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Original Article

Association between Characterizations of Bone Marrow Mesenchymal Stem Cells, Ejection Faction and Hospitalization Period in Patients with Severe Left Ventricular Dysfunction after Off-Pump Bypass Surgery

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Abstract

Background and Aim: Severe left ventricular dysfunction patients with ejection fraction ≤ 30% are at a higher risk for complications and mortality than patients with ejection fraction >30%. The death of cardio-myocytes at injured regions leads to myocardium dysfunction. Bone marrow-derived mesenchymal stem cells are undifferentiated cells that have been used for the regeneration of damaged cardio-myocytes. Due to the inherent capability of mesenchymal stem cells to improve cardiac functions, in this research, our objective was to explore the possible association of the mesenchymal stem cells proliferation rate with Coronary Artery Bypass Grafting outcomes in patients with severe left ventricular dysfunction after off-pump Coronary Artery Bypass Grafting. **Methods:** For investigating the possible association of mesenchymal stem cells proliferation with Coronary Artery Bypass Grafting outcomes (ejection fraction, hypertension risk, and the time of hospital stay), we collected bone marrow samples from 30 patients (18 men and 12 women) who underwent off-pump Coronary Artery Bypass Grafting at Afshar Hospital and Seyed Al-Shohada Hospital (Yazd, Iran). Mesenchymal stem cells were isolated and cultured; then, cells were counted after 4, 7, and 14 days using trypan-blue color, and doubling times were calculated. **Results:** There was an association between doubling time and ejection fraction after surgery. Ejection fraction in postoperative patients increased, but this association was not significant. Also, our study showed that the risk of hypertension is equal in male and female patients. There were no significant differences in mesenchymal stem cells doubling time with hypertension and the time of hospital stay in the ICU. **Conclusion:** Based on this study, we concluded that there was no significant relationship between the rate of mesenchymal stem cell proliferation and Coronary Artery Bypass Grafting outcomes in patients with severe left ventricular dysfunction.

Keywords: Severe Left Ventricular Dysfunction; Mesenchymal Stem Cell; Doubling Time; Ejection Fraction.

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Introduction

Severe left ventricular dysfunction has become very common in patients with ischemic cardiomyopathy and remains a remarkable risk factor for mortality and morbidity (1). Ejection fraction ≤ 30% has been selected as the cut-off point for severe left ventricular dysfunction (2). In ischemic circumstances, the death of cardio-myocytes at injured regions leading to myocardium dysfunction and necrosis (3, 4). Currently, Coronary Artery Bypass Grafting (CABG) is considered a standard treatment in patients with low ejection fraction (5). However, the therapeutic effects of CABG surgery in these patients are not easily predictable (6).

Furthermore, CABG does not restore the necrotic cells of injured heart tissues but only manages the clinical symptoms of the disease (1). In recent decades, many studies have proceeded to explore cells with the ability to differentiate and repair the damaged myocardium (7). Cells isolated from a wide variety of embryonic and adult tissues have been investigated for the regeneration and management of injured heart tissue (7-9). Among them, Human Bone Marrow Mesenchymal Stem Cells (BMSCs) have been particularly attractive because of their potential to differentiate into cardiomyocytes and coronary vessels (10). In a cell culture laboratory, isolated Mesenchymal Stem Cells (MSCs) were successfully differentiated into cardiomyocytes and characterized by cardiac markers expression (11). Several in vivo studies suggested that bone marrow-derived MSCs may be critically implicated in the protection of the structural and the improvement of myocardium function (10, 12, 13). Due to the importance of BMSCs in the repair of the cardiac structure and function, BMSCs may be related to CABG outcomes in patients with severe left ventricular dysfunction after off-pump CABG (14). In the present study, we examined the possible effects of MSCs in patients with severe left ventricular dysfunction (ejection fraction ≤ 30%) after off-pump bypass surgery and the improvement in ventricular performance. After CABG, the relationships between the doubling time of MSCs and ejection fraction, hypertension risk, and the hospital stay period were examined.

Methods

Patients and Study Design

The study protocol was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, and patients' consent was filled before entry to study. We enrolled 30 patients undergoing off-pump left ventricular CABG, who were referred to as Afshar and Seyed Al-Shohada Hospitals (Yazd, Iran). The inclusion criteria were as follows: patients with severe left ventricular dysfunction (ejection fraction < 30%). The exclusion criteria were as follows: ejection fraction > 30, patients with concomitant diseases such as severe valvular heart disease, diabetes, and infectious diseases. Then, the necessary tests, such as echocardiography, were evaluated by a physician. One cc bone marrow sample per patient was aspirated from the sternum during off-pump CABG and then transferred to a stem cell laboratory for isolation of MSCs and further evaluation.

