Quadriceps muscle atrophy and inflammatory markers in COPD patients

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Research article

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Abstract

Background

COPD (chronic obstructive pulmonary disease) is a systemic chronic inflammatory disease manifested by increased proinflammatory protein and cytokine levels. In this study, we aimed to investigate whether there is any correlation between systemic inflammatory mediators (CRP, ESR, PCT, IL-6, IL-1β, and TNF-α) and quadriceps muscle atrophy in patients with COPD.

Methods

The study group included forty patients with consecutive COPD followed at the Outpatient Pulmonology Unit of Imam Reza Hospital, Tabriz, Iran. Depth of quadriceps muscle was measured by B-mode ultrasonography using an 8 MHz 5.6 cm linear transducer array. Serum levels of CRP, PCT, IL-1β, IL-6 and TNF-α and the ESR were also measured. All patients were evaluated in three stages; the first day of mechanical ventilator admission, 48 hours later and at the seventh day.

Results

The results showed that the depth of quadriceps muscle on the seventh day after mechanical ventilator admission was significantly lower than of the first day ($P = 0.0008$). Statistical analysis showed a significant difference between the level of all systemic biomarkers on day 1 and day 7 ($P < 0.05$). The correlation study showed the significant negative associations between systemic inflammation markers and depth of quadriceps muscle. We found that the increased level of systemic inflammation biomarkers such as ESR, CRP and PCT were associated with reduced quadriceps depth in COPD patients. Moreover, evaluation of proinflammatory cytokines in COPD patients showed that the elevated IL-1β, IL-6, and TNF-α levels were associated with quadriceps atrophy.

Conclusion

The main finding of this study is the positive correlation of systemic biomarkers of inflammation with quadriceps muscle atrophy in COPD patients over 7 days of mechanical ventilator admission.

Background

Many patients with chronic obstructive pulmonary disease (COPD) are recognized with cachexia or skeletal muscle dysfunction [1]. COPD-related reduction in muscle strength plays an important role in poor exercise performance [2] and is associated with increased healthcare consumption and mortality [3]. Whilst the pathophysiology of skeletal muscle dysfunction and wasting is multi-factorial, inactivity is likely to play a key role. Evidence suggests that inactivity is probably linked to multi-factorial pathophysiology of skeletal muscle dysfunction and wasting. Reduced muscle strength is more readily

demonstrable in the lower limbs [4] of COPD patients/ Muscle weakness is more detectable in the lower limbs of patients with COPD and this can contribute to a “downward disease spiral” of loss of muscle strength, in which progressive dyspnoea leads to more sedentary lifestyle and locomotor muscles de-conditioning, and thus further limiting physical activities [5]. Of note, reduced exercise capacity correlates with increased COPD dyspnoea based on the MRC (Medical Research Council) dyspnoea scale with preserved whole body fat-free mass (FFM) indices/ [6]. The outcome of patient's pulmonary rehabilitation goals is to improve exercise performance which is assumed to increase muscle strength and endurance [7]. However, the international guidelines recommend the use of pulmonary rehabilitation for COPD patients with a MRC dyspnoea grade of > 3/ According to the international guidelines, patients with COPD, of MRC dyspnoea grade of > 3 are currently recommended for the use of pulmonary rehabilitation [8].

It has been noted that systemic inflammatory markers including IL-1 (interleukin-1), IL-6 (interleukin-6), TNF-α (tumor necrosis factor-α), CRP (C reactive protein) and PCT (procalcitonin) show an increase in COPD patients and are clearly associated to the systemic manifestations of the disease. CRP is an acute-phase protein biomarker of systemic inflammation that serves as a commonly used measure of disease severity. COPD patients often show high plasma levels of CRP that is a risk factor for increased mortality [9]. It is believed that CRP level is a measure of cardiovascular risk prediction and most likely to be of value in independently predicting death due to COPD [9]. Elevated levels of CRP are also associated with clinically important outcomes including exercise tolerance [10], health status [11], and muscle strength [12]. The synthesis of CRP is induced by inflammation associated cytokinessuch as IL-6 in hepatocytes [13]. Thus, IL-6 and CRP concentrations have been reported to correlate with COPD [13]. Furthermore, both IL-1β and TNF-αhave been demonstrated to implicate in the muscle wasting and weight reduction observed in patients with COPD [14] and consistently studies have found high circulating levels of TNF-α and its cognate receptors in COPD patients [14]. There is much evidence that IL-1β and TNF-α are cytokines associated with IL-6 and CRP levels [12].

