

MESENCHYMAL STEM CELL-BASED THERAPY FOR CHRONIC KIDNEY FAILURE RELATING LUPUS ERYTHEMATOSUS - A CASE REPORT

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ABSTRACT

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease, which is characterized by systemic multiple-organ involvement, relapses with large amount of autoantibodies. Their pathophysiology is multifaceted, involves complex hormonal-immunological-cellular interactions, and leads to damage in multiple cell types, which is often resistant to conventional therapy. Thus, novel strategies are needed to repair the renal parenchyma and preserve kidney function. Mesenchymal stem cells (MSC) confer renal protection through paracrine/endocrine effects, and to some degree possibly by direct engraftment. The patient was diagnosed with chronic kidney disease by standard methods for more than fifteen years. The patient agreed to the treatment of autologous adipose mesenchymal stem cell transplantation. The adipose mesenchymal stem cells were harvested by surgery, isolated with our enzyme protocol. The patient received one injection with $2,6 \times 10^6$ cells/kg for a total of 43kg of body weight. The patient with SLE do not receive prompt treatment, he get irreversible organ damage. After seven months, the preexisting renal insufficiency gradually ameliorated, including the decrease of creatinine and blood urea as well as the increase of estimated glomerular filtration rate. Lupus symptoms also reduced, followed by

the improvement of body movement and medication reduction. There was insufficient evidence of the clinical setting to show the efficiency of mesenchymal stem cells on the lupus nephritis relating to chronic kidney disease. This clinical trial highlights the feasibility and safety of mesenchymal stem cell treatments in renal failure-associated- autoimmune diseases.

Keywords: *Mesenchymal stem cells, adipose tissue, chronic kidney failure, lupus erythematosus, transplantation, autoimmune disease*

I. BACKGROUND

Lupus nephritis (LN) occurs in 12 to 69% of patients suffering from systemic lupus erythematosus (SLE), depending on case series. Up to 40% of patients with systemic lupus erythematosus (SLE) develop kidney disease, which represents a major cause of morbidity. Systemic lupus erythematosus (SLE), an autoimmune disease characterized by autoantibodies, is associated with various organ dysfunction, including lupus nephritis, resulting in chronic kidney disease (CKD) and kidney failure (1). CKD is typically characterized by the progressive loss of functional nephrons (2). Many patients with CKD get a high risk of cardiovascular diseases and death (3). For those who are ongoing to CKD, renal replacement's limited accessibility is a primary issue (3). In southeast Asia, CKD's prevalence is consistent with the estimates of 10-16% (3). Despite significant advances in supportive

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therapy, mortality rates still remain high and elevated (3).

Mesenchymal stem cells (MSCs) have been studied in several clinical research to alternative kidney disease models [3]. However, few reports exist of lupus nephritis related- CKD models and adipose tissue derive from MSCs (ATMSCs). We report a case using autologous ATMSCs therapy for a patient with CKD associated- systemic lupus erythematosus. Mesenchymal stem cells become a novel therapy for immune-mediated kidney injury and ATMSCs controllable disease CKD

II. CASE REPRESENTATION

Patient information

A 35-year-old male patient was diagnosed with stage 3B of chronic kidney disease relating to inherited systemic lupus erythematosus at the Hematology-Transfusing Center of Hue Central Hospital Hue, Vietnam, for eight years. For 14 months continuous checking up the disease, the serum chemistry revealed average elevated blood urea (BU) of 14.2 ± 2.3 mmol/L (normal, 2.76-8.07 mmol/L, creatinine (Cr) of 183.3 ± 5.4 μ mol/L (normal, 62-106 μ mol/L) and estimated glomerular filtration rate (eGFR) of 36.7 ± 1.2 (normal, >90 mL/min/1.73m²), and proteinuria of 0.49 ± 0.12 g/L (normal, 0 - 0.15 g/L). For systemic lupus erythematosus evaluation, the anti-dsDNA test shows a negative result, but the antinuclear antibody (ANA) test was positive ($OD_{\text{serum}}/OD_{\text{control}}$ was 2.2). The patient got chronic anemia with the

