doi.org/10.36721/PJPS.2021.34.3.SP.1227-1231.1

Efficacy and safety of mesenchymal stem cell in Chinese patients with chronic renal failure: A pilot study in Shandong province, China

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Abstract: This study designed to evaluate efficacy and safety profile of Mesenchymal stem cells (MSCs) versus Acetyl cysteine (NACys) in the Chinese patients with Chronic renal failure (CRF). The CRF patients having eGFR less than 60ml per minute per 1.73m^2 randomly assigned to MSCs (N=100) or NACys (N=100) (1:1) for 8 weeks. MSCs administered as intravenous infusion of marrow-derived autologous MSCs (1 × 106 to 2 × 106/kg) reperfusion, whereas, another group received NACys 600mg orally twice a day for 8 weeks. The efficacy variables include: creatinine; cystatin C; TGF-β levels; oxidants/reactive oxygen species production induced by TGF-β; collagen levels (type 1 and 4); urinary albumin/creatinine ratio and Glomerular area. Safety was also assesed. Both the treatments significantly decreased creatinine, cystatin C and reactive oxygen species from baseline, however, reduction in creatinine, cystatin C, and reactive oxygen species level from baseline was significantly higher in patient treated with MSCs (N=100) as compared to NACys (N=100). Moreover, improvement in renal and systemic functional parameters from baseline was significantly higher in patient treated with MSCs as compared to NACys. Overall, MSCs offer significantly greater improvement in renal function as compared to NACys in Chinese CRF patients.

Keywords: Acetyl cysteine, chronic renal failure, creatinine, Cystatin C, mesenchymal stem cells, renal function.

INTRODUCTION

Chronic renal failure (CRF) is a noteworthy cause and one of the foremost reasons of death worldwide, adding heavy financial burden to patient and their family. In most cases, the main cause of CRF were diabetes and hypertension (Gilbertson *et al*., 2015; Papazova *et al*., 2015). Patients with chronic metabolic diseases are highly susceptible for developing kidney diseases; uncontrolled diabetes and hypertension are associated with CRF (Diana *et al*., 2016). Even though substantial developments in treatment modalities of CRF including renal replacement remedies, however, CRF patients suffering with considerably reduced quality of life. Thus, there is greater demand of novel drug, which stimulate renal cellular healing and tissue restoration (Morigi *et al*., 2016, Villanueva *et al*., 2019). From last few decades, there has been noteworthy advancement in the area of reformative drug that allowed development of cell remedies appropriate for kidney healing process (Makhlough *et al*., 2018). Mesenchymal stem cells (MSCs) are indistinguishable cells that possess immune modulatory and tissue protective properties with capability to distinguish into several cell types (Roushandeh *et al*., 2017).

Treatment approaches that increase cellular reinforcement might offer worthy substitutes for CRF patients (Kim *et al*., 2017). The MSCs can be extracted from a range of soft tissue, segregate into numerous cell extractions that hold exclusive immune modulatory capability that improve swelling and immune reactions, establishing a favorable instrument to ease renal healing process (Urt-Filho *et al*., 2016). Recently, investigational studies have exposed the potential of MSCs to recover impairment of renal function in several pre-clinical simulations of CRF. Numerous studies showed the favorable efficacy and safety of MSCs in patients with CRF (Lin *et al*., 2016). However, several hurdles need to be resolved before clinical use of MSCs in patients with CRF.

In China, little information is available on effect of MSCs in patient with renal disease induced by chronic metabolic diseases. In addition, there are few controversy reports on the efficacy and safety of MSCs in patients with CRF. There was no study in Chinese patients with CRF evaluating efficacy and safety of MSCs compared to N-Acetyl cysteine (NACys). Also, no data on effect of MSCs on biomarkers serum such as creatinine and cystatin C in Chinese patients with CRF. Thus, the present preliminary investigation designed to evaluate efficacy and safety profile of MSCs versus NACys in the Chinese CRF patients of Shandong Province, China.

MATERIALS AND METHODS

Patients and ethics

In this pilot study, Chinese CRF patients who had eGFR less than 60 ml per minute per 1.73 m^2 who were meeting all eligibility criteria were enrolled at Linyi People's Hospital, Linyi, Shandong province, China after obtaining their written, inform consent form. Ethics committee approval was taken from institutional ethics committee of Linyi people's hospital (ref number: IEC/2020/Apr-

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192/LH/04). The exclusion criteria include: Subject with any infection or sepsis or sign of severe CRF/kidney injury, heart diseases, severe lung diseases, thyroid diseases, cancer, or with any other pathology, which consulting physician or doctor feels may affect the result of study or patients who were received forbidden concomitant medicines or experiencing any other surgery were excluded, in view of influence of study consequence and subject's safety were also excluded from the study.

