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SHORT RESEARCH ARTICLE

Correlation between the Respiratory Muscle Strength and Six-Minute Walking Distance and Oxidative Stress/Inflammatory Cytokines in Patients with Stable Chronic Obstructive Pulmonary Disease: A Preliminary Study

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ABSTRACT | Chronic obstructive pulmonary disease (COPD) relates to high oxidative stress and inflammation, which may impair physical activity of the patients. However, studies on the relationship of oxidative stress/inflammatory conditions with walking capacity or respiratory muscle strength in COPD patients are lacking. Therefore, the aim of the present study was to evaluate the correlation of respiratory muscle strength (PImax) and walking distance [6-minute walking distance (6MWD)] with oxidative stress parameters [total antioxidant capacity (TAC)], malondialdehyde (MDA), and nitric oxide (NO)] or inflammatory markers [tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)]. Twenty-nine stable COPD patients, diagnosed with mild severity, were evaluated twice, one month apart, and the results showed that PImax and the 6MWD had a significantly positive correlation with TAC ($r = 0.271$, $p = 0.04$; $r = 0.289$, $p = 0.028$; respectively). On the other hand, both PImax and the 6MWD had a significantly negative correlation with TNF-α ($r = –0.558$, $p < 0.001$; $r = –0.587$, $p < 0.001$; respectively) and IL-6 ($r = –0.588$, $p < 0.001$; $r = –074$, $p < 0.001$; respectively). In addition, the 6MWD showed a significantly negative correlation with MDA ($r = –0.37$, $p = 0.004$). Taken together, this study demonstrated a significant correlation of oxidative stress and inflammation with respiratory muscle strength and walking capacity in COPD patients.

KEYWORDS | Chronic obstructive pulmonary disease; Cytokines; Oxidative stress

ABBREVIATIONS | 6MWD, six-minute walking distance; CBC, complete blood count; COPD, chronic obstructive pulmonary disease; GSH, reduced form of glutathione; IL-6, interleukin-6; MDA, malondialdehyde; NO, nitric oxide; PImax, maximal inspiratory mouth pressure; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-alpha

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CONTENTS

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is currently the third most common disease causing death worldwide [1]. Low physical activity from dyspnea, skeletal muscle wasting, decreased respiratory muscle and limb strength [2, 3], decreased exercise capacity [4], and low health-related quality of life are among the chief sequalae of this disease [5]. Oxidative stress and inflammation have been suggested to be major factors involved in the pathogenesis of COPD [6]. In this context, increased lipid peroxide product malondialdehyde (MDA) [7], and decreased glutathione (GSH) and total antioxidant capacity (TAC) were observed in COPD patients [8, 9]. Many cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can be released in the chronic inflammatory stage of COPD, and may play a role in the disease process [10]. However, the relationship of oxidative stress and inflammatory conditions with walking capacity or respiratory muscle strength in COPD patients had not been previously investigated. Accordingly, the aim of this study was to evaluate the correlation of respiratory muscle strength [as indicated by maximal inspiratory mouth pressure (PImax)] and 6-minute walking distance (6MWD) with oxidative stress and inflammatory cytokines in patients with moderately severe COPD.

2. PARTICIPANTS AND METHODS

2.1. Participants

This study was carried out with 29 stable COPD patients who were ex-smokers and living at home in Sansai district, Chiang Mai, Thailand, where data collection or evaluation was performed at the Ban Tor-Public Health Center. The protocol in this study was approved by the Ethic Committee at the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand, and conducted in accordance with the Declaration of Helsinki (2001). The pulmonary function test was repeated twice and evaluated using spirometry (HI-105, Japan) [11]. All of the participants were permitted to receive medication of either a long-acting inhaled bronchodilator or long-acting inhaled steroids, according to the recommendations by the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The participants did not present evidence of any hospitalization for uncontrolled hypertension, unstable cardiac disease, recurrent symptoms of acute exacerbation, recurrent pneumothorax, thoracic or chest pain, any liver or renal dysfunction, or endocrinal abnormalities. They were not permitted to take supplements or any extra-nutrients such as multi-vitamins or antioxidant-rich diets within 6 months prior to data collection. All of the participants were asked to self-record their daily food, fluid, and supplement intake in a logbook, and be checked by weekly telephone calls. A stable condition of all the participants had to be confirmed before starting the protocol by screening complete blood count (CBC) at the AMS Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. Blood from the anterior cubital vein was taken and kept in sterile ethylenediamine tetracetic acid (EDTA) for evaluating oxidative stress markers (TAC, MDA, and nitric oxide), and inflammatory cytokines (TNF-α and IL-6). In addition, PImax and the 6MWD also were evaluated. Measurements of all the parameters in this study were taken twice, one month apart.

