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CLINICAL STUDY

Mesenchymal stem cells treatment in COVID-19 patient with multi-organ involvement

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ABSTRACT

The aim of this study is to evaluate the therapeutic effect of mesenchymal stem cells (MSCs) in a severe case of brain and multiple organ involvement in a patient with COVID-19. Here, a 51-year-old male patient with multi-organ involvement due to COVID-19 infection and developing cardiac arrest is presented. MSCs were transplanted to the patient four times systematically and once intrathecally. As a result, the application of MSCs has been found to have a healing effect on organs in this patient with severe COVID-19 infection. In addition, transplantation of MSCs both systematically and intrathecally is considered to be effective in the treatment of the central nervous system *(Tab. 2, Fig. 2, Ref. 24)*. Text in PDF *www.elis.sk*

KEY WORDS: mesenchymal stem cell, COVID-19, organ involvement.

Introduction

Clinical symptoms of COVID-19 infection include fever, cough, and myalgia or fatigue with pneumonia demonstrated on chest CT scan imaging (1). The clinical situation of patients varies from mild fever to acute respiratory distress syndrome (ARDS) and death (2, 3). Patients with severe disease were likely to display neurologic symptoms such as acute cerebrovascular diseases, impaired consciousness, loss of smell and taste, and skeletal muscle injury (2, 4).

Mesenchymal stem cells (MSCs) have been widely used in cell-based therapy, from basic research to clinical trials (5, 6, 7). Wharton's jelly mesenchymal stem cells (WJ-MSCs) have distinct advantages of being abundant, easy to obtain with minimal invasiveness, and readily cultured in a sufficient number for transplantation without ethical issues of allografting (8). The aim of the case report is to determine WJ-MSCs treatment's efficacy in a severe case of multiple organ failure and brain involvement in a patient with COVID-19 infection. In addition, another aim of the study is to get to understand the way in which MSCs are given, especially in patients with brain involvement.

Case report

This study was undertaken in the Bakirkoy Dr. Sadi Konuk Education and Training Hospital at Health Science University and Istinye University with the permission of the Ministry of Health. The study protocol was approved by the Ethical Committee. The informed consent was obtained from the patient's relatives.

A 51-year-old male patient started to complain of cough, myalgia, high fever (39.5 °C), and diarrhea on March 11, 2020. Upon the progression of symptoms, the patient was hospitalized in the infection service on March 16. Thorax tomography was performed and throat swab was taken. Radiological involvement related to bilateral COVID-19 infection was observed in both lungs (Fig. 1A). The patient had no previous chronic illnesses or additional pathologies in his medical records. A swab was taken, and the diagnosis of COVID-19 was made using the PCR method. A supportive therapy was initiated. Upon progression of respiratory distress during his daily follow-up, the patient was transferred to the intensive care unit (ICU).

During the ICU follow-up, the patient was intubated oro-tracheally due to high fever; DSS: 33-40 breaths per minute (bpm), low O_2 saturation, and gradually increasing respiratory distress. Due to hypoxemia in arterial blood gas (ABG) values and deterioration in P/F (Horowitz) values (< 150), the patient was placed in a prone position immediately after intubation. Methylpredniso-

