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Use of Mesenchymal Stem Cells in an Experimental Model of Metabolic Syndrome Complicated with Cardiomyopathy

Histopathological Effects of Mesenchymal Stem Cells Administration on Diabetic Cariomyopathy

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Abstract

Introduction: Obesity is a major global health issue. Most obese patients develop metabolic syndrome, a cluster of clinical features characterized by hypertension, insulin resistance and dyslipidemia. Development of ventricular dysfunction in patients with DM in the absence of CAD, valvular heart disease or hypertension is defined as diabetic cardiomyopathy. A major effort is under way to develop therapies aiming at regenerating the myocardium or to stimulate endogenous repair programs. MSC can differentiate into many mesenchymal cells as cardiomyocytes. The application of MSCs in the treatment of DC in recent years offers promising results.

Aim of the Work: This study is to evaluate the effect of intravenous administration of MSCs on the hearts of obese diabetic rats.

Methods: Sixty male mice were fed a regular diet up to one month of age. Then 20 mice were kept on a regular diet (healthy) and 40 mice switched to a high-fat diet (obese) until the end of the study (16 months of tested diet). Two months following MSC administration the following parameters will be evaluated as: Blood pressure, blood glucose, glycated hemoglobin, insulin, triglyceride and cholesterol, Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C). At the end of the study rats were sacrificed by cut throat and dissected to expose the hearts that were fixed in 10% formalin. Sections from the hearts were stained by (H&E) and Masson's trichrome.

Results: Blood glucose, cholesterol, triglyceride, glycated hemoglobin and insulin resistance was significantly decreased in MScs injected group when compared to obese and diabetic group ($p<0.00$, $p<0.00$). Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C) by real time PCR as molecular markers of cardiac hypertrophy significantly decreased ($p<0.01$, $p<0.01$, $p<0.01$, $p<0.01$). Cardiomyocyte hypertrophy and number of inflammatory cells was significantly decreased ($p<0.01$, $p<0.01$). Also fibrosis decreased but this was statistically insignificant ($p<0.069$).

Conclusion: MScs decrease blood glucose, glycated hemoglobin, insulin, triglyceride and cholesterol, Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C), cardiomyocyte hypertrophy, number of inflammatory cells and fibrosis in DC.

Key Words: *Mesenchymal stem cells – Diabetic cariomyopathy.*

Introduction

METABOLIC syndrome is a complex cluster of obesity-related complications. Dyslipidemia, hypertension, and diabetes or glucose dysmetabolism are the major factors constituting metabolic syndrome. These factors share underlying pathophysiological mechanisms. Severe obesity predisposes to metabolic syndrome and affected patients have an increased risk of cardiovascular disease and mortality. Heart disease remains a major cause of worldwide morbidity and mortality. Despite advances in clinical and surgical care of cardiac patients, current therapies are able to treat symptoms, delay clinical deterioration, and increase survival but are not effective in repair induction in a diseased heart [1-3]. This is the case of cardiomyopathy caused by metabolic diseases like diabetes. Therefore, a major effort is under way to develop therapies aiming at regenerating the myocardium or to stimulate endogenous repair programs [4].

Both types of DM increase the progression of atherosclerosis and the development of macrovascular complications such as coronary artery disease

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(CAD), peripheral artery disease (PAD), and stroke, and these patients have a two to four fold increased risk of fatal myocardial infarction (MI) [5]. Development of ventricular dysfunction in patients with DM in the absence of CAD, valvular heart disease or hypertension is defined as diabetic cardiomyopathy (DC) [6]. DC caused by hyperglycemia causes changes in the diabetic myocardium such as hypertrophy, apoptosis of cardiomyocytes, and abnormal myocardial matrix deposition. Specifically in DC, there are changes in the activity of matrix metalloproteases MMP-2 and MMP-9. Reduced MMP-2 activity results in increased collagen accumulation and increased activity of proapoptotic MMP-9 and subsequent cell apoptosis, capillary density reduction, and poor myocardial perfusion. Other pathological consequences include microcirculatory defects, and interstitial fibrosis [7].

Mesenchymal stromal cells (MSCs) have been highlighted as a new emerging regenerative therapy in recent years. MSCs are progenitors of all connective tissue cells and can differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [8] their differentiation into myocytes and neurons has been also proposed [9]. MSCs can be derived from many different organs and tissues such as bone marrow, adipose tissue, nervous tissue, amniotic fluid, umbilical cord, placenta, menstrual blood, and dental pulps [10]. MSCs express on their surface CD54/CD102, CD166, CD49, CD73, CD90, CD44 and CD105 [11]. MSCs play a role in tissue repair and regeneration as they are able to migrate and home to injured sites, where they act both by regenerating tissues and by secreting trophic factors and paracrine mediators. They also have remarkable immunosuppressive properties secreting cytokines and immunomodulatory substances [12].

The application of MSCs in the treatment of DC (in addition to other CVDs) has received much attention in recent years and MSCs do offer promising treatments due to their direct differentiation to cardiomyocytes and secretion of potent trophic and paracrine mediators, capable of inducing cardio regeneration and cardio protection [13].

This study is to evaluate whether the intravenous administration of MSC modifies cardiac dysfunction of obese rat.

