Cell therapy in patients with COVID-19 using Wharton’s jelly mesenchymal stem cells: a phase 1 clinical trial

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Abstract

Background: Mesenchymal stem cells (MSCs) have received particular attention because of their ability to modulate the immune system and inhibit inflammation caused by cytokine storms due to SARS-CoV-2. New alternative therapies may reduce mortality rates in patients with COVID-19. This study aimed to assess the safety and efficacy of injecting intravenous Wharton’s jelly-derived MSCs in patients with COVID-19 as a treatment.

Methods: In this study, five patients with severe COVID-19 were treated with Wharton’s jelly-derived mesenchymal stem cells (150 × 106 cells per injection). These patients were subject to three intravenous injections 3 days apart, and monitoring was done on days 0, 3, 6, and 14 in routine tests, inflammatory cytokines, and flow cytometry of CD4 and CD8 markers. A lung CT scan was performed on base and days 14 and 28. In addition, IgM and IgG antibodies against SARS-CoV-2 were measured before and after treatment.

Results: The results showed that IL-10 and SDF-1 increased after cell therapy, but VEGF, TGF-β, IFN-γ, IL-6, and TNFα decreased. Routine hematology tests, myocardial enzyme tests, biochemical tests, and inflammation tests were performed for all patients before and after cell therapy on base and days 3, 6, and 14, which indicated the improvement of test results over time. COVID-19 antibody tests rose in 14 days after WJ-MSC injection. The total score of zonal involvement in both lungs was improved.

Conclusions: In patients, the trend of tests was generally improving, and we experienced a reduction in inflammation. No serious complications were observed in patients except the headache in one of them, which was resolved without medication. In this study, we found that patients with severe COVID-19 in the inflammatory phase respond better to cell therapy. More extensive clinical trials should be performed in this regard.

Trial registration: IRCT, IRCT20190717044241N2. Registered April 22, 2020.

Keywords: COVID-19, WJ-MSC, Cell therapy

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**Introduction**

Coronavirus disease 2019 (COVID-19) has spread worldwide and was first detected in Wuhan, China, in December 2019 [1]. The virus has been widely distributed in different geographical areas [2]. SARS-CoV-2 primarily involves the respiratory system in addition to affecting other organs. Symptoms associated with lower respiratory tract infections, including fever, dry cough, and shortness of breath, have been reported in early case series isolated from Wuhan, China [3]. Signs such as headache, dizziness, general weakness, vomiting, and diarrhea were also observed [4]. According to 2020 guidelines of the WHO, severe coronavirus disease is defined as follows: an adolescent or adult with clinical signs of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of the following signs: respiratory rate > 30 breaths per minute; severe respiratory distress; or SpO2 < 90% in ambient air [5].

At present, there is no specific drug to treat COVID19. The pathogenesis of HcoV19 is mediated through the detection of the ACE2 receptor by the spike protein of this virus [6–8]. Unfortunately, the ACE2 receptor is abundantly present in human cells, especially alveolar type II (ATII) and capillary endothelium. Immune cells such as B and T lymphocytes as well as macrophages in the bone marrow, lymph nodes, thymus, and spleen are negative for ACE2 [9].

Up to now, adjuvant therapeutic strategies such as corticosteroid-mediated reduction of inflammation, treatment of congestion using plasma, administration of antibiotics to treat secondary bacterial infections, and antivirals, as well as antiviral therapies, plasma exchange, corticosteroids, and so on. Unfortunately, the patient’s condition exacerbated after a few days, during which the treatment team used WJ-MSC cells for the patient. This study found that WJ-MSC cells could be an ideal and practical option for treating COVID19 patients [32]. The immunomodulatory effects of MSCs are triggered via activation of TLR receptor on MSC, which is stimulated by pathogen-related molecules such as LPS or double-stranded RNA of viruses [33, 34] like HCOV19 [13]. MSCs secrete paracrine factors, including keratinocyte growth factor (KGF), angiopoietin-1 (Ang-1), prostaglandin E2 (PGE2), interleukin 10 (IL-10), and other tropic cytokines. These paracrine factors can increase alveolar fluid clearance, regulate epithelial and endothelial permeability of the lung, facilitate endothelial repair, and reduce inflammation [35]. MSCs can also release significant quantities of extracellular vesicles (EVs) that encapsulate cytokines, growth factors, signaling lipids, mRNAs, and functional microRNAs [36]. EVs are involved in cell-to-cell communication, cellular signal transduction, and metabolism, as well as local and long-distance immune modulation [37]. In this study, due to the low immunogenicity, easy accessibility, and unique capacity of WJ-MSCs to modulate the immune system to prevent cytokine storm and inflammation caused by SARS-CoV-2, the therapeutic potential of Wharton’s jelly mesenchymal stem cells was used for patients with COVOD-19.

