Influence of interleukin-1β gene polymorphism on the risk of myocardial infarction complicated with ischemic stroke

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Abstract. This study investigated the correlation between interleukin (IL)-1β-511C/T gene polymorphism and myocardial infarction (MI) complicated with ischemic stroke (IS). A total of 251 MI patients complicated with IS (observation group) and 200 healthy people (control group) were selected for the case-control study. IL-1β-511C/T gene polymorphism was detected via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotype distribution and allele frequency were compared between the two groups, and the correlation between gene polymorphism and MI complicated with IS, was analyzed after traditional risk factors were adjusted by using logistic regression method. The frequencies of CT and TT genotypes in the observation group were higher than those in the control group (P<0.05). The frequency of T allele in the observation group was significantly higher than that in the control group (P<0.05), but the frequency of C allele was obviously lower than that in the control group (P<0.05). According to results of logistic regression analysis, arrhythmia and high-density lipoprotein cholesterol (HDL-C) were associated with MI complicated with IS. In patients with arrhythmia, the risk of disease in carriers with IL-1β-511T gene was 1.7-1.8 times that in non-carriers [odds ratio (OR) = 1.742 and 1.839, P<0.05]. In patients with abnormal HDL-C, the risk of disease in carriers with IL-1β-511T gene was 2.0-2.2 times that in non-carriers (OR = 2.011 and 2.249, P<0.05). Besides, the risk of MI complicated with IS in carriers with CC genotype had no significant difference in patients with arrhythmia and abnormal HDL-C (P>0.05). IL-1β-511C/T gene polymorphism may be related to the risk of MI complicated with IS.

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Introduction

Myocardial infarction (MI) is a severe cardiovascular disease, which is often accompanied with increased activity of serum myocardial enzyme and progressive changes in electrocardiograms (ECGs), and may lead to arrhythmia, shock and heart failure (1). Ischemic stroke (IS) is a common complication of MI (2), which can be caused by a variety of factors. The incidence rate of IS is high in the elderly, and the disease severely affects the prognosis of MI patients (3). MI patients complicated with IS have a significantly higher mortality rate than patients with MI alone (4,5), which has attracted extensive attention in the clinic. Studies have shown that there are often abnormal ECG changes of patients with acute IS, and ECG changes of these patients are very sensitive with a very low specificity, suggesting that ECGs are insufficient to be used as a diagnostic criterion for IS (6). The major pathological basis of IS is atherosclerosis (7), and the relevant inflammatory response during atherosclerosis is mainly initiated jointly by interleukin (IL) and some other related factors (8,9).

IL is an important pro-inflammatory factor playing an important role in ischemic brain injury (10,11). It has been proved in studies that IL-1 gene polymorphism has a certain correlation with cerebral infarction (12,13). IL-1 family members include IL-1α, IL-1β and IL-1Ra, the first two of which can be involved in the senescence of vascular endothelial cells and inflammatory response of hypoxic-ischemic brain injury, thereby affecting the function of vascular endothelial cells and atherosclerosis process (14). Studies have revealed that there is C/T polymorphism in the IL-1β-511 locus (15), which may be related to the occurrence of IS (16). This study investigated the correlation between IL-1β-511C/T gene polymorphism and MI complicated with IS, so as to provide references for future research.

Table I. Primer sequences of IL-1β-511C/T amplification.

SNP	Primer sequence (5'-3')	Fragment length (bp)	Tm value
IL-1β-511C/T	F: TGGCATTGATCTGGTTCATC R: GTTTAGGAATCTTCCCACTT	304	58˚C

IL, interleukin; F, forward; R, reverse.

Table II. Enzyme digestion fragment length and genotyping.

SNP	Enzyme digestion site	Enzyme digestion fragment (bp)					
		CC			CT		TT
IL-1β-511C/T	*Ava* I	190	114	304	190	114	304

IL, interleukin.

