Serum Myostatin Level in COPD Patients with and without Sarcopenia : Application for Biomarker of Sarcopenia Diagnosis

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Serum Myostatin Level in COPD Patients with and without Sarcopenia -Application for Biomarker of Sarcopenia Diagnosis-

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Abstract

Background

Chronic obstructive pulmonary disease (COPD) has significant extrapulmonary effects including weight loss, nutritional abnormalities, and skeletal muscle dysfunction. Sarcopenia is one of several comorbidities experienced by COPD patients, which may negatively affect quality of life and increase mortality. Previous studies have reported that exercise may reduce the severity of sarcopenia; however, the mechanism is only partially understood. We hypothesized that levels of irisin and myostatin, one of the myokines, are correlated with sarcopenia in COPD patients.

Methods

A total of 39 male COPD patients were enrolled, in addition to 30 male never-smokers as a control group. Pulmonary function testing was performed on all participants, while body-composition analysis was performed on the COPD patients to measure muscle mass and diagnose sarcopenia. Serum irisin and myostatin levels were measured using a commercially available enzyme-linked immunosorbent assay kit.

Results

The COPD patients were divided into a sarcopenia group (n=9) and a non-sarcopenia group (n=30). Serum myostatin levels were significantly lower in COPD patients with sarcopenia than in those without sarcopenia, while there was no significant difference in irisin levels among COPD patients with and without sarcopenia.

Conclusions

Serum myostatin level was correlated with muscle mass and is a potential biomarker for sarcopenia in COPD patients.

Key Words: Sarcopenia; Irisin; Myostatin; Myokine; COPD

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common, preventable, and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases. COPD has significant extrapulmonary effects including weight loss, nutritional abnormalities, and skeletal muscle dysfunction. Skeletal muscle dysfunction is characterized by both sarcopenia and abnormal function of the remaining cells[1].

As the human body ages, skeletal muscle mass declines annually by 0.1% to 0.5% starting from 30 years old, with a dramatic acceleration after 65 years old. This gradual decrease in muscle mass is accompanied by a simultaneous reduction in strength. Excessive age-related loss of muscle mass and strength is often referred to as "primary sarcopenia"[2]. There is also a "secondary sarcopenia" related to disease. Sarcopenia is among the comorbidities present in COPD patients and may negatively impact quality of life. Therefore, early detection of sarcopenia can reduce the long-term care needs of this population, as well as improve quality of life and reduce morbidity and mortality[3]. Previous studies have reported that exercise may reduce the severity of sarcopenia; however, the mechanism is only partially understood.

Recently, myokines, such as irisin and myostatin, were discovered to be secreted from muscle tissue. Irisin is secreted by proteolytic cleavage of the membrane protein fibronectin type Ⅲ domain-containing protein 5 (FNDC5)[4]. Exercise triggers the cleavage of FNDC5 to secrete irisin into the bloodstream, which subsequently elevates energy expenditure in the subcutaneous adipose tissue through adipocyte browning[4]. The beneficial role of irisin in skeletal muscle metabolism has been described and it has been shown that irisin stimulates glucose uptake and lipid metabolism via activation of AMP-activated protein kinase[5,6].

On the other hand, myostatin, a member of the transforming growth factor-beta superfamily, has been established to be a negative regulator of skeletal muscle mass and is mainly expressed in skeletal muscles[7]. Myostatin inhibits satellite cell proliferation and differentiation in an autocrine and paracrine manner; conversely genetic deletion of myostatin leads to muscle hypertrophy in humans and mice[8]. While myostatin activation negatively regulates muscle growth, myostatin expression is downregulated after endurance and resistance exercise[9]. Myostatin is considered to be negatively correlated with muscle mass because it is a negative regulator of muscle growth. However a recent study reported that plasma myostatin levels were positively correlated with lean body mass[10].