TABLE 1. Characteristics, complete blood count, and pulmonary function test in 29 stable COPD participants

Parameter	Mean ± SD	Range (min–max)
Characteristics		
Age (years)	72.24 ± 9.34	46–91
Gender (males/females)	22/7	
BMI ($kg.m^{-2}$)	22.68 ± 3.78	16.22–30.73
Systolic blood pressure (mm Hg)	121.54 ± 6.5	100–132
Diastolic blood pressure (mm Hg)	72.76 ± 10.10	63–97
Complete blood count [reference range]		
WBC (10^3/µl) [4.5–11.5]	6.7 ± 1.3	5.5–9.2
RBC (10^6/µl) [3.8–5.3]	4.2 ± 0.4	3.9–4.9
Hb (g/dl) [10–16]	13.2 ± 1.4	10.2–15.7
Hct (%) [35–54]	41.5 ± 1.2	35.2–45.8
PLT (10^3/µl) [140–440]	269.0 ± 29.0	149–395
Pulmonary function test		
FVC (L)	2.11 ± 0.65	0.77–3.52
FVC (%predicted)	92.43 ± 14.5	71.43–125.25
FEV1 (L)	1.32 ± 0.55	0.31–2.37
FEV1 (%predicted)	62.75 ± 10.45	50–79.23
FEV1/FVC (%predicted)	66.52 ± 4.53	56.49–78.45

Note: BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; RBC, red blood cells; WBC, white blood cells.

2.2. Oxidative Stress and Cytokine Evaluation

The plasma from whole fresh blood was separated by centrifugation at 3,000 g for 10 min. Fresh plasma was used to evaluate the TAC, nitric oxide (NO), and MDA by the 2,2′-azinobis-(3-ethylbenzothi-azoline-6-sulfonic acid) (ABTS) decolorization [12], Griess reagent [13], and thiobarbituric acid (TBA)-reactive substances [14] assays, respectively. Residual plasma was pooled and frozen for determining TNF-α and IL-6 by using human ELISA-kits (Quantikine®, R&D systems, Minneapolis, MN, USA).

2.3. Respiratory Muscle Strength Test

Respiratory muscle strength was evaluated by measuring PImax at residual volume, using a portable hand-held mouth pressure meter (MicroRPM, Micro Medical, Kent, UK). The highest data output in units of centimeters in water (cm H_2O) from three repeated efforts, or a drop of more than 20% from the previous effort, was the criterion for stopping the test [15, 16].

2.4. Six-Minute Walking Distance Test

The 6MWD test was evaluated by following the guideline of the American Thoracic Society [17]. A modified protocol of the 20-meter straight walking test was performed in an indoor corridor. Participants were instructed to walk at a comfortable pace for 6 min under supervision of an experienced physiotherapist. They were allowed to stop and rest during the test if feeling strong or heavy dyspnea (equal to 6 from a maximal 10 on the Borge scale), in accordance with the guideline of the American College of Sport Medicine (ACSM, 2004) [18].

2.5. Statistical Analysis

All data were analyzed statistically for normal distribution, using the one-sample Kolomogorov–Smirnov test, before presenting as mean with standard deviation (SD), and minimal and maximal values. Correlation of PImax and the 6MWD tests with oxidative stress parameters and cytokines was analyzed by Pearson's correlation test. All the statistical analyses

TABLE 2. Parameters of PImax, 6MWD, TNF-α, IL-6, TAC, MDA, and NO measured twice in 29 stable COPD patients

Parameter	Mean ± SD	Range (min–max)
PImax (cm H_2O)	71.58 ± 23.09	25–135
6MWD (m)	324.98 ± 94.58	110–523
TNF-α (ng/ml)	10.08 ± 3.69	4.5–17.6
IL-6 (ng/ml)	7.76 ± 3.66	2.6–15.60
TAC (mM Trolox)	1.13 ± 0.06	1.0–1.31
MDA (μM)	6.52 ± 2.66	2.4–14.96
NO (μM)	5.0 ± 3.25	1.5– 0.35

were carried out using the statistical package for social science software (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 is considered statistically significant.

