Early growth response 3 is Associated with Prognosis in Patients with Coronary Heart Disease

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Research Article

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Abstract

Aim

To investigate the association between the expression of early growth response 3 (Egr3), the level of interleukin-6 (IL-6) and interleukin-1ß(IL-1ß) as well as prognosis in patients with coronary heart disease (CHD).

Methods

Clinical data of 119 patients who visited the Fifth Affiliated Hospital of Xinjiang Medical University with CHD symptoms and underwent coronary artery angiography (CAG) were collected. According to the CAG results and Gensini score, the patients were divided into a mild stenosis group (n = 56), severe stenosis group (n = 32) and control group (n = 31). Circulating serum levels of Egr3 and the pro-inflammatory cytokines IL-1ß and IL-6 were assessed by enzyme-linked immunosorbent assay.qRT-PCR was used to measure Egr3 gene expression in peripheral blood of patients. The incidence of the CHD clinical composite end point was followed for 1 year by examining outpatient medical records and inquiry by telephone.

Results

The level of Egr3 in CHD patients was affected by Gensini score (b = 7.170, t = 2.912, P = 0.005) and IL-6 (b = 26.248, t = 4.279, P < 0.001). There was a significant difference in Egr3 mRNA levels between the controls and CHD patients. Moreover, the mRNA levels of Egr3 was a contributing factor to the adverse cardiovascular prognosis in patients with CHD (HR = 1.036, 95% CI 1.014–1.058, P = 0.001).

Conclusions

Egr3 may interact with the inflammatory response resulting in CHD. Egr3 may aggravate the severity of coronary artery stenosis, indicating that Egr3 mRNA levels are a potential prognostic factor in patients with CHD.

1. Introduction

Coronary heart disease (CHD) is a pathological condition characterized by the accumulation of obstructive or non-obstructive atherosclerotic plaques in epicardial vessels. In response to damage, the vessel wall produces a chronic low-grade inflammatory response by forming a multi-molecular network of inflammatory factors [1]. The inflammatory factors interleukin-1ß (IL-1β) and interleukin-6 (IL-6) are

involved in all stages of atherosclerosis development [2–6]. Recent studies showed that IL-1ß, IL-6 and C-reactive protein are mainly involved in the inflammatory pathways of atherosclerosis [7–8].

Early growth response 3 (Egr3) is a member of a zinc finger transcription factor family and an essential regulator of gene expression. Our previous research screened for possible mutations and polymorphisms of the Egr3 gene and found that variations in Egr3 were associated with CHD in the Xinjiang region, China. The Egr3 gene may be an important contributor to the heterogeneity of CHD [9]. Egr3 mRNA and protein expression levels were significantly upregulated in plaques in the mouse atherosclerotic(AS) model[10]. The Egr3 transcription factor has a variety of functions, including the regulation of inflammatory responses. Studies about tumor of prostate [12], Systemic lupus erythematosus[13]and the study of using bronchial epithelial cells stimulated with cigarette smoke extract (CSE) [14] have shown that Egr3 may be related to multi-organ inflammation and play a direct or indirect role in regulating gene expression. It was suggested that IL-6 and IL-1ß were potential Egr3 target genes. Studies on vascular diseases have found that the expression of Egr3 was highly induced by treatment of endothelial cells with vascular endothelial growth factor (VEGF). Therefore, Egr3 mediates many inflammatory effects of VEGF [15]. Mouse models and in vivo findings confirmed that Egr3 induces the secretion of inflammatory factors such as IL-6 by regulating the plasma level of VEGF [16]. Egr3 may play an important role in CHD by mediating the inflammatory response in CHD.

The aim of this study was to explore the association between Egr3 protein levels and the degree of coronary artery stenosis in patients with CHD as well as the role of inflammation as a potential confounding factor of this association. In addition, quantitative real-time PCR (qRT-PCR) was used to measure the expression of Egr3 mRNA in patients with CHD, and the occurrence of adverse cardiovascular events was followed to explore the association of Egr3 expression with prognosis.

