PLASMA LEPTIN STATUS AND THE RELATIONSHIP BETWEEN DIFFERENT MEDICAL TREATMENTS USED IN ANKYLOSING SPONDYLITIS

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ABSTRACT

Objective: To determine plasma leptin level in ankylosing spondylitis (AS) and its correlation with disease activity measurements. To examine the effects of different treatment modalities on leptin levels.

Material and Method: One hundred eight patients diagnosed with AS according to New York Criteria and 65 healthy individuals were enrolled in the study. The Bath AS Disease Activity Index (BASDAI) was used for disease activity. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels, acute phase reactants, plasma leptin levels, serum interleukin-6 (IL-6) and serum tumor necrosis factor-α (TNF-α) levels were investigated.

Results: Plasma leptin levels in AS patients were statistically significantly lower compared with those in the healthy control group (p=0.003). There was no significant difference among sub-groups established on the basis of medical treatments and disease activity. No statistically significant correlation was determined between leptin level and disease activity parameters, radiological score and functional indices (p>0.05).

Conclusion: Plasma leptin was lower in AS patients compared with the control group. This is not correlated with disease activation and medical treatment utilized.

Key Words: Ankylosing spondylitis, leptin, TNF-α

ANKİLOZAN SPONDİLİTLİ HASTALARDA UYGULANAN FARKLI TEDAVİ ŞEKİLLERİ İLE PLAZMA LEPTİN DEĞERLERİ ARASINDAKİ İLİŞKİ

ÖZET

Amaç: Ankilozan spondilitli (AS) hastalarda plazma leptin seviyeleri ve hastalık aktivite ölçümleri arasindaki ilişkiyi belirlemek ve leptin düzeyleri üzerine farklı tedavi yöntemlerinin etkilerini incelemek.


Bulgular: AS’li hastalarda plazma leptin düzeyi sağlıklı kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı ölçüde düştü (p=0,003). Tibbi tedavi ve hastalık aktivitesi temelinde kurulan alt gruplar arasında anlamlı bir fark yoktu. Leptin düzeyi de hastalık aktivite parametreleri, radyolojik skoru ve fonksiyonel indeksler arasında istatistiksel olarak anlamlı bir korelasyon saptanmamıştır (p>0,05).

Sonuç: Kontrol grubu ile karşılaştırıldığında AS’li hastalarda plazma leptin seviyeleri düğük bulundu.

Anahtar Kelimeler: Ankilozan spondilit, leptin, TNF-α Nobel Med 2013; 9(2): 109-113
INTRODUCTION

Leptin is a 16-kDa cytokine-like peptide, correlated with obesity and secreted by adipose tissue. Adipose tissue was used to be regarded as a silent tissue with no function, but after the discovery of leptin in 1994, it was started to be seen as a secretory organ secreting several proteins, known as adipokines. Adipokines (such as leptin, adiponectin, resistin and visfatin) are proteins that play a role in several metabolic processes, energy metabolism, obesity and in the pathogenesis of various diseases.\(^2\) The correlation between obesity and musculoskeletal diseases has been shown through wide-ranging epidemiological studies. Obesity has been shown to be correlated with both mechanical loading and diseases such as osteoarthritis, back pain, gout, plantar fasciitis and inflammatory arthritis related to its effect on inflammatory pathways.\(^4\) The effects of adipokines on immune response and inflammation have previously been described. Leptin, adiponectin and resistin are the most studied adipokines. Leptin is regarded as a proinflammatory hormone in several studies. Effects on the immune system include an increase in monocyte/macrophage-activation related interleukin (IL)-6 and tumor necrosis factor (TNF)-α secretion, naïve T cells inducing Th1 proliferation, angiogenesis as a result of endothelial cell stimulation, chondrocyte stimulation, transforming-growth factor increase-related enhanced osteophyte formation and an increase in IL-2 and interferon (IFN)-γamma secretion. Levels of all three adipokines have been shown to rise in synovial fluid in joint diseases.\(^4\) Leptin has been shown to serve as a proinflammatory hormone in patients with rheumatoid arthritis, the most common inflammatory rheumatismal disease and to be significantly correlated with disease activation.\(^9\) Another study showed that leptin induced an increase in interleukin 1 receptor antagonist secretion in vivo, and it was suggested that leptin might have an immunomodulator effect.\(^10\) Studies that report a negative correlation between leptin levels and disease activation also exist.\(^11\)

Ankylosing spondylitis (AS) is a prototype of the spondyloarthropathies group of diseases. AS particularly affects the spine and sacroiliac joints. Enthesial regions are where AS and inflammation begin, and involvement of the region is characteristic of the disease.\(^12\) Studies have shown the migration of mononuclear cells and T lymphocytes in inflammation areas particularly in the sacroiliac joint and the increased levels of TNF-α and interferon gamma, which are the products of these cells. High levels of proinflammatory cytokines such as TNF-α and IL-6 have been determined in AS and correlated with disease activity.\(^13\) Some studies concerning leptin levels in AS have reported a correlation, while others have maintained the opposite.\(^1,15,16\)

The aim of our study is to investigate leptin levels in AS patients observed at our clinic. Its correlation with disease activity, and to research the correlation between leptin levels and different medical treatments.

