**The association between serum levels of growth differentiation factor-15 and activity of rheumatoid arthritis**

**Abstrac**t

Background: Macrophages play a crucial role in the pathogenesis of rheumatoid arthritis (RA). Growth differentiation factor-15 (GDF-15) acts as an autocrine regulator of macrophage activation. Objective: The aim of this study was to assess serum level of GDF-15 as a potential biomarker for detecting RA activity. Method: A total of 100 female RA patients and 55 age matched healthy control females were enrolled. The serum level of GDF-15 was measured using enzyme-linked immunosorbent assay by an eBioscience kit. Results: Serum levels of GDF-15 in RA patients with high, moderate, low and no disease activity were 989.0±161.9, 505.6±220.5, 349.2±155.9 and 349.0±144.0 pg/ml, respectively. GDF-15 with a cut-off value higher than 705 pg/ml was indicative of high RA activity with sensitivity of 96% and specificity of 92%. Conclusion: GDF-15 serum levels may be used as a biomarker to predict high RA disease activity.

**What is already known about this topic?**

Serum levels of growth differentiation factor-15 in rheumatoid arthritis have increased and are associated with disease activity.

**What does this article add?**

Serum growth differentiation factor-15 levels with a cut-off value higher than 705 pg/ml is indicative of severe disease activity in rheumatoid arthritis with a sensitivity of 96% and specificity of 92%.

**Keywords:** Growth differentiation factor-15 (GDF-15), rheumatoid arthritis, disease activity, cytokine, DAS28

**1. Introduction**

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases that affects approximately 1% of white people.1 RA most commonly present as inflammatory chronic polyarthritis which involves small and large joints in a symmetrical manner.1 However, sometimes RA present with unusual forms like acute or subacute arthritis, asymmetric arthritis, monoarthritis and palindromic form.1,2 RA is associated with decreased quality of life, increased morbidity and decreased survival of affected patients.1,3 Early diagnosis and treatment of RA improves disease outcomes.1 Biomarkers like C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) help measure RA disease activity and autoantibodies including rheumatoid factor (RF) and anticitrullinated peptides contribute to the early diagnosis of RA.1 However, the use of these biomarkers has limitations such as their relatively low sensitivity and specificity.4,5 For this reason, researchers are looking for new biomarkers for diagnosis and assessing activity of RA.

Macrophages play a crucial role in the pathogenesis of RA.1 They participate in inflammatory response and joint damage by multiple mechanisms. Growth differentiation factor-15 (GDF-15) also called the macrophage inhibitory cytokines-1 (MIC-1), is a member of the transforming growth factor beta (TGF-β) superfamily which acts as an autocrine regulator of macrophage activation.6 This factor is a stress response cytokine expressed in cardiomyocytes, adipocytes, endothelial cells, macrophages and smooth muscle cells in normal and pathologic conditions.7 GDF-15 has been shown to play a role in inflammatory events and apoptosis in damaged tissues. GDF-15 increases under inflammatory conditions and tissue damages. GDF-15, while its receptor has not been known yet, acts as an antagonist for β2 integrin. Integrin β2 action is essential for leukocytes migration into the inflammation site leading to continuation of inflammatory processes. This integrin is responsible for adhesion of leukocytes to the endothelium, which is essential for passing leukocytes from the vessels.8 GDF-15 inhibits leukocytes migration to the site of inflammation by blocking β2 integrin activation.8 GDF-15 is the first known cytokine that inhibits leukocyte function through direct interference with integrins function.8

GDF-15 has been known to be correlated with cardiovascular diseases, inflammation and malignancy, and detection of its serum levels have been shown to possess clinical benefits in different inflammatory and malignant diseases, such as colon, pancreas, prostate, ovary and thyroid malignancies, atherosclerosis, pulmonary thromboembolism, and systemic sclerosis.9-12 Furthermore, considering its inhibitory role in leukocyte function, it can be used as a therapeutic target for controlling inflammation in diseases with abnormal lymphocyte function.14

A few studies reported elevated serum GDF-15 levels in RA patients and the association between serum GDF-15 and RA activity.13,14 However, they did not characterize a cut-off value for discrimination between high and low disease activity. The aim of this study was to compare the serum level of GDF-15 in RA patients and healthy controls, and assess it as a potential biomarker for detecting RA activity.

