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DIFFERENCES IN VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AND MYOCARDIAL PERFUSION BETWEEN BEFORE AND AFTER PHASE II CARDIOVASCULAR REHABILITATION IN CORONARY ARTERY DISEASE PATIENTS AFTER COMPLETE REVASCULARIZATION: A PROTOCOL STUDY

J. W. Martha[1], A. H. S. Katamihardja[2], A. Nazir[3], N. Sylviana[4], D. K. Turbawaty[5], B. B. Tiksnadi[1]

[1]Department of Cardiology and Vascular Medicine, [2]Department of Nuclear Medicine, [3]Department of Physical Medicine and Rehabilitation, [4]Department of Biomedical Sciences, [5]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran – Bandung, Indonesia

РАЗЛИКИ В ЕКСПРЕСИЯТА НА СЪДОВИЯ ЕНДОТЕЛЕН РАСТЕЖЕН ФАКТОР И МИОКАРДНАТА ПЕРФУЗИЯ МЕЖДУ ПРЕ- И ПОСТФАЗА II СЪРДЕЧНО-СЪДОВА РЕХАБИЛИТАЦИЯ ПРИ ПАЦИЕНТИ С КОРОНАРНА АРТЕРИАЛНА БОЛЕСТ СЛЕД ПЪЛНА РЕВАСКУЛАРИЗАЦИЯ – ПРОТОКОЛНО ПРОУЧВАНЕ

Дж. У. Марта[1], А. Х. С. Катапихарджа[2], А. Назир[3], Н. Силвиана[4], Д. К. Турбауати[5], Б. Б. Тиксанди[1]

[1]Катедра по кардиология и съдова медицина, [2]Катедра по нуклеарна медицина, [3]Катедра по физикална медицина и рехабилитация, [4]Катедра по биомедицински науки, [5]Катедра по клинична патология, Факултет по медицина, Университет „Padjadjaran“ – Бандунг, Индонезия

Abstract.

Introduction: Cardiac rehabilitation may improve health outcomes in coronary artery disease (CAD) patients, but the mechanism is still unknown. Collateralization and improvement of microvascular flow is one of the hypotheses underlying this mechanism, however research on this is scant and inconclusive. Collateralization is affected by VEGF expression and has the effect of improving myocardial perfusion. Therefore, this study aimed to assess VEGF levels and myocardial perfusion before and after cardiac rehabilitation. **Methods and Results:** This study is a comparative study of patients who received cardiovascular rehabilitation treatment at Dr. Hasan Sadikin Hospital. The subjects of this study were consecutively selected from patients who received cardiovascular rehabilitation treatment at Dr. Hasan Sadikin Hospital. The primary outcome of this study is to assess the expression of VEGF A and myocardial perfusion in coronary artery disease patients who have undergone complete revascularization before and after phase II cardiac rehabilitation. **Conclusion:** This study will assess the beneficial effects of phase II rehabilitation in more detail as it uses proteomics parameters to determine the underlying mechanism and assesses myocardial perfusion as an outcome.

Key word: cardiovascular rehabilitation, myocardial perfusion, SPECT, VEGF

Address for correspondence: Badai Bhatara Tiksnadi, e-mail: badai.bhatara.tiksnadi@unpad.ac.id

Резюме.

Въведение: Сърдечната рехабилитация може да подобри здравните резултати при пациенти с коронарна артериална болест (CAD), но механизмът все още не е известен. Колатерализацията и подобряването на микроваскуларния поток е една от хипотезите, лежащи в основата на този механизъм, но изследванията за това са оскъдни и неубедителни. Колатерализацията се влияе от експресията на VEGF и има ефект за подобряване на миокардната перфузия. Ето защо в нашето проучване си поставихме за цел да се оценят нивата на VEGF и миокардната перфузия преди и след сърдечна рехабилитация. **Методи и резултати:** Представяме сравнително проучване на пациенти, преминали сърдечно-съдова рехабилитация в болницата „Д-р Хасан Садикин“. Субектите на проучване бяха последователно избрани сред пациенти, преминали сърдечно-съдова рехабилитация в болница „Д-р Хасан Садикин“. Основният резултат от това проучване е да се оцени експресията на VEGF A и миокардната перфузия при пациенти с коронарна артериална болест, които са претърпели пълна реваскуларизация преди и след фаза II сърдечна рехабилитация. **Заключение:** Това проучване ще оцени благоприятните ефекти от фаза II рехабилитация с повече подробности, тъй като използва протемични параметри за определяне на основния механизъм и оценява миокардната перфузия като резултат.

