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Case Report

Mesenchymal stem cell therapy in children with end-stage kidney disease: report of two cases

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Chronic kidney disease (CKD) is a major health problem worldwide, with increasing incidence and prevalence. While the incidence of CKD in children is relatively low, CKD contributes to major health problems and has many long-term effects.[1] Chronic kidney disease is characterized by a gradual decline in kidney function over time. *The Kidney Disease Improving Global Outcomes* (KDIGO) report defined CKD as an abnormality of renal structure or function with decreased glomerular filtration rate (GFR) that lasts more than three months. Chronic kidney disease is classified into 5 stages based on the GFR value.[2]

Patients with stage V CKD transition from progressive disease to irreversible, terminal, end-stage kidney disease (ESKD). To date, the standard of ESKD management has been kidney replacement therapy, consisting of hemodialysis (HD), peritoneal dialysis (PD), and/or kidney transplantation. Complexity and cost of kidney care have obvious consequences on the availability of kidney replacement therapy for children, especially in developing countries. Dialysis provides only partial replacement of renal functions, especially clearance and fluid balance, but does not cure the disease. Kidney transplantation is a curative management, but donor availability for pediatric patients remains challenging. **[Paediatr Indones. 2022;62:217-22 DOI: 10.14238/pi62.3.2022.217-22**].

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The development of tissue engineering and regenerative medicine provides hope as alternative therapies for ESKD patients. Mesenchymal stem cells (MSC) therapy provides good outcomes in treating several other diseases, such as myocardial infarction, diabetes, spinal damage, osteoarthritis, lupus, aplastic anemia, Parkinson's disease, rheumatoid arthritis, liver cirrhosis, and multiple sclerosis.[3,4] In vitro studies of stem cell therapy for kidney disease have been carried out in experimental animal models. Most studies show promising outcomes, including the evidence that stem cell could actively blunt immune responses, there for it can be used for allogenic transplant without tissue matching.[5] However, to date, study in humans has been limited to adult patients. Here we report two pediatric ESKD cases who underwent MSC therapy from umbilical cord MSCs (uc-MSCs).

The cases

Case 1

A 16-year-old girl with ESKD secondary to lupus nephritis on HD first had symptoms of pallor and low urine production at 10 years of age. She previously underwent multiple abdominal surgeries due to appendicitis and post-surgical complications.

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Afterwards, she became severely malnourished and deteriorated to the level of ESKD in need of kidney replacement therapy. Hemodialysis was initiated at the age of 10.5 years after intracranial bleeding due to uncontrolled hypertension. Hemodialysis was performed thrice per week, with durations of 3-4 hours per session. Fluid balance and anemia were the main challenges in her management. Those complications led to severe pulmonary hypertension (PH), dilated cardiomyopathy, malnutrition, and complicated ascites. She had multiple hospital admissions because of cardiac-related dyspnea and fluid overload. Thus, she became oxygen dependent. Sildenafil was given for PH treatment, ascites was tapped to reduce the abdominal pressure, and other supportive treatments were performed.

At the age of 14, she underwent living donor kidney transplantation which failed due to stenosis of the recipient's iliac vein. One month after the procedure, she experienced more severe cardiac symptoms, fluid overload, ascites, hypertension, and psychological problems. In addition, she complained of frequent syncope, bone pain, and repeated vascular access infection. Her family requested a second attempt of kidney transplant, however, her general condition was not suitable for major surgery.

Patients generally receive the standard treatment for ESKD, such as hemodialysis thrice a week, medicine, and supporting treatment. Since the patient could not have major kidney transplantation surgery due to her physical condition, we discussed MSC therapy with her and her parents. They agreed and provided written informed consent.

The uc-MSCs used in this report were isolated in our center from women who had a healthy pregnancy by the *Stem Cell Medical Technology Integrated Service Installation*, Dr. Cipto Mangunkusumo Hospital/ Universitas Indonesia Medical School. The uc-MSCs were collected in tubes with phosphate-buffered saline (PBS) as well as 100 U/mL penicillin and 100 μg/mL streptomycin as antibiotic supplementation. Before incubating in an atmosphere of humidified air with 5% CO2 in DMEM at 37°C in 25 cm^2 flasks, the uc-MSC were washed with PBS in a cell culture hood and cut longitudinally into 5 cm2 segments. Medium conditioning was done with 10% FBS or 10% CBS supplementation. Further processing involved rapid thawing and re-culturing inside a 25 cm tissue culture (T25) flask. Platelet lysate with 10% concentration in complete medium was used in the procedure. The harvested cells were washed, suspended, counted, and reseeded at 80-90% confluence using trypsin and 0.05% EDTA inside DMEM supplemented with either 10% FBS or 10% CBS.

