The Effect of Human Bone Marrow Mesenchymal Stem Cells on Diabetic Heart Failure Rats.

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Abstract: Aim: The purpose of this study was to investigate the effect of bone marrow mesenchymal stem cells (MSCs) on cardiovascular complications of type 1 diabetes mellitus (DM) in rats associated with heart failure. **Material and Methods:** BM-MSCs were derived from the human bone marrow. The MSCs were characterized morphologically and by RT-PCR for CD29 expression. They were then infused into rat tail vein which were they were made diabetic by IP injection of streptozotocin (STZ) and also we induce heart failure through injection of adramycin. The rats were divided into control, diabetic(D), and diabetic and heart failure(D_HF) plus MSC groups where D-HF rats injected with human bone marrow derived stem cells(BM-MSC). Serum glucose, insulin, and fibrinogen were estimated in all groups. Physiological cardiovascular functions :Systolic and diastolic blood pressure, echocardiography were assessed. Homing of BM-MSCs in cardiac tissue and histological examination were done at the end of the experiment. **Results:** Diabetic rats which received MSCs showed significantly lower serum glucose and increased serum insulin levels compared with the D- HF group. Improvement of cardiovascular performance was also observed in the D-HF group compared with the D group. Homing of stem cells was detected in cardiac tissues of the BM-MSC group. **Conclusions:** Human bone marrow harbors cells that have the capacity to differentiate into functional insulin-producing cells capable of controlling blood glucose level in diabetic rats. Furthermore, MSC transplantation can improve cardiac function in diabetic rats associated with heart failure.
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Key words: Human bone marrow derived mesenchymal stem cells, diabetes, steptozotocin, heart failure, adramycin, rats.

1. Introduction

Diabetes mellitus (DM) is a strong risk factor for cardiovascular complications. The development of macrovascular diseases (coronary artery disease, peripheral vascular disease, and stroke) in both diabetes mellitus type 1 (DMT1) and diabetes mellitus type 2 (DMT2) is 2–4 times more common compared to non-diabetics when all other cardiovascular risk factors are taken into consideration **[1, 2].** DM associated with characteristic structural and functional changes of the myocardium independent of coronary atherosclerosis is termed diabetic cardiomyopathy (DCM). DCM is a microvascular disease in which chronic hyperglycemia and elevated glycosylation end products are contributing factors [**3**]. Heart failure (HF) is more commonly seen in individuals with diabetic cardiovascular complications. In this context, DCM is increasingly recognized as a cause of HF **[4, 5].** Diabetes therefore independent of coronary artery disease, carries an increased risk of HF **[6].**

Mesenchymal stem cells (MSCs) are adult stem cells derived from different tissues including bone marrow and adipose tissue. They have the capacity of self renewal and differentiation into multiple cell lineages, including but not limited to chondrocytes, osteoblasts, myoblasts and adipocytes **[7].** Bone marrow derived mesenchymal stem cells (BM-MSCs) have been implicated in cardiovascular repair. In vitro studies have demonstrated their capacity to morphologically and functionally transdifferentiate into myocyte resembling cells **[8, 9]**. However, it is still unclear whether these observations contribute to their reparative effects following transplantation **[10, 11, 12].** This is further complicated by low rates of myocardial retention and survival **[13]**. Alternatively mesenchymal stem cells may facilitate endogenous repair processes where paracrine actions may underlie much of this reparative potential **[14, 15].**

In clinical settings, stem cell transplantation is a promising therapy for patients with heart failure where most conventional therapies cannot replace for the substantial cell loss. In this regards, human BM-MSCs have demonstrated a moderate efficacy probably due to paracrine effect of engrafted BM-MSCs on residual host cardiomyocytes [12] or neovascularization [13, 14]. However, data regarding the use of human BM-MSCs in diabetes with heart failure are still lagging. We previously reported that rat BM-MSCs improved cardiac function in animal

models of diabetes and cardiovascular complications **[16].**

Recently, human adipose-derived stem cells **(**AT-MSCs) have also gained great interest for their application in cardiac repair. Besides their ability to differentiate toward mesodermal, mesenchymal and neuronal phenotypes**[17]** their reduced immunogenicity **[18]** make them of special interest. In myocardial infarction, transplantation of AT-MSCs has been shown to induce neovascularisation, reduce infarct size and scar formation [**14].**

We therefore aimed to evaluate the potential effect of human BM-MSCs in an experimental model of diabetes and heart failure in rats.

2. Materials and Methods

1. Expansion of Bone Marrow MSCs:

Bone marrow cells were obtained from iliac crest aspirated from healthy donors giving their BM for allogeneic transplantation purposes, after informed consent, and were used in accordance with the procedures approved by the human experimentation and ethics committees of Faculty of Medicine, Cairo University. Samples were hydrostatically expelled from the bones with complete medium, consisting of Dulbecco's Modified Eagle's Medium (DMEM) containing selected lots of 10% calf serum and antibiotics (100 U/ml penicillin G, 100 µg/ml streptomycin, 0.25 mg amphotericin B, all obtained from Gibco laboratories) in a humidified atmosphere of 5% CO_2. The marrow plugs were disaggregated and the dispersed cells were centrifuged and re-suspended twice in complete medium. Mononuclear cell separation was done using Ficol Hypaque density gradient centrifugation. Mononuclear cell (MNC) layer was aspirated, washed in phosphate buffered saline, and cell count and viability performed. 1 million MNCs were plated in T25 flasks in complete medium as previously described. Cultures were incubated in 5% CO2 incubators at 37ºC. Medium was replaced every 3 days and the non-adherent cells discarded at day 5. Each primary culture was twice divided into three new plates and cultured until the cell density of the colonies grew to approximately 90% confluence**.** At this point, cells were dislodged using 5 mL 0.25% trypsin-EDTA for 5 minutes at 37ºC, followed by the addition of 1ml foetal calf serum to stop the action of trypsin. Cells were then washed by phosphate buffered saline (PBS) and subsequent counting and viability testing using trypan blue exclusion test was done.