**Materials and methods**

**Patients and study design**

This pilot study was performed in Shariati Hospital of Tehran and was approved by the Ethics Committee of
cells (150 × 10^6 cells) up to passage five were used for adherent ones proliferated. In the next step, 80% confluence was reached after 7 days of growth, and the cells were transferred to another flask. Finally, cultured in a 75-cm^2 flask and digested by enzyme cocktails, and then a MSC cell culture medium was added to them. The flasks were incubated at 37 °C for 2–3 days without shaking, and the tissue fragments were allowed to adhere. Non-adherent cells were washed away, but adherent ones proliferated. In the next step, 80% confluence was reached after 7–10 days of growth, and the cells were transferred to another flask. Finally, cultured cells (150 × 10^6 cells) up to passage five were used for each injection by transfer bags in GMP conditions together with heparin and human albumin serum. Confirmatory tests, including flow cytometry (CD73, CD34, CD90) and multiple lineage differentiation of MSCs (into fat, bone, and cartilage), were conducted according to the international society of cell therapy (ISCT). 150 × 10^6 cells were considered for each patient, injected intravenously (cephalic and basilic veins) three times a week for 3 days, namely days 0, 3, and 6 for 15–20 min. Before injection, 100 mg of hydrocortisone was injected into patients to prevent complications such as allergies. Patients were then monitored on days 0, 3, 6, and 14 for routine tests, inflammatory cytokines, and flow cytometry for CD4 and CD8 markers.

All the patients were assessed for adverse events through clinical examinations, measurement of vital signs, and routine tests. Moreover, on days 0, 3, and 6, vital signs (heart rate, respiration, blood pressure, body temperature, oxygen saturation) were recorded during the injection and 1 h after it. Routine hematological parameters, myocardial enzymes, biochemistry, and inflammatory tests were performed before and after cell therapy.

SDF-1, IL-10, VEGF, TGF-β, IFN-γ, IL-6, and TNFα levels were measured in serum samples on days 0, 3, 6, and 14 (ELISA R&D, USA). COVID-19 IgM and IgG antibodies were measured the day before treatment and 14 days after it (Ideal Tashkhis Atieh, Iran).

**Flow cytometry procedure**

Peripheral blood CD4 and CD8 markers were assessed by flow cytometry. Briefly, 100 μl of whole blood was poured into three separate test tubes, each containing 10 μl of Anti-Hu CD4 PE (Exbio, Czech Republic), CD8 FITC (Exbio, Czech Republic), and Anti-Hu antibodies. CD45 PerCP (Cytognos, Spain) was mixed well and incubated at room temperature for 30 min. The red blood cells were lysed using RBC lysis buffer solution (AP-RAD, Iran) for 5 min at 300g. The supernatant was then discarded, and the cells were suspended with 0.3–0.5 ml PBS. The samples were immediately read using flow cytometry (Sysmex Partec Pas III, Germany).

**Statistical analysis**

Some frequency tables and graphs were used to describe the individual data. The data were analyzed using the statistical package IBM SPSS version 26.0 (Statistical Package for the Social Sciences, Chicago, IL). The categorical variables are expressed as proportions and frequencies. Kolmogorov-Smirnov test was applied to test the normality distribution. To explore the independent nature of some categorical variables, a chi-square test was used. The comparison of the means between two related groups was made by paired T-test or Wilcoxon
signed ranks. Generalized estimation equation (GEE) analysis was applied to test the effect of time on longitudinal data.