2. Materials And Methods

2.1 Participants

A total of 119 patients who were admitted to the Cardiovascular Department and the General Practice Department of the Fifth Affiliated Hospital of Xinjiang Medical University urumqi Xinjiang China with CHD symptoms and who underwent a coronary artery angiography (CAG) were enrolled from September 2020 to December 2020. Based on the Gensini score, patients diagnosed with CHD were divided into two groups: a mild stenosis group (score ≤ 52, n = 32) and severe stenosis group (score > 52, n = 56). Patients who were admitted to the hospital but in whom CHD was excluded by CAG examination were enrolled as controls (n = 31). The exclusion criteria were as follows: past or present definite tumors, mental illness, myocarditis, cardiomyopathy, endocarditis, pulmonary heart disease, congenital heart disease, inflammatory disease, autoimmune disease, active infections, hematological system disease or lymphatic system disease.

2.2 Data documenting

The following data were collected after admission: (1) Baseline characteristics: age, sex, heart rate, blood pressure, body mass index(BMI), and smoking status. (2) Medical history: hypertension and diabetes. (3) Blood biochemical indexes: Level of White Blood Cell(WBC), fasting blood glucose(FBG),triglyceride(TG), total cholesterol(TC),Low Density Lipoprotein-C(LDL-C), High Density Lipoprotein-C(HDL-C) and cardiac ultrasound (left ventricular ejection fraction,LVEF), which were obtained by the laboratory and ultrasound department of the hospital. (4) CAG results (lesion location and stenosis degree of coronary artery).

2.3 Blood extraction and measurement of Egr3, IL-1ß and IL-6 levels in serum

Two milliliters of venous blood was obtained from each fasting subject in a blood collection tube without anticoagulant. Then, the blood was centrifuged at 1500 r/min for 30 min. Serum was separated and stored at − 80 ℃ for later use. The levels of Egr3, IL-1ß and IL-6 in serum were detected by enzyme-linked immunosorbent assay (ELISA). ELISA kits were provided by Shanghai Enzyme Linked Biology (Shanghai China)to measure levels of IL-6 (batch number Ml058097), IL-1β (batch number Ml058059)and Egr3 (batch number Ml622406). All ELISAs were carried out in accordance with the manufacturer instructions.The absorbance (OD value) was measured sequentially at a wavelength of 450 nm using a microplate reader (type K6600A, Beijing Kaiao technology Co.,Ltd.Beijing China).

2.4 Extraction of RNA and measurement of Egr3 mRNA in monocytes

Two milliliters of venous blood was obtained from each fasting subject in a blood collection tube with anticoagulant. Blood was then divided in 250 μL samples in 1.5 ml Eppendorf tubes. Red blood cell lysate was added in a ratio of 1:3 for 5 min at low-temperature to lyse the cells. After centrifugation, 1 ml Trizol was added to the white precipitate. The sample was then quickly frozen with liquid ammonia and stored at − 80 ℃. RNA concentrations were measured using a spectrometer (Hangzhou Langji, Q2000B,Zhejiang China). For the reaction mixture, the following reagents were used: total RNA, 7 μl; primer (0.1 μg/ul), 1 μL; 2 × TS Reaction Mix, 10 μl; TransScript @ RT/RI Enzyme Mix, 1 μl; gDNA Remover, 1 μl; and RNase-free water, added to a 20 μl of total volume. Reverse transcription was performed under the following conditions: 25 ℃ for 10 min, 42 ℃ for 30 min and 85 ℃ for 5 s. The primer sequences for Egr3 were F-TGCTATGACCGGCAAACTC and R-GGCTACAGAGAATGTAATGGACAT, and all RT-PCR primers were obtained by Tsingke Biotechnology Co., Ltd. (Beijing, China). The thermal cycling conditions for reverse transcription included an initial pre-incubation phase, which was as follows: 95°C for 5 min, 95°C for 5 s and 60°C for 35 s. The 2 − ΔΔCT method was used to calculate the difference of expression between controls and CHD patients. Each step was repeated three times.

2.5 Assessment of the degree of coronary stenosis

The Gensini score is an effective tool for assessing the severity of coronary artery disease [17], and includes the number, location, and degree of stenosis of coronary artery lesions. The total score is multiplied by a weighting factor reflecting the importance of the lesion location in the coronary circulation for each lesion[18],1 for 25% stenosis, 2 for 50%, 4 for 75%, 8 for 90%, 16 for 99%, and 32 for total occlusion. Each principal vascular segment was assigned a coefficient:5 points for left main coronary lesion; 2.5 points for proximal left anterior descending branch and left circumflex artery; 1.5 points for middle left descending artery lesion; 1 point for first diagonal branch and obtuse marginal branches and right coronary artery; 0.5 points for the second diagonal and posterolateral branch of the left circum-flex artery.