Materials and methods

General data. A total of 251 MI patients treated in the People's Hospital of Zhangqiu District (Jinan, China) from September 2014 to October 2017 were selected as observation group, including 136 males and 115 females with an average age of 62.8±5.4 years. The diagnostic criteria met the universal definition of MI in 2012. Patients with cerebral hemorrhage and space-occupying lesions were excluded. Another 200 healthy people receiving physical examination in People's Hospital of Zhangqiu District during the same period were selected as control group, including 101 males and 99 females with an average age of 61.2±6.8 years, and they had no recent inflammation. The study was approved by the Ethics Committee of People's Hospital of Zhangqiu District. Patients who participated in this research, signed an informed consent and had complete clinical data.

Research methods

Main reagents. Wizard whole blood deoxyribonucleic acid (DNA) extraction kit, Taq DNA polymerase, polymerase chain reaction (PCR) product purification and recycling kit, and restriction endonuclease NcoI were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Primers used in this study were all synthesized by Nanjing GenScript Biotechnology Corp. (Nanjing, China).

Specimen collection. After 2 ml fasting venous blood was collected, and ethylene diamine tetraacetic acid (EDTA) was added for anticoagulation in accordance with instructions of the Wizard whole blood DNA extraction kit. A total of 300 μl whole blood was added with 900 μl cell lysis solution, shaken fully and mixed evenly, followed by incubation at room temperature for 5 min and centrifugation at 13,000 x g at room temperature for 15 sec. Then the supernatant was discarded. The nuclear lysis solution was added and mixed evenly with the sediment, and the protein precipitation solution was added and mixed evenly, followed by centrifugation at 13,000 x g for 3 min. The supernatant was transferred into a new centrifuge tube, and 30 μl isopropanol was added to precipitate DNA. After centrifugation, the sediment was washed twice with 70% ethanol, and added with DNA dissolving solution to obtain the whole blood DNA.

IL-1β-511C/T amplification. The PCR primers of IL-1β-511C/T gene were designed according to the study of Li *et al* (17), and synthesized by Nanjing GenScript Biotechnology Corp. Primer sequences are shown in Table I. A total of 20 μl PCR system included 2 μl buffer, 2 μl dNTPs, 0.5 μl forward primers and 0.5 μl reverse primers, 1 μl Taq enzyme, 1 μl DNA, and 20 μl ddH_2O. PCR conditions are as follows: 95˚C for 5 min, 95˚C for 50 sec, 58˚C for 50 sec, and 72˚C for 1 min for a total of 30 cycles, and 72˚C for 10 min. According to instructions of the PCR product purification and recycling kit, the PCR products were purified and recycled for subsequent enzyme digestion assay.

Enzyme digestion reaction and genotype analysis. Enzyme digestion reaction was performed for the above-mentioned recycled PCR products. A total of 10 μl enzyme digestion reaction systems included 1 μl buffer, 1 μl restriction endonuclease *Ava* I, and 8 μl recycled PCR products. The reaction was performed at 37˚C for 6 h. The products after enzyme digestion were separated via 1.5% agarose gel electrophoresis. The product fragment size was detected by using the GeneSnap software of Syngene gel imaging system, based on which the different genotypes were determined. Each genotype was analyzed by using the fragment content (Table II).

Statistical analysis. Statistical Product and Service Solutions (SPSS) 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical processing. Measurement data are presented as mean ± standard deviation. t-test was used for the analysis between two groups, and Chi-square test was used for enumeration data. $P<0.05$ was considered to indicate a statistically significant difference. The correlation between IL-1β-511C/T and MI complicated with IS was detected via logistic regression analysis.

Results

Analysis of clinical data in both groups. All the participants of the study in both groups had complete clinical data (Tables III and IV). There were no significant differences in age, sex, history of hypertension and smoking history between the two groups ($P>0.05$), but the history of diabetes mellitus and arrhythmia had significant differences ($P<0.05$).

Table III. Comparison of clinical data between the two groups.