**Results**

In this research, patients underwent cell therapy using WJ-MSC. All patients needed oxygen masks, and four of them were admitted to ICU, and one patient remained out of ICU. All patients received common treatments, including heparin and dexamethasone. Preliminary characteristics of patients such as age, sex, weight, initial baseline symptoms, duration of symptoms before hospitalization and first cell injection, ICU hospitalization period following cell injection, comorbidities, disease severity, and common drugs are listed in Table 1. All the patients were monitored for vital signs such as pulse, respiration, heart rate, blood pressure, SO2, and fever at the time of injection and 1 h later. In this study, no serious complication associated with WJ-MSC stem cells was observed. Only patient no. 3 had a slight post-injection headache that resolved without any drug after half an hour. The above statements indicate that WJ-MSC is safe and tolerable for the patient. There was zero mortality rate within the first 28 days.

Flow cytometry analysis was performed on patient samples before and after cell therapy with WJ-MSC, and the upward trend of CD4 and CD8 markers is shown in Fig. 1, in which the improvement of lymphocyte population can be observed (more details of these markers are listed in the supplementary data file, i.e., Fig. S1a & b). In this study, the percentage of lymphocytes, absolute lymphocyte count, and CD4 and CD8 T cell ratio increased, indicating the improvement in immune system function after cell therapy with WJ-MSC (Table 2). Routine hematology tests (WBC, Hb, PLT, neutrophil and lymphocyte percentage, absolute lymphocyte count, ESR, and D-dimer), myocardial enzyme tests (CPK, LDH, and Troponin I), biochemical tests (ALT, AST, Cr, BUN, total and direct bilirubin, K, Na, and ferritin), and inflammation tests (ESR, CRP, and procalcitonin) were conducted for all patients before and after cell therapy on base and days 3, 6, and 14, which are shown in Table 2. The results indicated the improvement of test results over time. We statistically examined four of these tests that are more important for five COVID-19 patients of our study, including LDH, CRP, lymph count, and ferritin, among which ferritin showed a significant decrease (supplementary data, Fig. S2).

In this study, the trend of oxygen saturation percentage among patients over MSC injection days (admission day, days 0, 3, and 6; supplementary data, Fig. S3-a), at the time of injection, and 15, 30, 45, and 60 min after it is shown in Fig. 3-b (supplementary data).

In this research, we examined inflammatory cytokines, angiogenesis, and implantation in the days before and after therapy. The results indicated the improvement of test results over time. We statistically examined four of these tests that are more important for five COVID-19 patients of our study, including LDH, CRP, lymph count, and ferritin, among which ferritin showed a significant decrease (supplementary data, Fig. S2).

**Table 1** Demographic data

| Patient ID | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|------------|-----------|-----------|-----------|-----------|-----------|
| Gender     | Female    | Female    | Male      | Male      | Male      |
| Age        | 51        | 51        | 54        | 45        | 53        |
| Weight     | 94        | 70        | 88        | 95        | 102       |
| Initial vital signs (base) | | | | | |
| RR breaths/min | 22 | 28 | 42 | 36 | 28 |
| PR beats/min | 71 | 92 | 89 | 66 | 92 |
| Sys BP, mmHg | 141 | 103 | 124 | 139 | 138 |
| Dias BP, mmHg | 76 | 71 | 89 | 90 | 97 |
| Temp > 37.3 °C (baseline) | 37 | 37.3 | 36.7 | 37.4 | 36.2 |
| SO2 (without oxygen mask) | 87 | 70 | 79 | 80 | 89 |
| Other      |           |           |           |           |           |
| COVID type | Sev       | Sev       | Sev       | Sev       | Sev       |
| Comorbidities | Fatty liver disease | HTN | Damaged lung | No | Diabetes |
| Interval between symptoms onset and admission (days) | 6 | 10 | 11 | 13 | 4 |
| Interval between symptoms onset and first cell injection (days) | 10 | 14 | 13 | 17 | 7 |
| Duration of hospitalization in ICU after cell injection | 7 | 5 | 0 | 6 | 0 |
| Conventional therapy | Hep/Dexa | Hep/Dexa | Hep/Dexa | Hep/Dexa | Hep/Dexa/Ataz |
| RT-PCR at admission | + | + | + | + | + |

Sev severe, HTN hypertension, Hep heparin, Dexa dexamethasone, Ataz atazanavir, RT-PCR reverse transcription polymerase chain reaction
after cell therapy, as shown in Fig. 2. The results show that over 14 days before and after cell therapy, SDF-1 and IL-10 levels increased, but VEGF, TGF-β, IFN-γ, IL-6, and TNFα decreased.