Ключови думи: сърдечно-съдова рехабилитация, миокардна перфузия, SPECT, VEGF

Адрес за кореспонденция: Бадай Тикснади, e-mail: badai.bhatara.tiksnadi@unpad.ac.id

Introduction

Coronary artery disease (CAD) is a condition in which there is an imbalance between the supply and demand of oxygen to the myocardium. The most common cause of coronary artery disease is the formation of plaque in the lumen of the coronary arteries, which impedes blood flow. It is the leading cause of death worldwide. In 2012, approximately 17.5 million people died from cardiovascular disease, accounting for 31% of all mortality worldwide. This shows an increase in the number of deaths compared to data from 2008, when 17.3 million people died. By 2030, World Health Organization (WHO) estimates that deaths from cardiovascular disease will reach more than 23.6 million people each year [1].

The standard management for acute ST elevation myocardial infarct patients includes medical therapy with thrombolysis and primary percutaneous coronary intervention (pPCI), while for chronic disease, therapy includes elective percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). The goal of revascularization in CAD is to restore blood flow to the coronary arteries. In addition to emergency therapies that rescue the patients, there are also regenerative therapies that aim to restore the physiological and anatomical functions of the heart to improve the patient's quality of life, namely secondary cardiovascular rehabilitation [3]. Cardiac rehabilitation program is a secondary prevention program that can improve the quality of life, both in physical, social, and emotional aspects, so as to reduce the physiological and psychological impact of heart disease that is chronic and after the acute phase of cardiovascular disease. The selection of cardiovascular rehabilitation program should be based on the patient's clinical condition, degree of atherosclerosis, clinical factors, and availability of facilities after revascularization procedures [2].

Various studies have been conducted to see the effectiveness of cardiac rehabilitation programs in cardiovascular disease patients. These programs have been shown to be effective in reducing total mortality (13-26%) and cardiac mortality (26-36%), reducing morbidity, reducing the risk of further cardiac problems, reducing the number and cost of hospital visits (28-56%), improving functional capacity, increasing exercise tolerance, accelerating return to work, developing self-management capabilities, as secondary prevention [3-8]. The mechanisms that are thought to contribute to the improvement of the above outcomes have yet to be fully explained. One hypothesis is that it could be through improved myocardial perfusion, but there are very few studies that explain this mechanism. Myocardial perfusion may result from improved coronary microcirculation, increased collateralization, and improvement of myocardial ischemia status.

Myocardial perfusion is an imaging test to assess how adequately blood flows to the heart muscle. It is non- invasive and is assessed using a nuclear technique. There are several ways to assess myocardial perfusion, including single photon emission computed tomography (SPECT) and positron emission tomography (PET). SPECT examination is one of the most performed because radiopharmaceuticals are easier to obtain and more affordable. One way to improve myocardial perfusion is through physical exercise. Physical exercise is expected to increase can cause structural and functional changes in the heart, such as improvements in epicardial vasa vasoreactivity through improved endothelial function, increased vasodilatory capacity, stabilization of vascular smooth muscle phenotype, improved blood flow through the process of weakening resistant vasa and collateral formation so that myocardial perfusion becomes better [9]. In the study of Ozdemir et al. stated that patients with Rentrop grade 2-3 had a positive effect on myocardial perfusion and function but were not statistically significant compared to Rentrop grade 0-1 [10]. There is also another study that states that although not visible on angiography, collaterals can provide more than half of normal perfusion [11]. However, in a meta-analysis assessing the relationship between physical exercise and myocardial perfusion in animal subjects, it was found that physical exercise had a positive correlation with improved myocardial perfusion [12]. In another study conducted by Giallauria et al. also showed significant results between physical exercise for 6 months and reduced myocardial perfusion disability in patients with acute myocardial infarction [13]. From these studies, there are inconsistent results regarding perfusion improvement.