Groups	n	Age (years)	Male n (%)	Hypertension n (%)	Diabetes mellitus n (%)	Smoking n (%)	Arrhythmia n (%)
Control	200	61.20±6.80	101 (50.50)	113 (56.50)	30 (15.00)	62 (31.00)	5 (2.50)
Observation	251	62.80±5.40	136 (54.20)	140 (55.80)	71 (28.30)	76 (30.30)	176 (70.10)
χ^2		0.102	0.294	0.019	10.786	0.025	211.827
P-value		0.766	0.588	0.891	0.001	0.876	<0.001

Table IV. Comparison of blood routine and blood lipid data between the two groups (mean ± SD).

		Blood routine (10^9/l)					Blood lipid (mmol/l)			
Groups	n	RBC count	Hb (g/l)	WBC count	NEU count	PLT count	HDL-C	LDL-C	TC	TG
Control	200	4.05±0.53	115.54±18.85	10.35±4.53	8.03±4.85	235.41±68.33	1.45±0.25	2.78±0.57	4.78±0.83	1.38±0.45
Observation	251	4.13±0.45	110.36±15.48	9.87±3.23	7.59±2.89	231.28±70.19	1.32±0.21	2.66±0.62	4.59±0.92	1.58±1.03
t value		0.623	2.622	0.64	0.389	3.846	5.629	4.157	3.657	-0.597
P-value		0.597	0.117	0.588	0.735	0.061	0.03	0.053	0.067	0.611

RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; NEU, neutrophil; PLT, platelet; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Table V. Comparison of IL-1β-511C/T genotype and allele frequency between the two groups.

	Genotype frequency, n (%)			Allele frequency, n (%)	
Groups	CC	CT	TT	C	T
Control (n=200)	143 (71.50)	37 (18.50)	20 (10.00)	323 (80.75)	77 (19.25)
Observation (n=251)	134 (53.39)	80 (31.87)	37 (14.70)	348 (69.30)	154 (30.68)
χ^2		15.598		15.259	
P-value		<0.001		<0.001	

IL, interleukin.

Table VI. Logistic regression analysis of risk factors of MI complicated with IS.

Factor	OR	(95% CI)	P-value
Diabetes mellitus	1.038	0.997-1.031	0.192
Arrhythmia	0.147	0.027-0.337	0.026
HDL-C	1.231	1.067-2.358	0.031

MI, myocardial infarction; OR, odds ratio; HDL-C, high-density lipoprotein cholesterol; CI, confidence interval.

No significant differences were found in the blood routine examination at admission between the two groups (P>0.05). In blood lipid indexes, there was a significant difference in the high-density lipoprotein cholesterol (HDL-C) level between the two groups (P<0.05), and the level was obviously lower in the observation group than that in the control group. Other indexes had no significant differences (P>0.05).

Analysis of IL-1β-511C/T gene polymorphism in two groups. IL-1β-511C/T genotype distribution and allele frequency were significantly different between the observation and the control groups (P<0.05) (Table V). CT and TT genotype frequencies in the observation group were all higher than those in the control group. The frequency of T allele in the observation was remarkably higher than that in the control group, but the frequency of C allele was obviously lower than that in the control group.

Results of multivariate logistic regression analysis. The correlations of diabetes mellitus, arrhythmia and HDL-C with MI complicated with IS were analyzed via logistic regression analysis. It was found that arrhythmia and HDL-C were related to MI complicated with IS (P<0.05) (Table VI). The effect of IL-1β-511C/T gene polymorphism on MI complicated with IS

Table VII. Logistic regression analysis of IL-1β-511 locus.

