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Serum adiponectin as a predictor of laboratory response to anti-TNF-α therapy in rheumatoid arthritis

DOROTA SIKORSKA[1], RAFAŁ RUTKOWSKI[2], JOANNA ŁUCZAK[2], WŁODZIMIERZ SAMBORSKI[1], JANUSZ WITOWSKI[2]

[1]Department of Rheumatology and Rehabilitation, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Pathophysiology, Poznan University of Medical Sciences, Poznan, Poland

Abstract

Introduction: *While adiponectin is typically viewed as an anti-inflammatory mediator, such an activity of adiponectin in rheumatoid arthritis (RA) is not so obvious. In the present study we examined whether serum levels of adiponectin reflect the clinical phenotype of RA patients and/or correlate with severity of the disease and the response to anti-TNF-α therapy.*

Material and methods: *Twenty-one female RA patients qualified to receive anti-TNF-α treatment were prospectively assessed before and after 12 weeks of therapy. Patients underwent full clinical and biochemical assessment. Disease activity was assessed by the Modified Disease Activity Scores (DAS28). Serum concentrations of adiponectin were measured with an immunoassay. The individuals were divided into two subgroups according to whether their baseline serum adiponectin was below or above the median value. The subgroups did not differ in basic demographic, anthropometric, and clinical parameters.*

Results: *Anti-TNF-α treatment resulted in a significant clinical (DAS28) improvement in patients from both subgroups, but no significant differences between basal and post-treatment serum adiponectin concentrations were observed. However, patients with higher baseline adiponectin experienced a significant and more pronounced improvement in laboratory parameters of inflammation (ESR, CRP, neutrophil count, neutrophil-to-lymphocyte ratio).*

Conclusions: *It is possible that adiponectin exerts systemic anti-inflammatory effects independently of the local activity of RA.*

Key words**:** *rheumatoid arthritis, inflammation, adiponectin, adipocytokines, anti-TNF-α treatment.*

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Introduction

It is now evident that adipose tissue-derived adipokines may critically modulate inflammatory responses in various tissues and organs [1]. These include the cartilage and the synovium of rheumatoid arthritis (RA) patients [1-4]. Adiponectin is an abundant adipokine viewed typically as an anti-inflammatory mediator. However, adiponectin activities may go beyond this paradigm, given the complexity of its isoforms, receptors, and signal transduction pathways [2, 5, 6]. While the role of adiponectin in metabolic syndrome appears to be clearly anti-inflammatory [7], it may not be the same in RA [5, 6]. *In vitro* studies with chondrocytes and synovial fibroblasts have shown that adiponectin can induce pro-inflammatory cytokines, chemokines, and matrix proteases in these cells [8, 9]. Thus, the systemic role of adiponectin may be different from that being played locally in the inflamed synovium [10].

The pattern of changes in serum adiponectin that occur (if any) in RA is not clear [5, 6]. Some studies reported increased levels of adiponectin in RA [5, 6] and their correlation with clinical activity of the disease [11, 12]. However, other studies did not confirm these observations [13, 14], and some even found a negative correlation between adiponectin and the severity of disease [15, 16]. Some studies have described the association of serum adiponectin with radiographic features of RA progression [17, 18], but others have suggested a protective role of adiponectin in this respect [19]. Furthermore, it is not clear whether and how the levels of adiponectin are affected by treatment regimens for RA [20-22]. One may hypothesise that the role of adiponectin in RA changes over the course and stage

Correspondence: Dorota Sikorska, MD, PhD, Department of Rheumatology and Rehabilitation, Poznan University of Medical Sciences, 135/147 28 Czerwca 1956 St., 61-545 Poznan, Poland, e-mail: dorota_s8@wp.pl
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of the disease. In this respect, it has been demonstrated that serum levels of adiponectin increase with the disease duration. Moreover, it has been observed that there was a negative correlation between serum adiponectin and clinical activity of the disease in patients with long-lasting RA, but not in those with early disease [15].