**2. Methods**

*2.1 Participants*

A total of 100 female RA patients diagnosed by ACR/EULAR 2010 RA classification criteria were enrolled in the study. For control group, 55 healthy subjects were selected. Exclusion criteria were the presence of infectious diseases, other inflammatory diseases, renal insufficiency, liver disease and pregnancy. The activity of disease was calculated based on DAS28-ESR formula and patients according disease activity divided to 4 groups, including: i) high disease activity: DAS28-ESR >5.1; ii) moderate disease activity: DAS28-ESR >3.2 and ≤5.1; ii) low disease activity: DAS28-ESR ≥2.6 and ≤3.2; iv) disease in remission: DAS28-ESR <2.6.15

*2.2 GDF-15 analysis*

Three milliliters venous blood sample was obtained from all participants after overnight fasting. Blood samples were centrifuged immediately and then stored at -80 C° until analysis. The serum GDF-15 levels were quantitated by enzyme linked immunosorbent assay method using eBiOScience kit, according the manufacturer instructions.

*2.3 Data analysis*

We used SPSS software version 16.0 (SPSS, Inc., USA) for statistical analysis. The distribution of data was assessed using the Kolmogorov-Smirnov test. Continuous variables with normal and non-normal distribution were reported as mean ± standard deviation (SD) and median (25-75% interquartile range [IQR]), respectively. T-test was used in cases with normal distribution of data. Otherwise, the Mann-Whitney U test was used to compare two groups. ANOVA or Kruskal-Wallis test was used to analyze the association between GDF-15 serum level and RA activity. Additionally, Pearson correlation coefficient was used to express the correlation between this variable. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value and the optimal cut-off points of GDF-15 for predicting RA and RA disease activity. The area under the curve (AUC) value was calculated to determine the accuracy of the test. P values less than 0.05 were considered as statistically significant.

**3. Results**

The study included 100 female RA patients and 55 age matched healthy control females. The mean age of participants in the RA and control groups were 35.7 ± 9.4 and 36.4 ± 10.6 years, respectively (P = 0.701). The median (IQR) disease duration in the RA patients was 3 (1-7) years.

We compared the serum levels of GDF-15 in the RA and control groups (Figure 1). GDF-15 in the RA and control groups were 548.2±314.02 and 270.7± 167.9, respectively. Differences were significant (P=0.001). In addition, we compared the serum levels of GDF-15 in RA patients with different disease activity (Figure 2). Serum levels of GDF-15 in RA patients with high, moderate, low and no disease activity were 989.0±161.9, 505.6±220.5, 349.2±155.9 and 349.0±144.0 pg/ml, respectively.

We assessed the correlation between the serum levels of GDF-15 and patients age, disease duration and disease activity (Table 1). There was a significant and positive correlation between serum levels of GDF-15 and RA disease activity (Table 1, Figure 3). No significant association was observed between serum levels of GDF-15 and patients age and disease duration.

ROC analyses were performed to compare the ability of the GDF-15 to predict RA and activity of RA (Figure 4). GDF-15 with cut-off value higher than 272.5 pg/ml was indicative of RA with sensitivity of 82% and specificity of 62% (Figure 4A). GDF-15 with a cut-off value higher than 705 pg/ml was indicative of high RA activity with sensitivity of 96% and specificity of 92% (Figure 4B). Cut-off values for prediction of moderate and low RA disease activity were 360 and 307 pg/ml, respectively (Figure 4C and 4D). Sensitivity and specifity of this cut-off value for moderate RA activity was 68% and 64%, respectively. These figures were 60% and 61% for differentiation of low disease activity from disease in remission, respectively.

**4. Discussion**

Our study showed higher serum GDF-15 in patients with RA compared with healthy controls. We found a positive correlation between serum GDF-15 levels and RA activity. GDF-15 higher than 705 pg/ml was predictive of high RA activity with very good sensitivity of 96% and specificity of 92%. The sensitivity and specifity of serum GDF-15 for discrimination between moderate disease activity and low disease activity or disease in remission was not high. To the best of our knowledge, this is the first study to report a cut-off value for serum GDF-15 levels in differentiating severe RA activity from lower disease activity.

