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Original Article

# Mesenchymal stem cells are a rescue approach for recovery of deteriorating kidney function

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**KEY WORDS:**
chronic kidney disease, glomerulonephritis, MSCs, renal transplantation, tissue repair.

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**SUMMARY AT A GLANCE**

Mesenchymal stem cells may provide a new therapeutic tool for the treatment of chronic kidney disease by rescuing deteriorating kidney function.

**ABSTRACT:**

*Aim:* **Stem cell (SC) therapy for chronic kidney disease (CKD) is urgently needed. The use of mesenchymal stem cells (MSC) is a possible new therapeutic modality. Our work aimed to isolate human MSC from adult bone marrow to improve kidney functions in CKD patients.**

*Methods:* **In our study 30 patients with impaired kidney function were included, their ages ranged from 22 to 68 years. They included 10 inactive glomerulonephritis patients due to systemic lupus erythromatosus (SLE) (group I), 10 renal transplantation cases (group II) and 10 patients of other aetiologies as the control group. Fifty millilitres of bone marrow was aspirated from the iliac bone, for separation of MSC.**

*Results:* **There was a highly statistically significant difference between both CD271 and CD29 before and after culture with increase of both markers at end of culture, $P < 0.01$. Finally 50–70 million MSC in 10 mL saline ($0.7–1.0 \times 10^6$ MSC/kg body weight) were infused intravenously in two divided doses one week apart. There was a highly statistically significant difference between each of serum creatinine and creatinine clearance levels before and after MSC injection at 1, 3 and 6 months post-infusion with SLE cases showing a greater decline of their serum creatinine and elevation of mean creatinine clearance levels after injection than transplantation and control groups, $P < 0.05$.**

*Conclusion:* **Mesenchymal stem cells therapy is a potential therapeutic modality for early phases of CKD.**

## INTRODUCTION

Our work aimed to isolate human mesenchymal stem cells (MSC) from adult human bone marrow in the virtue of improving kidney functions in patients with chronic kidney disease (CKD) due to allograft nephropathy or inactive glomerulonephritis. CKD is a considerable cause of mortality and morbidity in Western countries, affecting about 11% of the adult population.[1] In developing countries it is estimated that 150 per million populations is the average incidence of CKD. The goal of therapy is to slow down or halt the progression of CKD into established kidney failure that necessitates one of the forms of renal replacement therapy; this may be dialysis, or ideally a kidney transplant. However, organ availability lags far behind demand due to the high cost and continuous lack of donors. This compelled us to seek a simpler and more attractive strategy for organ repair. The stem cells (SC) therapeutic modality allows self renewal and intervention in building and maintaining the structural and functional integrity of tissues.[2]

The development of stem cell therapies for kidney repairs, although in its infancy, is in a very promising era. Mesenchymal stem cells (MSC) are bone marrow populating cells constituting a rare population of adult stem cells with clonogenic, self-renewing ability and have the capacity to differentiate into a wide range of mesenchymal tissue types, including cartilage, bone, muscle, stroma, fat, tendon and other connective tissues.[3] MSC possess a very characteristic property that is adherent to plastic surfaces; this allows easy identification and collection for investigators. In some studies, the surface phenotype of an MSC has been investigated. No single antigen is exclusively expressed by MSC; unlike hematopoietic stem cells (HSC) they are negative for markers that include CD34, CD45 and CD14 and positive for CD166, CD105, CD271, CD29 and CD44.[4]

Mesenchymal stem cells are implicated in the reparative capacity of kidney injuries possibly by providing paracrine and/or endocrine factors that explain their positive effects on kidney injury. Evidence for this paracrine/endocrine process was suggested by Bi *et al*.[5] using a model of cisplatin-induced renal damage. This study showed that the apparent reparative function of MSC could be achieved via an intraperitoneal injection of the MSC-conditioned medium alone. MSC have been shown to secrete a number of growth factors. Imberti[6] suggest that this humoral function results from insulin-like growth factor-1 (IGF1), whereas Bi *et al*.[5] attributed it to a combination of human growth factor (HGF), IGF1 and epidermal growth factor (EGF). Of interest, Okuyama *et al*.[7] suggested that exposure of MSC to hypoxia induced the expression of vascular endothelial growth factor (VEGF) and VEGF-receptor 1 by MSCs so play a crucial role in the repair of hypoxic tissue.