Although peripheral muscle weakness has a multifactorial etiology, a key concept is that systemic inflammation is strongly linked to muscle dysfunction [15]. COPD patients suffering from decreased quadriceps muscle strength, during exacerbation, are more likely to have an increased plasma IL-β [15], IL-6 and TNF-α levels suggesting an inverse correlation between quadriceps muscle strength and systemic inflammatory mediators [12]. However, many studies have focused on lower limb muscles [16] [17] results of a previous study reported that relative intensity of movements in arm was lower in COPD patients than that of healthy control subjects [18]. Nevertheless, the contribution of systemic inflammatory biomarkers to strength in specific muscles is not clearly defined in these patients. The purposes of the present study was therefore to examine whether there is any correlation between mediators of systemic inflammation (CRP, ESR, PCT, IL-1β, IL-6 and TNF-α) and quadriceps muscle atrophy in patients with COPD.

Method

Study Patients

The study population included forty patients with consecutive COPD followed at the Outpatient Pulmonology Unit of Imam Reza Hospital, Tabriz, Iran. Eligibility criteria included patients that meet criteria for COPD based on the guidelines established in the Global Initiative for Chronic Obstructive Lung Disease. These criteria include an increase in FEV1 of 15% or 200 mL after inhalation of a β2-agonist and a post bronchodilator forced expiratory volume in the first second (FEV1)/forced vital capacity (FVC) ratio 0.70 . Patients diagnosed with other chronic diseases (ie, diabetes, renal failure, or cancer) or other respiratory disease (ie, asthma, bronchiectasis, bronchiolitis, or tuberculosis); and those with oral steroid use or exacerbations in the 3 months before enrollment in the study were excluded. All patients were evaluated in three stages; the first day of admission, 48 hours later and at the seventh day. Each participant signed a written informed consent form and was made aware of the study procedures based on ethical approval by the Research Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.134). Demographic data of patients are summarized in Table 1.

Muscle Thickness (MT) measurements by ultrasonography

Measurement of quadriceps muscle depth muscle were made by B-mode ultrasonography using an 8 MHz 5.6 cm linear transducer array (PLM805, Toshiba Medical Systems, Crawley, UK), as performed by de Bruin et al. [19]. The transducer was placed perpendicular to the long axis of the thigh on its superior aspect, three-fifths of the distance from the anterior superior iliac spine to the superior patellar border. This was the highest point in the thigh that the entire rectus femoris cross-section could be visualized in a single field in all subjects; other muscles of the quadriceps group could not be encompassed in this manner. During the imaging the participants were made to lie supine with one leg extended passively Care was taken using excess contact gel to ensure minimal distortion of the underlying tissue. Visual feedback was used to minimize oblique imaging to obtain the cross-section smallest images. Scanning depth was set to where the femur could be discerned for orientation. Muscle septa were delineated through the gentle contraction-relaxation cycles before image acquisition. Quadriceps muscle depth was measured by a planimetric technique which was taken following the inner echogenic line of the rectus femoris was outlined by a movable cursor on a frozen image. An average of three consecutive measurements within 10% was performed to outline the depth of quadriceps muscle.

Blood collection and analysis

Peripheral blood was collected early morning from each fasting participant at three time points (at first day, 48 hous later and at the day 7) and the plasma were then stored at -80°C. IL-1β, IL-6 and TNF-α were assessed by enzyme linked immunosorbent assay (ELISA) using high-sensitivity commercial kits (BioSource International Inc, Camarillo, CA, USA). CRP and PCT levels were measured by high-sensitivity particle-enhanced immunonephelometry (CardioPhase, Dade Behring Marburg GmbH, Marburg, USA), with a lower detection limit of 0.007 mg/L. All assessments were performed in duplicate.

Statistical analysis

Analyses were performed using GraphPad Prism1 Version 7.0 (GraphPad Software Inc., San Diego, CA, USA). - Results are reported as means and standard deviations or median interquartile range (25%–75%). Between groups analyses using ANOVA and Post HOC Pair wise comparison were employed. We performed an association analysis between inflammatory markers of ESR, PCT, CRP, IL-1β, IL-6, TNF-α and quadriceps muscle atrophy using multiple linear regression with robust standard errors with adjustment for potential confounders. P-values below 0.05 were considered statistically significant.