MATERIAL and METHOD

108 patients diagnosed with AS according to Modified New York Criteria and monitored by Karadeniz Technical University Medical Faculty Physical Medicine and Rehabilitation Department clinic were enrolled.\(^1\) Patients were given detailed physical examinations and divided into three sub-groups according to medical treatment received. Group 1 (n=47) consisted of patients administered with anti-tumor necrosis factor-α (TNF-α), Group 2 (n=37) of patients administered with only non-steroidal anti-inflammatory drugs (NSAID) and group 3 (n=22) of patients using sulfasalazine (SS) and NSAID. Patients receiving anti TNF-α treatment consisted of patients who were determined by improvement in at least 2 units from the Bath AS disease activity index (BASDAI) a 3-month evaluation. The control group consisted of 65 healthy adults chosen from patient relatives applying to the same clinic and matched in terms of age and gender. Patients with diabetes mellitus, hypertension, hyperlipidemia, atherosclerotic heart disease, previous chronic systemic diseases and chronic steroid users were excluded from the study.

Clinical and biochemical assessments

Patients were given detailed physical examinations. Disease activity was evaluated using BASDI, spinal morbidity using the Bath AS metrology index (BASMI), functional status using the Bath AS functional index (BASFI) and radiological examination using the Bath AS radiological indices (BASRI). The validity, reliability and repeatability and sensitivity to change of the indices used have been established.\(^18,19\) Patients with BASDAI values ≥4 were regarded as active.\(^20\) Serum and plasma specimens obtained from blood taken from individuals in the patient and control groups after at least 12 h of fasting and were kept at -80 °C until biochemical measurements were performed. Serum C reactive protein (CRP) levels were determined by immunonephelometric measurement (Dade-Behring II). Plasma leptin levels (biosoche, KAP2281), serum TNF-α (Biosoche, KAP1751) and interleukin (IL)-6 (Biosoche, KAP1261) levels were measured using ELISA.

Compatibility with normal distribution of data obtained by measurement was investigated using the Kolmogorov-Smirnov test. Student’s t test was used in comparing two-way distributed measurement data and the “Mann-Whitney U” test for data not normally distributed. In three-way --
comparisons, ANOVA (post-hoc Tukey) was used since this was compatible with normal distribution. The “chi-square test” was used in the comparison of qualitative data. Pearson correlation analysis was used in the correlation of normally distributed parameters in the analysis of leptin levels and disease, and Spearman correlation analysis for those not normally distributed. p<0.05 was regarded as significant.

Informed consent forms were received from patient and healthy controls. Ethics Committee approval was obtained prior to the study.

RESULTS

173 individuals (108 AS patients and 65 controls) were enrolled. Mean age of the AS patients was 36.4±11.2 years, and that of the controls was 38.2±13.0 (p= 0.330). 88 of the AS patients were men, and 20 were women; 49 of the control group were men and 16 were women. There was no statistical difference between the groups in terms of gender distribution (p=0.222). Patients’ symptom duration was 8.4±5.6 years, and length of first diagnosis 4.2±4.4 years. Patients’ chest expansion was 3.6±1.6 cm and Schober’s test 12.9±1.6 cm. AS patients’ clinical characteristics and the parameters used in disease evaluation are shown in Table 1.

When AS patients and the control group were compared in terms of plasma leptin levels, these were statistically significantly lower in the former (p=0.003). Patients were divided into three sub-groups on the basis of medical treatment. No statistically significant differences in ESR, CRP, IL-6 or leptin levels were determined among these sub-groups (p=0.486, p=0.258, p=0.342 and p=0.180, respectively). There was, however, a significant difference in TNF-α values (p=0.037). We classified disease activation as BASDAI<4 (n=50) and BASDAI ≥4 (n=49). When groups were evaluated in terms of ESR, CRP, TNF-α, IL-6 and leptin levels, there was a difference in ESR and CRP but no difference in terms of other parameters (p=0.0001, p=0.008, p=0.773, p=0.129, and p=0.405, respectively) (Figure 1). Although leptin levels were lower in in the group using biological agents, the difference was not statistically significant (Figure 2).

Correlation analysis revealed no significant correlation between leptin levels and the disease activity parameters BASDAI, ESR, CRP, TNF-α and IL-6. There was no significant correlation between the BASMI, BASRI and BASFI disease indices used in the study (p>0.05) (Table 2).

DISCUSSION

Plasma leptin levels were significantly lower in the AS patients in our study compared to the control group. Leptin level was not correlated with disease activity parameters.

