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Original Article

Effect of PCI on TGF-β/Treg mediated immune imbalance in coronary heart disease

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Abstract: Objective: This research aimed to investigate the level of TGF-β1/Treg pathway related factors in patients with coronary heart disease (CHD) after PCI, so as to evaluate the prognostic value. Methods: This is prospective research. Thereinto, 84 CHD patients undergoing PCI in our hospital were considered as an observation group, while 84 healthy people were regarded as a control group. The levels of CD3+CD4+T, CD3+CD8+T, CD19+B cells, CD4+CD25+Tr, Th1, Th2 and Th17 cells in the observation group and the control group before and after operation were tested by flow cytometry. The TGF-β11 and FoxP mRNA levels in the observation group and the control group before and after operation were examined by RT-PCR. The IL-2, IL-4, IL-8 and TNF-α levels in the observation group and the control group before and after operation were determined by ELISA. Six months later, patients were followed up and then divided into a poor prognosis group (61 cases) and a good prognosis group (23 cases). The TGF-β1 level of both groups was compared, and its clinical value in evaluating patients' prognosis was analyzed by ROC curve. Results: Compared with the control group, there was no remarkable difference in Th2 levels before and after operation in the observation group (P>0.05), but the levels of CD3+CD4+T, CD19+B cells, Th1, Th17, IL-2, IL-4, IL-8 and TNF-α were markedly higher, and the mRNA levels of CD3+CD8+T, CD4+CD25+TR, TGF-β1 and FoxP were markedly lower (P<0.05) in the observation group. Compared with before operation, the levels of CD3+CD4+T, CD19+B cells, Th1, Th17, IL-2 and TNF-α were markedly higher, while the levels of CD3+CD8+T, CD4+CD25+Tr, TGF-β1 and FoxP mRNA were dramatically lower (P<0.05) after operation. The mRNA level of TGF-β1 in the poor prognosis group was markedly lower than that in the good prognosis group (P<0.05). ROC curve revealed that the AUC of TGF-β1 in predicting the prognosis of patients was 0.828 when the cut-off value was 0.658 (P<0.05). Conclusion: The levels of TGF-β1/Treg in CHD patients changed after PCI, and thus it has certain clinical value in evaluating prognosis.

Keywords: Coronary heart disease, PCI operation, TGF-β1/Treg, immune imbalance

Introduction

Coronary heart disease (CHD) is a familiar cardiovascular and cerebrovascular illness. Its pathogenesis is coronary atherosclerosis (AS), in which lipids accumulate on the intima of arteries in the formation of plaques [1, 2]. When plaques increase continuously, the arterial lumen becomes narrow or even blocked, and then the blood flow is blocked, resulting in myocardial ischemia, hypoxia or necrosis, which eventually leads to angina pectoris [3-5]. In recent years, it has been found that immune factors play a vital part in the process of CHD and are relevant to AS formation and development. Treg is a type of T cell subset, and its main function is to suppress autoimmunity. Research shows that the imbalance of T cell subsets increases AS development, and TGF-β1 plays a vital central role in related cytokines [6-9]. At the moment, the main clinical treatment for CHD is percutaneous coronary intervention (PCI). PCI can treat diseases, improve patients' quality of life and reduce the risk of adverse cardiovascular events by effectively reconstructing blood supply. However, with the deepening of research, various problems have gradually emerged after PCI, among which in-stent restenosis is the most common. According to statistics, about 10%-30% of patients after PCI will have in-stent restenosis, which seriously affects their prognosis [10, 11]. Some

Table 1. Primer sequence

Primer	Sequence (5'-3')
TGF-β1 upstream	CACGATCATGTTCGACAACTCCTCC
TGF-β1 downstream	CTTCAGCTCCACAGAGAAGAACTGC
FoxP upstream	CTACGCCACGCTCATCCGCTGG
FoxP downstream	GTAGGGTTGGAACACCTGCTGGG
β-actin upstream	ACTCTTCCAGCCTTCCTTCC
β-actin downstream	CGTACAGGTCTTTGCGGATG

scholars have found that TGF-β1 is relevant to the occurrence of in-stent restenosis after PCI. Based on this, this research explored the changes of TGF-β1/Treg-mediated immune factors in CHD before and after PCI, aiming to provide relevant theoretical basis for clinical treatment and evaluation of prognosis. The present research report is as follows.

