

Serum levels and clinical significance of IFN- γ and IL-10 in patients with coronary heart disease

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Abstract. – **OBJECTIVE:** The present study aims to investigate the relationship of IFN- γ and IL-10 with the pathogenesis of coronary heart disease (CHD).

PATIENTS AND METHODS: A total of 128 patients with angiographically confirmed CHD were included in CHD group, while 106 patients with no angiographically confirmed coronary artery stenosis were included in the control group. The age and body mass index of patients were documented. Concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured. Moreover, serum levels of IFN- γ and IL-10 were determined by ELISA and mRNA levels of these two factors were measured by quantitative RT-PCR.

RESULTS: Compared to those of controls, concentrations of TG and LDL-C were significantly higher ($p < 0.01$) whereas HDL-C concentrations were significantly lower in CHD patients ($p < 0.001$). Correlation analysis revealed that serum IFN- γ level was significantly positively correlated with TG concentration in CHD patients ($r = 0.560$, $p < 0.05$), while the IL-10 level was negatively correlated with TG concentration ($r = -0.411$, $p < 0.05$). Both protein level and mRNA level of IFN- γ were higher in the serum of CHD patients than in controls. However, the protein level and mRNA level of IL-10 were significantly lower in CHD patients than in controls ($p < 0.001$).

CONCLUSIONS: IFN- γ and IL-10 are involved in the development of atherosclerosis of coronary artery and IL-10 may inhibit atherogenesis.

Key Words:

Coronary heart disease, Atherosclerosis, Interferon- γ , Interleukin-10.

Introduction

Coronary atherosclerotic heart disease, also known as coronary heart disease (CHD), is an atherosclerotic disorder featured by the forma-

tion of white plaques in the arterial intima initiated by lipid deposition due to aberrant lipid metabolism. Accumulation of atherosclerotic plaques causes the narrowing of the vascular lumen and blocks blood flow, which leads to cardiac ischemia and thereby result in angina. The initiation and development of CHD are affected by multiple factors, among which, inflammatory response plays an important role in the initiation and development of atherosclerosis^{1,2}. Currently, a variety of cytokines has been found to be involved in the chronic inflammation of the vessel wall^{3,4}. In the present study, serum levels of interferon-gamma (IFN- γ) and interleukin (IL)-10 in peripheral blood samples of patients with various types of CHD were measured, in an effort to explore the relationship between inflammation and CHD.

Patients and Methods

Patients

A total of 128 patients (73 males, 55 females, mean age of 65.35 ± 9.62 years) with angiographically confirmed CHD admitted to our hospital were included in this study as CHD group. The diagnosis of CHD was established when $\geq 50\%$ luminal narrowing was detected in at least one main coronary artery or its main branches. However, patients with acute myocardial infarction (MI) were not included. Besides, 106 patients (47 males, 59 females, mean age of 64.73 ± 7.95 years) with no stenosis of coronary arteries confirmed by coronary angiography during the same period were included as the control group. No prior history of diabetes mellitus, cerebrovascular disease, hepatic disease or hypertension was noticed in these patients. Moreover, patients from both groups had not experienced any severe medical conditions, including

serious acute and chronic infections, tumor, liver disease, renal dysfunction, autoimmune disease, thyroid dysfunction, cerebrovascular disease, surgery and trauma. No statistically significant differences were observed in ages and genders between two groups. Written informed consent was obtained from all patients.

Methods

A 6 ml fasting blood sample was collected from all patients the next morning of admission. The blood sample was divided into three samples placed in three tubes, 2 ml for each. Sample presenting severe hemolysis and lipemia were excluded. The first tube of blood sample was coagulated at room temperature, centrifuged at 2000 r/min for 8 min and placed at -80°C for the analysis of IFN- γ and IL-10. The second tube of the sample was treated with an anticoagulant agent for the analysis of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C). RNA was extracted from the third tube of a blood sample for evaluating the mRNA levels of IFN- γ and IL-10 by using RT-PCR.

Lipid Analysis

Serum concentrations of lipids, including TC, TG, HDL-C and LDL-C, were measured using Cobas C501 automated biochemical analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA).

Cytokine Detection

Serum levels of IFN- γ and IL-10 in CHD patients and healthy controls were measured by ELISA using the detection kit (eBioscience, San Diego, CA, USA) following manufacturer's instructions. Absorbance at 450 nm was measured using microplate reader and concentrations of cytokines were calculated using a linear regression formula.