Few studies have highlighted the potential role of GDF-15 in RA. In an investigation conducted by Brown et al.,13 serum levels of GDF-15 in subjects with RA was 1084 ± 687 pg/ml and in healthy participants was 487 ± 197pg/ml. In this study, serum levels of GDF-15 were associated with disease activity and joint erosion, and decreased after treatment.13 In 2017, Tanrıkulu et al. in a study on 46 RA patients reported higher GDF-15 levels in patients with active disease compared with patients in remission.14 Serum GDF-15 levels in active and RA in remission groups were 1749.8 ± 847.4 and 1069.9 ± 853.6 pg/ml, respectively. They reported a positive correlation between serum GDF-15 levels and morning stiffness, number of tender joints, ESR, and DAS28 score.14 In addition, they found a positive correlation between serum GDF-15 levels and carotid intima media thickness (CIMT) (r=543, P=0.001). Ärlestig et al. in a study on 681 RA patients analyzed the polymorphisms of the gene coding for GDF-15 (rs1058587).16 They found a higher atherothrombotic complications (myocardial infarction, angina pectoris with intervention, deep vein thrombosis and pulmonary embolism) in RA patients with GG genotype (odds ratio 3.75).

GDF-15 is mainly secreted by activated macrophages and is effective in cell growth and differentiation, intercellular signal transduction and apoptosis regulation.16,17 The role of GDF-15 in RA pathogenesis is poorly understood. Although, the serum level of GDF-15 is an independent predictor of erosion in RA and are positively correlated with CIMT, expression of this cytokine in RA synovium has not been demonstrated.14-16 It has been shown that GDF-15 has both pro-inflammatory and immunosuppressive properties.18,19 GDF-15 is an inhibitor of macrophage activation and suppress TNF-α production by macrophages stimulated by lipopolysaccharide.19 GDF-15 can suppress migration of leukocytes to inflammation site. This property of GDF-15 is evident after myocardial infarction which suppress leukocyte recruitment into the injured myocardium.18

The results of this study should be interpreted carefully because of the small sample size, the cross-sectional method of study and ignoring the effect of treatment on serum GDF-15 levels. A prospective study is necessary to assess the role of GDF-15 in predicting RA activity and prognosis of disease and effect of treatment on the serum levels of GDF-15.

**5. Conclusions**

GDF-15 serum levels may be used as a biomarker to predict high RA disease activity.

**Conflict of interest statement**

There is no conflict of interest.

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**Figure legends:**

Figure 1. Serum GDF-15 levels in RA and control groups.

GDF-15: Growth differentiation factor-15; RA: Rheumatoid arthritis

Figure 2. Serum GDF-15 levels in RA patients with various disease activity.

GDF-15: Growth differentiation factor-15; RA: Rheumatoid arthritis

Figure 3. Correlation diagram between activity of RA and serum GDF-15 levels.

GDF-15: Growth differentiation factor-15; RA: Rheumatoid arthritis

Figure 4. ROC analysis. A) ROC for the prediction of RA, The area under the ROC curve is 0.791; 95% CI: 0.716–0.867, P <0.0001; B) ROC for the differentiation of severe RA from moderate, low and no RA activity, The area under the ROC curve is 0.977; 95% CI: 0.950-1.000, P <0.0001; C) ROC for the differentiation of moderate RA activity from low and no RA activity, The area under the ROC curve is 0.730; 95% CI: 0.613–0.848, P <0.001, D) ROC for the differentiation of mild RA activity from RA in remission, The area under the ROC curve is 0.514; 95% CI: 0.348-0.681, P=0.861.

ROC: Receiver operating curve; RA: rheumatoid arthritis; CI: confidence interval

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|  | | Age | Disease  duration | DAS28 |
| GDF-15 | Pearson correlation | - 0.158 | -0.065 | 0.798 |
| P-value | 0.115 | 0.523 | 0.001 |

Table1. Linear correlation coefficient between age, disease duration

and disease activity in RA patients.

RA: rheumatoid arthritis; GDF-15: growth differentiation factor-15