

Elevated Serum Adiponectin Level in Patients with *Mycobacterium avium-intracellulare* Complex Pulmonary Disease

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Key Words

Mycobacterium avium-intracellulare complex · Body mass · Adiponectin · Leptin

Abstract

Background: Patients with *Mycobacterium avium-intracellulare* complex (MAC) pulmonary disease often suffer from weight loss. Adipokines are factors secreted by adipocytes, including leptin and adiponectin, as well as some inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6). Body mass index (BMI) is known to be inversely correlated with adiponectin and positively with leptin, TNF- α , and IL-6. **Objective:** We aimed to evaluate the levels of serum adipokines, including adiponectin, leptin, TNF- α , and IL-6 in patients with MAC pulmonary disease. **Methods:** Forty consecutive patients with MAC pulmonary disease (8 males; median age 62 years; median BMI 18.1) were examined. Serum levels of adiponectin, leptin, TNF- α , and IL-6 were measured with ELISA. Age-, sex- and BMI-matched healthy subjects served as controls. **Results:** Serum adiponectin was significantly elevated in patients with MAC pulmonary disease compared with the controls ($p < 0.01$). In both the patients and controls, serum adiponectin levels were inversely correlated with BMI ($p < 0.05$). No significant correlation was observed between serum adiponectin levels and C-reactive protein or lung function. Serum leptin levels,

which were positively correlated with BMI, did not differ between patients and controls. Serum levels of TNF- α and IL-6 were significantly greater in patients with MAC pulmonary disease than in controls. The levels of TNF- α and IL-6 were not correlated with BMI and other adipokines examined. **Conclusion:** The results of the present study indicate that, in patients with MAC pulmonary disease, adiponectin is inappropriately secreted and may play a role in the pathophysiology of the disease.

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Introduction

Pulmonary infection of nontuberculous mycobacteria, specifically *Mycobacterium avium-intracellulare* complex (MAC), has become an increasingly recognized clinical problem over the past 20 years [1, 2]. Besides chronic or recurring cough, patients with MAC pulmonary disease often have nonspecific symptoms, such as fatigue, malaise, fever, and weight loss [2]. Progressive weight loss in patients with pulmonary disease can be associated with weakened respiratory muscles, leading to respiratory failure with alveolar hypoventilation [3].

Adipokines are factors expressed and secreted by adipocytes into the systemic circulation; they include active molecules such as leptin and adiponectin, as well as some

inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6) [4, 5]. Adiponectin is an adipocyte-specific protein secreted by visceral fat tissue that has anti-inflammatory as well as anti-obesity effects [6–8]. In patients with metabolic syndrome, adiponectin levels in plasma decreased in proportion to the increase in body weight, and hypo-adiponectinemia correlated with both insulin resistance and atherosclerosis resulting in cardiovascular disease [6]. Although elevated plasma levels of adiponectin, which are negatively correlated with body mass index (BMI), have been revealed in patients with chronic obstructive pulmonary disease (COPD), the role of adiponectin in weight loss in patients with MAC pulmonary disease remains to be investigated [9].

Leptin is another adipocyte-derived hormone involved in regulating food intake and energy expenditure in humans [10]. It has been revealed that plasma leptin is elevated in obese patients and induces inflammatory responses [11]. On the contrary, plasma leptin levels are decreased in patients with COPD [9]. In addition, leptin is known to be an important host defense molecule against mycobacterial infection [12, 13]. Leptin-deficient mice displayed impairment of clearance of *Mycobacterium tuberculosis* and granuloma formation [12]. Elimination of *M. abscessus* from the lung was also delayed in leptin-deficient mice [13]. However, serum leptin level in patients with pulmonary MAC infection has not been reported.

In this study, we evaluated serum levels of adiponectin and leptin in patients with MAC pulmonary disease and healthy subjects. The serum levels of TNF- α and IL-6, which are known to be proinflammatory adipokines, were also examined.

