



Research Article

## Histological study on the effect of transplanted human umbilical cord blood CD34+ stem cells on albino rats subjected to myocardial infarction

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**Keywords:**

HUCB, CD34+ stem cells, myocardial infarction, transdifferentiation

**ABSTRACT**

**Background:** Heart failure is a significant burden to health care systems in the world. One of its major causes is myocardial infarction (MI). Recent developments in stem cells may offer ways to manage heart failure by replacing damaged cardiac muscle with healthy tissue. This study aimed at examining the regenerative effect of intravenously transplanted human umbilical cord blood CD34+ stem cell in a rat model of acute MI. **Methods:** Forty adult female rats were equally randomized into 5 groups. Groups I and II received saline alone or saline followed by isolation buffer respectively to serve as control groups. The other 3 groups were subjected to induction of acute MI using subcutaneous injection of isoprenaline hydrochloride. In groups III and IV, animals were sacrificed after one week and four weeks respectively. One week after induction of MI, animals in group V received intravenous injection of  $4 \times 10^6$  CD34+ stem cells separated from the human umbilical cord blood of male fetuses, and were sacrificed after 3 weeks from cell injection. At the end of the experiment, heart tissue was processed for both light and electron microscopic histological studies, and for PCR analysis of the male-specific SRY gene. **Results:** Light microscopic results of group III revealed increased diameter and necrosis of cardiomyocytes, decreased cross-striations, vascular congestion and mononuclear cellular infiltration. Group IV revealed multiple extensive fibrotic areas. Group V revealed smaller fibrotic areas compared to Group IV. Ultrastructural results confirmed findings of the light microscope. PCR analysis revealed that 63% of heart samples were positive for the presence of SRY gene. **Conclusion:** CD34+ stem cells can transdifferentiate into cardiomyocyte and regenerate the injured heart subjected to MI.

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### INTRODUCTION

Heart failure secondary to ischemic heart diseases constitutes the leading cause of death worldwide (World mortality report, 2015). Treatment of such cases provides some improvement of symptoms, but cannot reverse the condition of cardiac tissue from a diseased to a healthy state. The only therapeutic option to these patients is cardiac transplantation. However, searching

for compatible donors and immune suppression limit the application of this procedure and alternative therapies should be sought. (Boudoulas and Hatzopoulos., 2009).

Recent developments in stem cell biology and regenerative medicine may offer ways to manage heart failure by replacing damaged or lost cardiac muscle with healthy tissue (Furfaro and Gaballa., 2007). Skeletal myoblasts and bone marrow (BM)-derived cells have been the cell types most frequently used for the treatment of myocardial infarction because of their autologous nature and easy availability (Pinho-Ribeiro et al., 2010). However, these cell sources have limitations. For example cardiac calcifications were reported in patients following BM stem cell transplantation. Skeletal myoblasts have been associated with arrhythmias (Durrani et al., 2010). Due to limitations of these two cell types, the search for a

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more suitable cell has led investigators to study the repair potential of cord blood (CB) cells in acute MI. (Acosta et al., 2013).

Intravenous administration of stem cells is a practical mode of cell delivery because it does not require cardiac surgery or catheterization. However little information is available regarding the therapeutic potential of systemically delivered stem cells in myocardial infarction (Barbash et al., 2003). In addition, there is a paucity of histological studies that used umbilical cord blood stem cells. So, this study was designed to examine the regenerative effect of intravenously transplanted human umbilical cord blood CD34+ stem cell on the heart of adult albino rats subjected to myocardial infarction and to test if the stem cells can differentiate into cardiomyocyte.

## METHODS

Forty adult female rats weighing 150 to 170 gm (age 12-14 weeks), at the beginning of the experiment, were used in this study. The rats were housed under standardized conditions away from any stressful stimuli with 12-hr light/dark cycle, and free access to food and water for an acclimating period of one week. The animals were randomly divided into 5 groups each of 8 rats. Animals of group I (c1) received a subcutaneous injection of 1 ml saline for 2 days. Group II (c2) received a subcutaneous injection of 1 ml saline for 2 days followed one week later by I.V. injection of 1 ml isolation buffer (the solvent of CD34+ stem cells). Group III (AMI-1) were subjected to induction of acute myocardial infarction (AMI) by subcutaneous injection of isoprenaline hydrochloride (85 mg/kg/day) dissolved in 1 ml saline for 2 consecutive days, and were sacrificed after one week. Group IV (AMI-2) were subjected to induction of AMI as in group III, and animals were sacrificed at the end of the experiment. In group V, animals were subjected to induction of AMI followed one week later by I.V. injection of  $4 \times 10^6$  CD34+ stem cells dissolved in 1 ml Isolation buffer. Animals of all groups were sacrificed after 4 weeks except those in group III which were sacrificed after only one week. Separation of CD34+ stem cells, using human umbilical cord blood (UCB) of male fetus, was carried out according to the method described by Milteny et al. (1990) using immuno-magnetic separation technique.