Materials and methods

General data

From September 2018 to September 2020, 84 stable CHD patients undergoing PCI were regarded as the observation group, and 84 healthy people were placed in the control group. This research was approved by the Ethics Committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria of the observation group: (1) patients were ≥ 18 years old; (2) disease conformed to the Guidelines for Diagnosis and Treatment of Stable Coronary Heart Disease issued by Chinese Medical Association [12]; (3) PCI was needed; (4) patients and their families agreed to participate and signed an informed consent form.

Inclusion criteria of control group: (1) patients were ≥ 18 years old; (2) those who had undergone physical examination within the past 1 month; (3) patients and their families signed informed consent forms.

Exclusion criteria were as follows: (1) patients who were complicated with other cardiovascular and cerebrovascular diseases; (2) patients who were complicated with severe liver and kidney dysfunction; (3) patients were who complicated with infectious and immune system diseases or malignancies; (4) those who took immunosuppressants and other drugs that might affect the experimental results within the past six months; (5) also those with poor compliance.

Methods

In the control group, 5 mL × 3 tubes of venous blood was collected on an empty stomach in the morning. While in the observation group, 5 mL × 3 tubes of venous blood was taken on an empty stomach before operation (before receiving treatment) and the next morning after operation. The levels of CD3+CD4+T, CD3+CD8+T, CD19+B cells, CD4+CD25+Tr, Th1, Th2, and Th17 cells (Shanghai Beyotime Biotechnology Co., Ltd., product number: C1062S) were tested via flow cytometry. The first tube was centrifuged at 3000 r/min for 5 min, the serum was separated, and the levels of IL-2, IL-4, IL-8 and TNF-α were detected by ELISA (Shanghai Beyotime Biotechnology Co., Ltd., IL-2 product number: P1580, IL-4 product number: P1618, IL-8 product number: P1640). In the first tube, RNA was extracted from the peripheral blood with an RNA extraction kit (Taraka, Japan, product number: 9192), and then reversely transcribed according to a reverse transcription kit (Taraka, Japan, product number: 638313), and RT-PCR was conducted accrding to the instructions of the SYBR RT-PCR kit (RR036Q, Taraka, Japan). The reaction system was as follows: pre-denatured at 95°C for 30 s, 95°C for 5 s, 60°C for 30 s, with 35 cycles. The relative expression of TGF-β1 and FoxP mRNA in each group was determined by $2^{-\Delta\Delta Ct}$ method. The internal reference was β-actin, and each sample test was repeated three times to obtain the average value. Primers were synthesized by Beijing Dingguo Biotechnology Co., Ltd., China, and their sequences were shown in **Table 1**.

Follow-up

The deadline of follow-up after PCI in the observation group was 6 months after PCI, and the endpoint event was cardiac death. According to the occurrence of cardiac death, patients were divided into the poor prognosis group (61 cases) and good prognosis group (23 cases).