Mesenchymal stem cells being less immunogenic than other SC allows MSC to be a potential source for allogeneic cell therapy. Togel *et al*.[8] reported that renal protection by MSC in rats with acute renal failure (ARF) was attributed to proinflammatory cytokine inhibition and anti-inflammatory cytokine stimulation in post-ischemic kidneys rather than MSC differentiation into renal tubular epithelial cells, suggesting that MSC might also exhibit immunomodulatory properties. MSC have a unique ability to exert an inhibitory effect on a broad range of immune cells, including T, B, natural killer (NK) and dendritic cell proliferation *in vitro* and *in vivo*, this property provides immunosuppressive and anti-inflammatory activities, which is particularly beneficial in immune-mediated diseases such as glomerulonephritis.[9]

## METHODS

### Subjects

The present pilot study was conducted on a total number of 30 patients with deteriorating kidney function of various aetiologies; their ages ranged from 22 to 68 years (mean value 48.20 ± 15.25 years). They included 10 inactive SLE patients diagnosed pathologically as diffuse proliferative lupus nephritis (stage IV) and received therapy with corticosteroids and cyclophosphamide (two males and eight females), 10 renal transplantation cases with biopsy proven chronic allograft nephropathy (BPCAN), immunosuppressive regimens were cyclosporine (CsA), mycophenolate mofetil (MMF) and prednisolone (PRD) for all patients (seven males and three females) and control group with impaired renal functions due to other aetiologies. These patients were selected among cases of a private clinic; a written informed consent was taken from the patients. Criteria for diagnosis was based on detailed history taking, complete clinical examination and laboratory investigations including complete blood picture (CBC), kidney function tests, immune profile (ANA, Ds DNA, ENA, C3 and C4) and pathological examination of renal biopsy.

## Methods

### Sampling

Fifty mL of bone marrow (autologous from SLE patients; group I and allogenic from related donor for renal transplanted patients; group II) was aspirated from the iliac bone under local anaesthesia and placed in sterile tubes containing preservative free heparin (Sigma-Aldrich, St. Louis, MO, USA).

### Mononuclear cells isolation

The bone marrow aspirate was diluted with phosphate-buffered saline (PBS) containing 2 mM ethylenediaminetetraacetic acid (PBS/EDTA buffer). Mononuclear cells (MNC) were separated by density gradient centrifugation (density 1.077, GibcoBRL, Grand Island, NY, USA) at 1800 rpm for 20 min.

### Mesenchymal stem cells separation as previously described[10]

In brief the MNC were seeded at $2 \times 10^5$ cell count and plated in Dulbecco's modified Eagle's medium (DMEM) at a density of 1000 cells/cm$^2$ and supplemented with 10% heat-inactivated foetal calf serum (FCS), penicillin (100 U/mL), streptomycin (10 mg/mL), 100 µL amphotericin B (All from GibcoBRL) and 20 ng/mL basic fibroblast growth factor (b-FGF; R&D System, Minneapolis, MN, USA) and then incubated at 37°C in 5% $CO_2$. After one day, non-adherent cells were removed and adherent cells were cultured in the presence of mesenchymal media (Cambrex BioScience, Nottingham, UK) for 3 weeks, the media was changed ever 3–4 days. After reaching 90% confluence the MSCs were recovered by incubation with trypsin/EDTA and counted on hemocytometer. The cell count was adjusted at 1 million/mL (Fig. 1).

