Serum GDF-15 level in rheumatoid arthritis: relationship with disease activity and subclinical atherosclerosis

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ABSTRACT

Objectives: Growth differentiation factor (GDF)-15 was originally identified as a factor secreted by activated macrophages, and plays an important role in cell growth and differentiation. GDF-15 plays an important role in cell growth, signal transduction, and apoptosis regulation. The aim of this study was to evaluate the serum GDF-15 levels and their relationship with disease-related characteristics in patients with rheumatoid arthritis (RA).

Materials and methods: Forty-six patients diagnosed with RA and 36 demographically matched healthy control subjects participated in this study. GDF-15 levels were measured in blood samples from patients and controls. The disease activity score-28 (DAS28) was used to evaluate the disease activity of RA. The quality of life was evaluated using the disease-specific rheumatoid arthritis quality of life (RAQoL) scale. The health assessment questionnaire (HAQ) was used to evaluate the functional status. The degree of joint damage was assessed according to Larsen's method. Atherosclerosis was assessed by a cardiologist with the help of echocardiography according to the carotid intima media thickness (CIMT) method; vascular stiffness was assessed by using the flow mediated dilatation (FMD) method.

Results: Serum GDF-15 levels were significantly higher in RA patients when compared to the control subjects ($p<0.05$). RA patients were divided into two groups according to the disease activity; while 26 subjects (57%) were in the active group, 20 patients were in the non-active group (43%). Serum GDF-15 levels were significantly higher in the group that was considered to have an active disease. According to Pearson's correlation, serum GDF-15 levels were positively correlated with erythrocyte sedimentation rate (ESR) levels, morning stiffness, DAS28 score, tender joint count, and CIMT ($p<0.05$).

Conclusion: GDF-15 may play a role in the pathway of disease activity, joint involvement, and atherosclerosis in patients with rheumatoid arthritis.

Keywords: Rheumatoid arthritis; GDF-15; Disease activity; Morning stiffness; Atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by enhanced signals of the co-stimulatory molecules that are expressed on antigen presenting cells such as macrophages, dendritic cells, and B-lymphocytes[1,2]. Macrophages play a crucial role in various steps of RA pathophysiology. Inflamed rheumatoid synovium is characterized by the existence of many proinflammatory cytokines, that eventually induce joint destruction and repair. Joint repair may proceed via transforming growth factor-β (TGF-β) expressed in the synovium and subsynovial macrophages[3-5]. Another TGF-β superfamily cytokine, growth differentiation factor (GDF-15) has been implicated in chronic inflammatory pathways in RA[1,5]. Previous studies have revealed that serum levels of GDF-15 were higher in RA patients and reflected disease severity regardless of classic disease markers[1,5].

GDF-15 was originally identified as a factor secreted by activated macrophages, and plays an important role in cell growth and differentiation[5]. GDF-15 expression is generally low in resting tissues but may increase following an adaptive stress response to diverse cellular

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stress indicators such as hypoxia, inflammation, and tissue injuries[6]. The relationship of macrophage activation to GDF-15 gene expression suggests that GDF--15 may be an autocrine regulator of macrophage activation[5]. GDF-15 expression is increased after stimulation by proinflammatory cytokines, for instance, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 beta, or IL-6. Despite that the mechanism of GDF-15 is not fully clarified, it is believed that especially in inflammation, it can inhibit the later periods of macrophage activation, prevent the production of TNF-α induced by lipopolysaccharides, and also regulate the role of the proinflammatory cytokine IL-6[7-9]. In addition, GDF-15 influences the metabolism of carbohydrates and lipids. It plays multiple roles in various pathologies such as cardiovascular disease, obesity, inflammation, and cancer due to its anti-inflammatory and antiproliferative properties[10,11].

Previous studies have shown that GDF-15 is up regulated in the atherosclerotic vessel wall and GDF--15 is associated with infarct size in experimental heart attack models[7-12]. It has been reported that GDF-15 immunoreactivity is localized in macrophages of atherosclerotic carotid arteries and co-localizes with oxidized low-density lipoproteins. Furthermore, they have also revealed that the stimulation of apoptosis in cultured macrophages is related to increased GDF-15 expression[7].

