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The Effectiveness of Bax/Bcl-2 Gene Expression Ratio, Cardiac Function and Pulmonary Hemodynamics Indexes caused by Cardiopulmonary Rehabilitation Nursing

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ABSTRACT

This study aimed to observe the effects of cardiopulmonary rehabilitation nursing on gene expression, cardiac function and pulmonary hemodynamic indexes after percutaneous coronary intervention (PCI). A total of 104 coronary heart disease patients who received PCI treatment from January 2020 to January 2022 were selected by convenience sampling method. The patients were divided into control and observation groups by random number table method, with 52 cases in each group. The control group received routine nursing and the observation group received cardiopulmonary rehabilitation nursing. Cardiac function and pulmonary hemodynamics indexes were compared between the two groups. To evaluate gene expression, blood was taken from patients and healthy people after receiving complete information and obtaining consent. White blood cells were isolated by salting out. After RNA extraction and cDNA synthesis, the expression levels of Bcl2 and BAX genes were quantitatively measured by real-time PCR. Results showed that one month after discharge, the left ventricular end-diastolic diameter was decreased in the observation group compared with the control group and left ventricular ejection fraction and six minutes walk test grading were increased compared with the control group, showing a significant difference ($P< 0.05$). The pulmonary hemodynamic indexes were decreased in both groups compared with those at admission, but the pulmonary diastolic blood pressure, pulmonary systolic blood pressure, mean pulmonary artery pressure and pulmonary vascular resistance were lower in the observation group than in the control group of the same period, showing a difference ($P< 0.05$). The incidence of MACE was 1.92% (1/52) in the observation group, which was lower compared to the control group, showing a difference ($P< 0.05$). In this study, real-time PCR analysis showed that the expression ratio of Bcl2 to BAX genes in peripheral blood T cells in disease patients compared to healthy people was not different (P=0.07). In conclusion, Cardiopulmonary rehabilitation nursing for coronary heart disease after PCI can promote faster recovery of cardiac function, prolong exercise endurance and improve pulmonary hemodynamics indexes, demonstrating clinical reference value.

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Introduction

Due to recent aging in China, the number of coronary heart disease patients has been increasing year by year, leading to increasing demand for percutaneous coronary intervention (PCI) treatment (1). So far, PCI is generally considered an effective way to alleviate myocardial ischemia symptoms in coronary heart disease patients, demonstrating ideal clinical efficacy. However, there is still a large room for improvement in cardiac rehabilitation for patients after PCI. Most coronary heart disease patients are complicated with sleep disorders and low cardiopulmonary adaptability (2). Therefore, cardiopulmonary rehabilitation has gradually become an important field of clinical concern. Cardiopulmonary rehabilitation refers to cardiac rehabilitation and respiratory rehabilitation, which includes rehabilitation training of the whole body muscle system. However, most studies focus on cardiac rehabilitation, while respiratory rehabilitation is mostly applied to patients with chronic obstructive pulmonary disease, and no normative and mature cardiopulmonary rehabilitation nursing program has been formed for coronary heart disease patients. “Chinese Guidelines for Cardiac Rehabilitation and Secondary Prevention” points out that cardiac rehabilitation should be carried out through four stages in secondary prevention: early rehabilitation in the hospital, early rehabilitation outside the hospital, outpatient rehabilitation and long-term rehabilitation outside the hospital. Fan Min et al. (3) found that the knowledge, attitude and practice of cardiopulmonary specialists and nursing staff in cardiopulmonary rehabilitation were at a medium level and the nursing knowledge and behavior of some nursing staff demand improvement.

In several studies, a change in the ratio of Bcl2 to BAX gene expression was observed at the protein level in the cells of people with the disease compared to healthy people.

Therefore, our hospital carried out special cardiopulmonary rehabilitation nursing among coronary heart disease patients after PCI, which will help further improve the cardiac function and pulmonary hemodynamic indexes of coronary heart disease patients after PCI. Also, real-time PCR was used to quantify the expression levels of Bcl2 and BAX genes.

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Materials and Methods

Subjects

104 coronary heart disease patients who received PCI treatment in our hospital from January 2020 to January 2022 were selected by convenience sampling method.

