The association between CD69 and EGR1 levels, and CHD patients without reflow after PCI

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Abstract. Association between CD69 and EGR1 levels and coronary heart disease (CHD) patients without reflow after percutaneous coronary intervention (PCI) was investigated. Data of 156 patients undergoing PCI from September 2014 to December 2016 in People's Hospital of Hunan Province were analyzed, and they were divided into 72 cases in reflow group (group A) and 84 cases in no-reflow group (group B) according to the patient's postoperative blood flow. Another 70 volunteers undergoing normal physical examination in the same period were selected as control group (group C). The venous blood was extracted from patients before operation, at 5 min, 2 h and 6 months after operation, the serum CD69 and EGR1 levels were detected with flow cytometry and RT-qPCR methods, and association between CD69 and EGR1 levels and postoperative blood flow recovery was analyzed. CD69 expression before and 2 h after operation in group A was significant (P<0.05), and a difference in patients at 6 months after operation between group A, B and group C (P<0.05). The CD69 expression in group A was significantly lower than that in group B (P<0.05), but in group B was significantly higher than that in group C (P<0.05). The EGR1 expression in group A was significantly higher than that in group B (P<0.05), but in group B was significantly lower than that in group C (P<0.05). Multivariate logistic regression analysis revealed that CD69 (OR=6.424, P=0.025) and EGR1 (OR=3.684, P=0.013) were independent risk factors for patients without reflow after undergoing PCI. After undergoing PCI in CHD patients, if there was an increase in CD69 level and a significant decrease in EGR1 level in the early postoperative period, the patient may be suspected of having no-reflow and checked in time to improve the patient's therapeutic effect.

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Introduction

According to the data published by the World Health Organization (WHO), cardiovascular disease has become the number one cause of death at present, ranking second preceded only by malignant tumor in the number of deaths due to cardiovascular and cerebrovascular diseases in China (1). Coronary heart disease (CHD), which causes cardiac dysfunction in patients, is a very common disease in cardiovascular medicine and one of the leading causes of death worldwide (2). Studies have reported that the incidence of CHD has declined in developed countries, but increased in developing countries in recent years, which may be due to changes in dietary patterns caused by economic development (3). The consumption of high-fat diet and low cholesterol plays a very important role in the pathogenesis of CHD (4). According to the findings of the WHO in 2011, the incidence of CHD is ~8% in China and its mortality rate ranks second in the world (5). Therefore, the treatment of CHD has become one of the problems that medical researchers urgently need to solve.

At present, drug treatment and surgery are the methods for the clinical treatment of CHD. Surgical treatment mainly reconstructs blood supply through percutaneous coronary intervention (PCI) (6). PCI is a treatment method that clears narrow or even occluded coronary lumen using cardiac catheterization technique, thus improving myocardial blood flow perfusion. PCI is widely used in clinical practice due to its ideal effect on CHD, its minimal trauma to the patient, which minimizes the damage to the patient's recovery of myocardial blood flow reconstruction, and the emergence of lower price stents in recent years (7). Nevertheless, myocardial no-reflow still occurs to some patients after PCI in clinic. It is very complicated to cause postoperative no-reflow in patients, including inflammatory reaction, vascular injury, immune response, oxidative stress and other factors (7). CD69 is one of the molecules that first expressed on the surface of activated T lymphocytes of the immune system and is stable in normal human patients (8). Early growth response 1 (EGR1), which is one of the immediate early family members, exists extensively in human cells, and its family members all have zinc finger structure coding regions and have a high homology. Studies have shown that EGR1 plays an important role in cell growth, differentiation, proliferation and inflammatory reaction (9). In addtion, we found that CD69 and EGR1 had differential

expression in CHD patients through CEO database screening, but there was no clear conclusion whether they had an effect on the patient's postoperative blood flow.

Therefore, this study will analyze the association between CD69 and EGR1 levels and CHD patients without reflow after PCI.