erythrocyte average number was 3.82 ± 0.25 million/L. He usually used the erythropoietin and vitamin D monthly to improve the hematological indices. He also suffered myositis and mixed sensory-motor polyneuropathy that affects the pain when moving. In April 2020, the lupus nephritis got a poor prognosis, resulting in severe arthritis and fever. The patient gets restricted in movement by the swelling and inflammation in his limbs, with the BU 15.3 mmol/L, Cr 198 μ mol/L, eGFR 34.13 mL/min/1.73m² high proteinuria 0.68 g/L. Those symptoms were not reduced clinically after two months of continuous treatment with high cyclosporine and cyclophosphamide doses. Thus, the patient was designated for MSC transplantation based on the suitable clinical criteria in our project.

AT-MSCs preparation, transplantation and monitoring

The AT-MSCs were isolated from the removed adipose tissue with our enzyme protocol. Mononuclear cells were cultured in T175 flasks (Corning) with StemMACS (Miltenyi) containing 1% penicillin-streptomycin (Gibco) in an incubator at 37°C, 5% CO₂. When the MSCs achieved 80% confluence, the cells were harvested and re-plated until reaching the treatment dose due to the recipient's body weight. MSC characterization was evaluated based on criteria defined by the International Society for Cellular Therapy (ISCT) (Figure 1) (4).