### Flowcytometry

Flowcytometric analysis of surface expression of MSC using anti CD271, CD29 and CD34 monoclonal antibodies was done. MSC ($1 \times 10^6$ cells/100 µL) were suspended in PBS (GibcoBRL) containing 1% bovine serum albumin (BSA) and were stained with fluorochrome-conjugated mAb (anti-mouse mAanti-CD271, CD29 and CD34) (BD Biosciences, San Diego, CA, USA) for 20 min on ice. Flow cytometric analysis was performed using FACSCaliber (BD Biosciences) equipped with CellQuest Software (BD Biosciences). 10 000 cells were passed in front of the laser for each sample. Each sample was analyzed in duplicate. A cut off value at 20% was set to categorize samples as positive (Figs 2,3). A small panel of markers was used to identify hMSC and the functional assay for differentiation down specific lineage pathways was not performed.

### Mesenchymal stem cell injection

Fifty to 70 million MSC in 10 mL saline ($0.7–1.0 \times 10^6$ MSC/kg body weight) were infused intravenously in two divided doses 1 week apart.

### Detection of microchimerism

For allogenic transfused patients (group II) michrochimerism was identified by implementing polymerase chain reaction (PCR)

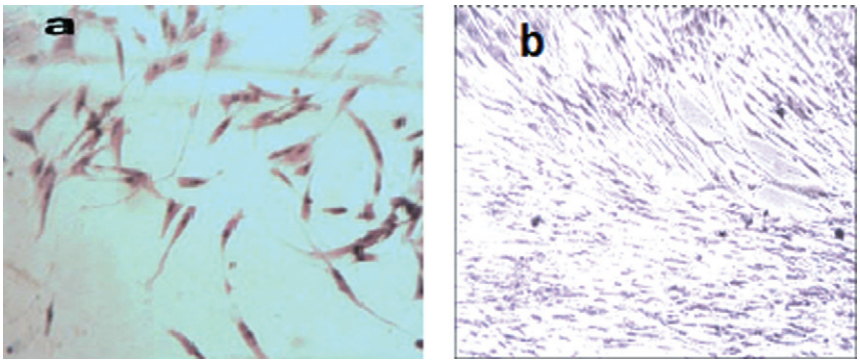


**Fig. 1** Photography of mesenchymal stem cells (MSC) in culture; (a) MSC 20% confluence, (b) MSC 90% confluence.

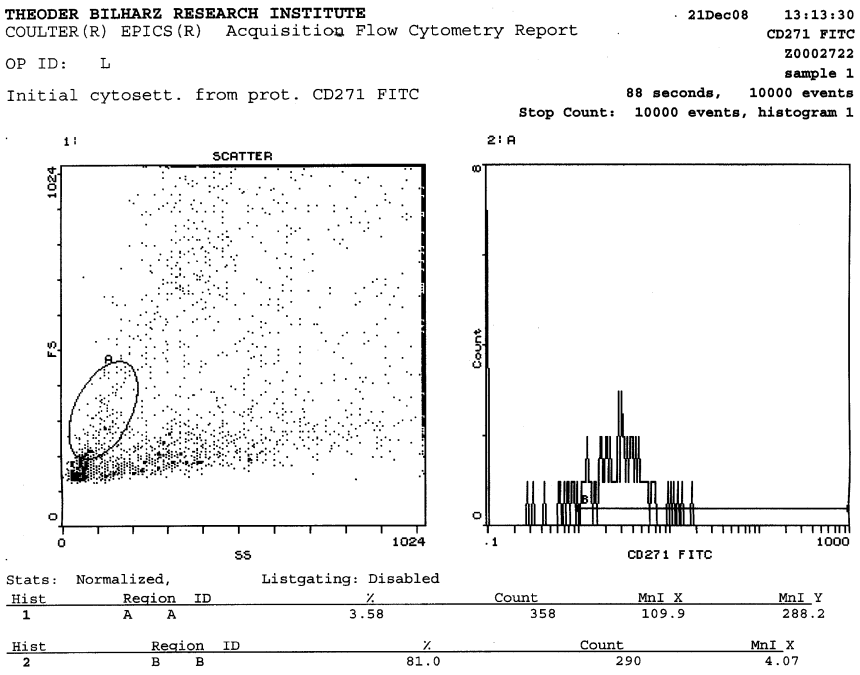


**Fig. 2** CD271 expression after culture by flowcytometry, revealing positive expression after culture.

amplification with sequence specific primers (PCR-SSP) molecular biology technique as previously described.[11]

### Follow up

Follow up of patients at 1, 3 and 6 months post-infusion by clinical assessment and laboratory work was done.

