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Relationship between serum adipokine levels and radiographic progression in patients with ankylosing spondylitis
A preliminary 2-year longitudinal study

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Abstract

The immunomodulatory effects of adipokines have been extensively studied in rheumatic diseases, and there is a paucity of information regarding their effects on bone metabolism.

The aim of this study was to investigate the relationships between serum adipokines levels and radiographic progression over 2 years in patients with ankylosing spondylitis (AS).

In this preliminary longitudinal study, we prospectively recruited 20 consecutive male patients with AS and 11 gender- and age-matched healthy subjects. At the baseline and 2-year follow-up, serum adiponectin, leptin, resistin, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and Dickkopf-1(DKK-1) levels were measured in AS patients using enzyme-linked immunosorbent assays; these measurements were only performed at the baseline for healthy controls. Radiographic progression was determined as the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) progression of ≥2 by comparing the baseline and 2-year follow-up radiographs.

All AS patients were naive to TNF-α blockers at the enrollment and during the 2-year follow-up period and their median disease duration was 51.5 months. At the baseline, the serum resistin, TNF-α, and IL-6 levels were significantly higher in AS patients than in controls. At the 2-year follow-up, the median mSASSS of AS patients was found to be significantly increased from the baseline (8–10.5, $P = .001$) and 7 (35%) AS patients showed radiographic progression. In AS patients, the leptin and resistin levels were significantly higher at the 2-year follow-up than at the baseline. The baseline resistin levels and changes in leptin levels from the baseline to the 2-year follow-up were significantly higher in AS patients with radiographic progression than in those without radiographic progression ($P = .002$ and .024, respectively). The baseline resistin levels and the increase in leptin levels during the follow-up period significantly correlated with changes in mSASSS ($\rho = 0.528$ and 0.559, $P = .017$ and .01, respectively). No association between changes in serum adipokine levels and disease activity in AS patients was observed.

Our findings suggest that leptin and resistin may contribute to the pathogenesis of new bone formation rather than to inflammatory processes and have the potential to be used as biomarkers of the structural outcome of AS.

Abbreviations: AS = ankylosing spondylitis, BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, BASMI = Bath Ankylosing Spondylitis Metrology Index, BMI = body mass index, CRP = C-reactive protein, DKK-1 = Dickkopf-1, ESR = erythrocyte sedimentation rate, HDL-C = high-density lipoprotein cholesterol, HLA-B27 = human leukocyte antigen B-27, IL-6 = interleukin-6, IQR = interquartile range, LDL-C = low density lipoprotein cholesterol, mSASSS = modified Stoke Ankylosing Spondylitis Spine Score, NSAIDs = non-steroidal anti-inflammatory drugs, OA = osteoarthritis, RA = rheumatoid arthritis, TG = triglyceride, TNF-α = tumor necrosis factor-α.

Keywords: adipokines, ankylosing spondylitis, bone, cytokines, osteoblasts

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1. Introduction

Ankylosing spondylitis (AS) is a chronic, progressive inflammatory arthritis that primarily affects the axial skeleton, that is, the spine and sacroiliac joint; however, peripheral joints are also commonly affected. In addition to axial inflammation, a key pathological hallmark is new bone formation in the form of syndesmophytes and spinal fusion resulting in spinal mobility limitation in patients with AS. Although inflammation is considered to impact quality of life in the early stage of the disease, as the disease progresses, new bone formation becomes the major factor determining the clinical outcome of AS.[1,2] The introduction of tumor necrosis factor-alpha (TNF-α) blocking agents has revolutionized the treatment of AS because of their high efficacy in reducing clinical and biological disease activity; however, the ability of TNF-α blockers to prevent new syndesmophytes development has not yet been fully determined.[3–5] In addition, the pathology underlying new bone formation in AS is not fully understood and biomarkers that predict disease progression are still lacking. Thus, there is a need for effective treatment strategies for and reliable predictors of structural damage in AS.

