Leptin is associated with disease activity but not with anthropometric indices in rheumatoid arthritis patients

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Abstract

Introduction: Leptin is a cytokine-like hormone which has a complex role in inflammation. However, the importance of leptin in the pathogenesis of rheumatoid arthritis (RA) is far from being fully elucidated. The aim of the study was to determine serum leptin levels in RA patients and to evaluate whether there is an association between disease activity, anthropometric indices and leptin levels.

Material and methods: This hypothesis-generating study included 55 RA patients and 25 matched healthy subjects. The serum leptin concentration was determined by enzyme-linked immunosorbent assay (ELISA).

Results: Median serum leptin level in RA patients of 27.4 ng/ml (14.5–54.9 ng/ml) was statistically significantly higher (p = 0.03) compared with the median leptin value of 16.3 ng/ml (9.6–38.8 ng/ml) determined in healthy controls. The serum leptin level in the high disease activity group was significantly higher (p < 0.0005) than that in the low disease activity group and in healthy controls. A significant difference (p = 0.001) in serum leptin level was also found when the high disease activity group was compared with the moderate disease activity group. In the RA group a statistically significant positive correlation (rho = 0.390; p = 0.003) was observed between serum leptin level and disease activity score (DAS28).

Conclusions: The present results show that serum leptin levels are increased and significantly associated with disease activity in patients with RA and may have a valuable role in the inflammatory reactions and pathogenesis of RA.

Key words: rheumatoid arthritis, disease activity, leptin.

Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, autoimmune disease characterized by synovial inflammation, cartilage damage and bone erosion [1]. The prevalence of RA in the general population is between 0.3% and 1% [2].

It is a disease with a multifactorial etiology in which genetic predisposition and autoimmune factors as well as various environmental and life style risk factors (e.g. infections by microbial agents, smoking, obesity) contribute to disease susceptibility [3].

Although the pathogenesis of RA remains unknown, it is commonly believed that the overproduction of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-17 (IL-17) plays a crucial role in the development of the disease [4].

The important role of adipokines, white adipose tissue (WAT) specific soluble proteins, in inflammation suggests that they may also contribute to the occurrence of RA [5].

Leptin is an obese (ob) gene product, a cytokine-like hormone synthesized predominantly by WAT, but also endothelial cells, T lymphocytes, bone marrow cells, spleen cells and platelets. Leptin belongs to the long-chain helical cytokine family and has structural similarity to IL-6, IL-12, IL-15, granulocyte colony-stimulating factor (G-CSF), oncostatin M (OSM), prolactin and growth hormone. The leptin receptor, Ob-R, shows sequence homology to members of the class I cytokine receptor (gp130) family, which includes the receptor for IL-6, IL-12, OSM and prolactin [6]. Although the main role of leptin is to regulate body weight by inhibiting food intake and stimulating energy expenditure [7], recent evidence has indicated that leptin also influences reproduction, glucose homeostasis, hematopoiesis, angiogenesis, osteogenesis, wound healing and inflammation [8]. Furthermore, leptin takes part in the regulation of both innate and adaptive immune responses.

In innate immunity, leptin induces chemotaxis of neutrophils and the release of oxygen radicals through direct and indirect mechanisms [9]. Leptin also increases phagocytosis by monocytes/macrophages, and activates and promotes macrophage cell chemotaxis [10].

In adaptive immunity, leptin stimulates secretion, maturation, and survival of thymocytes, proliferation of naive T cells and differentiation to Th1 phenotype [11, 12]. Leptin switches toward Th1 immune responses on memory T cells by increasing interferon-γ (IFN-γ) and TNF-α secretion and by stimulating production of immunoglobulin G2α (IgG2α) by B cells [13].

In recent years, a variety of studies have been carried out, and their results suggest that leptin could be a member of the cytokine network managing the inflammatory immune response and host defense mechanisms [14]. Essentially, the interactions between leptin and inflammation are bidirectional: leptin regulates the production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 and, on the other hand, these cytokines increase the synthesis and release of leptin.

To our knowledge, the data regarding the relationship between serum leptin levels versus the activity or severity of the disease in patients with RA are still conflicting [15]. Certain publications describe serum leptin levels to be up-regulated in patients with RA [16], whereas others could not confirm this observation [15].