Study treatment

Subjects who met eligibility criteria were randomly assigned to MSCs or NACys in Chinese CRF patients. The allocation ratio was 1:1. MSCs administered as intravenous infusion of marrow-derived autologous MSCs (1 × 106 to 2 × 106/kg) reperfusion, whereas, another group received NACys 600 mg orally twice a day for 8 weeks.

Efficacy and safety assessment

Demography and other characteristics data were collected. The following efficacy variables were assessed in both the treatment group (at baseline and after treatment): creatinine; cystatin C; TGF-β levels; oxidants/reactive oxygen species production induced by TGF-β; collagen levels (type 1 and 4); urinary albumin/creatinine ratio. In addition, effect on renal and systemic functional parameters including Glomerular area was compared between both the study drugs. Blood samples were centrifuged at 3,000 rpm for 10 min, and serums were separated and stored at -70 °C. Serum levels of TGF-β and TGF-β activity were measured using commercial ELISA kits (Bender MedSystems, Austria). Safety of both the study drugs was assessed after treatment.

STATISTICAL ANALYSIS

Based on a power of 80% and a type I error rate of alpha= 0.05 (2-tailed), a sample size of at least 99 patients in each group required to detect a clinically acceptable difference of 0.38 mg/dl in mean change in serum creatinine after treatment with a standard deviation of 0.70 mg/dl based on previous study (Tariq *et al*., 2008). The data that falls in numerical category, and showed bell shaped distribution were analyzed by student t test (unpaired t test) for the independent group, and paired t test for the dependent group. Non-normal data were analyzed using Mann Whitney test. Quantitative data were presented using Mean (SD). Categorical data were presented as percentage/proportion of patients and were analyzed using fisher exact test or chi-square test based on size of data. P values of less than 0.05 (level of statistical significance) will be considered as statistically significant. *P <0.05 compared to NACys, otherwise not statistically significant.

RESULTS

Data of 200 CRF patients were subjected for statistical analysis. Demography and baseline characteristics were found similar in the both the groups (table 1) as the difference was statistically non-significant. At baseline, there was no statistically significant difference between both the treatment groups for creatinine, cystatin C, TGF-β, reactive oxygen species level, collagen (type 1 and 4), renal and systemic functional parameters.

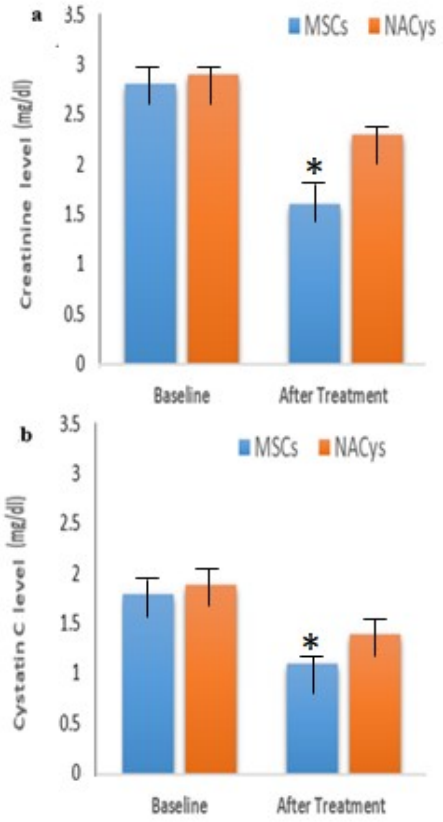

Fig. 1: Change in creatinine (1a) and cystatin C (1b) after treatment with MSCs and NACys in Chinese CRF patients

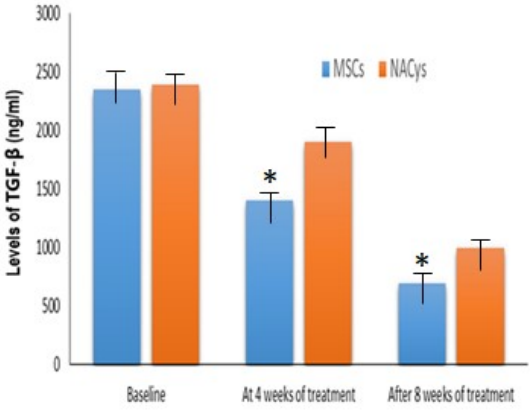

Fig. 2: Levels of TGF-β (ng/ml) after treatment with MSCs and NACys in Chinese CRF patients

Change in creatinine and cystatin C after treatment with MSCs and NACys in Chinese CRF patients is described in fig. 1a and 1b. At baseline, there was no statistical significant difference in creatinine and cystatin C. Both the treatments significantly decreased creatinine and cystatin C level from baseline, however, reduction in creatinine and cystatin C level from baseline was significantly higher in patient treated with MSCs as compared to NACys. Overall, treatment with MSCs offer significantly greater improvement as compared to NACys in Chinese CRF patients.

