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Original Article

Association between Bone Marrow Mesenchymal Stem Cell Characterization and Taking Inotropic Drugs in Patients with Severe Left Ventricular Dysfunction After Off-pump Bypass Surgery

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Abstract

Background and Aim: Left ventricular dysfunction is a frequent complication after coronary artery bypass graft (CABG) and is often treated with inotropic drugs to achieve suitable hemodynamic status. Bone marrow-derived mesenchymal stem cells (BMSCs) have potential effects on cardiac function. In this study, we aimed to identify the predictor role of MSCs in taking inotropic drugs in patients with severe left ventricular dysfunction undergoing CABG.
Methods: The study included 30 patients who underwent off-pump CABG at Afshar Hospital and Seyed Al-Shohada Hospital. For investigating the possible association of BMSCs proliferation rate with taking inotropic drugs, the bone marrow samples aspired from patients' sternum during surgery. MSCs were isolated and counted after 4, 7, and 14 days using trypan-blue color, and then doubling times were calculated.
Results: After cardiac surgery, the number of female patients who take inotropic drugs was significantly higher than men. Our data showed that the BMSCs doubling time in female patients who received inotropic drugs was less than that of male patients who received inotropic drugs ($p < 0.05$).
Conclusion: Based on this investigation, we concluded that there was a clear relevance between the MSCs' doubling time and the inotropic drug requirements in patients who received inotropic drugs.

Keywords: Mesenchymal Stem Cell; Bone Marrow; Severe Left Ventricular Dysfunction; Inotropic Drug; Doubling Time.

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Introduction

Permanent increasing myocardial infarction is one of the leading causes of death worldwide (1). Coronary artery bypass graft (CABG) is the most effective remedy for the cure of patients with myocardial infarction (2). One of the complication status in patients after myocardial infarction is the reductions in cardiac contractility, and therefore, improving the hemodynamic status and improving the cardiac function of patients during and after surgery is very important (2-4). Reduction in the myocardial muscle contraction can lead to systemic hemodynamic effects, including the loss of blood flow to vital organs (4). After cardiac surgery, patients often received inotropic drugs to maintain adequate hemodynamic status, including cardiac muscle contractility. These agents are a drug group that has an impact on myocardial contractility and improve cardiac function (5-7). Positive inotropic agents, including levosimendan and dopamine, strengthen the muscular contraction.In contrast, harmful inotropic agents, including calcium antagonists and ß-blockers, decrease the force of muscular contractions (8, 9). The detection of a predictor factor for taking inotropic drugs in

patients would be a valuable strategy for improving cardiac performance. Previous studies have described the functional benefits of bone marrow-derived mesenchymal stem cells (BMSCs) in patients with severe left ventricular dysfunction (10-12). BMSCs are adult multipotent stem cells that have a potential pattern in tissue repairment (13). These cells have been used in the regeneration of injured cardiac tissue and were able to differentiate into cardiomyocytes (14, 15).

Besides, many studies confirm the beneficial or protective effects of BMSCs in cardiac function after surgery (16, 17). Therefore, the current study was planned to detect BMSCs doubling time in patients with severe left ventricular dysfunction undergoing coronary artery bypass graft (CABG) who required and received inotropic drugs.

Methods

Patients and Study Design

The present study included 30 patients with severe left ventricular dysfunction. After approval by the Ethics Committee of Shahid Sadoughi University of Medical Sciences with this code (IR.SSU.MEDICINE.REC.1397.199), bone marrow samples from all patients with ejection fraction $\leq$30% who underwent CABG surgery at Seyed Al-Shohada hospital and Afshar hospital were collected. A written informed consent before study entry was filled by patients.

Culture and characterization of human BMSc

1 mL bone marrow samples had aspired from sternum during surgery from patients, and bone marrow MSCs were isolated at the cell culture laboratory. Briefly, isolated BMSCs were grown in minimum essential medium (αMEM) culture medium supplemented with 20% fetal bovine serum (FBS) containing 5% CO2 at 370C. For characterizing the BMSCs, the presence of hematopoietic markers CD34 and CD45 and mesenchymal markers CD90 and CD105 were examined by a flow cytometer (FACS Calibur Becton, Dickinson, USA). Briefly, BMSCs were detached (with trypsin/EDTA) from 25 cm^2 flasks after 14 days and counted.