Material and Methods

We did our research in the Stem Cell Research Unit at Biochemistry Department in Al-Kasr Al-Ainy, Cairo University, from January 2015 to August 2016.

Animals:

Sixty male mice were housed at constant temperature ($22 \pm 2^{\circ}$C) and humidity (60%), with a 12:12 hour light: Dark cycle and unrestricted access to food and water. When required, animals were lightly anesthetized with sevofluorane or 60mg/Kg ketamine plus 4mg/Kg xylazine. When sacrificed, animals were deeply anesthetized and received an overdose of ketamine/xylazine (60/4mg/Kg). Animal protocols were approved by the Ethics Committee of Sohag University.

Obesity induction:

All mice were fed a regular diet up to one month of age. Then 20 mice were kept on a regular diet (healthy) and forty mice switched to a high-fat diet (obese) until the end of the study (16 months of tested diet). Regular diet consisted of to 10 cal% fats, 20 cal% proteins and 70 cal% carbohydrates. High-fat diet consisted of to 60 cal% fat, 20 cal% proteins and 20 cal% carbohydrates [14].

MSC isolation and ex vivo expansion:

Six to eight week-old male mice were sacrificed by cervical dislocation. Bone marrow cells were obtained by flushing femurs and tibias with sterile PBS. After centrifugation, cells were resuspended in alpha-MEM supplemented with 10% selected fetal bovine serum and 80 ug/mL gentamicin and plated at a density of $1x10^6$ nucleated cells/cm^2. Non-adherent cells were removed after 72 hours by media change. When foci reached confluence, adherent cells were detached with 0.25% trypsin, 2.65mM EDTA, centrifuged and subcultured at 7,000 cells/cm^2. After two subcultures, adherent cells were characterized and transplanted [15].

Phenotype of administrated MSC:

Since there are currently no consensus markers for murine MSC as there are for human MSC [16], immunophenotyping was performed by flow cytometry analysis after immunostaining with monoclonal antibodies against CD45.2 (FITC-conjugated) from BD Pharmingen, USA, CD11b (PE-conjugated), Sca-1 (APC-conjugated) and CD90.2 (PE-conjugated).

MSC differentiation potential was assessed after cell exposure to standard adipogenic or osteogenic differentiation media for 14 and 21 days, respectively [17]. Images were acquired with a Nikon T1-SM microscope.

MSC intravenous administration:

A total of $0.5x10^6$ MSC were resuspended in 0.2mL of 5% mouse plasma and administered via the tail vein to lightly anesthetized mice. Control animals received 0.2mL of vehicle.

Biochemical evaluation:

Two months following MSC administration the following parameters will be evaluated:

1- Blood glucose, glycated hemoglobin, insulin, triglyceride and cholesterol after four hours of fasting, blood samples were collected from the tail vein of alert mice. Plasma glucose levels were determined. Plasma insulin levels and glycated haemoglobin were assayed. Plasma triglyceride and cholesterol levels were determined. Blood specimens were centrifuged immediately; serum and plasma samples were stored at –80ºC without repeated freeze thaw cycles until they were analyzed. Blood concentrations of cholesterol, triglycerides and fasting blood glucose were measured by routine laboratory techniques. Hemoglobin A1C is determined by chromatographic – spectrophotometric ion exchange methods using kits supplied by Bio Systems S.A, Barcelona, Spain, Cat. N.11045.

2- Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C) by real time PCR as molecular markers of cardiac hypertrophy. Genomic RNA was extracted using commercially available RNA extraction Kit. After extraction, genes were determined by polymerase chain reaction (PCR) through using the following primer for each gene:

1- *p300 gene PCR primer:*

- >XM_006242146.1 PREDICTED: Rattus norvegicus E1A binding protein p300 (Ep300), mRNA product length = 245
- Forward primer 1 CTGGACAGCAGATTGGAGCA 20.
- Reverse primer 1 AAGCTGCTGCTGGATGAGTT 20.

2- *Atrial natriuretic peptide (ANP) PCR primer NPPA natriuretic peptide A:*

- >NM_012612.2 Rattus norvegicus natriuretic peptide A (Nppa), mRNA product length = 242.
- Forward primer 1 CGTATACAGTGCGGTGTCCA 20.
- Reverse primer 1 ATCTATCGGAGGGGTCCCAG 20.

3- *Myocytes enhancer factor 2 (MEF2A and MEF2C) PCR primer:*

- >NM_001014035.1 Rattus norvegicus myocyte enhancer factor 2a (Mef2a), mRNA product length = 497.
- Forward primer 1 TGCATCTTGTGGAAAAGGAACAA 23.
- Reverse primer 1 GTATCAGGGTCTGGGCTGTC 20.
- >XM 006231743.1 PREDICTED: Rattus norvegicus myocyte enhancer factor 2C (Mef2c), transcript variant X13, mRNA product length = 349.
- Forward primer 1 GCAGCAAGAACACAATGCCA 20.
- Reverse primer 1 TGTGGGTATCTCGATGGGGT 20.

Cardiovascular parameter assessment at basal and stress conditions:

Mice were deeply anesthetized and placed in supine position on a thermo-regulated plate. Body temperature was monitored using a rectal thermometer and gaseous oxygen was supplied. Hemodynamic parameters were measured by cardiac catheterization. For cardiac function assessment under stress condition, a plastic tube was introduced into the jugular vein, and dobutamine was infused continuously at 12ng/g/min for two min. Dobutamine is a ß-adrenergic agonist with a high affinity for ß1-receptors expressed in the heart. When systemically administered, it increases cardiac demand, producing cardiac stress.