Results

In this study, 40 patients were studied. The mean and standard deviation of the subjects were 65.83 ± 15.6 years. 17 (42.5%) patients were female and 23 (57.5%) were male. It should be noted that this number included only patients who had been hospitalized for at least 7 days, and 40 patients, including those who had been discharged or died earlier than 7 days, were not due to patients who had died or died from the community We left the statistics and replaced the new patient. Participant characteristics are shown in table 1.

The results of quadriceps muscle atrophy

In this stage of the study, ultrasonography was performed to obtain the degree of atrophy of the quadriceps muscle of the patients, so that the patients underwent ultrasonography of the quadriceps muscle in the first day after mechanical ventilator admission, and once 48 hours later from admission and once on the seventh day, ultrasound was performed.

The average depth of quadriceps muscle 40 patients in ultrasound of the first day was 20.26 ± 6.976 in the second time of ultrasound 16.64 ± 6.248 and in the third time ultrasound was 14.33 ± 5.179, indicating a decrease in the depth and thickness of the muscle after admission. Statistical analysis of the results showed that the depth of muscle on the seventh day was significantly lower than the first day (P = 0.0008). However, the depth of muscle was not significantly different at the second time with ultrasound than the first time and in the third time, compared to the second time. The results of this step are presented in Fig 1 and Table 2.

The results of inflammatory factors evaluation

The results of the ESR test in the first day were 54.43 ± 27.06 in the second time, 41.83 ± 20.65 and in 7 days after admission, it was 37.15 ± 14.66. Statistical analysis showed a statistically significant difference between ESR on day 1 and day 7 (P = 0.0013) and day 1 compared to day 2 with P value = 0.025 (Fig 2a and Table2).

The results of the CRP test in the first day was 37.2 ± 18.026 in the second phase, was 26.14 ± 16.29 and 26.25 ± 11.26 at the 7 days after admission. Statistical analysis showed a significant difference between CRP level on day 1 and day 7 with P value = 0.0013 and CRP on day 1 and day 2 with P value = 0.022 (Fig 2b and Table2).

Also the result of the PCT test in the first day was 17.98 ± 14.19 for the second time, 7.348 ± 6.516, and at the final time, it was 6.925 ± 6.27. Statistical analysis showed a significant difference between PCT levels on day 1 and day 7 with a *P* value = 0.016 and PCT on day 1 and day 2 with a *P* value = 0.025(Fig 2c and Table2).

The results of proinflammatory cytokine evaluation

IL-1β secretion level of serum in the first day was 14.43 ± 8.04 pg/ml in the second day, 13.79 ± 6.61 pg/ml and in 7 days after admission, it was 16.1 ± 9.02 pg/ml. There was no statistically significant difference in the secretion level of IL-1b in three time (Fig 3a and Table 2).

IL-6 secretion level of serum in the first day was 3.96 ± 2.24 pg/ml in the second day, 3.65 ± 1.86 pg/ml and in 7 days after admission, it was 4.25 ± 2.44 pg/ml. There was no statistically significant difference in the secretion level of IL-6 in three time (Fig 3b and Table 2).

TNF-α concentration level of serum in the first time was 167.9 ± 65.08 pg/ml in the second time, 155.6 ± 85.12 pg/ml and in 7 days after admission, it was 194.7 ± 99.61 pg/ml. There was no statistically significant difference in the secretion level of TNF-α in three time of measurement (Fig 3c and Table 2).

Correlation between various inflammatory factors and quadriceps muscle atrophy

As manifested by the results, there was a positive correlation between the reduction of muscle depth in the quadriceps of patients and the Early rate of ESR, CRP and PCT. This correlation for ESR was statistically significant with *P* value <0.0001 and r = 0.698 and for CRP was statistically significant with *P* value =0.4nd to r = 0.53, Also, the correlation between quadriceps atrophy and PCT was statistically significant with *P* value <0.0001 and r = 0.786 (Fig 4).

In this study we also assessed correlation between early proinflammatory cytokine and quadriceps muscle atrophy. it was found that there was a positive correlation between the quadriceps atrophy of COPD patients and the early concentration of IL-1β, IL-6 and TNF-α. This correlation for IL-1b was statistically significant with *P* value =0.032 and r = 0.338 and for IL-6 was statistically significant with *P* value =0.0013 nd to r = 0.49, Also, the correlation between quadriceps atrophy and TNF-α was statistically significant with *P* value =0.005 and r = 0.43 (Fig 5).