2. Flow Cytomeric Analysis of Cell Surface Antigens

Analysis of cell surface molecules (CD34, CD44) and intracellular molecules (Oct 3/4) was performed on cultured stem cells after being detached and suspended in PBS. On the day of analysis, unattached cells in cultures were washed out with PBS, and adherent cells were trypsinized. After washing, cells were suspended in 0.5% BSA in PBS at a concentration of 4 × 104 per mL and incubated in blocking buffer (containing 25 µg/mL IgG) for 10 min followed by 40-min incubation with monoclonal antibodies labeled with fluorescein isothiocyanate (FITC) against CD34, CD44 (Beckman Coulter, France) and acquired onto FACSCalibur (Beckman Coulter, NE15106, USA). For intracellular molecules (Oct 3/4),the cells were incubated with phycoerythrin (PE)-conjugated antihuman/ mouse Oct 3 /4 monoclonal antibodies after fixation and permeabilization (R & D Systems) and acquired onto FACS Calibur (Beckman Coulter, NE15106, USA).

3. Stem cell labelling:

For tracking of stem cells in cardiac tissue, bone marrow derived mesenchymal stem cells were labelled using green fluorescent protein GFP (EzWay^{TM} Transfection Reagent, Komabiotech). For initial optimization of transfection, ratios of plasmid DNA and EzWayTM Transfection reagent were constructed according to manufacturer instructions, incubated for 30 minutes at room temperature and directly added to cultured cells in a final volume of 1000µl/100mm. Cells were then incubated for 24-48 hours to choose the highest transfection ratio prior to testing **[19, 20].**

4. Animals:

Forty male Wistar rats weighing 200-220 gms were used in this study. Animals were kept in the animal care facility of the Faculty of Medicine, Cairo University. Animals were kept in chip-bedded cages at room temperature under a 12:12-hr light –dark cycle and were given free access to standard rat chow and water for the entire duration of the study. The experimental protocol and procedures were approved by the Institutional Animal Care and ethical committee, kasr Al-Aini Faculty of Medicine, Cairo University.

Animals were randomly allocated into the following groups: Group 1, Control (C) group (n = 10) injected by vehicle of normal saline (0.2 ml i.p.), for six equal doses over 2 weeks. Group 2**,**Diabetic (D) group (n=10 rats) injected by a single dose of streptozotocin (STZ) to induce diabetes mellitus (65 mg/kg body weight i.p., MP Biomedicals, stored at +4^{o}C in sodium citrate buffer, pH 4.5) **[2].** Group3,Diabetes and heart failure group (D-HF group),(n =10) received single dose of streptozotocin (STZ, MP Biomedicals; 65 mg/kg body wt ip)**,** followed after four weeks by Adriamycin (doxorubicin hydrochloride, Pharmacia Italia;2.5mg/Kg body wt ip) dissolved in saline [**21**] to induce heart failure. Group 4, Bone marrow group

(BM-MSCs**),** (n= 10) received bone marrow derived mesenchymal stem cells(1 ml of 2,000,000stem cells intravenously in rat tail vein [**22]** following induction of diabetes and heart failure. At the end of the experimental study, blood samples were collected for blood glucose, serum insulin and fibrinogen (as a marker endothelial dysfunction marker). Heart samples were also collected for subsequent histopathological analysis and detection of stem cells homing.

5. Functional assessment by Echocardiography:

Was performed in all groups to evaluate the cardiac functions in vivo to provide a correct image of the state of the heart. The rats were lightly anesthetized with intra-peritoneal injection of ketamine hydrochloride (25 mg/kg, ip) and xylazine (5 mg/kg,ip.) **[23]**. Echocardiograms were performed with an echocardiography system equipped with a 12-MHz phased-array transducer (SONOS 5500; Philips Medical System, Best) placed over the left parasternal area and rocked through the heart from the apex to the base. Two-dimensional short axis view of the left ventricle and M-mode tracings were recorded to measure Left ventricular end-diastolic dimension (LVEDD) and Left ventricular end systolic dimension (LVSD). Fractional shortening (FS) was calculated from the composite LV internal diastolic (LVEDD) and LV internal systolic (LVSD) dimensions **[24].**

$$FS = \frac{\text{End-diastolic dimension} - \text{End-systolic dimension}}{\text{End-diastolic dimension}} \times 100\%$$

6. Noninvasive blood pressure measurements and echocardiography: were performed for all rats successively at the beginning, 4weeks after induction of diabetes, 4 weeks after induction of heart failure and then 4weeks following stem cells injection. The mean arterial blood pressure (ABP) was recorded in conscious rats using the tail-cuff method (Harvard 50–9331 Rectilinear Recording System; Harvard Apparatus, Kent, UK). At least three consecutive readings were obtained and averaged for each rat **[25]**

7. Immunofluorecence histochemistry

The presence of GFP protein in mouse tissue sections was assessed by a rabbit anti-GFP antibody. The human origin of these cells on mouse tissues was assessed by an antibody directed against human _2-microglobulin. Tissues were fixed in 4% neutral buffered formaldehyde for 16 hours at room temperature and were then embedded in paraffin. Sections (5 _m) were deparaffinized and rehydrated and then permeabilized for 5 minutes in 0.1% Triton/PBS at room temperature. Slides were incubated for 30 minutes with rabbit anti-GFP polyclonal antibody diluted at 1:1000. Fluorescence-labeled goat antirabbit immunoglobulin G (IgG) antibody was used. Immunofluorescence relativities were visualized

8. Histological examination:

The rats were sacrificed and hearts were harvested for following histological examination in order to determine the results of the stem cells transplantation. Tissue sections were underwent fixation, sectioning, and staining with hematoxylin-eosin to visualize the general morphology. These microscope slides were stored in a freezer after sectioning and staining in order to preserve the heart sections.