Adipokines such as leptin, adiponectin, resistin, and visfatin are bioactive molecules that are synthesized secreted primarily, but not exclusively by adipocytes; adipokines exert their functions in lipid metabolism, insulin sensitivity, and the regulation of energy balance.[6] With the increasing evidence of the immunomodulatory effects of adipokines, the role of these substances in the pathophysiology of chronic inflammatory diseases such as rheumatoid arthritis (RA) and AS has been studied extensively in recent times.[7–9] Previous investigations have suggested that serum adipokines levels are elevated in RA patients as compared with healthy controls and are associated with disease activity.[10] However, contradictory results have been reported on the association between adipokine levels and inflammatory status in patients with AS.[11] Leptin and adiponectin have been reported to stimulate osteoblast proliferation and bone mineralization in in vitro studies[12]; however, little attention has been directed to the role played by adipokines in the pathogenesis of new bone formation in AS. Although a recent clinical study showed that increased baseline visfatin levels are predictive of structural damage in AS patients,[13] longitudinal data investigating the relationship between the changes in serum levels and subsequent syndesmophyte formation are still lacking. Therefore, in the present 2-year longitudinal study, we examined the serum levels of adipokines, including adiponectin, leptin and resistin, and other cytokines such as TNF-α, interleukin-6 (IL-6), and Dickkopf-1 (DKK-1) in patients with AS and healthy controls and investigated the relationship between changes in the serum adipokines levels and radiographic progression.

2. Methods

2.1. Study design and subjects

In this preliminary 2-year longitudinal study, we prospectively recruited 20 consecutive male patients with AS and 11 gender- and age-matched healthy subjects from a university-affiliated rheumatology center in South Korea from January 2013 to December 2013. All subjects with AS met the modified New York criteria for AS[14] and were naive to biologic agents such as TNF-α blockers at the time of enrollment and during the 2-year follow-up period. Healthy controls had no history of any

rheumatic diseases and were not taking any drugs that could affect bone metabolism. The following patients with AS were excluded from the study: subjects with rheumatic diseases in addition to AS, patients with severe kidney or hepatic dysfunction, patients treated with bisphosphonates or other anti-osteoporosis medications, and patients who refused to participated in our study. Assessments including clinical and laboratory markers were performed at the baseline for both AS patients and healthy controls and at the 2-year follow-up for AS patients only (Fig. 1). For AS patients, radiographs are obtained at the baseline and at the 2-year follow-up (Fig. 1). All subjects voluntarily assented to participate in the study and provided written informed consent based on the Helsinki Declaration. Our study was approved by the Research and Ethical Review Board of the Pusan National University Hospital in Busan, South Korea (IRB no. 1302-004-015).

2.2. Clinical, laboratory, and radiographic assessments

At the baseline, demographic data such as age and the body mass index (BMI) and levels of laboratory markers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) TNF-α, IL-6, DKK-1, adiponectin, leptin, and resistin were obtained for both AS patients and healthy subjects. At the 2-year follow-up, CRP, ESR, TNF-α, IL-6, DKK-1, adiponectin, leptin and resistin levels, and BMI were measured only in the AS patients. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). All laboratory tests were conducted with blood samples obtained after overnight fasting. CRP levels were assessed by using the particle-enhanced immunoturbidimetric assay (Tina-Quant C-reactive protein assay; Roche Diagnostics, Zurich, Switzerland) with a P800 Module (Roche Diagnostics) and LDL-C, TG, and HDL-C concentrations were measured by using an enzymatic colorimetric reagent (Roche Diagnostics, Zurich, Switzerland) and a P800 Module (Roche Diagnostics); all measurements were conducted within 1 hour after blood collection. The remaining blood samples were centrifuged at 3000 rpm for 10 minutes (4°C) and the serum was separated and stored at -80°C to be used for quantitative measurements of adipokines and cytokines. The serum concentrations of TNF-α, IL-6, DKK-1, adiponectin, leptin, and resistin were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D System, Minneapolis, MN for TNF-α, IL-6, and leptin; Biomedica Medizinprodukte GmbH & Co. KG, Wien, Austria, for DKK-1, and BioVendor, Brno, Czech Republic for adiponectin and resistin) according to the manufacturer's protocol. The inter-assay coefficients of variation were 7.4% for
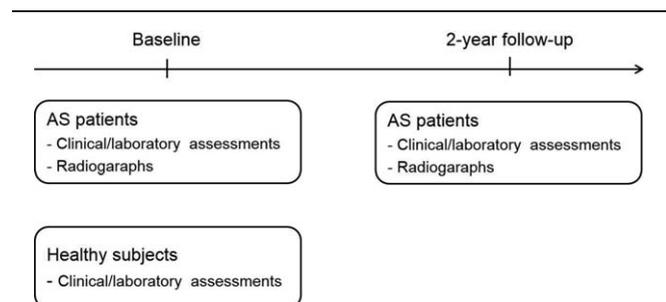
Figure 1. Schematic design of the present study.