Discussion

The main result here is the positive correlation of systemic inflammation biomarkers and quadriceps muscle atrophy in COPD patients over 7 days.

The results showed reduced quadriceps depth and increased systemic inflammation biomarkers such as ESR, CRP and PCT. The study further showed that the increased levels of IL-1β, IL-6, and TNF-α were correlated with quadriceps atrophy. A previous study however, has reported a negative association between systemic inflammation markers and quadriceps strength in stable COPD patients [12]. It has

already been indicated that inflammation in COPD patients can be explained by the elevation of CRP, IL-6, IL-8, and TNF-α in the circulation [20] [21]. The presence of local inflammation is a controversial issue in the management of COPD patients. There is still insufficient evidence supporting that local inflammation exists in the skeletal muscle of patients with COPD [22]. For example, according to an early investigation, the peripheral muscles atrophy of COPD patients have been detected by an increase in the TNF-α expression [23]. While none of the other investigators has reproduced these finding [24],[25]. Muscle proteolysis and inhibition of protein synthesis that may be a consequence of systemic inflammation may be responsible for skeletal muscle dysfunction [26]. The source of these circulating cytokines in muscles is thought to be either of systemic inflammation, activated leukocytes, and/or organ systems such as bone marrow or skeletal muscle itself [27]. Experiments have shown a causal link of inflammatory markers, such as high circulating levels of TNF-α [28] to muscle wasting which is due to enhanced activity of the ubiquitin proteasome pathway [29] or to the apoptosis induction [30]. Directly by compromising contractile function TNF-α may also function as a muscle regulator [31]. In line with these findings, we demonstrated a marked relationship between systemic TNF-α levels and quadriceps strength but not with muscle atrophy. Similarly, Yende et al [12] showed an inverse correlation between TNF-α and IL-6 levels with quadriceps strength. In that study, despite higher CRP levels in patients, CRP was not an independent predictor of quadriceps strength [13]. These observations support the notion that impairment in quadriceps muscle strength may be mediated through increased systemic inflammatory markers in patients with COPD. When comparing patients with mild/moderate COPD with those with severe/very severe disease, no difference was found between the groups, though a tendency to lower quadriceps depth was observed in severe and very severe patients.

Finally, it is necessary to point out that the current study had limitations. For example, it's better to be able to muscle strength by using the method one-repetition maximum (1 RM). In addition, we could examine more muscle or examine more specialized inflammatory mediums including different types of cytokines, and we could even evaluate the interactions between inflammatory markers and muscle weakness. In summary, our results are suggestive of an association between systemic inflammation markers and quadricep muscle atrophy in individuals with COPD.

Declarations

Acknowledgment

This is a report of a database from a thesis entitled “Relationship between onset of Quadriceps muscle atrophy with inflammatory markers among COPD patients under mechanical Ventilation” registered in the Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences, Tabriz, Iran.

Authors' contributions

MN wrote the manuscript and patients selected and management. MG contributed to wrote the paper and helped with ELISA tests. HM helped in the project design. MT contributed to the measurement of

quadriceps muscle depth. MA Supervised the project and performed the statistical analysis. HV contributed to the interpretation patients management and helped in the project design.

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Availability of data and materials

The data that support the findings of this project are accessible on request from the corresponding author. The data are not publicly accessible due to privacy or ethical limitations.

Consent for publication

All authors read the paper and approval for its publication.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethics approval and consent to participate

Each participant signed a written informed consent form and was made aware of the study procedures based on ethical approval by the Research Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.134)

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Tables

Table 1. Demographic data of patients (n = 40)

Characteristic	
Number of patients	40
Age (years)	65.83±15.6
Gender, male/female	23/17
BMI	25.54±5.43

Table 2. The results of inflammatory factors evaluation and quadriceps muscle atrophy in COPD patients over 7 days

	First time	Second time	Third time	*P* value 1 vs 2	*P* value 1 vs 3	*P* value 2 vs 3
ESR (mm)	54.43±27.06	41.83±20.65	37.15±14.66	0.025	0.0013	0.59
CRP	37.4±18.02	28.14±16.29	26.25±11.26	0.022	0.0046	0.84
PCT	14.19±17.98	7.348±6.516	6.925±6.27	0.025	0.016	0.98
IL-1b (pg/ml)	14.43±8.04	13.79±6.61	16.1±9.02	0.93	0.41	0.62
IL-6 (pg/ml)	3.96±2.24	3.65±1.86	4.25±2.44	0.82	0.85	0.44
TNF-α (pg/ml)	167.9±65.08	155.6±85.12	194.7±99.61	0.77	0.39	0.09
Muscle Depth	20.26±6.976	16.64±6.248	14.33±5.179	0.031	0.0008	0.3