9. Statistical Analysis:

Data was coded and entered using the statistical package SPSS (V.15.).Data was summarized using: Mean, SE, SD and range (minimum and maximum) for quantitative variables. Comparisons between groups were done using Analysis of Variance (ANOVA) and multiple comparisons by (Post-Hoc test, Bonferroni : conservative and non conservative types) for normally distributed quantitative variables. While quantitative variables which are not normally distributed were compared using non parametric tests (Kruskal-Wallis test, Mann-Whitney test). Correlation was done to test for linear relations between quantitative variables. P-values less than or equal to 0.05 were considered statistically significant.

3.Results

Adherent MSC was characterization using flowcytometric analysis. For tracking of stem cells in cardiac tissue, bone marrow derived mesenchymal stem cells were labeled using GFP. Homing of BM.MSC in cardiac tissues was detected using immunofluorecence histochemistry.

Our results showed a significant increase in body weight in control group (P<0.05). There was significant weight loss in diabetic and AD treated groups. After 4weeks of induction of diabetes and heart failure,treatment of diabetic or D-HF group with BM- MSCs resulted in a significant (P<0.05) increase in body weight 4weeks after treatment compared to pretreated level (Fig 1).

Table (1) showed that there is a significant reduction in both systolic and diastolic ABP in D-HF group after 4 weeks of administration of the adramycin. BM- MSCs treated group showed restoration of both systolic and diastolic ABP towards pretreated values.

Fig (2) showed significant increase in blood glucose level in diabetic and D-HF groups 4 weeks after induction of HF. Treatment of D-HF group with

bone marrow BM- MSCs resulted in a significant (P<0.05) decrease in blood glucose 4weeks after treatment compared to pretreated level.However, glucose level is still significantly less than control level. Insulin was also found to decrease after induction of diabetes and diabetic heart failure and increases significantly after treatment with BM-MSCs, *P*<0.05 (Fig 3),but it did not increase back to control level.

As a marker of endothelial dysfunction fibrinogen was found to increase in D and D-HF groups in comparison to control. Administration of BM- MSCs brings fibrinogen level back to control level (Fig 4).

***In vivo* cardiac function assessment: Echocardiography:**

Table (2) showed that there is no significant changes in LVEDD and posterior wall thickness followed administration of AD. However,. Administration of bone marrow derived stem cells resulted in a significant increase in FS % (Fig 2). LVESD (Table 2 and Fig 5A-C) also showed a significant (*P*< 0.05) increase in D-HF treated group compared to control. Administration of BM-SC also significantly reduced LVESD in comparison to heart failure group but not to control level (Fig 5 D).

Histopathologic analysis:

Histopathologic examination of cardiac tissue of control group (Fig 6 A) showed normal cardiac myocytes with connective tissue septa typical of intercalated discs. Single cardiomyocytes were stained acidophilic with central nuclei**.** Cardiac tissues from AD treated animals showed scattered areas of destructed myocytes, sarcoplasmic vacuoles and flat pyknotic peripherally located nuclei. There was also widening of intercellular spaces with interstitial hemorrhage. Marked mononuclear cell infiltrates was also observed. (Fig 6 B) Improvements were noticed in group treated with bone marrow derived stem cells (BM-MSCs) (Fig 6 C). The myocytes had normal staining pattern(moderate acidophilic) and the intercellular spaces were of average size. All the nuclei were centrally located, vacuolated pyknotic nuclei and interstitial hemorrhage could not be detected in all examined slides.

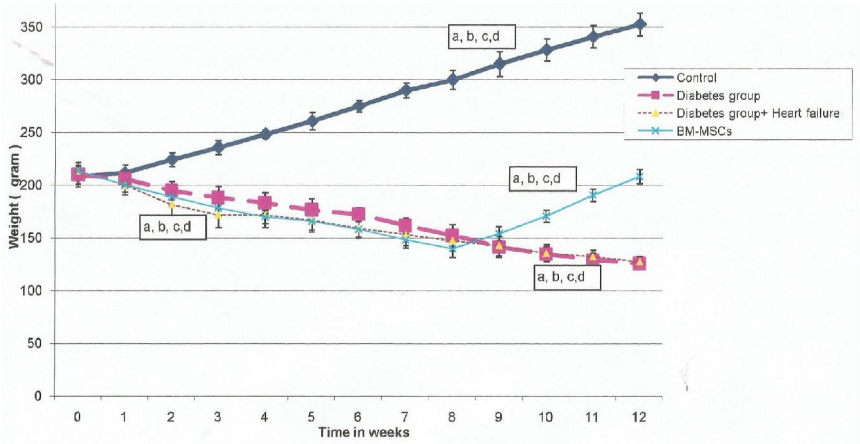

Figure (1). Weight gain (gm.)differences between groups at baseline, 8th and 12th week.

