Mesenchymal Stem Cell Infusion in Chronic Renal Failure Patients

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***Abstract*—The recently discovered therapeutic potential of mesenchymal stem cells (MSCs) has initiated development of various therapeutic options in a number of diseases. These therapeutic options may help in improving patients' quality of life, through preventing disease progression. *Methods:* Bone marrow samples from 11 chronic renal failure patients were cultured in appropriate culture medium to isolate MScs. MSCs obtained were identified by their plastic adherence property; positive expression of CD 271, CD 105 and negative expression of CD 34, CD 45 using flowcytometry. Harvested MScs were injected to the patients through transfemoral catheter every other week for six months. *Results:* The patients were followed up to detect any change in their laboratory tests. Follow up revealed a statistically significant improvement in blood urea, creatinine levels and GFR of p value 0.000. *Conclusion:* Stem cells are a promising therapeutic approach to ameliorate condition in chronic renal failure patients.**

***Index Terms*—chronic renal failure, autologous stem cell, transplantation**

I. Introduction

Acute and chronic kidney disease is a leading cause of morbidity and mortality worldwide with overall mortality rates between 50 and 80%. Shortage of compatible organs together with limited adaptability of current dialysis techniques led to urgent need to explore other alternatives [1]. There came hope that stem cells and regenerative medicine may provide regenerative options for kidney disease, where induction of repair may be achieved using endogenous or exogenous stem cells or the reprogramming of the organ to reinitiate development [2].

A number of studies showed that bone marrow (BM) represents a reservoir of stem cells that are physiologically INVOLVED in remodeling and repairing the kidney. BM can provide cells that integrate into the kidney and differentiate into new functional renal cells of a variety of types. There is evidence of engraftment and differentiation of stem cells during normal renal cellular turnover [3] and after acute and chronic damage [4]–[7].

The BM contains at least two populations of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stromal cells (MSCs), which provide stromal support for HSCs [8]. MSCs are able to self-renew and differentiate into bone, adipose and cartilage tissue and give rise to cells of multiple germ layers [9].

MSCs are non-immunogenic and display immunosuppressive properties, with the ability to INHIBIT maturation of dendritic cells and to suppress the function of memory T cells, B cells and NK cells. Such properties render MSCs suitable and attractive option for therapeutic application in several inflammatory and immune-mediated diseases, as well as in regenerative medicine [10], [11].

Autologous transplantation of BM-derived MSCs that can be easily harvested and expanded may be THE solution to limited donor organ [1].

Our study AIMED to evaluate the role of autologous BM- MSCs injection in the improvement of the patients' laboratory tests and quality of life as a reflection of regeneration of damaged renal tissue in chronic renal failure patients.

II. Materials and Methods

Sampling The study was conducted in Kasr Alainy hospitals; Cairo University on 11 chronic renal failure patients on regular hemodialysis. Sixty to eighty ml of bone marrow was aspirated from each patient under complete aseptic conditions and local anesthesia from multiple sites from the posterior iliac spine. BM collected was placed in sterile tubes containing preservative free heparin (Sigma-Aldrich, St. Louis, USA). The study was performed in accordance with the Helsinki Declaration, and the protocols were approved by the ethics committee of Cairo University. All participants provided informed consent before enrolment into the study.

Isolation and preparation of BM-MSCs The aspirated bone marrow was diluted with phosphate buffer saline containing 2 mM EDTA (PBS/EDTA buffer). Mononuclear cells (MNCs) were isolated by density gradient centrifugation at 1,700 rpm for 20 min (density 1.077, GibcoBRL, Grand Island, NY, USA) and washed in phosphate-buffered saline (PBS). Cells were seeded in $25m^2$ tissue culture flasks at a density of $5x10^5$ cells/ml in culture medium containing Iscove's modified dulbeco's medium with 1% L- glutamine, mesencult, 10% fetal calf serum and penicillin (10,000u/ml)/streptomycin(10mg/ml) (GIBCO). Cultures were maintained in a humidified atmosphere at 37℃ and 5% CO2 for three days without further handling. Then, the media and non-adherent cells were removed and 5 ml of fresh culture medium was

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added to the flask and incubated. The cells were examined every other day by inverted microscopy and medium change was performed every 3 days until the cells reached 70 % confluence. Cell harvest was performed at 70% confluence using Trypsin-EDTA and counted. 125,000 cells were suspended in 5ml fresh culture medium and cultured in 25m^2 tissue culture flasks with fresh culture medium changed every three days until cells reached 70 % confluence and cells were harvested and a second passage was performed. Cells are harvested after third passage. MSCs were identified by plastic adherence property, fibroblast-like morphology and flowcytometric analysis.

Isolated MSCs were collected in 15 ml sterile tubes and washed thrice with PBS.

Flow cytometric analysis of MSCs Analysis of surface expression of MSCs using anti CD271, anti CD105 (MSCs markers) and antiCD34, antiCD45 (exclusion marker) monoclonal antibodies was done. MSCs (1×10^5 cells) were suspended in PBS and were stained with fluorochrome-conjugated mAbs for 20 min on ice (antimouse mAanti-CD271, mAanti-CD105 and mAanti-CD34, mAanti- CD45; BD Biosciences, MN, USA). 10,000 events were analyzed for each sample. A cut off value at 20% was set to categorize samples as positive. Flowcytometric analysis was performed using a FACScan flowcytometer (Coulter Epics, Elite).

MSC injection The prepared cell suspension was injected into both renal arteries using transfemoral catheterization every other week for 6 months.

Follow-up Patients were followed by clinical assessment and laboratory tests.