At the end of the experiment, specimens from the left ventricle of the heart were fixed in 10% neutral buffered formalin solution and prepared for light microscopic examination. They were processed for 5  $\mu$ m thick paraffin sections and stained with HandE, Masson's trichrome, and Iron haematoxylin stains. Using the light microscope, histological sections were examined to assess the degree of cardiac affection. The percentages of necrotic cardiomyocytes were assessed

in HandE-stained sections in different groups. The number of necrotic cells was estimated and then divided by the total number of cells in the examined fields to get the percentage of necrotic cells. Five high power fields (HPF) were assessed per section and 3 sections were examined from each animal. Quantitative measurements were carried out using the image analyzer (Super eye- Heidi soft) to measure the following: 1- The color area percentage of the green colored collagen fibers in Masson's Trichrome stained sections and the black colored cross- striations in iron Haematoxylin stained sections. 2- The diameter of cardiomyocytes in transverse sections of HandE-stained sections. The image analyzer was calibrated for color and distance measurements before use.

A small piece of the left ventricle was excised from each animal to be used for electron microscopy. It was trimmed in the presence of glutaraldehyde into few mm pieces. These specimens were immediately fixed in 2.5% glutaraldehyde solution at 4°C for 24 hours, and then washed overnight in several changes of 0.1 M sodium phosphate buffer, pH 7.4 at 4°C. They were later post-fixed in 2% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 7.4 at room temperature. Specimens were dehydrated in ascending grades of cold ethanol and propylene oxide then embedded in Spurr's resin. Ultrathin sections (50 nm) were prepared on Reichert-June ultracut microtome and subsequently stained for 10 minutes with 3% aqueous uranyl acetate and for 10 minutes in lead citrate. Sections were then examined and photographed using Philips 400 transmission electron microscope.

To provide independent evidence that CD34+ stem cell can differentiate into cardiomyocyte after myocardial infarction, male-specific marker, SRY gene, (on the Y chromosome) was used to identify male donor-derived cells in female recipients. A small piece of the left ventricle was immediately frozen in liquid nitrogen and then transferred to -80°C, 4 weeks after myocardial infarction, and CD34+ stem cell transplantation (from group IV only), and then genomic DNA was isolated from the heart of female recipients for real time polymerase chain reaction (RT-PCR). DNA from normal human female and male was used as negative and positive controls, respectively. The primers used to amplify the **SRY** gene were 5'-CAGCTAACACTGATCTTTTC-3' and 5'-TTACTGAGCCAGAATCATAG-3'.

## Statistical Analysis

Results are presented as mean  $\pm$  standard deviation (SD) and compared using Student's t-test. Significance was set at  $p < 0.05$  for all comparisons. All statistical analyses were performed using SPSS-9 (Chicago, Illinois, USA) statistical analysis software.

**Ethical considerations**

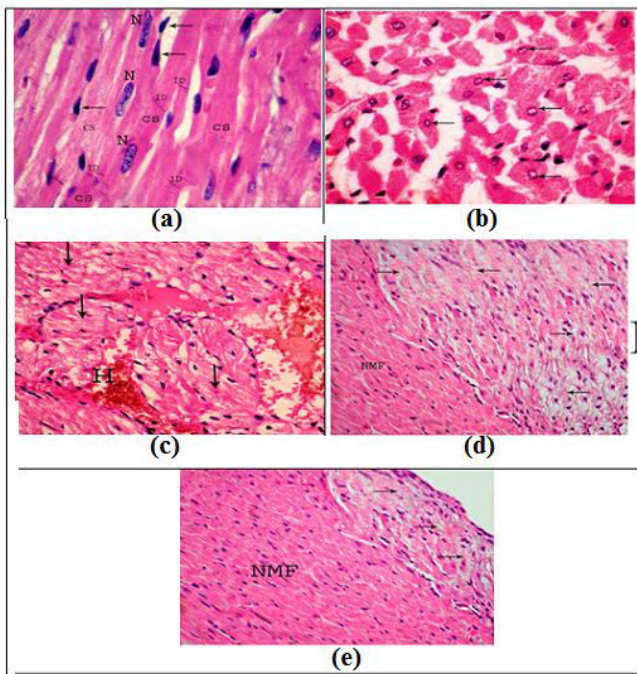
Informed consent was obtained from the pregnant women whose umbilical cord blood was obtained for this study. The guidelines of Suez Canal University in animal research were followed in this study. Approval of the research committee of the school was taken on the study protocol.

