Mesenchymal Stem Cell Secretome for Lung Fibrosis Post Covid-19 Infection-Case Report

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Abstract. Covid-19 has been a global health emergency and not only induced an unprecedented economic crisis in almost every country, but has led to social and educational crises. With the fast spreading and mutation rate of this virus, human lifestyle has been changed to mitigate the infection of SARS-CoV-2. However, this virus is still able to infect vaccinated people, thus reducing their quality of life for those who have post covid syndrome. In this case report, we report 3 case studies of Mesenchymal Stem Cell Secretome to reduce lung fibrosis post covid in humans. All patients who tested negative after being infected by SARS-CoV-2 have breathing difficulty and weakness as the symptoms of post covid syndrome, then intramuscular Mesenchymal Stem Cell Secretome injections were administered and patients were monitored closely to see its effectiveness. After 1 month from the last injection, all patients were able to breathe normally, shown by reduction of lung fibrosis sasasa.

Keywords: Covid-19 · Secretome · Lung fibrosis

1 Introduction

2019-nCoV or COVID-19 was first identified on Des 2019 in Wuhan, which then has spread all over China and worldwide in a matter of months. In January 2020, the WHO announced that the COVID-19 is the sixth global health emergency and categorized COVID-19 as pandemic across the globe, named it severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on 11 February 2020. Beside how easy SARS-CoV-2 can spread, the symptoms between each patient are quite diverse, in which a small population can show serious illness, but for others can only show mild or even no symptoms. This is one of the reasons why SARS-CoV-2 is uncontrollable and can spread to almost every country [1–4]. By the end of Des 2021, more than 300 million individuals have been infected in 226 countries and more than 5 million died worldwide. In Indonesia particularly, more than 4.2 millions cases have been reported and more than 144 thousand died.

The symptoms of COVID-19 can vary from asymptomatic, mild, or severe. Most of the cases include acute respiratory disease (ARDS) or pneumonia with different stages

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of severity. The asymptomatic without fever or ARDS, can be a carrier of SARS-CoV-2, which can transmit the virus to other people by direct contact. Patients with mild symptoms usually show acute respiratory disease like fever and cough with or without signs of pneumonia, and more than 30% of patients need oxygen therapy. Gastrointestinal symptoms like vomiting, nausea, or diarrhea have also manifested in some cases. Severely affected patients usually show fever, cough, dyspnea, sputum production and severe pneumonia, which most of them need oxygen therapy, and require mechanical ventilation [5].

From genomic analysis, it shows that SARS-CoV-2 infects human cells using the viral S spike protein which interacts with the angiotensir converting enzyme II (ACE2) similar to SARS-CoV [6]. This receptor is unfortunately widely expressed in the alveolar type II cells (AT2) which also express TMPRSS2 for S spike protein priming, an essential step for SARS-CoV-2 to infect the cell [7, 8].

It is well documented that severe SARS-CoV-2 infection will trigger excessive release of inflammatory cytokines or "cytokine storm". This immune system dysfunction will cause tissue damage, cellular compromise, organ dysfunction, and multi organ failure [9]. Compensatory anti-inflammatory response syndrome (CARS) will follow after systemic inflammatory response syndrome (SIRS) happens in response to dampen the proinflammatory state, orchestrating a fine-tuned balance of pro- and anti-inflammatory responses [10]. This so-called post covid-19 syndrome not only makes the patient lose immune competence, they are also susceptible to develop pulmonary fibrosis, leading to reduced pulmonary function and quality of life [11].

Mesenchymal stem cells (MSCs) and its derivatives (secretome and EV) have been proposed as a potential regime for treating Covid-19. Many researches have also shown that MSC and its derivatives are capable of orchestrating important immunological modulation functions in our body. MSCs play an important role due to their ability to secrete inflammatory factors, in which these inflammatory factors have shown supporting effects on the healing process of the host through paracrine action [12]. These paracrine factors consist of various proteins such as transcription factors, growth factors, cytokines, chemokines, which is important to promote revascularization and restore tissue damage. Anti-fibrotic cytokines and factors also detected in secretome, reducing collagen fiber and lung fibrosis caused by hyper inflammation [13]. MSC also can secrete angiopoietin 1 (Ang1) and keratinocyte growth factor (KGF) to restore the alveolar capillary barrier during lung infection [14]. Currently, there are 6 phase I/II clinical trials of MSC derivatives for Covid-19, in which 4 of them use EV and 2 using secretome (Table 1).