Culture of human BMSCs

Bone marrow samples (1mL) were obtained from the sternum during off-pump CABG. For the MSCs isolation from bone marrow, the aspirated bone marrow samples were diluted with αMEM cell culture medium containing 20% fetal bovine serum (FBS). In the laboratory, samples were transferred to 15 mL Falcons and then centrifuged at 12000 rpm for 5 minutes. After centrifugation, the supernatant was removed. The cells were grown in αMEM culture medium containing 20% FBS in an incubator containing 5% CO2 at 37◦C. For the Characterization of MSCs, the presence of surface markers of Hematopoietic Stem Cells (HSCs) (CD34, CD45) and MSCs (CD90, CD105) was evaluated by a flow cytometer (FACS Calibur Becton, Dickinson, USA).

Population-doubling time

For doubling time calculation, the cells were cultured in an αMEM supplemented with 20% FBS and incubated at 37◦C until arriving at 80% confluence. Then, cells were trypsinized, and the cell numbers were counted in 4, 7, and 14 days using trypan blue and hemocytometer. The relationships between the doubling time of MSCs /ejection fraction and between hypertension risk/ the hospitalization were examined. The relationships between MSCs doubling time/ejection fraction and between the risk of high blood pressure/the hospitalization period were examined.

Statistical analysis: The Graphpad Prism Version 8 software (GraphPad Software, San Diego, CA, USA) was used to analyze results. All data presented as means ± standard deviations. The results were analyzed by unpaired t-test.

Results

In this study, 18 men and 12 women with severe

left ventricular dysfunction were studied. In all 30 patients, 1 ml bone marrow from the sternum was collected during surgery for BMSCs isolation and doubling time calculation. The isolation of the MSCs was confirmed via flowcytometry method. MSCs express CD90 and CD105 markers. Also, these cells were negative for hematopoietic markers, CD34, and CD45 (data were not shown). MSCs' phenotype was shown in Figure 1.

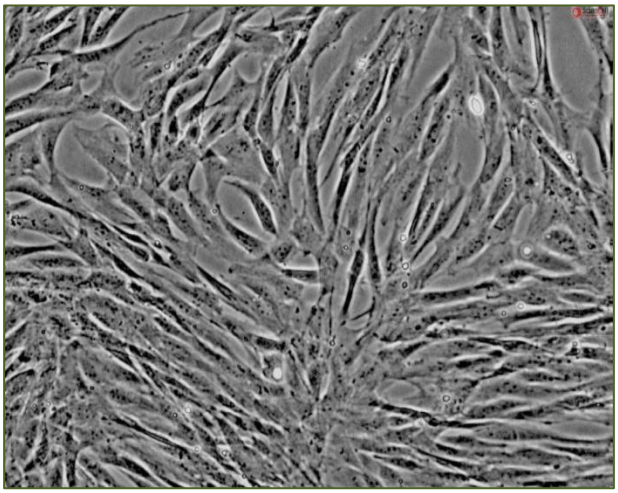

Figure 1. MSCs after extraction and culture.

The relationship between preoperative ejection fraction and the doubling time of BMSCs

Figure 2A shows the mean of preoperative ejection fraction. There was no significant difference in the percentage of ejection fraction in men and women. The mean ± deviation was 44.64 ± 2.5 in the male group and 43 ± 2.2 in the female group. Figure 2B shows the relationship between ejection fraction in male and female patients with a doubling time of BMSCs before CABG. There was no significant relationship between preoperative ejection fraction and MSCs doubling time (r2=0.0188).

The relationship between postoperative ejection fraction and the doubling time of BMSCs

Figure 3A shows the mean of postoperative ejection fraction in men and women. There was no significant difference in the percentage of ejection fraction in males and females, and the mean ± deviation was 46 ± 2.8 in the male group and 45.4 ± 2.1 in the female group. Figure 3B shows the relationship between ejection fraction in male and female patients with BMSCs doubling time after CABG. There was no significant relationship between preoperative ejection fraction and MSCs doubling time (r2=0.0252).

