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Original Article

Effects of secretome supplementation on interleukin-6, tumor necrosis factor-α, procalcitonin, and the length of stay in acute exacerbation COPD patients

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Abstract

Acute exacerbation chronic obstructive pulmonary disease (AECOPD) is associated with significant poor survival. Mesenchymal stem cells (MSC) therapy has been a promising treatment for COPD; therefore, it has the potential to be an additional therapy for AECOPD. Its potential is associated with its secretome since it has anti-inflammatory and immunomodulator activities. The aim of this study was to determine the effect of the secretome as an adjuvant therapy in reducing the levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), procalcitonin, and the length of stay in AECOPD patients. A clinical control trial study was conducted among 28 moderate and severe AECOPD patients who were hospitalized from January to February 2023. The control group (n=14) received standard therapy of AECOPD while the treatment group (n=14) received standard therapy plus secretome 1 ml twice daily for three days. The levels of IL-6, TNF-α, and procalcitonin were measured at admission and on the fourth day of treatment. The length of stay was calculated from the time the patient was admitted until the patient was discharged from hospital. The data were compared using a paired Student t-test, chi-squared test and Mann-Whitney test as appropriate. In the treatment group, the levels of IL-6, TNF-α and procalcitonin after the treatment reduced 13.09 pg/mL, 5.00 pg/mL and 751.26 pg/mL, respectively compared to pre-treatment. In contrast, the levels of IL-6, TNF-α and procalcitonin increased 48.56 pg/mL, 44.48 pg/mL and 346.96 pg/mL, respectively after four days of treatment. There was a significant reduction of IL-6, TNF-α and procalcitonin in treatment group compared to the control group with p=0.022, p=0.009 and p=0.001, respectively. However, there was no significant reduction of the length of stay (p=0.072). In conclusion, administration of secretome to AECOPD patients could reduce the levels of IL-6, TNF-α and procalcitonin.

Keywords: Secretome, acute exacerbation, COPD, procalcitonin, cytokine

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Introduction

Chronic obstructive pulmonary disease (COPD) is a lung disease associated with an exaggerated chronic inflammatory response in the airways and lung parenchyma to noxious gases or particles [1]. Global prevalence of COPD was 11.7% in 2010 [2] and it has the potential to become the third

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leading cause of death globally by 2030 if it is not treated comprehensively [3]. COPD is one of the four most serious non-communicable diseases, contributing to 60% of deaths in Indonesia [4]. Seventy percent of the overall health care costs is associated with COPD patients [5] along with the increase of COPD management annual cost in proportion to the number of COPD exacerbations [6]. The current standard management of COPD has not been able to modify long-term decline in lung function and reduce mortality; therefore, the development of new treatments is urgently needed [7].

Acute exacerbations of COPD (AECOPD) occurs in more than 50% of COPD patients in the Netherlands [8] and 23% of COPD patients in the United Kingdom had two moderate or severe exacerbations and 14% of patients had ≥3 exacerbations annually [9]. AECOPD is an important medical condition and a health care problem associated with significant poor outcome/survival [10] and it increases the likelihood of subsequent hospitalization related to COPD [11]. The management of AECOPD aims to minimize the negative impact of exacerbations, prevent further exacerbations, and reduce hospitalizations in ≤30 days after discharge from the hospital. Treatment failure of AECOPD occurs in 14–18% of patients which requires the development of new treatments [11]. AECOPD can increase inflammatory markers such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), procalcitonin (PCT), C-reactive protein (CRP), eosinophils and neutrophils [12].

One of promising therapies considered for the management of COPD is mesenchymal stem cells (MSCs) therapy which has the ability to suppress immune responses, maintain oxidative balance, and regulate the activity of matrix-degrading enzymes [13, 14]. MSC based therapy has been shown to be safe and well tolerated in clinical trials [15,16]. A placebo-controlled trial of MSC in moderate to severe AECOPD patients reported that there were no side effects and no increase in the frequency of AECOPD [15]. In addition, the administration of bone marrow-derived MSC (BM-MSC) resulted in a lung function increase in all COPD patients, especially in the first 30 days after administration [16]. The beneficial effects of MSC are largely ascribed to the secretome via paracrine effects [17]. However, presumptively there are no available studies assessing the effect of the secretome on inflammation and clinical improvement in AECOPD patients. The aim of this study was to determine the potential of the secretome as an adjuvant therapy in reducing levels of IL-6, TNF-α, and PCT, and the length of stay in AECOPD patients.