**RESULTS**

**Light microscopy:**

**Group I Control group 1 (C1):**

Examination of HandE stained sections from the control heart showed normal appearance of the cardiac myocytes with their centrally located nuclei (Fig.1a). The mean diameter of cardiac myocytes in this group was  $12.76 \mu\text{m} \pm 0.89$  (Table 1).



**Fig. 1** (HandE Stain), **(a):** Photomicrograph of a longitudinal section in the heart of a control (C1) rat showing branching and anastomosing muscle fibers with their central oval and vesicular nuclei (N), cross-striation (CS) and intercalated disks (ID). Darkly stained nuclei of the endomysial C.T (arrow) is shown in- between the muscle fibers (HandE  $\times$  1000). **(b):** Photomicrograph of a transverse section in the heart of a rat from AMI 1 group showing necrotic muscle fibers with pale cytoplasm and chromatin margination in many nuclei (arrow) (HandE  $\times$  1000) **(c):** Photomicrograph of a section in the heart of a rat from AMI 1 group showing extensive hemorrhage (H) and disintegrated muscle fibers (arrow) (HandE  $\times$  400). **(d):** Photomicrograph of a section in the heart of a rat from AMI 2 group showing large pale stained area of fibrosis (arrows). Small area of normal cardiac muscle fiber (NMF) is shown. (HandE  $\times$  400). **(e):** Photomicrograph of a section in the heart of a rat from stem cell group showing small pale stained area of fibrosis (arrows). Large area of normal cardiac muscle fiber (NMF) is shown (HandE  $\times$  400). Using Masson's trichrome stain,

the green stained endomysial connective tissue surrounding the muscle fibers appeared scanty (Fig.2a). The mean color area percentage of collagen in this group was  $0.122 \pm 0.0149$  (Table 2). Longitudinal sections, stained with Iron haematoxylin, showed a branching and anastomosing cardiac muscle fibers. The darkly stained cross-striations were obviously seen. Nuclei of cardiac myocytes were oval central and pale stained (Fig. 3a). The mean color area percentage of the black iron haematoxylin stain in this group was  $0.143 \pm 0.057$  (Table 2).

**Table 1.** Cardiomyocytes' diameter, and percentage of necrosed cardiomyocytes in the study groups.

Groups	Mean cardiomyocyte diameter ( $\mu\text{m}$ )	mean percentage of necrosed cardiomyocytes
Group I	$12.76 \pm 0.89$	$5.78 \% \pm 2.38$
Group II	$12.58 \pm 0.58$	$5.70 \% \pm 2.36$
Group III	$18.72 \pm 1.49^*$	$36.6\% \pm 3.84^*$
Group IV	$14.08 \pm 0.33^*$	$11.2\% \pm 0.74^*$
Group V	$13.73 \pm 0.39^* \bullet$	$8.70\% \pm 0.96^* \bullet$

Data are presented as Mean  $\pm$  SD. \* Statistically significant compared to Group I:  $p < 0.05$ .  $\bullet$  Statistically significant compared to Group IV:  $p < 0.05$

**Table (2):** The mean color area percentage of green collagen fibers & black cross striations in the different study groups.

Group	mean color area percentage of iron haematoxylin stain	mean color area percentage of collagen
Group I	$0.143 \pm 0.057$	$0.122 \pm 0.0149$
Group II	$0.143 \pm 0.067$	$0.124 \pm 0.0148$
Group III	$0.084 \pm 0.008^*$	$0.172 \pm 0.0157^*$
Group IV	$0.0482 \pm 0.011^*$	$0.323 \pm 0.097^*$
Group V	$0.076 \pm 0.005^* \bullet$	$0.238 \pm 0.041^* \bullet$