TNF-α, 6.4% for IL-6, 7% for DKK-1, 5.8% for adiponectin, 5.4% for leptin, and 7% for resistin.

The following additional data were collected from AS patients at the baseline and at the 2-year follow-up: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), disease duration, concurrent medications, and lateral view radiographs of the lumbar and cervical spine. The presence of human leukocyte antigen B-27 (HLA B-27) in AS patients was also determined at the baseline. Structural damage to the spine was assessed by determining the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS).[15] Specifically, the anterior corners of the vertebrae (C2 lower to T1 upper and T12 lower to S1 upper) in radiographs were scored as follows: squaring, erosion, or sclerosis (1 point); nonbridging syndesmophyte (2 points); and bridging syndesmophyte (3 points). By comparing the baseline and 2-year follow-up radiographs, radiographic progression over 2 years was determined as mSASSS progression of ≥2 in at least 1 vertebral corner.[3] Radiographic images were scored and interpreted by a single highly experienced rheumatologist (Suh) who was blinded to the clinical and laboratory data.

2.3. Statistical analyses

Data are presented as the median (interquartile range; IQR) for continuous variables and numbers (percentages) for categorical variables. Because the number of study subjects was small, we assumed that all continuous variables were distributed nonparametrically. To compare the continuous variables, the Mann–Whitney U test (between AS patients and healthy subjects) and the Wilcoxon signed-rank test (between the baseline and the 2-

year follow-up in AS patients) were used. The Chi-squared test, Fisher exact test, or McNemar test was used to compare categorical variables, as appropriate. Spearman rank correlation coefficient was calculated to investigate the relationship between mSASSS progression and adipokine levels in AS patients. A P value of less than .5 was considered statistically significant. All statistical analyses were carried out using SPSS software version 18.0 (SPSS Inc. Chicago, IL) and STATA version 11.1 for Windows (StataCorp LP, College Station, TX).

3. Results

The clinical and laboratory characteristics at the baseline and at the 2-year follow-up are summarized in Table 1. At the baseline, the median (IQR) age and disease duration in patients with AS were 41 (32–43.5) years and 51.5 (13.5–107.8) months, respectively. Eighteen (90%) patients had positive results for HLA B-27. The median (IQR) BASDAI and mSASSS were 4.5 (2.4–6.2) and 8 (2.3–17.8), respectively, and all AS patients were taking nonsteroidal anti-inflammatory drugs (NSAIDs) at the baseline. The age and BMI at the baseline did not differ significantly between AS patients and healthy controls. The serum levels of CRP, ESR, TNF-α, IL-6, and resistin were significantly higher in patients with AS than in the healthy controls. Serum DKK-1 levels in AS patients tended to be lower than that in the healthy controls, but the difference was not statistically significant. The adiponectin, leptin, leptin/BMI, LDL-C, TG, and HDL-C were similar between patients with AS and healthy controls.

At the 2-year follow-up, the mSASSS of AS patients was found to be significantly increased from the baseline [8 (2.1–17.8) to 10.5 (3.3–24.5), P =.001, Table 1, Fig. 2A], and 7 (35%) AS

Table 1

Clinical and laboratory characteristics at baseline and 2 years follow-up in patients with ankylosing spondylitis and healthy subjects.