2.6 Assessment of coronary end point events

All patients were followed at 1,6 and 9 months and 1 year by reviewing the outpatient medical records and inquiries by telephone after CAG. The composite end point was major adverse cardiovascular event (MACE) or hospitalization for heart failure. MACE was defined as cardiac death, recurrent myocardial infarction or accidental target vessel revascularization. Revascularization of target vessels was defined as any repeated revascularization of a stenosis of at least 50% in diameter in the presence of ischemic signs or symptoms or of a stenosis of at least 70% in diameter in the absence of ischemic signs or symptoms [19].

2.7 Statistical Methods

All data were imported in Excel(Microsoft Washington D.C.USA),and SPSS25.0(SPSS Inc.,Chicago,IL,USA) was used for statistical analysis. The mean and standard deviation (SD) were calculated to describe the normally distributed variables, and the median with interquartile range (IQR) was used to expound the skewed-distributed variables. The independent sample t-test was used for comparison of quantitative data between two groups, and one-way ANOVA was used for comparisons among multiple groups. Non-normal quantitative data were compared using the Wilcoxon test.Categorical variable were compared by the Chi-square test.Spearman correlation was used for correlation analysis. Multivariate analysis was performed using significant variables of univariate analysis to explore the factors affecting the relationship between Egr3 levels and CHD. Cox multivariate regression was used to analyze the independent risk factors affecting the prognosis of CHD. Based on Egr3 gene expression levels, patients were divided into an up-regulated group and normal expression group using the optimal diagnostic cut-off value of ROC. Patients were followed for 1 year for the incidence of the composite end point, and the cumulative incidence was evaluated by Kaplan-Meier analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Baseline data

There were statistically significant differences in gender, FBG,WBC count and hypertension history across the three groups ($P < 0.05$), as shown in Table 1. There were no significant differences in other parameters across the three groups. In this study, CHD was treated with lipid-lowering drugs in outpatient setting and at the emergency department based on chest distress, chest pain and other symptoms. Therefore, there were no differences in blood lipid levels compared with the control group.

Table 1
Comparison of baseline data across the three groups

	Control group (n = 31)	CHD group(n = 88)		*P*
		Mild stenosis group (n = 56)	severe stenosis group (n = 32)	
Age(years)	62.03 ± 5.9	63.59 ± 9.85	64.16 ± 12.02	0.443
Gender(meal/femeal)	10/21	26/30	24/8	0.002
Smoking history (n/%)	7/22.6	16/28.6	14/43.8	0.159
BMI(Kg/m2)	25.95 ± 4.04	25.28 ± 2.90	25.06 ± 3.04	0.531
TC(mmol/L)	4.5 ± 1.07	4.28 ± 1.10	4.10 ± 1.13	0.360
LDL(mmol/l)	2.80 ± 0.88	2.61 ± 0.95	2.55 ± 0.98	0.542
HDL(mmol/l)	1.59 ± 0.37	1.51 ± 0.28	1.41 ± 0.29	0.068
TG(mmol/l)	1.77 ± 1.05	1.77 ± 1.27	1.77 ± 1.03	0.998
FBGmmol/l)	5.3(4.9 6.0)	6.27(5.33 8.12)	6.5(5.48 8.03)	0.010
WBC(10^9/L)	5.98 ± 1.25	6.67 ± 1.73	7.02 ± 1.74	0.038
HR(beats/min)	80.87 ± 15.74	76.38 ± 13.26	73.66 ± 11.07	0.102
LVEF(%)	65.13 ± 4.70	64.11 ± 5.62	61.75 ± 7.73	0.075
HyP(n/%)	13/41.9	43/76.8	22/68.8	0.004
DM(n/%)	8/25.8	19/33.9	14/43.8	0.335
Notes: FBG: fast blood glucose; HR: heart rate; LVEF: left ventricular ejection fraction				

3.2 Comparison of serum levels of Egr3, and the inflammatory factors IL-1ß and IL-6

Levels of Egr3, IL-1ß and IL-6 in peripheral blood of patients in the three groups were analyzed (Table 2 and Fig. 1). The levels of serum Egr3 in the severe stenosis group (2067.74 ± 1200.77 pg/mL) were higher than that in the mild stenosis group (1585.07 ± 929.50 pg/mL). The levels of IL-6 were higher in the severe stenosis group (48.89 ± 17.4 pg/mL) than in the mild stenosis group (40.3 ± 18.43pg/mL) and the control group (39.66 ± 16.19 pg/mL). These difference were statistically significant (P < 0.05). No significant statistical difference was found in IL-1ß levels across groups.