The relationship between these myokines and muscle mass in COPD patients, however, has been unclear. We hypothesized that myostatin and irisin levels are correlated with sarcopenia in COPD patients. To address this hypothesis, we measured serum myostatin and irisin levels, and evaluated the relationships between these myokines and sarcopenia and muscle mass.

Methods

Subjects

Thirty-nine male COPD patients were recruited prospectively in the outpatient department of Osaka City University Hospital (Osaka, Japan) between April 2016 and December 2016. All COPD patients were stable outpatients and diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease guidelines[11]. They had no history of malignant diseases, pulmonary comorbidities and oral corticosteroid therapy. We recruited male never-smokers older

than 70 years as controls from a group of healthy volunteers at Medcity21, medical examinations clinics between March 2016 and October 2017.

This study was approved by the Ethics Committee of Osaka City University Hospital (approval No. 3330), and all patients provided informed written consent for participation. All procedures were performed according to the guidelines of the Declaration of Helsinki.

Body composition analysis (BIA)

Body-composition analysis was performed in the COPD patients to measure BMI, muscle mass (MM), fat-free mass (FFM), FFM index (FFMI), and skeletal muscle mass index (SMI), using bioelectrical impedance analysis using the InBody 3.0 system analyzer (InBody, Seoul, South Korea). We didn't check their ingestion of food or drink before BIA. SMI is defined as appendicular skeletal muscle mass (ASM) divided by the square of height. ASM is calculated by summing the muscle mass of the four limbs. Sarcopenia was defined according to the Asian Working Group for Sarcopenia (AWGS) standard, with SMI <7 kg/m^2 and grip strength <26 kg.

Myokine measurements

For each subject, peripheral venous blood was drawn at the rest and serum samples were deep frozen and kept at -80℃ until the measurement. Serum irisin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, Burlingame, CA, USA) according to the manufacturer's protocol. Serum myostatin levels also were measured using an ELISA kit (R&D systems Inc., Minneapolis, USA) according to the manufacturer's protocol.

Statistical analysis

Data are expressed as mean±standard deviation unless otherwise indicated. Baseline differences were determined using Student's t test or the Mann-Whitney U test. Group of data that failed tests for normality and equal variance were analyzed by the nonparametric Kruskal-Wallis analysis of variance followed by Dunn's test. Associations between continuous variables were described by Spearman's correlation coefficients for variables that were not normally distributed. Statistical analyses were performed using JMP version 10.0.0 (SAS Institute, Cary, NC, USA) for Windows (Microsoft Corporation, Redmond, WA, USA). In all statistical analyses, $p<0.05$ was considered to be statistically significant.

Results

Patient characteristics

The characteristics of the 39 COPD patients and 30 healthy controls are summarized in Table 1. There were no significant differences in age or BMI between the COPD patients and healthy controls. As shown in Table 1, there was no significant difference in FVC and %FVC between the two groups. However, FEV_1 was significantly lower in COPD patients than in healthy controls (1.8±0.6 vs 2.7±0.4, respectively; $p<0.0001$) and FEV_1 (% predicted) was also significantly lower in COPD patients than in healthy controls (66.4±21.1 vs 97.3±14.6, respectively; $p<0.0001$).

COPD patients were divided in two groups, the sarcopenia group (n=9) or the non-sarcopenia (n=30) group in accordance with AWGS standards (Table 2). There was no significant difference in FVC, FEV_1, FEV_1/FVC or BMI between COPD patients with and without sarcopenia. However, MM, FFMI, and SMI were significantly lower in COPD patients with sarcopenia than in those without sarcopenia ($p<0.0005$).

Table 1. Characteristics of all participants

	COPD	healthy controls	p value
Subject No.	39	30	
Age (years)	74±8	74±3	NS
BMI (kg/m^2)	23.3±3.6	23.0±2.9	NS
FVC (L)	3.3±0.8	3.4±0.5	NS
%FVC (%)	100.2±16.9	98.8±13.7	NS
FEV_1 (L)	1.8±0.6	2.7±0.4	<0.0001
%FEV_1 (%)	66.4±21.1	97.3±14.6	<0.0001
FEV_1/FVC (%)	51.9±13.9	78.8±3.9	<0.0001

COPD, chronic obstructive pulmonary disease; BMI, body mass index; FVC, forced vital capacity; and FEV_1, forced expiratory volume in 1 second.