Inclusion criteria: (i) Age 40~75 years old; (ii) Accord with the "Chinese Guidelines for Cardiovascular Disease Prevention (2017)" (4), PCI was performed for the first time; (iii) Normal cognitive function. Exclusion criteria: (i) Patients with hepatorenal insufficiency or tumor; (ii) Patients with new angina pectoris or severe arrhythmia within 8h after treatment; (iii) Patients who are in critical condition and are not conscious; (iv) Patients with other chronic diseases who need to restrict their activity. In order to guarantee the accuracy of the sample size, the sample size was calculated by substituting pre-experimental results into the formula: $n = (u_{\alpha/2} + u_{\beta})^2 (\sigma_1^2 + \sigma_2^2) / \delta^2$, $\chi \mp S$, $\chi \pm S$. In calculation, n=48. Considering the 10% loss of follow-up rate, the single sample size was set as 52 cases, and the total sample size was 104 cases. SPSS 24.0 software was used to generate a random number table, and the included objects were set as the control group and the observation group according to the sequence of random numbers. This study was reviewed and approved by the Medical Ethics Committee of our hospital. The contents of this study followed the "Helsinki Declaration". The subjects agreed to participate in this study and signed the informed consent. There was no statistical significance in the comparison of general data between the two groups (P> 0.05), indicating strong comparability.

Blood sampling and Real-time PCR reaction

2 ml of peripheral blood was taken from each person (coronary heart disease patients and control) in this study and EDTA anticoagulant was used to prevent blood clotting. In order to lyse red cells and separate white blood cells, the salting out method was used. To extract RNA from white blood cells, the RNA Extraction kit was used, and after extracting the RNA, its concentration and purity were determined using a biophotometer. Then cDNA was synthesized. Next, the difference in the expression level of BAX and Bcl2 genes was investigated by real-time PCR method. The expression of genes was compared to the reference gene B2M (microglobulin-β2).

The reaction was performed in the Applied Biosystems 7500 (USA) according to the following program: an initial denaturation step at 95°C for 30 seconds and 35 cycles at 95°C for 5 seconds and 60°C for 34 seconds.

Next, the temperature conditions of the separation curve formation stage included 95°C for 15 seconds, 60°C for 30 seconds and 95°C for 15 seconds. The sequence of the primers used is mentioned in Table 1. All experiments were repeated at least three times and the results were analyzed using the ΔΔCT equation.

Nursing methods

Routine nursing was given in the conventional group

At hospital admission, explain the precautions related to PCI to patients, such as the expected therapeutic effect, intraoperative process and postoperative complications. 1d after surgery, the patients were absolutely bedridden and passively mobilized the joint and major muscle group to eliminate concerns. 2d after surgery, the patients were urged to eat independently, exercise the contralateral puncture limb for 10min, and rest for 15min after the exercise. 3d after surgery, patients walked 30m slowly with the nurse assistance, and the nursing staff introduced the factors influencing postoperative cardiac function to the patients. 4d after surgery, the nursing staff assisted the patients in dressing and 90m walking, patients could watch health guidance videos, read newspapers and periodicals for less than 1h. 5d after surgery, the patients were asked to sit in a chair for washing and conduct slow walking. Patents walked no less than 100m a day for no more than 2h. 6~7h after surgery, the patients were instructed to continue with cardiac exercise rehabilitation to strengthen their self-management ability in cardiac rehabilitation.

The observation group was given cardiopulmonary rehabilitation nursing based on the control group

The cardiopulmonary rehabilitation nursing plan was designed and formulated according to the related contents of cardiac and pulmonary rehabilitation nursing in the "Guidelines of the American Heart Association for Cardiac Rehabilitation" (5) and "Chinese Expert Consensus on Perioperative Management of Enhanced Recovery after Surgery" (6). Three nursing staff in the Department of Cardiology served as the leader who undertook the whole process of rehabilitation nursing for patients from admission to discharge. Cardiopulmonary rehabilitation nursing mainly includes cardiac function rehabilitation nursing and pulmonary function rehabilitation nursing. On the day of admission, the patients were mainly treated with pulmonary function rehabilitation nursing. Preoperative sputum discharge training and abdominal breathing training were given, 10~15min each time, 2 groups /d. Avoid continuous cough during the training process. After the postoperative removal of the pipeline, assist the patients in turning over, guide the patients in abdominal breathing and sputum discharge training. During the training, patients should clasp the back with their hand, for 10~15min, 2 groups /d. 1 d after surgery, mainly postoperative cardiac function rehabilitation was given. Patients were instructed to lie in bed, do active and passive limb exercises, eat independently, wash their face on the bed, wash their hands

Table 1. Primers used to perform real-time PCR.