COVID-19 antibody test in the days before treatment and 14 days after it are shown in Fig. S4 (Supplementary data, Fig. S4).

CT scan
Table 3 lists CT scan results of parenchymal abnormalities, type of GGO opacities, zonal involvement, total score, and cardiomegaly. None of the patients had pleural effusion. One patient showed mild (patient no. 2) pericardial effusion before cell injection and 14 days after it but was free of pericardial effusion on day 28. Mild P4 emphysema was observed on days 14 and 28. None of the patients showed pulmonary fibrosis. Patient no. 5 showed mild bronchiectasis the day before cell therapy and at day 14. All patients (except for patient no. 3) showed mild to moderate cardiomegaly. In patient no. 1, the mosaicism pattern was completely resorbed on day 28, patient P2 showed severe mosaicism on day 28, patient no. 3 had moderate mosaicism on day 14 and mild mosaicism on day 28, and finally, patient no. 4 showed severe mosaicism on days 14 and 28. Patient no. 5 had no mosaicism. Chest computerized tomography (CT) images of the two COVID-19 patients are demonstrated in Fig. 3a, b.

Discussion
Most patients show a tolerant response to COVID-19 infection. When the virus enters the lungs, immune system cells are recalled to the infected area to defend the body against the virus and elicit an immune response [39]. In some cases, the increase in cytokines secreted by this response leads to cytokine storm, followed by inflammation, tissue damage, secondary infection, and ARDS, leading to death [40, 41]. Therefore, inhibition of cytokine storms in patients with COVID-19 can be crucial in treating these patients [24].

Studies have revealed that mesenchymal stem cells are involved in improving lymphocyte populations mainly through dendritic cells and shifting immune system cells’ response from Th1 to Th2 [42]. TCD8 cells are of high importance in killing virus-infected cells [43]. Various investigations have indicated that in patients with severe COVID-19, both TCD4 and TCD8 are reduced [45]. In our study, both TCD4 and TCD8 cells were recovered after cell therapy. A clinical trial was conducted in China on seven patients with COVID-19 pneumonia injected with mesenchymal stem cells. Patients who showed no improvement in symptoms compared to conventional treatment were recruited [13]. MSCs inhibit the overactivation immune system and promote endogenous repair by improving the microenvironment. In this study, it was stated that after IV injection of MSCs, these cells accumulated in the lungs and could improve the pulmonary microenvironment, prevent pulmonary fibrosis, and improve lung function [13]. According to Cao team reports, serum levels of IL-2, IL-7, GCSF, IP10, MCP1, MIP1A, and TNFα were considerably higher in ICU patients compared to ordinary people [12]. This research showed that IV injection of MSCs improves inflammation in patients with severe COVID-19. Besides, increasing IL-10 and VEGF promotes lung repair [13]. In a study by Lanzoni et al., the safety and, to some extent, the efficacy of hUC-MSC cells in ARDS patients induced by COVID-19 was evaluated. Patients received 100 ± 20 × 10^6 cells in two doses intravenously. They took drugs such as remdesivir,