Relationship between the hospitalization period and BMSCs doubling-time

Figure 4A shows the mean of hospitalization period in male and female patients in the ICU (day). We found no significant difference between the mean hospitalization period in the ICU in men and women, and the mean ± SD was 8.1 ± 2.8 in the male group and 8.5 ± 5 in the female group. Figure 4B shows the relationship between the hospitalization period in male and female patients with a BMSCs doubling time after CABG. There was no significant relationship between hospital stay time in the ICU and MSCs doubling time (r2=0.034).

Relationship between hypertension and doubling time of BMSCs

Figure 5A shows that among 18 male patients in this study, ten patients had hypertension, while among 12 female patients in this study, five patients suffer hypertension. Therefore, the percentage of women and men with hypertension did not differ. The odds ratio was 1, meaning that the chances of hypertension in male and female patients were equal. Figure 5B illustrates that there was no considerable difference in BMSCs doubling time in female patients with hypertension risk (196 ± 55) and male patients with hypertension risk (238 ± 93). On the other hand, there was no significant difference between BMSCs doubling time in males (224 ± 47) and female (205 ± 61) patients without hypertension.

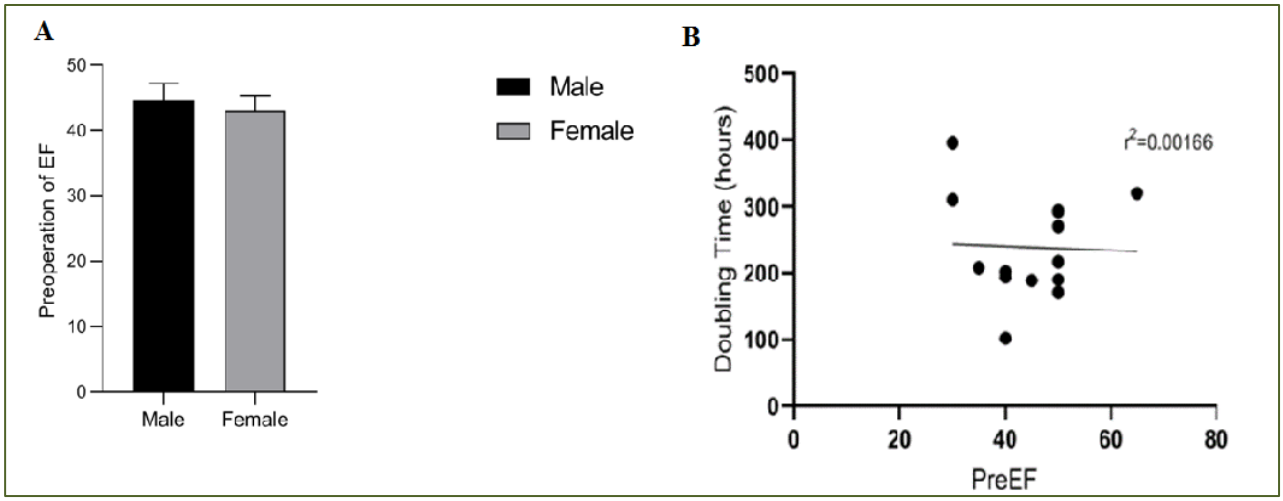

Figure 2. A) The relationship between gender and preoperative ejection fraction. The data is shown as a mean ± standard deviation **B)** the relationship between preoperative ejection fraction (EF) and BMSCs doubling time.