Table 2
Comparison of serum Egr3, IL-1β and IL-6 levels across the three groups

	Control group(n = 31)	Mild stenosis group(n = 56)	Severe stenosis group(n = 32)	*P*
Egr3(ng/L)/100	1675.03 ± 1154.12	1585.07 ± 929.50	2067.74 ± 1200.77	0.148
IL-6(pg/mL)	39.66 ± 16.19	40.3 ± 18.43	48.89 ± 17.4	0.0561
IL-1β(pg/mL)	41.1 ± 27.93	39.63 ± 21.52	44.9 ± 22.96	0.6034
Egr3, IL-1ß, and IL-6 in peripheral blood of patients in each group were compared, the data was mean ± SEM, p 0.05.				

3.3 A multi-factorial linear regression model with serum Egr3 as the dependent variable

Spearman correlation was used to analyze the relationship between serum Egr3 and the inflammatory factors IL-1ß and IL-6, as well as the Gensini score and clinical data in patients with CHD. Serum Egr3 was positively correlated with Gensini scores (r = 0.278, P = 0.009), IL-6 (r = 0.0.454, P < 0.001) and IL-β (r = 0.275, P = 0.01). IL-6 was positively correlated with IL-1β (r = 0.400, P < 0.001). IL-1ß was positively correlated with the Gensini score (r = 0.216, P = 0.043). To examine whether serum Egr3 is independently correlated with the above factors, the relationships between the serum Egr3 levels in the CHD group and factors were analyzed by multi-factorial linear regression with serum Egr3 as the dependent variable and gender, FBG, WBC, hypertension, Gensini score, IL-6 and IL-1β as independent variables. The results showed that the Gensini score (b = 7.170, t = 2.912, P = 0.005) and IL-6 (b = 26.248, t = 4.279, P < 0.001) were independently correlated with serum Egr3 levels (Table 3).

Table 3
Multiple linear regression analysis of relationship between serum Egr3 and related indicators

variable	b	b standard error	t	*P*	95%CI
Constant	10.151	620.034	0.016	0.987	-1223.756 ~ 1244.057
Gender(male/female)	334.183	213.910	1.562	0.122	-91.511 ~ 759.877
FBG(mmol/l)	0.529	34.784	0.015	0.988	-68.694 ~ 69.751
WBC(10^9/L)	-44.514	61.226	-0.727	0.469	-166.358 ~ 77.330
Hypertension	70.145	234.981	0.299	0.766	-397.483 ~ 537.773
Gensini score	7.170*	2.462	2.912	0.005	2.270 ~ 12.071
IL -6(ng/L)	26.248*	6.134	4.279	0.000	14.040 ~ 38.455
IL-1β(ng/L)	2.500	5.365	0.466	0.642	-8.177 ~ 13.177
Note: * independently correlated with Egr3, P < 0.05					

3.4 Egr3 expression in the three groups

Patients without Egr3 mRNA expression or in whom tests failed to detect Egr3 mRNA up to three times were excluded. There was a statistically significant difference in Egr3 mRNA levels between the control group(1.03 ± 0.01) and all groups of patients (2.417 ± 0.408). Egr3 mRNA levels were significantly different across the groups (P < 0.05). Egr3 mRNA levels were significantly different between the control group and the mild stenosis group (P < 0.05) (Table 4 and Fig. 2).

Table 4

Comparison of Egr3 mRNA levels across groups

Group	Egr3 mRNA	H	*P*
Control group(n = 29)	1.03 ± 0.01	4.95	0.0094
Mild stenosis group(n = 26)	3.03 ± 0.71		
Severe stenosis group(n = 27)	1.83 ± 0.39		
* Pairwise comparisons between the control group and the mild disease group. P < 0.05.			

3.5 Multivariate regression model to identify the prognostic factors of patients with CHD

Patients with CHD were followed for 12 months for outcomes and the incidence of composite end point events were recorded. First,univariate Cox regression analysis was performed. Then, the statically

significant factors, including history of hypertension, serum levels of Egr3, IL-6 and IL-1β, Egr3 mRNA and Gensini score were included in multivariate Cox regression as covariables (Table 5). The results suggested that Egr3 mRNA (HR = 1.036, 95% CI 1.014–1.058, P = 0.001) was a contributing factor to adverse cardiovascular outcomes in patients with CHD.