2 Preparation

2.1 MSC Isolation and Expansion

Umbilical cord was collected from a cesarean delivery donor, which tested negative from HCV, HIV, HBsAg, Syphilis, and CMV. MSC then was isolated using a nonenzymatic method [15]. MSC then incubated in α-MEM (Gibco) with 10% FBS supplementation for 21 days in 37 °C and 5% CO2. Medium was changed every 3 days until it reached 80% confluency, then MSC was subcultured until reach the total cell required for Secretome

Table 1. The current clinical trial using secretome/exosome

ID	Sample Size	Intervention	Comparison Group	Phase
NCT04798716	55	Escalating dose 2/4/8 × 10^9/mL EV	Placebo	I/II
NCT04493242	120	8×10^{11} of EV and 1.2×10^{12} of EV	Placebo (Saline)	II
NCT04602442	90	0.5–2 × 10^{10} of EV	Placebo	II
NCT04747574	35	1/5/10/100 × 10^8 of EV	No placebo	I
NCT04753476	48	1 mL of Secretome every 12 h for 3 days	standard Covid-19 therapy	II
NCT05019287	29	5 mL of Secretome every day for 5 days	Placebo (Saline)	I/II

production. MSC was tested positive for CD73, CD90, and CD105, negative for CD 14, CD 19, CD34, CD 45, HLA-DR.

2.2 Secretome Preparation

Secretome was collected from a Hypoxic-conditioned Mesenchymal Stem Cell after 3 days starving period in GMP Facility in basal medium. Secretome was filtered with 0.45 μm and 0.22 μm then tested for sterility, mycoplasma and phenol red residue. Secretome was stored in −80 °C until administration.

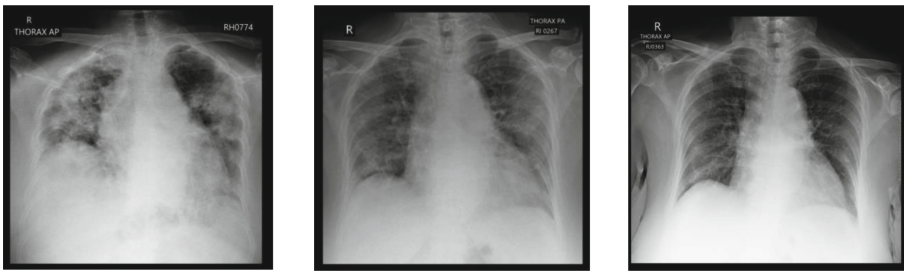
3 Case Report

3.1 Case 1

A 72-years old female patient, who was diagnosed covid previously and already tested negative by PCR came with breathing difficulty and felt weak. Lab testing showed the oxygen saturation was 85%, Hb 11.1, D-Dimer 3.7, CRP 23, pulmonary infiltrate was observed in thorax radiograph (Fig. 1a). The patient then was diagnosed with pulmonary duplex and agreed to use secretome as adjuvant therapy. 1.5 ml Secretome was injected intramuscular for 14 days and the patient was closely observed for 14 days. After 7 days the lab testing showed Hb 10.4, D-Dimer 0.4, and CRP 0.8, pulmonary infiltrate was reduced (Fig. 1b), and no more breathing difficulty or other symptoms after 1 months secretome injection (Fig. 1c).

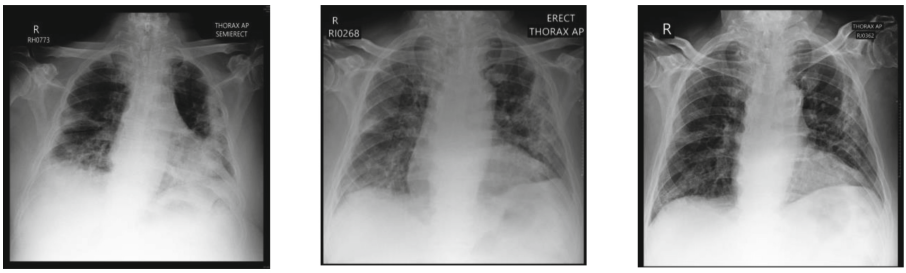
3.2 Case 2

A 73-years old male patient also diagnosed covid previously and already tested negative by PCR have trouble with breathing. Lab testing showed the oxygen saturation was below 80%, Hb 13.1, D-Dimer 1.7, CRP 201.8, pulmonary infiltrate was also observed



(a) (b) (c)

Fig. 1. Case 1. (a) first thorax radiograph. (b) after 14 days of secretome last injection. (c). 1 month after the last secretome injection.