| Variables | AS (n=20) | | Controls (n=11) | Baseline vs 2 y | Baseline vs controls |
	Baseline	2 y		P	P
Age, y, median (IQR)	41 (32–43.5)	—	34 (31–40)	—	0.123
mSASSS	8 (2.3–17.8)	10.5 (3.3–24.5)	—	0.001	—
CRP, mg/dL, median (IQR)	0.26 (0.06–0.56)	0.07 (0.03–0.36)	0.03 (0.03–0.07)	0.243	0.002
ESR, mm/h, median (IQR)	14 (5.8–26)	12.5 (4.8–19.3)	7 (2–11)	0.444	0.044
BMI, kg/m^2, median (IQR)	25.4 (22.9–27.2)	25.6 (23.3–27.2)	22.9 (20.9–24.2)	0.248	0.079
TNF-α, pg/mL, median (IQR)	12 (7.7–36.2)	8.1 (5.6–18.6)	5.8 (3–8.1)	0.191	0.003
IL-6, pg/mL, median (IQR)	4.7 (2.7–11.4)	4.7 (0.9–6.2)	1 (0.3–1.4)	0.272	0.002
DKK-1, pg/mL, median (IQR)	769 (492–843)	515 (390–778)	1019.3 (365–1260)	0.017	0.087
Adiponectin, μg/mL, median (IQR)	5.5 (3.8–7.7)	5.7 (3.9–8.4)	7.9 (4.3–13.5)	0.351	0.169
Leptin, ng/mL, median (IQR)	3.8 (2.5–4.9)	4.3 (3.5–5.5)	2.9 (2.8–5.5)	0.048	0.919
Leptin/BMI, ng m^2/mL kg, median (IQR)	0.14 (0.9–0.18)	0.16 (0.12–0.24)	0.13 (0.12–0.23	0.03	0.761
Resistin, ng/mL, median (IQR)	5.3 (4.5–7.1)	6.6 (4.9–11.6)	3.8 (1.9–5.1)	0.03	0.049
LDL-C, mg/dL, median (IQR)	112 (96.3–149)	—	107 (93–158)	—	0.73
TG, mg/dL, median (IQR)	140.5 (82.8–201.8)	—	132 (74–275)	—	0.984
HDL-C, mg/dL, median (IQR)	48.5 (40.3–54)	—	58 (47–65)	—	0.066
Disease duration, months, median (IQR)	51.5 (13.5–107.8)	—	—	—	—
HLA-B27, n (%)	18 (90)	—	—	—	—
BASDAI	4.5 (2.4–6.2)	3.2 (2.4–4.6)		0.083	
BASFI	2.4 (0.6–4)	1.4 (0.1–3.2)		0.033	
BASMI	2.8 (1.6–4.5)	3 (2.1–4.4)		0.906	
Concurrent medications					
NSAIDs	20 (100)	13 (65)			
SSZ	19 (95)	10 (50)			
Glucocorticoids	1 (5)	2 (10)			