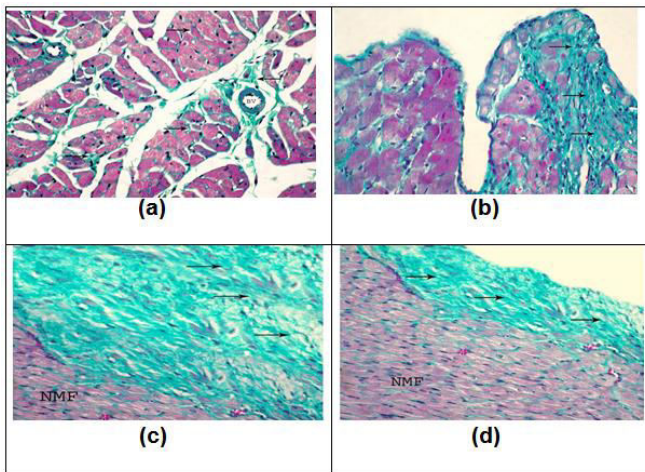
Data are presented as Mean  $\pm$  SD. \*Statistically significant compared to Group I:  $p < 0.05$ .  $\bullet$  Statistically significant compared to Group IV:  $p < 0.05$

**Group II control group 2(C2):**

Using all previous stains, the cardiac tissue appeared similar to that of control group I where no changes were detected (Table 1, 2).

**Group III (AMI-1):**

HandE stained sections from group III showed that 36.6% of cardiomyocytes were necrotic (Table 1) They showed pale cytoplasm and their nuclei were pyknotic or having chromatin margination (Fig.1b) . The cells appeared swollen with increased diameter. The mean diameter of cardiac myocytes in this group was  $18.72 \pm 1.49$  which was significantly increased compared to the

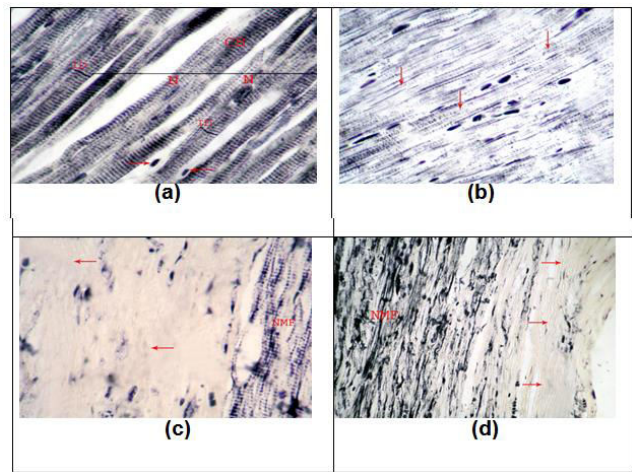


**Fig. 2:** (Masson's Trichrome stain **(a)**): Photomicrograph of a transverse section in the heart of a control (C1) rat showing scanty green stained endomysial C.T (arrow). Blood vessels (BV) are shown in the C.T (Masson's trichrome  $\times 400$ ). **(b)**: Photomicrograph of a section in the heart of a rat from AMI 1 group showing minimal increase of collagen fibers (arrow) (Masson's trichrome  $\times 400$ ). **(c)**: Photomicrograph of a section in the heart of a rat from AMI 2 group showing large green stained area with marked increase of collagen fibers (fibrosis) (arrow). Small area of normal cardiac muscle fiber is shown (NMF) (Masson's trichrome  $\times 400$ ). **(d)**: Photomicrograph of a section in the heart of a rat from stem cell group showing small green stained area with increased collagen fibers (fibrosis) (arrow). Large area of normal cardiac muscle fiber (NMF) is shown (Masson's trichrome  $\times 400$ ).

control group (Table 1). Marked hemorrhage between the muscle fibers was also observed (Fig.1c). In some sections, mononuclear cellular infiltration was noticed. Sections stained with Masson's trichrome technique revealed minimal increase in collagen fibers in the endomysial connective tissue surrounding the cardiac myocytes (Fig.2b). The mean color area percentage of collagen in this group was  $0.172 \pm 0.0157$  which was significantly increased compared to the control group (Table 2). Iron haematoxylin stain revealed decreased cross striation in longitudinal sections of cardiac muscle fibers in this group (Fig.3b). The mean color area percentage of the black iron haematoxylin in this group was  $0.084 \pm 0.008$  which was significantly decreased compared to the control group (Table 2).