The aim of this study was to investigate serum GDF--15 levels in patients with RA and to reveal the relationship of laboratory/clinical parameters and disease activity with GDF-15 levels in patients with RA.

MATERIALS AND METHODS

STUDY POPULATION

Forty-six patients, who presented to Outpatient Clinics of Physical Medicine and Rehabilitation of Dicle University Hospital in Turkey, between October 2014 and July 2015, diagnosed with RA according to the classification criteria of the 2010 American College of Rheumatology, were included in this study[13]. The 36 healthy controls were sought to be demographically matched to the patient group with emphasis on age, gender, and body mass index. The patients and healthy controls were between the ages of 18-65 years. The present study was approved by the local ethics committee. All the recruited subjects signed an informed consent form before participating in the study.

ASSESSMENT OF CLINICAL VARIABLES

Patients' demographic and clinical characteristics including age, gender, body mass index, educational level, disease duration, tender-swollen joint count, and the physician's and patient's global assessments were determined. Pharmacologic therapies use (non-steroidal anti-inflammatory drugs (NSAIDs)), disease modifying anti-rheumatic drugs (DMARDs), anti-TNF, or corticosteroids and smoking status were recorded. The duration of the morning stiffness (minutes) was also noted. The disease activity (DAS28) scores were evaluated using the disease activity score calculator[14]. The erythrocyte sedimentation rate (ESR) was measured through the Westergren method (mm/h) and the serum C-reactive protein (CRP) level was determined with the help of nephelometry (mg/dl). The RF titers were also measured through the nephelometric immunoassay method (IU/ml). The anti-cyclic citrullinated peptide (CCP) antibody titers were analyzed by the ELISA assay (Orgentec, Mainz, Germany) in line with the manufacturer's instructions (U/ml). Demographic and clinical data were obtained from patient interviews, chart reviews, physical examinations, and patient questionnaires.

EXCLUSION CRITERIA

RA patients using anti-TNF drugs, patients with any systemic diseases other than RA including patients with infectious or endocrine-related arthropathy, pregnant or lactating patients, coronary artery disease, diabetes mellitus, renal disease, malignancy, and patients with systemic infection were excluded from the study.

BIOCHEMICAL ANALYSES

Fasting blood samples were immediately centrifuged at 4000 rpm for 10 min. Subsequently, the sera were transferred to an Eppendorf tube for storage at -80 C° until analysis. GDF-15 serum concentration was measured by enzyme linked immunosorbent assay (pg/ml).

FUNCTIONAL DISABILITY, RADIOLOGICAL SCORE AND THE QUALITY OF LIFE

The quality of life was evaluated using the disease-specific rheumatoid arthritis quality of life (RAQoL) scale[15]. The health assessment questionnaire (HAQ) was used to evaluate the functional status[16]. Standardized radiographs of both the hands and feet were performed for all RA patients and the degree of joint damage was assessed by a radiologist according to

Larsen's method[17].

Atherosclerosis was assessed by a cardiologist with the help of echocardiography according to Carotid intima media thickness (CIMT) method; and also vascular stiffness was assessed by using flow mediated dilatation (FMD) method.

Measurement of the carotid intima-media thickness (CIMT) of the left and right common carotid arteries was obtained at the far wall and 1 cm from the bifurcation by using a high resolution B-mode carotid ultrasonography (Vivid S6, GE Vingmed Ultrasound, Horten, Norway) by a single trained sonographer, according to standardized method[18]. Individual results correspond to the mean of the left and right CIMT in mm.

The ultrasonographic (Vivid S6, GE Vingmed Ultrasound, Horten, Norway) evaluations of the patients' endothelial function and flow-mediated endothelial--dependent vasodilation were evaluated by flow-mediated vasodilation (FMD) measurement in the brachial artery of the non-dominant arm according to standardized method[19].