Table 5
Multivariate Cox analysis for the composite end point

Constant	b	*SE*	*Wald*	*P*	*HR*	*HR*95%CI
Hypertension	-1.167	0.600	3.786	0.052	0.311	0.096 ~ 1.009
Gensini score	0.012	0.007	3.001	0.083	1.012	0.998 ~ 1.025
IL -6(ng/L)	-0.002	0.018	0.016	0.900	0.998	0.962 ~ 1.034
IL-1β(ng/L)	0.006	0.015	0.133	0.715	1.006	0.976 ~ 1.036
Egr3(ng/L)	0.000	0.000	0.696	0.404	1.000	1.000 ~ 1.001
Egr3mRNA	0.036	0.011	10.725	0.001	1.036	1.014 ~ 1.058

3.6 Follow-up results and Kaplan-Meier survival analysis

Follow-up analysis was done in patients with CHD. Based on the Egr3 mRNA levels in the control group, the optimal truncation value for Egr3 mRNA was calculated using the ROC curve. The sensitivity and specificity of Egr3 mRNA were relatively high when the threshold value was 2.38mmol/L. Therefore, based on the value of 2.38, patients were classified according to the Egr3 mRNA expression levels: Egr3 mRNA ≥ 2.38 was taken to be the upregulated group (N = 20) and Egr3 mRNA < 2.38 was taken to be the normal expression group (N = 35). The Kaplan-Meier survival analysis showed that the incidence of the composite end point in the upregulated group (45%) was higher than that in the normal expression group (22.9%). There was no statistically significant difference in the incidence of the composite end point between the two groups (Fig. 3).

4. Discussion

CHD is characterized by high prevalence, high mortality and high disability rate. CHD causes great physical and mental pain to individuals, hinders economic and social development and increases the burden of global public disease. In response to the Healthy China strategy, there is an urgent need for the prevention and treatment of CHD. There are many hypotheses about the pathogenesis of CHD. At present, the hypothesis of the endothelial damaging response is one of the most important hypotheses to explain the pathogenesis of CHD. Excessive inflammatory and fibrous hyperplasia reactions after intima injury play an extremely important role in the occurrence and development of coronary artery disease.

Egr3 is an transcription factor activated immediately in response to multiple mitotic signals. To date, Egr3 has been studied mainly in the development of the central nervous system, cancer, angiogenesis, regulation of fibrotic processes and immunity [21–23]. Furthermore, Egr3 expression was shown to be upregulated during myocardial hypoxia in mice [24]. In addition, Egr3 increases the risk of acute coronary events by stimulating the activity of matrix metalloproteinase 9 (MMP9) [23]. Egr3 was also one of the most highly inducible genes in human VEGF-treated coronary endothelial cells. It was suggested that Egr3 may be a potential drug target for the treatment of pathological angiogenesis and vascular inflammation[25]. Our study found that increased Egr3 levels were associated with a higher Gensini score, suggesting that Egr3 has an effect on the degree of coronary stenosis.The level of Egr3 mRNA in CHD patients was higher than that in the control group. Egr3 protein levels were significantly different between the control group and the mild stenosis group ($P < 0.01$) but were not significantly different between the severe stenosis group and the mild stenosis group ($P = 0.66$). This may be due to gene transcription efficiency, post-transcription degradation rate, translation efficiency and protein degradation rate. Therefore, the association between expression levels of Egr3 mRNA and protein are not necessarily linear. Future experiments that examine regulatory factors that inhibit Egr3 protein transcription or accelerate protein degradation may provide insights. These in-depth studies of the molecular mechanism of Egr3 affecting CHD may provide new ideas for the exploration of targeted drugs.

Egr3 is a transcription factor involved in the transforming growth factor-beta (Tgf-β)-induced fibrosis of myocardial fibroblasts and can increase the secretion of fibrotic proteins [26]. This process may be initiated by the death of cardiomyocytes (CMs) in the ischemic region of the heart due to coronary stenosis or occlusion. Egr3 then activates myocardial fibroblasts to form chronic fibrotic fatigue tissue to compensate for the loss of CMs, resulting in pathological myocardial fibrosis and hypertrophy. Myocardial fibrosis can lead to cardiac dysfunction, and left ventricular dysfunction can accelerate the progression of heart failure. In addition, left ventricular fibrosis can induce arrhythmia, which is a risk factor for cardiogenic death, thereby seriously affecting the prognosis of patients [27]. Studies have shown that [28] targeted interventions of Egr3 mRNA expression in patients with CHD can exert endogenous anti-fibrosis effects, inhibit ventricular remodeling and improve the prognosis of patients with CHD. In this study, patients with CHD were followed for 1 year, and the incidence of the CHD composite end-point in the group with upregulated Egr3 mRNA expression (45%) was higher than that in the group with normal expression (22.9%). Upregulated Egr3 mRNA was associated with poor cardiovascular outcomes in patients with CHD but survival analysis did not yield statistical differences. Long-term follow up may be required to demonstrate statistical findings.

