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Original Article

Serum interleukin-6 promoter polymorphism as a predictor of angina in patients with coronary artery spasm angina: a case-report study

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Abstract: Inflammation is closely related to coronary vasospasm angina (CVA). Interleukin-6 is a multifunctional cytokine with dual effects of pro-inflammatory and anti-inflammatory actions. This study observed whether IL-6 gene polymorphism in patients could be a predictor for CVA. The serum samples of 30 patients with CVA and 30 healthy people were examined via PCR-RFLP to assess the genotype frequency. The distribution of IL-6 promoter 634 C/G genotype in the control group was 59.58% of those with CC type, 37.15% with CG type and 3.45% with GG type; while in the CVA group it was 37.14%, 48.15%, and 14.71%, respectively ($P < 0.01$). The frequency of the G allele was significantly higher in the control group (38.79% vs. 21.88%, $P < 0.01$). Compared with wild type CC, the CVA risk for G allele carriers (CG + GG genotype) was increased. The IL-6 promoter for the G allele increased the risk of coronary vasospasm angina pectoris, which can be used as a predictor of angina pectoris.

Keywords: Interleukin-6, polymorphism, PCR, coronary artery spasm angina pectoris

Introduction

Coronary artery spasms can cause angina, the occurance of which is called coronary artery spasm angina (CASA). According to the Chinese expert consensus on the diagnosis and treatment of coronary artery spasm syndrome [1], CASA can be divided into typical and atypical. The typical type of CASA is variant angina. Variant angina pectoris is common in China, but it is difficult to capture the characteristic ST-T elevation in ECG changes during the attack of variant angina pectoris. Therefore, it is not uncommon to treat patients with acute variant angina pectoris as those with acute coronary syndrome in clinical practice. Patients with chronic variant angina pectoris with prolonged course of disease are often missed, misdiagnosed or even mistreated for a long time because it is difficult to capture the ECG changes of the transient ST-T elevation.

Cardiac troponin and creatine kinase, markers of myocardial cell necrosis, are valuable tools for the diagnosis and risk stratification of new ACS. However, many ACS patients are likely to have ischemic events even without myocardial necrosis due to unstable shifts. A large number of inflammatory mediators are produced in the process of the inflammatory response [2]. Some inflammatory markers have important guiding significance for the diagnosis, treatment and prognosis of ACS.

Among these markers, the most studied is C-reactive protein (CRP). CRP concentration is elevated in UA and AMI patients. There is strong evidence that CRP is a reliable and important indicator for the risk of ischemic heart disease. In addition to liver cells, IL-1, IL-6, and TNF-a can also stimulate the cells that make up the atherosclerotic block to produce CRP, so the degree of local CRP in the coronary artery is significantly higher than that of circulating CRP.

IL-6 is a strong independent marker of increased mortality in patients with ACS, and it is helpful for guiding treatment. For example, early inter-

ventional therapy in patients with elevated IL-6 levels can reduce 12-month mortality by 65%, while early interventional therapy in patients with low IL-6 levels is not beneficial compared with conservative treatment. In addition, even if troponin T levels are not elevated, elevated levels of IL-6 and IL-1Ra 48 h after admission in ACS patients is associated with poor prognosis during hospitalization.

Coronary artery spasm angina pectoris (CVA) is the starting link of a variety of ischemic cardiovascular diseases [1-3], and it is one of the important causes of cardiac arrest outside the hospital. Its exact mechanism is still unclear, but it is closely related to the inflammatory response. The levels of IL-6 and hs-CRP in serum of CVA patients are correlated with the severity of CVA [3].

IL-6 is a 23.7 kDa multi-effect cytokine with anti-inflammatory and pro-inflammatory effects. Environmental and genetic effects make the serum IL-6 concentrations vary greatly among different individuals. The polymorphism of three single nucleotides in the IL-6 promoter region -597G/A, -634C/G and -174G/C affected IL-6 transcription.