Bearing in mind the current disagreement over leptin involvement in RA, we generated the hypothesis that the inflammatory process and disease activity in RA are related to serum leptin levels.

Material and methods

Study population

This hypothesis-generating study included an initial sample of 63 RA patients and 31 randomly selected age- and gender-matched apparently healthy, medication-free, asymptomatic volunteers from the general population without clinical or biochemical signs and symptoms of RA or any other disease.

Rheumatoid arthritis patients diagnosed according to the 1987 American College of Rheumatology (ACR) revised classification criteria [17] were hospitalized in the Clinic for Heart, Blood Vessels and Rheumatism, University Clinical Center Sarajevo (UCCS), between January 1st, 2011 and June 30th, 2011.

The inclusion criteria included patients above the age of 18 years, either sex, fulfilling the ACR criteria for RA. The exclusion criteria were concomitant diseases including other rheumatic diseases, malignancy, active local or systemic inflammation caused by bacterial and viral infections, thyroid dysfunction, liver or kidney disease, current smokers, patients receiving statins, enzyme-inducing drugs or enzyme-inhibiting drugs.

This study population has been analyzed previously in a study where serum nitric oxide (NO) concentration was determined [18].

Eight RA patients were excluded from the initial sample: three because of diabetes mellitus, one because of hyperlipidemia and 4 patients refused to participate. A total of 55 RA patients fulfilled the inclusion criteria and were recruited to the study as the RA group for leptin determination. Twenty-five of 31 healthy individuals agreed to participate in a subsequent study as a control group for leptin determination.

Based on the stage of disease activity evaluated by the disease activity score (DAS28), patients with RA (RA group) were divided into three subgroups: low disease activity (DAS28 ≤ 3.2; n = 14), mod-

erate disease activity (3.2 < DAS28 ≤ 5.1; n = 19) and high disease activity (DAS28 > 5.1; n = 22).

The study protocol was approved by the Ethical Committee of the UCCS, registered under number 0305-33957. All participants signed informed written consent after the explanation of the study procedure. All procedures were conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki for human subjects.

Physical examination

Detailed history was taken from patients using a specially prepared questionnaire that included questions relevant to RA such as disease duration, morning stiffness and drug taking history. At the time of blood sampling all the patients were receiving disease-modifying antirheumatic drugs (DMARDs). Methotrexate (MTX) was the most frequently prescribed DMARD, used by 51 patients (36 used MTX alone, 5 in combination with sulfasalazine, 3 in combination with cyclosporin, 7 in combination with TNF-α inhibitors) and the remaining 4 patients received sulfasalazine (2 used sulfasalazine alone, 2 in combination with cyclosporin). All patients underwent thorough clinical examination with special attention to articular examination.

The disease activity in RA patients was determined by an experienced physician with the use of DAS28. DAS28 is a mathematical formula with four variables: $DAS28 = 0.56 \times \sqrt{(TEN28)} + 0.28 \times \sqrt{(SW28)} + 0.70 \times Ln(ESR) + 0.014x$ (GH). (TEN28: 28 joint count for tenderness, SW28: 28 joint count for swelling, Ln(ESR): the natural logarithm of Westergren's erythrocyte sedimentation rate (ESR); GH: general health or patient's global assessment of disease activity on the visual analog scale (VAS) of 100 mm) [19].

Anthropometric measurements

Anthropometric measurements were obtained, including height, weight, waist and hip circumference. Height was measured to the nearest 0.5 cm using a stadiometer. Weight in light clothing and without shoes was measured to the nearest 0.1 kg on a digital scale. Waist circumference was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape. The hip circumference was measured at the maximum circumference of the hip with the subjects in the standing position [20]. In the European Union waist circumference ≥ 94 cm in men and ≥ 80 cm in non-pregnant women are used as cut-offs for central obesity [21].

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). According to World Health Organization (WHO) criteria, BMI values in the range 18–25 kg/m^2 were considered as normal weight, 26–29 kg/m^2 as overweight, and BMI equal to or greater than 30 kg/m^2 as obese [22].