Patients and methods

Patient data. In this study, 156 CHD patients undergoing PCI from September 2014 to December 2016 in People's Hospital of Hunan Province (Changsha, China) were collected and divided into reflow group (group A) and no-reflow group (group B) according to the patient's postoperative blood flow. Seventy-two patients in group A, including 35 males and 37 females, aged from 45 to 78 years with a mean age of 65.3±10.3 years; 84 patients in group B, including 44 males and 40 females, aged from 45 to 79 years with a mean age of 66.9±9.9 years; both groups of patients met the CHD diagnostic criteria of American Society of Cardiology (10). Another 70 volunteers for normal physical examination in the same period were selected as control group (group C), including 34 males and 36 females, aged from 45 to 78 years with a mean age of 65.3±10.3 years. This study was approved by the Medical Ethics Committee of People's Hospital of Hunan Province, and patients or their family members signed the informed consent.

Inclusion and exclusion criteria
Inclusion criteria. Patients who were not treated with gluco-corticoid within 1 month after undergoing PCI; patients who do not suffer from autism, memory impairment or hearing impairment; patients who cooperate with follow-up and patients who have complete clinical information.

Exclusion criteria. Patients who suffer from malignant tumor, chronic infection, pulmonary embolism, or immune dysfunction; patients who have received blood transfusion before operation and have been treated with thrombolytic therapy; patients who suffer from acute myocarditis or peri-carditis.

Methods
Main reagents and instruments. TRIzol, 2X SYBR-Green qPCR mix, RevertAid™ Premium First Strand cDNA Synthesis kit, Hank's liquid, penicillin-streptomycin double antibodies and PBS were purchased from Invitrogen; Thermo Fisher Scientific, Inc. (cat. nos. 15596018, 4309155, K1621, 88284, 15070063 and 10010049; Waltham, MA, USA); reverse transcription kit from Takara Biotechnology Co., Ltd. (RNA to cDNA EcoDry™ Premix Double Primed; cat. no. 639549; Beijing, China); quantitative PCR kit from Takara Bio, Inc. (Otsu, Japan); CD69 monoclonal antibody (mouse anti-human phycoerythrin marker), CD3 monoclonal antibody (mouse anti-human activating protein), CD4 monoclonal antibody (BD Horizon™ BV510 mouse anti-human) and PE-labeled mouse IgG1 CD69 isotype control from BD Biosciences (dilution, 1:400; cat. nos. 560738, 552852, 562971 and 557908; Franklin Lakes, NJ, USA); erythrocyte splitting liquor from Tiangen Biotech Co., Ltd. (cat. no. RT122; Beijing, China); ABI Prism 7900 PCR instrument from Applied Biosystems

(Thermo Fisher Scientific, Inc., Foster City, CA, USA), BD FACSCanto™ II flow cytometry from BD Biosciences.

Patient biochemical indicators and clinical data collection. In this study, the patient's sex, age, smoking, alcoholism and biochemical indicators were collected: Expressions of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), lipoprotein a (Lpa), fasting blood glucose (FBG), glycosylated hemo-globin (HbA1c), high-sensitivity C-reactive protein (hs-CRP) and serum creatinine (Scr).

Sample collection. In this study, 5 ml of fasting venous blood was extracted from patients in group A and B at 1 h before, 5 min, 2 h and 6 months after operation twice. They were collected using EDTA-Na2 for detection of the expression levels of CD69 and EGR1. Fasting venous blood (5 ml) was extracted from patients in group C at the same period twice.

CD69 detection. The venous whole blood collected with EDTA-Na2 tube was mixed with Hanks solution at 1:1, and then the mixture was superimposed on the surface of the 8 ml lymphocyte separating liquid, and centrifuged at 543 x g for 15 min at 25°C. The lymphocytes (circular milky white) on the second layer were collected after centrifuga-tion, put into a test tube (containing 10 ml of Hanks solution), mixed and centrifuged again at 543 x g for 20 min at 25°C. The supernatant was discarded, the precipitate was left, and the cells were resuspended and washed. Finally, the cell concentration was adjusted to $1x10^6$/ml using 10% RPMI-1640 medium, the resuspended suspension was added to a 24-well plate (1 ml for per well), and 100 μl/ml of penicillin, 100 μl/ml of streptomycin and a stimulant PHA 20 μg/ml were added and mixed. The mixture was incubated (37°C, 5% CO_2 incubator for 20 h), resuspended and adjusted to $1x10^6$/ml using PBS. Two cell suspensions (100 μl) were taken into the sample tube, and one was added with each 10 μl of CD3, CD4, and CD69 monoclonal antibodies and another with each 10 μl of CD3 monoclonal antibody, CD4 monoclonal antibody and CD69 isotype. They were incubated for 20 min without light. After staining, 2 ml of erythrocyte splitting liquor were added to mix and stand for 10 min to avoid light and lysed. The lysed tube was centrifuged at 1,370 x g for 5 min at 25°C. The supernatant was discarded, the cells were resuspended with added 2 ml of PBS, and the resuspended suspension was centrifuged again at 1,370 x g for 5 min at 25°C. Non-tuberculous antibodies were removed, the supernatant was discarded, and 0.5 ml of PBS was added for resuspension. Flow cytometry was used for detection of the expression level of CD69 in CD3+CD4+ T cells.