The girl received MSC therapy when she was 15.5-year-old with a total dose of 24 million (1.3 million unit/kg body weight), divided into three administrations with a 1-week interval. This MSC therapy was given peripherally with intravenous access and no drugs were administered afterward. No side effects were seen after MSC therapy. Improvement was seen in her decreased need of oxygen such that she could be weaned from 4-5 liters per minute (Lpm) to 1-2 Lpm. Marked reduction of pressure gradient showing improvement of pulmonary hypertension (89 mmHg before MSC therapy *vs.* 42 mmHg after MSC therapy) was also recorded despite the sildenafil therapy. We also noted a 20% body weight gain, from 18 kg to 22 kg, within 6 months after MSC therapy. Before therapy, the patient had difficulty gaining weight. Other complaints such as bone pain and frequent fainting was also markedly reduced. However, improvement was seen in only nutritional status and cardiac performance, with no effects on kidney function, as documented by either for pre-HD urea level or urine production. Clinical, laboratory, and echocardiography parameters of our first case are shown in **Table 1**.

Case 2

Our second ESKD case was a 13.5-year-old boy on HD due to bilateral contracted kidney disease, discovered by kidney ultrasound when he was 6 years old. At that time, he had experienced frequent seizures for 6 months and normocytic normochromic anemia. He started HD therapy at 12 years of age due to volume overload and uremia. He had repeated fluid overload episodes along with decreased urine production that put him on HD sessions thrice per week. The HD duration was 3 hours per session and intradialytic complications were recorded, such as abdominal cramping and hypotension. Hypertension, dilated cardiomyopathy, and anemia complicated his course of disease. His condition was generally considered to be too unstable to be a candidate for kidney transplantation.

Based on the patient's condition and discussion

Table 1. Clinical, laboratory, and echocardiographic parameters in Case 1

Parameters	MSC therapy	
	Before	After (6 months)
Urine production, mL/day	0	0
Pre-HD urea level, mg/dL	253	230
HD frequency and duration, times/week (duration, hours)	3 (3)	3 (3)
Body weight, kg	18	22
Oxygen therapy, Lpm	4-5	1-2
Echocardiography		
Ejection fraction, %	78	60
Fractional shortening	45	30
E/A ratio	0.8	3.0
RV systolic TAPSE, mm	12	10
TR pressure gradient, mmHg	89	42
Dilatation RA/RV	Yes	Yes
Sildenafil dose	4x10 mg/ day	4x10 mg/ day

HD=hemodialysis; RV=right ventricle; RA=right atrium; TAPSE=tricuspid annular plane systolic excursion; TR=tricuspid regurgitation

Table 2. Clinical, laboratory, and echocardiographic parameters in Case 2

Parameters	MSC therapy	
	Before	After (6 months)
Urine production, mL/day	50	250
Pre-HD urea level, mg/dL	230	171
HD frequency and duration, times/week (duration, hours)	3 (3)	2 (3.5)
Body weight, kg	23	22
Oxygen therapy, Lpm	-	-
Echocardiography		
Ejection fraction, %	62.1	51.1
Fractional shortening, %	32.6	25.8
IVSd, cm	1.2	0.827
IVSs, cm	0.98	1.12
LVIDd, cm	3.4	4.23
LVIDs, cm	2.3	3.14
LVPWd, cm	1.7	1.12
LVPWs, cm	2.2	1.32
EDV (Teich), mL	47.5	79.9
ESV (Teich), mL	18.0	39.1
IVS fractional thickening, %	-17.0	35.4

