

## Mesenchymal Stem Cell-Mediated Restoration of Ventricle Function

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### ABSTRACT

*Mesenchymal Stem cells (MSCs) based interventions in heart failure cases have gained significant interest among researchers and clinicians over the last decade. The ease of their isolation, expansion, high proliferation rate and storage has made MSCs an attractive candidate. MSCs transplanted in patients with ischemic heart disease have been demonstrated to play a significant role in cardiac regeneration through plethora of processes including angiogenesis, myogenesis, immune modulation, anti-apoptotic- and anti-fibrotic-activities. In present study, we have discussed a case of a male patient with severe ventricular dysfunction after an incidence of myocardial infarction. The primary clinical findings demonstrated that the patient had dilated left ventricle (LV) with global LV hypokinesia. The LV ejection fraction (LVEF) was 25%-30%, which is significantly lower than a healthy individual. The patient was treated with adipose derived MSCs, injected through intravenous route. A significant recovery was observed post 5 months with LVEF of 35%. After nine months of follow-up, LVEF improved to 50 % with fair LV systolic function. The LV size was normal, and no regional wall motion abnormality was observed. The study suggests that MSCs infusion can help in restoration of ventricular function.*

### Keywords

Mesenchymal stem cells, Cardiac regeneration, Ventricle function, Ejection fraction, Stem cells.

### Introduction

Heart failure (HF) is one of the primary causes of morbidity and mortality across the world. The severe loss of cardiomyocytes post-acute myocardial infarction is irreversible and subsequent damages in cardiac tissues lead to HF. A reduced ejection fraction where LVEF is  $\leq 40\%$  is termed as LV global hypokinesia. It reflects as a uniform decrease in amplitude of ventricle wall motion. The condition adversely affects quality of life and survival of patients. Despite development in area of interventional cardiology, surgery, medications, supportive care and rehabilitation, survival rate has not improved significantly. Consequently, efforts in alternative strategies led to identification and development of stem cell (SCs) based approaches. Role of SCs in regenerative medicine has been evaluated over the last decade and several clinical studies have

demonstrated their significant clinical efficacy. Mesenchymal stem cells (MSCs) are the most investigated and deployed SCs for clinical purposes. These are adult progenitor cells found throughout body including adipose tissue, bone marrow, liver, heart, kidney, gut, umbilical cord, placenta, dental pulp and amniotic fluid [1]. MSCs are immune privileged cells marked by low expression of human leukocyte antigen (HLA) class I and absence of HLA-DR molecules extending their suitability for allogeneic transplants. Due to immune tolerance property, MSCs offer specific clinical advantage and, therefore, are referred as “universal donors” [2]. MSCs are potential candidates capable of triggering cardiac regeneration through direct differentiation into cardiomyocytes, smooth muscles cells and endothelial cells which replace damaged cells. Further, MSCs also secrete soluble factors which have significant impacts on cardiac remodeling, fibrosis, immune regulation and apoptosis of cardiomyocytes. In the case report discussed here, is an ischemic heart disease patient with LV dysfunction who was treated with adipose derived MSCs.

## Case Report

### Patient

A 65-year-old male patient with ischemic heart disease had suffered myocardial infarction in March 2017 and later heart failure. During that period, the patient had received standard medications including ACE inhibitors, diuretics and anti-platelets but no noticeable improvement was realized. The global LVEF was recorded to be 27% in July 2018. The patient came to our center in September 2018. The patient was investigated with 2D echo color Doppler performed in long and short axis with apical and sub costal views. Investigations revealed a dilated LV with severe left ventricular systolic malfunction. The Global LVEF was 25% - 30% with global hypokinesia. Mild mitral regurgitation was observed, and no pericardial effusion was found. All the four valves were structurally normal. The patient had history of diabetes and hypertension. After performing pre procedural major investigations at our center, the patient was found to be fit for MSC-transplant.

### Preparation of adipose derived MSCs

Liposuction was performed under local anesthesia by an experienced plastic surgeon on a willing healthy donor. About 200 ml of lipo-aspirate was collected from the donor under aseptic conditions from subcutaneous abdominal adipose tissue. Collected lipo-aspirate was immediately transferred to the laboratory and processed under a bio-safety laminar air flow chamber. All the reagents were purchased from Invitrogen (Paisley, UK). The MSCs isolation from lipoaspirate involved standard enzymatic digestion method with 0.1% type I collagenase. The digested lipo-aspirate was centrifuged in a density gradient centrifuge at 900g and resultant pellet was suspended in and washed three times with phosphate buffered saline at 300g. Finally, cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a humidified chamber containing 5% CO<sub>2</sub>. The media was changed after every 3 days. The cells were sub-cultured when they became 80% confluent and then re-plated in new cell culture flasks. MSCs were expanded up to passage 2 and then maintained in serum free media (MSC-SFM) for 24 hours. After that, the cells were harvested and cryopreserved until further use. Morphological and phenotypical properties of MSCs were assessed by phase-contrast microscopy and flow cytometric analyses of MSC specific markers (Figure 1).