Histological and morphometric evaluations:

At the end of the study rats were sacrificed by cut throat and dissected to expose the hearts that were fixed in 10% formalin. After fixation longitudinal sections involving both ventricles myocardium were randomly obtained, processed, embedded in paraffin and were cut at 5 micron thickness for histopathological examination with light field microscopy.

The specimens were stained by Hematoxyline and Eosin (H&E) and Masson's trichrome and examined using an Olympus CX41 RF microscope connected to a computer through Olympus digital Camera E-330. Photomicrographs were obtained and processed using cell^B software. Morphometry was conducted using the same software.

To detect hypertrophy of cardiomyocytes the transverse trans nuclear widths of randomly selected cardiomyocytes were measured after calibrating the system in (H&E) stained sections. The mean value of 100 LV cardiomyocytes represents each sample **[18]**.

The number of inflammatory cells was determined by counting them in 10 fields (400x) per heart in hematoxylin-and eosin-stained sections after calibrating the system **[19]**.

To assess fibrosis, the sections were stained with Masson's trichrome and 20 randomly selected fields per section were analyzed (200x). After each field was scanned and computerized with a digital image analyzer, collagen volume fraction was calculated as the sum of all areas containing connective tissue divided by the total area of the image [20].

Statistical analysis:

Data was analyzed using SPSS computer program version 22.0. Quantitative data was expressed as means ± standard deviation, median and range. Qualitative data was expressed as number and percentage. The data were tested for normality using Shapiro-Wilk test. The nonparametric Mann-Whitney test and Kruskal-Wallis test were used for data which wasn't normally distributed. Independent Samples *t*-test and One-way analysis of variance test were used for normally distributed data. Spearman's correlation was used for testing of correlation between different quantitative variables. Chi-Square test was used for comparison between qualitative variables. A 5% level was chosen as a level of significance in all statistical tests used in the study.

Results

We begin our study on sixty rats (20 control and 40 tested animals), the tested animals were fed on high fat diet for 16 months. Fourteen months after high fat diet only 30 rats were alive of the tested animals; 16 rats were injected with MScs intravenously and the remaining 14 rats were injected with the vehicle only. Two months after injection (at the end of the study) we only had 46 animals; 18 (healthy or control group), 14 metabolic syndrome complicated with cardiomyopathy (obesity induced diabetic cardiomyopathy group), and 14 (MScs injected group).

Biochemical results:

Blood glucose, glycated hemoglobin, insulin, triglyceride and cholesterol were significantly increased in diabetic group when compared to the control group, also significantly increased in MScs injected group in comparison to the control group but significantly decreased in MScs injected group when compared to diabetic group ($p<0.00$, $p<0.00$, $p<0.00$, $p<0.01$, $p<0.00$) (Table 1).

Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C) by real time PCR as molecular markers of cardiac hypertrophy were significantly increased in diabetic group when compared to the control group, also significantly increased in MScs injected group in comparison to the control group but significantly decreased in MScs injected group when compared to diabetic group ($p<0.01$, $p<0.01$, $p<0.01$, $p<0.01$) (Table 1).

By H&E staining, hearts of the control group showed single, oval and centrally located nuclei of cardiomyocytes with regularly arranged cardiac myofibrils (Fig. 1) in comparison to the diabetic group where the nuclei were hypertrophied and deformed in sizes and shapes and the myofibrils were found to be in disarrayed, fragmented and degenerated with decrease in their staining intensity and vacuolization and infiltrated by inflammatory cells (lymphocytes and histiocytes) (Figs. 2-4) compared to the control group. The MScs injected group showed the same changes observed in obesity induced diabetic cardiomyopathy group but to a lesser extent.

Fat cells were observed inside the cardiomyocytes and in between the muscle bundles forming aggregates much in diabetic group compared to the MScs treated group but they were nearly absent in the control group.

Mean cardiomyocyte width, as a marker of cardiomyocyte hypertrophy was significantly increased in diabetic group when compared to the control group, also significantly increased in MScs injected group in comparison to the control group but significantly decreased in MScs injected group when compared to diabetic group $p<0.01$ (Table 1, Graph 1).

The number of inflammatory cells was significantly increased in diabetic group when compared to the control group, also significantly increased in MScs injected group in comparison to the control group but significantly decreased in MScs injected group when compared to diabetic group $p<0.01$ (Table 1, Graph 2).

We use Masson's trichrome to stain fibrosis (stained green). Fibrosis was assessed by collagen volume fraction in perivascular area, between muscle bundles and in subendocardial region. Fibrosis was absent in the control group but was detected in both the diabetic and the MScs treated groups. Collagen volume fraction was higher in diabetic group than in the MScs injected group but this was statistically insignificant $p<0.069$ (Table 1, Graph 3, Figs. 5-10).

Table (1): Histopathologic and biochemical parameters among the studied groups.