Collateralization and improvement of microvascular flow is one of the important factors in improving myocardial perfusion. This process is triggered by proteomic expression. Parameters that help proteomic expression in the formation process consist of Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), and Platelet-derived Growth Factor (PDGF). VEGF can trigger the process of angiogenesis in coronary blood vessels, with the subtype that plays the most role is VEGF A. A study by Skrypnik et al. explained the effect of cardiac rehabilitation for 2 weeks can increase the expression of VEGF A. However, a study conducted by Gao et al. showed a decrease in VEGF A expression in cardiac rehabilitation patients after being followed for 1 year. This condition may be due to the improvement of ischemia conditions so that VEGF A levels have decreased [14, 15].

Patients undergoing cardiac rehabilitation generally have already undergone revascularization, so ischemia is presumed to be absent. Whether this mechanism remains the reason for rehabilitation in this population remains unknown. Currently, there is limited research investigating the mechanism of benefit of cardiac rehabilitation in CAD patients who have undergone complete revascularization. Therefore, the aim of this study is to investigate the differences in VEGF-A and myocardial perfusion in patients with CAD who have had complete revascularization before and after phase II cardiovascular rehabilitation in order to determine the specific benefits of phase II cardiac rehabilitation.

Methods and results

Study Design and Subjects

This study uses an intervention design by comparing before and after treatment of coronary artery disease patients who underwent complete revascularization at Dr. Hasan Sadikin Hospital (RSHS) Bandung. Complete revascularization is defined as post PCI with TIMI flow 3 and no planned stagging PCI (all lesions with > 70% stenosis in LAD, LCx, and RCA or > 50% in LM have been successfully performed stenting) or in post CABG patients. Patients will receive intervention in the form of moderate intensity continuous exercise. The intervention will be performed 12 times with a frequency of twice a week. All patients will be tested for miRNA 92a and 126, vascular endothelial growth factor (VEGF) A, myocardial microalternation index (MMI), and myocardial perfusion using single photon emission computed tomography (SPECT) before and after the cardiac rehabilitation intervention. Major adverse cardiac events (MACE) will be monitored for up to one year after the intervention. The subjects of this study were consecutively selected from patients who received cardiovascular rehabilitation treatment at Dr. Hasan Sadikin Hospital. The population of this study was coronary artery disease patients aged ≥ 18 years who had undergone complete revascularization at Dr. Hasan Sadikin Hospital. Patients who did not complete Phase II cardiovascular rehabilitation, had ambulatory problems such as inability to walk due to stroke or other limb motor dysfunction, a history of stroke, hypothyroidism or hyperthyroidism, and a history of cancer or chemotherapy were excluded from this study. Based on statistical calculations, the minimum sample size in this study was 34 samples, but because this study followed the main study where the planned sample size was 25 samples.

Data Collection

Patients who met the inclusion and exclusion criteria were included in this study. Prior to Phase II cardiac rehabilitation, patients were assessed for demographic data, history of hypertension, diabetes, dyslipidemia, family history of heart attack and smoking status, vital signs, body mass index (BMI), quality of life assessment, lifestyle screening and functional capacity. Patients were then evaluated for VEGF and myocardial perfusion according to the evaluation protocol. (Fig. 1).