In the present exploratory study, we have attempted to determine whether serum levels of adiponectin reflect somehow the clinical phenotype of RA patients and/or correlate with severity of the disease and the response to anti-TNF-α therapy.

Material and methods

Patients

Twenty-one consecutive Caucasian female patients starting anti-TNF-α therapy for RA were enrolled into the analysis. The study was confined to females because adipokine levels differ significantly between the sexes [7], and female patients made up the majority of our RA patient population. The inclusion criteria were as follows: 1) the diagnosis of RA according to the American-European Consensus Group classification criteria [23], 2) the unsuccessful treatment for RA with synthetic disease-modifying anti-rheumatic drugs (sDMARDs) according to the current guidelines [23-25]. Exclusion criteria were: 1) heart failure (NYHA class ≥ II), 2) respiratory, kidney, or liver failure, 3) an acute or opportunistic infection in the last three months, 4) a documented HIV infection, 5) cancer (including a disease identified and cured in the past five years), 6) demyelinating diseases, and 7) pregnancy. The study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences (No. 1067/15), and written, informed consent was obtained from all participants.

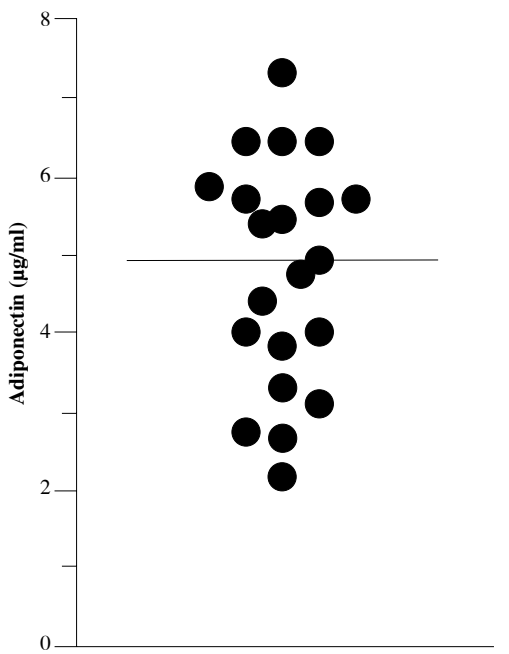

Fig. 1. Serum adiponectin levels in RA patients before the commencement of anti-TNFα therapy. The horizontal bar represents the median value

Treatment

Patients received anti-TNF-α treatment (adalimumab, certolizumab, etanercept, golimumab, or infliximab) as per standard protocols [25]. Nine patients (43%) continued to receive methotrexate, according to the European League Against Rheumatism (EULAR) recommendations [24, 25]; the 12 remaining patients did not tolerate methotrexate. In patients with severe symptoms, corticosteroids (≤ 5 mg prednisone/day) and non-steroid anti-inflammatory drugs (NSAIDs) were allowed, but such patients were included in the analysis only when these drugs were administered at the same doses for at least four weeks before and during the whole anti-TNF-α therapy.

Assessment of disease activity

Patients underwent full clinical and biochemical assessment before and after 12 weeks of anti-TNF-α therapy. Disease activity was assessed by the Modified Disease Activity Score (DAS), which includes different 28-joint counts and erythrocyte sedimentation rate ($DAS28_{ESR}$) [26]. The therapeutic response was according to the EULAR defined by the EULAR criteria [24].

Laboratory analysis

Blood samples were collected in a fasting state at the time of clinical examination. Serum obtained was aliquoted and stored at –80°C until measured for adiponectin, leptin, and visfatin using DuoSet Immunoassay kits (R&D Systems; Minneapolis, MN, USA), as per manufacturer's instructions. All other laboratory tests were performed routinely by the hospital central laboratory.