Gene name	Primer	Primer sequence (5'→ 3')	Product length (bp)
Bcl2	Forward	TTGTGGCCTTCTTTGAGTTCGGTG	114
	Reverse	GGTGCCGGTTCAGGTACTCAGTCA	
BAX	Forward	CCTGTGCACCAAGGTGCCGGAACT	99
	Reverse	CCACCCTGGTCTTGGATCCAGCCC	
B2M	Forward	TGCTGTCTCCATGTTTGATGTATCT	86
	Reverse	TCTCTGCTCCCCACCTCTAAGT	

and defecate in the bed pan, and sit up by raising the pillow. With the assistance of medical staff, patients sit for 15 min, 2~3 times/day. 2d after surgery, the improvement of the whole muscular system was the primary task. According to the muscle strength grading of Manual Muscle Testing (MMT) (7), when the MMT grading was less than grade 3, the patients should be instructed to perform passive joint activity training of the limbs, twice a day. When the muscle strength was in grade 3~4, the patients should gradually transit from a sitting position to independent sitting, and the muscle strength training should change from active exercise to independent sitting. The patients should be instructed to carry out arm lifting, knee bending and leg lifting with holding hands, which should gradually transit to the resistance exercise of grip ring, 8~10 times per group, 2~3 groups/d. When muscle strength > level 4, assist the patients in dumbbell lift and lower limb cycling training, 8~10 times/group, 2 groups/d. 3~4 d after surgery, mainly cardiopulmonary synchronous nursing was given. Patients received cardiopulmonary breathing training through a breathing exercise. The steps were as follows: calm breathing → standing position (inhale), leaning forward (exhale) → single upper arm lifting (inhale) → hands pressing on abdomen (exhale) →laterally raising upper limb (inhale)→ hands pressing abdomen (exhale) → holding head (inhale) → turning body (exhale) → standing with upper limbs lifting (inhale) → crouching position (exhale) →slow lip contraction abdominal breathing → calm breathing. 3 min after the end of breathing training, the patients were instructed to wipe the body from the bedside, wash and dress, read for no more than 15 min, and sit up for 15 min, 2~3 times /min. 5d after surgery and before discharge, the intervention was given from 3 links: warm-up nursing sports nursing and rehabilitation nursing. During the warm-up nursing, the nursing staff told patients to warm up before physical activities. Activity time was 5min to avoid skeletal muscle injury or electrocardiogram changes in the body amid sudden movement. During the exercise nursing period, the nursing staff told the patients to exercise for 10min by supine active treadmill exercise or sitting active treadmill exercise, 2 times /d. When the patients could walk more than 4 steps independently, assist the patients in indoor walking. When the patients could walk more than 5 steps, walking training and pedal training could be carried out. During rehabilitation nursing, slow walking and lower limb stretching exercises should be carried out at a low intensity tolerable by patients, which should continue for 5min.

At the time of discharge, the rehabilitation physicians in both groups formulated corresponding exercise plans. During the stay at home, the nursing staff instructed the patients to carry out breathing training and daily life training every day. The exercise lasted 30 min, 2 times/ d. The patients should return to the hospital for reexamination 1 month after discharge, which should continue until 6 months after discharge.

Observation indexes

Differences in general data. After admission, gender, age, MMT muscle strength grade, previous medical history, number of lesions, NYHA grade, surgery duration and hospital stay were investigated and recorded using the general data questionnaire.