#### **Group IV (AMI- 2):**

Sections stained with HandE showed that the mean percentage of necrotic cardiomyocytes was  $11.2\% \pm 0.74$  (Table 1) and the mean diameter of cardiac myocytes was  $14.08 \pm 0.33$ . Some animals of this group showed mononuclear cellular infiltration and hemorrhage. Multiple large pale stained areas of fibrosis were seen (Fig.1d) in 100% of the animals of this group. Masson's trichrome stain showed marked increase of collagen fibers (fibrosis) compared to the

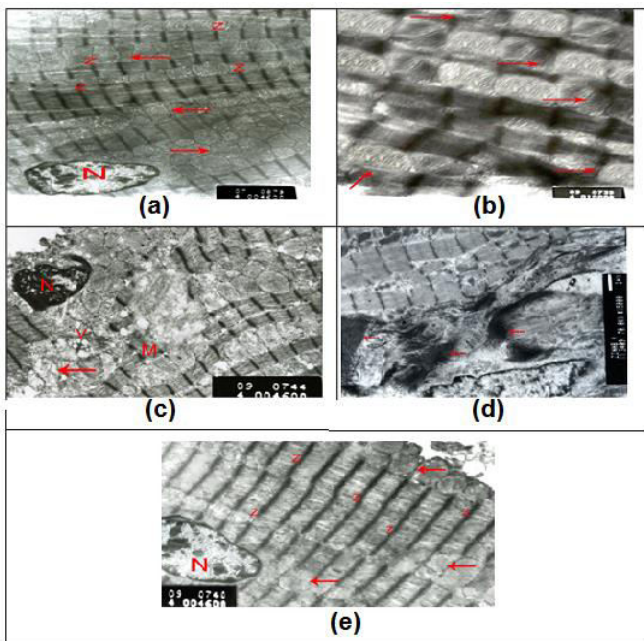


**Fig. 3. (Iron Haematoxylin stain **(a)**):** Photomicrograph of a longitudinal section in the heart of a control (C1) rat showing muscle fibers with their pale black nuclei (N), darkly black cross-striations (CS) and intercalated disks (ID). Darkly black endomysial C.T nuclei (arrow) are shown among the muscle fibers (iron haematoxylin  $\times 1000$ ). **Fig. 3(b)**: Photomicrograph of a section in the heart of a rat from AMI 1 group showing decrease in cross-striations (arrow) (iron haematoxylin  $\times 1000$ ). **Fig. 3(c)**: Photomicrograph of a section in the heart of a rat from AMI 2 group showing large area with marked loss of cross-striations (arrow). Small area of normal muscle fibers (NMF) with obvious cross-striations is shown (iron haematoxylin  $\times 1000$ ). **Fig. 3(d)**: Photomicrograph of a section in the heart of a rat from stem cell group showing small area with loss of cross-striations (arrow). Large area of normal muscle fibers (NMF) is shown (iron haematoxylin  $\times 400$ ).

control group (Fig.2c). The mean color area percentage of collagen in this group was  $0.323 \pm 0.097$  which was significantly increased compared to control group (Table 2). Iron haematoxylin stain revealed multiple large areas of cardiac muscles with marked loss of cross- striations (fig.3c) in 100% of the animals of this group. The mean color area percentage of the black iron haematoxylin was  $0.0482 \pm 0.011$  (Table 2).

#### **Group IV stem cell group (S):**

Sections stained with HandE showed marked improvement in the microscopic structure of the cardiac myocytes. Number of necrotic cardiomyocytes was markedly less than in AMI 2 group. The mean percentage of necrotic cardiomyocytes was  $8.70\% \pm 0.96$  (Table 1). The mean diameter of cardiac myocytes was  $13.73 \pm 0.39$  (Table 1). Few animals of this group showed mononuclear cellular infiltration and hemorrhage. Multiple pale stained areas of fibrosis (fig.1e) were still observed in all animals of this group, however they were markedly smaller in diameter compared to the AMI 2 group. Masson's trichrome stain showed moderate increase in collagen fibers (fig. 2d) compared to control group, however they were markedly smaller than that observed in AMI 2 group. The mean color area percentage of collagen in this



**Fig. 4** (Electro-micrographs): (a): Electromicrograph of a section of control (C1) heart showing, regular arrangement of myofibrils with regularly arranged Z lines (Z). Abundant mitochondria (arrow) are shown to occupy fusiform spaces. The cardiac myocyte nucleus (N) appears light and oval ( $\times 4600$ ). Fig. 4(b): higher magnification of the previous section showing, regularly arranged myofibrils. Numerous mitochondria (arrow) with intact outer membrane and closely packed cristae are shown ( $\times 10000$ ). Fig. 4(c): Electromicrograph of a section in the heart of a rat from AMI 1 group showing, loss of regular arrangement of the myofibrils (M). Cardiac myocyte nucleus appears smaller and dark. Degenerated mitochondria (arrow) and vacuoles (V) are shown in the cytoplasm ( $\times 4600$ ) Fig. 4(d): Electromicrograph of a section in the heart of a rat from AMI 2 group showing, an area of fibrosis (arrow) ( $\times 5000$ ). Fig. 4(e): Electromicrograph of a section in the heart of a rat from stem cell group showing, regular arrangement of myofibrils with regularly arranged Z lines (Z) and numerous mitochondria (arrow) in-between. Lightly stained cardiac myocyte nucleus (N) is also shown ( $\times 4600$ ).

group was  $0.238 \pm 0.041$  (Table 2). Iron haematoxylin stain revealed multiple areas with loss of cross-striations in 100% of animals of this group, however they were markedly smaller compared to the AMI 2 group (fig.3d). The mean color area percentage of the black iron haematoxylin in this group was  $0.076 \pm 0.005$  (Table 2).