Table (1): ABP differences between groups at baseline, 8th and 12th week. Check letters with Yazeed

	Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Groups	**Base**	**8th w**	**12th w**	**Base**	**8th w**	**12th w**
Control						
Mean	114.17	126.67	126.67	78.33	83.33	83.33
±SD	±4.92	±8.16(a-b)	±8.16(a)	±4.08	±8.16(a-b)	±8.16(a)
Diabetic						
Mean	123.33	121.67	125.00	80.83	83.33	83.33
±SD	±8.16	±7.53(c-d)	±8.37	±6.65	±7.53 (c-d)	±5.16(c)
D-HF						
Mean	123.33	100.00	85.00	84.17	59.17	50.00
±SD	±5.16	±8.94(a-c)	±5.48(a-d)	±4.92	±8.01(a-c)	±5.48(a-c-d)
BM-MSCs						
Mean	124.17	91.67	123.33	78.33	50.83	79.17
±SD	±11.14	±7.53(b-d)	±5.16(d)	±7.53	±10.68(b-d)	±3.76(d)

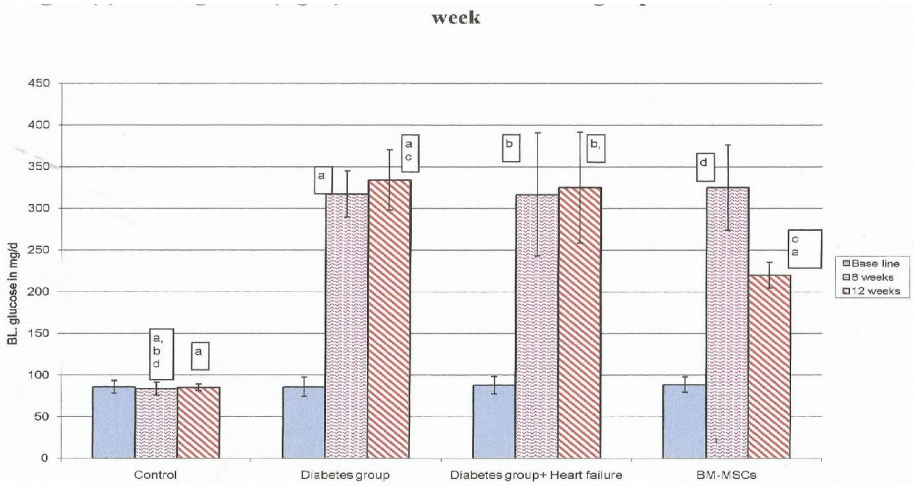

Figure (2). Blood glucose (mg/dl) level differences between groups at baseline, 8th and 12th week.

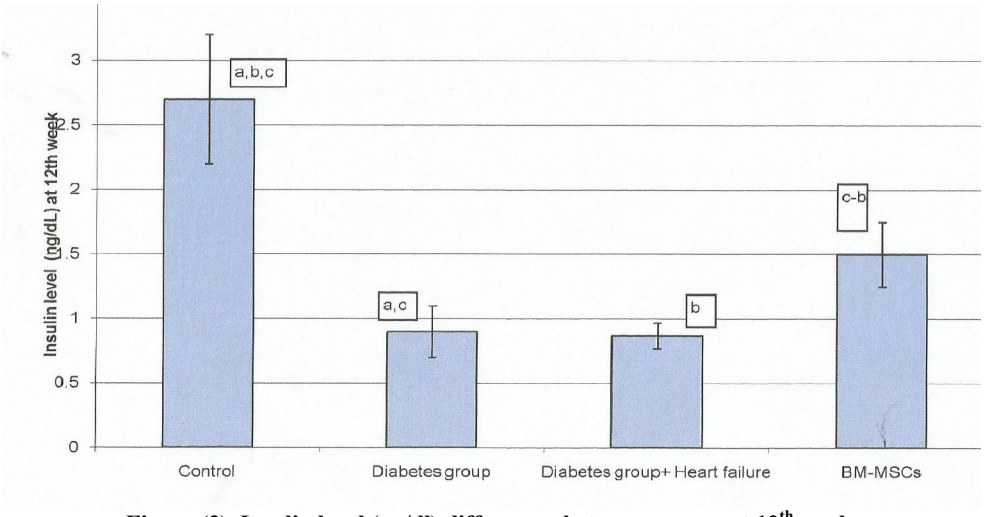

Figure (3). Insulin level (ng/dl) differences between groups at 12th week.