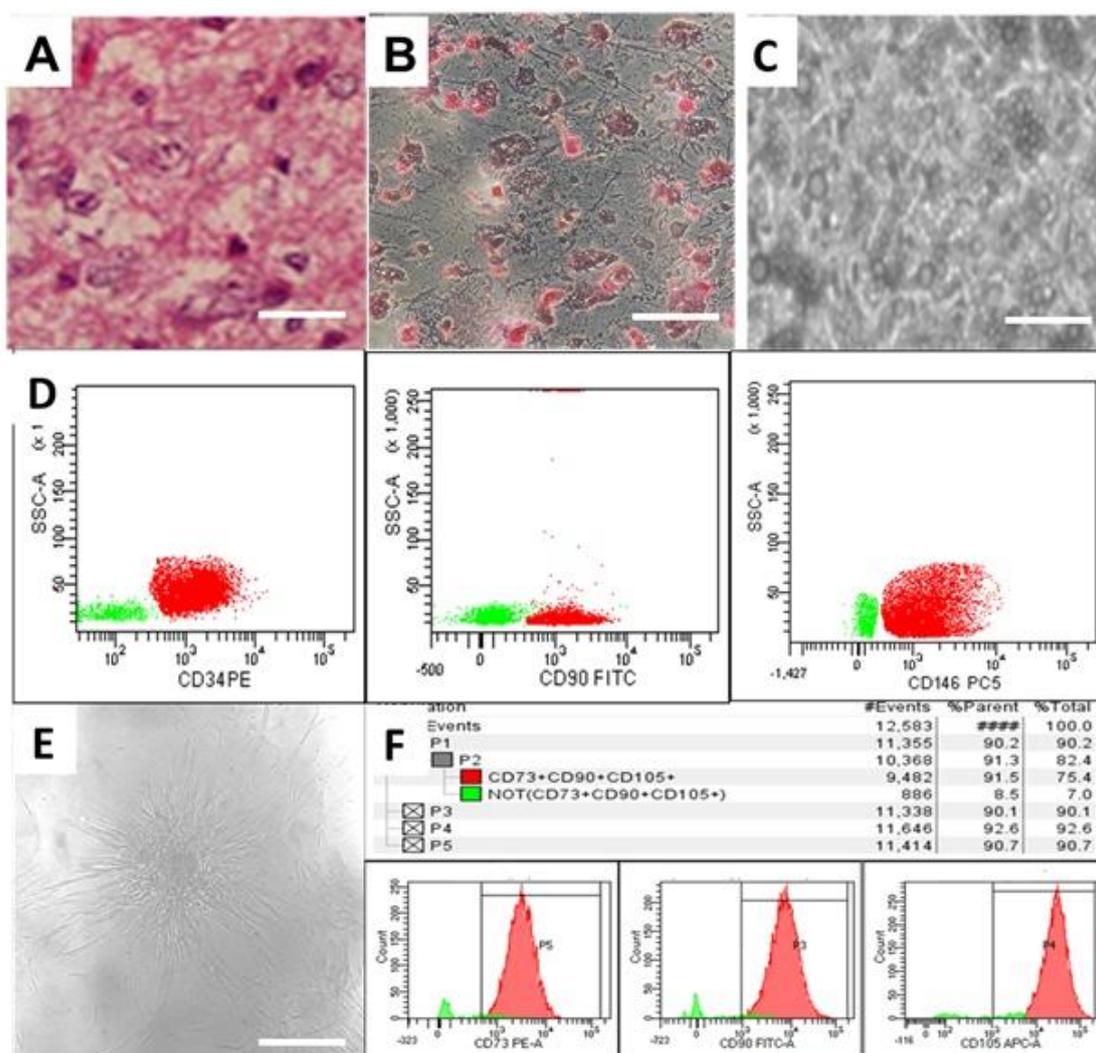


Figure 1. ATMSCs from CKD patient show normal expression of phenotypic markers and multilineage capacities. Osteogenic differentiation was validated after 21 culture days by Alizarin Red staining (A). Chondrogenic differentiation was confirmed after seven culture days by safranin O staining (B). Adipose differentiation was characterized by the formation of lipid droplets that were positive on oil red O staining (C). CKD AT-mononuclear cells positive expression (CD34, CD90, CD146) analyzed by flow cytometry (D). CFU-F evaluation after MSC culture (E). CKD AT-MSCs was positive expression for MSC markers (CD73, CD90, CD105) and were negative for other markers such as CD34, CD45 (data not shown) (F). Scale bar: 50 μ m

The cells were then injected intravenously into the systemic circulation. The patient received one injection with the number of MSCs was 2.6×10^6 cells/kg for 43 kg body weight in June 2020. Safety and tolerability

were also followed up by testing endotoxin and the patient reactions during the transplantation.

Significant improvements of CDK after transplantation

Two weeks after MSC therapy, serum chemistry performed revealed blood urea (BU) of 14 mmol/L, creatinine of 180 umol/L, and eGFR of 37.43 mL/min/1.73m². There was a significant reduction of lupus testing via the negative ANA result ($OD_{\text{serum}}/OD_{\text{control}} \leq 1$). The patient gets reduced joint pain and no fever and can walk, albeit limited. The erythrocyte induces improved with 4.51 million cells/L and reduced to use erythropoietin.

Seven months after the MSC therapy, serum chemistry shows significantly lower data. Although BU was slightly reduced at 13 mmol/L, creatinine(150 umol/L) and eGFR

(46.19 mL/min/1.73m²) were remarkably improved, which are the cause of the rapid deterioration of renal deficiency. The ANA testing still gets the negative result for lupus, and hematological data indicate the amelioration of erythrocytes and lymphocytes at the normal ranges. The patient reduced the doses of drugs for lupus and hematology after one month of MSCs treatment. Although these data are still out of the standard range of normal physiology, the patient shows a notable improvement of renal functions and pain by lupus effects (**Figure 2**).