The waist-to-hip ratio (WHR) was determined by dividing the waist measurement by the hip measurement. The waist-to-height ratio (WHtR) was determined by dividing the waist measurement by the height measurement [23].

Laboratory analysis

Blood samples were collected by venipuncture between 8.00 and 10.00 a.m. in all patients and controls, following an overnight fast. Serum was separated by centrifugation and kept at –80°C until estimation.

Leptin is exceptionally stable in frozen serum samples, over 2 years at –20°C and over five freeze-thaw cycles [24]. Serum leptin concentration was determined by sandwich enzyme-linked immunosorbent assay (ELISA) technique, using a commercially available quantitative reagent kit (DRG Leptin EIA-2395, Germany) based on the sandwich principle with a limit of detection of 1.0 ng/ml at the Department of Immunology, UCCS, Sarajevo. Positive and negative controls were included in each test run.

Statistical analysis

The distribution of the variables was determined with the Shapiro-Wilk test and normality plots. An unpaired Student t-test or Mann-Whitney U-test was used to compare the difference between two groups, as appropriate. The correlations between the variables were assessed by Spearman's test. P-values less than 0.05 were considered statistically significant.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 13 (IBM, Chicago, Illinois, USA), and the results are presented as tables or figures. Values with normal distribution were expressed as mean ± standard deviation, while those without normal distribution were shown as median and interquartile range.

Results

The baseline characteristics of RA patients and healthy controls are shown in Table I. The distribution of age, gender, BMI, waist circumference (WC), WHR, and WHtR were not statistically significantly different between the groups. The median disease duration in RA patients was 6 years (4.0–14.0 years).

Median serum leptin level of RA patients was 27.4 ng/ml (14.5–54.9 ng/ml) and was statistical-

ly significantly higher (p = 0.03) compared with median serum leptin level in healthy controls of 16.3 ng/ml (9.6–38.8 ng/ml) (Figure 1).

Median serum leptin level in the group of patients with high disease activity of 58.65 ng/ml (27.35–97.25 ng/ml) was significantly higher (p < 0.0005) than that in the low disease activity group of 19.15 ng/ml (10.62–28.57 ng/ml) and in healthy controls of 16.3 ng/ml (9.6–38.8 ng/ml). A significant difference (p = 0.001) in serum leptin level was also found when the high disease activity group was compared with the moderate disease activity group (58.65 ng/ml (27.35–97.25 ng/ml) vs. 22.1 ng/ml (9.0–48.8 ng/ml)). Comparing the serum leptin levels between other groups showed no significant difference (Figure 2).

A statistically significant positive correlation was observed between serum leptin level and BMI (rho = 0.697; p < 0.0005), WC (rho = 0.590; p = 0.002), and WHtR (rho = 0.510; p = 0.009) in the control group. No correlation was observed between serum leptin level and anthropometric indices (BMI (rho = 0.141; p = 0.306); WC (rho = 0.225; p = 0.108); WHR (rho = 0.169; p = 0.237), WHtR (rho = 0.058; p = 0.684)) or disease duration (rho = 0.083; p = 0.546) in the RA group (Table II).

A statistically significant positive correlation was observed between serum leptin levels and DAS28 score (rho = 0.390; p = 0.003) in the RA group (Figure 3).

Discussion

Although rheumatoid arthritis is a common autoimmune disorder, it is also associated with a chronic inflammatory response. Recently, it was shown that adipocytes surrounding the RA joints secrete adipokines that may regulate inflammatory and immune processes [25]. However, the mechanism of leptin performance, as a pleiotropic molecule, within arthritic inflammation remains unclear. Currently published data regarding the association between plasma/serum leptin levels and RA are contradictory.