EGR1 detection. A vacuum blood collection tube (EDTA-Na2) was used for the extraction of 5 ml of fasting venous whole blood from the patient and the extracted blood was centrifuged at 1,370 x g at 25°C for 30 min. The supernatant was discarded, transferred to an EP tube and placed in a refrigerator at -80°C for preservation. The frozen plasma was extracted for total RNA extraction using TRIzol reagent, and the extraction procedure was performed according to the kit instruction. The UV spectrophotometer was used for detection of RNA

Table I. Primer sequences.

Genes	Upstream primer	Downstream primer
EGR1	5'-CCCTTGCTCCCTTCAATGCT-3'	5'-CGAAATCCATGGCACAGACAC-3'
GAPDH	5'-AGCCACATCGCTCAGACA-3'	5'-TGGACTCCACGACGTACT-3'

concentration and purity, and the quality of total RNA extracted was analyzed using 1% denaturing agarose gel electrophoresis. Reverse transcription procedure was performed strictly in accordance with the reverse transcription kit instruction. EGR1 primers detected by RT-qPCR were designed and synthesized by Shanghai Shenggong Biotechnology Co., Ltd. (Shanghai, China) (Table I). The reaction system was configured using the PCR reaction kit (2X SYBR Green qPCR Mix) as follows: Upstream primer 0.2 μl, downstream primer 0.2 μl, cDNA 1 μl, 2X SYBR Select Master Mix 5 μl, supplemented with double distilled water to 10 μl; ABI PRISM 7900 PCR instrument was used for amplification. Reaction conditions were pre-denaturation at 95°C for 2 min, 95°C for 15 sec, 60°C for 60 sec, 95°C for 15 sec, a total of 40 cycles. GAPDH was used as an internal reference in the experiment for a total of three times. The results were analyzed using $2^{-\Delta Cq}$ method (11,12).

Statistical analysis. In this study, SPSS 20.0 (IBM Corp., Armonk, NY, USA) statistical software package was used for analysis of the collected data, and GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA) for drawing data images. All measurement data in this study are expressed as mean ± standard deviation (mean ± SD). The Student's t-test was used for comparison between the two groups and variance analysis for analysis between groups. The counting data are expressed in rate (%) and the χ^2 test for comparison between groups. Logistic multivariate regression was used for analysis of the association between CD69, EGR1 and coronary artery disease. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of data between two groups of patients. Through comparison of the clinical data between two groups of patients, it was found that there was no statistical difference in sex, age, smoking and alcoholism between the groups (P>0.05). Through further comparison of the biochemical indicators between groups, it was found that there was no statistical difference in TG, TC, HDL-C, LDL-C, HbAlc and FBG in patients between group A and group B (P>0.05), but a statistical difference in the expression levels of Lpa, hs-CRP and Scr between the two groups (P<0.05) (Table II).

Comparison of the CD69 expression at different time-points between groups. The CD69 expression in groups was detected with flow cytometry. Through comparison of the CD69 expression before operation, at 5 min, 2 h and 6 months after operation and at different time in the same group between the two groups, it was found that there was no significant difference in the CD69 expression before and at 5 min after operation in

Table II. Clinical data of patients [n (%)].