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, BASMI = Bath Ankylosing Spondylitis Metrology Index, BMI = body mass index, CRP = C-reactive protein; ESR = erythrocyte sedimentation rate, HDL-C = high-density lipoprotein, LDL-C = low-density lipoprotein cholesterol, mSASSS = the modified Stoke Ankylosing Spondylitis Spine Score progression, NSAIDs = nonsteroidal anti-inflammatory drugs, SSZ = sulfasalazaine, TG = triglyceride, TNF-α = tumor necrosis factor-alpha.
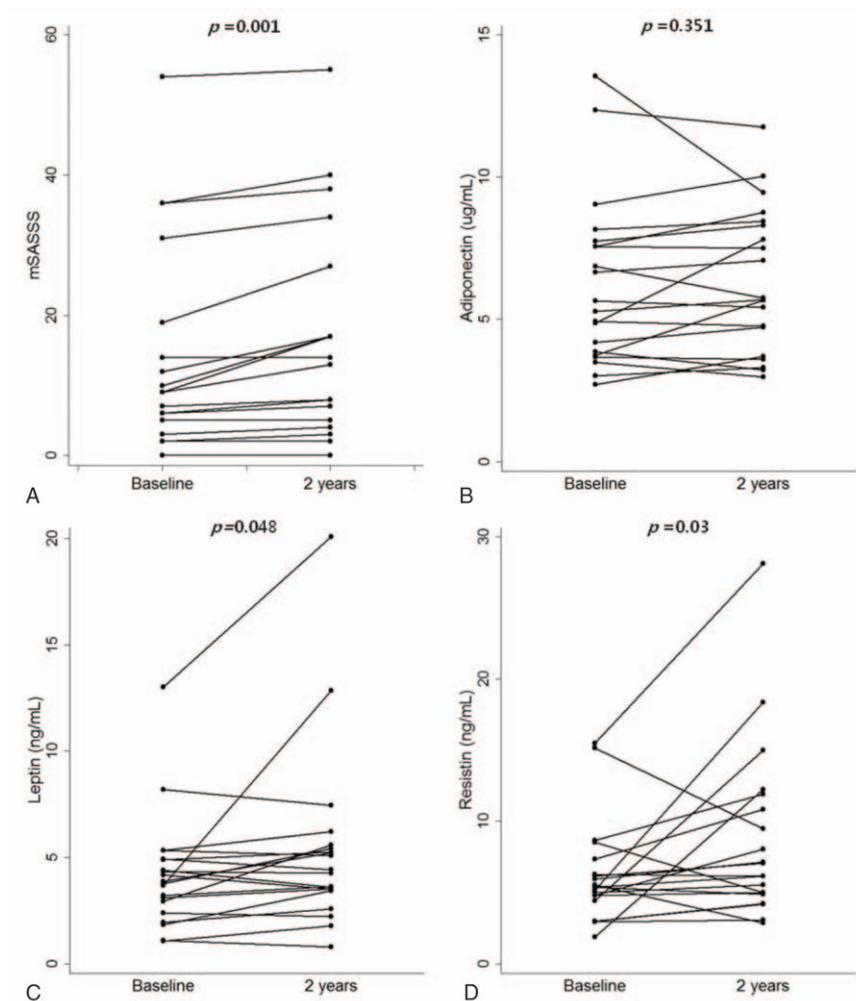
Figure 2. Changes in the modified Stoke Ankylosing Spondylitis Spine Score and serum adipokine levels in patients with ankylosing spondylitis during the follow-up period.

patients showed radiographic progression (mSASSS, ≥2). Adipokine, leptin, leptin/BMI, and resistin were significantly higher at the 2-year follow-up than that at baseline, whereas adiponectin levels at the baseline and the 2-year follow-up did not differ (Table 1 and Fig. 2B–D). As compared to the baseline values, the serum DKK-1 concentrations significantly decreased at the 2-year follow-up, but there were no significant changes in the TNF-α, IL-6, ESR, and CRP levels of patients with AS (Table 1). In addition, the BASFI of AS patients decreased significantly, while the BASDAI and BASMI did not change after 2 years (Table 1).

Table 2 summarizes the comparisons of clinical and laboratory markers according to the presence of radiographic progression in AS patients at the 2-year follow-up. As expected, AS patients with radiographic progression had higher baseline mSASSS and BASMI than did patients without radiographic progression (P=.024 and .046, respectively). Baseline resistin levels and changes in leptin and leptin/BMI from the baseline to the 2-year follow-up period were significantly higher in patients with radiographic progression than in those without radiographic progression (P=.024, .002, and .001, respectively). Otherwise, baseline values or changes in TNF-α, IL-6, DKK-1, adiponectin,

ESR, and CRP levels did not differ significantly between AS patients with and without radiographic progression. The use of sulfasalazine or glucocorticoids was not found to be associated with radiographic progression over 2 years (data not shown).

Table 3 summarizes the correlations between changes in mSASSS and clinical/laboratory variables in patients with AS at the 2-year follow-up period as calculated by using the Spearman correlation analysis. Baseline serum resistin and TNF-α levels showed significant positive correlations with changes in mSASSS (ρ=0.559 and 0.45, P=.01 and .047, respectively). In addition, changes in leptin and leptin/BMI levels between the baseline and 2-year follow-up were positively associated with increases in mSASSS (ρ=0.528 and 0.56, P=.017 and .01, respectively). Age at the baseline was also positively correlated with an increase in mSASSS in AS patients (ρ=0.53, P=.016). No other significant correlations were observed between changes in mSASSS and laboratory parameters, such as IL-6, DKK-1, adiponectin, ESR, and CRP. In addition, changes in the BASDAI did not correlate with changes in the levels of adipokines, such as adiponectin, leptin, leptin/BMI, and resistin (data not shown). However, delta serum TNF-α levels were positively correlated with changes in BASDAI (ρ=0.70, P<.001).