Methods

Study design and setting

A clinical trial was conducted at three hospitals, Universitas Sebelas Maret Hospital, Surakarta, Dr. Moewardi General Hospital, Surakarta, and Dr. Soehadi Prijonegoro General Hospital, Sragen, Indonesia from January to February 2023. Twenty-eight moderate and severe AECOPD patients were divided into treatment and control groups. The treatment group received AECOPD standard therapy plus secretome 1 ml/12 hours intramuscular for three days while the control group received AECOPD standard therapy only. Patients were followed up until the end of hospitalization (recovered and discharged). Blood samples were collected on the first and fourth day of hospitalization to determine the levels of IL-6, TNF-α, and PCT. The length of stay was assessed from admission time until the patient was discharged. This study was registered at Thai Clinical Trials Registry (TCTR20230712001).

Sample size and randomization

The sample size was calculated using the sample formula for the mean difference test of two independent populations which resulted in and rounded up to nine subjects. In this study, 10% of them were expected to be dropped out and therefore the minimum of research subjects were ten samples of each treatment and control group. The number of samples used in this study was 14 samples per group and the total sample was 28 samples. The AECOPD patients were randomly divided into treatment or control according to the order of entering the hospital. This research was approved by the Health Research Ethics Committee of Dr. Moewardi Hospital Surakarta prior to conducting the study.

Patients

The study subjects were AECOPD patients who were hospitalized at Universitas Sebelas Maret Hospital, Dr. Moewardi General Hospital, and Dr. Soehadi Prijonegoro General Hospital from January 2023 to February 2023. The inclusion criteria were patients with clinically diagnosed AECOPD, aged more than 40 years old and had the ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) less than 0.7. Exclusion criteria were AECOPD patients requiring intensive care unit (ICU) and ventilator care; patients with pleural effusion, lung cancer and human immunodeficiency virus (HIV).

Intervention

In the treatment group, the patients received intramuscular secretome 1ml/12hours daily for three days as well as AECOPD standard therapy. The control group received AECOPD standard care and a placebo. The secretome was obtained from umbilical cord mesenchymal stem cell (UC-MSC) cultures. UC-MSC were cultured to 80% confluency using complete growth media. The culture medium was obtained and centrifuged 500 × g for five minutes to remove impurities. The supernatant was removed and cell pellet was then filtered and stored at -80°C. The conditioned medium (CM) then thawed and transferred into sterile vials and carried out in a cool box at 2–8°C before used. Secretome containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with pharmaceutical heparin-treated human platelet lysate.

End points

The IL-6, TNF-α and PCT as inflammatory markers were measured on the first day of admission (before treatment) and the fourth day of treatment. The measurements of IL-6, TNF-α, and PCT were conducted with enzyme-linked immunosorbent assay (ELISA) technique. The quantitative detection kits used were Human IL-6 ELISA kit (DE2132) (Demeditec Diagnostics GmbH, Kiel, Germany), TNF- α ELISA kit (DE75111) (Demeditec Diagnostics GmbH, Kiel, Germany) and Procalcitonin Human ELISA kit (E-EL-H1492) (Elabscience Biotechnology Co., Wuhan, China). Physical examination and clinical improvement were evaluated daily and length of stay was calculated as the number of days the patient was being admitted.

Statistical analysis

The categorical data was presented with frequency distribution in percentage while numerical data with mean ± standard deviation (SD). Differential tests were performed based on type and distribution of data. Chi-squared/Fisher exact test was used to compare patient characteristics between treatment and control groups. The normality test was carried out using Shapiro-Wilk test. Student t-test or Mann-Whitney test were used to compare two groups as appropriated. The analysis was executed with SPSS 25 for Windows (SPSS Inc, Chicago, USA).

Results

Characteristics of patients

A total of 14 samples per group was recruited and followed during the study (**Figure 1**). The basic characteristics of the 28 AECOPD patients included in this study are presented in **Table 1**. Most of the subjects of this study was male in both groups (71.4% or 10 patients). The average age of patients in the treatment and control group were 63.07±12.2 and 62.93±11.3 years, respectively. The proportion of exacerbations in patients in the treatment group and the control group was different. The patients with a severe degree of exacerbation dominated the treatment group (7 patients or 50%) while the moderate degree dominated in the control group (8 patients or 57.1%). Both severe and moderate degrees of smoking history were dominant in the control group (4 patients or 35.7% each) while only severe history was in the treatment group (8 patients or 57.1%). However, gender, age, exacerbation severity, and smoking history of the patients did not differ significantly between the two groups.