Groups	Group A (n=72)	Group B (n=84)	χ^2/t	P-value
Sex				0.748
Male	35 (48.61)	44 (52.38)		
Female	37 (51.39)	40 (47.62)		
Age (years)				0.626
≥60	45 (62.50)	49 (58.33)		
<60	27 (37.50)	35 (41.67)		
Smoking				0.630
Yes	35 (48.61)	45 (52.38)		
No	37 (51.39)	39 (47.62)		
Alcoholism				0.703
Yes	15 (20.83)	20 (23.81)		
No	57 (79.17)	64 (76.19)		
TC mmol/l	1.62±0.85	1.58±0.77	0.308	0.758
TG mmol/l	4.68±0.92	4.72±0.84	0.284	0.777
HDL-C mmol/l	1.29±0.32	1.32±0.28	0.625	0.533
LDL-C mmol/l	2.24±0.74	2.29±0.61	0.463	0.644
Lpa mg/l	263.60±56.50	225.80±57.40	4.130	0.001
FBG mmol/l	5.74±1.35	5.63±0.99	0.585	0.559
HbA1c %	6.35±1.00	6.23±0.95	0.768	0.444
hs-CRP mg/l	5.54±2.57	3.76±2.41	4.460	0.001
Scr umol/l	73.50±16.40	60.30±15.60	5.145	0.001

patients between group A and group B (P>0.05), but a statistical difference at 2 h after operation between them that was significantly decreased in group A (P<0.05), and a difference at 6 months after operation in patients between group A and group B (P<0.05). Through pairwise comparison, it was found that there was a difference in the CD69 expression between group A and group B and it significantly decreased (P<0.05), there was no difference between group A and group C (P>0.05), and group B was significantly higher than that in group C (P<0.05). There was a difference in the CD69 expression at different time-points in patients in group A (P<0.05). It significantly decreased before operation when compared with that at 5 min after operation, and significantly increased when compared with that at 6 months after operation, and there was a difference between groups (P<0.05), but no difference when compared with that at 2 h after operation in group A (P>0.05); there was a difference in the CD69 expression at 5 min after operation when it significantly increased and at 2 h and 6 months after operation in group A (P<0.05), and a difference between at 2 h after operation when it was significantly
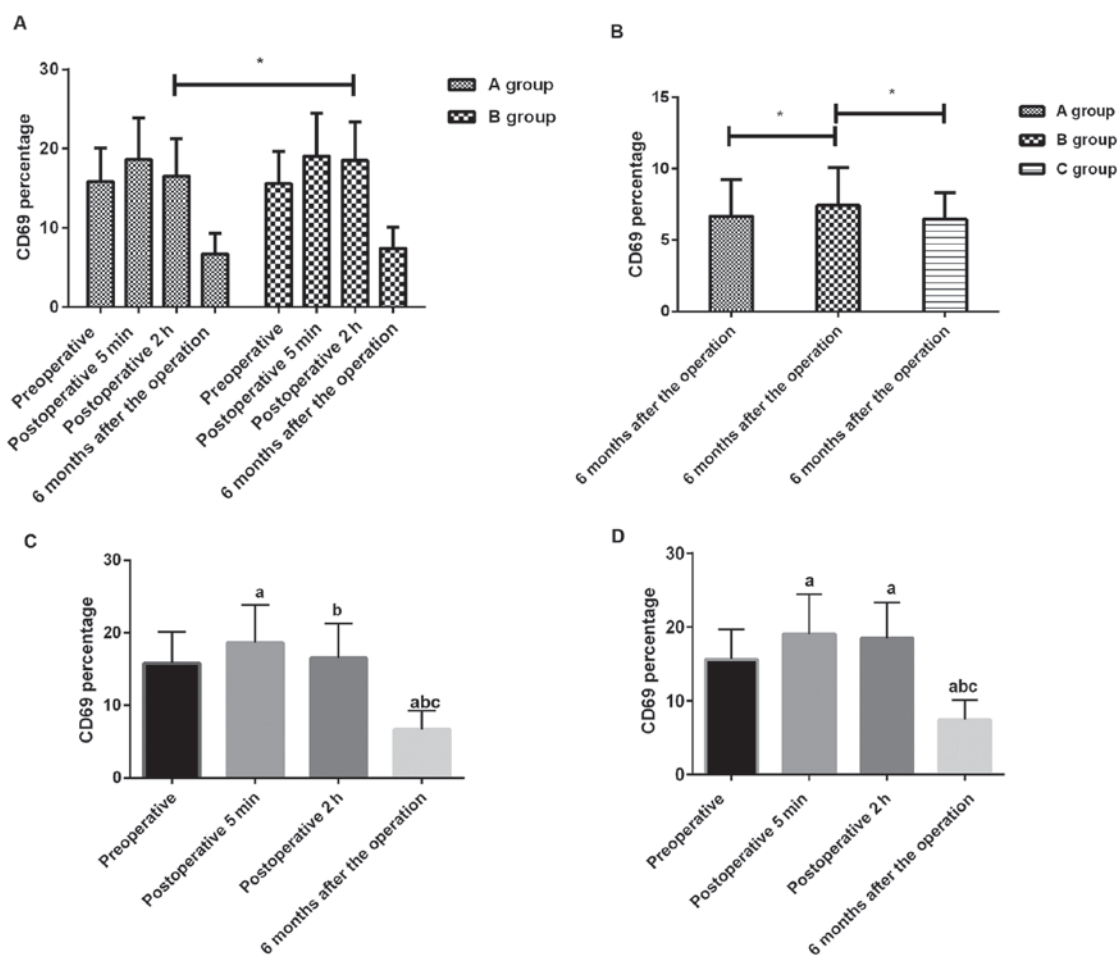