Statistical analysis

Statistical analysis was performed using STATISTICA 10.0 software (StatSoft Polska, Kraków, Poland). Because the data obtained did not consistently display a normal distribution (as assessed by the Shapiro-Wilk test), they were analysed with nonparametric statistics using the Wilcoxon test and the Mann-Whitney test for paired and unpaired data, respectively. Categorised data were analysed with the χ^2 test. Correlations between variables were analysed with the Spearman's rank correlation coefficient. The data are presented as medians and interquartile ranges or as percentages, as appropriate. Differences were considered significant at $p < 0.05$.

Table 1. Characteristics of subgroups according to baseline serum adiponectin (ADPN) concentration

	ADPN at baseline ≤ median (*n* = 11)	**ADPN at baseline > median (*n* = 10)**	***p***
Demographic characteristics			
Age, years	53 (39-60)	61 (50-66)	0.217
Disease duration, years	8 (3-16)	8 (6-18)	0.458
BMI, kg/m^2	24.7 (22.8-28.1)	23.1 (21.8-23.2)	0.307
Clinical parameters of rheumatoid arthritis activity			
DAS28			
Before treatment	5.3 (5.0-6.0)	5.9 (4.5-6.4)	0.860
After treatment	3.6 (3.1-4.5)[*1]	3.6 (2.8-4.0)[*2]	0.526
Change	–1.8 (–2.1-[–0.7])	–1.8 (–3.0-[–1.5])	0.460
Non-responders (based on DAS28), *n* (%)	2 (18)	0 (0)	0.156
TEN28			
Before treatment	10 (7-18)	8 (7-11)	0.477
After treatment	2 (1-4)[*3]	2 (1-3)[*4]	0.857
Change	–6 (–10-[–4])	–7 (–10-[–4])	0.971
SW28			
Before treatment	5 (3-7)	6 (5-9)	0.436
After treatment	0 (0-4)[*5]	2 (0-6)[*6]	0.303
Change	–3 (–6-[–2])	–3 (–7-[–1])	0.971
VAS			
Before treatment	70 (68-80)	61 (58-83)	0.230
After treatment	42 (29-68)[*7]	43 (39-50)[*8]	0.751
Change	–30 (–46-[–12])	–22 (–35-12)	0.359
Laboratory parameters of rheumatoid arthritis activity			
Erythrocyte sedimentation rate			
Before treatment, mm/h	22 (12-30)	26 (16-42)	0.647
After treatment, mm/h	20 (15-30)	10 (7-20)[*9]	0.084
Change, mm/h	–5 (–14-6)	–10 (–30-[–2])	0.130
C-reactive protein			
Before treatment, mg/l	6.2 (2.3-19.4)	8.8 (1.9-15.8)	0.915
After treatment, mg/l	6.7 (3.7-16.3)	0.2 (0.1-1.6)[*10]	0.001
Change mg/l	–0.2 (–10-6)	–6.5 (–15.7-[–1.8])	0.130
Leukocytes			
Before treatment, 10^3/µl	8.8 (6.0-9.5)	10.3 (7.6-13.9)	0.113
After treatment, 10^3/µl	7.9 (6.8-8.8)	9.3 (6.6-11.2)	0.324
Change, 10^3/µl	–1 (–1.2-0.9)	–1.1 (–2.8-[–0.2])	0.245
Neutrophils			
Before treatment, 10^3/µl	4.8 (3.4-6.3)	6.8 (4.2-9.2)	0.170
After treatment, 10^3/µl	4.9 (3.4-5.8)	4.8 (2.5-6.5)[*11]	0.888
Change, 10^3/µl	–0.6 (–1.2-0.6)	–1.8 (–3.9-[–0.8])	0.038

Table 1. Cont.

