

# Assessment of Adipokines, CXCL16 Chemokine Levels in Patients With Rheumatoid Arthritis Combined With Metabolic Syndrome

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## ABSTRACT

**OBJECTIVE:** Rheumatoid arthritis (RA), which is a chronic systemic inflammatory disease, is associated with accelerated atherosclerosis and an increased risk of cardiovascular disease (CVD), but the causal factors have yet to be completely elucidated. The studies show that the prevalence of metabolic syndrome (MetS) was significantly higher in RA patients compared to the population. In RA and MetS inflammation and atherosclerosis are closely linked. The level of chemokines and adipokines, which may play a role in the development of atherogenesis in RA with MetS patients is currently unknown. In this study, we investigated the level of chemokine C-X-C motif chemokine ligand 16 (CXCL16) and adipokine in RA with MetS patients and assessed the association of biomarkers with clinical and biochemical activity scores of RA and components of MetS.

**METHODS:** Blood serum of 298 people (48—patients with RA and MetS, 82—with RA without MetS, 105—with MetS, 63—control group without both RA and MetS) was tested for (CXCL16), Resistin, Leptin and Fibroblast Growth Factor 21 (FGF21) levels by fluorescent antibody technique. Statistical analysis was performed using SPSS version 18.0.

**RESULTS:** The biomarker study showed the highest level in the RA with MetS patient group; but as compared with the RA group the differences were insignificant. CXCL16 (Me = 426.2 pg/ml (Q<sub>25-75</sub> 250.5-527.6), resistin (Me = 8685.4 pg/ml (Q<sub>25-75</sub> 6480.8-13629.1), and FGF21 (Me = 443.6 pg/ml (Q<sub>25-75</sub> 772.9-916.3) proved to be significantly augmented in RA with MetS patients group, and in RA without MetS patients group (Me = 312.7 (Q<sub>25-75</sub> 199.4-517.7) pg/ml; Me = 8265.3 (Q<sub>25-75</sub> 5779.7-13340.5) pg/ml; Me = 412.4 (Q<sub>25-75</sub> 300.4-497.4) pg/ml, respectively) as compared with MetS patients group (Me = 189.4 (Q<sub>25-75</sub> 130.3-280.6) pg/ml; Me = 5364.8 (Q<sub>25-75</sub> 2368.9-10160.9) pg/ml; Me = 133.2 (Q<sub>25-75</sub> 76.2-268.6) pg/ml, respectively; *P* = <.001). Leptin level in all groups was higher than in the control group, but there were no differences between groups. The correlation analysis found a positive relationship between the leptin level and the waist circumference (*r*<sub>s</sub> = 0.39; *P* = .007) in the RA with MetS patients, the association of biomarkers with DAS28 score and ESR did not have any statistical significance. Conclusions: The augmented chemokine, resistin and FGF21 in the RA with MetS patients proves the systemic inflammation which is the basis of RA; the augmented leptin is linked to the abdominal obesity. These data are somewhat of an explanation of the increased risk of the CVD development in RA with MetS people. A differentiated specification can be useful to assess the cardiovascular risk of patients and justify prompt personalized treatment.

**KEYWORDS:** Rheumatoid arthritis, metabolic syndrome, biomarkers, CXCL16, Leptin, Resistin, FGF21

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## Introduction

Cardiovascular disease (CVD) continues to be a pressing global health problem. In Kazakhstan's healthcare system, the circulatory diseases (CD) are also the dominants in the morbidity and mortality rates, totaling 2595.7 and 174.8 instances per 100,000 population respectively in 2017, with the myocardial infarction and stroke taking up 74% of the CD-associated deaths.<sup>1</sup> The inflammation proved to be crucial in the development and progression of atherosclerosis, which is the key factor of cardiovascular complications.