Figure 1. (A) CD69 expression at different time-points in group A and group B. Detection of flow cytometry found that there was no significant difference in the CD69 expression before and at 5 min after operation in patients between group A and group B (P>0.05), but a statistical difference between them at 2 h after operation in group A was significantly lower (P<0.05). (B) CD69 expression at 6 months after operation in group A, B and C. Detection of flow cytometry found that there was a difference in the CD69 expression at 6 months after operation in patients between group A, group B and group C (P<0.05). Through pairwise comparison, it was found that there was a difference in the CD69 expression between group A and group B and it significantly decreased (P<0.05), but no difference between group A and group C (P>0.05), and group B was higher than that in group C (P<0.05). *P<0.05. (C) There were differences in the CD69 expression at different time-points in patients in group A (P<0.05). The CD69 expression decreased before operation when compared with that at 5 min after operation, and increased when compared with that at 6 months after operation, and there was a difference between groups (P<0.05), but no difference when compared with that at 2 h after operation in group A (P>0.05); there was a difference in the CD69 expression at 5 min after operation when it significantly increased and at 2 h and 6 months after operation in group A (P<0.05), and a difference between 2 h after operation when it significantly increased and at 6 months after operation in group A (P<0.05). (D) There were differences in the CD69 expression at different time-points in patients in group B (P<0.05). The CD69 expression decreased before operation when compared with that at 5 min and 2 h after operation in group B (P<0.05), and increased when compared with that at 6 months after operation, and there was a difference between groups (P<0.05); there was a difference in the CD69 expression between 5 min after operation when it significantly increased and at 6 months after operation in group B (P<0.05), but no difference when compared with that at 2 h after operation (P>0.05), and there was a difference between 2 h after operation when it significantly increased and at 6 months after operation in group B (P<0.05). a, represents that there is a difference when compared with before operation (P<0.05); b, represents that there is a difference when compared with 5 min after operation (P<0.05); and c, represents that there is a difference when compared with 2 h after operation.