	ADPN at baseline ≤ median (n = 11)	ADPN at baseline > median (n = 10)	p
Lymphocytes			
Before treatment, 10^3/μl	1.9 (1.6-2.4)	2.0 (1.5-3.0)	0.832
After treatment, 10^3/μl	2.1 (1.6-2.4)	2.8 (2.2-3.6)	0.016
Change, 10^3/μl	0.0 (–0.1-0.1)	0.7 (0.0-1.6)	0.090
Neutrophil-to-lymphocyte ratio			
Before treatment	2.1 (1.6-3.6)	2.9 (2.3-5.8)	0.275
After treatment	2.3 (1.6-3.4)	1.5 (0.9-2.5)*[12]	0.084
Change	–0.1 (–0.5-0.6)	–1.3 (–4.4-[–0.3])	0.026
Adipokines			
Leptin			
Before treatment, pg/ml	706 (555-848)	628 (434-870)	0.724
After treatment, pg/ml	641 (557-904)	644 (605-762)	0.860
Change, pg/ml	–36 (–207-260)	82 (–161-231)	0.549
Visfatin			
Before treatment, ng/ml	75 (56-98)	77 (68-94)	0.762
After treatment, ng/ml	64 (58-91)	74 (56-104)	0.596
Change, ng/ml	0 (–21-3)	2 (–12-10)	0.698

*p – ADPN < median vs. ADPN > median; *before vs. after; data presented as medians (and interquartile ranges); [1]p = 0.004; [2]p = 0.005; [3]p = 0.003; [4]p = 0.008; [5]p = 0.009; [6]p = 0.015; [7]p = 0.006; [8]p = 0.037; [9]p = 0.014; [10]p = 0.008; [11]p = 0.007; [12]p = 0.012; DAS28 – 28-joint disease activity score; TEN28 – the number of tender joints; SW – the number of swollen joints; VAS – visual analogue scale of pain*

Results

Twenty-one consecutive patients qualified to receive anti-TNF-α therapy for RA were analysed. Baseline serum adiponectin concentration in this patient population was found to range from 2.24 μg/ml to 7.33 μg/ml (Fig. 1).

In order to assess whether concentrations of adiponectin characterise the patients' status, the individuals were divided into two subgroups according to whether their baseline serum adiponectin was below or above the median value (4.95 μg/ml). The patients thus stratified did not differ in basic demographic, anthropometric, and clinical parameters (Table 1).

Twelve weeks of anti-TNF-α treatment resulted in a significant clinical improvement (as assessed by DAS28, TEN28, SW28, and VAS indexes) in patients from both groups. Only two patients (10%) were identified as EULAR non-responders, and they were found to belong to the subgroup with low baseline adiponectin. Nevertheless, this pattern of distribution did not yield a formal statistical significance (p = 0.156). Interestingly, however, patients with higher baseline adiponectin experienced a significant and a more pronounced improvement in several laboratory parameters of RA activity (ESR, CRP, neutrophil count, neutrophil-to-lymphocyte ratio) (Table 1). They achieved lower CRP values and a more favourable neutrophil-to-lymphocyte ratio after treatment (Table 1). Interestingly, serum baseline adiponectin levels correlated with final CRP only in patients with RA duration ≥ 8 years (n = 11; r = –0.79, p = 0.003).

The levels of adiponectin did not change significantly within the groups following the treatment (Fig. 2A). Regarding other adipokines, leptin and visfatin did not differ between the groups either at baseline or after 12 weeks of anti-TNF-α therapy (Fig. 2B and Fig. 2C).

Discussion

The main observation of the present study was that RA patients with higher serum adiponectin experienced a more pronounced biochemical improvement in response to anti-TNF-α therapy compared with patients with lower adiponectin concentrations. This was reflected by a greater decrease in CRP, neutrophil counts, and ESR, together with a greater increase in lymphocyte counts. These effects seemed to be independent of classic clinical indices of RA activity, which improved in both groups to a similar extent. It is possible that adiponectin plays different roles in serum and in the synovial fluid [10]. *In vitro* studies have shown proinflammatory effects of adiponectin on chondrocytes and synoviocytes [8, 9], but serum adiponectin may still