Rheumatoid arthritis (RA) being a chronic systemic inflammatory disease associates with an increased cardiovascular risk

(CVR) which is comparable to the risk of type 2 diabetes.<sup>2</sup> The link between atherosclerosis and RA is still incompletely understood. Circulating biomarkers in the pathophysiology of atherosclerosis, including inflammation, are the subject of study in rheumatoid arthritis.<sup>3-5</sup>

Metabolic syndrome is a cluster of traditional risk factors for CVR, including abdominal obesity, hypertension, high triglycerides, and low levels of high-density lipoprotein cholesterol.<sup>6,7</sup> The meta-analysis has shown that the metabolic syndrome is associated with a 2-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality rates.<sup>8</sup> Several studies report that patients with RA have a



significantly higher prevalence of the metabolic syndrome (MtS) compared to the general population.<sup>9</sup> The metabolic syndrome (MtS) is believed to increase the risk of CVR in patients with RA.<sup>10</sup> The metabolic syndrome has also been found to increase the risk of having moderate-to-high disease activity in RA patients.<sup>11</sup>

Systemic inflammation is assumed to be a common pathogenetic link of rheumatoid arthritis and metabolic syndrome. The exact pathogenesis of chronic inflammation is not yet completely studied. The adipocytokines and chemokines have been found to be among the major hallmarks for the pathogenesis of chronic inflammation. Chemokines play an important role in recruitment and functioning of immune cells, and angiogenesis in rheumatoid arthritis.<sup>12</sup> The stimulation of chemokine receptors aggravates an inflammatory response in adipose tissue. By regulating macrophage recruitment and M1/M2 phenotype switch they cause the insulin resistance.<sup>13</sup> Inhibition of CCR5 expression reduces the aggregation of pro-atherogenic cytokines to the site of arterial injury). Adipokines produced by white adipose tissue contribute to hepatic insulin sensitivity, skeletal muscle, and adipose tissue insulin sensitivity and to metabolic syndrome. Proinflammatory adipokines also participate in the regulation of the immune response and endothelial function, and have an effect on the synovial membrane and cartilage.<sup>14</sup>

The link between metabolic syndrome and rheumatic diseases is complex. Several studies have shown that there was no association between leptin and resistin levels and the RA disease activity,<sup>15,16</sup> other studies report about association of leptin and resistin levels and the clinical and laboratory scores of RA disease activity.<sup>17-19</sup> Increased FGF21 levels in RA patients compared to osteoarthritis patients was associated with BMI but not with RA disease activity.<sup>20</sup>

Since the biomarkers may have several participation roles and ways, we can assume that the RA together with the MtS enhances the biological effects of biomarkers. There are few studies on adipokine status in RA patients with MtS.<sup>21,22</sup> We did not find any comprehensive study of adipokine and CXCL16 chemokine levels in RA patients with MtS compared to RA patients without MtS, and MetS patients.

Thus, the objective of our study was to investigate the level of chemokine CXCL-16 and adipokines (leptin, resistin, FGF21) in RA patients with MtS.

## Material and Methods

### Subjects

We carried out an observational cohort study. The study was carried out at the level of 2 municipal outpatient clinics (#1 and #3) in Karaganda city (Kazakhstan). Inclusion criteria: age from 25 to 65 years, patients with verified rheumatoid arthritis, metabolic syndrome. The control group consisted of persons without metabolic syndrome and rheumatoid arthritis, corresponding to gender and age. Exclusion criteria: previous

myocardial infarction, history of acute cerebrovascular accident, end-stage cardiac, renal, liver failure, pregnancy.

In total, the study included 299 people. Of these, 48 are patients with rheumatoid arthritis in combination with metabolic syndrome (RA with MtS), 84 people are patients with rheumatoid arthritis without metabolic syndrome (RA without MtS), 105 people are people with metabolic syndrome without rheumatoid arthritis (MtS), 62 people made up a control group (Control).

Patients were examined in the setting of the Karaganda Medical University. All participants submitted a written informed consent prior to any study-associated procedure. The study was approved by the Institutional Review Board of the Karaganda Medical University (registration number 47 of August 10, 2018). The study was carried out in accordance with the Good Clinical Practice Guidelines (GCP) and the Declaration of Helsinki.

### *Diagnosis of rheumatoid arthritis and determination of activity*

The diagnosis of rheumatoid arthritis was made on the basis of the 2010 ACR/European League Against Rheumatism (EULAR)/American College of Rheumatology criteria for RA.<sup>23,24</sup> RA activity was determined by the Disease Activity Score 28-ESR (DAS28-ESR).