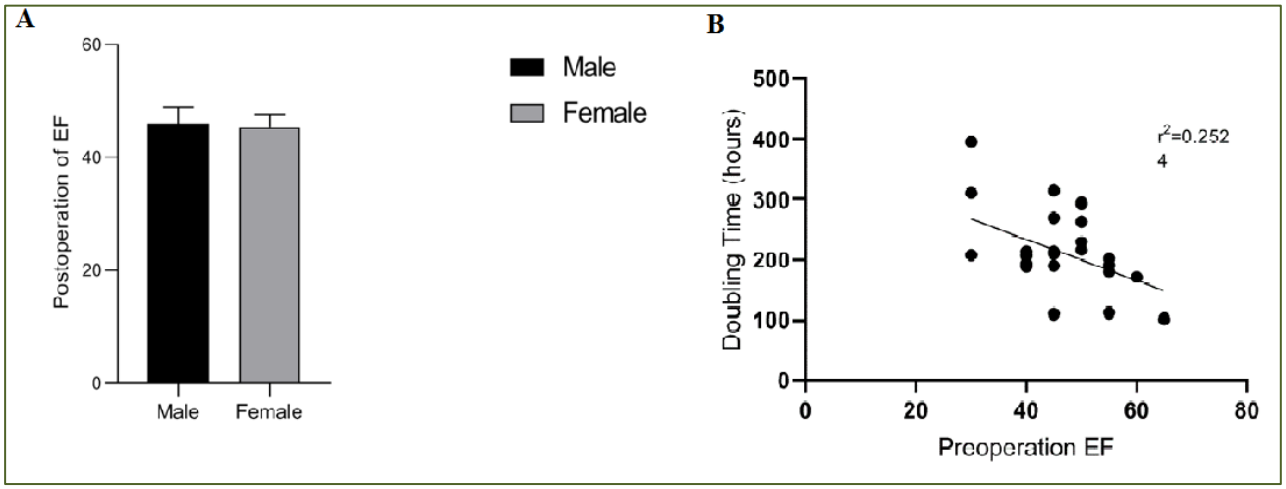

Figure 3. A) The relationship between gender and postoperative ejection fraction. The data is shown as a mean ± standard deviation. **B)** The relationship between postoperative ejection fraction (EF) and BMSCs doubling time.

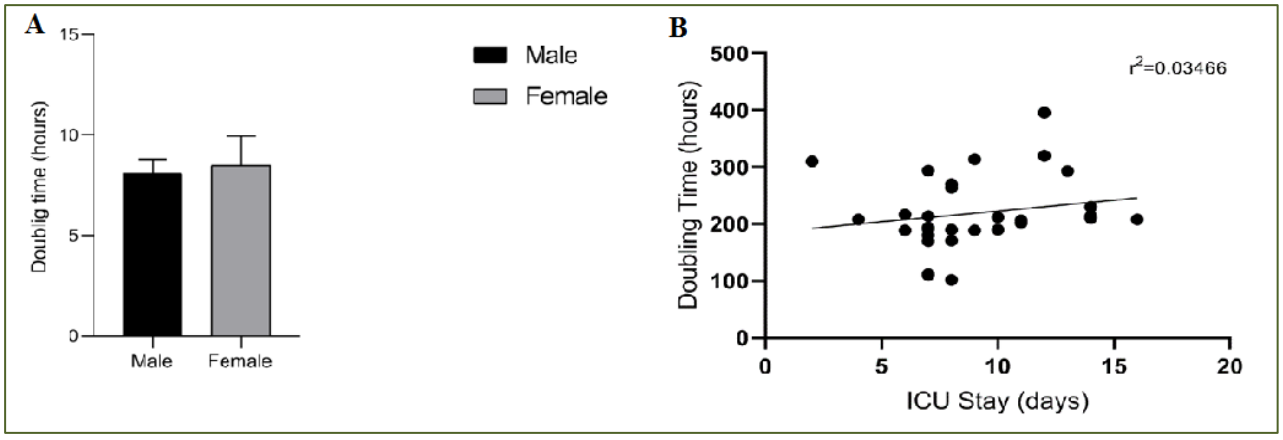

Figure 4. A) The relationship between gender and hospital stay in the ICU (day) **B)** The relationship between hospitalization period in the ICU and BMSCs doubling time.