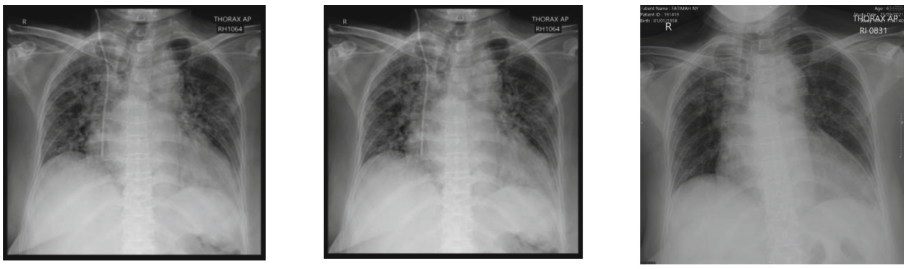
(a) (b) (c)

Fig. 2. Case 2. (a) first thorax radiograph. (b) after 14 days of secretome last injection. (c). 1 month after last secretome injection.

in thorax radiograph (Fig. 2a) and had to be admitted to hospital with a diagnosis of lung fibrosis. The patient also agreed to use secretome as adjuvant therapy. 1.5 ml Secretome was injected intramuscular for 14 days and the patient was closely observed for 14 days. After 7 days of secretome injection, the lab testing showed D-Dimer 0.9, CRP < 0.5, pulmonary infiltrate was still present (Fig. 2b), and no more breathing difficulty after 1 months secretome injection (Fig. 2c).

3.3 Case 3

The A 63-years old female patient, who was already tested negative by PCR after being diagnosed with covid still had problem with breathing. Lab testing showed the oxygen saturation was 88%, Hb 11.7, CRP 23.7, D-Dimer 1.3, pulmonary infiltrate was observed in both lungs (Fig. 3a). The patient was diagnosed with pulmonary duplex and lung fibrosis agreed to use secretome as adjuvant therapy. 1.5 ml Secretome was injected intramuscular for 14 days and the patient was closely observed for 14 days. After the last secretome injection the lab testing showed Hb 12.2, D-Dimer 0.4, CRP 2.3, pulmonary infiltrate was still present (Fig. 3b), and no more symptoms or oxygen help after 3 weeks of secretome injection (Fig. 3c). In your paper title, if the words “that uses” can accurately replace the word using, capitalize the “u”; if not, keep using lower-cased.



(a) (b) (c)

Fig. 3. Case 3. (a) first thorax radiograph. (b) after the secretome last injection. (c). 3 weeks after last secretome injection.

4 Discussion

"Cytokine storm" that leads to multi organ failure is one of the causes of death in covid-19 patients and is associated with an increase of D-dimer and CRP levels in blood [16]. Patient with covid-19 infection have increased level of IL-2, IL-4, IL-6, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP1A, TNF-α, IFN-γ [17]. Several anti-inflammatory mediators are produced to reduce this inflammatory imbalance. These mechanisms will then create a favorable environment for the body to recover and for homeostasis to return. Moreover, inflammatory mediators such as ACTH could trigger a negative-feedback to downregulate the production of proinflammatory cytokines and survival pathways of granulocytes. Upon apoptosis, neutrophils also express soluble mediators that attract phagocytes and eat me signals that allow the identification of the dying cell to be engulfed [10, 18]. This event, unfortunately, will dampen the immune system that leads to patient susceptibility to 2nd infection such as pneumonia and ARDS, which in some cases will progress to lung fibrosis or post-COVID interstitial lung disease [10, 19, 20].

MSC has been widely known to have immunomodulatory ability due to the presences of the set of bioactive factors they produce. These bioactive substances or secretome include soluble proteins, free nucleic acids, lipids and extracellular vesicles are known to play an important role in the regulation of various physiological and biological processes such as cell communication, tissue development, homeostasis, and cell regeneration [21, 22].

MSC also releases extracellular vesicles (EV) that are involved in their immune regulatory actions. This EV has been shown to deliver protein, lipid and nucleic acid content from cell to cell as intercellular communication and translated into proteins resulting in an alteration of target cell behavior [23]. Several studies indicated that EV from MSC has anti-apoptotic properties and has potential to increase the regeneration of the tissue [24–26].

In this case report, 3 patients which has been tested negative after infected by SARS-CoV-2, develop lung fibrosis and have common symptoms such as difficulty to breathe, weak, low oxygen saturation, and opacity on the lung, MSC Secretome then administered intramuscularly to give the sustained release and prolonged the effect from the soluble factors. After 14 days of secretome injection regimen, the opacity on the lung is reduced

as well as the inflammation markers. Oxygen saturation becomes normal and no more symptoms in 3–4 weeks after secretome injection.