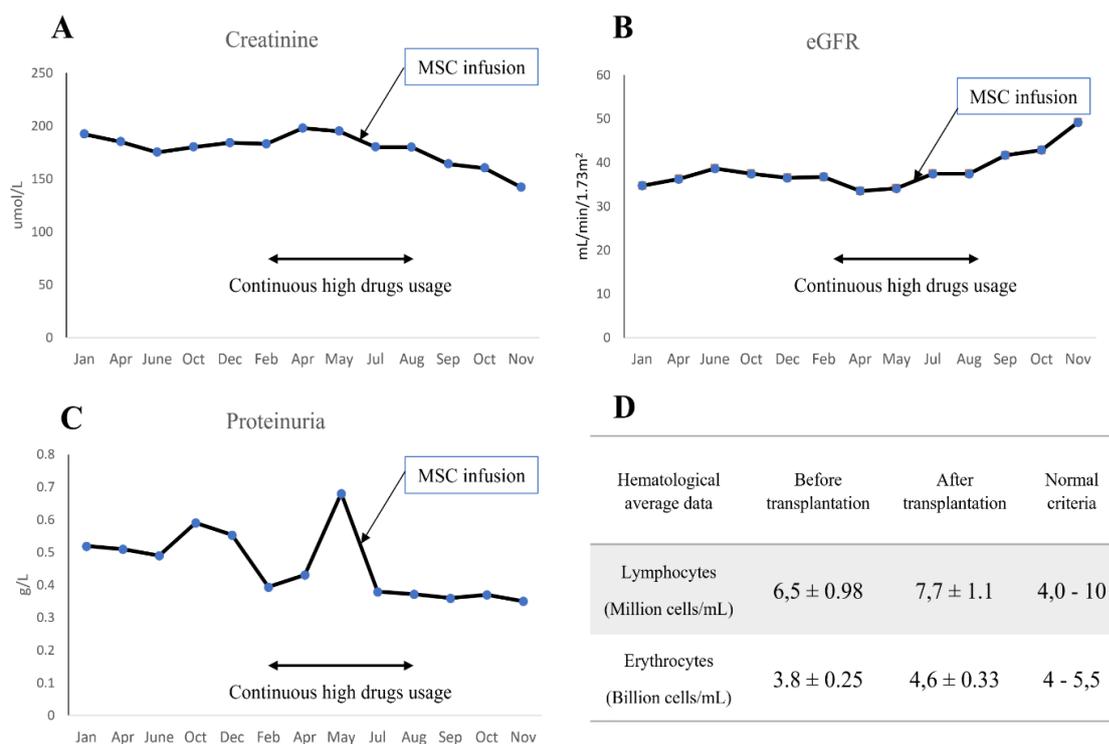


Figure 2. The data for the improvement of the kidney after AT-MSCs transplantation. Serum creatinine (A). eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) study equation(B), proteinuria (C), and the hematological indices (D) before and after MSC infusion.

III. DISCUSSION

Regenerative medicine has shown much promise for kidney repair. Mesenchymal stem cells (MSC) have become the preferred cell type, because a large number of MSC can be obtained relatively easily from adult sources like bone-marrow or adipose tissue, and because of their prominent anti-inflammatory properties. Indeed, MSCs possess homing - the ability to migrate to

MSCs interact with HSCs, leading to chemokines secretion that contribute to the HSC niche improvement and support the long-term growth of HSCs (9). We observed the improvement of hematological induces for our patients, and he can stop using the aforementioned- medications for his anemia. It could be that the MSCs migrate to the damaged sites and ameliorate the HSC niche, finally increase the downstream differentiation of hematopoietic lineage. MSC seem to be suited to target multiple pathways contributing to chronic kidney injury.

SLE leads to many immune cells malfunction, including B cells, T cells, and monocytes, resulting in immune cell deficiencies, plasma cell activation, and autoantibody (8). MSC and their environment can release dozens of active biological factors that decrease apoptosis, reduce inflammation and fibrosis formation (10) MSCs exhibit immunomodulatory properties, such as shifting T cells expansion to regulatory phenotypes (11).