Egr3 plays a key role in inflammatory responses of various pathogenic processes [29]. The NFκB-Egr3 complex formed by Egr3 and the NF-κB subunits p50 and p65 may regulate the expression of inflammatory genes in the pathogenesis of CHD. Moreover,the NFκB-Egr3 complex can activate the promoter sequence of IL-6 and IL-1β gene expression [23, 30]. The results of this study showed that serum Egr3 level was correlated with IL-6 levels. Currently, targeting of IL-1β, IL-6 and other inflammatory factors have been clinically verified for the treatment of atherosclerosis and cardiovascular diseases[31]. This

study suggests that Egr3 may be an upstream biomarker,which regulate the IL-6 expression, making inhibition of the Egr3 pathway an attractive potential target for the treatment of CHD.

5. Limitations Of Our Study And Prospects

This study has several limitations. First, few studies have been conducted in this field, and multi-center prospective studies with larger sample sizes should be carried out to verify the effect of Egr3 on CHD. Second, Egr3 levels may change during the course of the disease, and this study did not account these potential changes. The study lacked adjustment for unknown covariables, which may affect the outcome of prognosis. In addition, a longer follow up is needed to study the prognosis of CHD. Finally, the molecular mechanism of Egr3 in regulating inflammatory response and angiogenesis has to be clarified in future studies to provide a theoretical basis for delaying the progress of CHD. Targeted intervention of Egr3 may delay or reverse the occurrence and development of atherosclerotic plaques and prevent and treat CHD, providing a new direction for the discovery of new clinical intervention targets.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from each participant prior to enrollment.

Consent for publication

All authors gave final approval of the version for publication, and agreed to be accountable for all aspects of the study.

Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

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Author Contributions

Zumureti Abudukeyimu and Xia Li are co-first authors. Xia Li and Junyi Luo were responsible for the conception and design of the study. Kairui Zhu, Siqing Wang, Juxing Ma and Xiaomei Lin collected the samples and analyzed the basic clinical data. Fangliu and Mengge Xu were involved the statistical analysis. Xia Li and Junyi Luo interpreted the results and critically revised the manuscript. All authors contributed to data analysis, drafting, and revising the manuscript.