increased and at 6 months after operation in group A (P<0.05). There were differences in the CD69 expression at different time-points in patients in group B (P<0.05). The CD69 expression significantly decreased before operation when compared with that at 5 min and 2 h after operation in group B (P<0.05), and significantly increased when compared with that at 6 months after operation, and there was a difference between groups (P<0.05); there was a difference in the CD69 expression between at 5 min after operation when it significantly increased and at 6 months after operation in group B (P<0.05), but no difference when compared with that at 2 h after operation (P>0.05), and there was a difference between 2 h after operation when it significantly increased and at 6 months after operation in group B (P<0.05) (Fig. 1).

Comparison of the EGR1 expression at different time-points between groups. The EGR1 expression in groups was detected with RT-qPCR. Through comparison of the EGR1 expression before operation, at 5 min, 2 h and 6 months after operation between the two groups, it was found that there was no significant difference in the EGR1 expression before and at 5 min after operation in patients between group A and group B (P>0.05), but a statistical difference between them in it that at 2 h after operation in group A was significantly higher than that in group B (P<0.05), and a difference in it at 6 months after operation in patients between group A, group B and group C (P<0.05). Through pairwise comparison, it was found that the EGR1 expression in group A was significantly higher than that in group B (P<0.05), and it in group B was
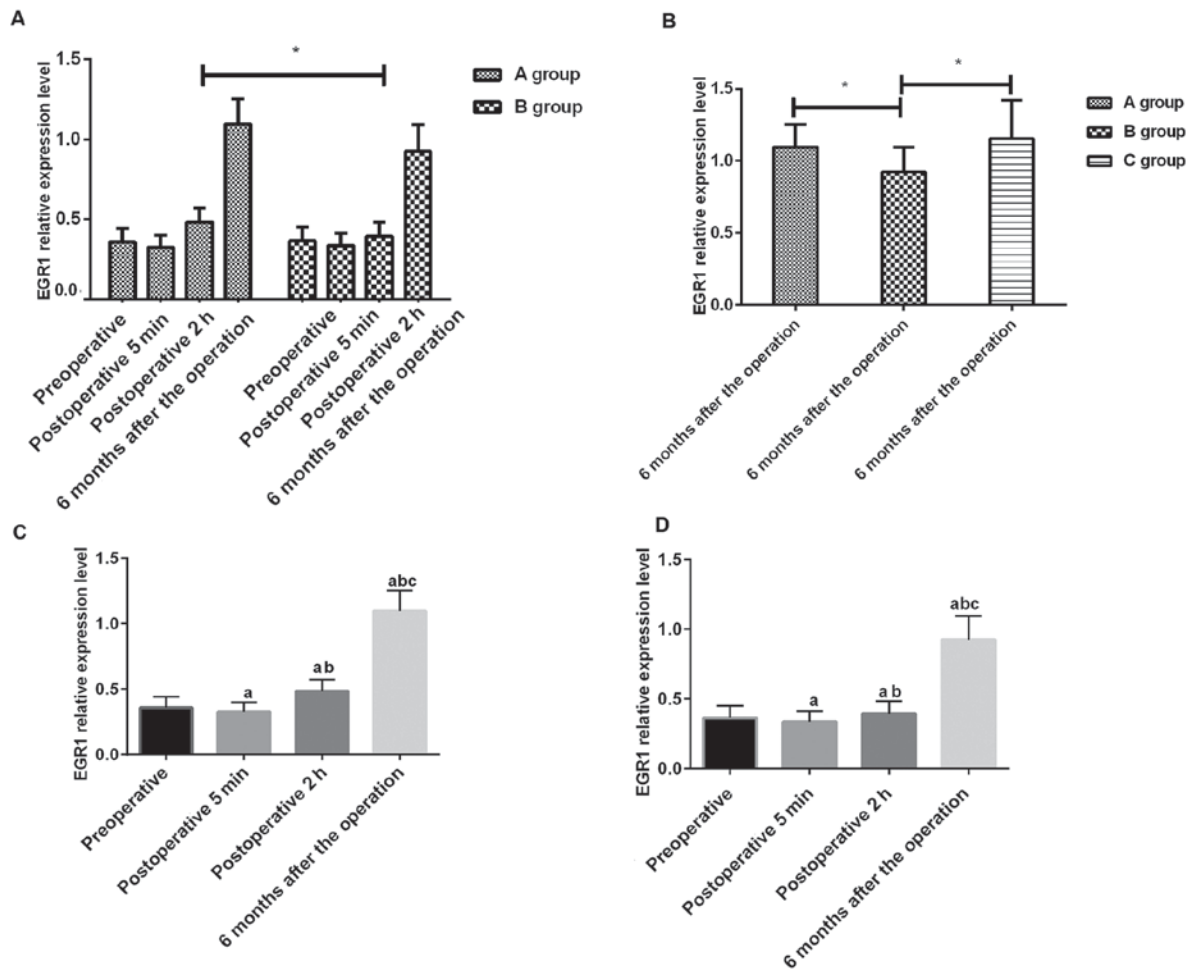
Figure 2. (A) EGR1 expression at different time-points in group A and group B. Detection of RT-qPCR found that there was no significant difference in the EGR1 expression before and at 5 min after operation in patients between group A and group B (P>0.05), but a statistical difference between them at 2 h after operation in group A was significantly lower (P<0.05). (B) EGR1 expression at 6 months after operation in group A, B and C. Detection of RT-qPCR found that there was a difference in the EGR1 expression at 6 months after operation in patients between group A, group B and group C (P>0.05). Through pairwise comparison, it was found that there was a difference in the EGR1 expression between group A and group B and it significantly increased (P<0.05), but no difference between group A and group C (P>0.05), and group B was higher than that in group C (P<0.05). *P<0.05. (C) There were differences in the EGR1 expression at different time-points in patients in group B (P<0.05). It decreased before operation when compared with that at 5 min after operation, and increased at 2 h after operation when compared with that at 6 months after operation, and there was a difference between groups (P<0.05); there was a difference in the EGR1 expression between 5 min after operation when it significantly decreased and at 2 h, 6 months after operation in group A (P<0.05), and a difference between at 2 h after operation when it significantly decreased and at 6 months after operation in group A (P<0.05). (D) There were differences in the EGR1 expression at different time-points in patients in group B (P<0.05). It decreased before operation when compared with that at 2 h and 6 months after operation in group B (P<0.05), and increased when compared with that at 5 min after operation, and there was a difference between groups (P<0.05); there was a difference in the EGR1 expression between 5 min after operation when it significantly decreased and at 2 h, 6 months after operation in group B (P<0.05), and a difference between 2 h after operation when it significantly decreased and at 6 months after operation in group B (P<0.05). a, represents that there is a difference when compared with before operation (P<0.05); b, represents that there is a difference when compared with 5 min after operation (P<0.05); and c, represents that there is a difference when compared with 2 h after operation.