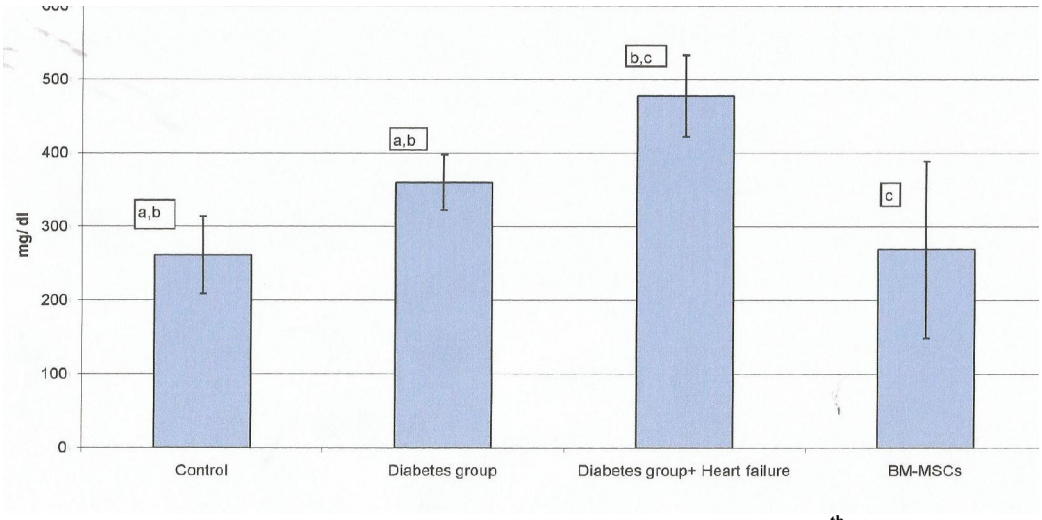

Figure (4) Fibrinogen (mg/dl) level differences between groups at 12th week.

Table (2) Results of Echocardiography Examination.

		Control mm		Diabetes mm		Diabetes + Heart failure mm		BM-MSCs mm		P
LVEDD	**Base**	3.9±	1.5	3.8±	1.2	3.6±	0.6	3.9±	0.7	0.799
	8W			3.5±	0.7	3.6±	0.8	4.8±	1.5	0.245
	12 W			3.7±	0.5	4.6±	1.6	4.2±	1.1	0.26
LVESD	**Base**	1.1±	0.2	1.3±	0.3	1.2±	0.3	1.2±	0.5	0.56
	8 W			1.4±	0.4	1.7±	0.9	1.9±	1.0	0.27
	12 W			a1.5±	0.2	a-b 2.7±	0.2	b 1.7±	0.4	0.03
Posterior wall	**Base**	1.7±	0.6	1.1±	0.5	1.1±	0.3	1.5±	0.5	0.315
	8 W			1.1±	0.4	1.1±	0.3	1.5±	0.4	0.403
	12 W			1.4±	0.6	1.2±	0.3	1.7±	0.4	0.517

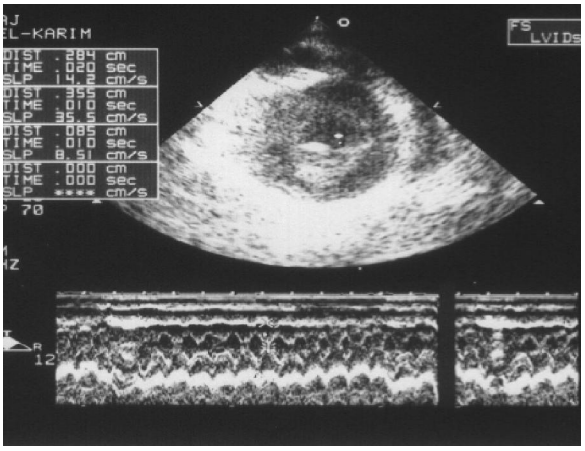

Fig (5 A): Control group at base line. Echocardiography showed the following values : FS: 83.3 %, LVEDD= 4.8 mm, LVESD= 0.8mm and post wall thickness = 2.8mm.

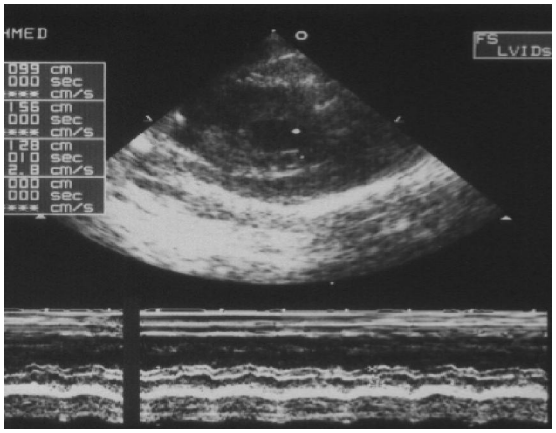

Fig (5C) Diabetes -Heart failure group at the 8thweek of the study before BMSC injection. Echocardiography showed the following values : FS: 32.5%, LVEDD: 4.00mm, LVESD: 2.7mm and, post. wall thickness : 0.9mm

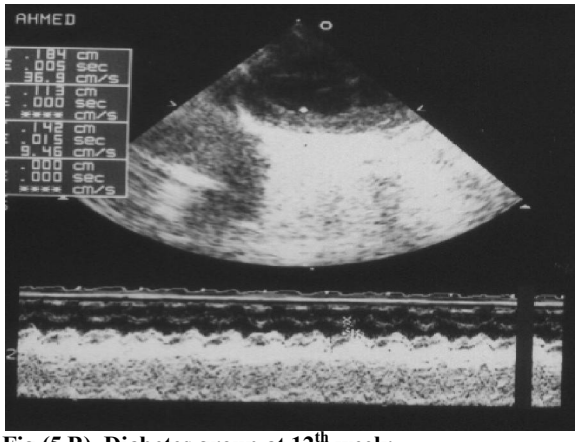

Fig (5 B). Diabetes group at 12th week: Echocardiography showed the following values;: FS: 56.4%, LVEDD: 3.9mm, LVESD: 1.7 mm and post. wall thickness : 0.7 mm. LVED D is not displayed in picture.