Leptin is an important adipokine shown to play a role in inflammation. Leptin levels in studies regarding rheumatoid arthritis (RA) have been determined as

<table>
<thead>
<tr>
<th>Table 1: Demographic, clinical and biochemical variable data of patients with AS and controls.</th>
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<tr>
<td><strong>AS (n=108)</strong></td>
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<tr>
<td><strong>Gender (M/F)</strong></td>
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<td><strong>Age (years)</strong></td>
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<td><strong>ESR (mm/h)</strong></td>
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<td><strong>CRP (mg/dl)</strong></td>
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<td><strong>IL-6 (pg/ml)</strong></td>
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<td><strong>BASDAI</strong></td>
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<td><strong>BASFI</strong></td>
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<td><strong>Symptom duration (years)</strong></td>
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<td><strong>First diagnosis (years previously)</strong></td>
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<td><strong>Schober’s test (cm)</strong></td>
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<td><strong>Chest expansion (cm)</strong></td>
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BASDAI: Bath AS Disease Activity Index, BASMI: Bath AS Metrology Index,  BASFI: Bath AS Functional Index, BASRI: Bath AS Radiographic Index, CRP: C reactive protein, ESR: Erythrocyte sedimentation rate

![Figure 1: Leptin values in patients with ankylosing spondylitis and control groups](image1)

![Figure 2: Leptin levels after treatment in patients with ankylosing spondylitis](image2)
higher compared to the control groups, while different results have been reported in AS studies. Similar results to ours have been reported, although there are other studies that have reported the opposite.

Sarı et al. compared serum leptin levels, insulin resistance and body composition in AS patients with a healthy control group. They reported that leptin levels were significantly lower than those in the control group. Toussirot et al. investigated the levels of adipokines (leptin, adiponectin and ghrelin) in AS, and reported lower leptin levels compared to the control group, but no difference in adiponectin levels were found. However, it was suggested that the significantly higher ghrelin levels could mean that ghrelin might play a role in the pathogenesis of AS.

No correlation was determined between leptin and disease activity parameters in either study.

Some studies have reported a significant correlation between leptin levels and disease activity. Park et al. conducted a prospective investigation of leptin levels and disease parameters in AS patients. One significant difference between that study and the other study is that it consisted of newly diagnosed and DMARD naive patients. At initial analysis, leptin levels were higher than those in the control group and were correlated with BASDAI, CRP and IL-6 level. It was suggested that leptin might play a role in the pathogenesis of AS. Patients were started on nonsteroid anti-inflammatory and disease modifying drugs. At a second evaluation conducted after an average 31 months of monitoring, there was a significant decrease in disease activity parameters (BASDAI, sedimentation, CRP) and leptin levels.

Another study by the same authors was intended to investigate the hypothesis that leptin mRNA expression increases from peripheral blood mononuclear cells (PBMCs) of patients with active AS and whether it stimulates pro-inflammatory cytokine production. AS patients’ leptin, IL-6 and TNF-α mRNA expressions of PBMCs were significantly higher compared to the controls. This study concluded that leptin production rose and its stimulation of PBMCs significantly enhanced the production of pro-inflammatory cytokines in patients with active AS, suggesting that it has pro-inflammatory effect in AS pathogenesis.

In our study, analysis in terms of BASDAI (<4 inactive and ≥4 active) revealed no statistically significant difference in leptin levels among the groups (p>0.05). We determined no significant correlation between leptin and CRP and IL-6. In contrast to Park et al.’s study, our patients had been ill for an average duration of 4.2±4.4 years and were receiving medical treatment (biological drugs, salazopyrin, NSAID). In their study, Sarı et al. determined a significant correlation between BASRI and leptin. We determined no correlation between BASRI, BASFI and BASMI scores and leptin levels. In conclusion, there was no correlation shown in our study between leptin and activity and functional status.

Biological agents are drugs that act by targeting different pathways on immune system mechanisms. The most frequently used of these are drugs that make TNF-α blockage. TNF-α is a key cytokine that plays a role in inflammation. Anti TNF-α drugs have been shown in many studies to have positive effects on disease activity and progression. The effects on leptin levels of biological agents frequently used in the treatment of inflammatory diseases have been investigated in various studies. The effects of biological treatment compared with initial levels. Some studies have reported a significant increase in adiponectin levels after one year in 99 female patients with RA using biological treatment compared with initial levels. In another study, Derdemizis et al. determined no significant difference in leptin and adiponectin levels after 6 months of infliximab treatment. In conclusion, leptin levels in RA were high, and the effects of medical treatment on leptin were debatable.

We found no studies in the literature examining the effect of biological agents on leptin in AS. In contrast to other studies, our patient group contained a high number of subjects (50%) using biological drugs. Leptin levels were lower in the biological treatment group when patients were grouped according to medical treatment, though this was not statistically significant.
Leptin levels were higher in the group using NSAID and SS compared to the group receiving biological treatment. However, there is a need for wide-ranging and comprehensive studies involving pre and post treatment analysis in order to support these data. We consider our study a fore-runner to such research.

There are certain limitations to our study. No evaluation was made of patients pre and post treatment. However the preponderance of patients receiving biological treatment among the treatment groups was significant. In conclusion, plasma leptin levels were low in our AS patients. This is not correlated with medical treatment or clinical and laboratory parameters. Leptin levels were lower in the group receiving biological treatment. There is now a particular need for wide-ranging studies investigating the role of leptin in the AS clinical process and the effects of biological treatments.

REFERENCES