### *Metabolic syndrome*

Metabolic syndrome was classified according to the criteria of the International Diabetes Federation (IDF, 2009) based on the presence of central obesity (waist circumference (WT)  $\geq 94$  cm for Caucasian males and  $\geq 80$  cm for women in combination with any 2 of the following 4 factors: triglycerides (TG):  $\geq 150$  mg/dl (1.7 mmol/l), or specific treatment for the disorder; a decrease in the content of high density lipoproteins (HDL cholesterol):  $< 40$  mg/dl (1.03 mmol/l) in men and  $< 50$  mg/dl (1.29 mmol/l) in women, or specific treatment for this disorder; increased blood pressure (BP): systolic blood pressure (SBP)  $\geq 130$  or diastolic blood pressure (DBP)  $\geq 85$  mm Hg, or treatment of previously diagnosed hypertension; increased fasting plasma glucose:  $\geq 100$  mg/dl (5.6 mmol/l), or previously diagnosed type 2 diabetes mellitus.<sup>25</sup>

### *Questionnaire*

The questionnaire was comprised of questions about gender, age of the respondent, socio-demographic aspects (nationality, marital status, education background, occupation). The "employed" category of the "occupation" line included the civil servants, private sector workers, and entrepreneurs. The "unemployed" category included pensioners and actually unemployed people. The questionnaire also comprised the questions about daily 30-minute physical activity (PA), active smoking at that point.

### Physical examination

We measured the waist circumference (WC) by standard method to verify the metabolic syndrome. Blood pressure (BP) was measured 3 times in both hands after 15 minutes of rest. The final result of the measurement was the arithmetic mean of the 3 measurements. The BMI was calculated in all patients.

### Laboratory diagnostics

Glucose was measured using the Accu-Chek Active system in capillary blood after 12 hours of fasting. The high-density lipoprotein cholesterol was determined by selective precipitation method. Blood samples for laboratory evaluation of biochemical parameters were obtained after participants' overnight fast. Cholesterol, (TG), uric acid levels in the blood were determined by enzymatic colorimetric method with selective protection without precipitation. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald Equation. The erythrocyte sedimentation rate was determined (ESR) by Panchenkov's method.

To determine soluble CXCL16, Leptin, Resistin and FGF21 in blood serum, a multiplex immunofluorescence assay was performed using XMap technology (examination on the surface of fluorescently coded magnetic particles known as MagPex—microspheres). For this, a Bioplex 3D device and a set of reagents Milliplex Human Cardiovascular disease Magnetic Bead, Bio-Plex Pro Human Diabetes, Bio-Plex RBM Hu Metab Panel 2 were used. A variant with a 16-hour incubation was used. The results were evaluated using the patented Luminex Xponent v. 4.0.846.0 and Bioplex Manager 6.1. CXCL16 minimum detectable concentration 13.2 pg/ml intra-assay CV <10% inter-assay CV <20%. FGF-21 low limits of qualification 25 pg/ml low limits detection 11 pg/ml intra-assay CV 16% inter-assay CV 19%. Leptin Low limits of qualification 11.5 pg/ml low limits detection 3.1 pg/ml intra-assay CV 3% inter-assay CV 4%. Resistin Low limits of qualification 2.3 pg/ml low limits detection 1.3 intra-assay CV 3% inter-assay CV 4%.

### Statistical processing

Statistical processing of the material was carried out using the statistical package for social sciences (SPSS for Windows, version 23.0, SPSS Inc., Chicago, Illinois, USA). All continuous variables were checked for normal distribution using the Kolmogorov–Smirnov criterion. In our study, all variables had an abnormal distribution, so the data is presented as median and IQR. Categorical values were represented as numbers and percent. Comparisons between groups were made using the Kruskal–Wallis test. Significance level was calculated by the following formula:  $P = 1 - .951/n$ , where  $n$  is the number of comparisons made. The number of paired comparisons between the 4 groups was 6, so the significance level is  $P = 1 - .951/6 = .0085$ . The 2-tailed correlation analysis was

carried out using Spearman's method. The significance level for correlation analysis assumed to be  $P \leq .005$ .

### Study results

The characteristics of the surveyed are presented in Table 1. Females dominated in all groups, the mean of age did not differ significantly in the groups RA with MtS, RA without MtS and MtS. The mean of age in the Controls was lower than in the other groups. The analysis of socio-demographic points showed that married people predominated in all groups. The proportion of people with higher education was higher in the MtS group (49.4%) compared to other groups. Persons with disabilities were noted in RA with MtS—41.6% and in RA without MtS—41.5%. Despite the presence of rheumatoid arthritis, we found no differences between groups in the frequency of daily 30-minute PA. The percentage of active smoking in the groups did not differ and did not exceed 18%. It should be noted that in the RA without MtS group, the median BMI did not belong to normal values and amounted to 25.9 kg/m<sup>2</sup>.