III. Results

This study included 11 chronic renal failure patients on regular hemodialysis. Six patients were males (55%) and five were females (45%) with age range from 20 to 63 years old with a mean of 35.82 ± 13.81, disease duration was between 1 and 12 years with mean 5.36 ± 3.295.

Exclusion criteria included; acute renal failure, presence of infection, diabetes mellitus, heart failure or liver failure.

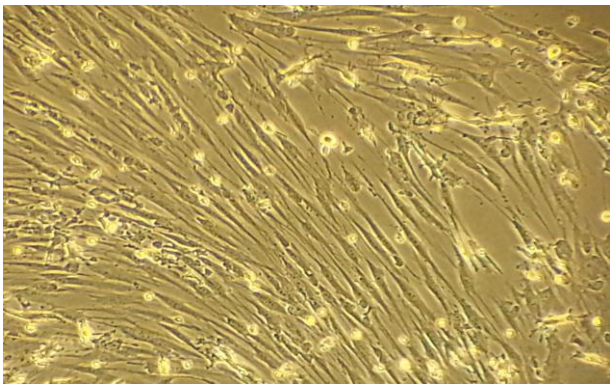

Figure 1. Mesenchymal stem cells. Magnification 10X

All the patients were on regular hemodialysis with a mean urea level of 135.83 ± 59.426 mg/dl, mean creatinine level of 7.777 ± 1.5718 mg/dl and mean GFR of 9.55 ± 3.078 ml/min/1.73m^2.

MSC characterization MSC after isolation from BM by their plastic adherence property consisted of a heterogeneous cell population with a predominant spindle-shaped morphology and were able to form fibroblast-like colonies (Fig. 1).

By FACS analysis, cultured cells were positive for CD271, CD105 and negative for CD34, CD45.

Follow up of the patients After Six month of MSCs injection the laboratory tests of the patients were estimated to detect any change as an outcome to MSCs injection. There was a highly significant statistical change in urea, creatinine and GFR levels with a mean ±SD of 104.15 ± 49.559 mg/dl, 7.745 ± 4.4491 mg/dl and 11.45 ± 5.165ml/min/1.73m^2 respectively (Table I).

IV. Discussion

Based on the unique ability of stem cells to differentiate and self-regenerate and the capability of MSCs to differentiate in all the three germ layers (9), stem cell therapy provided hope for patient through tissue regeneration especially in diseases where currently available therapies are ineffective [12].

The exact mechanism of action of MSCs in repair of kidney damage is not yet well known, but several studies on animal models suggested a number of hypotheses that allowed the use of MSCs in human therapeutic trials in order to explore new alternatives to reduce the suffering of such patients.

Transplantation of BM-MSCs or stromal cells from rodents has been identified as a strategy for renal repair in experimental models of acute kidney injury (AKI). The human BM-MSCs infusion decreased proximal tubular epithelial cell injury and ameliorated the deficit in renal function, resulting in reduced recipient mortality. Infused BM-MSCs became localized predominantly in peritubular areas and acted to reduce renal cell apoptosis and to increase proliferation. BM-MSCs also induced protection against AKI-related peritubular capillary changes consisting of endothelial cell abnormalities, leukocyte infiltration, and low endothelial cell and lumen volume density. These findings indicate that human MSCs of bone marrow origin have the ability to prolong survival in AKI [13].

When injected after injury, MSCs are capable of selectively homing into the kidney and to accelerate morphological and functional repair of the injured nephrons. MSCs most **likely** act by paracrine or endocrine mechanisms related to the production of mediators and growth factors with immunosuppressive, anti-inflammatory, antiapoptotic and proliferative effects. Bi *et al* [14] also supported this hypothesis by demonstrating that humoral factors secreted by MSCs, and not the local presence of these cells, are responsible for the renoprotective effect of MSC-based therapy, suggesting an endocrine action. Denoting that, local recruitment helps in increasing the intrarenal concentration of paracrine factors released by MSCs.

TABLE I. LABORATORY DATA OF THE PATIENTS BEFORE AND AFTER MSCS INJECTION

	Mean ±SD	P-Value	Significance
Urea- before	135.83 ±59.426		
Urea- after	104.15 ±49.559	0.000	H.S.
Creatinine- before	7.777 ±1.5718		
Creatinine- after	7.745 ±4.4491	0.000	H.S.
GFR- before	9.55 ±3.078		
GFR- after	11.45 ±5.165	0.000	H.S.

Kunter *et al* [15] demonstrated that intrarenal administration of BM-MSCs can be used as cell therapy in the anti-Thy1.1–mediated model of antibody-mediated mesangiolysis and glomerular capillary destruction. The study demonstrated that these beneficial effects are not mediated through replacement of damaged glomerular cells by differentiated MSCs but rather are caused by paracrine effects. The authors stated that these actions are specific for MSCs and dependent on local delivery.

In a recent study by Villanueva et al [16] on chronic kidney disease (CKD) rat model, they demonstrated that a single intravenous infusion of MSCs was able to enhance renal reparative processes and markedly improve renal function. Further several studies have proved the ability of MSCs in improving laboratory tests in CKD animal models, leading to, reduction in plasma creatinine levels [17], improvement of proteinuria [18], renal fibrosis [19], glomerulosclerosis, macrophage infiltration [20], improvements of renal filtration [21] and the reduction of pro-inflammatory cytokines [22].

Although all the previous studies were carried on animal models and little information is available on human trials, our study on chronic renal failure patients showed promising results that may provide a new hope for those patients suffering from such morbid disease in improving patients' quality of life and leading to less frequent hemodialysis.

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