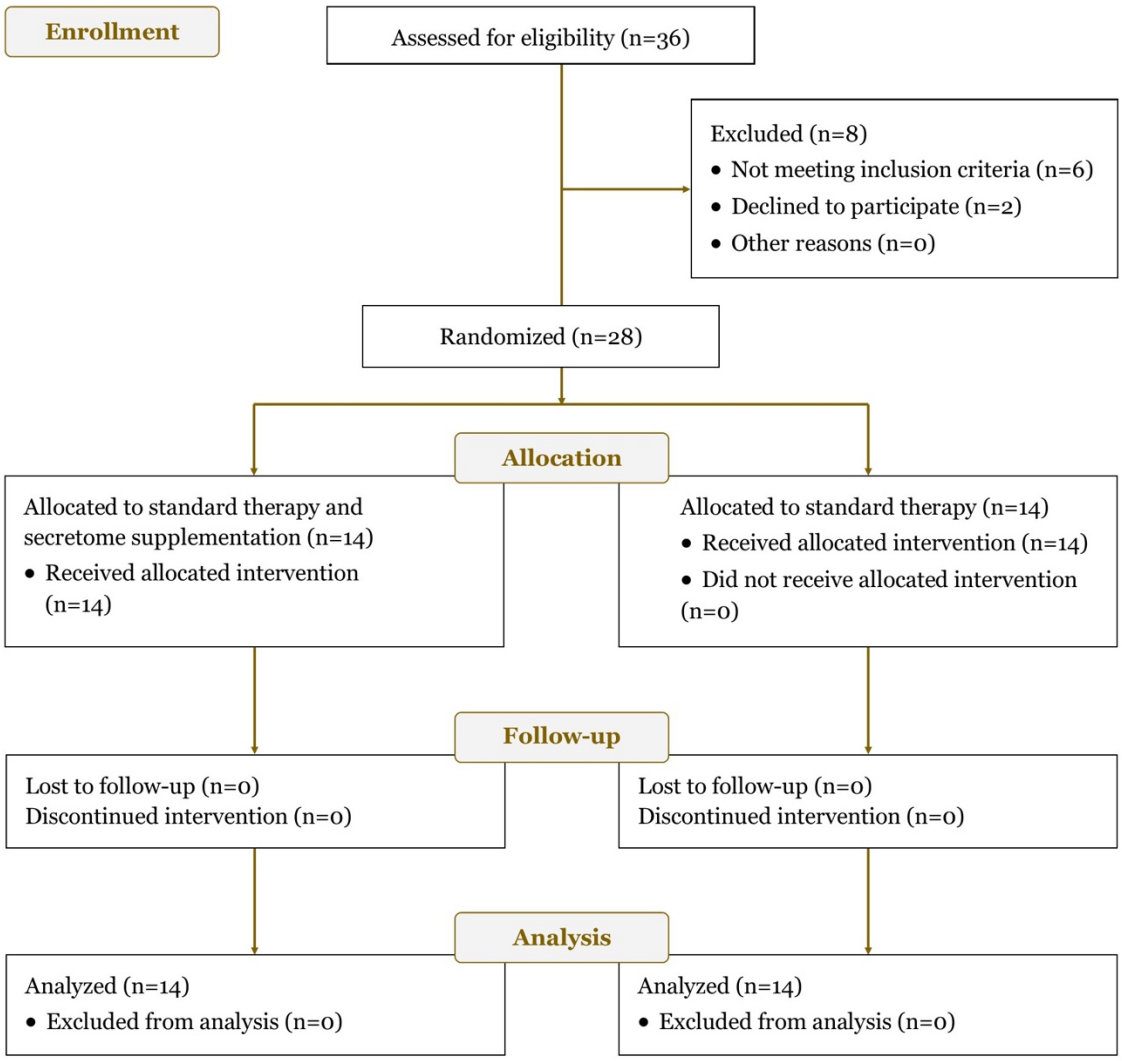

Figure 1. CONSORT flow diagram of the study.

Table 1. Basic characteristics of patients (n=28)

Patient characteristics	Group		*p*-value
	Treatment (n=14) Mean ± standard deviation	Control (n=14) Mean ± standard deviation	
Sex			1.000
Male	10 (71.4%)	10 (71.4%)	
Female	4 (28.6%)	4 (28.6%)	
Age			0.975
Mean±SD	63.07±12.23	62.93±11.30	
Median (Min-max)	64.00 (44-85)	60.50 (44-83)	
Severity grade of exacerbation			0.242
Mild	1 (7.1%)	2 (14.3%)	
Moderate	6 (42.9%)	8 (57.1%)	
Severe	7 (50.0%)	4 (28.6%)	
History of smoking			0.458
Passive smoker	4 (28.6%)	4 (28.6%)	
Mild	0 (0.0%)	0 (0.0%)	
Moderate	2 (14.3%)	5 (35.7%)	
Severe	8 (57.1%)	5 (35.7%)	

