Serum leptin levels do not correlate with disease activity in rheumatoid arthritis

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ABSTRACT

Objectives: Leptin is a fat tissue hormone, which effects energy expenditure, food intake, hematopoiesis, osteogenesis, angiogenesis, reproductive and immune systems. We aimed to determine serum leptin levels and investigate the association between disease activity and other in parameters in rheumatoid arthritis (RA) patients.

Methods: Patients with RA (n=106), as the study group, healthy controls (n=52) and osteoarthritis (OA) patients (n=37), as a control group, were enrolled to the study. RA patients were categorized in four different groups according to DAS28 scores: remission, low (LDA), moderate (MDA) or high (HDA) disease activity.

Results: No significant difference was present between the body mass indices of the three groups. Mean leptin levels in RA patients, OA group and healthy individuals were 25.60±13.41, 23.03±11.51 and 23.81±12.85 ng/ml, respectively and no significant difference was present between the groups. Nine of (8.5%) RA patients were in remission, 16 (15.1%) were in LDA, 40 (37.7%) in MDA and 41 (38.7%) were in HDA. Leptin levels did not correlate with DAS28 scores of RA patients (r=-0.12, p=0.11). Mean leptin levels in RA patients in remission was 32.65±7.28; in LDA 23.94±10.94; in MDA 26.73±14.92 and in HDA 23.59±13.50 ng/ml (p=NS). No associations were observed between leptin levels and CRP, ESR, RF positivity and disease duration.

Conclusions: Our study revealed no correlation of disease activity and serum leptin levels. Therefore leptin does not seem to be an appropriate biomarker to monitorize inflammation in RA.

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INTRODUCTION

Leptin is a peptide hormone mainly produced by adipocytes, initially described in 1994 as a regulator of body weight by inhibiting food intake and stimulating energy consumption[1,2]. Later, studies on the functions of white adipose tissue (WAT) and adipokines showed that leptin also influence endocrine system, maturation of the reproductive system and immunity[3]. Leptin receptor (Ob-R) is a member of class I cytokine receptor superfamily and has a comparable structure with IL-6 receptor[4].

Leptin is found to be effective on both innate and adaptive immunity. It stimulates the activation, phagocytosis and cytokine release of monocytes and macrophages[5]. Leptin also induces proliferation and activation of T cells and protects them from apoptosis, altering T cell differentiation to Th1 phenotype[6]. Studies with leptin deficient mice (ob/ob) revealed less severe arthritis than controls[7]. Leptin deficient mice were found to be resistant or less susceptible to immune-mediated inflammatory diseases[8]. Several changes in immune system were demonstrated in leptin deficient humans. Number of CD4+ Tcells were reduced in circulation, proliferation and cytokine release of T cells were deteriorated and these changes were reversed after recombinant leptin was administered[9]. In RA patients, disease activity decline after fasting, which is in correlation with decreases in serum leptin levels and a switch to Th2 cytokine release[10]. Proinflammatory cytokines like Tumour Necrosis Factor-alpha (TNF-α) and Interleukin 1-beta (IL1-β) have also stimulatory effects on leptin release[6,11]. The increase in serum leptin levels during infections and inflammatory pathologies suggest that this adipokine takes part in immune reac-

tions[8,12,13]. Hyperleptinemia via increased inflammatory cytokines may be a potential cause of rheumatoid cachexia. Leptin can be a possible biomarker that could associate with disease activity in RA patients and a target molecule for new therapeutic approaches.

With this background, the aim of this study was to assess serum leptin levels in patients with RA, comparing to osteoarthritis (OA) patients and healthy controls, and to determine whether serum leptin levels correlate with disease activity.

MATERIAL AND METHODS

A total of 106 consecutive RA patients (F/M: 88/18, mean age: 48.2±12.3 years) followed in the Department of Rheumatology, Kartal Education and Research Hospital, Istanbul between 2009-2010 years were enrolled to the study. All patients were older than 18 years old and fulfilled the 1987 American College of Rheumatology criteria for RA[14]. The mean duration of disease was 6.2±6.8 (1-45) years. The control group consisted of 37 OA (F/M: 31/6, mean age: 50.7±6.7 years) patients and 52 healthy individuals (F/M: 41/11, mean age: 46.0±12.7 years). The Ethical Committee of Kartal Education and Research Hospital approved the design of the study and all patients gave written informed consent according to the Declaration of Helsinki.