STATISTICAL ANALYSES

The Kolmogorov–Smirnov test was used to confirm that the data was normally distributed in both groups. Descriptive statistics for continuous variables were expressed as the mean ± standard deviation, and categorical variables were expressed as number and percentages. The chi-square test was used to assess differences in categorical variables. The independent samples t-test was applied to evaluate statistical difference in continuous variables between the two groups. Correlations between different continuous variables were evaluated by Pearson's or Spearman's correlation analyses, depending on the distribution of the variables. The level of statistical significance was set at a two--tailed p-value of 0.05 or less. All statistical analysis was performed using SPSS for Windows (Version 21.0).

RESULTS

A total of 46 patients diagnosed with RA and 36 demographically-matched healthy controls were recruited for the study. The clinical and demographic characteristics of the patients and healthy control subjects are listed in Table I. Other than two subjects who were being followed-up without any drugs, 44 patients with RA were taking medications, including NSAID, DMARDs (methotrexate, hydroxychloroquine and/or sulphasalazine), and steroids or their combinations. There were no significant differences between the RA patients and healthy controls in terms of gender, mean age, and mean BMI ($p>0.05$). The serum GDF-15 levels were significantly higher in patients with RA when compared to the control group ($p<0.05$) (Table I).

RA patients were divided into two groups according to the disease activity (DAS28<2.6 considered as non--active, DAS28>2.6 considered as active group): 26 subjects (65%) were in the active group, and 20 patients were in the non-active group (35%). Serum GDF--15 levels were significantly higher in the group that was considered to have an active disease (Table II). Serum GDF-15 levels were positively correlated with, ESR level, morning stiffness, DAS28 score, tender joint count, and CIMT (Table III). Effects of smoking and age to the correlation between CIMT and GDF-15 were evaluated with covariance analyses. As a result factors (such as smoking and age) were not effects correlation between GDF15 and CIMT.

DISCUSSION

In the present study, the mean serum GDF-15 levels were higher in RA patients, when compared to the healthy control subjects. In our study population, level of ESR, DAS-28 score, morning stiffness, and tender joint count were positively correlated with serum GDF-15 levels. Furthermore, GDF-15 levels were significantly higher in the group that was considered to have an active disease. To the best of our knowledge, this is the first study indicating the relationship between serum GDF-15 level and disease activity evaluated with DAS28 score in RA patients. Our finding of an increased GDF-15 levels in patients with active disease and also the relationship between GDF-15 levels with disease related variables may be explained by increased macrophage and cytokine levels in inflammatory serum via inflamed rheumatoid synovium in RA patients. GDF-15 expression is increased after stimulation by proinflammatory cytokines such as TNF-α, IL-1b, or IL-6 in the rheumatoid synovium[8]. Furthermore, signalling pathways are activated in the pathogenesis of RA, including activator protein 1/NF-kappa beta and p53 pathways. Activation of the p53 pathway is related to chronic RA disease[1,20,21]. Additionally, activation of the p53 pathway is associated with the suppression of IL--6 production in rheumatoid synovium and eventual-

TABLE I. CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS WITH RA (MEAN±SD OR %)

Disease characteristics	RA patients (n=46)	Healthy controls (n=36)	p
Age (years)	44.21 ± 8.40	41.44 ± 10.79	0.173
Male/female	34/12	24/14	0.090
BMI	27.83 ± 5.18	25.97 ± 6.27	0.069
GDF-15 (pg /ml)	1465.92 ± 902.05	993.23 ± 955.01	0.024
Disease duration (moths)	88.32 ± 92.14		
WBC	8.73 ± 2.45		
ESR (mm/h)	15.36 ± 12.17		
CRP (mg/dl)	1.33 ± 3.02		
Tender joint count	3.23 ± 4.37		
Swollen joint count	0.56 ± 1.40		
Morning stiffness (min)	27.39 ± 45.07		
Anti-CCP (U/ml)	175.30 ± 613.19		
RF (IU/ml)	132.75 ± 456.85		
RAQoL	16.60 ± 7.50		
HAQ	0.82 ± 0.48		
Larsen score	15.96 ± 21.41		
CIMT (mm)	0.59 ± 0.21		
FMD basal	3.49 ± 0.50		
FMD post ischemia	3.84 ± 0.49		
Difference of FMD	(9%)		
DAS28	2.81 ± 1.21		
Smoking status	13 (28%)		
Drug usage (n, %)			
NSAID	30 (65%)		
Methotrexate	32 (69%)		
Sulphasalasine	5 (10%)		
Leflunomide	8 (17%)		
Hydroxychloroquine	15 (33%)		
Corticosteroid	27 (59%)		
No treatment	2 (0.4%)		