3. RESULTS

The results of patients' characteristics, CBC, and pulmonary function test are shown in **Table 1**. The blood pressure results were within the reference range indicating a stable cardiovascular condition before collection of all the parameters in this study. Moreover, results of the CBC were within the reference value before starting the protocol. Pulmonary function test indicated a moderate severity.

The results of PImax, 6MWD, TNF-α, IL-6, TAC, MDA, and NO are presented in **Table 2**. The correlation analysis showed that PImax and 6MWD had a significantly low direct linear relationship with TAC ($r = 0.271$, $p = 0.04$; $r = 0.289$, $p = 0.028$; respectively) **(Figure 1**). On the other hand, PImax and 6MWD showed a significantly moderate inverse linear relationship with TNF-α ($r = –0.558$, $p < 0.001$; $r = –0.587$, $p < 0.001$; respectively) and IL-6 ($r = –0.588$, $p < 0.001$; $r = –074$, $p < 0.001$; respectively) (**Figure 2**). Furthermore, a significantly low inverse linear relationship between 6MWD and MDA was observed (**Figure 3**). However, PImax did not correlate with NO ($r = 0.207$, $p = 0.12$), and neither did the 6MWD ($r = –0.185$, $p = 0.164$) (figure not shown).

4. DISCUSSION

This preliminary study showed the correlation between parameters of physical assessment and biochemical markers that relate to oxidative stress and inflammatory conditions in COPD patients with moderate severity. Due to the small sample size of 29 participants, evaluation was carried out twice, one month apart, for confirmation of the validity of the results. Up to now, no study had investigated the correlation between oxidative stress or inflammatory conditions and physical function among COPD patients. Previous studies only reported that increased lipid peroxide product MDA [7] and decreased GSH or TAC were found in COPD patients [8, 9], as were inflammatory markers such as TNF-α and IL-6 [10]. This study demonstrated a significantly inversed correlation of PImax and 6MWD with cytokines (TNF-α and IL-6). The possible correlation of respiratory muscle and leg muscle strength with inflammation is of interest. The results of this study were in line with those in a previous one that showed a correlation between TNF-α and COPD severity [19] and suggested that a higher level of TNF-α might predict not only worse lung severity, but also possible respiratory muscle strength or walking capacity. Furthermore, the results of the present study also showed a possive correlation between TAC and walking distance, and a negative correlation between MDA and walking distance, which together suggested that low inflammation, low lipid peroxidation, and high antioxidant status might improve walking capacity. The causal relationship between them, however, warrants further investigation.

This study did not show a correlation of PImax and 6MWD with NO. As a vasodilator, NO may affect muscle strength via modulating blood flow. A previous study proposed that NO was associated with pathological pathways and possibly predicted airway obstruction depending on disease severity of COPD [20]. The reason for the lack of a correlation between

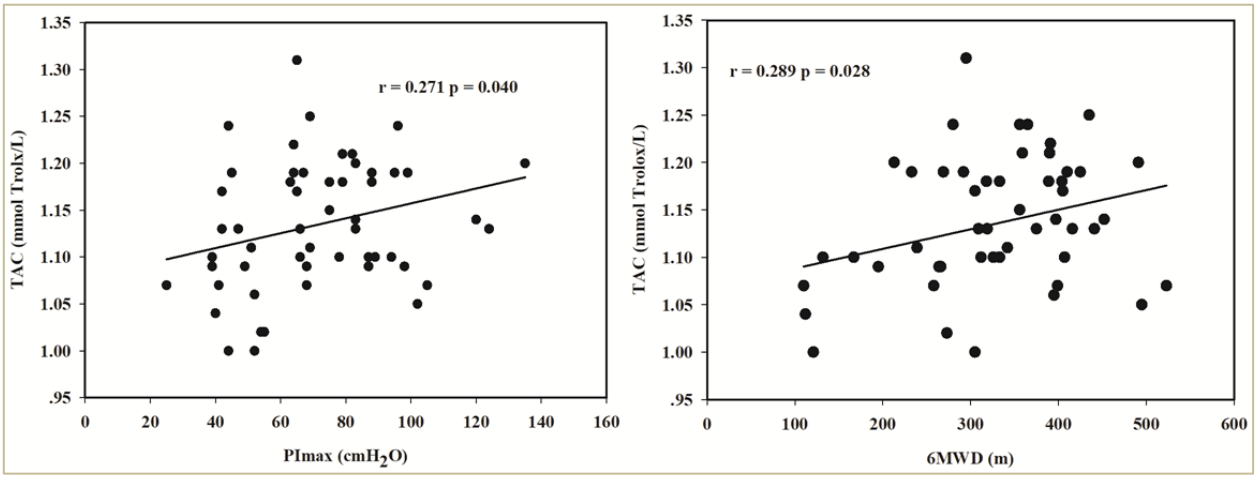

FIGURE 1. Correlation between PImax (cm H_2O)/6MWD (m) and TAC (mM Trolox). There were 58 data points from two repeated measurements in 29 COPD patients. Data were statistically analyzed by the Pearson correlation test.