Table 2. Characteristics of COPD patients with and without sarcopenia

	non-sarcopenia	sarcopenia	p value
Subject No.	30	9	
GOLD stage Ⅰ, n	9	1	
GOLD stage Ⅱ, n	12	7	
GOLD stage Ⅲ, n	8	1	
GOLD stage Ⅳ, n	1	0	
FVC (L)	3.5±0.9	2.9±0.3	NS
%FVC (%)	101±18	97±10	NS
FEV_1 (L)	1.8±0.7	1.6±0.6	NS
%FEV_1	66±20	67±23	NS
FEV_1/FVC (%)	51±14	53±14	NS
Age (yr)	73±8	80±5	<0.05
BMI (Kg/m^2)	23.8±3.2	21.7±3.6	NS
MM	46.5±6.2	37.1±3.5	<0.001
FFMI	17.8±1.5	15.6±1.5	<0.0005
SMI (Kg/m^2)	7.3±0.8	6.1±0.6	<0.0005

COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; BMI, body mass index; MM, muscle mass; FFMI, fat free mass index; and SMI, skeletal muscle mass index.

Relationships between myokine levels and body composition

In COPD patients, serum irisin levels were positively correlated with FFMI ($r=0.36$, $p<0.05$) and SMI ($r=0.40$, $p<0.01$), but not significantly correlated with MM ($r=0.32$, $p>0.05$) (Fig. 1). However, serum myostatin levels were positively correlated with all body composition indices including MM ($r=0.44$, $p<0.005$), FFMI ($r=0.50$, $p<0.01$), and SMI ($r=0.54$, $p<0.005$) (Fig. 2).

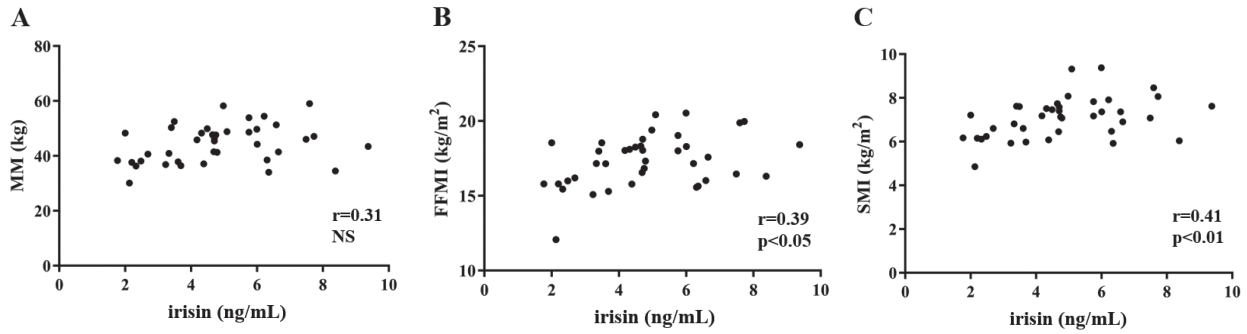

Figure 1. Correlations of serum irisin levels with MM, FFMI, and SMI. A, There was no significant correlation between serum irisin levels and MM. B and C, There were significant positive correlations between serum irisin levels and FFMI and SMI. MM, muscle mass; FFMI, fat free mass index; and SMI, skeletal muscle mass index.