Parameter	Metabolic syndrome complicated with cardiomyopathy (A) (N=14)	MScs treated (B) (N=14)	Control (c) (N=18)	*p*-value	p_1	p_2	p_3
Glucose (mg/dl):							
Mean±S.D.	229.3±35.8	150.9±21.5	90.6±9.9	0.000*	0.000*	0.000*	0.000*
Median (Range)	218 (192-293)	145 (124-180)	87 (81-112)				
Insulin (ng/ml):							
Mean±S.D.	3.7±0.9	1.9±0.3	2.4±0.3	0.000*	0.000*	0.001*	0.000*
Median (Range)	4.03 (2.5-4.7)	1.9 (1.4-2.5)	2.4 (2.02-3.01)				
Cholesterol (mg/dl):							
Mean±S.D.	238.9±34.8	177.9±15.4	153±16.5	0.000*	0.000*	0.000*	0.000*
Median (Range)	226 (201-312)	180 (153-204)	151 (132-181)				
Triglycerides (mg/dl):							
Mean±S.D.	103.3±17.5	89.1±9.9	71.6±12.09	0.000*	0.000*	0.000*	0.016*
Median (Range)	109 (73-121)	91 (75-103)	73 (48-91)				
HBA 1 c (%):							
Mean±S.D.	5.5±1.4	3.9±0.2	4.04±0.3	0.000*	0.000*	0.000*	0.000*
Median (Range)	4.9 (4.1-8.2)	4.03 (3.6-4.3)	4.03 (3.7-4.6)				
P300:							
Mean±S.D.	10.3±4.4	3.5±0.89	1.2±0.28	0.000*	0.000*	0.000*	0.01*
Median (Range)	11.5 (4.2-14.9)	3.01 (2.3-4.8)	1.04 (1-1.9)				
ANP:							
Mean±S.D.	11.04±2.8	4.1±1.06	1.9±0.9	0.000*	0.000*	0.000*	0.01*
Median (Range)	10.7 (5.4-13.6)	3.7 (3.1-6)	2 (1-3.2)				
MEF2A:							
Mean±S.D.	10.7±3.2	3.3±1.3	1.1±0.2	0.000*	0.000*	0.000*	0.01*
Median (Range)	10.2 (5.9-16.2)	2.6 (1.8-6.4)	1.04 (1-1.5)				
MEF2C:							
Mean±S.D.	8.02±4.3	3.4±1.3	1.2±0.3	0.000*	0.000*	0.000*	0.01*
Median (Range)	8.1 (1.8-12.7)	3.01 (2.05-5.03)	1.03 (0.9-1.7)				
Mean cardiomyocyte:							
Mean±S.D.	17.6±.94	15.3±1.4	10.8±1.2	0.000*	0.000*	0.000*	0.01*
Median (Range)	18 (16-19)	15 (13-17)	11 (9-13)				
Inflammatory cells:							
Mean±S.D.	573.6±197.1	180.6±50.9	16.8±7.9	0.000*	0.000*	0.000*	0.01*
Median (Range)	610 (270-915)	185 (80-250)	16 (8-34)				
Collagen volume:							
Mean±S.D.	0.18±0.04	0.14±0.04	0.001±.002	0.000*	0.000*	0.000*	0.069
Median (Range)	0.19 (0.13-0.21)	0.16 (0.07-0.19)	.00 (.00-.007)				

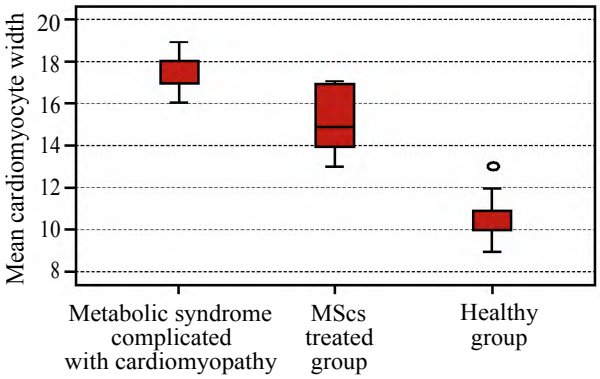
p-value compared the three groups and was calculated by Kruskal Wallis test.

p_1: Compared metabolic syndrome complicated with cardiomyopathy (A) and control (C) groups and was calculated by Mann–Whitney test.

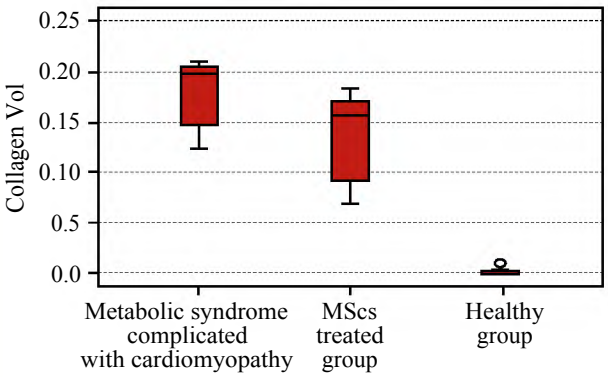
p_2: Compared MScs treated (B) and control groups and was calculated by Mann-Whitney test.

p_3: Compared metabolic syndrome complicated with cardiomyopathy (A) and MScs treated (B) groups and was calculated by Mann–Whitney test.