| Variables                  | Normal Range       | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|---------------------------|--------------------|-----------|-----------|-----------|-----------|-----------|
| **Routine blood tests**   |                    |           |           |           |           |           |
| WBC count (×10^9/L)       | 3400–12,500        | 10940     | 14910     | 16710     | 14220     | 9600      |
| Hb (g/L)                  | M: 14–18 F: 12–16 | 12.1      | 12.7      | 15.1      | 13.7      | 11.5      |
| PLT count (×10^9/L)       | 150,000–450,000    | 146       | 195       | 227       | 218       | 233       |
| Neutrophil (%)            | 45–75              | NA        | 88        | 88        | 70        | 90        |
| Lymphocyte (%)            | 20–40              | NA        | 2         | 10        | 19        | 4         |
| LYM count (×10^3/L)       | 0.4–1.0            | 592       | 298.2     | 1671.8    | 2701.8    | 384       |
| ESR (mm/h)                | M: 0 to 20 F: 0 to 25 | 96       | 73        | 23        | 26        | 104       |
| **Myocardial enzymes**   |                    |           |           |           |           |           |
| CPK (U/L)                 | 5 to 25            | 21.5      | 38        | 10        | 168       | 161       |
| Troponin I (ng/ml)        | ≤0.1               | 0.1       | 1.4       | 1.4       | 0.1       | 0.3       |
| LDH (U/L)                 | 240–480            | 723       | 939       | 462       | 374       | 860       |
| **Biochemical indicators**|                    |           |           |           |           |           |
| Total Bilirubin (mg/dL)   | 0.1–1.2            | 0.26      | 0.7       | 1         | 0.6       | 1.1       |
| Direct Bilirubin (mg/dL)  | Up to 0.3          | 0.1       | 0.3       | 0.3       | 0.1       | 0.3       |
| ALT (U/L)                 | M: up to 41 F: up to 31 | 55      | 29        | 40        | 43        | 98        |
| AST (U/L)                 | M: up to 38 F: up to 31 | 23.5     | 20       | 25       | 20       | 68        |
| BUN (mmol/L)              | 7–20               | 6         | 23        | 24        | 16        | 23        |
| Cr (μmol/L)               | M: 0.7–1.4 F: 0.6–1.3 | 0.8      | 0.8       | 0.7       | 0.8       | 0.5       |
| K (mmol/L)                | 3.6–5.2            | 4.1       | 4.8       | 4.8       | NA        | 4.1       |
| Na (mEq/L)                | 135–145            | 139       | 137       | 138       | NA        | 144       |
| **Inflammatory markers**  |                    |           |           |           |           |           |
| CRP (mg/L)                | Up to 10           | 60.5      | 6         | 3         | 0.48      | 107       |
| Procalcitonin (ng/ml)     | <0.10              | 0.17      | 0.01      | 0.03      | 0.08      | 0.02      |
| Ferritin (ng/ml)          | M: 24 to 336 F: 11 to 307 | 1979    | 959       | 231       | 169       | 798       |