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Figures

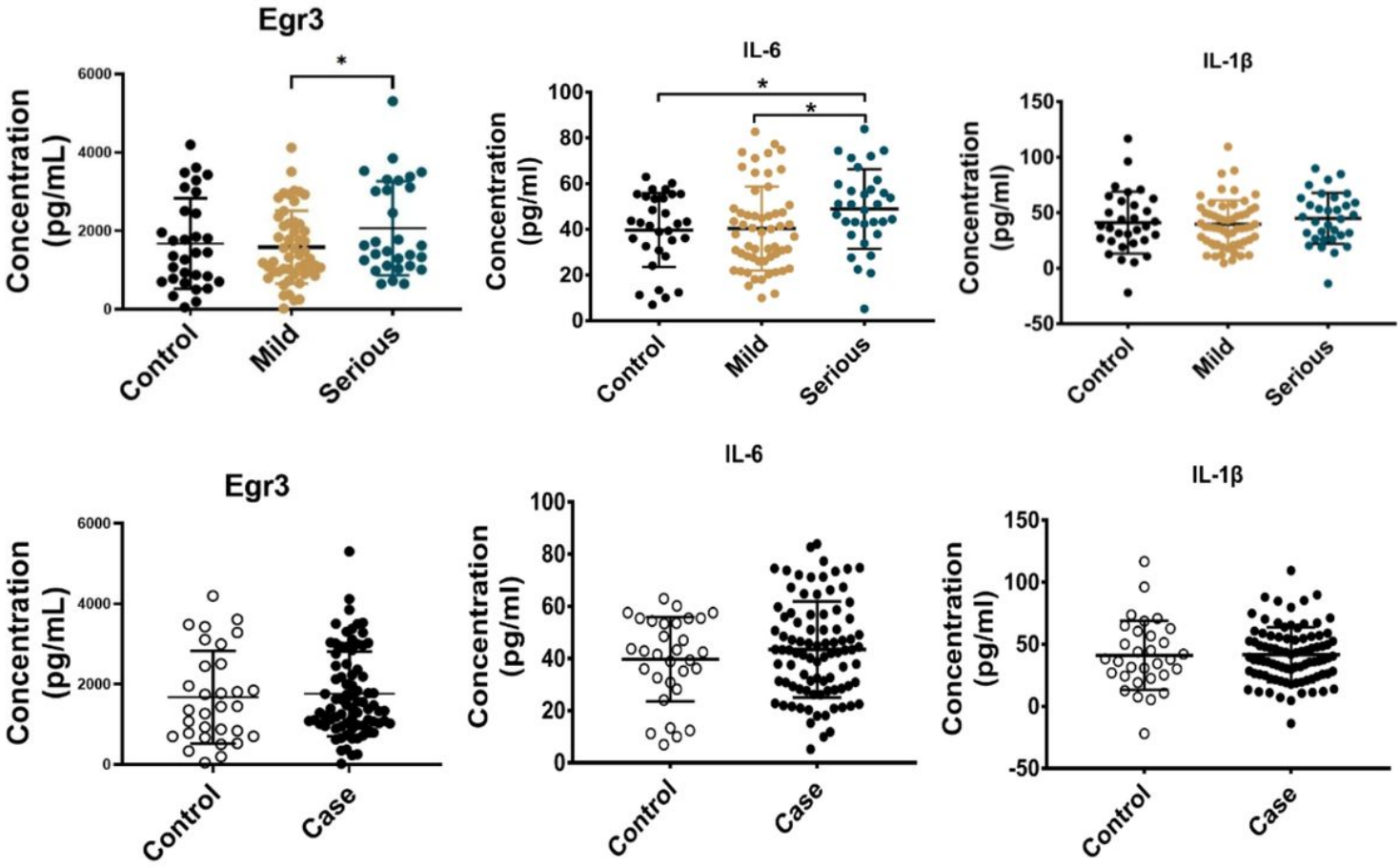

Figure 1

Egr3, IL-1ß, and IL-6 in peripheral blood of patients in each group were compared, the data was mean ± SEM, p 0.05.