An analysis of the parameters of carbohydrate and lipid metabolism and biomarkers is presented in Table 2. Perhaps, due to the “lipid paradox” in rheumatoid arthritis, we did not find changes in lipid metabolism, while in the MtS group there were the highest blood glucose, triglycerides, low density lipoproteins and the lowest high density lipoproteins. Cholesterol levels did not differ between groups.

Analysis of biomarker levels showed the following results: adipokine levels have the lowest values in Control. It is clearly seen that the levels of biomarkers increase from the Control group to the RA with MtS group and acquire the highest rates in the RA with MtS group.

In a pairwise comparison between groups Table 3, it was found that in the group of patients with RA with MtS, the glucose level was higher in patients with RA without MtS and was lower than in patients with MtS. Comparison of lipid metabolism indices showed that the HDL-C level in patients with RA with MtS and RA without MtS was higher than in the group with MtS and Controls. The triglyceride level in all groups was higher than the level of the control group, but in patients with MtS it was higher than in the group with RA without MtS. The level of biomarkers had the highest values in the group of patients with RA with MtS, but compared with the group of patients with RA without MtS, this increase in biomarkers did not have statistically significant differences. When comparing biomarkers of the group with RA with MtS with the group of patients with MtS, it was found that the level of chemokine CXCL16, resistin and FGF21 was significantly higher in RA with MtS.

Correlation analysis of biomarkers with clinical and laboratory parameters showed that in the RA with MtS group, the leptin level had a positive relationship with WC, the resistin level—with the age of the patients (Table 4). FGF21 levels were negatively associated with HDL. We found no correlations

**Table 1.** Characteristics of patients with RA with MtS, RA without MtS, MtS and controls.

CRITERIA	RA WITH MTS (N=48)	RA WITHOUT MTS (N=82)	MTS (N=105)	CONTROLS (N=63)	P
Age, years Me (Q <sub>25</sub> -Q <sub>75</sub> )	57 (52-62)	53.0 (47.0-59.0)	56.0 (47.5-60.0)	42.0 (38.0-49.5)	.004
Sex, n (%)					
Female	36 (75)	59 (72)	67 (63.8)	38 (60.3)	.257
Male	12 (25)	23 (28)	38 (36.2)	25 (39.7)	
Marital status, n (%)					
Single	4 (8.4)	6 (7.4)	10 (9.5)	12 (19.0)	.001
Married	29 (60.4)	64 (78.0)	77(73.3)	47 (74.6)	
Divorced	5 (10.4)	6 (7.3)	8 (7.7)	3 (4.8)	
Widower/widow	10 (20.8)	6 (7.3)	10 (9.5)	1 (1.6)	
Education background, n (%)					
Secondary	9 (18.8)	7 (12.3)	4 (3.9)	18 (28.6)	<.001
Vocational secondary education	25 (52.1)	48 (58.5)	49 (46.7)	29 (46.0)	
Higher	14 (29.1)	24 (29.2)	52 (49.4)	16 (25.4)	
Occupation, n (%)					
Employed	13 (27.2)	23 (28.0)	63 (60)	42 (66.6)	<.001
Unemployed	15(31.2)	25 (30.5)	42 (40)	21 (33.4)	
Disabled	20 (41.6)	34 (41.5)	0 (0)	0(0)	
FA, n (%)	23 (47.9)	54 (65.9)	61 (58.1)	38 (60.3)	.167
Smoking, n (%)	8 (16.7)	13 (15.9)	18 (17.1)	12 (19)	.967
WC, cm Me (Q <sub>25</sub> -Q <sub>75</sub> )					
Female	98 (90.0-102.7)	90.0 (81.0-98.0)	100 (89-110)	72.5 (68.7-75.2)	<.001
Male	99.5 (95.0-108.5)	90.0 (80.0-92.0)	107.5 (100-118.5)	80.0 (78.5-91.0)	
BMI, kg/m <sup>2</sup> Me (Q <sub>25</sub> -Q <sub>75</sub> )	29.2 (25.9-32.7)	25.9 (21.9-29.3)	30.1 (27.3-34.5)	22.8 (20.8-24.6)	<.001
SBP, mm Hg Me (Q <sub>25</sub> -Q <sub>75</sub> )	135 (120-148)	120 (113.7-130.0)	130.0 (120.0-140.0)	110.0 (100.0-120.0)	<.001
DBP, mm Hg Me (Q <sub>25</sub> -Q <sub>75</sub> )	80 (80-90)	70.0 (70.0-80.0)	80.0 (80.0-90.0)	70.0 (65.0-80.0)	<.001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FA, physical activity; SBP, systolic blood pressure; WC, waist circumference.