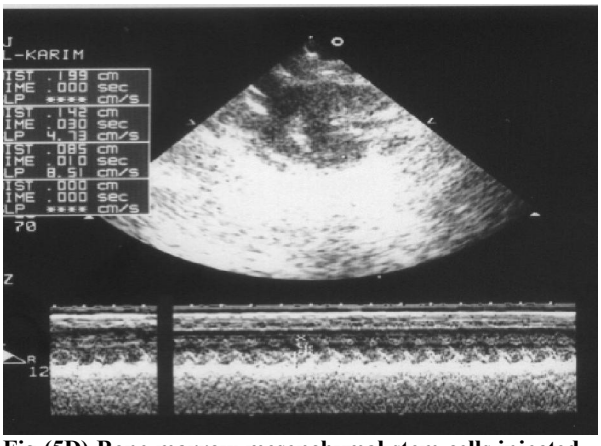

Fig (5D) Bone marrow mesenchymal stem cells injected group at 12th week. Echocardiography showed the following values : FS: 61.5 %, LVEDD: 3.4mm, LVESD: 1.3mm and post. wall thickness : 1.4mm

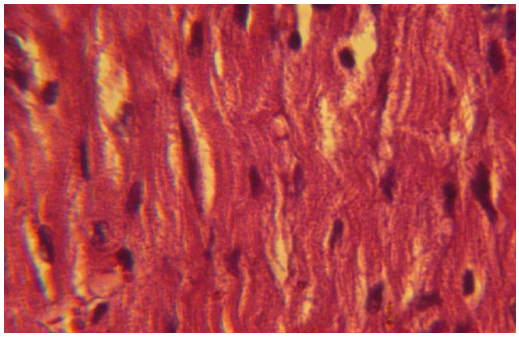

Fig (6A) Control group: longitudinal section in cardiac tissues, the myocytes stained moderate acidophilic with apparent intercalated disc. H&EX400

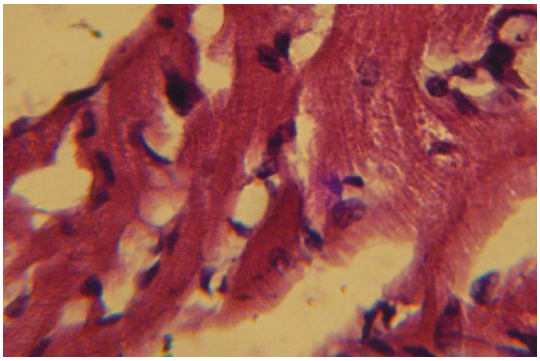

Fig (6 B) AD group : Cardiac tissues revealed intracellular vacolation, homogenous acidophilic sarcoplasm, destruction of myofibers and flat pyknotic nucleus. H&EX400

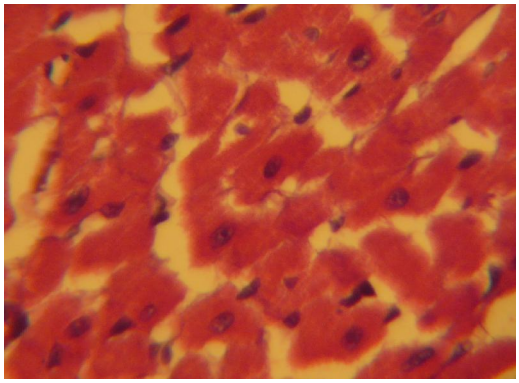

Fig (6 C) BM-MSCs treated group : Cardiac tissues revealed unremarkable intercellular spaces. All the nuclei are central. H&EX400.

4.Discussion:

Hypertension, obesity, dyslipidaemia, microalbuminuria, endothelial dysfunction and diabetic cardiomyopathy are amongst the many factors that contribute to the high prevalence of cardiovascular disease in human diabetes. In both type I and type II cardiovascular diseases especially remain the major cause of a 2-3 fold increase in such complication over age and gender matched non diabetic patients **[26].**

Since diabetes and cardiovascular disease lead to high mortality and severe morbidity with its impact on society and economy and since treatments are just supportive and not curative, myocardial infarction and cardiomyopathy are still without effective treatment. Accordingly the search of curative treatments to restore pancreatic and cardiac functions in diabetes mellitus remained a major goal in medical therapy.

Regenerative medicine is the field of science that attempts to change the course of chronic disease and in many instances will regenerate tired and failing organ systems or congenital defects **[27]** by creating living functional tissues and stimulating irreparable organs to heal themselves **[28].** It has the potential to solve the problem of organ transplantation including availability of donors and rejection. Examples include: cell therapies, induction of regeneration by biologically active molecules and tissue engineering **[29].**

Mesenchymal stem cells have a number of attractive characteristics, including (a) the ease with which they can be cultured to the high numbers needed for transplantation, (b) apparent potential for mediating both myocardial and vascular repair, and (c) immunoregulatory properties, which may enable their use as an allogeneic treatment. Animal studies of MSC transplantation in myocardial infarction and ischemic cardiomyopathy have reported wide-reaching biological and functional benefits that include attenuation of myocardial scar and infarct size, improved regional and global ventricular function, restoration of myocardial mechano-energetics, and increased vascular density and myocardial perfusion. There has also been evidence of benefit in models of nonischemic, dilated cardiomyopathy, arrhythmia, and valvular disease **[30, 31.**

In the present study we evaluated the effect of bone marrow derived stem cells on cardiovascular complications in diabetic rats complicated with heart failure as reflected on their effect on cardiac and pancreatic functions, aiming to derive a therapeutic interventional model that can effectively be used specifically in regenerating cardiac and pancreatic tissue during the course of diabetes mellitus.