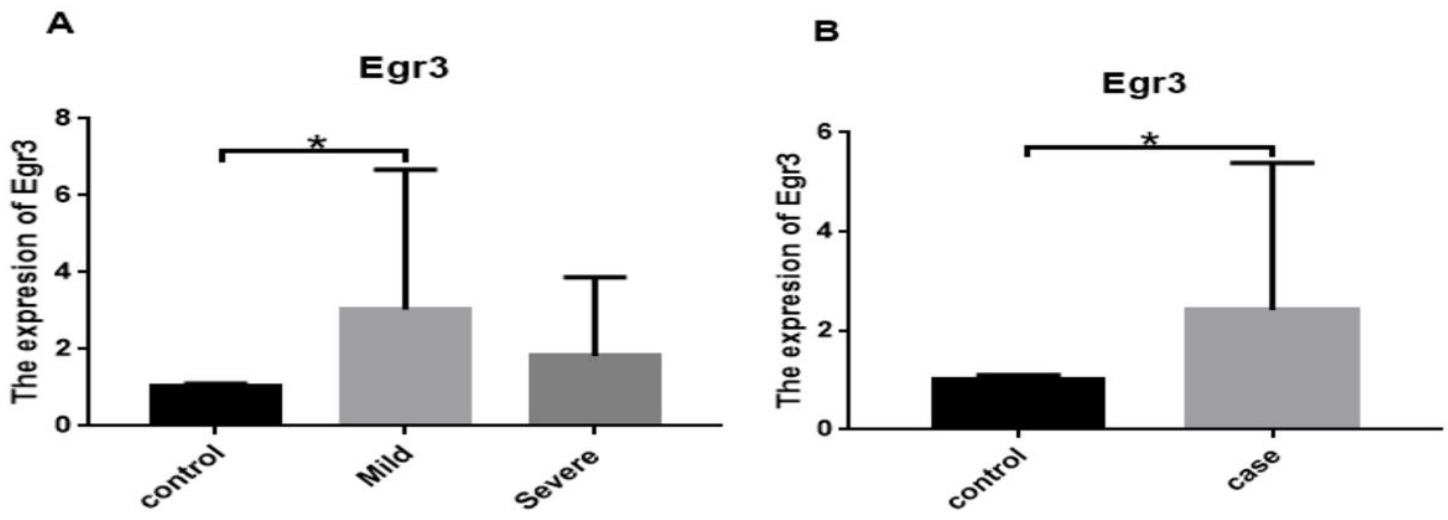

Figure 2

Egr3 expression

A. Comparisons between the control group, mild disease group and severe disease group

B. Comparison between the control group and patient groups.

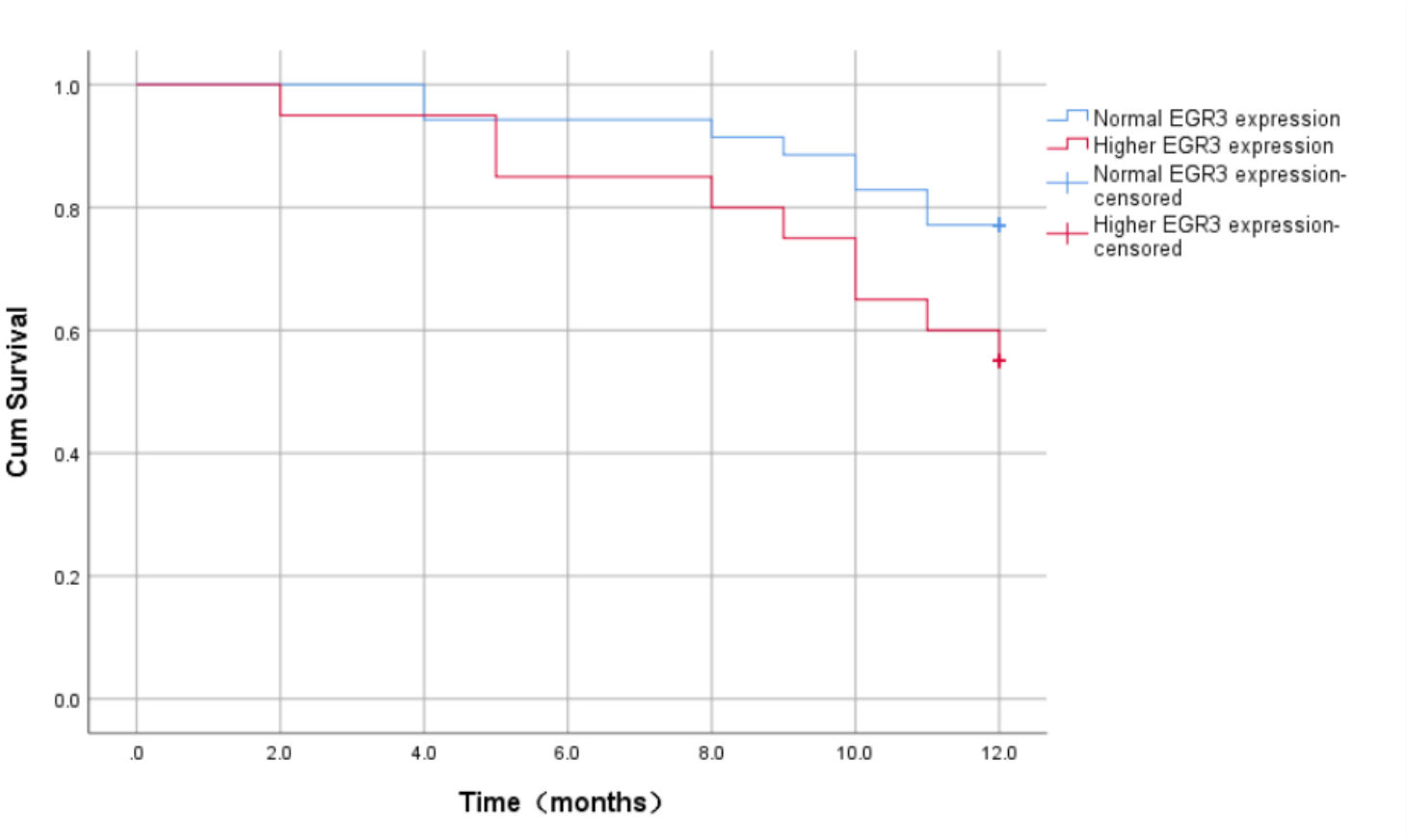

Figure 3

Comparison of composite end point at follow-up between the two patient groups.