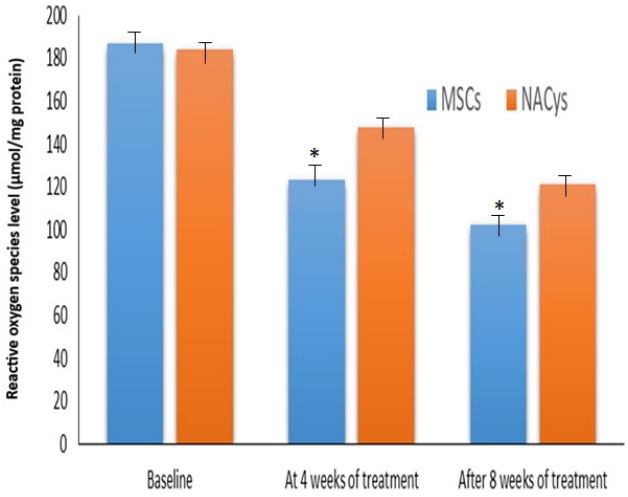

Fig. 3: Reactive oxygen species level (µmol/mg protein) after treatment with MSCs and NACys in Chinese CRF patients

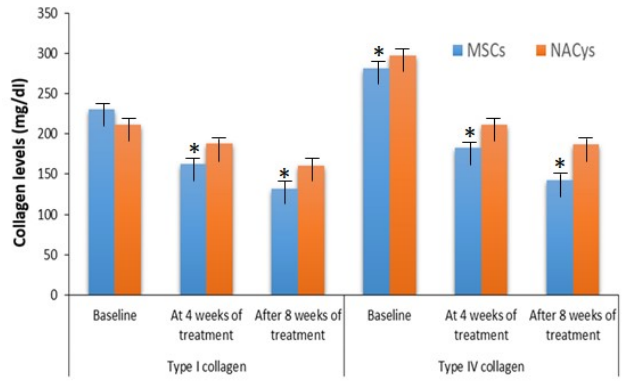

Fig. 4: Collagen levels (type 1 and 4) after treatment with MSCs and NACys in Chinese CRF patients

Change in TGF-β levels after treatment with MSCs and NACys in Chinese CRF patients is described in fig. 2. Both the treatments significantly decreased TGF-β levels from baseline at week 4; however, reduction in TGF-β levels from baseline were significantly higher in patient treated with MSCs as compared to NACys after 4 and 8 weeks of treatment.

Change in reactive oxygen species level after treatment with MSCs and NACys in Chinese CRF patients is described in fig. 3. Both the treatments significantly decreased reactive oxygen species level from baseline at week 4; however, reduction in reactive oxygen species level from baseline was significantly higher in patient treated with MSCs as compared to NACys after 4 and 8 weeks of treatment.

Change in type 1 and 4 collagens after treatment with MSCs and NACys in Chinese CRF patients is described in fig. 4. Both the treatments significantly improved type 1 and 4 collagen level from baseline at week 4; however, improvement in type 1 and 4 collagen level from baseline was significantly higher in patient treated with MSCs as compared to NACys after 4 weeks of treatment.

Comparison of renal and systemic functional parameters after treatment with MSCs and NACys in Chinese CRF patients is described in table 2. Both the treatments significantly improved renal and systemic functional parameters from baseline at week 4; however, improvement in renal and systemic functional parameters from baseline was significantly higher in patient treated with MSCs as compared to NACys after 4 weeks of treatment.

DISCUSSION

Since, there are few controversy reports on the efficacy and safety of MSCs in patients with CRF as the clinical usage of MSCs is a key topic of debate for the management of renal disease. This is the first report that evaluated the efficacy and safety profile of MSCs in the Chinese patients with CRF. The results of present investigation showed that both the treatments significantly decreased creatinine, cystatin C, and reactive oxygen species from baseline, however, reduction in creatinine, cystatin C, and reactive oxygen species level from baseline was significantly higher in patient treated with MSCs as compared to NACys. Moreover, improvement in renal and systemic functional parameters from baseline was significantly higher in patient treated with MSCs as compared to NACys. Our results are consistent with the earliest reports, which revealed that the MSCs significantly improved renal function after 12 months of treatment as compared to antibody therapy in patients with last stage of renal disease who were undergoing renal transplant (Chen *et al*., 2016, Christian *et al*., 2020). In addition, that published study revealed that MSCs reduced frequencies of renal transplant rejection and complications during renal transplant by decreasing the susceptibility of adaptable infection (Chen *et al*., 2016, Christian *et al*., 2020). Another study reported that there were no significant adverse events associated with MSCs and was considered well tolerated without compromising survival of patients undergoing renal transplant (Pan *et al*., 2017, Christian *et al*., 2020). In addition, in that published article, MSCs was found to be effective in pre-