**Electron microscopy**

**Group I control group 1 (c1):**

The cardiac muscle fibers in this group showed regular arrangement of the myofibrils with their alternating light and dark bands. The cardiac myocytes nuclei were lightly stained, oval and occupied a central position in the cell (Fig.4a). The mitochondria were abundant and occupied a large volume of the cardiac myocyte

(Fig.4b). Nuclei of endomysial connective tissue cells were darkly stained. The transverse intercalated discs, which are specialized junctions between the cardiac myocytes, appeared at intervals.

**Group II control group 2 (c2):**

No changes were detected in this group by electron microscopic examination and it is similar to c1 group .

**Group III (AMI-1):**

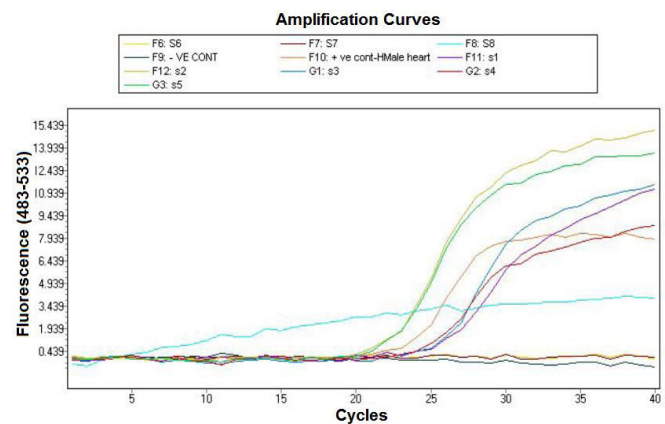
Examination of this group revealed marked necrotic changes affecting the cardiac myocytes. They showed disruption and breakdown of the regular arrangement of the myofibrils. The cardiac myocytes nuclei were smaller and more condensed. The mitochondria were degenerated and appeared swollen with loss of their cristae and some of them had ruptured membrane. Vacuoles were obviously seen in the sarcoplasm (fig.4c).

**Group IV (AMI- 2):**

Multiple large areas of extensive fibrosis were seen (fig.4d).

**Group V stem cell group (S):**

In this group, minimal necrotic changes were detected. However, some areas of fibrosis were still seen. Most of the cardiac myocytes showed nearly normal structure of regularly arranged myofibrils with numerous normal mitochondria in-between and lightly stained centrally located nuclei (fig.4e).



**Fig. 5:** The Amplification curves of positive and negative sample.

**PCR analysis results**

The PCR product could be amplified from the regenerating hearts of five female rats (fig. 5). So 63% of heart samples were positive for the presence of **SRY** gene. The PCR product could not be amplified from the regenerating hearts of three female rats (fig. 5). So 37% of heart samples were negative for the presence of **SRY** gene. The PCR product was also detected in DNA from normal human males but was absent in DNA from

normal human untransplanted females, demonstrating the specificity of the assay.

## DISCUSSION

In this study we used isoprenaline to provide a simple, non-invasive way to induce myocardial injury. This method caused histopathological, biochemical, hematological and oxidative stress markers changes similar to those observed in myocardial infarction secondary to coronary occlusion (Lobo Filho et al., 2011). Isoprenaline-induced myocardial infarction in albino rats serves as a well standardized animal model for myocardial infarction of human beings to study the beneficial effects of many drugs and cardiac functions (Sivakumar et al., 2007)

Light microscopic examination of the rats' hearts, one week after subcutaneous injection of isoprenaline (AMI 1 group), showed a significant myocardial infarction. The most important pathological change of this infarction was necrosis of cardiomyocytes. The cardiomyocytes were also swollen and showed a significant increase in diameter compared to that of the control group. There is also a decrease of cross striations and minimal increase of collagen fibers (fibrosis) denoting beginning of the healing process by formation of fibrous scars. This was in addition to extensive hemorrhage and mononuclear cellular infiltration which were detected in high percentage in this group. These findings were confirmed by image analysis and by the ultrastructural results

Our findings are in accordance with previous studies (Furfaro and Gaballa, 2007; Kawamoto et al, 2006) which detected severe hemorrhagic infarction in mice and rats after ligating the left anterior descending coronary artery for few minutes. The isoprenaline used in this study to induce myocardial infarction was previously used by other investigators (Brooks and Conrad, 2009; Banjeree et al., 2003). However, they confirmed the myocardial infarction based on the determination of diagnostic marker enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), but no histological studies were used.