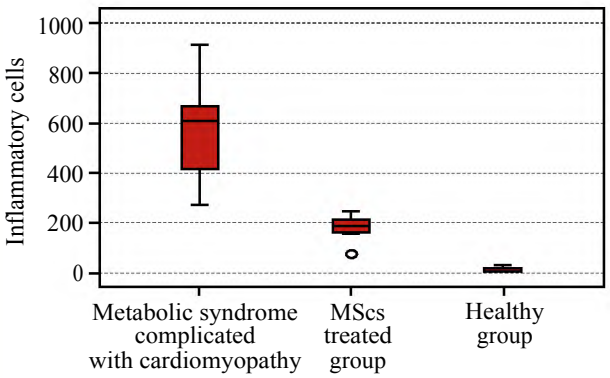
*Statistically significant.



Graph (1): Mean cardiomyocyte width in the three studied groups.



Graph (3): Collagen volume fraction in the three studied groups.



Graph (2): Number of inflammatory cells in the three studied groups.

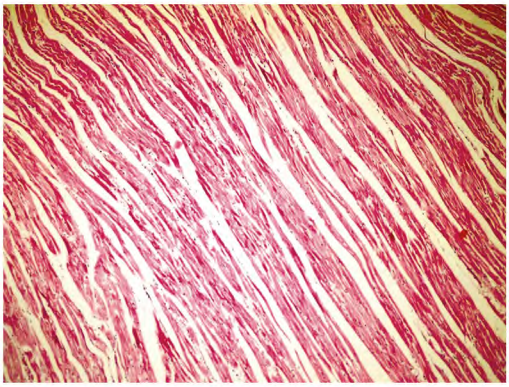


Fig. (1): Cardiomyocytes of the control group infiltrated by lymphocytes (arrow) H&E x 40.

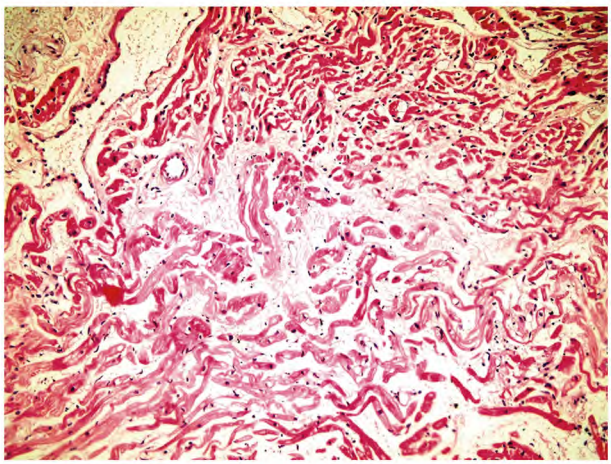


Fig. (2): Cardiac myofibrils in diabetic induced cardiomyopathy group are disarrayed, fragmented, degenerated with decrease in their staining intensity and infiltrated by lymphocytes H&E X 100.

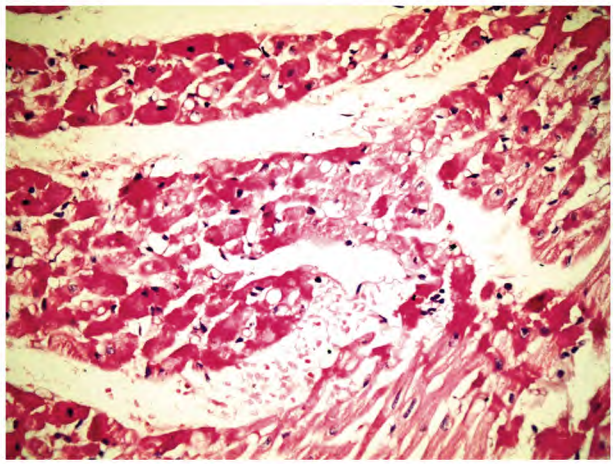


Fig. (3): Cardiomyocytes of the diabetic induced cardiomyopathy group show vacuolization of the cytoplasm (arrow) H&E X 200.

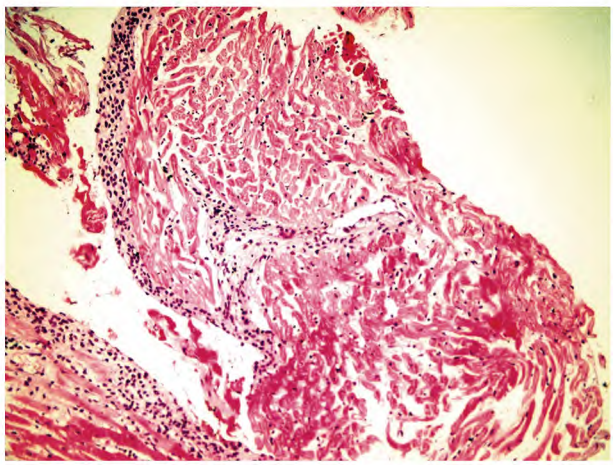


Fig. (4): Cardiomyocytes of the diabetic induced cardiomyopathy group infiltrated by lymphocytes (arrow) H&E X 100.