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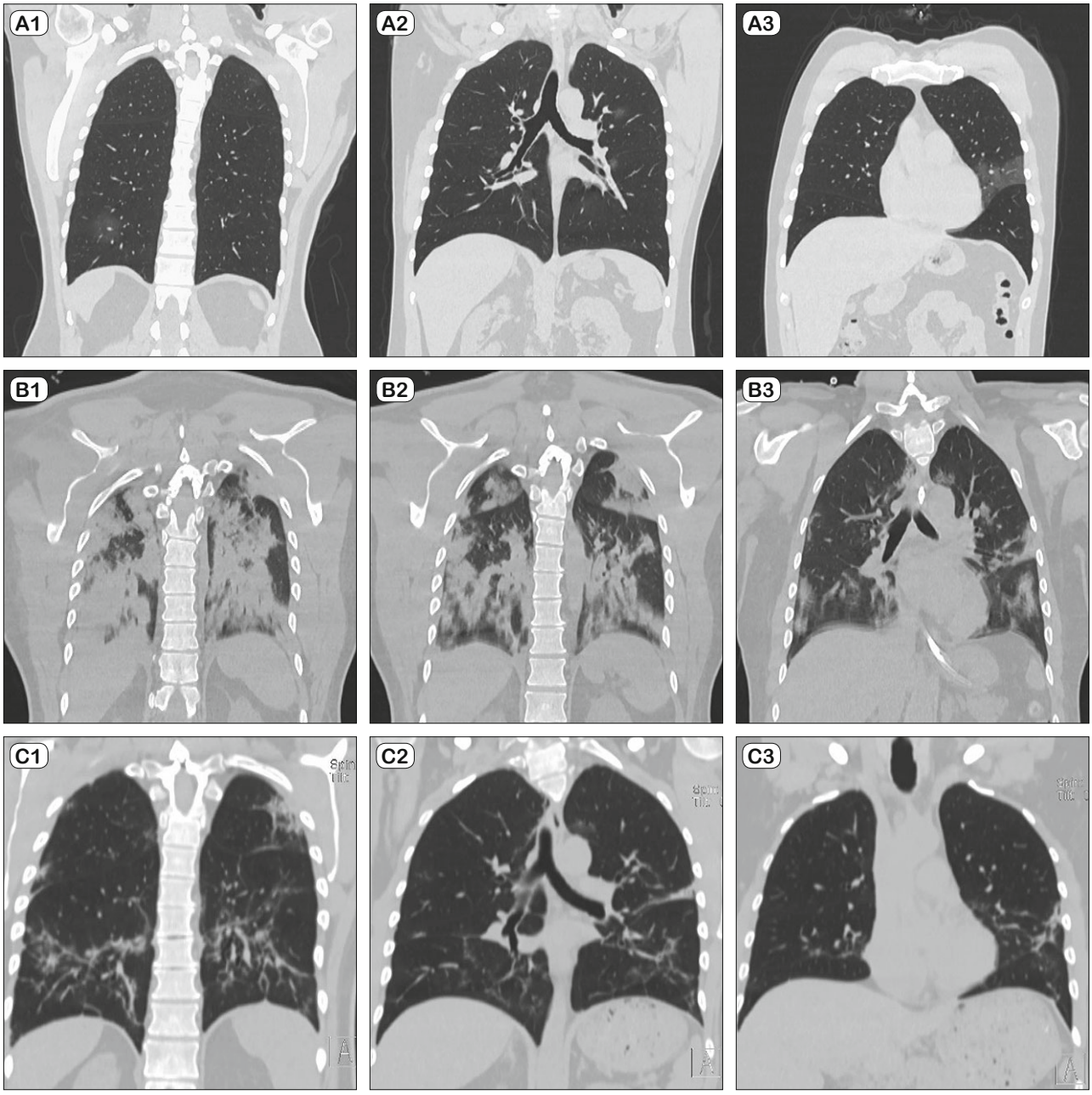

Fig. 1. Radiological evaluation of patient lungs. A1–3: focal ground-glass opacity in the lower part of the lung observed on day 5 after first symptom onset. B 1–3:on day 10 day after first symptom onset, it is observed that lesions increase in both upper and lower lung lobes. It is seen that the lesions are converted into consolidation form and merge. C 1–3: on day 25 after first symptom onset, interlobular and intralobular septal thickening is observed in parenchyma of both lungs. In his previous film, the common consolidation areas and patched ground-glass densities are observed to be resorbed.

lone was added to the treatment of the patient for 5 days. In the prone position, CPR was applied to the patient who developed sudden cardiac arrest for 10 minutes. Two hours after the arrest, a targeted temperature management was started. Body temperature was adjusted to 33 degrees. It was sedated to be between 20 and 40 according to patient state index. The antiviral treatment of the patient was changed to Avigan. The patient was thought to have a cytokine storm. On March 25, the patient was treated with tocilizumab (IL-6 antagonist) for 2 days.

Echocardiography performed after cardiac arrest revealed a global dyskinesia compatible with myocarditis, and ejection fraction (EF) of 25 % with severe apical akinesia. The patient deve-