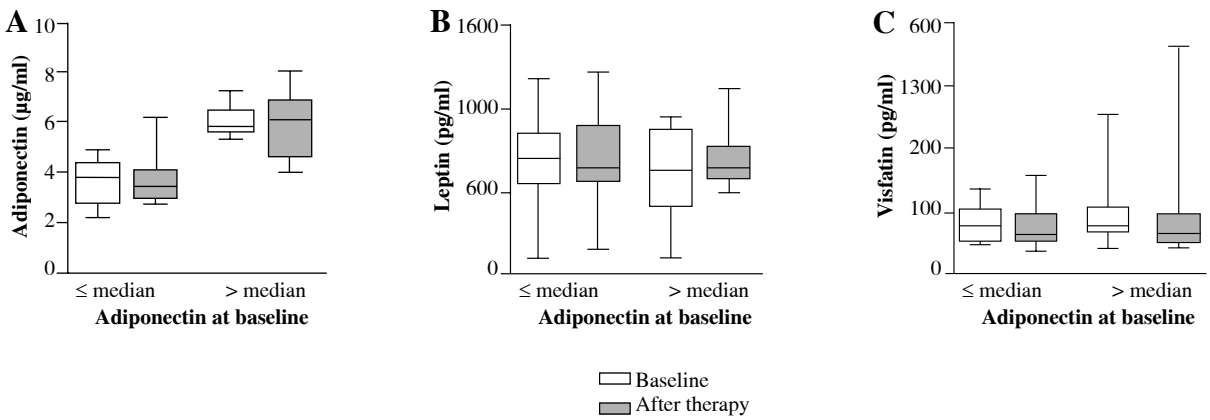

Fig. 2. Adiponectin, leptin, and visfatin levels before and after 12 weeks of anti-TNFα therapy in patients stratified at baseline according to adiponectin levels (as described in Methods). Boxes represent median values and interquartile ranges, and whiskers represent the ranges of values

have some systemic anti-inflammatory effects in patients with RA [27-29].

Previous studies assessing the association between adiponectin and other inflammatory biomarkers produced unequivocal results. A recent meta-analysis failed to demonstrate a significant correlation between serum levels of adiponectin and CRP [13]. However, some studies reported a clear relationship between circulating adiponectin and CRP [27]. Moreover, some [21, 30], but not all [22, 31], studies have demonstrated that anti-TNF-α therapy in RA was associated with an increase in serum adiponectin levels. A few studies suggested that the level of serum adiponectin in RA is related to disease duration [15, 32]. In this respect, we have observed that serum adiponectin levels correlated with CRP only in patients with RA duration ≥ 8 years.

The net effect of adiponectin activity may depend on the proportions of low and high molecular weight isoforms [8]. In this respect, the proinflammatory activities of adiponectin are attributed to a high molecular weight isoform, whereas anti-inflammatory effects are thought to be exerted by low molecular adiponectin [33]. The immunoassay employed in the present study was designed to measure total (i.e. both low and high molecular weight) human adiponectin, Therefore, future studies will need to assess specifically the role of different adiponectin isoforms in RA.

Moreover, they will need to compare adipokine levels separately in female and male RA patients. This is because the sexes differ generally in the amount of adipose tissue and consequently in the magnitude of adipokine release [7, 27]. In this respect, we have recently demonstrated that some changes in serum adipokine levels in obese patients occur in men but not in women [34]. Furthermore, it would be interesting to compare the levels of adiponectin in serum to those in the synovial fluids because it may discriminate between systemic and local adiponectin activities.

An obvious limitation of our study is the small number of patients analysed and the fact they were all treated in a single centre. Thus, our observations should be viewed as preliminary and should be validated in a much larger and heterogeneous independent patient population.

Conclusions

Patients with higher serum adiponectin experienced a more pronounced biochemical improvement in response to anti-TNF-α therapy, compared with patients with lower adiponectin concentrations. These effects seemed to be independent of classic clinical indices of RA activity.

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The authors declare no conflict of interest.

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