Cardiac function. Color Doppler ultrasound was used to evaluate the left ventricular morphology and cardiac function of the patient upon admission and after 1 month of nursing. Average values of 3 consecutive cardiac cycles were measured, Left Ventricular Ejection Fraction (LVEF) was measured by the two-plane Simpson method, and Left Ventricular end Diastolic Diameter (LVED) was recorded. Meanwhile, at 6 months of nursing, the patient's cardiac function was evaluated using the six minutes walk test (6MWT) by strictly following the draft standard of the six minutes walk test (8). According to the 6MWD grading criteria, the patient's cardiac function status was determined after nursing, which was divided into severe cardiac function abnormality of < 150m, moderate cardiac function abnormality of 150~450m, and mild cardiac function abnormality of > 450m.

Pulmonary hemodynamic indexes. During the same period of cardiac function evaluation, patients' pulmonary artery diastolic pressure (PADP), Pulmonary arterial systolic pressure (PASP), Mean pulmonary artery pressure (MPAP) and Pulmonary vascular resistance (PVR) indexes were measured by Doppler ultrasound detector.

Follow-up and data collection. The patients were followed up for 6 months after discharge to collect the incidence of severe arrhythmia, angina pectoris, heart failure and cardiovascular death. The incidence of Major Adverse Cardiovascular Events (MACE) was calculated and compared between the two groups for data collection.

Statistical methods

SPSS 24.0 software was used for data processing, measurement data conforming to normality was represented by $\bar{x} \pm s$, independent sample t-test was used for comparison between groups, repeated measurement ANOVA was used for comparison at different time points within the group, count data was described by frequency, and χ^2 test was used for comparison between groups, with $P < 0.05$ indicating statistical significance.

Results

Comparison of general data between the two groups

There was no statistical significance in the comparison of gender, age, MMT muscle strength grade, previous medical history, number of lesions, NYHA grade, surgery duration and hospital stay between the two groups (P> 0.05), indicating strong comparability (Table 2).

Comparison of cardiac function between the two groups

Before the intervention, LVED and LVEF levels were not statistically significant between the two groups (P> 0.05). At 1 month after discharge, LVED was decreased in the observation group compared with the control group, and LVEF and 6MWT were increased compared with the control group of the same period, showing a statistically significant difference (P< 0.05) (Table 3).

Comparison of pulmonary hemodynamic indexes between the two groups

The pulmonary hemodynamic indexes were decreased in both groups compared with that at admission, but PADP, PASP, MPAP and PVR were all lower in the observation group than in the control group of the same period, showing a statistically significant difference (P< 0.05)

(Table 4).

Comparison of the incidence of MACE between the two groups

The incidence of MACE was 1.92% (1/52) in the observation group, which was lower compared to the control group, showing a statistically significant difference ($P< 0.05$) (see Table 5).

Bax/Bcl-2 Gene Expression

The concentration and purity of the extracted RNA were determined by a biophotometer. The concentration of RNA was about 230 μg/ml and the absorption ratio of 260/280 was between 1.8-2 that it showed the desired quality of RNA. Then, by analyzing the results of real-time PCR, the average expression level of the studied genes was determined. According to Table 6, the average level of Bcl2 gene expression in coronary heart disease patients was 0.72 compared to control subjects (0.59) and did not show a significant difference (P>0.05). Also, the mean difference in BAX gene expression in coronary heart disease patients (0.60) compared to control subjects (0.66) was not significant (P>0.05). On the other hand, the mean expression ratio of BAX/Bcl2 genes in coronary heart disease patients was increased by 1.1 compared to control subjects (0.90). But this increase was not statistically significant (P=0.08) (Table 6).

Discussion

For patients undergoing PCI, good cardiac function rehabilitation is an important objective of clinical interven-

Table 2. Comparison of general data between the two groups.

Group	Gender		Age (year)	MMTmuscle strength grade (level)			Previous medical history			
	Male	Female		<3	3~4	>4	Diabetes	Hypertension	Hyperlipidemia	None
Control (52)	24	28	69.06±4.26	17	15	20	12	14	12	14
Observation (52)	27	25	68.63±4.34	17	20	15	12	11	17	12
χ^2/t	0.343		-0.502			1.429			1.376	
P	0.558		0.617			0.490			0.711	

Group	Number of lesions			NYHA grade		Surgery duration (min)	Hospital stay (d)
	Single branch	Double branches	Three branches and above	Grade II	Grade III		
Control group (52)	21	20	11	27	25	304.48±25.08	7.35±1.51
Observation group (52)	21	11	20	23	29	300.15±25.49	7.27±1.48
χ^2/t	5.226			0.610		-0.873	-0.262
P	0.073			0.435		0.385	0.794

Table 3. Comparison of cardiac function indexes between the two groups (point).