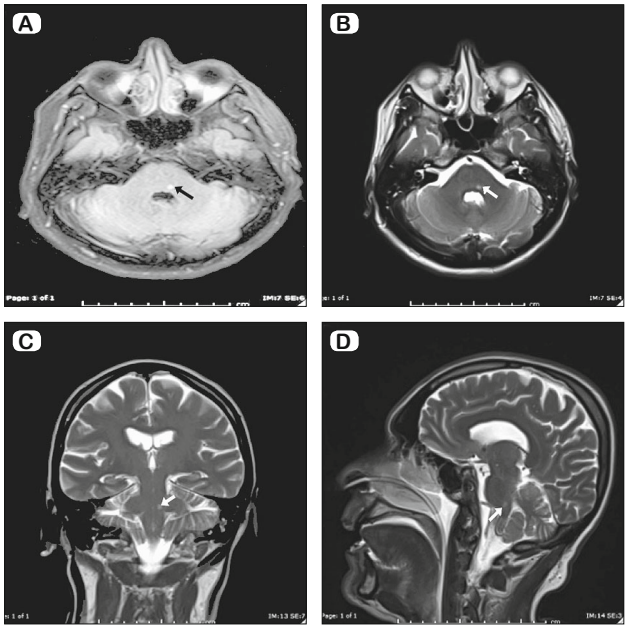

Fig. 2. Radiological evaluation of the patient brain. A-D Focal intensity increased in T2-weighted levels in the posterior side of the pons. The lesion is marked with an arrow.

loped a severe bilateral pneumonia (Fig. 1B), ARDS, and multiple organ failure, while no response was received.

The patient did not wake up and did not show any clinical improvement, even though his medical sedations were closed after the entire treatment period. On monitorization, delta waves were observed, and contrast enhanced cranial MR and CT were taken. Hyperintense signal change in T2A-FLAIR sequence in pons was observed in cranial MR (Fig. 2). After the general condition of the patient had not improved as a result of treatment, it was decided by the medical committee to perform MSC transplantation. WJ-MSCs were transplanted to the patient four times intravenously (IV). No side effects were observed in the patient. After stem cell transplantation, the patient underwent control echocardiography. Cardiac activity and myocardial structure were found to be normal. EF was evaluated as 60 %.

On April 07, 2020, the patient was extubated with high-flow nasal cannula. Upon neurological examination, the patient was observed to be orientated and cooperative. After 5 days of follow-up, the patient was taken to medical service. The patient was diagnosed with upper gastrointestinal bleeding. After the patient's anti-thromboembolic treatment had been discontinued, his vital signs were stable, and it was decided to follow him up at the clinic. The patient was discharged from hospital on April 20, 2020 as symptom-free and with no complaints. Information about the patient's laboratory examination results are given in Table 1.

Drug treatment of the patient was made on the basis of international literature and guideline prepared by the scientific committee related to this issue and affiliated to the ministry of health. The MSCs were slowly drawn into the syringe without pressure, suspended in 250 ml of 0.9 % NaCl, and then given intravenously over 1 hour (Tab. 2).

The levels of alanine transaminase, aspartate transaminase, total protein, albumin, total bilirubin, direct bilirubin, ferritin, triglycerides, D-dimer, troponin I, myoglobin, procalcitonin, ammonia, c-reactive protein, pro B-type natriuretic peptide, creatine kinase, and alkaline phosphatase were determined in venous blood samples using Beckman Coulter AU5800 analyzer (Beckman Coulter, Brea, CA, USA). The complete blood count was analyzed with ADVIA 2120i autoanalyzer (Siemens Healthcare Diagnostics, Erlangen, Germany).

For coagulation assay, all analytical procedures were carried out on a random-access coagulation analyzer (Beijing Succeeder Technology Inc. China).

Flow cytometric analyses were performed with Navios cytometer (BECLS)-Kaluza Software. Whole blood was stained with anti-human FITC-CD45, PE-CD4, ECD-CD8, PE-CY5.5-CD3 (Beckman Coulter, Brea, California) antibodies.

All samples of WJ-MSCs as cell therapy medicinal products were isolated, expanded, and analyzed in cGMP-certified facility at Liv Hospital Center for Regenerative Medicine and Stem Cell Manufacturing (LivMedCell). Human WJ-MSCs were prepared and tested as described in our previous clinical trials (8, 9, 10).

Cryopreserved vials from each donor were thawed and mixed in the same tubes before seeded at a cell density of 4,000 cells/cm^2. After harvest at the fourth passage, quality control tests were performed, such as flow cytometry analysis, endotoxin, rapid microbiological and sterility tests. The final product was prepared as 3×10^6 cells/kg/dose of allogeneic WJ-MSCs pooled from three donors.

Discussion

The central nervous system (CNS) is not immune to alterations that lead to neurological disease resulting from acute, persistent or latent viral infections (11). In some circumstances, opportunistic viral pathogens such as human corona viruses can avoid the immune response and cause more severe respiratory diseases or even spread to other tissues including the CNS (12). The detection of HCoV-RNA in human brain samples clearly demonstrates that these respiratory pathogens are naturally neuroinvasive in humans, and suggests that they establish a persistent infection in human CNS (13). On March 4, 2020, researchers from Beijing Ditan Hospital, China, first described a confirmed patient with 2019-nCoV, whose cerebrospinal fluid (CSF) tested positive for 2019-nCoV by gene sequencing, suggesting a need to consider direct infection when patients with 2019-nCoV present with neurological disorders (14).