### Statistical analysis

Quantitative values are expressed as mean ± SD and were compared using Student's *t*-test. Qualitative data were compared using $\chi^2$ test. Pearson's correlation coefficients for the different variables were calculated. A *P*-value <0.05 was considered a significant. A *P*-value <0.01 was considered as highly significant. The SPSS statistical package 15 was used.

### Declaration of ethics

This study was approved by the review board of our hospitals and written informed consent was obtained from all patients according to Helsinki guidelines of research ethics.

## RESULTS

Demographic and laboratory data of all patients at diagnosis are shown in Table 1.

On comparing all groups at baseline; regarding demographic data, all patients were between 22 to 68 years, all groups were comparable in age ($P = 0.690$) and gender ($P = 0.090$). On clinical examination before MSC injection, anaemic manifestations were evident in most patients of all groups with less frequency among group I patients; however, this difference was not statistically significant ($P > 0.05$).

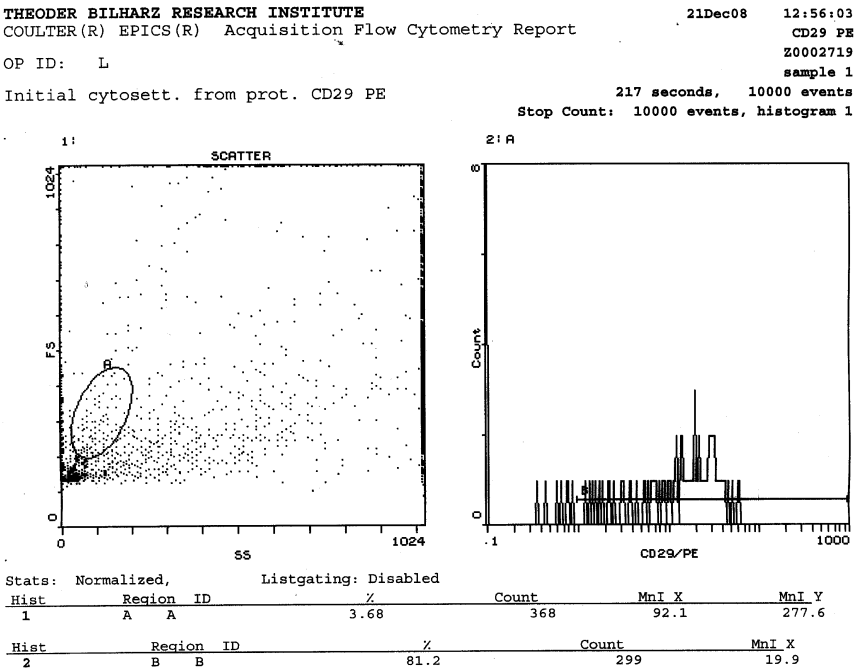


**Fig. 3** CD29 expression after culture by flowcytometry, revealing positive expression after culture.

**Table 1** Clinical and laboratory data of all groups at diagnosis

| Items | Patients (No. 30) | | | *P*-value |
|---|---|---|---|---|
| | Group 1 (No. 10) | Group II (No. 10) | Controls (No. 10) | |
| Clinical data: | | | | |
| Age (years): | | | | |
| Range | 22–58 | 25–68 | 28–65 | 0.690 |
| Mean ± SD | 32.29 ± 12.20 | 35.98 ± 13.90 | 31.33 ± 12.09 | NS |
| Sex: | | | | |
| Males (No; %) | 2; 20% | 7; 70% | 8; 80% | 0.090 |
| Females (No; %) | 8; 80% | 3; 30% | 2; 20% | NS |
| Anaemic manifestations: | | | | |
| Present | 5; 50% | 7; 70% | 8; 80% | 0.656 |
| Absent | 5; 50% | 3; 30% | 2; 20% | NS |
| Laboratory data: | | | | |
| Creatinine (mg/dL) | | | | |
| Range | 1.20–4.60 | 2.12–6.50 | 1.90–5.50 | 0.106 |
| Mean ± SD | 2.35 ± 1.07 | 3.62 ± 1.59 | 2.82 ± 1.18 | NS |
| Creatinine clearance (mL/min) | | | | |
| Range | 7.60–68.60 | 9.90–49.00 | 11.90–55.00 | 0.115 |
| Mean ± SD | 39.92 ± 17.59 | 25.05 ± 12.55 | 32.02 ± 15.48 | NS |
| Hb (g/dL) | | | | |
| Range | 8.50–13.00 | 8.25–12.50 | 7.20–11.85 | 0.322 |
| Mean ± SD | 10.85 ± 2.25 | 10.25 ± 1.98 | 9.45 ± 1.88 | NS |
| Albuminuria/proteinuria/hematuria (No; %) | | | | |
| Present | 2; 20% | 0; 0% | 0; 0% | 0.09 |
| Absent | 8; 80% | 10; 100% | 10; 100% | NS |

NS, not significant; SD, standard deviation.

Regarding baseline laboratory data, two out of 10 patients in group I presented with albuminuria/proteinuria/hematuria, none of the other patients presented with these findings. all patients suffered from impaired kidney functions and lowering of haemoglobin (HB) levels; however, there was no statistically significant difference between the three groups regarding mean value of serum creatinine, creatinine clearance and HB levels ($P > 0.05$).