The results of our study showed that the serum leptin level was significantly higher in RA patients

Table I. Baseline characteristics of control group and RA group

Variables	Control group (n = 25)	RA group (n = 55)
Ages [years]	50.6 ±7.4	54.9 ±11.8♦
Sex (F/M)	23/2	51/4♦
Disease duration [years]	NA	6.0 (4.0–14.0)
BMI [kg/m^2]	27.0 (22.9–30.2)	26.0♦ (22.6–28.4)
WC [cm]	86.0 ±12.9	91.3 ±12.1♦
WHR	0.80 (0.70–0.90)	0.85♦ (0.79–0.91)
WHtR	0.50 (0.45–0.60)	0.53♦ (0.48–0.59)

Data are presented as mean ± SD; median (25th and 75th percentiles); BMI – body mass index, WC – waist circumference, WHR – waist-to-hip-ratio, WHtR – waist-to-height ratio, RA group – patients with rheumatoid arthritis, n – number of cases, NA – not applicable, NS – not significant, p – probability, ♦p = NS – compared with control group.

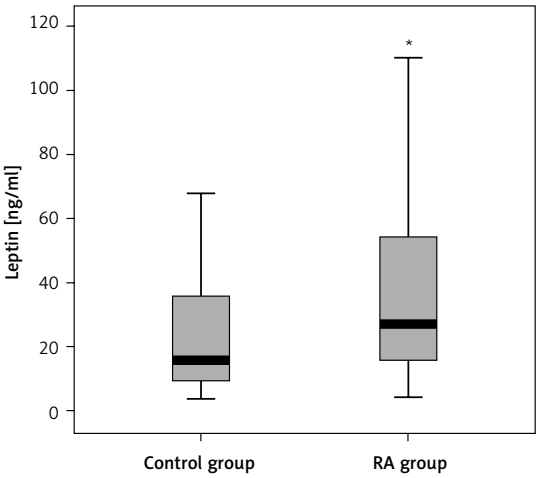

Figure 1. Box-and-whisker plots of serum leptin levels (ng/ml) in the RA group and healthy controls. The solid horizontal lines denote the median value, the box represents the 25% and 75% interquartile ranges, and the whiskers represent minimum and maximum values

**p = 0.03 compared with control group.*

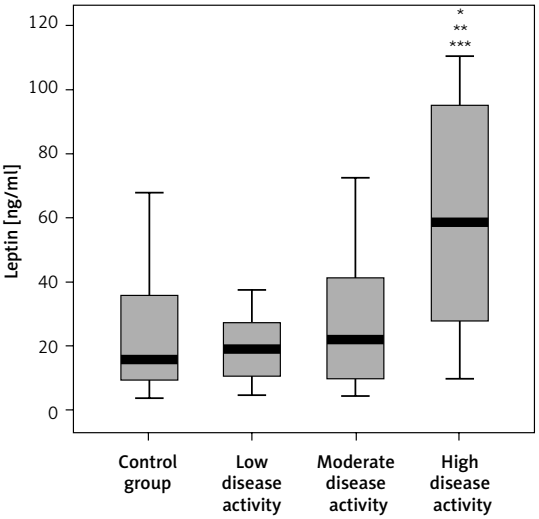

Figure 2. Box-and-whisker plots of serum leptin levels (ng/ml) in the RA patients with different disease activity and healthy controls. The solid horizontal lines denote the median value, the box represents the 25% and 75% interquartile ranges and the whiskers represent minimum and maximum values

**p < 0.0005 compared with control group, ** p < 0.0005 compared with low disease activity, ***p = 0.001 compared with moderate disease activity.*

Table II. Correlations of serum leptin levels with disease duration and anthropometric indices in control group and RA group

Variables	Leptin [ng/ml]			
	Control group (n = 25)		RA group (n = 55)	
	Rho	*P*-value	Rho	*P*-value
Disease duration [years]	NA		0.083	0.546
BMI [kg/m^2]	0.697	< 0.0005	0.141	0.306
WC [cm]	0.590	0.002	0.225	0.108
WHR	0.200	0.337	0.169	0.237
WHtR	0.510	0.009	0.058	0.684

BMI – body mass index, WC – waist circumference, WHR – waist-to-hip-ratio, WHtR – waist-to-height ratio, RA group – patients with rheumatoid arthritis, n – number of cases, NA – not applicable, rho – Spearman correlation coefficient, p – probability.