In this study, MSCs transplantation was successfully performed both systemically and intrathecally. Here, after the first 2 transplants had been given systematically, the patient was awakened and extubated. Later, the patient who developed neurological symptoms was reintubated. After that, the transplantation of MSCs was performed systematically for the third time, as well as an intrathecal stem cell transplantation. We thought the blood brain barrier (BBB) was an obstacle here. Neurologically, the dia-

Tab. 1. Treatment days and laboratory findings are given; * stem cell transplantation, ** tocilizumab administration, * steroid treatment.**

Daily follow-up	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
				*													
				**	**	***	*	***		*			*				
				***	***		***										
AST (U/L)	**115**	**489**	**344**	**200**	**75**	**76**	**72**	**75**	**113**	**51**	28	40	35	28	23	32	39
ALT (IU/L)	**110**	**369**	**551**	**551**	**325**	**257**	**245**	**263**	**160**	**109**	**110**	**98**	**76**	**55**	**52**	**91**	**67**
LDH (U/L)	**641**		**1.144**	**750**	**857**	**522**		**507**		**603**	**449**	**407**	**366**	**324**	**315**	**347**	202
CK (U/L)	**1.320**	**1.132**		**211**	**171**	**243**		**210**		**279**	100	72	28	91	57	151	**298**
CRP (mg/L)	**133**	**69**	**50**	**21**	**12**	**8**			4	4.7	3	1.6	1		1.2	1	1
PCT (ng/mL)	0.57	0.25	0.13			0.06		0.06	0.04	0.03	0.03		0.03	0.04	0.03	0.05	0.05
D-dimer (µg/mL)		**0.71**			**2.05**		**1.49**		**2.75**	**5.71**	**3.13**		**1.19**		**1.21**	**0.64**	0.44
Pro-BNP (ng/L)				**14500**	**10700**			**4500**		**16400**			**3380**		**2290**	**1470**	**1150**
Myoglobin (ng/mL)						**149**						78	72		52	45	58
Troponin (pg/mL)		**578**	**677**	**313**		**507**	**507**			**1062**	**322**		**445**		**318**		**72**
Ferritin (µg/L)	**1.474**	**3.491**		**3.585**					**967**	**876**		**633**		**483**		**444**	333
Fibrinogen (mg/dL)					**462**					335	363	343	340	386	**442**	**429**	
Triglycerides (mg/dL)				**485**	**357**			**203**	**215**								
Lymphocytes count ($\times 10^9$/L)	**0.47**	**0.5**	**0.58**	**0.8**	1.31	1.89		1.72					1.79	2.3	2.3	1.69	22.49
Neutrophil count ($\times 10^9$/L)	3.28	**8.06**	**7.52**	**8.72**	**10.57**	**15.88**		**10.36**					**8.61**	6.23	3.95	2.77	**10.4**
WBC ($\times 10^9$/L)	4.2	10.1	9.5	**10.7**	**13**	**19.3**		**14.1**					**12.1**	**10.2**	7.6	5.8	**14.3**
Eosinophils ($\times 10^9$/L)	**0**	0.04	**0**	**0**	**0.01**	0.04		0.09					0.17	0.17	0.24	0.23	0.24
Basophils ($\times 10^9$/L)	**0.01**	0.04	0.05	0.04	0.09	0.11		0.04					0.05	0.05	0.05	0.04	0.05
Monocytes ($\times 10^9$/L)	0.47	0.48	**1.41**	**1.08**	**1.03**	**1.44**		**1.84**					**1.44**	**1.44**	**1.15**	**1.07**	**1.25**
Ammonia (µg/dL)					**128**	**130**		**114**			**105**	**98**		88	88		

AST – aspartate transaminase, ALT: alanine transaminase, LDH: lactate dehydrogenase, CK – creatinine kinase, CRP – c-reactive protein, PCT – procalcitonin, BNP – B-type natriuretic peptide

gnosis of brain involvement was demonstrated by MR and proved by COVID-19 antibodies in the spinal fluid. Upon examination of CSF samples, protein and IgG levels were elevated while albumin levels were found low. The CSF result is compatible with previous SARS-CoV-2 infection studies (15). The patient's vital signs were improved, especially after intrathecal and systemic MSCs transplantations. After the patient had been extubated, his neurological symptoms regressed, consciousness restored, and he could speak. Most neurologic manifestations occurred early in the illness (the median time to hospital admission was 1-2 days) (16). In our patient, the time period between hospitalization and determining the neurological symptoms was approximately 11 days.