Figures

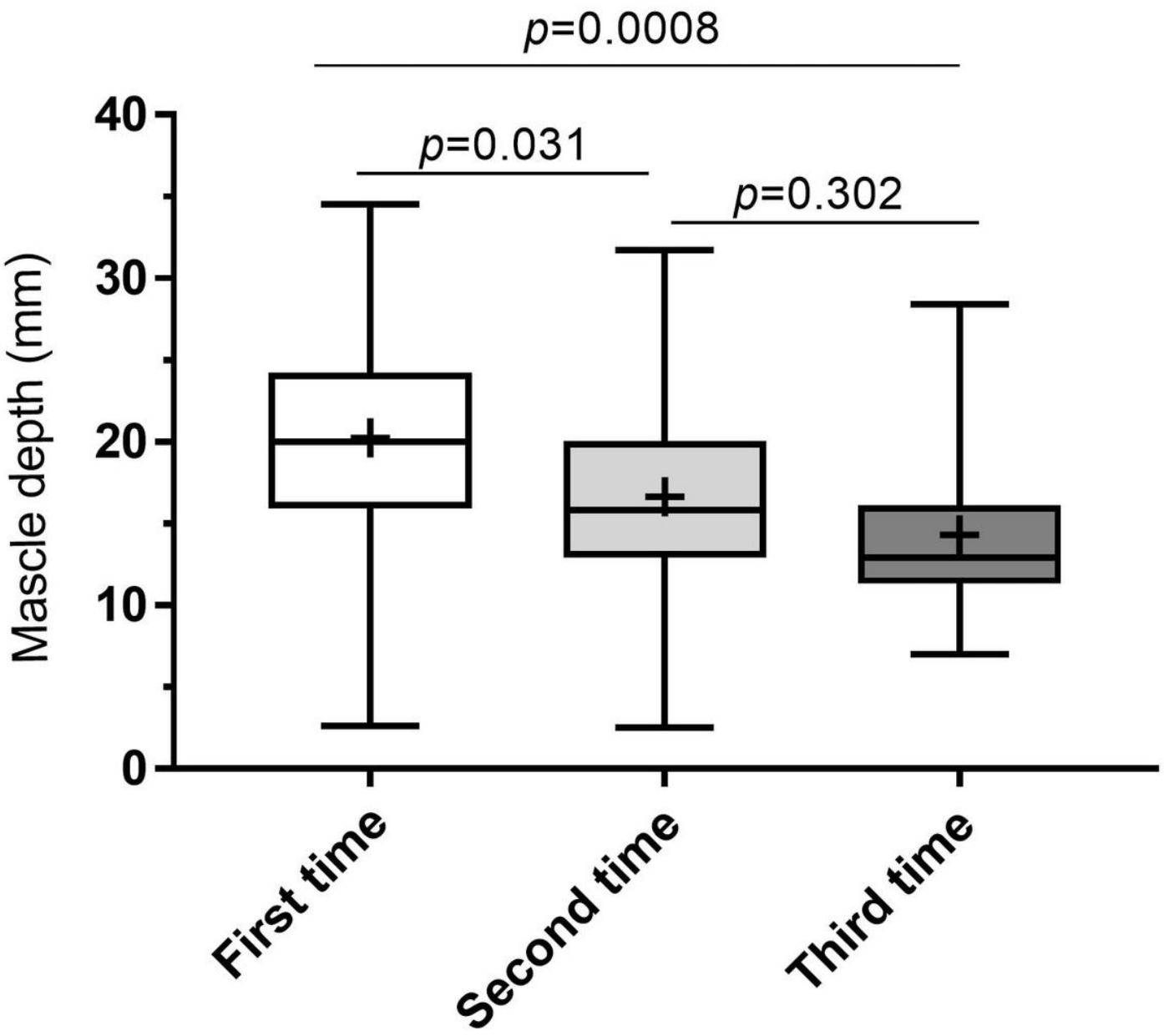

Figure 1

Ultrasonography was performed to obtain the degree of atrophy of the quadriceps muscle of the patients, statistical analysis of the results of this study showed that the depth of muscle on the seventh day was significantly lower than the first day (P = 0.0008).