In our study we chose a model of adriamycin induced heart failure in association with experimental diabetes since rat models of diabetes mellitus show high resistance to CVS complications which have been only demonstrated over extended durations. This was confirmed by our study that after 3 months we could not find any cardiovascular complications detected by regular time fixed monitoring of arterial blood pressure (every 4 weeks), by echocardiography and by heart perfusion at the end of the experimental

period(unpublished data). Michael Wei, et al, 2003 detected that rats do not develop atherosclerosis and remain normotensive -unlike human diabetics- at least over a 24 weeks observation period. However, these characteristics of the chronic STZ-diabetic rat allow investigation of hyperglycaemia-induced changes which are independent of the development of atherosclerosis and hypertension.

Echocardiographic differences in performance have been detected in the two widely used diabetic rat models, viz STZ-diabetic Wistar **[32]** and STZ-diabetic Sprague-Dawley **[33]** rats. The differences in DCM susceptibility and cardiac performance may underlie the manifestation of cardiac functional abnormalities in these models of type 1 diabetes.

Various toxic cardiac agents (catecholamines, adriamycin, amphetamine, and radiation) can cause a significant deterioration of cardiac function and eventually heart failure **[34, 35].** Adriamycin induced heart failure has been chosen as its mechanism in inducing cardiac alterations is somewhat similar to those associated with diabetic cardiomyopathy **[36]**. Extracellular matrixes with increased cardiac fibrosis **[37],** excessive generation of reactive oxygen species **[38],** as well as cardiac inflammation **[39],** and finally cardiac and endothelial cell necrosis/apoptosis are suggestive mechanisms for diabetic cardiomyopathy **[40].** Similarly adriamycin induced myocardial alterations include oxidative stress, mitochondrial DNA damage, intracellular calcium overload, inhibition of protein synthesis, disturbance of myocardial adrenergic function, cytokine release, myofibrillar degeneration,endothelial and cardiomyocyte apoptosis **[41, 42].**

Measurement of body weight showed that all over the our study period control rats had normal weight gain while all groups subjected to heart failure and diabetes had significant weight loss. **43, 44, 45];** found a significant loss of body weight in diabetic rats. Also a significant reduction in body weight in diabetes and heart failure was observed by Li and colleagues**[46]**. Meanwhile, Dong **[47]** reported an increase in body weight of diabetic rats 4 weeks after injection of allogenic BM_MSCs. Stem cells treatment in our study displayed a significant increase in body and it was positively correlated with insulin level. (r= !!!).

Diabetic –HF group showed significant decrease in both systolic and diastolic blood pressure in comparison with control group. This was supported by one of the most recent studies used adriamycin for induction of heart failure done by Kalender and his colleagues[**48].** A drop in both systolic, diastolic and mean pressure concomitant with reduction of all myocardial contractile parameters assessed by isolated heart perfusion studies(unpublished data). Treatment with stem cells showed significant improvement in blood pressure, this was concomitant to improved myocardial contractility showed by increased FS%.

During the study pancreatic function and possible recovery was monitored by measurement of fasting serum blood glucose as well as single insulin measurement done at the end of the study period. Significant diagnostic levels of blood sugar and insulin;which were negatively correlated, were observed in D and D-HF groups. We also detected a significant reduction in blood glucose level;which negatively correlated to insulin levels; 4weeks following bone marrow stem cell injection. In BM-MSC group, serum insulin level was correlated positively to improved body weight and cardiac parameters. Yang [**49]** similarly reported a significant reduction in blood glucose levels after one week following MSC transplantation in STZ- induced diabetes in rats.

Chen Li-Bo **[50]** found that MSCs could successfully differentiate in vitro into pancreatic islet β-like cells. These cells were morphologically similar to pancreatic islet cells. More importantly, they could also transcript, translate and excrete insulin. **Lee [51]** detected that in STZ-induced diabetic NOD/SCID mice that were repeatedly transplanted with human MSCs via intra- cardiac infusion showed increased production of endogenous β cells and higher levels of mouse circulating insulin. This was associated with decreased blood glucose level. More recently, in a model of murine STZ-induced diabetes, concomitant administration, via a single injection, of BM cells with syngeneic or semi-allogeneic MSCs normalized blood glucose and serum insulin levels, and allowed regeneration of recipient-derived pancreatic insulin-secreting cells due to the immunosuppressive effect of MSCs on the β cell-specific T-lymphocyte response **Urbán[52].**

At the end of the experimental study we further evaluated the effect of experimental diabetes and bone marrow derived stem cells on endothelial function assessed by measuring serum fibrinogen level. Estimation of serum fibrinogen has been repeatedly used as a biomarker for endothelial dysfunction in diabetes mellitus **[53, 54].** Diabetes induced endothelial dysfunction as detected by elevated levels of fibrinogen was confirmed in groups D and D-HF groups, in accordance with Giovanni *et al.* **[55]** and Barillari *et al.* **[56].**

Vascular endothelial dysfunction is a common sequel for diabetes mellitus. Oxidative stress and oxidative damage with lipid peroxidation of endothelial cell membranes are important

contributing factors. A direct link to insulin deficiency has been elucidated by Oelze **[53] .** Where Insulin therapy to long established (6weeks) STZ-diabetic rat model, completely normalized blood glucose, body weight, vascular dysfunction and oxidative stress after 2weeks therapy. This was also associated with attenuation of increased cardiac reactive oxygen and nitrogen species formation in diabetic rats. Following bone marrow stem cell therapy, the present study demonstrated a significant improvement in fibrinogen level which was negatively correlated to insulin levels.