Materials and Methods

Patients

Forty consecutive patients with MAC pulmonary disease were enrolled in this study. MAC pulmonary disease was diagnosed according to the diagnostic criteria of the American Thoracic Society and the Infectious Diseases Society of America [14]. All patients were clinically stable, namely with negative culture and no change on chest radiography for at least 6 months, and 33 were not under treatment at the time of evaluation. Seven patients were undergoing treatment, mostly with clarithromycin, rifampicin, and ethambutol. The median duration of therapy was 9 months. This study included 40 healthy subjects with no documented pulmonary disease as controls. They were randomly selected from the university employees who underwent blood examination on a health check-up and were in the same BMI category closest to the nearest integer. The age, sex and BMI of the control subjects were matched with those of the patients with MAC pulmonary disease. The Institutional Review Board of our university ap-

proved the study and all subjects provided written informed consent.

Blood Sampling and Analysis

Blood samples were obtained from the antecubital vein after an overnight fast. The blood was centrifuged immediately at 4°C and serum samples were stored at -80°C pending analysis. Serum adiponectin, leptin, TNF- α , and IL-6 were measured using an enzyme-linked immunosorbent assay method (Quantikine; R&D Systems, Minneapolis, Minn., USA). The minimum detectable doses of adiponectin, leptin, TNF- α , and IL-6 were 0.246 ng/ml, and 7.8, 0.19 and 0.11 pg/ml, respectively.

Statistical Analysis

SPSS 15.0 software (SPSS Inc., Chicago, Ill., USA) was used for statistical analyses. Data are presented as median score with interquartile range (IQR) in parentheses. Comparisons of parameters between the patients and control subjects were done by the nonparametric Mann-Whitney U test since the data were not normally distributed. The relationships between variables were analyzed by the Spearman rank-order correlation test. The slope of the linear regression lines was compared using analysis of covariance. The gender and smoking status of the subjects were compared using the χ^2 test. Statistical significance was defined as $p < 0.05$.

Results

Characteristics of the Subjects

The clinical characteristics of patients with MAC pulmonary disease and healthy control subjects are summarized in table 1. There was no significant difference in age and smoking status between control subjects and patients with MAC pulmonary disease (table 1). None of the patients and control subjects was HIV positive. Since the average BMI of the healthy Japanese population is estimated at 22.8, the BMI of the study subjects was lower than the average regardless of MAC infection [15].

Serum Adiponectin Levels in Patients with MAC Pulmonary Disease

Serum adiponectin levels in patients with MAC pulmonary disease and control subjects were 15.9 (IQR: 11.4–26.6) and 10.8 (IQR: 7.8–18.1) $\mu\text{g/ml}$, respectively (table 2). There was a significant difference in serum adiponectin levels between the patients and controls ($p < 0.01$). In 23 patients with BMIs < 18.5 , the serum adiponectin level was as high as 22.8 (IQR: 13.1–32.6) $\mu\text{g/ml}$. There was a significant difference in serum adiponectin levels between patients and controls with BMIs < 18.5 ($p < 0.05$). In the subjects with BMIs > 18.5 , the serum adiponectin levels in MAC patients and controls were 11.7 (IQR: 9.3–15.6) and 8.6 (IQR: 7.7–11.0) $\mu\text{g/ml}$, re-

Table 1. Characteristics of the study subjects

Characteristics	Control subjects (n = 40)	Patients with MAC pulmonary disease (n = 40)	p value
Age, year	56 (51–64)	62 (50–71)	0.08 ¹
Male/female	7/33	8/32	0.73 ²
BMI	18.3 (17.4–19.3)	18.1 (16.3–19.6)	0.40 ¹
Smoker/nonsmoker	6/34	3/37	0.29 ²
Lung function test			
%VC	N/A	86 (73–98)	–
FEV _{1,0} %	N/A	72 (66–78)	–
Serum markers			
TP, g/dl	7.2 (7.0–7.4)	7.5 (7.2–7.9)	<0.01 ¹
TC, mg/dl	205 (165–221)	211 (195–241)	<0.05 ¹
CRP, mg/dl	N/A	1.13 (0.58–5.16)	–

Data are presented as median score with interquartile range in parentheses. TP = Total protein; TC = total cholesterol; CRP = C-reactive protein.

¹ Mann-Whitney's U test.

² χ^2 test.

Table 2. Serum adipokine levels of the study subjects

Adipokines	Control subjects (n = 40)	Patients with MAC pulmonary disease (n = 40)	p value ¹
Adiponectin, $\mu\text{g/ml}$	10.8 (7.8–18.1)	15.9 (11.4–26.6)	<0.01
Leptin, ng/ml	2.63 (1.63–4.29)	1.84 (0.63–4.68)	0.17
TNF- α , pg/ml	0.37 (0.30–0.44)	0.49 (0.36–0.69)	<0.01
IL-6, pg/ml	1.14 (0.95–1.47)	2.43 (1.28–6.26)	<0.001

Data are presented as median score with interquartile range in parentheses.