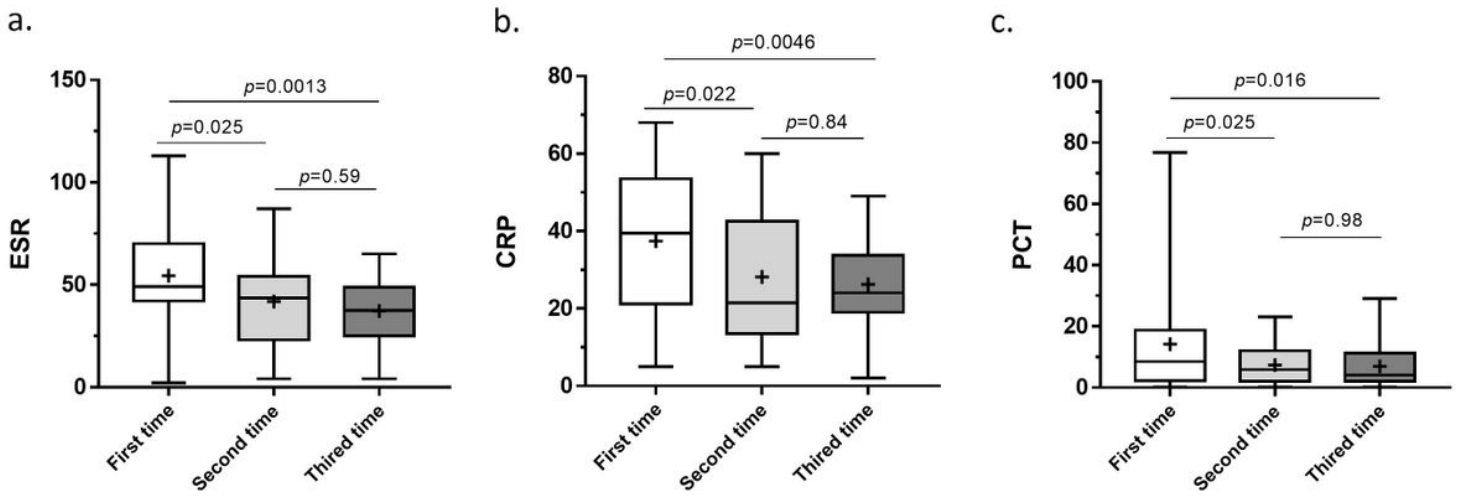

Figure 2

The results of inflammatory factors evaluation, statistical analysis showed a statistically significant difference between all three factors on day 1 and day 7.