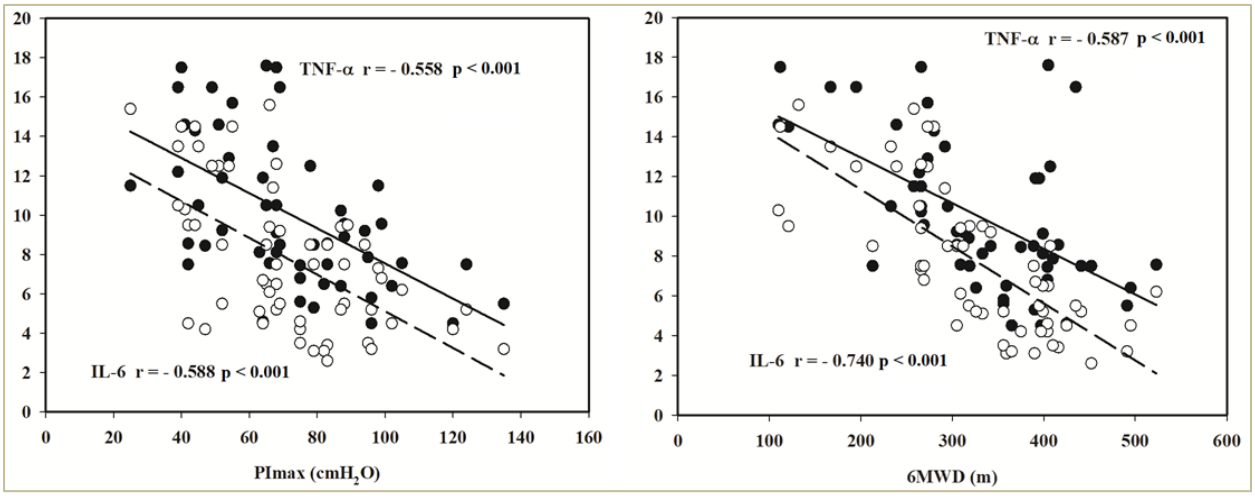

FIGURE 2. Correlation between PImax (cm H_2O)/6MWD (m) and TNF-α (ng/ml)/IL-6 (ng/ml). There were 58 data points from two repeated measurements in 29 COPD patients. Data were statistically analyzed by the Pearson correlation test.

NO and physical capacity in this study was unclear. Other researchers reported that NO did not differ among severe COPD patients because there was no inducible NO synthase in any muscle, when compared to non-COPD patients [21]. Nevertheless, the results of the present study revealed a significant correlation between oxidative/inflammatory stress parameters and the physical functionality of the COPD patients, which might provide new insight into the pathophysiology of COPD and the development of mechanistically based intervention for this disorder.

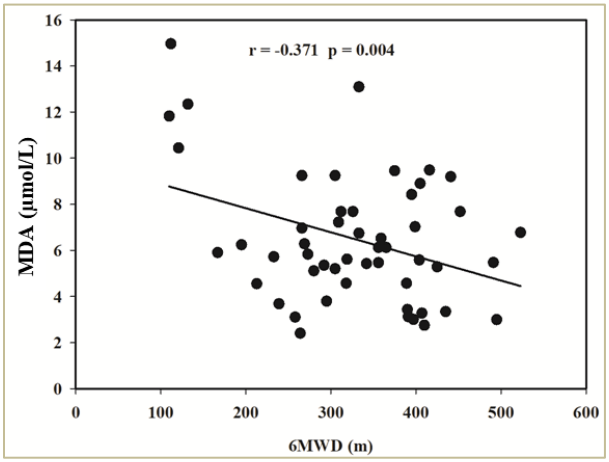

FIGURE 3. Correlation between 6MWD (m) and MDA (µM). There were 58 data points from two repeated measurements in 29 COPD patients. Data were statistically analyzed by the Pearson correlation test.

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