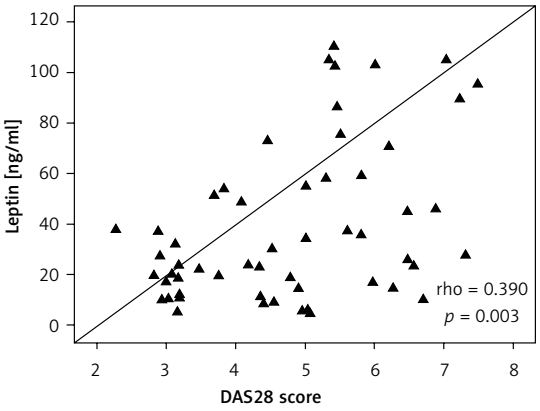

Figure 3. Correlation between serum leptin levels and DAS28 score in RA group

compared with the healthy controls. The obtained results are consistent with the results of Seven *et al*. [26], who found significantly higher serum and synovial fluid leptin levels in RA patients than in the control group. Our study also confirmed results reported by Sarraf *et al.* [27], who reported that in the process of chronic inflammatory diseases, pro-inflammatory cytokines (IL-1 and TNF-α) increase obese gene expression and leptin secretion.

According to the results of Rho *et al.* [28], the higher concentrations of leptin in patients with RA than controls can be attributed to differences in inflammation rather than BMI.

Leptin has pro-destructive and pro-inflammatory effects on cartilage. Human chondrocytes express the leptin receptor Ob-Rb and, when acting together with IFN-γ, leptin stimulates NO production in the joint cavity. NO contributes to the destruction of cartilage by inhibiting the synthesis of collagen and proteoglycans and enhancing apoptosis of chondrocytes [29]. However, in our previous study we did not find a statistically significant difference between serum NO values in RA patients and healthy controls [18].

In the present study, there was no correlation between serum leptin levels and anthropometric measurements (BMI, WC, WHR and WHtR) in the group of patients with RA, but positive correlations between serum leptin levels and BMI, WC and WHtR were found in healthy individuals.

Our results are in agreement with the findings of Seven *et al.* [26], who also reported no correlation between plasma leptin concentration and BMI in RA patients and suggested that regulation of leptinemia is very complex and that weight is not the only major regulator of leptin concentration. On the other hand, a positive correlation between serum leptin concentration and BMI in patients with RA was found in a study by Allam *et al.* [30].

Leptin was not found to be significantly correlated with disease duration in the current study. This is in agreement with the findings of Popa *et al.* [31], who also found that serum leptin levels in RA patients were not related to disease duration.

Although the majority of studies determined high serum leptin levels in patients with RA, some other studies did not support these results. In contrast to our results, Hizmetli *et al.* [32] reported no differences between serum levels of this adipokine in RA and healthy subjects. Targojska-Stdpniak *et al.* [33] also found that mean serum leptin concentration in their RA patients remained within the normal range. Tokarczyk-Knapik *et al.* [34] reported lower mean serum leptin concentration in patients with RA than in the control group. These authors suggested that the physiologic relation of serum leptin to body fat stores is not present in patients with RA.

In our study, patients with high disease activity showed statistically significantly higher serum leptin levels then healthy controls and patients with low and moderate disease activity.

This is in agreement with the findings of Lee *et al.* [35], who also reported significantly higher serum leptin levels in RA patients with high dis-

ease activity, as well as positive correlations between leptin and DAS28 score. Similarly, Seven *et al.* [26] reported that RA patients with moderate disease activity had significantly higher leptin levels than those with low disease activity.

In the present study, a significant positive correlation was found between serum leptin levels and DAS28 score. The obtained results suggest that adipose tissue leptin secreting activity is increased in patients as the disease progresses, which may be associated with the pro-inflammatory role of leptin in RA.

Further, longitudinal prospective studies involving a larger population are needed to confirm the findings of the present study and to better understand the role of leptin in the pathogenesis of rheumatoid arthritis.

In conclusion, we observed that serum level of leptin in patients with RA was significantly higher than that in the control group. Serum level of leptin in the RA patients was correlated with disease activity but not with BMI or disease duration. Our findings indicate that leptin itself has an important role in the inflammatory process in RA patients and may be a valuable marker in monitoring disease activity.

Conflict of interest

The authors declare no conflict of interest.

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