The present study was undertaken to investigate whether the polymorphism in serum IL-6 are correlated to pro-inflammatory effects.

Material and methods

Subjects

This protocol was conducted in The Affiliated Hospital of Qingdao Binhai University, and Ningxia People's Hospital. This study was performed in accordance with the Declaration of Helsinki, and approved by the review board and Ethics Committee of Qingdao Binhai University Affiliated Hospital (2020ACU009). Written informed consent was provided by all participants.

Inclusion criteria

The main criterion for inclusion was a diagnosis of CASA by the Chinese expert consensus according to the diagnosis and treatment of coronary artery spasm syndrome [1]: along with 30 outpatients patients with CVA who had been diagnosed and enrolled in this study, all patients were aged 28 to 40 years old (29.85±3.63 years). The course of illness was 5 to 6 years (6.33±6.33 years). The ratio of men to women was 16/14. 1) At rest and/or fatigue, angina-like episodes of 12-lead electrocardiogram (ECG), dynamic electrocardiogram (DCG) in two leads above ST segment rise or fall more than 0.1 mV, or negative U wave. 2) Sublingual nitroglycerin can be used. 3) There was no obvious obstructive coronary disease after treatment with nitroglycerin in the coronary artery (the stenosis of the main coronary artery cavity was less than 50%). 4) Signed informed consent.

Exclusion criteria

The common exclusion criteria of the two groups were acute coronary syndrome, hypertrophic cardiomyopathy, valvular disease, left ventricular dysfunction (ejection fraction < 50%), tumor, kidney, liver or thyroid disease. 1) No chest pain symptoms. 2) ECG is normal. 3) No history of angina and smoking. 4) Signed informed consent.

Control group

A total of 30 healthy subjects with the same age and gender at the same time period were enrolled in the group: aged 28 to 40 years old (28.12±3.23 years). The course of illness was 0 years. The ratio of men to women was 16/14.

Laboratory methods

Fasting for more than 10 hours the previous night, venous blood was drawn, and stored at 4°C after which it was centrifuged for 30 minutes, at a speed of 2500 rpm, and then stored in -80°C freezer. TC, HDL-C, LDL-C, TG, hs-CRP and IL-6 were examined.

Genomic DNA of peripheral blood leukocytes was extracted by the salting-out method. The IL-6-634C/G genotype was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).

Statistical analysis

All measurement data were expressed as mean ± standard deviation (x ± SD). Student' s t test was used for two groups of continuous variables statistics; the genotypes and allele frequencies were directly counted, and the distri-

Table 1. IL-6-634 C/G genotype and allele frequency in CVA patients

Group	n	Genotype frequency (%)			Allele frequency (%)	
		CC	CG	GG	C	G
Control	30	59.58	37.15	3.45	78.02	21.98
CVA	30	37.14	48.15	14.71	61.11	38.79
P			0.006		0.005	

Table 2. Relative risk between IL-6-634C/G genotype and CVA

Genotype	OR (95% CI)	P	OR (95% confidence interval)	P
CC	1.00		1.00	
CG	2.086 (0.8763-4.9657)	0.0914	2.0091 (0.8443-4.7808)	0.1096
GG	6.9 (1.769-26.9134)	0.0123	4.9091 (1.3215-18.2367)	0.0289
CG + GG	2.4957 (1.0949-5.6888)	0.0256	2.2838 (1.0026-5.2026)	0.0447

bution differences of alleles and genotypes were analyzed by χ^2 test. Intra-group and inter-group differences were compared by t test followed by analysis of variance. SPSS 11.0 software was used for data processing. $P < 0.05$ indicated that the difference was significant (two sided).

Results

Comparison of serum IL-6 between the two groups

The expression of serum IL-6 in healthy subjects was low, and the concentration of IL-6 in the CVA group was higher than that in control group. There was a significant difference between the two groups ($P < 0.05$).