significantly lower than that in group C (P<0.05), and there was no difference between group A and group C (P>0.05). There were differences in the EGR1 expression at different time-points in patients in group B (P<0.05). It significantly decreased before operation when compared with that at 5 min after operation, and significantly increased at 2 h after operation when compared with that at 6 months after operation, and there was a difference between groups (P<0.05); there was a difference in the EGR1 expression between 5 min after operation when it significantly decreased and at 2 h, 6 months after operation in group A (P<0.05), and a difference between 2 h after operation when it significantly decreased and at 6 months after operation in group A (P<0.05). There were differences in the EGR1 expression at different time-points in patients in

group B (P<0.05). It significantly decreased before operation when compared with that at 2 h and 6 months after operation in group B (P<0.05), and significantly increased when compared with that at 5 min after operation, and there was a difference between groups (P<0.05); there was a difference in the EGR1 expression between 5 min after operation when it significantly decreased and at 2 h, 6 months after operation in group B (P<0.05), and a difference between at 2 h after operation when it significantly decreased and at 6 months after operation in group B (P<0.05) (Fig. 2).

Correlation analysis. Through inclusion of Lpa, hs-CRP, Scr, CD69 and EGR1, logistic multivariate analysis found that CD69 (OR=6.424, P=0.025) and EGR1 (OR=3.684, P=0.013)

Table III. Logistic regression analysis.

Variables	Regression coefficient	SD	P-value	OR	95% CI
CD69	1.94	0.54	0.025	6.424	3.585-12.517
EGR1	1.64	0.44	0.013	3.684	1.598-11.584
Lpa	1.25	0.18	0.084	2.849	2.154-3.484
hs-CRP	1.36	0.21	0.184	3.251	2.658-3.894
Scr	1.84	0.36	0.098	4.251	3.548-4.846

were independent risk factors for patients after undergoing PCI (Table III).

Discussion

With the development of society, the increasing incidence of CHD disease has posed a serious threat to people's lives and health, and the number of deaths due to CHD in Western countries accounts for 30% of the total number and 50% or more of cardiovascular deaths (13). The mortality of CHD has gradually declined year by year, despite the mandatory intervention of risk factors and effective secondary prevention, but it still remains one of the most common causes of death in the world. Studies have shown that the number of death due to CHD exceeds that of all tumors, ranking first in the cause of death (14). The main cause of CHD is due to the formation of atherosclerosis on which chronic inflammation has an induced effect. The report of Zhou *et al* (15) showed that inflammatory stimuli can accelerate the formation of atherosclerosis.