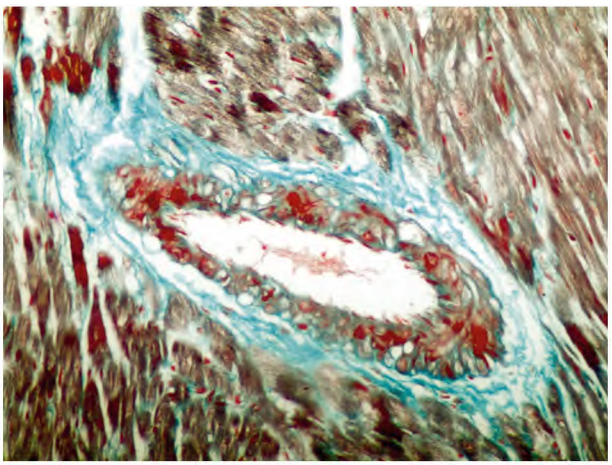


Fig. (5): Perivascular fibrosis in obesity induced diabetic cardiomyopathy group (masson trichrome stain) X200.

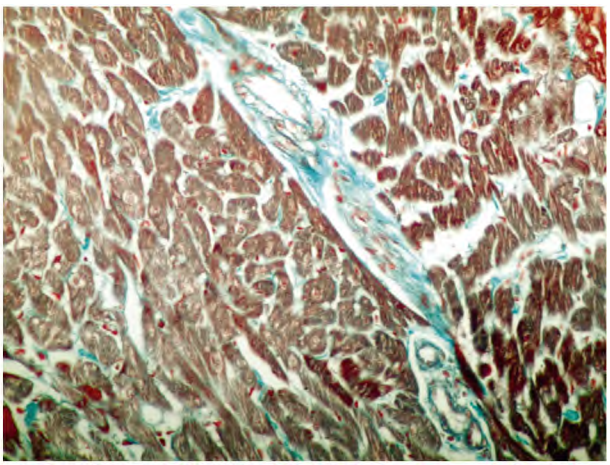


Fig. (8): Perivascuar fibrosis in MScs treated group (masson trichrome stain) X 200.

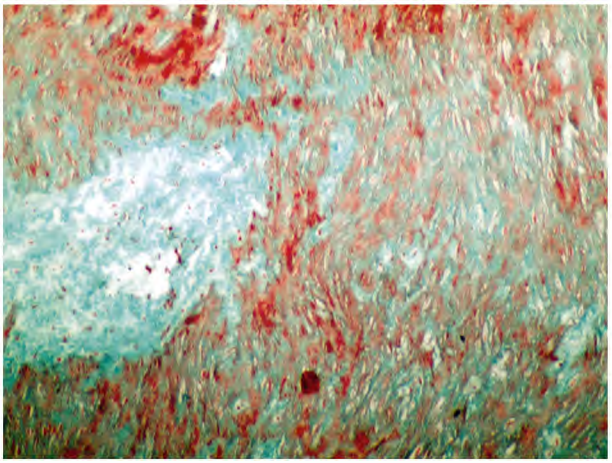


Fig. (6): Fibrosis between muscle bundles in obesity induced diabetic cardiomyopathy group (masson trichrome stain) X 100.

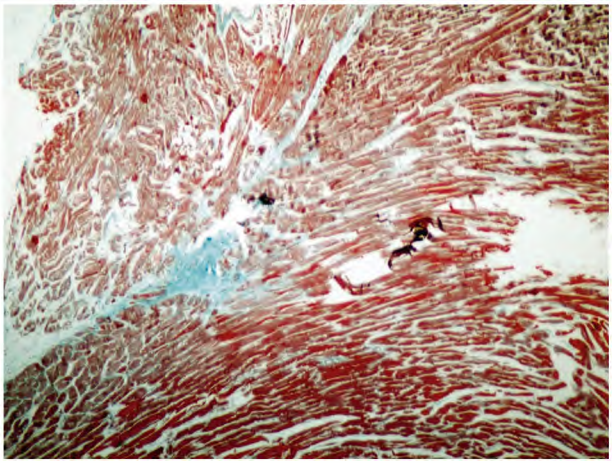


Fig. (9): Fibrosis between muscle bundles in MScs treated group (masson trichrome stain) X 100.

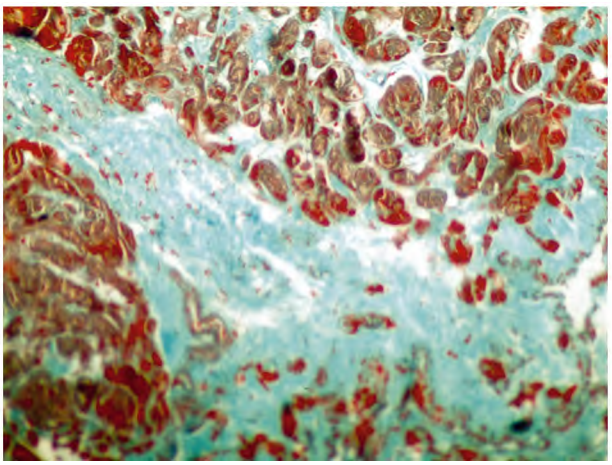


Fig. (7): Fibrosis between muscle bundles in obesity induced diabetic cardiomyopathy group (masson trichrome stain) X 200.