Table 2

Comparisons of clinical and laboratory characteristics according to the presence of radiographic progression in patients with AS.

Variables	Radiographic progression (n = 7)	No radiographic progression (n = 13)	P
Age, y, median (IQR)	42 (36–53)	35 (32–41.5)	.067
Disease duration, mo, median (IQR)	75 (7–96)	38 (14–116)	.757
mSASSS	12 (9–31)	5 (2–10.5)	.024
CRP, mg/dL, median (IQR)	0.46 (0.19–0.58)	0.24 (0.04–0.9)	.211
ESR, mm/h, median (IQR)	14 (10–44)	12 (2–24)	.275
BMI, kg/m^2, median (IQR)	25.5 (22.8–30.5)	25.3 (23.2–27)	.877
TNF-α, pg/mL, median (IQR)	17.7 (11.7–51)	8.8 (6.2–27.1)	.351
IL-6, pg/mL, median (IQR)	5.8 (3.5–17.9)	4.5 (1.5–7.1)	.491
DKK-1, pg/mL, median (IQR)	26.7 (19.1–35.1)	30.2 (18–32.7)	.999
Adiponectin, μg/mL, median (IQR)	5.2 (3–7.5)	6.6 (4–8.6)	.275
Leptin, ng/mL, median (IQR)	3.2 (1.8–3.9)	4.3 (2.7–5.1)	.241
Leptin/BMI, ng m^2/mL kg, median (IQR)	0.13 (0.06–0.16)	0.17 (0.1–0.21)	.311
Resistin, ng/mL, median (IQR)	7.4 (5.4–15.1)	5 (3–6)	.024
BASDAI	5.2 (2.9–6.2)	3.6 (1.2–6.3)	.536
BASFI	3.5 (2.1–4)	1.8 (0.4–3.8)	.241
BASMI	4.6 (2.8–6)	2.8 (1.3–3.4)	.046
Delta CRP, mg/dL, median (IQR)	−0.18 (−0.3 to 0.27)	−0.03 (0.85–0.21)	.817
Delta ESR, mm/h, median (IQR)	−3 (−24 to 7)	1 (−19 to 4)	.877
Delta BMI, kg/m^2, median (IQR)	0.4 (−0.2 to 1.3)	0 (−0.9 to 1.3)	.485
Delta TNF-α, pg/mL, median (IQR)	−6.1 (−27.9 to 2.4)	−0.5 (−8 to 1.9)	.183
Delta IL-6, pg/mL, median (IQR)	− 5.1 (−17.9 to 2.7)	−4.1 (−6.6 to 0)	.643
Delta DKK-1, pg/mL, median (IQR)	−3.7 (−13 to 1.7)	−4.5 (−9.5 to 0.2)	.817
Delta adiponectin, μg/mL, median (IQR)	0.3 (−0.2 to 0.9)	0.3 (−0.6 to 0.8)	.999
Delta leptin, ng/mL, median (IQR)	1.6 (0.7–7)	−0.2 (−0.6 to 0.5)	.002
Delta leptin/BMI, ng m^2/mL kg, median (IQR)	0.05 (0.03–0.14)	0 (−0.2 to 0.02)	.001
Delta resistin, ng/mL, median (IQR)	3.2 (−2.7 to 3.5)	0.9 (0.2–5.8)	.938
Delta BASDAI	−1.2 (−2.9 to 0.4)	−0.2 (−2.1 to 1.4)	.135
Delta BASFI	−1.2 (−2.7 to 0.6)	−0.4 (−1.9 to 0.6)	.241
Delta BASMI	−0.2 (−2.2 to 0.6)	0 (−0.7 to 1.5)	.183

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, BASMI = Bath Ankylosing Spondylitis Metrology Index, BMI = body mass index, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, HDL-C = high-density lipoprotein, LDL-C = low-density lipoprotein cholesterol, mSASSS = the modified Stoke Ankylosing Spondylitis Spine Score progression, TG = triglyceride, TNF-α = tumor necrosis factor-alpha.