**Table 2** Statistical comparison of both CD271 and CD29 percentages in patients before and after mesenchymal stem cells (MSC) culture

| Items | Before culture | After culture | *P*-value |
|---|---|---|---|
| CD271 (%) | | | |
| Range | 2.00–7.00 | 60.00–90.00 | 0.001 |
| Mean ± SD | 4.25 ± 1.68 | 73.70 ± 9.78 | HS |
| CD29 (%) | | | |
| Range | 4.00–9.00 | 72.00–95.00 | 0.001 |
| Mean ± SD | 6.25 ± 2.11 | 78.30 ± 11.38 | HS |

HS, highly significant; SD, standard deviation.

**Table 3** Correlation of mesenchymal stem cells (MSC) dose with age and sex and fold change of laboratory data of patients before and after injection

| Item | r value | *P*-value | Significance |
|---|---|---|---|
| Age | –0.057 | 0.81 | NS |
| Sex | 0.080 | 0.73 | NS |
| Creatinine fold decrease | –0.670** | 0.001 | HS |
| Creatinine clearance fold increase | 0.334 | 0.150 | NS |
| CD271 fold increase | 0.334 | 0.150 | NS |

**Correlation is highly significant at the 0.01 level (2-tailed). HS, highly significant; NS, non significant.

### Statistical comparison of both CD271 and CD29 percentage in all patients before and after MSC culture

There was a highly statistically significant difference between both CD271 and CD29 percentages before and after MSC culture with increase of both levels at the end of the culture ($P < 0.001$, Table 2, Figs 2,3).

### Correlation of MSC dose with clinical and laboratory data of all patients before and after injection

There was a highly statistically significant correlation between MSC dose and creatinine fold decrease after injection ($P < 0.001$). However there was no statistically significant correlation between MSC dose and each of creatinine clearance, CD271 and CD29 fold increase, age and sex prevalence (Table 3 and Fig. 4).

During follow up, regarding clinical data, few cases of group I are still having anaemic manifestations in comparison to the larger number of cases of group II and the control group. There were significantly less frequent anaemic manifestations in group I patients compared with the other two groups (*P* 0.044, 0.035 and 0.039 at 1, 3 and 6 months respectively). Regarding laboratory data, the disappearance of albuminuria/proteinuria/hematuria in two patients of group I was encountered. Also there was significant improvement of kidney function tests in group I patients compared with the other two groups with lowering of serum creatinine (*P* 0.002, 0.001 and 0.001 at 1, 3 and 6 months, respectively) and elevation of creatinine clearance levels (*P* 0.039, 0.033 and 0.017 at 1, 3 and 6 months, respectively). Although there was no statistically significant difference between different groups as regards HB level, higher HB levels were noticed among group I patients than other groups (*P* 0.436, 0.904, 0.760 at 1, 3 and 6 months, respectively (Table 4)).