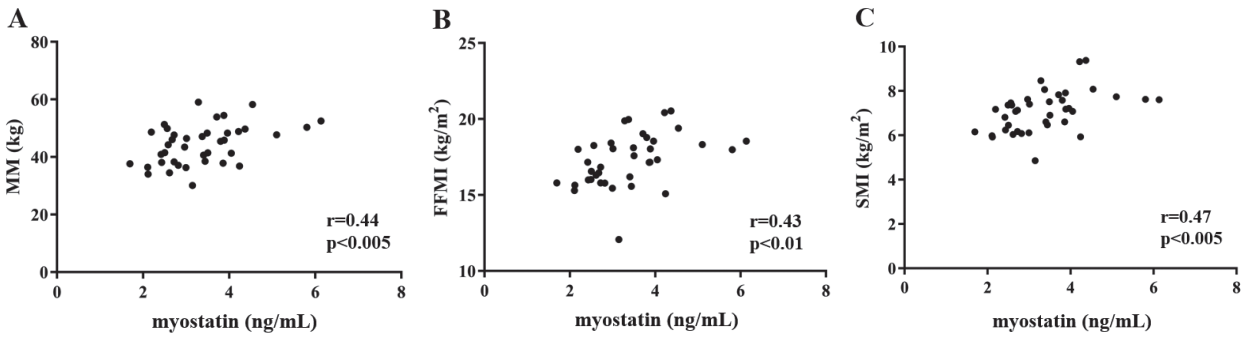

Figure 2. Correlations of serum myostatin levels with MM, FFMI, and SMI. A-C, There were significant positive correlations between serum myostatin levels and MM, FFMI, and SMI. MM, muscle mass; FFMI, fat free mass index; and SMI, skeletal muscle mass index.

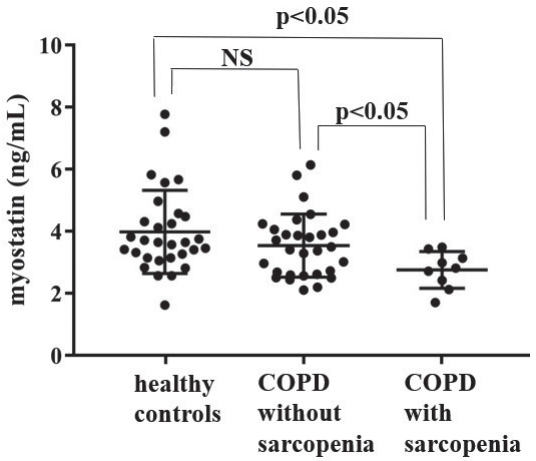

Figure 3. Comparison of serum myostatin levels between healthy controls and COPD patients with and without sarcopenia. There was a significant difference in serum myostatin levels between healthy controls and COPD patients with sarcopenia. COPD, chronic obstructive pulmonary disease.

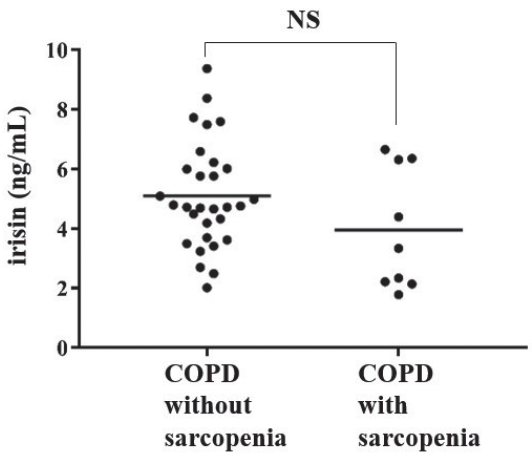

Figure 4. Comparison of serum irisin levels between COPD patients with and without sarcopenia. There was no significant difference in serum irisin levels between COPD with and without sarcopenia. COPD, chronic obstructive pulmonary disease.