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IL-6-634 C/G genotype and allele frequency

As shown in **Table 1**. The distribution of IL-6 promoter 634 C/G genotype in the control group was 59.58% with CC type, 37.15% with CG type and 3.45% with GG type, while that in the CVA group was 37.14%, 48.15%, and 14.71%, respectively ($P < 0.01$). The frequency of the G allele was significantly higher in the control group (38.79% vs. 21.88%, $P < 0.01$).

Relative risk of IL-6-634C/G genotype and CVA

See **Table 2**. After adjusting age, gender, blood pressure, BMI, blood lipid level and IL-6, the relative risk OR = 2.2838, 95% CI = 1.0026-5.2026, $P < 0.05$.

This indicated that there were differences between CC, CG, GG and CG + GG of different gene expression types. Compared with wild type CC, the risk of CVA in G allele carriers (CG + GG genotype) increased.

There was no significant relationship between IL-6 genotype and age, gender, blood pressure, body mass index, left ventricular ejection fraction and blood lipid levels in the two groups. The level of CG + GG type IL-6 was higher in CVA group ($P < 0.05$).

Discussion

The present study showed major findings based upon comparative clinical investigation revealing that IL-6-6-634C/G has CVA risk. Compared with wild type CC, the serum IL-6 level of CG + GG in G allele carriers was significantly increased, which increased the risk of CVA. This study supported the view that mild inflammatory response can lead to CVA. The IL-6 promoter G allele increased the risk of coronary vasospasm angina pectoris, which can be used as a predictor of angina pectoris.

IL-6 is closely related to CVA. IL-6 is an important regulator of C-reactive protein in acute phase of inflamation. Activation of endothelial cells leads to dysfunction of endothelial cells

and stimulation of synthetic fibrinogen leading to CVA [3, 7, 8]. High levels of serum IL-6 and hs-CRP suggest poor prognosis of CVA, and several lines of evidence suggest that slow coronary flow phenomenon seems to be an early form of atherosclerosis and low-grade inflammation plays a role in the development of SCF.

Both IL-6 promoter-572C/G and -597G/A have polymorphism distribution characteristics. The IL-6 gene-597G/A locus had GG and GA genotypes, and the frequency of -597G/A genotype was GG [46 (8.55%)] and GA [492 (91.45%)]. There were only GG and GC genotypes in IL-6 gene promoter-174 in the study population, no CC genotype was found, and the genotype was mainly GG.

Interleukin-6 (IL-6) is a pleiotropic cytokine, which functions as a mediator of inflammatory response, and it has both pro-inflammatory and anti-inflammatory properties. IL-6-634C/G polymorphism played a role in SCF in Han Chinese [9].

IL-6 promoter region-174G/C and -597G/A loci are closely linked, while the 174C loci is rare, and 634C is only found in the eastern Asian population, with 174C being common in people with white skin; this study also suggests that CVA is related to this allele in the Asian population [6].

IL-6-174 promotor polymorphism may modulate the effects of alcohol on carotid atherosclerosis. These data support the hypothesis that inflammation forms part of the intermediate causal pathway between alcohol intake and atherosclerosis [10]. A polymorphism located in the promoter region (-174 G/C) of interleukin 6 (IL-6) has been linked to early onset of type 1 diabetes (T1D) and increased body mass index (BMI) [11, 12].

The limitations of this study were that it was a case-control study performed retrospectively, with cross-sectional analysis, no prospective observation, and only coronary vasospasm angina being considered without further analysis of whether atrial fibrillation could be related and no investigation of the final outcome. Second, the control group was only selected from “normal people” who had no clinical symptoms. Due to the limitation of conditions, no examination of their coronary artery was carried out, and it was impossible to confirm that the control group had no coronary atherosclerosis and other lesions. Therefore, further investigation on these topics are worth further clinical research.

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Disclosure of conflict of interest

None.

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