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corticosteroids, hydroxychloroquine, and tocilizumab together with the cells. In this study, they stated that hUC-MSC cells are safe in these patients and significantly reduce side effects and death and improve recovery time compared to the control group [46]. In another research by Hashemian et al., both PL-MSC and UC-MSC cells were used in ARDS patients. Patients received $200 \times 10^6$ cells at three doses through intravenous infusion with IVIG, ribavirin, and favipiravir. In these patients, factors such as IL-6, IL-8, TNF-$\alpha$, INF-$\gamma$, IL-4, IL-10, and CRP were also evaluated. This research stated that placental and umbilical cord stem cell injections are safe and can quickly improve respiratory symptoms reducing inflammatory factors in several patients [47].

**Fig. 2** Inflammatory cytokines. SDF-1 : stromal cell-derived factor 1, IL-10 : Interleukin 10, TGF-$\beta$1 : Transforming growth factor beta 1, IFN-$\gamma$ : Interferon gamma, TNF-$\alpha$ : Tumor necrosis factor alpha, IL-6 : Interleukin 6, VEGF : Vascular endothelial growth factor
| Parameters | Patient- 1 (WJ-MSC treatment) | Patient- 2 (WJ-MSC treatment) | Patient- 3 (WJ-MSC treatment) | Patient- 4 (WJ-MSC treatment) | Patient- 5 (WJ-MSC treatment) |
|------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|            | Base | Day 14 | Day 30 | Base | Day 14 | Day 30 | Base | Day 14 | Day 30 | Base | Day 14 | Day 30 |
| CT parenchymal abnormalities: | | | | | | | | | | | | |
| a- GGO | b | a, c | a | a, h, d, e | a, c, d, e | a, c | a, c | a, d | a | b | a, b, e | a | a, b, d, g | a, b, d | NA |
| b- Consolidation | | | | | | | | | | | | |
| c- Reticular pattern | | | | | | | | | | | | |
| d- Mixed pattern | | | | | | | | | | | | |
| e- Linear opacities | | | | | | | | | | | | |
| f- Multifocal GGO of rounded morphology | | | | | | | | | | | | |
| g- Reverse halo sign or other findings of organizing pneumonia | | | | | | | | | | | | |
| Type of GGO opacities: | | | | | | | | | | | | |
| a- Pure GGO | a | a | a | d | c, d | d | a | a | d | a | a, d | a, d | b | a | NA |
| b- Crazy paving: GGO + intralobular lines | | | | | | | | | | | | |
| c- Irregular lines and interfaces with architectural distortion + GGO | | | | | | | | | | | | |
| d- Streaky pattern | | | | | | | | | | | | |
| Zonal involvement: | | | | | | | | | | | | |
| a- Right upper: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | a (3) | a (2) | a (0) | a (3) | a (4) | a (3) | a (2) | a (3) | a (2) | a (4) | a (5) | a (1) | a (2) | a (5) | NA |
| b- Right middle: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | b (3) | b (2) | b (0) | b (4) | b (2) | b (2) | b (2) | b (3) | b (1) | b (4) | b (5) | b (2) | b (3) | b (5) | |
| c- Right lower: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | c (3) | c (1) | c (0) | c (4) | c (2) | c (2) | c (2) | c (3) | c (2) | c (4) | c (3) | c (2) | c (3) | c (5) | |
| d- Left upper: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | d (3) | d (2) | d (1) | d (3) | d (4) | d (3) | d (2) | d (3) | d (2) | d (4) | d (5) | d (1) | d (3) | d (5) | |
| e- Left middle: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | e (3) | e (1) | e (0) | e (4) | e (2) | e (2) | e (2) | e (3) | e (1) | e (4) | e (5) | e (2) | e (4) | e (5) | |
| f- Left lower: (0–5% = 1, 5–25% = 2, 26–50% = 3, 51–75% = 4, 76–100% = 5) | f (3) | f (2) | f (1) | f (4) | f (3) | f (2) | f (3) | f (3) | f (2) | f (4) | f (3) | f (2) | f (3) | f (5) | |
Table 3 CT evaluation (Continued)

| Parameters                                           | Patient- 1 (WJ-MSC treatment) | Patient- 2 (WJ-MSC treatment) | Patient- 3 (WJ-MSC treatment) | Patient- 4 (WJ-MSC treatment) | Patient- 5 (WJ-MSC treatment) |
|------------------------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                                      | Base  | Day 14 | Day 30 | Base  | Day 14 | Day 30 | Base  | Day 14 | Day 30 | Base  | Day 14 | Day 30 |
| Total score of zonal involvement in both lungs:      | 18    | 10     | 2      | 22    | 17     | 14     | 13    | 18     | 10     | 24    | 26     | 10     |
| Cardiomegaly: (Yes/No)                               | Yes   | Yes    | Yes    | Yes   | Yes    | Yes    | No    | No     | No     | Yes   | Yes    | Yes    |
| Severity (mild, moderate, severe)                     | (mild)| (mild) | (mild) | (mild)| (mild) | (mild) | (mild)| (mild)| (mild)| (mild)| (mild)| (mild)| (mild)|
| a. Highly suggested                                   | a     | b      | d      | a     | b      | c      | a     | a      | b      | a     | b      | b      |
| b. Indeterminate                                     |       |        |        |       |        |        |       |        |        |       |        |        |
| c. Inconsistent                                      |       |        |        |       |        |        |       |        |        |       |        |        |
| d. None                                              |       |        |        |       |        |        |       |        |        |       |        |        |