About 10^6 Cell suspension was distributed into aliquots and centrifuged at 1500 rpm for 5 min, and then, the pellets were resuspended in PBS. Samples were incubated with appropriate antibodies, including anti CD90, CD105, CD34, and CD45 for 1 hour and read by a flow cytometer.

BMSCs doubling time

For evaluating the relationship between taking inotropic drugs and BMSCs proliferation rate, doubling times for all patients were assessed. Briefly, 10^4 cells were plated in 6-well plates, and then cells were trypsinized and counted in 4, 7, and 14 days applying trypan blue color and a hemocytometer.

The acquired results from the counted BMSCs were plotted, and doubling times were calculated from the log phase of the growth curve.

Statistical analysis

All results were figured out by the Graphpad Prism Version 8 software (GraphPad Software, San Diego, CA, USA). All consequences are presented as mean±standard deviation (SD). The mean differences between groups were compared using the two-way ANOVA. Statistical significance difference was indicated as $p < 0.05$.

Results

In this study, 30 patients (18 men and 12 women) with severe left ventricular dysfunction were included. For appraising the possible relevancy between MSCs proliferation rate and taking inotropic drugs, a 1 mL bone marrow sample from the sternum of patients was aspired for BMSCs isolation and doubling time calculation during surgery. Human BMSCs are shown in Figure 1. For confirmation of human BMSC, a flow cytometry method was used. The flow cytometry diagrams are displayed in Figure 2.

The cells were positive for mesenchymal markers CD105 and CD90 and negative for hematopoietic markers CD34 and CD45. The expression rate of CD105 and CD90 markers was 99.2% and 98.7%, respectively (Figure 2A and Figure 2B). Also, the expression of CD34 and CD45 markers was 0.249% and 0.945%, respectively (Figures 2C and 2D).

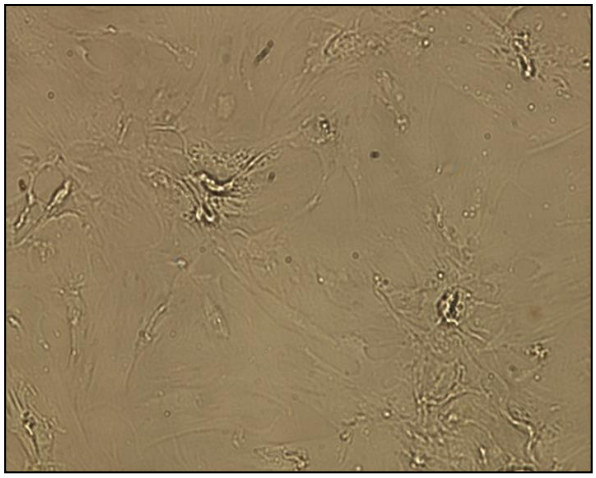

Figure 1. BMSCs after isolation and culture**.**

The association between postoperative inotropic drugs requirements and BMSCs doubling-time

Figure 3A indicates that in 18 male studied patients, three of them received inotropic drugs after surgery, while nine female patients received inotropic drugs after surgery. Therefore, the percentage of women female patients who require inotropic medication was higher than that of male patients with similar circumstances (p=0.0024). The odds ratio in this study was 15, meaning that taking inotropic drugs in female patients is 15 times more often than men (Figure 3A). Figure 3B represents that the mean of BMSCs doubling time in female patients (205 ± 65) who received inotropic drugs was less than that of male patients (327 ± 59) who received inotropic drugs ($p < 0.05$). There was no difference in BMSCs doubling time in males (211 ± 54) and females (196 ± 16) patients who did not use medication.