Statistical analysis

SPSS 22.0 was applied for statistical analysis. The counting data were represented as cases and percentages (n, %), and the measurement data were shown in mean ± standard deviation ($\bar{x}$ ± sd). The inter-group comparison was made

Table 2. Comparison of general data between both groups ($\bar{x}$ ± sd)

Group	Observation group (n=84)	Control group (n=84)	χ^2/t	P
Age (years)	57.4±3.3	57.1±3.8	0.546	0.586
Gender (n)			0.223	0.636
Male	49	52		
Female	35	32		
History of hypertension (case)			0.835	0.361
Have	22	17		
No	62	67		
History of diabetes (case)			0.453	0.501
Have	13	10		
No	71	74		
Smoking history (case)			1.143	0.285
Have	24	18		
No	60	66		
History of drinking (example)			1.273	0.259
Have	21	15		
No	63	69		
BMI (kg/m^2)	22.7±2.4	22.3±2.5	1.058	0.292

Table 3. Comparison of immune cell levels between observation group and control group

Group	Before operation (n=84)	After operation (n=84)	Control group (n=84)
CD3+CD4+T (%)	33.54±3.52$^{\#\#\#}$	37.89±3.27$^{\#\#\#,***}$	28.35±4.41
CD3+CD8+T (%)	22.85±3.13$^{\#\#\#}$	20.05±2.24$^{\#\#\#,***}$	25.97±3.92
CD4+CD25+Tr (%)	14.82±3.43$^{\#\#\#}$	11.17±2.39$^{\#\#\#,***}$	17.21±4.87
CD19+B (%)	6.94±0.71$^{\#\#\#}$	10.22±1.13$^{\#\#\#,***}$	4.33±0.29
Th1 (%)	15.64±3.34$^{\#\#\#}$	19.88±3.97$^{\#\#\#,***}$	6.81±1.34
Th2 (%)	0.83±0.19	0.82±0.23	0.79±0.24
Th17 (%)	2.23±0.41$^{\#\#\#}$	2.97±0.44$^{\#\#\#,***}$	1.84±0.32

Note: Compared with the control group, $^{\#\#\#}$P<0.001; compared with before operation, ***P<0.001.

by independent-samples t test, and the intra-group comparison was assessed via paired-samples t-test. The counting data were compared by χ^2 test. ROC curve was drawn to analyze the clinical value of TGF-β1 level in the observation group in evaluating prognosis.

Results

Comparison of general data between both groups

The results revealed that there was no statistical difference in the general data such as age and gender between both groups (P>0.05; **Table 2**).

Comparison of immune cell level between the observation group and control group

Compared with the control group, there was no obvious difference in Th2 before and after operation in the observation group (P>0.05), but the levels of CD3+CD4+T, CD19+B cells, and Th1 and Th17 were higher, while those of CD3+CD8+T and CD4+CD25+Tr were lower (P<0.001). Compared with before operation, patients had higher levels of CD3+CD4+T, CD19+B cells, and Th1 and Th17, but lower levels of CD3+CD8+T and CD4+CD25+Tr after operation (P<0.001; **Table 3**).

Comparison of TGF-β1 and FoxP mRNA levels between both groups

The results revealed that the TGF-β1 and FoxP mRNA levels in the observation group were lower than those in the control group before and after operation (P< 0.001). Compared with before operation, the levels after operation were significantly lower (P<0.001; **Table 4** and **Figure 1**).

Comparison of IL-2, IL-4, IL-8 and TNF-α levels between both groups

Compared with the control group, the levels of IL-2, IL-4, IL-8 and TNF-α in the observation group were higher before and after operation (P<0.001). Compared with before operation, the IL-4 and IL-8 levels after operation were not remarkably different (P>0.05), but those of IL-2 and TNF-α were higher (P<0.001; **Table 5**).

Comparison of TGF-β1 mRNA level between the poor prognosis group and good prognosis group

Compared with the good prognosis group, the TGF-β1 mRNA levels in the poor prognosis

Table 4. Comparison of TGF-β1 and FoxP mRNA levels between the two groups

Group	TGF-β1	FoxP
Before operation (n=84)	0.84±0.12$^{###}$	0.72±0.04$^{###}$
After operation (n=84)	0.72±0.14$^{###,***}$	0.48±0.04$^{###,***}$
Control group (n=84)	1.03±0.03	0.94±0.05

Note: Compared with the control group, $^{###}$P<0.001; compared with before operation, ***P<0.001.