The basic characteristics of RA patients are summarized in Table I. Disease activity was assessed with the disease activity score 28-ESR (DAS28-ESR) and patient's global assessment (PGA) of disease activity (visual analog scale: 0-100 mm). Rheumatoid Factor (RF) was positive in 55 (51.9%) patients. Tender joint count (TJC), swollen joint count and patient's global assessment of disease activities were 4(0-24), 1.5(0-28) and 45.5±27.2 (0-100) respectively. Nine patients were in remission, 16 patients were in low (DAS28: 2.6-3.2), 40 were in moderate (DAS28: 3.2-5.1) and 41 in high disease activity groups (DAS28: >5.1).

Twenty-one RA patients (19.8%) were taking nonsteroidal anti-inflammatory drugs (NSAIDs), 63 (59.4%) were on methotrexate, 21 (19.8%) on leflunomide, 31 (29.2%) on hydroxychloroquine, 33 (31.1%) on sulfasalazine and 15 (14.2%) were on anti-TNF-α therapies. Forty-five (42.5%) RA patients were on low dose corticosteroid treatment.

Serum samples were centrifuged at 2000G for 10 min and stored at -80°C. Serum leptin levels were measured by AssayMax Human Leptin ELISA Kit with a detection limit < 150 pg/ml, according to the manufacturer's instructions. Body mass index was calculated as weight/height^2 (kg/m^2). The normal range of ESR was <20 mm/h and CRP was <5 mg /L.

STATISTICS

Statistical analyses were performed by using the Software Statistical Package Sciences (SPSS) for Windows version 16.0. Demographic and clinical data were expressed as mean±standart deviations. Continuous variables were compared using Student's t test or One-way ANOVA and /or nonparametric Mann-Whitney U tests, whenever the data did not appear to have normal distribution. Correlation analyses was performed by Pearson's coefficient. For all tests p values less or equal to 0.05 were considered significant.

RESULTS

Mean leptin levels in RA patients, osteoarthritis group and healthy individuals were 25.6±13.4 ng/ml, 23.0±11.5 ng/ml and 23.8±12.8 ng/ml, respectively, and no significant differences were present between these three groups. In RA group, mean leptin serum levels in women and men were 25.5±13.7 ng/ml and 25.8±12.0 ng/ml, respectively and the difference was not statistically significant. Leptin concentrations did not correlate with BMI both in women (r=0.02, p=0.98) and men (r=0.35, p=0.15) with RA. There were also no significant correlations between leptin levels and age, RF positivity, disease duration, ESR and CRP levels (Table II). Different therapies including DMARDs, corticosteroids or anti-TNF-α therapies also did not effect serum leptin levels (Table III).

RA disease activity was not associated with serum leptin levels in our study (r=-0.12, p=0.18). Mean serum leptin levels in RA patients with DAS28<2.6 (remission) was 32.6±7.2 ng/ml, in LDA 23.9±10.9 ng/ml, in MDA 26.7±14.9 ng/ml and in HDA 23.5±13.5 ng/ml (p=NS) (Table II).

Mean BMI of three study groups were 28.6±6.3 in RA, 33.1±5.4 in OA and 28.8±6.0 in healthy controls. OA patients had higher BMI than the other groups. However, no correlation was observed between leptin levels and BMI in any group.

TABLE I. CHARACTERISTICS OF RA PATIENTS

Female n (%)	88 (83)
Age mean (± SD)	48.2 (±12.3)
Disease duration median (min-máx)	4 (1-45)
RF >15ıu/mL n (%)	55 (51.9)
ESR mean (± SD)	38.4 (±24.2)
Positive CRP, >5 mg/L n (%)	44 (41.5)
BMI mean (± SD)	28.6 (±6.3)
DAS28>5.1 n (%)	41 (38.7)

RF:Rheumatoid factor
ESR:Erythrocyte sedimentation rate (mm/h)
CRP:C reactive protein
BMI:Body mass index (Kg/m^2)
DAS28:Disease activity score

TABLE II. LEPTIN SERUM CONCENTRATIONS IN RA PATIENTS (NG/ML) (MEAN±SD)

		Leptin levels [ng/mL (mean+-SD)]	P value
Sex	Male	25.8±12.0	*n.s.
	Female	25.5±13.7	
Disease duration	<10 years	26.1±13.5	n.s.
	> 10 years	23.3±13.1	
CRP (<5 mg/L)	Positive	24.4±14.6	n.s.
	Negative	27.1±11.5	
Disease activity score	<2.6	32.6±7.2	n.s.
	2.6-3.2	23.9±10.9	
	3.2-5.1	26.7±14.9	
	>5.1	23.5±13.5	
RF (cut off > 15ıu/mL)	Positive	26.4±14.9	n.s.
	Negative	24.6±11.6	