Effect of secretome on IL-6, TNF-α and PCT levels

The comparisons of IL-6, TNF-α and PCT value between treatment and control groups are presented in **Table 2**. In the control group, IL-6 level increased by 48.56 pg/mL compared to baseline (20.31 vs 68.87 pg/mL). In contrast, the IL-6 levels decreased by 13.09 pg/mL (48.90 pg/mL vs 35.81 pg/mL) in the treatment group. The IL-6 level differences (the changes between post-treatment and pre-treatment) were statistically significant between control group and treatment group (p<0.022) (**Table 2**).

Table 2. Comparisons of IL-6, TNF-α and PCT between treatment and control groups

Variables	Phase	Group		*p*-value
		Treatment Mean±SD	Control Mean±SD	
IL-6 (pg/mL)	Pre	48.90±81.72	20.31±10.87	0.662 [a]
	Post	35.81±64.51	68.87±101.47	0.060 [a]
	p-value	0.140 [b]	0.028 [b*]	
	Delta changes (post-pre)	-13.09±110.01	48.56±102.33	0.022 [a]
TNF-α (pg/mL)	Pre	13.81±13.39	7.08±3.71	0.024 [a]
	Post	8.80±6.28	11.56 ±8.58	0.448 [a]
	p-value	0.084 [b*]	0.030 [b*]	
	Delta changes (post-pre)	-5.00±13.34	4.48±8.30	0.009 [a*]
PCT (pg/mL)	Pre	1,177.01±1614.64	394.44±663.56	0.066 [a]
	Post	425.75±645.21	741.40±862.65	0.520 [a]
	p-value	<0.001 [b *]	<0.001 [b *]	
	Delta changes (post-pre)	-751.26±1268.53	346.96±561.13	<0.001 [a*]

[a] Analyzed using Mann-Whitney test
[b] Analyzed using paired Student t-test
* Statistically significant at p=0.05

The mean of pre- and post-treatment TNF-α levels were 7.08 and 11.56 pg/mL, respectively among patients within control group indicating an increase (**Table 2**). In contrast, in the treatment group, the mean TNF-α post-treatment was significantly reduced compared to pre-treatment (13.81 vs 8.80; with a mean reduction of 5.01). The difference in change of TNF-α levels between control group and treatment group was statistically significant with p<0.009, suggesting that secretome could reduce TNF-α level in AECOPD patients (**Table 2**).

The average level of PCT in the control group increased, from 394.44 pg/mL to 741.40 pg/mL after four days. In contrast, there was a significant reduction in PCT levels with an average of 751.26 pg/mL in patients in the treatment group; mean values of pre-treatment (1,177.01 pg/mL) and post-treatment (425.75 pg/mL) (**Table 2**). The difference in change PCT levels between control group and treatment group was statistically significant (p<0.001), suggesting that secretome was effective in reducing PCT levels in AECOPD patients.

Effect of secretome on length of stay

The mean length of stay of AECOPD patients within treatment group and control group was 5.21±1.31 days and 6.36±1.86 days, respectively. The length of stay in the treatment group was slightly shorter than control group but not statistically significant (p=0.072).

Discussion

The decrease in IL-6 levels in the secretome treatment group of this study suggested that secretome might be supplemented as additional therapy in AECOPD patients. It was in accordance with a previous study which reported that administration of secretome at a dose of 1 ml/12 hours for three days can control inflammation in COVID-19 patients [18]. The elevated IL-6 level indicates a systemic inflammatory response such as dyspnea and lung function, and can be used as an early indicator of inflammation in infection [19]. Particularly, the IL-6 level was significantly higher in AECOPD patients compared to the stable COPD patients and can be used as a predictor of patients with AECOPD within 48 hours [20].

The expression of IL-6 is decreased by the IL-10 contained in secretome that is able to inhibit the NF-κB pathway [17]. The main mechanisms of the secretome immunomodulatory effects are

cell-to-cell interactions and paracrine signaling [21]. Secretome can suppress the activity of proinflammatory M1 macrophages through p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) [17]. Therefore, it could reduce inflammation in patients with AECOPD.