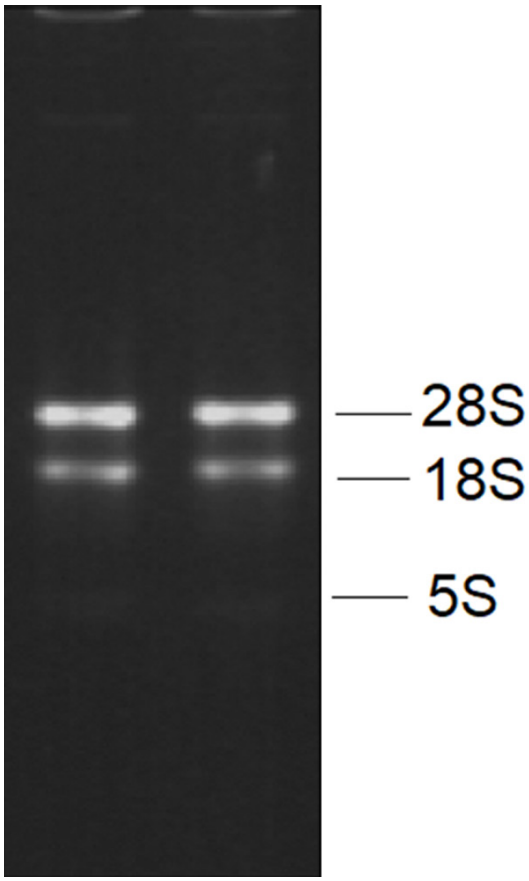

Figure 1. RNA electrophoresis diagram.

group were lower after operation (0.593±0.172 vs. 0.759±0.096, t=5.600, P<0.001).

ROC curve results

The results manifested that when the cut-off value was 0.658, the AUC of TGF-β1 was 0.828 (P<0.001; **Table 6** and **Figure 2**).

Discussion

Immune cells play a vital role in CHD development and progression. Studies have shown that the imbalance of T cell subsets is involved in AS plaque inflammation and immune response processes. T lymphocytes can be divided into CD4+ and CD8+ subsets. Among them, CD4+CD25+TR in CD4+ cells mainly play a benign regulatory role in cellular immunity [13-15]. CD8+ cells, on the other hand, maintain the stability of the immune response together with CD4+. Th1 in CD4+ cells mainly secrete pro-inflammatory factors, while Th2 secretes anti-inflammatory factors. The two can regulate and inhibit each other. Furthermore, Treg cells can suppress the immune response, while Th17 cells take part in the process of AS plaque rupture, which occurs in the damaged blood vessels by recruiting related cytokines, and finally brings about the occurrence of acute coronary syndrome [16, 17]. CD19 is a CD antigen molecule on the surface of B cells. Research has shown that the level of CD19+ molecules in the peripheral blood of patients with acute myocardial infarction is markedly increased. Based on this theory, this research investigated the expression of T-lymphocyte subsets and related inflammatory factors in CHD patients. Compared with normal people, the levels of CD3+CD4+T, CD19+B cells, Th1, Th17, IL-2 and TNF-α in CHD patients were all higher, while the mRNA levels of CD3+CD8+T, CD4+CD25+Tr, TGF-β and FoxP were lower, which further confirmed that T-lymphocyte subsets were relevant to CHD.