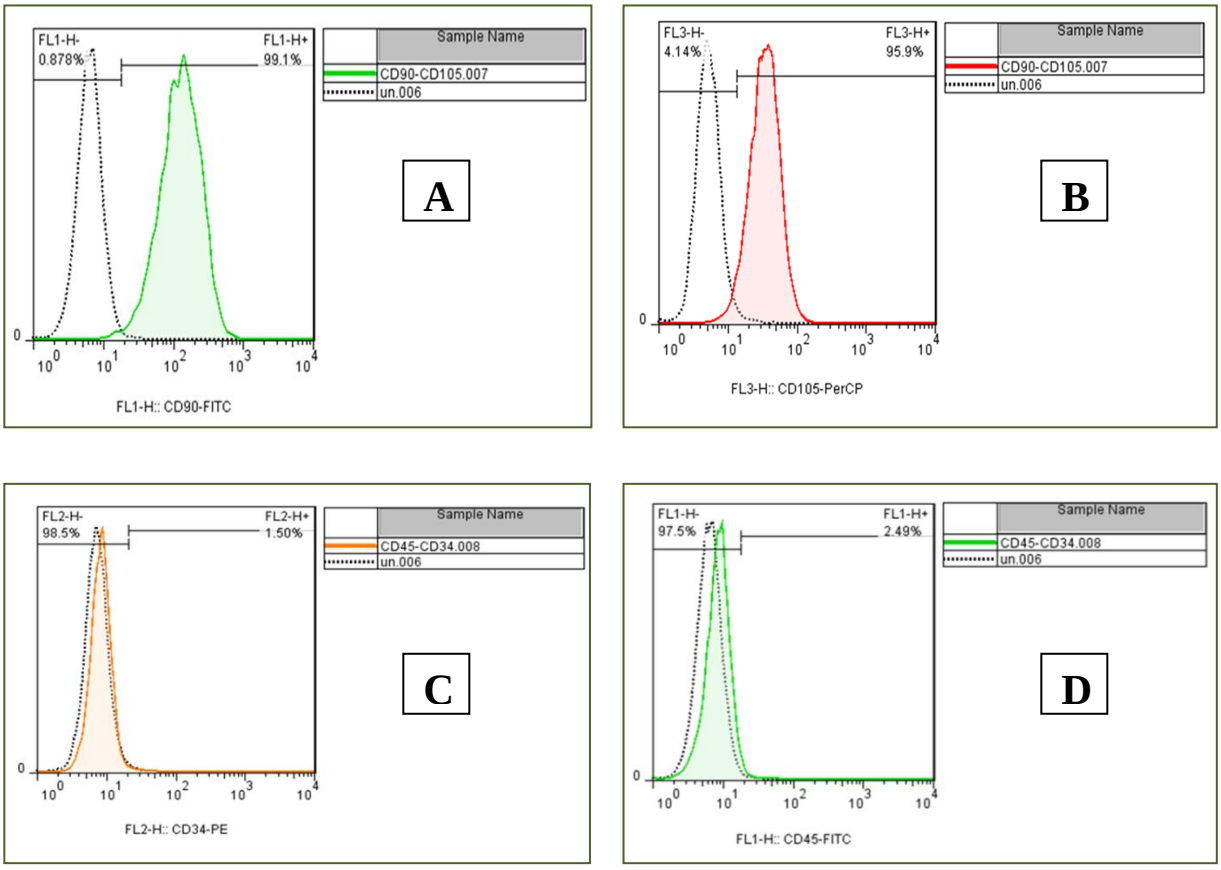

Figure 2. A) Flow cytometry diagram of CD90 mesenchymal antibody. **B)** Flow cytometry diagram of CD105 mesenchymal antibody. **C)** Flow cytometry diagram of CD34 hematopoietic antibody. **D)** Flow cytometry diagram of CD45 hematopoietic antibody.