Several investigators who explained the isoprenaline induced myocardial necrosis suggested an altered mitochondrial function and free radical-mediated tissue damage as important pathological events in isoprenaline induced cardiotoxicity (Rajadurai and Prince, 2007; Padmanabhan and Mainzen Prince, 2006). Isoprenaline treated rats showed significant increase in mitochondrial lipid peroxidation products and lysosomal enzymes, and significant decrease in mitochondrial antioxidants, mitochondrial protein, mitochondrial enzymes and respiratory chain enzymes. These alterations associated the mitochondrial

degeneration and dysfunction with inadequate ATP synthesis. Brega et al. (2001) postulated that, myocardial tissue is highly dependent on energy supplied by normal mitochondrial function. Therefore defects of energy production in addition to direct toxic effects of free radicals disturb maintenance of the cell and renewal of cellular proteins which lead to loss of structural integrity and disruption of the myofibrils. It was also reported that myocardial necrosis induced by isoprenaline is probably due to a primary act on the sarcolemmal membrane. This is followed by stimulation of adenylate cyclase, activation of Ca<sup>++</sup> and Na<sup>+</sup> channels, loss of selective permeability with exaggerated Ca<sup>++</sup> inflow, excess of excitation-contraction with inadequate energy which lead to cellular death (Zhang et al., 2008). The formation of cytoplasmic vacuoles, observed in this study, could be explained by expansion of cytoplasmic membranous components caused by intracellular water and electrolyte redistribution (Brega et al., 2001). Increased cardiomyocytes diameter, hemorrhage and cellular infiltration found in our study can be explained by a response to myocardial necrosis induced by isoprenaline where irreversible microvascular injury developed resulting in endothelial cell necrosis and detachment with subsequent vascular leakage and tissue edema (Boudoulas and Hatzopoulos, 2009) The observed increased cardiomyocytes diameter in this study was most probably due to edema. With prolonged injury, vascular rupture developed leading to extensive hemorrhage. Cells in and around the affected necrotic areas up-regulate and secrete cytokines and chemokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), interleukin (II)-1 $\beta$ , II-6 or II-8, which trigger an immediate and massive infiltration of circulating leukocytes into the ischemic core (Boudoulas and Hatzopoulos, 2009).

The present study also revealed that 4 weeks after injection of rats with isoprenaline (AMI 2 group), sections of the heart showed multiple fibrous scars seen in all animals. These scars replaced most of the necrotic tissues. This indicated that, most of the damaged regions of the myocardium have healed by fibrous scars. Ultrastructural results of this group confirmed the extensive fibrosis. Masson's trichrome and Iron haematoxylin stains also confirmed the presence of these fibrotic areas. The Fibrosis detected in our study can be explained by the study of Konstantinos et al. (Boudoulas and Hatzopoulos, 2009) who reported that damaged zones of the myocardium were progressively replaced by the ingrowth of highly vascularized granulation tissue, which progressively becomes less vascularized and more fibrous. They added that, in most instances, scarring is well advanced by the end of the fourth week.

Results of the present study revealed that human umbilical cord blood CD34+ stem cells treatment of rats subjected to isoprenaline induced myocardial infarction (stem cell group) resulted in less marked histopathological changes compared to the AMI 2 group. This was evident by the reduction of mononuclear cellular infiltration, number of swollen and necrotic cardiomyocytes in this group compared to AMI 2 group. Although fibrous scars were seen in all animals of this group, yet they were markedly smaller in size compared to the AMI 2 group. All these findings indicated that, intravenous administration of HUCB CD34+ stem cells had an efficient homing mechanism to the infarcted myocardium, had the ability to regenerate the damaged myocardial regions and markedly contributed to the reduction of infarction size, improved the structural damage, and prevented expansion of scars. Hemorrhage was the only feature that appeared in high percentage in this group. This may be due to leakage from regenerating capillaries which remain leaky until the endothelial cells differentiate and form intercellular junctions (Boudoulas and Hatzopoulos, 2009). Ultrastructural results of this group confirmed those of the light microscope which revealed some areas of fibrosis in addition to minimal necrotic changes compared to the AMI 2 group.