TGF-β plays a central part in the process of immune imbalance [18, 19]. Recently, research has confirmed that TGF-β1 is relevant to AS development and progression [20]. TGF-β1 is a protective factor of the coronary artery, it promotes the formation of AS and the remodeling after myocardial infarction. It has been found that TGF-β1 can suppress IL-2 and TNF-α expression, thereby promoting the apoptosis of neutrophils involved in the process of vascular injury and inhibiting the expression of matrix metalloenzymes, thus stabilizing plaques [21, 22]. This research also found that compared with the normal population, the TGF-β1 level in CHD patients is decreased. At present, the clinical efficacy of PCI in CHD treatment has been affirmed. Nevertheless, the operation is invasive, which may stimulate the local blood vessels of patients during operation, and then lead to systemic inflammatory reactions. Studies have shown that the inflammatory reaction after PCI is one of the risk factors of cardiac

Table 5. Comparison of levels of IL-2, IL-4, IL-8 and TNF-α between the two groups

Group	IL-2 (ng/L)	IL-4 (ng/L)	IL-8 (ng/L)	TNF-α (ng/L)
Before operation (n=84)	52.54±7.81$^{\#\#\#}$	59.98±9.84$^{\#\#\#}$	67.56±12.92$^{\#\#\#}$	93.42±21.21$^{\#\#\#}$
After operation (n=84)	64.99±8.84$^{\#\#\#,***}$	61.87±10.52$^{\#\#\#}$	70.08±11.54$^{\#\#\#}$	128.23±27.14$^{\#\#\#,***}$
Control group (n=84)	13.97±3.38	21.34±5.23	18.75±4.46	12.94±3.37

Note: Compared with the control group, $^{\#\#\#}$P<0.001; compared with before operation, ***P<0.001.

Table 6. ROC curve results

Cutoff value	AUC	95% CI		P	Sensitivity	Specificity
0.658	0.828	0.721	0.934	<0.001	0.918	0.625

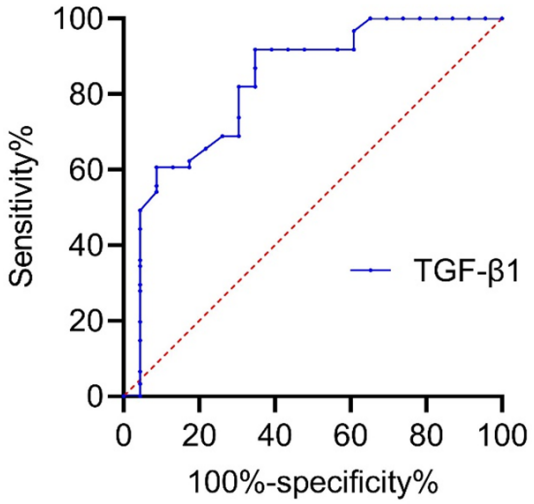

Figure 2. ROC curve results.

death [23, 24]. In addition, some studies have shown that TGF-β1 may also affect the restenosis of CHD patients after treatment. The main pathological mechanism of postoperative vascular restenosis is the migration, proliferation and differentiation of vascular smooth muscle cells, which results in intimal hyperplasia. TGF-β1 has a bidirectional regulation on vascular smooth muscle cells, which can promote their proliferation and migration. At a certain concentration, it can inhibit this process. This research revealed that the levels of CD3+CD4+T, CD19+B cells, Th1, Th17, IL-2 and TNF-α were higher than those before operation, while those of CD3+CD8+T, CD4+CD25+Tr, TGF-β1 and FoxP mRNA were lower. Furthermore, this research found that the TGF-β1 mRNA level of patients with poor prognosis was lower than that of those with good prognosis. ROC curve revealed that when the cut-off value was 0.658, the AUC of TGF-β1 for predicting the prognosis of patients was 0.828. This suggests that there may be an immune imbalance after PCI, and TGF-β1 has good clinical value in evaluating patients' prognosis.

However, there are still some limitations. This research only discussed the changes of related factors before and one day after operation, but did not analyze their dynamic changes. In addition, it is a single-center study with a small-sample size. Hence, follow-up still needs to be confirmed through a multi-center and larger-sampling survey.

In general, the levels of TGF-β1/Treg in CHD patients change after PCI, and TGF-β1/Treg has certain clinical value in evaluating patient prognosis.

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Disclosure of conflict of interest

None.

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