A study by, Zhang **[57]** detected that significant increase of serum insulin levels leads to endothelial cell protection, and this is accompanied with enhanced myogenesis, angiogenesis, and attenuation of cardiac remodeling, all of which are crucial for the improvement of cardiac function in diabetic animals.

To evaluate bone marrow stem cell therapy on cardiac performance in the present study, all experimental groups were subjected to baseline echocardiographic examination. Cardiac functional changes and response to stem cells was further monitored. Four weeks after Ad administration our work showed significant reduction in FS% and significant increase in LVESD.This agrees with reports of Ueno and colleagues**[58]** who found reduced FS % value after 4 weeks of adriamycin administration(a dose similar to that used in the present study)

Bone marrow stem cell injection to AD-HF group improved myocardial contractility 4 weeks after injection. This was reflected by a higher FS% and reduction in LVESD.

In accordance with our data, Zhang and coworkers**[36]** studied cardiac performance 4weeks after bone marrow mesenchymal stem cell injection in a rabbit model of Adriamycin induced cradiomyopathy. EF% increased in transplanted group in comparison with those in the control group. At the same time point taken for cardiac functional examination, histopathological analysis of cardiac tissues was also performed and showed positive staining for differentiated cardiac- like cells. Similarly Zhang and colleagues**[37]** utilized a model of STZ-diabetic cardiomyopathy. Intravenous MSCs transplantation into this model significantly increased myocardial arteriolar density and decreased the collagen volume resulting in improved cardiac function.

Indeed, a beneficial effect of stem cells administration has been reported in experimental models of myocardial infarction. Zhang **[59]** reported beneficial effects of tail vein transplanted MSC stem cells in a rat model of acute myocardial infarction. Echocardiographic examination was 70% higher in treated groups. Their study also concluded that stem cell effects were mediated primarily through preservation, not regeneration of cardiac myocytes within the infarct zone.

Regarding comparative analysis of both subtypes of mesenchymal stem cells van der Bogt**[60]** evaluated the effect of bone marrow, adipose tissue and fibroblast intramyocardial injection into a mice model of myocardial infarction. Cardiac function was monitored by echocardiography 2, 4 and 6 weeks following infarction and invasive pressure- volume loop analysis at the time of sacrifice. Results of their study showed that at the 6th week of stem cell therapy both adipose and bone marrow stem cells showed equal trends towards improvements in LVFS%. Similarly stroke work and cardiac output derived from pressure volume loops showed similar results for both bone marrow and adipose derived stem cells. An important finding from this study, however, is that both BMMSC and ATMSC did not tolerate cardiac environment and based on their quantitative measurements using bioluminescence imaging (BLI) signals, both cell types have been reported to die at the 6th week of transplantation.

Despite growing data concerning stem cell potential effects in myocardial ischemia there is no comparative functional data evaluating in -vivo behaviour of both subtypes regarding their potential effect in diabetic cardiomyopathy.

The present study evaluated the in vivo effect of BM-MSC in ameliorating cardiac dysfunction in diabetic cardiomyopathy associated with induced heart failure. Results of our present work showed that stem cell survive and 4 weeks after their administration associated with significant improvement in cardiac contractility and fractional shortening during this short term duration of treatment.

Xiaohong Wang *et al.* **[61]** used bone marrow-derived multipotent progenitor cell transplantation into hearts with acute myocardial infarction via a transarterial catheter using a swine model and found improvement in ejection fraction after 4 weeks of treatment detected by MRI accompanied by a significant decrease in scar size, low engraftment rate and myogenic differentiation. The improvement in cardiac function was explained by a paracrine and trophic effects of stem cells transplantation ie, the cytokines released from the engrafted stem cells act on the host myocytes and spare them from apoptosis.

Our work also showed that bone marrow derived stem cells were able to home in the diseased cardiac tissue and diabetic pancreatic tissue

(unpublished data).This was detected by fluorescence microscopy showing green fluorescence of the GFP as shown in the results in which the transplanted cells were distinguishable from the cells of the recipient by the GFP expression **[22].**

We have examined the tissue of the heart for pathology for more confirmation of our results so as to detect if there was any regeneration in these tissue compared by the group that did not receive stem cells.

For the pathology of the heart,improvements were noticed in groups treated with bone marrow derived stem cells on pathological bases. Vacuolation, pyknotic nuclei and interstitial hemorrhage could not be detected. The myocytes had normal staining pattern and the intercellular spaces were of average size. All the nuclei were centrally located. This support the possibility that stem cells were capable of scavenging the myocardium form permanent damage. This was compared by adriamycin induced heart failure group 3 which produced a massive change in the myocardium showing a varying degree of vacuolar changes **[62]** in the cardiac muscle fibres. The vacuolated cells were found to be more towards the endocardial surface of the heart. In addition, necrosis of cardiac muscle fibres with isolated cells showing features of hypertrophy in between the necrotic and fragmented muscle fibres was seen.

So, the pathology indicates the potential of mesenchymal stem cells to regenerate the cardiac tissue not completely but regaining the physiological function in the form of blood glucose and insulin level and cardiac contractility.

Concluding remarks:

BM-MSCs successfully ameliorated cardiac function of diabetic hearts. The short term amelioration might be a direct effect of paracrine mediators controlled by stem cells. Also, stem cells were able to protect the myocardium at risk from permanent damage. More animal experimental work is needed to detect the doses, side effects and survival to step to clinical applications safely and successfully. Studies should be further extended to optimize the benefits of stem cells studies to be applied clinically.

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3/5/2013