between CXCL16 and adipokines with DAS28, ESR. As well as in the RA with MtS group, in the RA without MtS group, a significant positive correlation was found between the level of leptin and WC (Table 5). HDL levels were accompanied by increases in CXCL16 and resistin. We did not find significant correlations of biomarkers with activity on the DAS 28-ESR scale and ESR level.

## Discussion

Our study was the first to compare such biomarkers as CXCL16, leptin, resistin, and FGF21 in RA patients with MetS compared to RA patients without MetS, and MetS patients.

We found a high level of CXCL16 in the RA with MetS group compared to MetS group and no difference in RA without MetS group. Studies of CXCL16 levels in RA patients are conflicting: some authors have found highly elevated CXCL16 levels in serum and in synovial fluid in RA patients compared to osteoarthritis patients, and the control group.<sup>26,27</sup> In another study, the chemokine CXCL16 levels were the same as in the control group,<sup>28</sup> but the authors noted that CXCL16 was highly elevated in patients with seropositive RA compared to seronegative ones. We have not found any significant correlation relationship between CXCL16 and ESR, the RA disease activity score. While Zhang et al, Li et al in their independent studies have shown a correlation of the CXCL16 levels with

**Table 2.** Indicators of carbohydrate, lipid metabolism and the level of biomarkers in patients with RA with MTS, RA without MTS, MTS and controls.

PARAMETER, ME (Q <sub>25</sub> -Q <sub>75</sub> )	RA WITH MTS (N=48)	RA WITHOUT MTS (N=82)	MTS (N=105)	CONTROL (N=63)	χ <sup>2</sup>	P
Serum cholesterol, mmol/l	5.3 (4.7-6.8)	5.9 (4.7-7.5)	5.5 (4.6-6.9)	5.4 (4.5-6.7)	1.79	.616
Glucose, mmol/l	5.7 (5.2-6.0)	5.2 (5.0-5.7)	6.1 (5.6-7.9)	5.4 (5.1-5.7)	74.2	<.001
TG, mmol/l	1.2 (0.8-2.3)	1.06 (0.6-1.4)	1.5 (0.9-2.1)	0.7 (0.5-1.1)	45.0	<.001
HDL-C, mmol/l	1.43 (1.07-2.93)	1.6 (1.3-2.6)	0.96 (0.81-1.12)	0.99 (0.19-1.45)	97.0	<.001
LDL-C, mmol/l	2.5 (2.0-3.9)	3.0 (2.3-4.0)	3.3 (2.9-4.5)	1.3 (1.1-1.5)	13.9	.003
CXCL16, pg/ml	426.2 (250.5-527.6)	312.7 (199.4-517.7)	189.4 (130.3-280.6)	274.4 (221.0-362.9)	72.9	<.001
Leptin, pg/ml	16536.8 (8148.6-31648.4)	12186.5 (5207.1-25070.1)	11184.0 (6100.7-31916.1)	4230.6 (1799.6-8129.4)	46.8	<.001
Resistin, pg/ml	8685.4 (6480.8-13629.1)	8265.3 (5779.7-13340.5)	5364.8 (2368.9-10160.9)	4886.9 (3127.2-6730.9)	40.3	<.001
FGF21, pg/ml	443.6 (772.9-916.3)	412.4 (300.4-497.4)	133.2 (76.2-268.6)	126.1 (45.1-232.4)	89.8	<.001

Abbreviations: CXCL16, C-X-C motif chemokine ligand 16; FGF21, fibroblast growth factor 21; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglycerides.

**Table 3.** Comparative analysis between groups in terms of carbohydrate and lipid metabolism and the level of biomarkers.