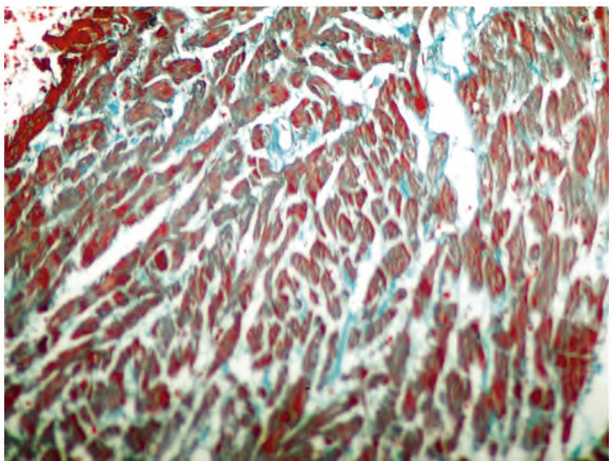


Fig. (10): Fibrosis between muscle bundles in MScs treated group (masson trichrome stain) X 200.

Discussion

Diabetic cardiomyopathy is revealed by cardiac remodeling (concentric hypertrophy), fibrosis, progressive diastolic and systolic dysfunction and impaired contractile reserve in stress test performance [21]. These cardiac alterations have been related to increased oxidative stress, altered calcium homeostasis, progressive mitochondrial dysfunction starting with a reduction of ATP production, activation of apoptotic signals (release of cytochrome c) and lipotoxicity (apoptosis-induced by ceramide) [22]. It has been reported that VEGF, HGF, FGF and matrix metalloproteinases produced by MSC promote myocardium regeneration and improve functionality in animal models of acute myocardial infarction [23]. It has also been suggested that MSC might contribute to the management of diseases where tissue damage is linked to oxidative stress directly [24] or through the secretion of IGF-1, a factor that inhibits oxidative stress production in cardiomyocytes [25].

In our study we found statistically significant decrease in biochemical paramaters (glucose, insulin, glycated hemoglobin, cholesterol and triglycerides) in MScs injected group compared to diabetic group. Gene expression of p300, atrial natriuretic peptide (ANP) and myocytes enhancer factor 2 (MEF2A and MEF2C as molecular markers of cardiac hypertrophy and the mean cardiomyocyte width, as marker of cardiomyocyte hypertrophy and number of inflammatory cells was significantly decreased in MScs injected group when compared to diabetic group. Also fibrosis decreased in MScs injected group when compared to diabetic group but this was statistically insignificant.

Our results are similar to those of Zhang et al., who demonstrated in their study that intravenous administration (into the femoral vein) of bone marrow-derived MSCs to rats with type 1 DM complicated by DC resulted in improved cardiac function of the treated animals through increased angiogenesis and attenuated cardiac remodeling. Transplanted MSCs differentiated into cardiomyocytes and improved angiogenesis and myogenesis. MMP2 activity increased, MMP-9 activity decreased, and there was reduced collagen content in the diabetic myocardium [26].

More positive results were seen in a rodent model of dilated cardiomyopathy (DCM). MSC transplantation (5×10^6 cells by injection into the rat myocardium) resulted in induction of myogenesis and angiogenesis and secretion of large amounts of angiogenic and anti-apoptotic factors (VEGF, HGF, adrenomedulin (AM), and IGF-1). A comparison of conditioned medium from MSC versus mononuclear cells MNC revealed MSCs secreted fourfold more VEGF than MNCs. Transplanted MSCs differentiated into cardiomyocytes, vascular endothelial cells, and smooth muscle cells and there was improvement in cardiac function, inhibition of ventricular remodeling, and a decrease in collagen volume in the myocardium with a reduction in myocardial fibrosis when compared to untreated tissue [27].

Also Li et al., in their study found that intramyocardial transplantation of MSC had a protective effect on the diabetic myocardium and DC, and anoxic pre-conditioning (AP) of MSCs was found to enhance this protective effect. In a rodent model of DC, 2 weeks after MSC administration, MSC and AP-MSC groups increased the fractional shortening of the diabetic heart. AP-MSC groups increased myocardial capillary density, attenuated myocardial fibrosis, and inhibited cardiac cell apoptosis [28].

Khan et al., found that Pre-conditioning of diabetic MSCs with medium from cardiomyocytes exposed to oxidative stress and high glucose (HG/H-CCM medium) also had a beneficial effect on cardiac tissue regeneration. In a mouse model of DM, autologous MSCs preconditioned with HG/H-CCM exhibited up regulated gene expression of angiogenic and cardiac markers. When these cells were implanted by intramyocardial injection into the hearts of STZ-induced diabetic mice (approximately 0.1×10^6 MSC/animal), cardiac function was markedly improved. Pre-conditioned MSCs demonstrated increased homing ability, increased expression of angiogenic and cardiac markers and paracrine mediators, such as IGF-1, HGH, SDF-1, and FGF-2. Four weeks after transplantation reduced fibrosis, apoptosis, and increased angiogenesis was observed in the mouse diabetic hearts [29].

Shabbir et al., mentioned that intramuscular delivery of MSCs resulted in improved ventricular function in a hamster heart failure model with enhancement of the density of capillaries and myocytes and a reduction in apoptosis and fibrosis [30].