¹ Mann-Whitney's U test.

spectively. There was also a significant difference in serum adiponectin levels between the patients and controls with BMIs >18.5 ($p < 0.05$). In patients with MAC pulmonary disease, serum adiponectin levels did not differ between those undergoing medical treatment and those that did not (data not shown). Serum adiponectin levels were inversely correlated with BMI in both patients with MAC pulmonary disease ($\rho = -0.61$, $p < 0.001$) and control subjects ($\rho = -0.42$, $p < 0.01$) as shown in figure 1. The slope of the linear regression lines did not differ significantly between the two groups. In patients with MAC pulmonary disease, there was no correlation between the serum levels of adiponectin and lung function param-

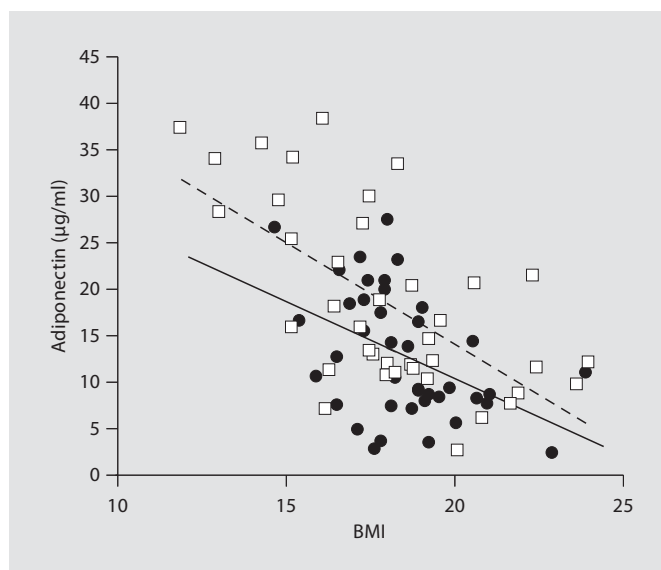


Fig. 1. Serum adiponectin levels in healthy subjects and patients with MAC pulmonary disease. ● = Healthy subject; □ = patients with MAC pulmonary disease; — = linear regression line for healthy subjects; - - - = linear regression line for patients with MAC pulmonary disease.

ters (data not shown). In control subjects and patients with MAC pulmonary disease, there was no correlation between the serum levels of adiponectin and total cholesterol or total protein (data not shown). In patients with MAC pulmonary disease, the serum adiponectin levels were not correlated with the serum levels of C-reactive protein (data not shown).

Serum Leptin Level in Patients with MAC Pulmonary Disease

Serum leptin levels in patients with MAC pulmonary disease and control subjects were 1.84 (IQR: 0.63–4.68) and 2.63 (IQR: 1.63–4.29) ng/ml, respectively (table 2). There was no significant difference in serum leptin levels between the patients and controls. Serum leptin levels were positively correlated with BMI in both patients with MAC pulmonary disease ($\rho = 0.62$, $p < 0.001$) and control subjects ($\rho = 0.46$, $p < 0.01$). The slope of the linear regression lines did not differ between the two groups.

Serum TNF- α Level in Patients with MAC Pulmonary Disease

Serum TNF- α levels in patients with MAC pulmonary disease and control subjects were 0.49 (IQR: 0.36–0.69) and 0.37 (IQR: 0.30–0.44) pg/ml, respectively (table 2).

There was a significant difference in serum TNF- α levels between the patients and controls ($p < 0.01$). No correlation was observed between serum TNF- α level and BMI, leptin, or adiponectin (data not shown).

Serum IL-6 Level in Patients with MAC Pulmonary Disease

Serum IL-6 levels in patients with MAC pulmonary disease and control subjects were 2.43 (IQR: 1.28–6.26) and 1.14 (IQR: 0.95–1.47) pg/ml, respectively (table 2). There was a significant difference in serum IL-6 levels between the patients and controls ($p < 0.001$). No correlation was observed between serum IL-6 level and BMI, leptin, or adiponectin (data not shown).