4. Discussion

In the present study, baseline serum resistin levels were significantly higher in patients with AS than in healthy subjects. Leptin and resistin levels increased significantly from the baseline to the 2-year follow-up in AS patients. The increase in leptin levels as well as the high baseline serum resistin concentrations were associated with radiographic progression (mSASSS ≥2) and changes in mSASSS during the follow-up period. Our findings suggest that increases in serum leptin levels may reflect structural damage proportionate to disease progression, and baseline resistin levels may be predictive of new bone formation in AS patients. Further, no significant association was observed between changes in DKK-1 levels or proinflammatory cytokines, such as TNF-α and IL-6, and radiographic progression. Thus, we speculate that leptin and resistin may be related to the pathogenesis of structural damage and can be used as biomarkers of the clinical outcome of AS.

Leptin, a 16-kDa protein encoded by the *ob* gene and produced mainly by adipocytes, plays a physiological role in the regulation of body weight by increasing satiety and energy expenditure. Circulating levels of leptin are known to be correlated with body fat and BMI. Recently, as the pro-inflammatory properties of leptin have been recognized, its contribution to the pathogenesis and clinical outcome of AS has been widely studied. However, in accordance with our findings, a recent meta-analysis reported no significant differences in plasma/serum leptin levels between AS patients and healthy subjects.[8] Further, a previous study

reported no association between leptin levels and AS disease activity,[16–18] although 1 study reported conflicting results.[19] In a cross-sectional study conducted by Kim et al,[20] they found that serum leptin levels adjusted by BMI were associated with the presence of syndesmophytes, and this finding is consistent with the positive correlation observed between changes in serum leptin levels and mSASSS in AS patients in our longitudinal data analysis. However, the increase in serum leptin levels did not coincide with the changes in disease activity in the present study. Considering all these findings, we hypothesize that leptin is primarily involved in new bone formation rather than inflammation in AS.

The role of leptin in bone metabolism is complex. In the central pathway, after binding to its hypothalamic receptor, leptin inhibits osteoblast differentiation and enhances osteoclast activation by increasing sympathetic activity,[21] whereas, in the peripheral pathway, leptin increases bone formation and inhibits bone resorption by directly acting on osteoblast and osteoclast.[21] In osteoarthritis (OA), leptin appears to enhance bone formation by inducing osteoblast proliferation and differentiation while inhibiting the adipogenic differentiation of bone marrow cells.[7,12] Further, increased production of leptin in subchondral osteoblasts in OA patients is reported to be correlated with bone formation markers, such as alkaline phosphatase and osteocalcin.[22] In addition, hypothalamic leptin gene therapy also decreases marrow adipose tissue and enhances osteoblast perimeter in ob/ob mice.[23] Similar to its role in OA, our findings indicate that leptin promotes new bone

Table 3

Correlations between the changes of mSASSS and clinical/laboratory variables in patients with ankylosing spondylitis during the study period.

Variables	Spearman correlation coefficient	P
Age, y	0.53	.016
Disease duration, mo	−0.081	.735
CRP, mg/dL	0.159	.503
ESR, mm/h	0.075	.752
BMI	0.183	.441
TNF-α, pg/ml	0.45	.047
IL-6, pg/mL	0.208	.38
DKK-1, pg/mL	0.216	.36
Adiponectin, ug/mL	−0.339	.143
Leptin, ng/mL	−0.114	.631
Leptin/BMI, ng m^2/mL kg	−0.114	.633
Resistin, ng/mL	0.559	.01
BASDAI	0.09	.707
BASFI	0.243	.302
BASMI	0.332	.152
Delta CRP, mg/dL	−0.059	.806
Delta ESR, mm/h	0.068	.775
Delta BMI, kg/m^2	−0.029	.903
Delta TNF-α, pg/ml	−0.321	.168
Delta IL-6, pg/mL	−0.165	.488
Delta DKK-1, pg/mL	−0.078	.745
Delta adiponectin, μg/mL	0.058	.808
Delta leptin, ng/mL	0.528	.017
Delta leptin/BMI, ng m^2/mL kg	0.56	.01
Delta resistin, ng/mL	−0.168	.48
Delta BASDAI	−0.323	.165
Delta BASFI	−0.208	.378
Delta BASMI	−0.261	.266