Table 1: Demographic and baseline characteristic of Chinese CRF patients

Outcome variable	MSCs (N=100)	NACys (N=100)	P values
Age	64.5 (2.1)	61.2 (3.1)	>0.05
BMI	25.1 (2.1)	24.6 (3.4)	>0.05
Gender (M/F), %	65/35	68/32	>0.05
Serum creatinine	2.1 (0.5)	2.4 (0.8)	>0.05
Stage of CRF, % of patients			
Stage 1	20	22	>0.05
Stage 2	40	38	
Stage 3	30	27	
Stage 4	10	13	
Concomitant disease, % of patients			
Nephropathy*	87	93	>0.05
Hypertension**	100	100	
Type 2 Diabetes***	100	100	

Values are as mean (SD) for numerical variables except categorical variables such as Gender, Stage of CRF and Concomitant disease variable. P values calculated by Man Whitney test for numerical variables, whereas chi-square test for categorical variables. P values more than 0.05 considered as non-statistical difference.*defined by increased urinary albumin excretion (microalbuminuria: urinary albumin excretion (UAE) >20µg/min or/and ≤199µg/min and macroalbuminuria: ≥200µg/min) in the absence of other renal diseases; **defined as a systolic blood pressure above 140 mmHg or a diastolic blood pressure above 90 mmHg; *** defined as fasting glucose ≥ 7.0mmol/L, 2-h glucose ≥ 11.1mmol/L, or HbA1c ≥ 6.5%.

Table 2: Comparison of renal and systemic functional parameter safter treatment with MSCs and NACys in Chinese CRF patients

Outcome variable	MSCs (N=100)	NACys(N=100)	P value
Urinary Albumin: Creatinine (µg/mg)			
Baseline	1.41 (0.8)	1.38 (0.6)	<0.005
End of study visit (8 weeks)	1.11 (0.2)	1.23 (0.6)	
BP (SBP/DBP), mmhg			
Baseline	140/95	130/90	<0.005
End of study visit (8 weeks)	125/85	127/88	
GFR, ml/min			
Baseline	1.2 (0.4)	1.3 (0.7)	<0.005
End of study visit (8 weeks)	0.4 (0.3)	0.9 (0.2)	
Proteinuria, mg/24hr			
Baseline	24 (2.3)	26.3 (0.4)	<0.005
End of study visit (8 weeks)	14 (1.5)	21 (2.3)	
Urinary Sodium/potassium ratio			
Baseline	0.9 (0.3)	0.86 (0.6)	<0.005
End of study visit (8 weeks)	0.5 (0.2)	0.72 (0.7)	
Aldosterone, ng/dl			
Baseline	356 (12.3)	376.3 (12.4)	<0.005
End of study visit (8 weeks)	279 (8.5)	311 (13.3)	
TBARS, nmol/24hr			
Baseline	146 (8.3)	173.3 (9.4)	<0.005
End of study visit (8 weeks)	69 (3.5)	112 (8.3)	
Glomerular Area, %			
Baseline	18.2 (3.1)	17.9 (2.1)	<0.005
End of study visit (8 weeks)	26.2 (2.3)	22.3 (1.1)	

Values are as mean (SD) for all variables. P values calculated by Man Whitney test. P values less than 0.05 considered as statically significant difference.

venting immune rejection of renal transplant by restricting memory T-cell, that encourage studies evaluating MSCs in patients with stage 4 kidney disease patients who are undergoing kidney transplantation (Pan *et al*., 2017, Christian *et al*., 2020). Our study results are consistent with published studies, which reported MSCs help preserve renal function and attenuate renal injury in CRF (Pan *et al*., 2017, Christian *et al*., 2020).

Overall, the results of this study showed that MSCs has acceptable risk-benefit ratio, and the results of this study recommend the use of MSCs in Chinese CRF patients. The safety profile of MSCs was similar in published studies (Chen *et al*., 2016, Pan *et al*., 2017; Christian *et al*., 2020). Since the present study was the first study, thus, the result of this study will become benchmark for future studies in this area. Our finding related to the effect of MSCs on renal function in CRF patients is consistent with the previously reports results irrespective of indication studied. The only limitation of the present preliminary investigation was that the study was conducted at single center, thus results could not be generalized to whole Chinese population.

CONCLUSION

Compared to NACys, MSCs treatment demonstrated significantly greater improvement in renal function in Chinese CRF patients. The finding of present study may benefit to scientific community and helps to design large clinical trial to evaluate the efficacy and safety profile of MSCs versus standard therapy in Chinese patients with CRF across globe.

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