Our results of stem cell group were in accordance with those found by Furfaro and Gaballa., (2007) who reported that intravenous injection of  $4 \times 10^6$  cord blood cells into mice 24 h post-MI resulted in decrease in both infarct size and collagen deposition three weeks after treatment. Contrary to our results, Pinho-Ribeiro et al. (2010) reported that fresh human umbilical cord blood (HUCB) cells therapy did not prevent cardiac remodeling or improve the cardiac function of infarcted rats after intramyocardial injection of  $2 \times 10^5$  HUCB cells. This discrepancy with our results may be due to the low dose of HUCB cells used in their study which means a dose-dependent effect of transplanted HUCB cells.

Different mechanisms were suggested for the CD34+stem cells inducing cardiac repair after myocardial infarction. One of these mechanisms is the translineage differentiation of adult human stem cells into cardiomyocytes when introduced into heart by intramyocardial injection or via the circulation (Orlic et al., 2001; Jackson et al., 2001). Transformations into cardiomyocytes replace damaged tissues, limit fibrosis and promote myocardium repair in the infarct region and consequently improve heart function. Another mechanism is that transplantation of human CD34+ stem cells into rats with myocardial infarction, lead to incorporation of stem cells into site of myocardial injury, differentiation into mature endothelial cells (ECs) (vasculogenesis) (Jackson et al., 2001), increase

capillary density, inhibition of myocardial fibrosis and apoptosis, and preserved left ventricular function. It was stated that human CD34+ cells not only undergo transdifferentiation into endothelial cells but also have been shown to express angiogenic growth factors in a paracrine way to stimulate neovascularization at the site of the cell graft and participate in angiogenesis, thus alleviating myocardial ischemia (Kawamoto et al., 2006). Another suggested mechanism is that CD34+ cells had transformation potential not only into cardiomyocytes but also into ECs and smooth muscle cells (SMCs) which lead to heart regeneration through both cardiomyogenesis and vasculogenesis with functional and histological recovery from myocardial infarction (Iwasak et al., 2006)

The mechanism which was tested in the current study is the postulation of transformation of the CD34+ stem cell into cardiomyocyte using PCR analysis. To provide independent evidence that CD34+ stem cells can transform into cardiomyocytes after myocardial infarction, male-specific marker, SRY gene located on the Y chromosome, was used to identify male donor-derived cells in female recipients using real time polymerase chain reaction (RT-PCR). PCR was done for the stem cell group only four weeks after induction of myocardial infarction and CD34+stem cell transplantation. The results revealed that, 63% of the regenerating hearts of the female recipients' rats, who received male CD34+stem cell, contain male-specific SRY gene positive cardiomyocytes. Presence of male cardiomyocytes in female recipients indicates that the new cardiac cells originated from the donor male CD34+ stem cell. This finding support the conclusion that, transformation of the CD34+ stem cell into cardiomyocyte is one of the underlying mechanisms through which the CD34+ stem cell transplantation leads to cardiac repair after myocardial infarction. (Iwasak et al., 2006)

Different research groups have reported contradictory results about the mechanisms of the apparent transformation of stem cells into cardiomyocyte. Some investigators attributed this transformation to the transdifferentiation potential of stem cells. Others have demonstrated that this apparent transformation is a result of cell fusion between donor cells and the cardiomyocytes of the recipient. Alvarez-Dolado et al.(2003) reported that cell fusion in a mouse model was responsible for transformation of bone marrow-derived cells into cardiomyocytes. No transdifferentiation was observed in their study. Beltrami et al. (2003) demonstrated that adult cardiac stem cells differentiated into mature cardiomyocytes by transdifferentiation independent of cell fusion. However, another study suggested that, both cell fusion and transdifferentiation may account for the transformation of human CD34+ cells into

cardiomyocytes in the injured hearts of mice. The authors of that study added that discrepancy about the mechanism of the transformation of stem cells into cardiomyocyte could result from differences in the approaches used to distinguish fusion from transdifferentiation. (Zhang et al., 2004)

Results obtained from this study indicated that, intravenous infusion of H UCB CD34+ stem cells had the ability to regenerate the injured heart subjected to myocardial infarction. Based on the PCR results, we can suggest that transformation of the CD34+stem cell into cardiomyocyte is one of the underlying mechanisms through which cardiac repair occurs..

#### ACKNOWLEDGEMENT

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