correlation between BMI and CRP in the control group

B. Correlation between BMI and CRP in patients with CTD in the control group



A



B

Figure 4

A. Correlation of leukocyte count and TNF-α in patients with CTD and undernourishment

B. Correlation of lymphocyte count and TNF-α in patients with CTD and undernourishment

Table 3. **Characteristics of adipokines in studied groups**

Characteristics	Patients with CTD and signs of undernourishment	Patients with CTD without signs of undernourishment	Control group	P
Leptin 0,51±0,17		0,88±0,15	2,25±0,20	<0,01
Leptin receptors	7,35±0,45	6,24±0,56	5,91±0,35	<0,02
Resistin	4,58±0,24	7,64±0,60	5,50±0,34	<0,01
Adiponectin	13,38±0,45	9,54±0,46	10,59±0,53	<0,01

Table 4. **The frequency of LEPR mutations in studied groups**

Parameters	I group, n (%)	II group, n (%)	Patients with CTD, n (%)	Control group,n (%)
LEPR mutations (Arg223Gln) A/A	14 (41,18 %)**	3(10,0 %)	17(26,56%)	12(40,0%)
LEPR mutations (Arg223Gln) A/G	12(35,29 %)**	20(66,67 %)	32(%50,0)*	18(60,0%)
LEPR mutations (Arg223Gln) G/G	8(23,5 3%)	7(23,33 %)	15(23,44%)**	0

Comment:
* statistically significant differences comparing with the control group,
** statistically significant differences comparing with the II group.

diators. Adiponectin, soluble leptin receptor (LEPR) levels in patients with CTD and low body weight were significantly higher than in patients with CTD without undernourishment and in the control group, and the concentrations of resistin and leptin were consequently lower in patients with CTD and undernourishment comparing with patients with CTD without signs of undernourishment and the control group (Table 3).

Investigation of the frequency of LEPR (Arg223Gln) mutations in patients of studied groups revealed that 73,44% of abnormalities of leptin metabolism in CTD are genetically determined.

LEPR (Arg223Gln) G/G mutations were found in 23,44% of patients with CTD (p<0,05) and undernourishment, Arg223Gln A/G mutation were present in 50,0% of patients (p<0,05), and they were absent in the groups of comparison and control (Table 4).

Patients with LEPR G/G genotype had body weight 16,5±0,3 kg lower (p<0,0001) comparing with A/G genotype (II group), and their body weight was 10,5±1,3 kg lower comparing with the patients having AA genotype (p<0,0001).

This study demonstrated that the presence of undernourishment in patients with CTD is accompanied with evident immunological changes like leucopenia, lymphocytopenia, lower CRP levels, and high concentration of proinflammatory cytokines (IL-1, IL-6). IL-6 changes in patients with CTD and low body weight correlate with the degree of BMI reduction (negative correlation of moderate power), and the severity of leucopenia correlates with TNF-α concentration (significant direct correlation). These changes have been registered together with imbalanced levels of key mediators regulating energetic homeostasis (leptin, leptin receptors, resistin, adiponectin) and in 73,44% of cases they were associated with polymorphism of soluble leptin receptor gene (Arg223Gln)G/G.

Discussion

It is known that fat tissue is not only the largest energy storage in the organism, but it also possesses para-, auto-, and endocrine activity secreting big number of hormones called adipokines, between which there are leptin, adiponectin, resistin, grelin, insulin-like growth factor-1 etc.

Apart from it, adipocytes, like T-lymphocytes and macrophages, produce many cytokines and trigger the chain of inflammatory processes, and inflammation processes become stable, systemic and highly intensive.

IL-6 is one of proinflammatory cytokines produced by adipocytes that reveals its action not only in fat tissue, but also at systemic level [6]. IL-6 acts as a potent activator of hypothalamic-pituitary-adrenal axis and consequently lead to cachexia. Changes of leptin and IL-6 concentrations correlate with decrease of CD8+ T-lymphocytes count and increase of CD4= lymphocytes number that can participate in pathogenesis of autoimmune diseases.

It is known that TNF-α also reflects the severity of muscle and fat tissue depletion and that it is produced by activated neutrophils and mononuclear phagocytes. Apart from it, TNF-α is the key mediator of antitumoral immunity, and TNF-α reduction can reflect not only activation of antimicrobial immunity, but also indicate a certain degree of oncological risk [7].

Thus, increased levels of proinflammatory cytokines like IL-6 together with low concentration of C-reactive protein may indicate the presence of slowly developing latent inflammatory process or the

risk of autoimmune or allergic diseases, and reduced concentration of TNF-α together with leucopenia may be a sign of infectious diseases or cancer.

Conclusion

The presence of undernourishment in patients with CTD is accompanied with evident abnormalities of key adipokines' levels like reduced concentration of leptin and resistin in peripheral blood, increased concentration of soluble leptin receptors and adiponektin levels.

Apart from it, imbalanced immunological parameters like leucopenia and lymphocytopenia, lowered CRP levels and higher concentrations of proinflammatory cytokines like IL-1 and IL-6 have been registered.

Detected immunological changes in patients with CTD may be used not only as diagnostic criteria of severity of undernourishment, but also as factors determining different risks of associated pathologies. Increased IL-6 levels combined with low CRP concentration can be a sign of latent inflammatory process, autoimmune and allergic diseases, and decreased concentration of TNF-α associated with leucopenia can be an evidence of the risk of infectious or oncologic diseases.

Conflict of interest: None declared.

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