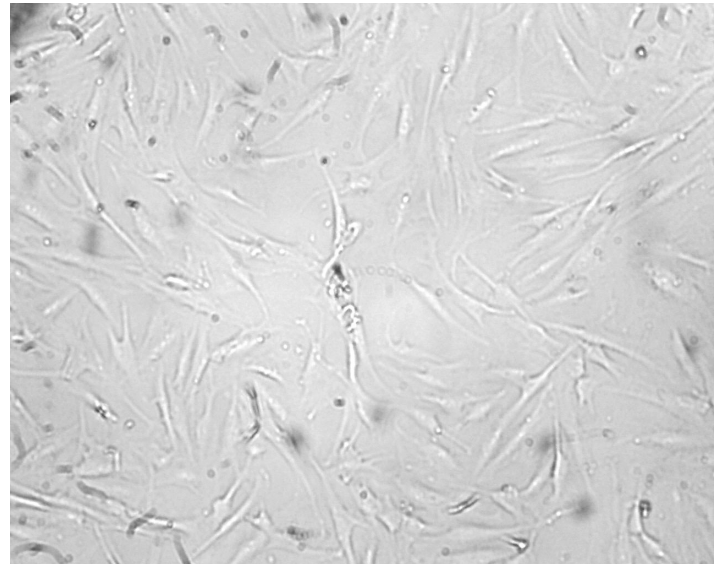
### Cell Infusion

Before infusion, cells were thawed, washed with and re-suspended in clinical grade isotonic (0.9%) normal saline. The cell viability was measured by trypan blue dye exclusion method and found to be > 95%. The MSCs were administered through intravenous route. One million cells/kg body weight were infused in each cycle. Three infusions of MSCs were carried out at intervals of 1 month. After transplant patient was kept 24 hours under observation.

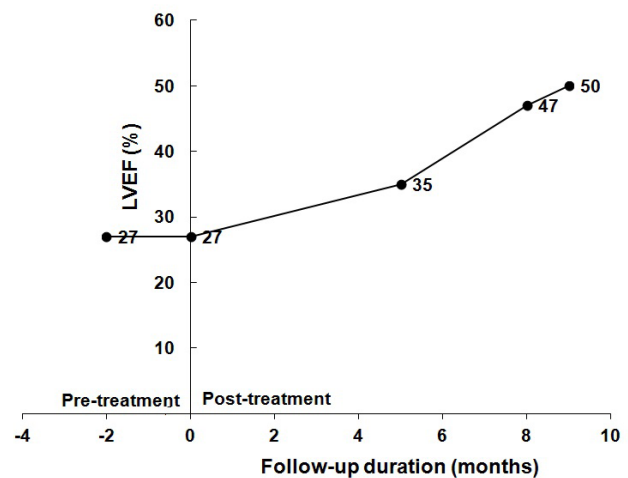
### Treatment, Follow-Up and Outcome

The patient was evaluated at 5th, 8th and 9th month post 1st infusion. The echocardiography analysis post 5 months revealed partial improvement in LVEF which was 35% in comparison to

27% at time of admission. This indicated mild Global hypokinesia of LV. Further follow-ups at 8th and 9th month demonstrated LVEF of 45-50% and 50% respectively (Figure 2). At 9th month, LV systolic function improved significantly with normal LV size. No RWMA (regional wall motion abnormality) was observed at rest. This improvement was observed upto a period of 26 months post which decline in LVEF was detected at 40% (data not shown).



**Figure 1:** Phase contrast microscopic image demonstrating spindle shaped MSCs (x 200).



**Figure 2:** Analysis of LVEF change post MSCs transplant.

Pre-treatment echocardiographies were performed at 2 months and 1 day prior to 1<sup>st</sup> MSCs transplant. Post treatment LVEF (%) were recorded at 5 months, 8 months and 9 months post 1<sup>st</sup> MSCs transplant.

### Discussion

Ischemic heart diseases and ventricular dysfunctions are the major causes of cardiac ailment-related mortalities. Blockade in coronary artery leads to interrupted blood supply causing infarction and progressive hypoxic conditions. Under anaerobic

**Report**

Reg.ID	00100100017958	OPD ID	00100100017428	Bill/Rec.No.	00100100021275
Name of Patient	Mr. Rajesh Baburao	Lab ID	00100100127956	Bill Date	19/09/2018
Address	Ahmedabad				
City	Ahmedabad				
Age	65 Yrs	Gender	Male		
Department	Health Check Up	Ref.Doctor	GIO STAR		

**2D Echo Colour Doppler**

**OBSERVATION:**

2 D Echo and color flow studies were done in long and short axis, apical and Sub costal views.

1. Dilated LV with Global LV hypokinesia.
2. Normal RV and RA. Concentric LVH.
3. All Four valves are structurally normal.
4. Severe LV systolic dysfunction. LVEF = 25-30%.
5. Reduced LV Compliance.
6. Mild TR. Mild MR. No AR.
7. Mild PAH. RVSP = 38mmHG.
8. Intact IAS and IVS.
9. No Clot. No Vegetation.
10. No pericardial effusion.