SD: Standard deviation, RA: Rheumatoid arthritis, BMI: body mass index, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, WBC: White blood cell, DAS: Disease activity score, RaQoL: RA Quality of Life, CCP: Cyclic citrullinated peptide, RF: Rheumatoid factor, HAQ: Health assesment questionnaire, CIMT: Carotid intima media thickness, FMD: Flow mediated dilatation, NSAIDs: non steroidal anti-inflammatory drugs

ly could lead to diminished serum CRP levels[22]. In accordance with these results, also in the present study, higher ESR and higher DAS28 levels were correlated with higher GDF-15 levels in patients with RA. However, no significant correlation was observed between the GDF-15 and CRP levels. These findings also indicate that GDF-15 may play a role in the pathway of rheumatoid synovitis independent of familiar inflammatory markers.

In the present study, GDF-15 levels were not correlated with joint erosions (measured by the Larsen score). Only a limited number of studies to date have investigated GDF-15 levels in patients with rheumatic diseases[1,23]. Previous studies investigating GDF-15 levels in RA patients provided evidence indicating a potential role for GDF-15 in the pathogenesis of RA. The study conducted by Brown *et al.* found that serum GDF-15 levels were independently associated with disease severity and joint erosions in patients with RA. Authors also emphasized that the allelic variation of

TABLE II. DISEASE CHARACTERISTICS IN PATIENTS WITH ACTIVE AND NONACTIVE RA

Disease characteristics	Non-active group (n=20)	Active group (n=26)	P
GDF-15	1069.88 ± 853.59	1749.79 ± 847.43	0.013
ESR (mm/h)	8.50 ± 5.77	20.61 ± 13.24	<0.001
CRP (mg/dl)	0.55 ± 0.44	1.93 ± 3.93	0.012
WBC	8.76 ± 2.63	8.72 ± 2.36	0.951
RF	172.13 ± 661.38	102.45 ± 201.62	0.654
Anti-CCP	191.33 ± 694.14	162.96 ± 572.82	0.882
Age (years)	42.00 ± 8.99	45.95 ± 7.89	0.117
BMI	26.91 ± 4.02	28.55 ± 4.67	0.217
Disease duration (moths)	90.02 ± 97.18	87.86 ± 92.30	0.944
Tender joint count	0.30 ± 0.50	5.07 ± 3.75	<0.001
DAS28	1.73 ± 0.44	3.71 ± 0.89	<0.001
Morning stiffness (min)	6.50 ± 12.80	43.46 ± 36.73	0.003
RAQoL	13.80 ± 7.95	18.76 ± 6.69	0.026
HAQ	0.66 ± 0.47	0.95 ± 0.46	0.045
Larsen score	15.95 ± 30.03	15.96 ± 11.83	0.999
CIMT (mm)	0.47 ± 0.15	0.65 ± 0.22	0.065
FMD basal	3.49 ± 0.60	3.49 ± 0.47	0.991
FMD post ischemia	3.78 ± 0.52	3.87 ± 0.49	0.713

SD: Standard deviation, RA: Rheumatoid arthritis, BMI: body mass index, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, WBC: White blood cell, DAS: Disease activity score, RaQoL: RA Quality of Life, CCP: Cyclic citrullinated peptide, RF: Rheumatoid factor, HAQ: Health assesment questionnaire, CIMT: Carotid intima media thickness, FMD: Flow mediated dilatation, NSAIDs: non steroidal anti-inflammatory drugs