Various possible mechanisms of MSC-mediated cardiac improvement have been suggested including transdifferentiation, somatic reprograming, and direct electro physical coupling [31], but precise delineation of the functional consequences of MSC administration remains to be elucidated. MSCs administration can also result in improvements in

cardiac function through secretion of paracrine mediators, such as Bcl-2, HSP20, and hypoxia-regulated heme oxygenase-1, hypoxic Akt-regulated stem cell factor, VEGF, HGF among others **[32]**, Recent evidence strongly suggests these factors affect remodeling, regeneration, and neovascularization leading to improvements in myocardium contractility and viability. Release of trophic mediators by MSCs had also been suggested **[33]**.

Opposite to our results, Calligaris and Conget, 2013, in their study on a mouse model of obesity-induced DC. IV administration of allogeneic bone marrow-derived MSCs with a single dose of 0.5 x 10^6 or three consecutive monthly doses of 0.5 x 10^6 MSCs did not result in improved cardiac function when assessed 4 months later but rather has a neutral effect on DC. The observed effects may be as a result of the route, time, and dose used but possibly also to issues with efficient homing and engraftment of the tail vein administered cells **[34]**.

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استخدام الخلايا الجذعية الوسيطة في نموذج تجريبي من متلازمة التمثيل الغذائى معقدة مع اعتلال عضلة القلب

مقدمة: السمنة هى قضية صحية عالمية رئيسية. معظم مرضى السمنة المفرطة يعانون من متلازمة التمثيل الغذائى، مجموعة من الخصائص الاكلينيكية التى تتميز بارتفاع ضغط الدم، ومقاومة الانسولين وخلل فى توزيع دهون الدم. حدوث الخلل البطينى فى المرضى الذين يعانون من زيادة سكرالدم فى غياب امراض الشرايين التاجية، وأمراض صمامات القلب أو ارتفاع ضغط الدم يعرف بأنه اعتلال عضلة القلب السكرى. ويجرى حالياً بذل جهود كبيرة لتطوير علاجات تهدف إلى تجديد عضلة القلب أو تحفيز برامج الإصلاح الذاتية. الخلايا الجذعية الوسيطة ممكن ان تتحور الى خلايا عضلية قلبية. استخدام الخلايا الجذعية الوسيطة فى علاج اعتلال عضلة القلب الناشئ عن السمنة ومرض السكرى فى السنوات الأخيرة تقدم نتائج واعدة.

الهدف من العمل: هذه الدراسة هى لتقييم تأثير الحقن الوريدى للخلايا الجذعية الوسيطة على قلوب الفئران المصابين بالسمنة المفرطة ومرض السكرى.

الطريقة: تم تغذية ستين من الفئران الذكور نظام غذائى منتظم إلى شهر واحد من العمر. ثم تم الاحتفاظ بعدد٢٠من الفئران مع اتباع نظام غذائى منتظم (صحية) و٤٠ الفئران تحولت إلى اتباع نظام غذائى عالى الدهون (السمنة) حتى نهاية الدراسة (١٦ شهرا من النظام الغذائى). بعد شهرين من الحقن الوريدى للخلايا الجذعية الوسيطة سيتم تقييم المعلمات التالية : ضغط الدم، الجلوكوز فى الدم، الهيموجلوبين السكرى (جليكوزيلاتد هيموجلوبين)، الأنسولين، الدهون الثلاثية والكوليسترول، التعبير الجيني من p300، الببتيد الناتريوتريك الأذينى (ANP) و ميوسيتس محسن عامل ٢ MEF2A (و MEF2C). في نهاية الدراسة تم ذبح الفئران عن طريق قطع الحلق وتشريحها لاخذ القلب التى تم حفظها فى الفورمالين ١٠٪. مقاطع من القلوب تم صباغتها باستخدام (H & E) وثلاثى ألوان ماسون.

النتائج: انخفضت نسبة الجلوكوز فى الدم، والكولسترول، والدهون الثلاثية، والهيموجلوبين السكرى، ومقاومة الأنسولين بشكل ملحوظ فى مجموعة الفئران التى تم حقنها بالخلايا الجذعية الوسيطة بالمقارنة مع مجموعة البدناء المصابة بالسكرى ($p<0.00$، $p< 0.00$) التعبير الجينى عن طريق تفاعل البوليميريز المتسلسل من p300، الببتيد الناتريوتريك الأذينى (ANP) و ميوسيتس محسن عامل ٢ (MEF2A) و (MEF2C,الدال على تضخم عضلة القلب قلت كذالك بشكل ملحوظ فى المجموعة التى تم حقنها ($p< 0.01$، $p< 0.01$، $p< 0.01$، $p<0.01$) تضخم وعدد الخلايا الالتهابية انخفض بشكل ملحوظ ($p<0.01$، $p<0.01$). كما انخفض التليف ولكن هذا كان غير مهم إحصائياً ($p<0.069$).

الاستنتاج: الخلايا الجذعية الوسيطة تقلل نسبة الجلوكوز فى الدم، الهيموجلوبين السكرى، الانسولين ،الدهون الثلاثية ،الكولسترول، التعبير الجينى المتسلسل من p300، الببتيد الناتريوتريك الأذينى (ANP)، ميوسيتس محسن عامل ٢ (MEF2A) و (MEF2C)، تضخم عضلة القلب ،عدد الخلايا الالتهابية والتليف فى مرض اعتلال عضلة القلب السكرى.