Group	LVED (mm)		LVEF (%)		6MWT (case)		
	At admission	1 month after discharge	At admission	1 month after discharge	<150m	150~450m	>450m
Control group (52)	49.90±6.22	47.29±4.66*	50.75±6.47	60.44±4.11*	20	31	1
Observation group (52)	50.27±6.68	44.21±3.71*	49.81±6.37	63.37±3.56*	14	26	12
t/χ^2	0.289	-3.727	-0.748	3.873		6.759	
P	0.773	<0.001	0.456	<0.001		0.009	

Table 4. Comparison of pulmonary dynamics indexes between the two groups ($\bar{x}$±s).

Group	PADP (mmHg)		PASP (mmHg)		MPAP (mmHg)		PVR (dyn·s/cm)	
	At admission	1 month after discharge	At admission	1 month after discharge	At admission	1 month after discharge	At admission	1 month after discharge
Control group (58)	27.27±3.52	22.37±2.40*	57.92±9.33	39.23±5.51*	37.12±7.41	29.54±4.56*	621.35±20.41	386.77±35.76*
Observation group (58)	27.85±3.77	15.42±1.90*	58.13±7.72	31.12±3.62*	37.40±8.41	22.40±2.30*	616.23±20.86	282.54±24.38*
t	0.807	-16.339	0.126	-8.877	0.186	-10.062	1.264	-17.369
P	0.422	<0.001	0.900	<0.001	0.853	<0.001	0.209	<0.001

Table 5. Comparison of MACE incidence between the two groups [n (%)].

Group	Severe arrhythmia	Angina pectoris	Heart failure	Cardiovascular death	Incidence
Control group (52)	1	2	3	1	7(13.46)
Observation group (52)	0	1	0	0	1(1.92)
χ^2					4.828
P					0.028

Table 6. Expression level of BAX, Bcl2 and BAX/Bcl2 genes at the transcriptional level in coronary heart disease patients compared to healthy people (control), (mean ± standard deviation).

Gene	disease people	healthy people	P
BAX	0.60 (±0.16)	0.66 (±0.18)	0.19
Bcl2	0.72 (±0.31)	0.59 (±0.26)	0.11
Bcl2/BAX	1.10	0.90	0.08

tion. However, PCI not only incurs high treatment costs but also has potential risks of easy readmission and non-standard clinical operations, resulting in uneven long-term patient prognosis (9). There is a high risk of readmission after PCI, and a more optimized discharge plan for such patients will help reduce the risk of readmission (10). Fu C et al. (11) believed that rehabilitation exercise after PCI could reduce the risk of coronary artery restenosis and avoid lumen loss at the later stage of the stent coronary artery segment. A Chinese scholar found that personalized exercise training in cardiac rehabilitation could improve cardiac function and exercise endurance, which is an important means of secondary prevention after PCI (12). Therefore, in the perioperative period of PCI for coronary heart disease patients, individualized exercise rehabilitation nursing can be used to reduce the risk of postoperative readmission and promote the improvement of cardiac function indexes. Meanwhile, in the process of rehabilitation exercise, as exercise tolerance increases, the degree of myocardial ischemia is aggravated in coronary heart disease patients, resulting in the disorder of oxygen supply and demand, leading to secondary functional movement disorders in the myocardial ischemic area. In recent years, it is advocated clinically that attention should be paid to the improvement of cardiac function and pulmonary function at the same time.