GDF-15 was associated with earlier erosive disease[1]. The role of GDF-15 in RA pathogenesis remains controversial, and conflicting data have already been produced through from different experiments regarding rheumatoid synovium. The effects of GDF-15 were not limited to proinflammatory properties, but also included immunosuppression[24,25]. Despite the fact that the major function of GDF-15 is not certain, the relationship of macrophage activation to GDF-15 suggests that GDF-15 may be an autocrine regulator of macrophage activation. On the other hand, recombinant GDF-15 protein was able to inhibit lipopolysaccharide--induced TNF-α production, suggesting a role for GDF-15 in inhibition of macrophage activation[5]. Furthermore GDF-15 might mediate an anti-inflammatory effect by reducing the migration of leukocytes to areas of inflammation[3]. The rheumatoid synovium is characterized by the presence of many proinflammatory cytokines that eventually induce joint destruction. Joint repair may proceed via TGF-β expressed in the synovium and subsynovial macrophages. Another TGF-β superfamily cytokine, GDF-15, has been implicated in chronic inflammatory pathways in rheumatoid arthritis[1,5]. As a result, the conflicting results regarding the relationship between the Larsen score and GDF-15 levels may be explained by the various and conflicting effects of GDF-15 on rheumatoid inflammation, and also the relatively less aggressive course of our rheumatoid arthritis patients.

This study also revealed that serum GDF-15 levels were positively correlated with CIMT. This finding is consistent with recent data indicating that increased serum levels of GDF-15 reflect endothelial activation and vascular inflammation, regarding the development of atherosclerosis[26]. Previous studies revealed that GDF--15 is upregulated in the atherosclerotic vessel wall and GDF-15 is associated with infarct size in experimental heart attack models. GDF-15 expression is increased after stimulation by proinflammatory cytokines and also lipopolysaccharides[8,12,27]. It has been reported that GDF-15 immunoreactivity is localized in the macrophages of atherosclerotic carotid arteries and co-localizes with oxidized low-density lipoproteins. Furthermore, they have also shown that the induction of apoptosis in cultured macrophages correlates with increased GDF-15 expression[27,28]. In addition, GDF-15 deficien-

TABLE III. CORRELATION BETWEEN THE GDF-15 LEVEL WITH DISEASE CHARACTERISTICS IN PATIENTS WITH RA

Disease characteristics	r	p
Age (years)	0.207	0.167
BMI	0.203	0.177
Disease duration (months)	0.127	0.399
Morning stiffness (min)	0.353*	0.016
WBC	-0.047	0.755
ESR (mm/h)	0.333*	0.024
CRP (mg/dl)	0.126	0.400
RF	-0.094	0.534
Anti-CCP	-0.039	0.795
RAQoL	0.097	0.522
HAQ	0.119	0.436
Tender joint count	0.330*	0.026
Swollen joint count	0.083	0.585
DAS28	0.407*	0.005
Larsen score	0.130	0.396
CIMT (mm)	0.543*	0.001
FMD basal	0.303	0.059
FMD post ischemia	0.191	0.186

Correlations were evaluated by Pearson's or Spearman's correlation analyses, * p<0.05
SD: Standard deviation, RA: Rheumatoid arthritis, BMI: body mass index, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, WBC: White blood cell, DAS: Disease activity score, RaQoL: RA Quality of Life, CCP: Cyclic citrullinated peptide, RF: Rheumatoid factor, HAQ: Health assesment questionnaire, CIMT: Carotid intima media thickness, FMD: Flow mediated dilatation, NSAIDs: non steroidal anti-inflammatory drugs

cy shows a significant reduction in atherosclerosis formation in mice models[10]. However, the findings of this study support the idea that GDF-15 may be a considerable indicator for cardiovascular diseases especially atherosclerosis.

One limitation of this study is its cross-sectional design. Prospective studies are needed in order to fully reveal the relationship between clinical findings of RA and GDF-15 levels.

CONCLUSION

In conclusion, our study indicated that serum levels of GDF-15 were significantly higher in patients with RA compared to the control group. Furthermore, high levels of GDF-15 were associated with disease activity, level of ESR, morning stiffness, tender joint count, and CIMT in RA patients. These findings suggests that GDF-15 might be associated with pathway in RA. Further studies including larger series should be conducted on this subject.

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