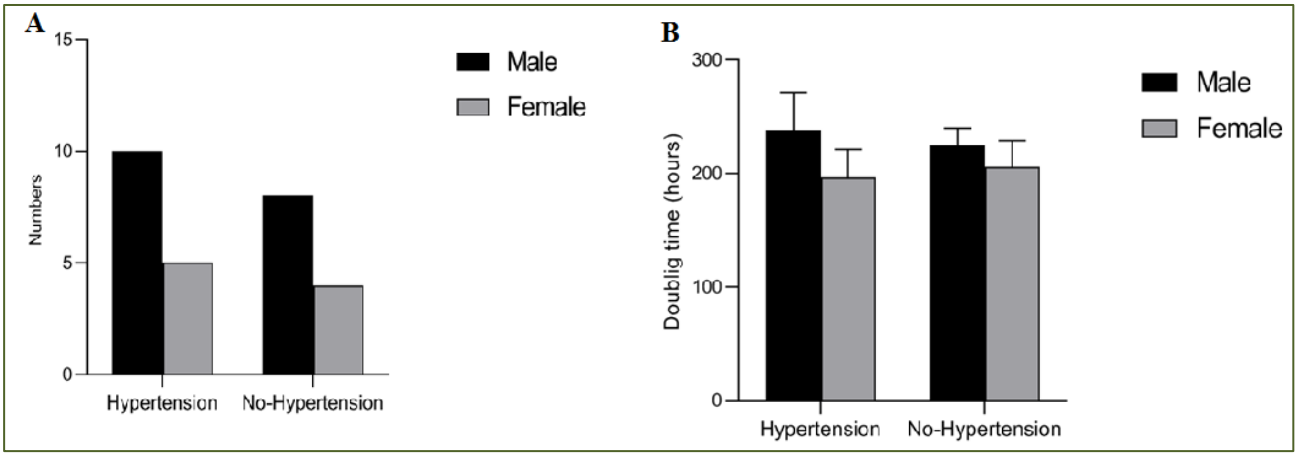

Figure 5. A) Relationship between hypertension in male and female patients. **B)** Relationship between doubling time of BMSCs in patients with hypertension. Data were presented using a two-way ANOVA statistical test and mean ± standard deviation.

Discussion

The main findings of our study can be summarized as follows: no significant differences were found between BMSCs doubling time and any of these 3 CABG outcomes: ejection fraction, hypertension risk, and the time of hospital stay in patients with severe left ventricular dysfunction after off-pump CABG.

According to the American Society of Echocardiography's Guidelines and Standards Committee, severe left ventricular dysfunction has been described with an ejection fraction of less than 30% (2). Many clinical kinds of research have shown relevant evidence that patients with an ejection fraction < 30% are at higher death risk than patients with ejection fraction > 30% (15). In the last decade, stem cell therapy has been used as a new option for the repair of damaged myocardium and the improvement of cardiac function (16-18). Several in vitro and in vivo studies have confirmed the capability of human BMSCs to differentiate into the cardio-myocytes (3). In 2016, Valentina et al. reported that the ability of stem cells to proliferation and differentiation decreased with aging. They also reported that the ability of MSC to regenerate damaged tissues such as myocardium is relevant to their paracrine ability and not to their ability to differentiate into cardiomyocytes (19-21). Due to the importance of BMSCs in tissue repair and regeneration, the features of BMSCs may be related to CABG outcomes in patients with severe left ventricular dysfunction after off-pump CABG. In the current study, MSCs were isolated from aspirated sternum bone marrow of patients undergoing CABG, using a small aspiration needle. Consistent with previous reports, isolated MSCs expressed positive markers CD90, and CD105, but did not show negative markers CD34 and CD45 (22-24). Then, we investigated the possible association between isolated MSCs doubling time and ejection fraction, risk of hypertension, and the hospitalization period in patients with severe left ventricular dysfunction (ejection fraction ≤ 30%) after off-pump bypass surgery. There was no significant relationship between the doubling time of MSCs and preoperative ejection fraction. There is an association between doubling time and ejection fraction after surgery. After surgery, the ejection fraction was increased, but this association was not significant. Any similar study has not yet examined the relationship between hospitalization and MSC characteristics. Here, the relationship between hospitalization period and MSC doubling time was investigated, but no remarkable relationship was obtained between the patient hospitalization period in ICU and MSC doubling time.

In hypertensive rats, intravenous injection of MSCs reduces the pressure on the ventricles and the symptoms of hypertension. MSCs can increase the gene expression of nitric oxide synthase (NOS) and

reduce the average blood pressure by producing endothelial progenitors (25). Therefore, due to the MSCs' potential capability to improve hypertension and its symptoms, we investigated the relationship between the MSCs' doubling time and the hypertension risk in patients. Our study suggested that hypertension risk is equal in male and female patients. There were no noticeable differences in BMSCs female patients with and without hypertension, as well as male patients with and without hypertension. The lack of a significant association between hypertension and doubling time can be attributed to the patients aging (50-70 years).

Conclusion

Despite the role of MSCs in the repair of damaged cardiac tissue, the results of the present study claimed that there was not a clear relationship between the rate of MSCs proliferation and CABG outcomes (ejection fraction, hypertension risk, and the time of hospital stay) in patients with severe left ventricular dysfunction. It is recommended that this study be performed on a larger specimen group.

Acknowledgment

Not to declare.

Ethics

The study protocol was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences with this code: IR.SSU.MEDICINE.REC.1397.199.

Conflict of Interest

The authors declare that they have no conflict of interest.

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