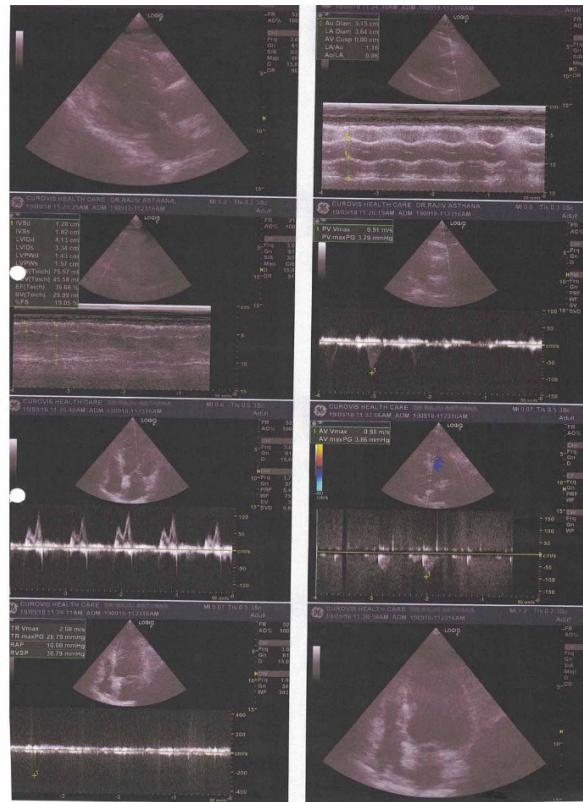
**CONCLUSION**

1. Dilated LV with Severe LV systolic dysfunction.
2. Concentric LVH. Reduce LV Compliance
3. Mild TR with Mild PAH. Mild MR. No AR
4. Global LV hypokinesia.

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A



B

**Report**

Reg.ID	00100100023277	OPD ID	00100100023092	Bill/Rec.No.	00100100027771
Name of Patient	Mr. Rajesh Baburao	Lab ID	00100100172161	Bill Date	09/07/2019
Address	AHMEDABAD				
City	Ahmedabad				
Age	66 Yrs	Gender	Male		
Department	Health Check Up	Ref.Doctor	GIOSTAR-USA		

**2D Echo Colour Doppler**

**OBSERVATION:**

2 D Echo and color flow studies were done in long and short axis, apical and Sub costal views.

1. Normal LV size. No RWMA at rest.
2. Normal RV and RA. Concentric LVH.
3. All Four valves are structurally normal.
4. Fair LV systolic function. LVEF = 50%.
5. Stage I diastolic dysfunction.
6. Trivial TR. Mild MR. No AR.
7. No PAH. RVSP = 30 mmHG.
8. Intact IAS and IVS.
9. No Clot. No Vegetation.
10. No pericardial effusion.

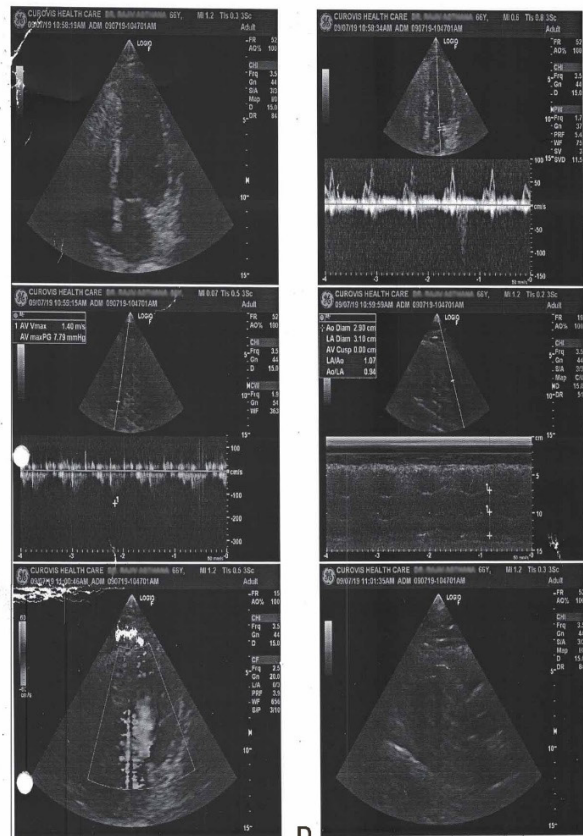
**CONCLUSION**

1. Normal LV size with Good LV systolic function.
2. Concentric LVH. Stage I diastolic dysfunction
3. Trivial TR with No PAH. Mild MR. No AR
4. No RWMA at rest.