### Microchimerism

Microchimerism (MC) was proved in many of group II patients after donor specific (DS)-MSCs transfusion by examination of human leucocyte antigen (HLA) class II antigens (HLA-DR) by PCR-SSP molecular biology technology. In one patient, HLA typing before transfusion of MSC was DR7, DR13 (6), DR53, DR52. HLA typing of related donor was DR4, DR13 (6), or DR14 (6), DR52, DR53. After transfusion of MSC HLA typing of the patient became DR4, DR7, DR13 (6), DR52, DR53.

## DISCUSSION

Chronic kidney disease is increasing with an annual rate of 6–8% in the US alone. In developing countries it represents about 150 per million populations. Up to date dialysis and transplantation are the only treatment modalities. However, new therapeutic options like SC therapy may provide additional regenerative options for kidney disease. Such new treatment might involve induction of repair using endogenous or exogenous stem cells or the reprogramming of the organ to re-initiate development.[12]

The bone marrow (BM) contains at least two populations of stem cells, HSC and MSC. Over the last decade, there has been a rising interest in the use of MSC for various clinical applications like cellular therapy and immunosuppression. Owing to feasibility of isolation, identification, *ex vivo* expansion of MSC, combined with their differentiation into various cell types of mesenchymal origin and cells of endodermal and ectodermal lineages through transdifferentiation and their impressive record of safety in clinical trials provide these cells superiority to the repair of other organs like liver, bone and kidney[13]

Our results revealed a highly statistically significant difference between both CD271 and CD29 before and after MSC culture with increase of CD271 and CD29 levels at the end of culture $P < 0.001$. Our findings matched with the opinion of Bühring *et al*.[14] who stated that so far CD271 is one of the most specific markers for BM-derived MSC. Also Sagrinati *et al*.[4] stated that unlike HSC, MSC are negative for markers that include CD34, CD45 and CD14 and positive for CD166, CD105, CD271, CD29 and CD44.

Also, there was a highly statistically significant correlation between MSC dose and creatinine fold decrease after injection (*P*-value <0.01). In two patients who received smaller

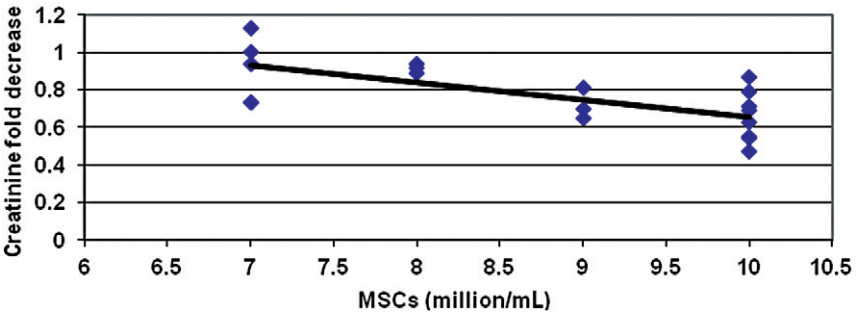


**Fig. 4** Correlation between mesenchymal stem cells (MSC) dose and creatinine fold decrease after MSC injection in group I patients, $P < 0.001$.