The results of this study showed contrasting TNF-α levels between the control and treatment groups. TNF-α level increased 63.27% in control while decreased 36.20% in the treatment group. The differences between the two groups were statistically significant, suggesting that secretome could reduce TNF-α levels in AECOPD patients. The decrease in the treatment group was in accordance with the previous study which reported that giving secretome at a dose of 1 ml/12 hours for three days could control inflammation in COVID-19 patients [18]. Another study showed that secretome and MSCs supplementation suppressed cytokine production, increased the expression of macrophage marker M2, and increased the phagocytic capacity of monocyte-derived macrophage (MDM) in acute respiratory distress syndrome (ARDS) mouse model [22]. In addition, COPD patients with high levels of C-reactive protein (CRP), TNF-α and IL-6 experienced clinical improvement after mesenchymal stromal cell infusions treatment [15]. The exosome stem cell administration could reduce the levels of IL-6, TNF-α, and chemokine (C–X–C motif) ligand (CXCL) in rats exposed to cigarette smoke [23].

Secretome supplementation causes TNF-α decrease through the activation of NF-κB pathway [24]. It activates IL-8 gene transcription and enhances IL-8 release from airway epithelium and neutrophils which causes the inflammatory activity reduction of M1 macrophages and pro-inflammatory activity increase of M2 macrophages [24], thereby increasing the damage to lung tissues [25]. TNF-α is a powerful proinflammatory cytokine involved in the pathogenesis of AECOPD and it can induce the onset of COPD [25]. Severe infection can trigger the production of TNF-α in large quantities that cause systemic reactions in AECOPD patients [26].

PCT levels in the secretome treatment group decreased 63.82% compared to the control group which had an 87.81% increase. The PCT level decrease on the fourth day of hospitalization in the treatment group demonstrated the role of the secretome in reducing inflammation in AECOPD patients.

Secretome administration is able to inhibit NF-κB activity and therefore it will inhibit the release of pro-inflammatory cytokines, including PCT [18]. It has been known that AECOPD patients had higher PCT values compared to the stable COPD [27, 28] up to nine times [29]. Serum PCT level has a good capability in distinguish bacterial from non-bacterial infection [30] as it has been shown to be elevated in bacterial infections but remains low in viral infections and other inflammatory conditions, thus, it has been proposed that PCT could be useful in the evaluation of bacterial infection in AECOPD patients [31]. This is important to provide guidance to clinicians in determining causative of exacerbations so they can provide more appropriate therapy [31].

The average length of stay in AECOPD patients was between 6.7 and 8.7 days [32, 33]. The stay period more than seven days was associated with an increases the likelihood of developing acute respiratory acidosis [34]. In the present study the AECOPD patients receiving secretome treatment stayed for 5.2 days. Although it was shorter than the control group, it was not statistically significant. One of the reasons for this finding is the decision to discharge the AECOPD patients influenced by many factors.

Owing to the potent ability to modulate immune and inflammatory responses, MSCs are emerging as a prospective tool of cell-based therapy in immune disorder as well as inflammatory disease. The therapeutic effects of MSCs are largely owed to their secretome, which is rich in growth factors, cytokines, extracellular vesicles and exosomes [35,36]. The effect of the secretome in AECOPD patients is by suppressing the inflammatory response to avoid damage and restore cellular function to homeostasis [37].

The result of this study showed that administration of secretome can reduce the levels of IL-6, TNF-α, and PCT compared to control group. This can be caused by secretome that is able to reduce the activity of inflammatory mediators derived by M1 macrophages such as TNF-α and IL-6 which resulted in the inflammation reduction in patients with AECOPD. In a subsequent phase 1 and 2 clinical study, four doses MSC were shown to significantly alleviate the severity of COPD symptoms [38]. The secretome supplementation has a potential to be used as a preventative

approach as it is reported that administration of BM-MSC injections reduced the risk of hospitalization for COPD patients [35].

There some limitation of this study. The sample size was relatively small for clinical trial and lack of analysis on confounding factors such as comorbidity that could affect the levels of IL-6, TNF-α and PCT, as well as the length of stay of the AECOPD patients.

Conclusion

This study suggests that the additional therapy of intramuscular secretome 1ml/12 hours for three days could reduce inflammation markers such as IL-6, TNF-α and PCT in patients with AECOPD. Therefore, the administration of secretome could be an alternative option as an anti-inflammatory in cases of AECOPD. However, further study is needed to determine a more optimal secretome dose to reduce inflammation in AECOPD patients. It is also necessary to conduct a discussion and assessment related to patient comorbidities that can affect the severity of AECOPD patients.

Ethics approval

This study was approved by the Health Research Ethics Committee Dr. Moewardi General Hospital Surakarta, Indonesia (421/IV/HREC/2022). All patients voluntarily signed the informed consent.

Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the first author on request.

How to cite

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