PARAMETER	RA WITH MTS AND RA WITHOUT MTS		RA WITH MTS AND CONTROL		RA WITHOUT MTS AND MTS		RA WITHOUT MTS AND CONTROLS		MTS AND CONTROL			
	U	P	U	P	U	P	U	P	U	P		
Serum cholesterol, mmol/l	1714.0	.311	2404.0	.786	1496.0	.924	3788.0	.35	2233.0	.243	3120.5	.68
Glucose, mmol/l	1275.5	.001*	1671.0	.001*	1082.5	.01	1504.5	<.001*	2333.0	.317	1323.5	<.001*
TGL, mmol/l	1438.0	.011	2288.0	.41	793.0	<.001*	2652.5	<.001*	1957.0	.018	1425.5	<.001*
HDL-C, mmol/l	1776.5	.414	936.0	<.001*	1003.0	.003*	1054.0	<.001*	1203.0	<.001*	1858.0	<.001*
LDL-C, mmol/l	1840.0	.67	1284.0	.04	986.0	.04	2341.0	.002*	1770.0	.033	2061.0	.61
CXCL16, pg/ml	1465.0	.15	844.0	<.001*	304.0	<.001*	2259.0	<.001*	1008.0	<.001*	2706.0	.096
Leptin, pg/ml	1540.5	.09	2203.0	.428	458.0	<.001*	3755.0	.357	1121.0	<.001*	1302.0	<.001*
Resistin, pg/ml	1836.0	.826	1520.0	<.001*	623.0	<.001*	2572.0	<.001*	1093.0	<.001*	2721.5	.401
FGF21, pg/ml	525.5	.034	301.0	<.001*	111.0	<.001*	1048.0	<.001*	436.0	<.001*	2838.0	.222

Abbreviations: CXCL16, C-X-C motif chemokine ligand 16; FGF21, fibroblast growth factor 21; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglycerides. \*Significance level P < .0086.

**Table 4.** Correlations between the studied adipokines and clinical and biochemical parameters в rpyrnye RA with MtS.

	CXCL16 <i>r; P</i>	LEPTIN <i>r; P</i>	RESISTIN <i>r; P</i>	FGF21 <i>r; P</i>
Age	0.144; .352	0.117; .435	0.345; .018*	-0.334; .095
WC	0.075; .630	0.39; .007*	0.172; .247	-0.107; .602
SBP	0.048; .758	0.130; .383	0.053; .726	-0.234; .25
TG	0.031; .843	-0.047; .753	-0.050; .74	-0.276; .172
HDL-C	0.087; .576	0.135; .366	0.278; .058	-0.521; .006*
LDL-C	-0.157; .31	0.171; .255	-0.194; .197	-0.091; .664
DAS28-ESR	0.132; .403	0.034; .823	0.183; .23	0.009; .966
ESR	-0.069; .684	-0.047; .777	0.012; .942	-0.2; .338

Abbreviations: DAS28-ESR, Disease Activity Score evaluated using erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.

\*Significance level  $P \leq .05$ .

**Table 5.** Correlations between the studied adipokines and clinical and biochemical parameters в rpyrnye RA without MtS.

	CXCL16 <i>r; P</i>	LEPTIN <i>r; P</i>	RESISTIN <i>r; P</i>	FGF21 <i>r; P</i>
Age	0.253; .025*	0.073; .519	0.006; .96	0.056; .68
WC	0.018; .877	0.291; .009*	0.101; .373	-0.063; .644
SBP	0.096; .401	0.083; .464	-0.005; .967	-0.091; .5
TG	0.139; .221	0.075; .508	0.067; .554	0.143; .29
HDL-C	0.241; .033*	0.138; .225	0.411; <.0001*	-0.043; .754
LDL-C	-0.058; .611	-0.008; .944	-0.198; .078	-0.088; .514
DAS28-ESR	0.085; .457	0.56; .526	0.035; .755	0.096; .476
ESR	0.183; .131	-0.098; .03*	-0.073; .55	0.015; .916

Abbreviations: DAS28-ESR, Disease Activity Score evaluated using erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.

\*Significance level  $P \leq .05$ .

BMI, RA disease activity and bone erosion.<sup>26,27</sup> The positive correlation that we have found between the CXCL16 and WC and the negative one with the HDL-C levels in MetS is confirmed by studies of other authors who have found the correlation between CXCL16 and WC, triglyceride levels and diastolic blood pressure.<sup>29</sup> In the RA, a direct correlation relationship between chemokine and CXCL16, and HDL-C can be associated with a “lipid paradox” where the HDL-C does not perform its usual positive role in the prevention of atherosclerosis, but becomes a proinflammatory marker.