	Arrhythmia				HDL-C			
IL-1β-511 genotype	n	OR	95% CI	P-value	n	OR	95% CI	P-value
CC	59	1.213	0.519-2.304	0.651	51	0.874	0.484-1.852	0.752
CT	80	1.742	1.036-2.029	0.029	89	2.011	1.062-2.353	0.009
TT	37	1.839	1.098-2.249	0.017	43	2.249	1.634-2.548	<0.001

IL, interleukin; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio; CI, confidence interval.

was analyzed. Results of logistic regression analysis (Table VII) showed that in patients with arrhythmia, the risk of disease in carriers of IL-1β-511T gene (n=117) was 1.7-1.8 times that in non-carriers (n=59) [odds ratio (OR) = 1.742 and 1.839, P<0.05]. In patients with abnormal HDL-C, the risk of disease in carriers of IL-1β-511T gene (n=132) was 2.0-2.2 times that in non-carriers (n=51) (OR =2.011 and 2.249, P<0.05). Besides, the risk of MI complicated with IS in carriers of CC genotype had no significant difference in patients with arrhythmia and abnormal HDL-C (P>0.05).

Discussion

As a common complication of MI, IS has different pathogenesis and complex and diversified symptoms, bringing difficulties to clinical prediction (18). The main reason for MI complicated with IS is cardiogenic cerebral embolism, which occurs more easily in patients accompanied with atrial arrhythmia or intracardiac mural thrombus. Besides, hypotension, reflex cerebral arterial spasm, and simultaneous thrombosis in cerebral artery and coronary artery are also important causes of MI complicated with IS (19).

IL can act on multiple systems in the body, which possesses extensive biological effects and can mediate inflammatory reactions and participate in immune regulation, lipid metabolism and other physiological processes (20). In the IL-1 family, IL-1α and IL-1β, through inhibiting the endothelial cell proliferation, can induce the expression of adhesion molecules, lead to aggregation of monocytes and lymphocytes, promote thrombosis, and accelerate the formation of atherosclerosis (21). IL plays an important role in IS. Studies have manifested that there are polymorphisms in the IL-1α-889-C/T and IL-1β-511C/T loci, which have been found to be able to affect the activity of IL-1, and change the occurrence and development of hypertension and coronary heart disease, by affecting the inflammatory response (22). In this study, results revealed that both IL-1β-511C/T genotype distribution and allele frequency were significantly different between the observation and the control groups (P<0.05). The frequencies of CT genotype and TT genotype in the observation were higher than those in the control group, and the frequency of T allele in the observation was significantly higher than that in the control group (P<0.05), which were consistent with results obtained previously (23). According to results of logistic regression analysis, arrhythmia and HDL-C were associated with MI complicated with IS. In patients with arrhythmia, the risk of disease in carriers with IL-1β-511T gene was 1.7-1.8 times that in non-carriers (OR = 1.742 and 1.839, P<0.05). In patients with abnormal HDL-C, the risk of disease in carriers with IL-1β-511T gene was 2.0-2.2 times that in non-carriers (OR =2.011 and 2.249, P<0.05). Besides, the risk of MI complicated with IS in carriers with CC genotype had no significant difference in patients with arrhythmia and abnormal HDL-C (P>0.05). The above results indicate that the MI complicated with IS is related to the increase of IL-1β-511 T allele frequency. IL-1β-511 T may indirectly affect the occurrence of IS through affecting arrhythmia and HDL-C content. However, some studies also argued that there is no correlation between IL-1β-511C/T polymorphism and IS (24), which may be related to the sample size, regional differences and dynamic development of disease. In addition, the precipitating factors of MI may be different from those of stroke caused by other factors, thus leading to differences in results.

In conclusion, results of this study suggest that the IL-1β-511C/T gene polymorphism may be involved in the occurrence and development of MI complicated with IS. However, clinical cases in only one region were observed in this experimental study, which had certain limitations, so studies involving more regions are needed to confirm the conclusion. Moreover, this study broadens thoughts for the research on MI complicated with IS at the level of gene polymorphism, and provides more possibilities for the prediction and treatment of MI complicated with IS.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LC and FL wrote this manuscript and collected specimen. LL and LY were responsible for PCR. ZW, JZ and QM contributed

to enzyme digestion reaction and genotype analysis. All authors read and approved the final study.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of People's Hospital of Zhangqiu District (Jinan, China). Patients who participated in this research had complete clinical data. 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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