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, BASMI = Bath Ankylosing Spondylitis Metrology Index, BMI = body mass index, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, HDL-C = high-density lipoprotein, LDL-C = low-density lipoprotein cholesterol, TG = triglyceride, TNF-α = tumor necrosis factor-alpha.

formation in AS, although the underlying mechanism is unclear. Imaging studies have shown that focal fat infiltration of the spine visible on magnetic resonance imaging is a strong predictor of new syndesmophyte formation in AS patients.[24–26] Thus, considering the association between fat and adipokines, we speculate that leptin may upregulate osteoblast proliferation and downregulate adipocyte differentiation by acting directly on or within the vicinity of focal fat lesions in the spine, subsequently stimulating the progression of the lesions to syndesmophytes. However, this hypothesis warrants further investigation.

Resistin, which is produced by nonadipocyte, resident inflammatory cells and expressed in macrophages in humans, can produce pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1, by binding to toll-like receptor 4.[12,27] Resistin is reported to have a strong effect on the osteoclastogenesis and weakly induce osteoblast recruitment,[28] but its exact role in bone metabolism is less elucidated as compared with that of leptin. In line with our findings, a previous pilot study reported higher serum resistin levels in AS patients than in healthy controls.[29] However, no significant relationship between serum resistin levels and the disease activity of inflammatory makers in AS patients was found in previous studies.[29,30] Our study showed that baseline resistin concentrations correlated with radiographic progression, suggesting its potential role in new bone formation in AS; however, conflicting results have also been reported by Syrbe et al.[13] Therefore, further studies are needed

to elucidate the relationship between resistin and bone metabolism in AS.

The changes in the circulating levels of adipokines and cytokines over 2 years in AS patients under conventional treatment were investigated in this study. In the AS patients in our study, serum leptin and resistin levels increased significantly as the disease progressed, irrespective of changes in disease activity. In previous studies, anti-TNF-α agents were not found to significantly modulate changes in serum leptin and resistin levels in patients with AS,[17,18,30] suggesting that TNF-α, a key pro-inflammatory cytokine in the pathogenesis of AS, may not be a critical regulator of the production or action of adipokines. This notion also supports the lack of association between changes in disease activity and serum leptin and resistin levels over the 2-year follow-up period in our study. In addition, it is presumed that TNF-α blockers and conventional treatments, such as NSAIDs, may differently affect the production of adipokines in AS patients. Thus, the biological and clinical implications of temporal changes in serum adipokine levels induced by specific treatments and their effect on the outcome of AS may be an interesting topic for further research.

Some limitations of the present study warrant further discussion. First, due to the small sample size, we could not perform multivariable analyses to investigate the independent association between adipokines and radiographic progression in AS patients. Therefore, our results need to be confirmed in future studies with larger sample sizes. Second, the effect of medications, such as NSAIDs, on the interplay between adipokines and structural damage was not fully assessed. NSAIDs are reported to reduce radiographic progression in AS patients,[31] although the available data are conflicting.[32] However, this is an observational study and not a randomized trial, and we considered that it was impractical to discontinue NSAIDs during the study period because of ethical issues. Third, 2-year follow-up data were not collected for the healthy subjects. Thus, comparison of the changes in serum adipokine levels between AS patients and controls was not possible. Fourth, only male subjects were recruited, and therefore, any sex-related differences in the role of adipokines in new bone formation could not be evaluated. A previous study reported that leptin levels were higher in females than in males, even after adjusting for confounding factors,[33] suggesting that leptin may play a more significant role in female patients with inflammatory diseases. Further investigation is needed to confirm this notion.

5. Conclusion

High baseline resistin concentrations and increases in serum leptin levels were correlated with radiographic progression of AS over 2 years. Although a significant association between these adipokines and disease activity was not observed, our findings suggest that they may contribute to the pathogenesis of new bone formation rather than to inflammatory processes. These adipokines have the potential to be used as therapeutic targets and biomarkers of the structural outcome of AS. However, our findings are preliminary, and further large-scale studies are required to confirm them.

Acknowledgments

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