In mice model of idiopathic pulmonary fibrosis, MSC soluble factors can reduce collagen deposition by reducing collagen I and SMA-α, which was mainly deposited in the fibrotic area of the lung after 12–14 weeks of injection. These soluble factors also can reduce inflammation by reducing inflammatory cells like neutrophil and lymphocytes [27].

In wound healing model in mice, exosomes secreted by umbilical cord MSC can reduce scar formation caused by myofibroblast aggregations and myofibroblast accumulation by inhibition of transforming growth factor-b2/SMAD2 pathway in a dependent manner. Overexpression of this pathway has been associated with excessive scarring and tissue fibrosis [28]. Another study in a liver fibrosis model in mice, exosome from adipose-derived MSC can increase autophagy activation and reduce TGF-β1-induced liver fibrosis by inhibiting the STAT3/Bcl-2/Beclin 1 pathway [29].

5 Conclusion

Secretome has been known for having regenerative ability, in this case study we found that not only does secretome can reduce inflammation, these soluble factors are also capable of reducing lung fibrosis. Even though the full mechanism is not yet revealed, it is worth noting that secretome has potential as a new therapy for lung fibrosis and further research need to be conducted to find the full mechanism.

Author Disclosure. e authors declare that they have no competing financial interest and the protocol has followed WMA Declaration Of Helsinki regarding human subject. All authors contributed to the content, drafting, and critical review of the manuscript.

References

1. H. Zhu, L. Wei, P. Niu, "The novel coronavirus outbreak in Wuhan, China," Glob Health Res Policy, vol. 5, no.6, 2020.
2. D. Sharma, F. Zhao, "Updates on clinical trials evaluating the regenerative potential of allogeneic mesenchymal stem cells in COVID-19," NPJ Regen Med, vol. 6, no. 1, pp. 37, 2021.
3. C.R. Harrell, B.P. Jovicic, V. Djonov, V. Volarevic, "Therapeutic Potential of Mesenchymal Stem Cells and Their Secretome in the Treatment of SARS-CoV-2-Induced Acute Respiratory Distress Syndrome," Anal Cell Pathol (Amst). 2020:1939768.
4. Y. Chen, Q. Liu, D. Guo, "Emerging coronaviruses: Genome structure, replication, and pathogenesis," J Med Virol, vol. 92, no. 10, pp. 2249, 2020.
5. A. Klimczak, "Perspectives on mesenchymal stem/progenitor cells and their derivatives as potential therapies for lung damage caused by COVID-19," World J Stem Cells, vol. 12, no. 9, pp. 1013-1022, 2020.
6. R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E.C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, "Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding," Lancet, vol. 395, no. 10224, pp. 565-574, 2020.