In recent years, ideal progress has been made in the treatment of CHD owing to the explosive development of medical level and equipment (16). As a main method for the treatment of CHD patients in clinic currently, compared with previous surgeries, PCI has a clear treatment advantage that causes less harm to patients and can reach directly to the lesions (17). However, patients treated with PCI will undergo no-reflow phenomenon that will lower the blood flow rate in the coronary artery, and more serious blood flow obstruction, which makes it unable to achieve the desired effect on the patient during the administration method. The occurrence of no-reflow has a serious effect on the patient's treatment effect and prognosis. However, there is no clear statement about the occurrence of no-reflow phenomenon after PCI (18). Some studies (19) have proposed that no-reflow is caused by the participation of multiple factors, among which the main ones are gene regulation and leukocyte aggregation.

In this study, through detection of the expression of CD69 and EGR1 at different time-points in PCI patients, it was found that there was no significant difference in the CD69 expression before and at 5 min after operation in patients between group A and group B, but at 2 h after operation in patients in group A was significantly lower than that in group B, suggesting that the CD69 expression may be related to the patient's reflow status. As one of the phytohemagglutinin-like receptor family members that activate the early leukocyte receptor, CD69 can

express rapidly after activation and be detected on the surface of different activated leukocyte subset. CD69 regulates the synthesis, differentiation and inflammatory response of cells. For example, CD69-deficient mouse can cause acute onset of collagen-induced arthritis due to the overreaction of T-cell and B-cell to collagen (20). Therefore, we speculated that the overexpression of CD69 may be due to the accumulation of white blood cells in patients with no-reflow after PCI. Patients in group A, group B and normal control group (group C) were detected at 6 months after operation. There was a difference in the CD69 expression between group A in which it was significantly decreased and group B, and a difference between group B and group C, but no difference in patients between group A and group C. Thus, we can initially demonstrate that there is a certain association between CD69 and the patient's reflow status. In addition, detection of the EGR1 expression in plasma of patients found that there was no difference in the EGR1 expression before and at 5 min after operation when it decreased between group A and group B, but at 2 h after operation in group A was significantly higher than that in group B. EGR1, which is widely expressed in human cells and has the ability to express rapidly, belongs to the immediate early family members that have a high homology (21).

Studies have shown that the EGR1 expression plays a key role in cell proliferation, cell differentiation and inflammatory response in signal pathways. For example, EGR-1 can induce the expression of T-cell growth factor, IL-2 and TNF-α, and TNF-α has an important effect on the body's emergency defense response and anti-infection non-specific cellular immune response (22). The damaged blood vessels need to be reconstructed in the course of PCI, and the damage of coronary endothelial cells causes massive release of inflammatory factors (23), while the EGR1 expression may increase due to regulation of the release of inflammatory factors. Through comparison between CD69 and EGR1 in blood of patients in the two groups at 6 months after operation compared with those in the same period, it was found that there was a difference between them. There was no difference in the expression of CD69 and EGR1 in patients between group A and group C, suggesting that the recovery of the patients after operation was good, and there was a difference in patients between group B in which they increased and group C. Through longitudinal comparison of the expression of CD69 and EGR1 in the two groups of patients, it was found that indicators of them gradually returning to normal levels over time, and there was a difference in patients between group A in which the recovery was better than group B. Therefore, logistic regression analysis of CD69 and EGR1 revealed that they are independent risk factors for patients with or without reflow after operation, which also proved the importance of CD69 and EGR1 in PCI operation.

However, there are some flaws in this study. First of all, only detection of the EGR1 expression was performed, but detection and prediction of downstream regulated genes were not performed. The way in which EGR1 regulated its factors was unknown to us, and the number of patients in this study was relatively small and did not play a representative role. Therefore, we hope to increase the number of samples in future studies, predict the target genes through dual luciferase reporters, and increase the number of detection items to

further validate the results of this study, thus obtaining more results to corroborate this conclusion.

In conclusion, after undergoing PCI in CHD patients, if there was an increase in CD69 level and a significant decrease in EGR1 level in the early postoperative period, the patient may be suspected of having no-reflow and checked in time to improve the patient's therapeutic effect.

Authors' contributions

YX drafted the manuscript. YX and JP were mainly devoted to collecting and interpreting the data. YX, JP, QL and CG revised the manuscript. JP and QL were responsible for the concept and design of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of People's Hospital of Hunan Province (Changsha, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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