*n.s: non significant
RF:Rheumatoid factor

TABLE III. SERUM LEPTIN LEVELS OF PATIENTS ACCORDING TO CURRENT TREATMENT AGENTS

Treatment agents		Leptin levels [ng/mL (mean+-SD)]	P value
Steroid	Treated	23.1±13.2	ns
	non-treated	27.4±13.3	
NSAIDs	Treated	28.2±13.9	ns
	non-treated	24.9±13.2	
Hidroxychloroquine	Treated	25.9±12.6	ns
	non-treated	25.4±13.8	
Sulfasalazine	Treated	24.9±10.8	ns
	non-treated	25.8±14.4	
Methotrexate	Treated	24.4±13.7	ns
	non-treated	27.3±12.8	
Leflunomide	Treated	28.6±10.9	ns
	non-treated	24.8±13.9	
Anti-TNF therapies	Treated	24.5±12.8	ns
	non-treated	25.7±13.5	

NSAIDs: Non steroidal anti-inflammatory drugs,TNF: tumor necrosis factor

DISCUSSION

Leptin's function in immune system as an immunomodulatory and a pro-inflammatory cytokine have been investigated in several studies. Serum leptin levels are observed to be increased both in autoimmune diseases such as SLE and autoinflammatory diseases such as Behcet's disease[15,16]. Leptin levels also correlate with disease activity in Behcet's disease. However the role of leptin in RA pathogenesis is insufficiently studied and association of leptin with disease activity in RA is controversial.

We could not demonstrate an elevation of serum leptin levels in RA and OA in our study, as demonstrated in some studies[17-19]. However, Tokarczyk-Knapik et al.[20] showed decreased while Otero et al.[12] and Bokarewa et al.[21] reported increased levels in RA. Furthermore, leptin concentrations were observed to be associated with decreased radiographic damage in one study[22].

We observed no association of leptin with disease activity or acute-phase response (ESR, CRP) in RA, similar to some other studies[17,20,23]. In contrary, Targo ska-Stepniak et al. observed a positive correlation between leptin levels and DAS28, TJC and ESR in patients with long-standing RA and in erosive disease[24]. Rho et al. demonstrated that CRP inhibits leptin's binding to its receptors and prevent leptin from signaling *in vitro*, suggesting that high concentrations of CRP

may lead to leptin resistance in RA[25].

In our study, we have also evaluated the effects of current treatments on serum leptin levels. Different treatments did not seem to have any effect on serum leptin levels in our study. Gunaydin et al.[26], similarly, observed no difference of serum leptin levels in patients treated with Methotrexate (MTX). In contrast, Bokarewa et al.[21] found higher leptin levels in patients treated with MTX, compared to other DMARDs. When serial measurements are done in patients starting anti--TNFα therapy with adalimumab, although clinical activity and markers of inflammation decreased, serum levels of leptin and adiponectin did not change[27]. However, in another study, leptin levels were also found similar between baseline and 2 weeks after anti-TNFα therapy[19].

In vitro studies revealed that inflammatory cytokines especially TNF-α, may have dual effects on leptin secretion. In acute inflammatory situations such as sepsis or major surgeries, increased TNF-α and IL-1β stimulate leptin release[28]. As a result of higher leptin concentrations, anorexia and cachexy may develop in acute inflammatory processes, similar to active RA patients. However, leptin levels decrease during chronic inflammation[11,29]. The decreasing effect of chronic inflammation on leptinaemia may be counterpoised with inflammatory mediators increased during the flares of the disease. Suppressive effect of chronic inflammation on leptin levels may be one of the reasons of increased susceptibility to infections in RA patients, especially on anti-TNFα therapies[30].

The main limitation of our study is its cross-sectional nature. Longitudinal studies may demonstrate fluctuating levels of leptin better than our study. Also, we have measured only serum concentrations of leptin while some studies showed increased levels in the synovial fluid of RA patients[21,31].

In conclusion, in our study, serum leptin levels were not increased in RA patients and did not correlate with disease activity. Different factors such as BMI, chronicity of inflammation and other concomitant chronic diseases may effect leptin levels. Therefore further studies are needed to elucidate the relationship between leptin and inflammation.

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