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C



D

**Figure 3:** Pre and post MSC transplant echocardiograph analysis.

The 2D echo color flow studies were done in long and short axis with apical and sub-costal views. Pre- treatment reports and scan (A & B) indicate a dilated LV with global LV hypokinesia. Nine months post treatment reports and scan (C & D) demonstrate an improved LV function.



conditions in extracellular environment cardiomyocytes adopt the glycolytic pathway for energy generation. This results into lactic acid accumulation leading to a significant drop in intracellular pH. The hypoxic and acidic environment triggers extensive cardiomyocytes death and tissue necrosis. The damaged tissue is replaced by a non-vascular collagenous scar resulting into cardiac remodeling leading to reduced cardiac function, HF and death of patient [3]. SC-based interventions for myocardial infarction have gained significant attention in last decade. The approach is aimed towards generation of new functional cardiomyocytes, reduction of scar and neo-vascularization of afflicted heart. MSCs play significant role in cardiac regeneration directly by differentiating into cardiomyocytes replacing the dead ones and by secretion of immunomodulatory molecules, cytokines and metalloproteinase. MSCs can be isolated from different sources, including adipose tissue. The effect of adipose derived adult MSCs transplant has been examined in HF models.

Miyahara et al. [4] observed that transplantation of monolayered adipose derived MSCs in scarred myocardium lead to reduction in scar thickness, formation of new blood vessels and generation of cardiomyocytes in rat models. In present study, an ischemic heart patient with dilated LV and global LV hypokinesia approached at our center and decided to undergo MSCs transplant. Initially, the patient had received standard medications for a period of > 1 year, however, no noticeable improvement was observed. The Global LVEF of patient was 25% - 30%, an indicative of severely low ventricular pumping capacity. The patient was administered with 3 doses of adipose derived MSCs through intravenous route. After each transplant, patient was kept under observation for 24 hours. The transplant was well tolerated and no adverse events were observed. The safety of MSCs transplant has been confirmed in several clinical studies [5,6]. MSCs are immunoprivileged, due to absence of HLA-DR molecule, facilitating escape from immune surveillance and avoid host rejection. After first follow-up at 5 months, echocardiography analysis demonstrated LVEF of 35%, which indicated mild global hypokinesia. Further, after 2nd and 3rd follow-up evaluation at 8th and 9th month, LVEF increased to 50% (Figure 3). All segments of left ventricle were working normally. This significant cardiac functional recovery may be mediated through alteration in gene regulation and secretion of cytokines, chemokines and growth factors by transplanted MSCs.

Du et al. [7] demonstrated that MSCs modulate microenvironment of stressed cardiac tissues through down-regulation of pro-inflammatory cytokines TNF- $\alpha$  and IL-6 and up-regulation of anti-inflammatory cytokines IL-10. MSCs secrete angiogenic vascular endothelial growth factor (VEGF) and arteriogenic basic fibroblast growth factor (bFGF). VEGF increases capillary wall permeability, induces cell proliferation, migration and vascularization. On other hand, bFGF promotes smooth muscle formation as part of angiogenesis [8]. MSCs release metalloproteinases which can alter the deposited matrix in damaged sections of heart. Myocardial infarction induced damage triggers deposition of collagen causing scar formation and ventricular remodeling which adversely affect cardiac function and pumping capacity. Metalloproteinases,

secreted by MSCs, can trigger matrix degradation and down-regulate type I and type III collagen synthesis, leading to reverse ventricular remodeling [9]. Studies suggest that MSCs may impart cardiac repair through secretion of extracellular vesicles termed exosomes. These vesicles are composed of proteins, mRNA, miRNA and lipid molecules. The exosomes mediate inter-cellular communications through exchange of genetic materials and signaling proteins. They can promote angiogenesis through transfer of mRNA and reduce the cardiac infarct size [10].

The case discussed here demonstrates restoration of ventricular function in a patient treated with adipose derived MSCs. MSCs offer therapeutic effects in diseased organs through paracrine secretion. Their mode of action entails improvement in blood supply; recruitment of endogenous stem cells to infarct regions of the heart and reinstatement of ventricular remodeling. Further, the immune privileged character and immune modulating capability make MSCs a safe candidate for allogenic transplants also. However, the desired outcome for a prolonged period can only be achieved if transplanted cells contribute to biological functions and the engraftment survives successfully.

### Acknowledgements

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