Over the last few years, the role of the adipokines in the RA pathogenesis has been studied, but the results remain conflicting. We have found the elevated leptin levels in RA patients with MetS as compared to the control group, but it is not substantially different from that in the RA group and MetS group. Most studies have also shown elevated leptin levels in the blood

of RA patients compared to the controls. Leptin is assumed to be a proinflammatory adipokine, since it promotes production of cytokines, such as tumor necrosis factor, IL-6 reactive oxygen species, and is a cause for production of macrophage-derived chemokine. Some studies have shown the relationship between elevated leptin level and RA disease activity, RA disease duration, radiographic joint damage.<sup>30-32</sup> We have not found an association of leptin levels with RA disease activity indicators. In our study we have found positive correlation relationship between leptin levels and WC in all groups, which is stronger in RA patients with MetS group ( $r=0.39$ ;  $P=0.007$ ). We believe that our findings stress the negative role of central obesity, found to be in most patients with RA and in all patients with MetS. In contrast, a study by Fioravanti A, et al. no correlations between leptin levels and clinical and biochemical parameters were found.<sup>33</sup>

Our findings on the elevated resistin levels in the RA patients with or without MetS, are consistent with the findings of Kontunen et al.<sup>34</sup> We have not found more comparative data of resistin levels in the RA patients with MetS. Meta-analysis have shown that in most studies the resistin level in RA patients is higher than that in the control group.<sup>35</sup> Several studies have found the association of resistin level with inflammation indicators such as C-reactive protein, erythrocyte sedimentation rate, IL-6, IL-1, as well as with white blood cells count in RA patients.<sup>30,34-38</sup> We have not found any correlation between the resistin levels and the DAS28 activity score. We have also shown that resistin levels do not correlate with WC, unlike leptin levels, what coincides with the findings of other researchers.<sup>39</sup> A positive correlation relationship between resistin and patients' age was found in RA with MetS group. The age seems not affect the resistin levels regardless of body fat mass, but the elevated level of that adipokine is associated with an increased risk of CVD in older men and women.<sup>40</sup>

The FGF21 studies at RA are few. The increase in the FGF21 level in RA, regardless of the presence of MetS, coincides with the results of a study in which the authors note that serum FGF-21 levels were significantly higher in RA patients and did not correlate with the disease activity.<sup>20</sup> In contrast to this work, we have not found any positive correlation between the FGF-21 and the BMI, but have found a negative relationship with the HDL-C level. It is assumed that an augmented FGF-21, especially in seropositive RA patients, can indicate a compensatory response to inflammation, as well as a manifestation of co-morbidity.<sup>41</sup> It can be assumed that elevated FGF-21 in the RA with MetS group is due to high comorbidity rates as compared to other groups of patients.

The study has some limitations. Firstly, the study was a cross-sectional one, therefore the cause-effect relations have not been considered. Secondly, the study included just part of all RA disease activity indicators, visual signs of joint damage, which hinders the identification of a close relationship between biomarkers and the disease activity. Thirdly, the study included a relatively small number of RA with MetS patients.

## Conclusion

Thus, the results of our study made it possible for the first time to evaluate the features of changes in the level of inflammation biomarkers, adipokine status in patients with RA and MS compared with patients with RA without MS and with patients with MS. An increase in the level of chemokine CXCL16, resistin, and FGF21 in patients with RA, regardless of the presence of MS, confirms the importance of systemic inflammation, which underlies RA; an increase in the level of leptin is more associated with the presence of abdominal obesity. These data may, to a certain extent, explain the existing increased risk of CVD in RA with MS. Differentiated refinement can be useful in assessing patients' CVRs and justifying early personalized treatment.

## Author Contributions

LGT invented and developed the research, formulated the hypothesis and the purpose of the research, supervised the analytical part of the research, wrote part of the manuscript text. AAS recruited patients into the study, questioned patients, formed a database of the obtained results, carried out statistical processing of the results obtained, and participated in writing the text of the manuscript. DAK participated in the processing of the results obtained, in the formation of the presentation of the obtained data in the form of tables. JPZ reviewed the final version of the manuscript. LLA guided the process of determining the laboratory parameters and writing the corresponding section in the Materials and Methods of the manuscript. RSD supervised the results of this work, reviewed the final version of the manuscript. All authors participated in the discussion of the results and the formation of the final version of the manuscript.

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