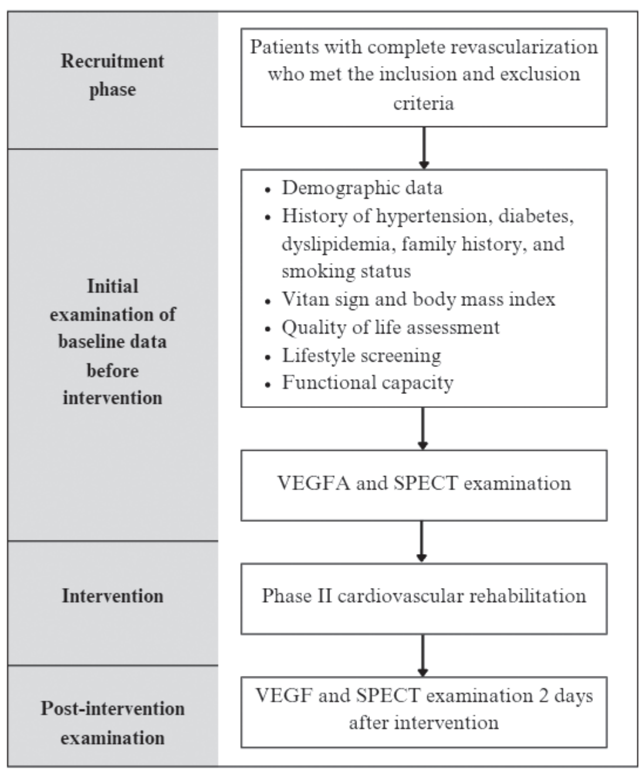

Fig. 1. Data collection and research procedures

Outcomes

The primary outcome of this study is to assess the expression of VEGF A and myocardial perfusion in coronary artery disease patients who have undergone complete revascularization before and after phase II cardiac rehabilitation. As secondary outcomes, this study also assesses the patient's blood pressure, body mass index, functional capacity, and quality of life based on Cantril's Ladder of Life and PHQ-2 before and after cardiac rehabilitation. Patients will be followed for 1 year regarding mortality and rehospitalization due to cardiovascular problems.

The expected outcome of this study is that there are differences in VEGF expression after cardiovascular rehabilitation. VEGF levels may decrease due to improvement in ischemic conditions. This decreased VEGF level is also expected to be related to the results of myocardial perfusion examination, where there is expected to be a decrease in ischemic burden after cardiovascular rehabilitation. However, because studies on VEGF are still inconclusive, it is possible that VEGF levels in this study increased due to the incomplete angiogenesis process, so that ischemic conditions still occur.

Phase II Cardiac Rehabilitation Protocol

Phase II of cardiac rehabilitation takes place after the patient is discharged from the hospital. This phase aims to restore the patient's function as optimally as possible, control risk factors, and provide additional education and counseling regarding a healthy lifestyle. The duration of the cardiac rehabilitation program is six weeks, with the sessions held twice a week, consisting educational sessions along with supervised aerobic exercise, using ergocycle or treadmill devices with the FITT (frequency, intensity, time, type) principle. The aerobic exercise session starts with a low intensity on a scale of 7 for the first 5 minutes as a warm-up, followed by 30 minutes of moderate intensity, determined by a heart rate reserve of 50-80% (based on maximal treadmill test at entry test) and a Borg scale rating of 11-15, with a continuous exercise approach. In addition to supervised exercise sessions in the hospital, patients are also asked to exercise at home 1-3 times per week at a similar intensity to the hospital exercises. Home exercise activities are recorded in a logbook. During these sessions, the subjects' symptoms, blood pressure, pulse, and oxygen saturation were closely recorded. In addition, a home exercise regimen was prescribed to each patient. There were personalized health education sessions for each patient covering topics such as cardiac anatomy, exercise, sexual activity, and healthy diet through educational video programs.