MSCs are also able to inhibit B cell proliferation and differentiation *in vitro*, followed by suppressing antigen-specific IgG1 and IgM secretion (12). These traits are advantages of this therapy for lupus nephritis as they would not further need immunosuppressive drugs (13). Indeed, our patient tapered to use the anti-inflammatory

injured tissues by various signals, including chemokines and matrix metalloproteinases (5, 6). Following injury, MSCs preferentially go across the endothelium and go into the injured tissue bed (6). Several direct publications revealed abnormal HSCs in SLE, resulting in the change of immune cells' fate or exertion of an irreversible modification in these cells' development (7, 8).

and immunosuppressive drugs after one month due to the improvement of hematology and inflammation in the limbs' joint. There was a significant change of ANA test to negative result and increased hematology to improve lupus nephritis.

When locating in the damaged kidney, MSCs might reverse fibrosis and improve injured renal structures by releasing growth factors (14). ATMSCs secrete specific cytokines to improve tubular cell necrosis following renal failure amelioration (8). These capabilities were able to impede activation of p38 MAPK, then inhibit the apoptosis pathway (8). Human MSCs are the repertoire of vascular endothelial growth factor, which can stabilize blood vessels' network then improve the BUN, serum creatinine index (15). Mesenchymal stem cells become a novel therapy for immune-mediated kidney injury. We observed the gradual improvement of kidney function through creatinine and eGFR after MSC transplantation. Compared with the standard therapeutic drugs for refractory lupus nephritis, including cyclophosphamides and corticosteroids, our therapy also has a long-term effect on the patient to improve the symptoms, including urine protein <1.0 g/l and serum creatinine <25%. These changes relate to considerable tubular epithelial injuries and proteinaceous casts in the tubules (16). However, the proteinuria and

BU data only reduced after one month of infusion and remained high. It could be the long-term damage of the kidney that takes a long time for the final healing of the kidney.

Apart from lupus nephritis, SLE also affects many organs, including osteoarthritis, myositis, and neurodegeneration. MSCs are supported to enhance synovial inflammation and protect cartilage from chronic degradation (17, 18). Cytokines released from MSCs together with ECM proteases and growth factors and transforming growth factor- β can initiate cartilage repair, resulting in chondrogenic proliferation (17, 18). These factors comprise a vital part of the MSCs secretome and enhance cartilage improvement. After delivering MSCs, functional improvement is also reported in some research with an improvement of neurodegenerative diseases. Transplanted MSCs support neuroprotection and neovascularisation and induce axonal sprouting by producing cytokines and neurotrophic factors (19). Our patient gets gradual enhancement of his movement without pain by inflammation and arthritis.

Autologous bmMSCs proved to functionally abnormally in some disorders such as lupus (20), limiting their clinical application. SLE-bmMSCs a senescent phenotype when being reduced proliferation ability,), increased DNA damage and reactive oxygen species (ROS) resulting in cell cycle blocking (21). Adipose tissue is a crucial MSC source, with proliferative capability up to 1000 times higher than bmMSCs (22). According to Villanueva *et al.*, the safety and efficacy of autologous ATMSCs for CKD treatment was proved after infusion and until 1-year tracking, with no adverse effect in all patients and a significant increase of eGFR (23).

We conduct autologous ATMSC transplantation mainly for safety reasons and the clinical trial results from Villanueva *et al.* (23) and the increasing use of this cell type. Our first step research has successfully shown a future pilot study's safety and feasibility to improve the patient's clinical outcome suffering from lupus nephritis.

IV. CONCLUSION

In summary, AT- MSCs can ameliorate the tissue damage progression in CKD relating to lupus erythematosus and have been investigated in clinical studies. Our patient saw a remarkable improvement in the lupus symptoms which associated with the chronic disease and complication such as arthritis and myolitis. Those beneficial effects of stem cell transplantation can be applied in clinical trials. We attempt to find the optimal cell dose and improve the transplantation procedure to standardize the MSCs transplantation as an innovative therapy for CKD's regenerative treatment.

ABBREVIATIONS

ANA: Antinuclear antibody
BU: Blood urea
CKD: Chronic kidney disease
eGFR: estimated Glomerular filtration rate
HSC: Hematopoietic stem cell
LN: Lupus nephritis
MSC: Mesenchymal stem cell
SLE: Systemic lupus erythematosus

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