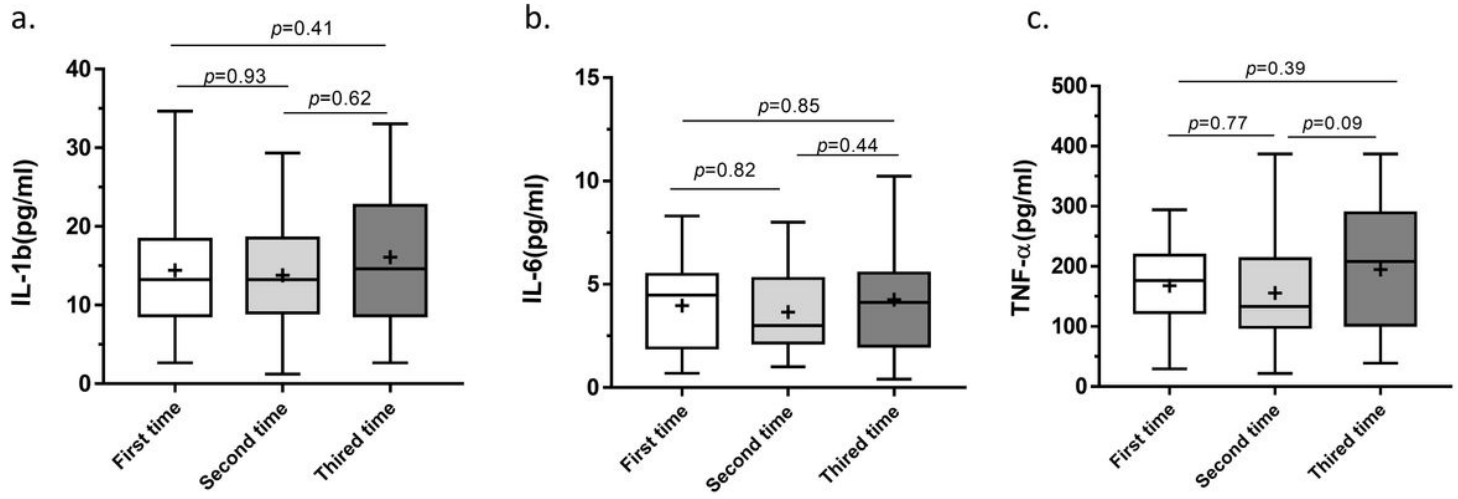

Figure 3

The results of proinflammatory cytokine evaluation, There was no statistically significant difference in the secretion level of proinflammatory cytokine.

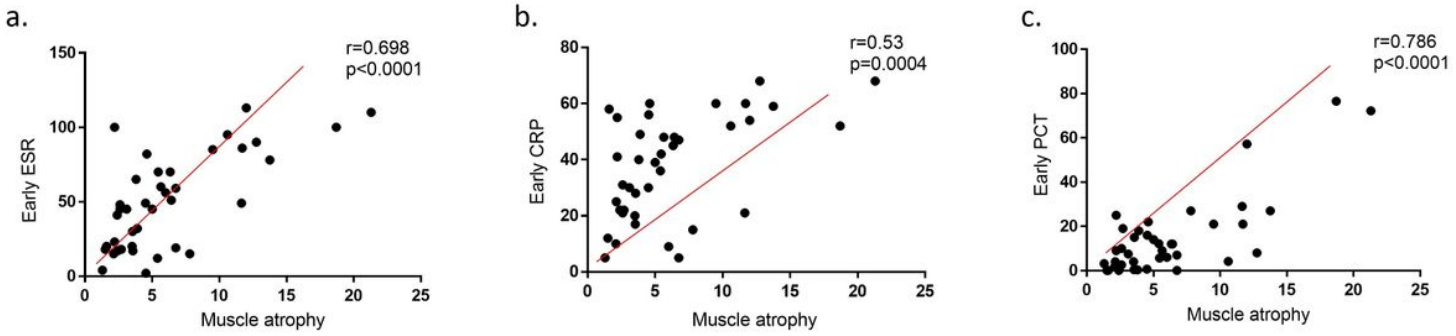

Figure 4

The results of correlation between various inflammatory factors and quadriceps muscle atrophy showed a positive correlation between the reduction of muscle depth in the quadriceps of COPD patients and the Early rate of ESR, CRP and PCT.

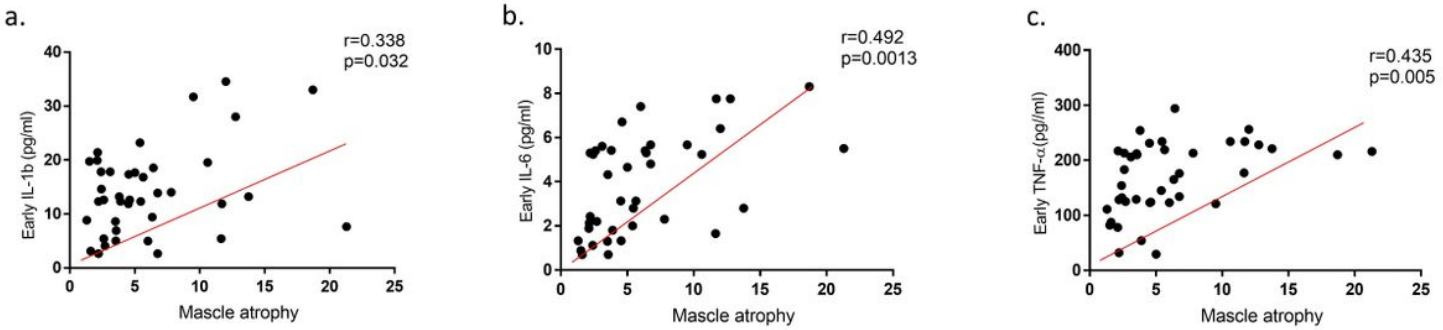

Figure 5

The results of correlation between proinflammatory cytocine and quadriceps muscle atrophy showed a positive correlation between the quadriceps atrophy of COPD patients and the Early concentration of IL-1b, IL-6 and TNF-α.