Cardiopulmonary rehabilitation nursing is an individualized exercise rehabilitation program guided by nursing staff. Increasing cough training and respiratory training before PCI can help to avoid the risk of postoperative lung infection and pulmonary function decline, which lays the foundation for early exercise training. Studies suggest that inspiratory muscle training can improve the hemodynamic indexes, respiratory function and postural balance of elderly female patients (13). An Austrian study on outpatient cardiac rehabilitation model intervention showed (14) that with the extension of intervention duration, patients' maximum exercise amount increased, with both HDL and blood glucose improved. An animal study found that individualized exercise could not only reduce pulmonary hypertension but also increase blood vessel relaxation signal, which provides an auxiliary means for further developing an optimal exercise regimen. Therefore, cardiopulmonary rehabilitation nursing should focus on exercise rehabilitation to improve patients' cardiac and pulmonary function after PCI.

The results of this study confirm that cardiopulmonary rehabilitation nursing can improve patients' LVEF and LVED levels more quickly and optimize the 6MWT grading at 6 months after surgery. Such result indicates that cardiopulmonary rehabilitation nursing can improve cardiac function, ventricular remodeling and exercise endurance of coronary heart disease patients after PCI. The previous exercise intervention was to reduce patients' anxiety, daily inform patients to carry out the corresponding exercise. It can guarantee that patients complete the corresponding exercise under the supervision of nursing staff, but patients will barely perform continued rehabilitation training without supervision and after discharge, which impairs the nursing effect. Patients in the control group lacked pulmonary function rehabilitation training and had no cough or respiratory training before surgery, so the risk of postoperative lung infection was increased, and the risk of the delayed start time of postoperative exercise rehabilitation was aggravated. During the implementation of cardiopulmonary rehabilitation nursing, muscle strength evaluation should be added, and exercise training modes should be regularly adjusted through individualized nursing guidance to improve patients' flexibility in physical strength and functional adaptation parameters, thereby improving patients' cardiac function. Morucci (15) found that regular physical activity could regulate oxidative activity and cortisol levels, while cortisol levels are closely related to cardiac function in coronary heart disease patients.

At the same time, cardiopulmonary rehabilitation nursing can effectively reduce PADP, PASP, MPAP and PVR levels in patients. Silva et al. (16) found that early moderate-intensity continuous aerobic exercise could prevent harmful remodeling of the right heart and lung, with pulmonary artery resistance and dysfunction increased in single cardiomyocyte contraction and Ca_2 cycling models. Low aerobic exercise can improve pulmonary hypertension and reduce pulmonary vascular resistance (17). El-Sayed et al. (18) found that inspiratory muscle training could effectively improve forced vital capacity and promote the improvement of pulmonary function. Further comparison of MACE events between the two groups showed that the incidence of MACE was lower in the observation group. Hence, cardiopulmonary rehabilitation nursing can reduce the risk of MACE after PCI. Previously, the respiratory muscle was only regarded as the primary respiratory regulating muscle, and its role in cardiac function was often overlooked. As a kind of tissue that can promote venous and lymphatic reflux, the respiratory muscle can regulate the left ventricular after-load hemodynamic index and improve cardiac autonomic tension (19). Therefore, by adding nursing measures such as respiratory training, res-

piratory exercise (20-30) and passive joint activity training of limbs in cardiopulmonary rehabilitation nursing, it is possible to effectively improve pulmonary hemodynamic indexes and reduce the risk of prognostic MACE.

Apoptosis is an important physiological process that, by causing programmed death, causes the development of organs, tissue homeostasis, and the removal of abnormal or dangerous cells from the body (31). Incorrect regulation of apoptosis causes various diseases such as cancers, neurodegenerative disorders, coronary heart disease, immunodeficiency syndromes and inflammatory bowel diseases (32). In this study, the expression ratio of BAX/Bcl2 genes was determined to be higher in affected people than in healthy people, but this increase in BAX/Bcl2 gene expression was not significant.

The proper regulation of apoptosis in the body is necessary to maintain the health of the person, and in various studies, an increase in the ratio of Bcl2 to BAX protein levels has been observed in various diseases (33-35). There are several reports regarding the relationship between coronary heart disease and apoptosis (36-39).

To conclude, cardiopulmonary rehabilitation nursing after PCI can promote faster recovery of cardiac function, prolong exercise endurance and improve pulmonary hemodynamic indexes, demonstrating clinical reference value.

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