IVSd=intraventricular septum thickness at end-diastole; IVSs=intraventricular septum thickness at end-systole; LVIDd=left ventricular internal dimension at end-diastole; LVIDs=left ventricular internal dimension at end-systole; LVPWd=left ventricular posterior wall thickness at end-diastole; LVPWs=left ventricular posterior wall thickness at end-systole; EDV=end-diastolic volume; ESV=end-systolic volume; IVS=intraventricular septum

among the medical team, parents, and patient, MSC therapy was chosen as subsequent treatment. His parents provided written informed consent. Mesenchymal stem cell therapy was given at the age of 13.5 years by injecting 20 million cells (1 million unit/ kg) intravenously once via femoral venous access. No adverse reaction occurred during or after MSC therapy. After MSC therapy, the patient had a significant clinical improvement in urine production from 50 mL/day to 250 mL/day, such that the frequency of lung edema significantly reduced. Hemodialysis frequency was then reduced from 3 times per week (duration of 3 hours) to 2 times per week (duration of 3.5 hours). His overall condition also improved so he could perform daily activities without dyspnea or fatigue. The patient's pre-HD urea decreased from 230 mg/dL before MSC therapy to 171 mg/dL, however, there was no significant improvement in creatinine and urea levels. A summary of clinical, laboratory, and echocardiographic parameters of case 2 is shown in T**able 2**.

Discussion

Mesenchymal stem cells have been shown to be an ideal candidate for cell-based therapies for preservation and regeneration of human kidneys, as well as other damaged tissues and organs. These cells have been isolated from several different tissues including bone marrow, peripheral blood, adipose tissue, placenta, amniotic fluid, and umbilical cord blood. As multipotent stem cells, the first MSCs isolated from bone marrow were capable of differentiating into tissue of mesodermal origin only. Surprisingly, results from hundreds of animal studies and many human studies reported that MSCs were able to engraft and differentiate into functional cells of tissues that did not originate from mesoderm. The MSC therapy has been shown to be effective in myocardial infarction, kidney disease, corneal damage, as well as in lung, brain, and spinal cord injuries, and in graft versus host disease. Mesenchymal stem cells has great potential in the field of regenerative care in animals, because the cells are easily isolated and developed, have immunosuppressive capacity, and do not increase the risk of teratoma.[6,7]

Pre-clinical reports and clinical trials of stem cells used for the treatment of kidney disease are increasing rapidly. The main mechanism through which MSCs

contribute to tissue regeneration seems to be the local production of soluble factors that act through endocrine and paracrine pathways.[6] It is generally accepted that reno-protective activity of stem cells in acute and chronic kidney disease models is due to cytokine secretion and other molecules induced by stem cells inhibiting inflammation and endogenous stimulation of repair processes including angiogenesis. One mechanism of action for stem cells is through extracellular vesicles (EV). In the host cells of the kidney, EV can transfer genetic information that promotes regenerative processes. Then EV can "trap" the ligand on the EV membrane surface receptor. For example, vascular endothelial growth factor (VEGF) is trapped by VEGF receptors on the EV surface that block VEGF from the host glomerular cell receptor and inhibit pathological VEGF signals involved in the process of kidney damage.[8]

In CKD, accumulating experimental evidence indicates that MSC treatment can reduce renal dysfunction through revascularization, as well as reduced inflammation, oxidative stress, apoptosis, and fibrosis. Soluble factors are involved in different processes including: (1) immune system signaling such as IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1/CCL2), and TGF-β; (2) extracellular matrix remodelers such as tissue inhibitor of metalloproteinases-2 (TIMP-2), fibronectin, periostin, collagen, and metalloproteinase inhibitors; (3) growth factor and regulators such as insulin-like factor 1 (IGF-1), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF). This effect occurs independently of the source of the MSCs (adMSC, ucMSC, or bmMSC) and injury model (ischemia reperfusion, IgA nephropathy, and unilateral ureteral obstruction).[6]

An animal CKD model showed ,that MSCs localize in damaged kidney cells, acting to limit podocyte migration and leakage, repair glomerular endothelial cell damage, and reduce the formation of glomerular fibrotic and sclerotic lesions.[6] Furthermore, it has been observed that human MSCs have an anti-inflammatory effect, which is superior in umbilical cord mesenchymal stem cells (ucMSC) compared to other stromal cells. These anti-inflammatory effects reduce macrophage infiltration and induce polarization of proinflammatory M1 macrophages to anti-inflammatory M2 macrophages.[9]