**Table 4** Comparison between the three groups regarding clinical and laboratory data at follow up

| Items | Patients (No. 30) | | | *P*-value |
|---|---|---|---|---|
| | Group 1 (No. 10) | Group II (No. 10) | Controls (No. 10) | |
| Anaemic manifestations after 1 m | | | | |
| Present | 3; 70% | 7; 70% | 8; 70% | 0.044 |
| Absent | 7; 30% | 3; 30% | 2; 30% | S |
| Anaemic manifestations after 3 m | | | | |
| Present | 2; 20% | 6; 60% | 7; 70% | 0.035 |
| Absent | 8; 80% | 4; 40% | 3; 30% | S |
| Anaemic manifestations after 6 m | | | | |
| Present | 2; 20% | 6; 60% | 7; 70% | 0.039 |
| Absent | 8; 80% | 4; 40% | 3; 30% | S |
| Creatinine (mg/dL) after 1 m | | | | |
| Range | 1.10–4.10 | 1.40–4.50 | 1.60–4.90 | 0.002 |
| Mean ± SD | 1.85 ± 0.52 | 2.25 ± 0.81 | 3.20 ± 0.98 | S |
| Creatinine (mg/dL) after 3 m | | | | |
| Range | 1.01–2.90 | 1.25–3.12 | 1.60–3.85 | 0.001 |
| Mean ± SD | 1.65 ± 0.48 | 2.05 ± 0.75 | 3.00 ± 0.95 | S |
| Creatinine (mg/dL) after 6 m | | | | |
| Range | 0.8–2.05 | 0.9–2.25 | 1.25–3.05 | 0.001 |
| Mean ± SD | 0.94 ± 0.25 | 1.05 ± 0.33 | 1.49 ± 0.50 | S |
| Creatinine clearance after 1 m (mL/min) | | | | |
| Range | 13.55–82.29 | 12.95–62.10 | 10.55–59.12 | 0.039 |
| Mean ± SD | 55.15 ± 19.22 | 41.05 ± 17.65 | 35.25 ± 13.35 | S |
| Creatinine clearance after 3 m (mL/min) | | | | |
| Range | 15.65–95.85 | 13.05–75.23 | 11.00–65.45 | 0.033 |
| Mean ± SD | 62.05 ± 21.02 | 49.22 ± 19.05 | 39.02 ± 15.11 | S |
| Creatinine clearance after 6 m (mL/min) | | | | |
| Range | 21.00–117.00 | 26.00–78.00 | 17.15–69.25 | 0.017 |
| Mean ± SD | 72.33 ± 25.08 | 55.28 ± 20.95 | 43.02 ± 17.55 | S |
| Hb (g/dL) at 1 m | | | | |
| Range | 9.20–13.00 | 8.95–12.65 | 7.85–11.90 | 0.436 |
| Mean ± SD | 10.95 ± 2.89 | 10.30 ± 2.15 | 9.55 ± 2.05 | NS |
| Hb (g/dL) at 3 m | | | | |
| Range | 9.50–13.50 | 8.05–12.80 | 7.90–12.20 | 0.904 |
| Mean ± SD | 11.00 ± 2.95 | 10.50 ± 2.25 | 9.88 ± 2.15 | NS |
| Hb (g/dL) at 6 m | | | | |
| Range | 10.30–13.90 | 8.20–13.00 | 8.00–12.50 | 0.670 |
| Mean ± S | 11.05 ± 3.05 | 10.62 ± 2.55 | 10.00 ± 2.18 | NS |

NS, non significant; S, significant; SD, standard deviation.

MSC count there was a rise in their serum creatinine 6 months after infusion following initial improvement, so more frequent sessions and higher MSC doses are required. However there was no statistical significant correlation between MSC dose and each of creatinine clearance fold increase, CD271 fold increase and age and sex prevalence. Of important interest in the clinical application of MSC is the establishment of the optimum dosing that will achieve the

best results with the least or absent side effect. In our study, about $0.7–1.0 \times 10^6$ MSC/kg body weight is achieved, *in vitro* studies show that immunosuppressive effects occurred at (MSC:PBMC)-ratios of (1:10) or higher.[15] These high concentrations of MSC may not be achievable in clinical applications, as the majority of MSC may disappear as a result of distribution to other organs and because of the cell-loss caused by immunologic or mechanical stress after infusion.[15] A more recent multi-centre trial showed that doses of $0.5 \times 10^6–9 \times 10^6$ cells per kg body weight did not lead to adverse side-effects. In contrast to our results, in this study, the effect of MSC appeared to be independent of the dose.[16]