a. Highly suggested
b. Indeterminate
c. Inconsistent
d. None
SDF-1 factor is a cytokine that plays a crucial role in organ formation and repair after injury [48]. In a number of MSC implantation studies, it has been shown that increasing SDF-1 levels after tissue injury plays an essential role in the accumulation of mesenchymal stem cells at the site of injury [49–51]. Increased SDF-1 also increases CXCR4 expression on mesenchymal stem cells and plays a vital role in transplantation of these cells into damaged tissue [52]. Another study indicated that using SDF-1 along with WJ-MSC cells increased the migration of these cells in vitro [53]. VEGF is a significant factor in acute lung injury and ARDS, and the increase of that can be seen in acute inflammation and hypoxia [54, 55]. In addition to angiogenesis, VEGF increases vascular leakage and permeability [56, 57]. Studies have indicated that VEGF concentrations increase in patients with COVID19 admitted to the ICU and those outside it [3]. In our research, VEGF was gradually reduced after WJ-MSC cell therapy. In one of our patients (no. 1) in the days before cell therapy, there was a bloody sputum complaint, which may be due to increased level of VEGF and subsequently increased permeability of small pulmonary arteries. The rise in VEGF level was more prominent in this patient than in other patients, which resolved after receiving the first dose of cells within 24 h. Mesenchymal stem cell injections reduce TGF-β, IFN-γ, and TNFα, and related factors associated with pulmonary fibrosis were reduced, including TGF-β and IFN-γ. In this research, it seems that the time of cell injection in these patients is of high importance, and it can be stated that the optimal time for cell injection is the second week of the disease, namely the second phase of disease or hyper inflammation [60] beginning on days 7 to 15.

**Conclusions**

This study showed that the injection of Wharton’s jelly-derived stem cells was safe and well-tolerated by the patient. From our perspective, the timing of stem cell injections in patients with severe COVID19 is critical. It seems that it is better to inject these cells in the inflammatory phase, and for this purpose, we should check inflammatory tests for the patient before cell injection. Considering the behavior of mesenchymal stem cells, it seems that paying attention to the precise protocols of isolation, culture, proliferation, an appropriate number, manner, and time of proper injection into humans can be the beginning of a new treatment strategy in COVID19. Further studies should be conducted to prove the effective outcomes using control and treatment groups to indicate these significant differences. It is also necessary to increase the sample size and use randomization methods in further studies to indicate these cells’ positive function and improve the disease.
Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13287-021-02483-7.

Additional file 1: Figure S1. Flow cytometry. a. Patient 2, Lymphocyte regeneration, A. control, B. base (before WJ-MSC injection), C. day3, D.day6 and E. day 14 after WJ-MSC injection. b. Patient 4, Lymphocyte regeneration, A. control, B. base (before WJ-MSC injection), C. day3, D.day6 and E. day 14 after WJ-MSC injection. Figure S2. The result of the laboratory data. GEE analysis was also applied to show the effect of time on change of some main variables such as CRP, Lymph count, Ferritin and LDH. The results showed a significant change during time just for ferritin with p-value, 0.008. Figure S3. O2 Saturation. Generalized Estimating Equation (GEE) Analysis. GEE modeling was used to show the effect of day (0, 3 and 6) and time of injection (start, min15, min30, min45 and min60) on O2sat change. Baseline values were entered to the GEE model as a covariate variable. The overall mean O2sat during three days had a same trend but in return, injection time has a significant effect on O2sat as mean O2sat at start is different to other injection time (p-value=0.001). Mean O2sat at injection time is: 91.3, 92.3, 92.3, 92.5 and 93.1. a. Mean of O2sat during time. b. Change of O2sat by patients. Figure S4. SARS-CoV2 Abs. Paired Comparison Analysis. Mean SARS-CoV2 IgM and SARS-CoV2 IgG at baseline and end of study, were compared using Wilcoxon Signed-Ranks. The line charts related to both antibodies are shown in Fig.S4.

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Authors’ contributions
MS proposed the initial idea, study design, and writing of the manuscript. MS, AV, RA, AS, ZK, MSH, VS, BKH, SHA, NA, IS, and JV were responsible for the manuscript’s reference selection and writing. MS, NA, ZK, and RA took care of the patients and performed the follow-up checks. MS, NA, AV, and BHK collected and analyzed the data. MS, IS, IV, and NA contributed to the critical paper of the manuscript. MS and LA analyzed the CT. All authors read and approved the final manuscript.

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Availability of data and materials
All of the data generated and analyzed during this study are included in our manuscript.

Declarations
Ethics approval and consent to participate
Written informed consent was obtained from each patient or the patient’s legally authorized surrogate before the conduct of study-specific procedures.

Consent for publication
Not relevant

Competing interests
The authors declare that they have no competing interests.

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