Mesenchymal stem cells-based therapy has been widely studied for the treatment of kidney disease and has been shown to improve kidney function and recovery of damaged kidney tissue in animal studies and clinical trials.[8] However, MSC-based therapy is limited by low endurance of the cells when used to treat severe kidney disease. Several factors, such as anoikis, ischemia, inflammation, and the production of reactive oxygen species (ROS) reduce the effectiveness of MSC-based therapy.[10] Because of its therapeutic potential, MSCs from bone marrow or other tissues are considered to be an effective way to treat several human diseases. Although several findings have suggested that MSC-based therapy is beneficial in a pre-clinical CKD model, the experimental design of our study varies greatly in terms of CKD model, cell type and dose, route of administration, and parameters of kidney outcomes, making it difficult to measure the real therapeutic potential of MSC and application method to patients.[6]

In our first case, kidney function parameters of urine production and blood urea level did not respond to MSC therapy. In contrast, a previous study showed a satisfactory clinical response in 40 adult patients with active SLE who received two doses of ucMSCs at days 0 and 7.[11] Thirteen patients (32.5%) had a major clinical response and eleven patients (27.5%) had a partial response. *The Systemic Lupus Erythematosus Disease Activity Index* (SLEDAI) score, *British Isles Lupus Assessment Group* (BILAG) score, and renal function showed significant improvement, with peak improvement at the sixth month of MSC therapy. The effect slowly reduced after that, indicating the necessity to repeat MSC therapy after certain period of time.[11] In our first case, her advanced stage of illness when receiving cell therapy may have been the reason no kidney restoration was observed.

Cardiovascular improvement occurred, namely in PH as a long-term complication of CKD, as shown by decreased oxygen therapy and increased ability to perform daily activities. A systematic review of pre-clinical animal studies including 29 articles reported effectivity of stem cell therapy for pulmonary arterial hypertension.[12] One clinical report including 2 adult cases of PH receiving stem cell therapy also showed clinical improvement. Reduction of oxygen therapy and increased activity index in one patient were noted after stem cell therapy, while another patient had improved clinical or PH condition.[13]

In our second case, kidney improvement was observed in the form of increased urine production, as well as reduced blood urea level and HD frequency per week. However, cardiovascular function showed no response. We did not find clinical study of MSC therapy in patient with contracted kidney. However, a clinical study on a 13-year-old patient with recurrent focal segmental glomerulosclerosis (FSGS) after kidney transplantation who had responded poorly to conventional therapy, underwent MSC therapy from an allogeneic spinal cord. Before treatment of stem cells, the patient needed weekly plasmapheresis to achieve a targeted urine protein-creatinine ratio. After three stem cell therapy treatments without side effects, the patient had stable kidney function and target proteinuria was achieved without plasmapheresis. In addition, some inflammatory cytokines declined, and remained low after one year.[14]

Therapeutic effects of MSC in ESKD patients on regular dialysis cannot be evaluated from GFR. We defined kidney improvement by interdialytic urea generation rate before and after MSC therapy in same patient. Detailed mechanisms of how MSC improved cardiovascular performance by reduction of oxygen demand are not known. We also cannot explain the mechanism of these cells on increased weight gain. Previous studies have yet to provide conclusive evidence of a significant role for cytokines or soluble proteins on organ repair as a mechanism of MSC.[8]

From these cases we observed that peripheral MSC therapy in the pediatric population with ESKD may have a potentially beneficial effect in increasing kidney function by reducing blood urea level and weekly HD frequency in Case 2, and by reducing cardiac complications of ESKD in Case 1. Our cases also demonstrate the safety and tolerability of MSC-based therapy at a dose of 1 million cells/kg BW for children with ESKD. After observing these encouraging cases, we have enormous hope for MSC therapy use in children with ESKD who have limited therapy options due to their general condition. Improvement in any organ system in this complicated disease is precious enough to continue with future therapeutic development, especially as this option has a good safety profile. However, a larger clinical study, especially in the pediatric population, is needed to confirm the therapy effect of MSC in CKD patients. Additionally, other factors should be taken into consideration in the clinical application of stem cell therapy, such as appropriate selection of cell types, number of cells required, and route of administration.

Conflict of interest

None declared.

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