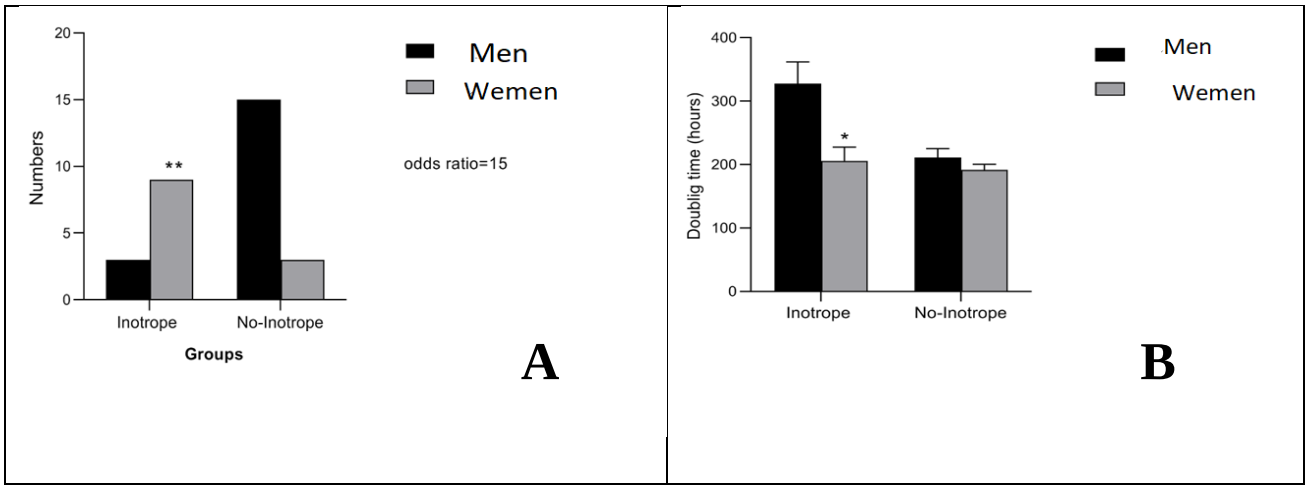

Figure 3. A) The inotropic drug requirements in male and female patients. **B)** The association between MSCs doubling time and taking inotropic drugs in males and females. The data is shown as a mean ± standard deviation. Statistical significance difference was indicated as $p < 0.05$.

Discussion

The main results of this study can be summarized as follows: after surgery, the mean of BMSCs doubling time in female patients who received inotropic drugs was less than that of male patients. There was no difference in the BMSCs doubling time in male and female patients who did not receive inotropic drugs.

Left ventricular dysfunction with low ejection fraction is joint in patients undergoing CABG surgery (18, 19). Despite advancements in surgical techniques, the management of patients with severe left ventricular dysfunction undergoing CABG remains challenging, and patients with ejection fraction <30% are at a higher risk for complications after surgery. Therefore, early diagnosis of perioperative variables to identify higher-risk patients play an active role in the management process of patients. In patients undergoing CABG surgery, low ejection fraction is associated with requirements for inotropic support (18, 20). It is not surprising that the prediction of the inotropes' requirements might be a strong mortality predictor.

In many studies, BMSCs have been used to improve heart function as a new option for cardiac disease therapy (21, 22).

BMSCs are adult multipotent stem cells that can differentiate into a variety of cells, including cardiomyocytes (23, 24). There are specific markers BMSCs surface. According to studies conducted by other researchers and us, the results of flow cytometry indicated that the expression of CD105 and CD90 mesenchymal markers are specific features of these cells (25).

Numerous studies have reported that BMSCs can be useful in improving infarcted myocardium. Transplantation of stem cells into the infarcted region leads to the regeneration of cardiomyocytes and the improvement of contractile function of the damaged myocardium (26, 27). Therefore, it is expected that the proliferation rate of MSCs will have a significant relationship with inotropic taking requirements and in patients who need inotropic administration, doubling time would be shorter. Our study proved that the average doubling time in female patients with inotropic use is less than male patients with inotropic use. But doubling time in female and male patients without inotropic use was not significantly changed.

Conclusion

We concluded that in patients who require inotropic administration, doubling time would be shorter. The results of the present study showed that the rate of MSCs proliferation in female patients who received inotropic drugs were significantly less than that of male patients in the same situations.

The conclusion of this investigation may help cardiothoracic surgeons to prospectively identify

those patients who will likely require inotropic support after surgery.

Conflict of Interest

The authors declared no conflict of interest.

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Ethics

This project was approval by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (Code: IR.SSU.MEDICINE.REC.1397.199).

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