Furthermore, there was a highly statistically significant difference between each of serum creatinine levels and creatinine clearance levels before and after MSC injection with lowering of mean serum creatinine and increase of mean creatinine clearance levels after 1, 3 and 6 months post-infusion in patients who received MSC than controls ($P < 0.05$). The data obtained in this study confirmed with those reported by Togel and Westenfelder[17] and Chhabra and Brayman[18] who confirmed the ability of stem cells to differentiate into glomerular and tubular cells, in addition, they exhibit immunomodulation potential and secrete growth factors that can induce tubular and vascular cell proliferation thus mediating renal tissue regeneration. In addition Morigi *et al*.[19] supported this opinion by saying that MSC engrafted in damaged kidneys and differentiate into tubular epithelial cells, thereby restoring renal structure and function in a cisplatin-induced renal injury model. Meanwhile, Togel *et al*.[20] suggested that MSC treatment is associated with improvement of renal function independent of differentiation into target cells, but rather through complex paracrine effects. Furthermore, Pino and David[21] reported that MSC do not integrate long-term or differentiate into renal tubule cells; however, when administered after injury, adult stem cells, particularly MSC, have been shown to exert therapeutic action through complex paracrine and endocrine action including the secretion of growth factors, cytokines, mitogenic, antiapoptotic, anti-inflammatory, vasculogenesis and angiogenesis factors.

On following up the patients at 1, 3 and 6 months after MSC injection, all patients were re-evaluated, clinically and through laboratory testing. Clinically, almost anaemic manifestations in group I patients showed greater improvement compared with the other two groups. Regarding laboratory testing, although there was no statistically significant difference between different groups as regards HB level, higher HB levels were noticed among group I patients than the other two groups. Meanwhile, there was significant improvement of kidney function tests in both SLE and transplantation groups greater than that obtained for the control group with lowering of mean serum creatinine and elevation of mean creatinine clearance levels; however, this improvement was more elicited in the SLE group than the transplantation group. Also the disappearance of albuminuria/proteinuria/hematuria was elicited in SLE patients. This could be explained by the study of Bartholomew *et al*.[22] who demonstrated that MSC administration *in vivo* could prolong skin graft survival owing to their immunosuppressive properties. They suggested that MSC might exhibit immunomodulatory and nonspecific anti-inflammatory properties. MSC modulate the immune system through interaction with a broad range of immune cells. They exert a profound inhibitory effect on T cell proliferation *in vitro* and *in vivo*. Similarly, MSCs were shown to exert such effects on B cells, dendritic cells and natural killer cells suggesting that some of the beneficial effects of MSC might reflect their immunosuppressive and anti-inflammatory activities. These immunomodulatory properties of MSC have been the basis for their use in treating conditions characterized by immunologic dysregulation such as Crohn's disease, rheumatoid arthritis and graft-*versus*-host-disease (GVHD) after allogeneic HSC transplantation.[23]

Microchimerism (MC) is defined by the presence of circulating cells, bidirectionally transferred from one genetically distinct individual to another. It occurs either physiologically during pregnancy, or iatrogenically after blood transfusion and organ transplants.[24] The migrated cells may persist for decades. MC was proved in many group II patients after DS-MSC transfusion by examination of HLA class II antigens (HLA-DR) by PCR-SSP molecular biology technology. In one patient HLA typing before transfusion of MSC was DR7, DR13 (6), DR53, DR52. HLA typing of related donor was DR4, DR13 (6), or DR14 (6), DR52, DR53. After transfusion of MSC HLA typing of the patient became DR4, DR7, DR13 (6), DR52, DR53. This haemopoietic MC confirms the persistence of donor MSC cells in the renal transplanted group for the development and maintenance of immunological tolerance.

We can conclude that owing to high cost and continuous lack of donors, a novel therapy of regenerative medicine that creates an improvement over dialysis and renal transplantation is a mandatory option in the last decades for CKD. BM derived MSC are feasible and a readily accessible source of stem cells for renal-based cellular therapy; however, the adoption of this new promising and potential era necessitates early diagnosis of chronic renal disease before end-stage renal failure is reached. The dose and frequency of this treatment is still to be defined in further clinical studies.

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