VEGF Examination Protocol

Venous blood sampling was performed more than 48 hours after the last exercise in patients before undergoing a cardiovascular rehabilitation program and 48 hours to 12 days after the last exercise in patients after completing a cardiovascular rehabilitation program. Patients were asked to avoid exercise for at least 2 days prior to testing. This avoids the acute effects of post-exercise VEGF elevation [16]. To avoid post-rehabilitation detraining effect, blood test should be done before 12 days [17]. Blood samples were taken from venous blood up to 10 ml, which was then collected in a yellow tube or clot activator tubes which contains a gel separator. The sample was then separated to obtain serum by centrifugation for 30 min. The separated serum samples were centrifuged at 3000 rpm for 15 min, then divided into aliquots and stored in a freezer at -80º C. VEGF levels are measured by enzyme-linked immunosorbent assay (ELISA) according to the provided protocol. ELISA was selected as the VEGF assay method because it has high sensitivity and specifici-

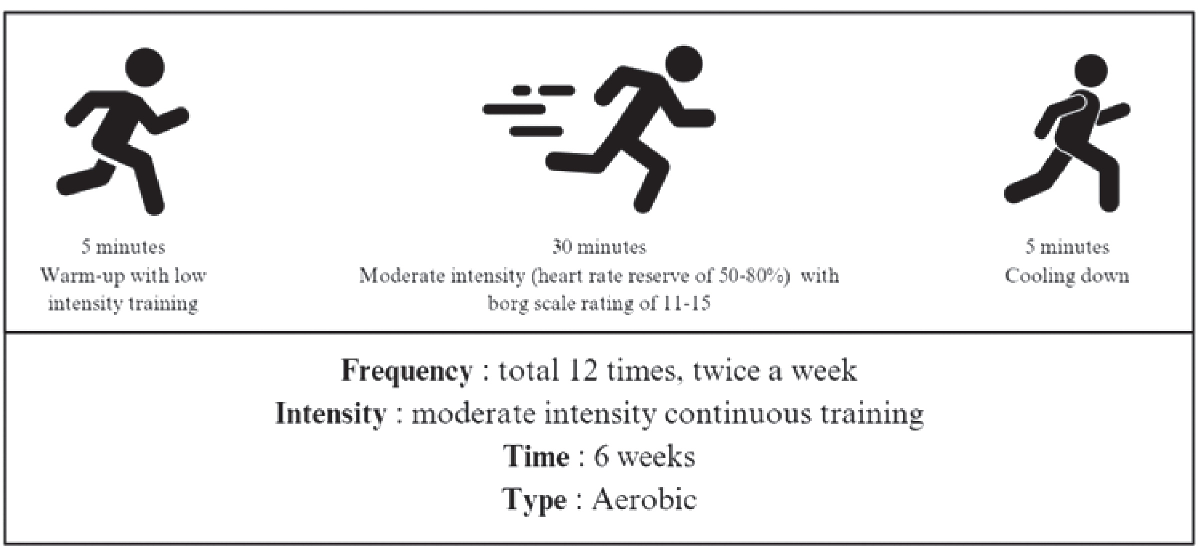

Fig. 2. Summary of moderate intensity continuous training protocol

ty, assesses quantitatively, and is the most used technique. Alternatives to ELISA for VEGF examination are immunohistochemistry (IHC) and real-time polymerase chain reaction (qPCR). IHC is not performed because it uses tissue as the sample, while qPCR does not directly test for VEGF protein levels. Other tests that can be used to assess VEGF include mass spectrometry and bioassays, but they are expensive, infrequent and take more time to perform.

Myocardial Perfusion Examination Protocol

Myocardial perfusion was assessed by SPECT. The examination was performed using the two-day protocol, with physical stress or pharmacological stress (adenosine) and at rest performed on different days. Physical stress was performed with the modified Bruce method and increased every three minutes. The radiopharmaceutical used was Tc-99m sestamibi, administered intravenously when peak stress was achieved. Myocardial perfusion imaging was performed before and three months after the intervention. Prior to the s tudy, several preparations were made, including discontinuation of beta-blockers one day before the study, calcium channel blockers (verapamil/diltiazem) two days before the study, nitrates two days before the study, no coffee/tea/chocolate 24 hours before the study, and no fat-rich food two hours before the study.