Discussion

In the present study, we showed that serum adiponectin levels in patients with MAC pulmonary disease were significantly higher than in healthy subjects with comparable BMI. In both healthy controls and patients with MAC pulmonary disease, the serum adiponectin levels were inversely correlated with BMI. In contrast, serum leptin levels, which were positively correlated with BMI, did not differ between the MAC patients and controls. Our data provide the basis for speculating that the elevated serum adiponectin level was associated not only with decreased BMI but also with chronic inflammation due to pulmonary MAC infection.

In obese subjects, the adiponectin level is decreased and inversely correlated with both body weight and fat mass [6, 16, 17]. In contrast, it has been reported that plasma adiponectin levels are elevated in underweight subjects such as patients with anorexia nervosa [18] and cachexia [19]. In this study, serum adiponectin levels were inversely correlated with BMI in both healthy controls and patients with MAC pulmonary disease. Although the slope of the linear regression lines was not different, the serum adiponectin level in patients with MAC pulmonary disease was significantly higher than in control subjects with comparable BMI. Even in the subjects with BMIs >18.5 , serum adiponectin levels were significantly higher in the patients than in the controls. We therefore speculated that, in patients with MAC pulmonary disease, adiponectin might be inappropriately secreted and associated with the pathogenesis or disease course.

Tomoda et al. [9] reported that plasma levels of adiponectin were elevated in patients with COPD and correlated with body weight loss and lung hyperinflation. Al-

though some of the patients evaluated in the present study exhibited impaired lung function, the median values of the lung function parameters were within the normal ranges. In addition, no significant correlation was observed between the adiponectin levels and lung function parameters. We speculated that, in patients with MAC pulmonary disease, the increase in serum adiponectin might not be related with the impaired lung function.

In the present study, the role of elevated adiponectin was not elucidated. Previous studies revealed that adipocytes secrete a variety of cytokines, including TNF- α and IL-6, which have detrimental effects on chronic inflammation or infection, and weight loss results in a reduction of inflammatory mediators [5, 20]. Moreover, obesity is associated with an increase in adipose tissue macrophages, which also participate in the inflammatory process through the elaboration of cytokines [4]. Adiponectin is known to have an anti-inflammatory effect, to suppress TNF- α expression and increase the expression of anti-inflammatory mediators such as IL-10 and IL-1 receptor antagonists [21, 22]. On the other hand, elevated adiponectin levels are reported in chronic inflammatory/autoimmune diseases that are unrelated to increased adipose tissue such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes, COPD, and cystic fibrosis [9, 23]. Proinflammatory effects of adiponectin have been reported in tissues such as joint synovium [24] and colonic epithelium [25]. We speculated that adiponectin might exert differential functions in various inflammatory conditions. In this study, serum TNF- α levels were greater in patients with MAC pulmonary disease than in controls, which indicates that, in case of MAC infection, elevated adiponectin levels in the patients were not likely to result in the inhibition of TNF- α production. However, since we did not evaluate the levels of anti-inflammatory cytokines, it may be possible that adiponectin might increase expression of IL-10 and IL-1 receptor antagonist, leading to increased host susceptibility to MAC infection.

The major limitation of this study is that a proportional hazards method was not used to evaluate the risk ratio for death because of the limited number of study subjects. The association between the adipokine levels and disease progression also remains to be evaluated. We believe that these issues should be subjects of future investigations.

In this study, we were unable to measure percent body fat, but we found a close correlation between percent body fat and BMI in a cohort of 594 Japanese subjects [$r = 0.594$, $p < 0.0001$, unpubl. data]. In addition, it was previously

reported that the correlation between plasma adiponectin level and BMI ($r = -0.32$) was more significant than between adiponectin level and percent body fat ($r = -0.26$) [26]. Although we did not measure percent body fat in this study, we consider that the BMI characterized the adiposity status of the study subjects well.

In conclusion, serum adiponectin levels are elevated in patients with MAC pulmonary disease and inversely correlated with BMI, whereas serum leptin levels did not differ between the patients and control subjects. Although adiponectin is known to have anti-inflammatory effects

on obesity-associated inflammation, the inappropriately secreted adiponectin in MAC pulmonary disease might have proinflammatory effects as in other chronic inflammatory diseases that are not associated with obesity and play a role in the pathophysiology of the disease.

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