Sarcopenia and myostatin levels

Serum myostatin levels in healthy controls were significantly higher than COPD patients with sarcopenia (4.01±0.25 ng/mL vs 2.76±0.20 ng/mL; $p<0.05$) but there was no significant difference in serum myostatin levels between healthy controls and those without sarcopenia. Serum myostatin levels were significantly lower in COPD patients with sarcopenia than in those without sarcopenia (2.76±0.20 ng/mL vs 3.53±0.19 ng/mL; $p<0.05$) (Fig. 3). There was no significant difference in serum irisin levels between COPD patients with sarcopenia and those without sarcopenia (3.94±0.68 ng/mL vs 5.10±0.33 ng/mL; $p>0.1$) (Fig. 4).

Discussion

In this study, we examined body composition and sarcopenia in COPD patients, and revealed that irisin and myostatin, one of the myokines, were positively correlated with muscle mass parameters including FFMI and SMI. Sarcopenia is a comorbidity in COPD, and is characterized by the loss of skeletal muscle mass and strength, which is associated with low exercise capacity, lower quality of life, and increased mortality[12,13].

The assessment of body composition measured using bioelectrical impedance analysis (BIA) or dual energy X-ray absorptiometry (DXA) are among the diagnostic methods for sarcopenia. BIA measures the resistance to flow of an electrical current as it passes through the body and is reflective of body composition. DXA calculates body density and body composition by irradiating the body with two X-ray beams. Early detection of sarcopenia in COPD patients is important. However, in many clinics and hospitals, body composition cannot be measured due to a lack of these devices; thus early detection of sarcopenia is difficult. Alternatively, we focused on myokine levels, such as irisin and myostatin, to be potential biomarkers of sarcopenia.

Myostatin is mainly produced by skeletal muscles and served as a negative regulator of muscle growth and it is released into plasma in a form of precursor protein, which can be cleaved into mature myostatin by bone morphogenetic-1/tolloid family of metalloproteases. Some studies have reported that postnatal inhibition of myostatin unequivocally increased skeletal muscle mass in adults and older mammals[14,15]. Another study reported that weekly injection of a neutralizing antibody to myostatin for 4 weeks significantly increased the relative weights of individual muscles by up to 17% in aged mice and improved indices of muscle performance and whole-body metabolism[16]. The other study reported that blocking myostatin might be a therapeutic target to combat sarcopenia and inhibiting myostatin-activin A signaling is therefore a highly promising therapeutic strategy to combat muscle loss in older people[17]. Therefore, myostatin was considered to be a negative regulator of muscle growth.

However, a recent study demonstrated a positive correlation between plasma myostatin levels and lean body mass[11]. Our study also revealed a positive correlation between serum myostatin levels and muscle mass. We speculate that hypertrophied muscle may secrete more myostatin as negative feedback to inhibit excessive muscle hypertrophy. Our study demonstrated that myostatin levels were significantly lower in COPD patients with sarcopenia than in those without sarcopenia. This result suggests that myostatin may be a potential biomarker for sarcopenia in COPD patients.

Previous studies have also reported that serum C1q, C-terminal agrin fragment, and p53 codon 72 polymorphism may be also be potential biomarkers of sarcopenia[18-20]. On the other hand, there was no significant difference in serum irisin levels between COPD patients with and without sarcopenia.

Considering the different results between myostatin and irisin, we speculated that myostatin would be mainly correlated with muscle mass but irisin would be correlated with muscle mass and other metabolic disorder such as diabetes mellitus. As a result, serum irisin levels might be influenced by not only muscle mass but also other metabolic factors.

In conclusion, myostatin is a potential biomarker of sarcopenia in COPD patients. However, there were some limitations to this study, among which include its single-center, cross-sectional design and limited sample size; therefore, causal relationships could not be determined. Second, this study enrolled only male COPD patients; therefore, it also remains unknown whether these results apply to female COPD patients. Third, twenty-four out of thirty-nine COPD patients overlapped with those in the previous report[21] which our group reported. Fourth, we didn't estimate the difference in amount of muscle mass between COPD patient and healthy controls because we didn't measure body composition in healthy controls.

Further studies are needed to better understand the pathophysiology of sarcopenia in COPD and to determine other potential biomarkers.

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All authors have no COI to declare regarding the present study.

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