Statistical Analysis

The research data will be analyzed quantitatively by measuring the levels of VEGF and SPECT of coronary artery disease patients after complete revascularization undergoing Phase II cardiovascular rehabilitation. All quantitative data were statistically analyzed by Kolmogorov-Smirnov normality test, and if the distribution was normal, paired t-test was used to compare VEGF and SPECT levels before and after Phase II cardiovascular rehabilitation. Multivariate analysis will be performed to determine the dependent factors for changes in VEGF and SPECT levels. Descriptive summaries will be presented for all patients and for subgroups of patients by reporting risk factor prevalence and management records. Bivariate analysis will be performed on categorical and numerical variables, and where possible, multivariate analysis will be performed on variables that are significant in the bivariate analysis using linear regression. The analysis in this experiment used a 5% significance level with a 95% confidence level. A significant difference was considered to be a significant if the p-value was < 0.05. The analysis was performed using IBM SPSS computer software version 25.0 for Windows system.

Ethics and Dissemination

This study has been approved by the ethics committee of Dr. Hasan Sadikin Hospital, Bandung with the ethical code DP.04.03/D.XIV.6.5/325/2024.

Discussion

This study will assess in more detail the mechanisms of the beneficial effects of cardiac rehabilitation in patients with coronary artery disease who have undergone complete revascularization. The underlying mechanisms will be examined using proteomic expression parameters and assessing myocardial perfusion using SPECT.

Vascular endothelial growth factor (VEGF) is a protein that regulates the signaling of new blood vessel formation. VEGF has 5 families, namely VEGF A, VEGF B, VEGF C, VEGF D, and placental growth factor and 3 receptors, namely VEGFR-1, VEGFR-2, and VEGFR-3. In cardiomyocyte, the dominant VEGF component is VEGFA. VEGF A functions as vasculogenesis, which is the process of forming new blood vessels (de novo) and angiogenesis, which is the process of forming new blood vessels from existing blood vessels. In conditions of ischemic heart disease, cardiomyocyte will release VEGF A as a result of inflammation, mechanical stress, and the release of cytokines, which then binds to VEGFR-1 and VEGFR-2 on the surface of cardiomyocytes. The study conducted by Tieqiang Zhao et al. examined the relationship of VEGF A to angiogenesis in post-myocardial infarction conditions. This study compared VEGF levels in the acute phase after myocardial infarction using animal studies. This study reported that there was an increase in VEGF A levels after two hours post-infarction and a peak at 12 hours, then began to decline on the first day and returned to normal values after day 2 [16]. A study by Skrypnik et al. stated that after cardiac rehabilitation for 2 weeks, VEGF levels in patients with acute coronary syndrome increased significantly [14]. B Hoier et al. also stated the similar result in their study on the relationship between acute physical exercise and VEGF levels, where after acute physical exercise VEGF levels increased sevenfold higher [18]. However, studies conducted by Darren R. Brenner et al. and Yang Gao et al. reported that there was no significant relationship between cardiac rehabilitation and VEGF levels [15, 19]. Another study conducted by Lee et al. showed opposite results where there was a decrease in VEGF levels in the group that performed physical exercise for 3 months [20]

The inconclusive conclusion of the beneficial effects of cardiac rehabilitation on VEGF expression prompted the authors to conduct a study to find the underlying mechanism in patients with coronary artery disease who have been completely revascularized, which is expected to resolve the condition of ischemia in these patients. Practically, this study can help determine whether cardiovascular rehabilitation has a central benefit on the cardiovascular system with a moderate intensity rehabilitation protocol. The results of this study can help educate patients who refuse cardiac rehabilitation after complete revascularization that it still has a function in helping the cardiovascular system through the process of angiogenesis.

Conclusion

This study will assess the beneficial effects of phase II rehabilitation in more detail as it uses protemic parameters to determine the underlying mechanism and assesses myocardial perfusion as an outcome.

No conflict of interest was declared

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