7. I. Hamming, W. Timens, M.L. Bulthuis, A.T. Lely, G. Navis, H. van Goor, "Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis," J Pathol, vol. 203, no. 2, pp. 631-7, 2004.
8. M. Hoffmann, H. Kleine-Weber, N. Krüger, M. Müller, C. Drosten, S. Pöhlmann, "The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells," bioRxiv, vol. 2001, no. 2031, p. 929042, 2020.
9. M.J. Delano, P.A Ward, "The immune system's role in sepsis progression, resolution, and long-term outcome," Immunol Rev, vol. 274, no. 1, pp. 330-353, 2016.
10. M.A. Sugimoto, L.P. Sousa, V. Pinho, M. Perretti, M.M. Teixeira, "Resolution of Inflammation: What Controls Its Onset," Front Immunol, vol. 26, no. 7, pp. 160, 2016.
11. H. Shi, X. Han, N. Jiang, Y. Cao, O. Alwalid, J. Gu, Y. Fan, C. Zheng, "Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study," Lancet Infect Dis, vol. 20, no. 4, pp. 425-434, 2020.
12. S. Maxson, E.A. Lopez, D. Yoo, A. Danilkovitch-Miagkova, M.A. Leroux, "Concise review: role of mesenchymal stem cells in wound repair," Stem Cells Transl Med, vol. 1, no. 2, pp. 142-9, 2012.
13. A. Mahajan, S. Bhattacharyya, "A brief review on potential application of mesenchymal stem cell and secretome in combating mortality and morbidity in COVID-19 patients," Biomed J, vol. 44, no. 1, pp. 63-73, 2021.
14. J.W. Lee, A. Krasnodembskaya, D.H. McKenna, Y. Song, J. Abbott, M.A. Matthay, "Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria," Am J Respir Crit Care Med, vol. 187, no. 7, pp. 751-60, 20113.
15. C. De Bruyn, M. Najar, G. Raicevic, N. Meuleman, K. Pieters, B. Stamatopoulos, A. Delforge, D. Bron, L. Lagneaux, "A rapid, simple, and reproducible method for the isolation of mesenchymal stromal cells from Wharton's jelly without enzymatic treatment." Stem Cells Dev, vol. 20, no. 3, pp. 547-57, 2011.
16. Waqas Ullah, Nishanth Thalambedu, Shujaul Haq, Rehan Saeed, Shristi Khanal, Shafaq Tariq, Sohaib Roomi, John Madara, Margot Boigon, Donald C. Haas & David L. Fischman, "Predictability of CRP and D-Dimer levels for in-hospital outcomes and mortality of COVID-19," Journal of Community Hospital Internal Medicine Perspectives, vol. 10, no. 5, pp. 402-408, 2020.
17. B. Hu, S. Huang, L. Yin, "The cytokine storm and COVID-19," J Med Virol, vol. 93, no. 1, pp. 250-256, 2021.
18. K.S. Ravichandran, "Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums," J Exp Med, vol. 207, no. 9, pp. 1807-17, 2010.
19. N.S. Ward, B. Casserly, A. Ayala, "The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients," Clin Chest Med, vol. 29, no. 4, pp. 617-25, 2008.
20. Z.F. Udwadia, P.A. Koul, L. Richeldi, "Post-COVID lung fibrosis: The tsunami that will follow the earthquake," Lung India, vol. 38, no. 1, pp. 41-S47, 2021.
21. F.J. Vizoso, N. Eiro, S. Cid, J. Schneider, R. Perez-Fernandez, "Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine," Int J Mol Sci, vol. 18, no. 9, pp. 1852, 2017.
22. G. Maguire, P. Friedman, D. McCarthy, R. Friedman, A. Maniotis, "Stem cell released molecules and exosomes in tissue engineering," Proc. Eng, vol. 59, pp. 270–278, 2013.
23. S. Bruno, M.C. Deregibus, G. Camussi, "The secretome of mesenchymal stromal cells: role of extracellular vesicles in immunomodulation," Immunology letters, vol. 168, no. 2, pp. 154-8, 2015.
24. B. Zhang, Y. Shi, A. Gong, Z. Pan, H. Shi, H. Yang, H. Fu, Y. Yan, X. Zhang, M. Wang, W. Zhu, H. Qian, W. Xu, "HucMSC Exosome-Delivered 14-3-3ζ Orchestrates Self-Control of the Wnt Response via Modulation of YAP During Cutaneous Regeneration," Stem Cells, vol. 34, no. 10, pp. 2485-2500, 2016.

25. H. Wang, R. Zheng, Q. Chen, J. Shao, J. Yu, S. Hu, "Mesenchymal stem cells microvesicles stabilize endothelial barrier function partly mediated by hepatocyte growth factor (HGF)," Stem Cell Res Ther, vol. 8, no. 1, pp. 211, 2017.
26. Z.Z. Jiang, Y.M. Liu, X. Niu, J.Y. Yin, B. Hu, S.C. Guo, Y. Fan, Y. Wang, N.S. Wang, "Exosomes secreted by human urine-derived stem cells could prevent kidney complications from type I diabetes in rats," Stem Cell Res Ther, vol. 6, no. 7, pp. 24, 2017.
27. M. Choi, T. Ban, T. Rhim, "Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell," Mol Cells, vol. 37, no. 2, pp. 133-9, 2014.
28. S. Fang, C. Xu, Y. Zhang, C. Xue, C. Yang, H. Bi, X. Qian, M. Wu, K. Ji, Y. Zhao, Y. Wang, H. Liu, X. Xing, "Umbilical Cord-Derived Mesenchymal Stem Cell-Derived Exosomal MicroRNAs Suppress Myofibroblast Differentiation by Inhibiting the Transforming Growth Factor-β/SMAD2 Pathway During Wound Healing," Stem Cells Transl Med, vol. 5, no. 10, pp. 1425-1439, 2016.
29. Y. Qu, Q. Zhang, X. Cai, F. Li, Z. Ma, M. Xu, L. Lu, "Exosomes derived from miR-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagy activation," J Cell Mol Med, vol. 21, no. 10, pp. 2491-2502, 2017.