After transplanting MSCs through intravenous infusion, a part of the MSCs accumulate in the lung, which could potentially improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis, and improve the lung function (2, 6, 14). They promote tissue and organ regeneration via secreting a variety of paracrine factors, conferring anti-inflammatory, immunomodulatory, angiogenic, antifibrotic, and structural reparative properties (17, 18). BM-MSCs significantly improved the efficiency of cardiomyogenesis and cardiac function (19, 20). When the treatment of patients with organ failure due to COVID-19 infection is examined, no treatment can be seen to improve the organ damage. Our patient had cardiac involvement due to COVID-19 infection. In our patient who underwent echocardiography after cardiac arrest, the ejection fraction was very low (25 %). In echocardiography performed after systematic transplantation of MSCs, this rate increased to the level of 60 %. It is our opinion that the transplantation of MSCs has a healing effect on the heart. After MSCs transplantation had been performed for the fourth time, the patient's heart functions have returned to normal. Here, we think that MSCs have a positive and accelerating effect upon other healing mechanisms of the body.

MSCs engraftment was observed in the injured lung and engraftment rates increased with the extent of tissue injury (6). In a study by Leng Z et al, 7 patients with COVID-19 infections were given MSCs systematically, which had a healing effect (20).When the thorax CTs of the patient were examined in our case study, it was observed that the upper and lower lobes

Tab. 2. Drug and mesenchymal stem cell management.

			Route of administration
Drug	Ritonavir + Lopinavir	2X400mg	Oral
	Oseltamivir	2X75mg	
	Azithromycin	1X500mg	
	Favipiravir	2X1600mg/2X600mg	
	Hydroxychloroquine	2X200mg/	
	Methylprednisolone	2X100mg	Intravenous
	Tocilizumab	1X400mg	
MSC	1st application/ Day 1	$3X10^6$/kg	Intravenous
	2nd application/ Day 3	$3X10^6$/kg	
	3rd application/ Day 6	$3X10^6$/kg	
	4th application/ Day 9	$2X10^6$/kg + $1X10^6$/kg	Intravenous + intrathecal

of both lungs were commonly held, especially in the second thorax CT. In our patient, the lesions seen in both lungs had ground-glass appearance, and areas of consolidation were compatible with COVID-19 infection (21, 22). After the MSC transplantation for the last time in our patient, bilateral lung symptoms regressed on control thorax CT. After the patient had been discharged, there was no problem in his medical checkups.

After the first MSC transplantation in our patient, the values of AST, ALT, LDH, CK, pro-bnp, ferritin, triglyceride, fibrinogen, ammonia, and myoglobin began to decrease. The second time the MSCs had been given, CRP reached normal values (Tab. 1). We thought that MSCs were related to the immunomodulatory effect on cytokine storm. On day 2 after MSC transplantation, the lymphocyte count reached the normal level. What is important here, is that reaching the efficacy of lymphocyte count on day 2 was achieved by the administration of MSCs and tocilizumab at the same time. In the literature, it is seen that the normal level of the number of lymphocytes was reached on day 5 in the study of Xiaoling Xu et al (23). In our study, the increase in lymphocytes was thought to be related to MSCs transplantation. It was observed that the number of TH-2 cells increased, and the number of TH-1 cells decreased in the immune modulation after MSC transplantation. After the first MSC transplant, the proportions of CD4+T cells and CD8+T cells were 66 % and 26.7 %, respectively. After 3 MSC transplantation, the proportion of CD4+T cells was 42.9 % and 39.1 %, while that of CD8+T cells was 18.7 % and 22 %, respectively. After the second and third MSC transplantations, it was found that the proportion of CD4+T cells was 42.9 % and 39.1 % while that of CD8+ cells was 18.7 % and 22 %, respectively. In previous studies, the decrease in T lymphocytes has been shown to be due to co-death in T cells infected with the virus (6, 24).

Learning points

The transplantations of MSCs, both systematically and intrathecally, were effective in the treatment of the central nervous system. This activity is related to the fact that MSCs administered intrathecally can easily cross the blood brain barrier. Combining both ways of MSC treatment in multi-organ and brain involvement due to COVID-19 infection will increase the effectiveness of treatment.

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