Assessment of IFN- γ and IL-10 mRNA Levels in Peripheral Blood Sample

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation separation (Takara, Otsu, Shiga, Japan). The isolated PBMCs were homogenized with 1 ml Trizol (Takara, Otsu, Shiga, Japan) and then total RNAs were extracted. Subsequently, the isolated RNAs were dissolved in 25 μl of diethyl pyrocarbonate (DEPC) water and its concentration and purity were determined using UV spectrophotometer. Afterwards, cDNA was generated using reverse transcription kit (Fermentas, Hanover, MD, USA) and stored at -20°C . RT-PCR was performed with PCR system (Takara, Japan). The PCR conditions were denaturation at 95°C for 20s followed by 40 cycles of 60°C for 20s and 70°C for 1s. The primer sequences for PCR amplification were designed and synthesized by Shanghai Invitrogen Biotechnology Co., Shanghai, China (Table I).

Statistical Analysis

Data analysis was performed by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as $\pm$ SD. Differences between two groups were analyzed using Student's *t*-test. Qualitative data were analyzed using χ^2 test. The relationship between two variables was analyzed using Pearson correlation analysis. $p < 0.05$ was considered statistically significant.

Results

Basic Characteristics and Lipid Levels

Statistical analysis showed that no significant differences were detected in gender, age, body mass index (BMI) and TC levels between two groups ($p > 0.05$). Compared to those of control group, levels of TG and LDL-C were higher

Table I. Primer sequences for real-time Q-PCR.

Genes		Primer sequences (5'→3')
β -actin	Forward	CCTCTATGCCAACACAGTGC
	Reverse	GTACTCCTGCTTGCTGATCC
IFN- γ	Forward	AAAGAGATAATCTGGCTCTGC
	Reverse	GCTCTGAGACAATGAACGCT
IL-10	Forward	GGACTTTAAGGGTTACCTGGGTTGCC
	Reverse	GCCTTGATGTCTGGGTCTGGTTCTC

Table II. Basic characteristics and biochemical data ($\bar{x} \pm SD$).

Parameters		CHD group	Control group	<i>t</i> or χ^2	<i>p</i>
Age (years)		65.35 $\pm$ 9.62	64.73 $\pm$ 7.95	0.532	0.596
Gender (%)	Male	73 (57.1)	47 (44.3)	1.183	0.236
	Female	55 (42.9)	59 (55.7)	1.181	0.119
BMI (kg/m ²)		23.62 $\pm$ 1.89	23.14 $\pm$ 2.12	1.831	0.068
TC (mmol/L)		4.83 $\pm$ 1.26	4.76 $\pm$ 1.12	0.442	0.656
TG (mmol/L)		1.47 $\pm$ 0.76	1.22 $\pm$ 0.81	2.431	0.015
HDL-C (mmol/L)		1.38 $\pm$ 0.36	1.52 $\pm$ 0.28	3.272	0.001
LDL-C (mmol/L)		2.94 $\pm$ 0.88	2.58 $\pm$ 0.75	3.331	0.001

whereas HDL-C levels were lower in CHD group ($p < 0.001$) (Table II).

Serum Levels of IFN- γ and IL-10

ELISA analysis showed that serum levels of IFN- γ were significantly higher whereas IL-10 levels were significantly lower in CHD group than those in the control group ($p < 0.001$) (Table III). In addition, correlation analysis revealed that serum IFN- γ level was positively correlated with TG concentration with statistical significance ($r = 0.560$, $p < 0.05$), while the IL-10 level was negatively correlated with TG concentration ($r = -0.411$, $p < 0.05$).

IFN- γ and IL-10 mRNA Levels in Peripheral Blood

Q-PCR results demonstrated that IL-10 level in peripheral blood of CHD patients was significantly lower than that of healthy controls (0.77 ± 0.26 vs. 1.08 ± 0.22 , $p < 0.001$), whereas IFN- γ mRNA level of CHD patients was significantly higher than that of healthy controls (1.26 ± 0.32 vs. 0.92 ± 0.25 , $p < 0.001$) (Table IV).

Discussion

Coronary atherosclerosis is the major pathological basis of CHD. Pathogenesis of atherosclerosis mainly involves lipid metabolism disorder accompanied by chronic inflammation of arterial wall^{5,6}. Chronic inflammation in the vessel wall has been shown to play a critical role in promoting the formation, development and rupture of atherosclerotic plaque^{4,7,8}. Both pro-inflammatory cytokines and anti-inflammatory cytokines are involved in chronic inflammation in the vessel wall⁴, among which pro-inflammatory IFN- γ and anti-inflammatory IL-10 are more representative. Moreover, the roles of IFN- γ and IL-10 in atherogenesis are closely associated with macrophages.

Macrophages have been shown to play a central role in all stages of atherogenesis⁹. Formation of atheroma is initiated by the recruitment of monocytes to the intima, followed by differentiation into macrophages induced by inflammation. Macrophages can uptake modified LDL in the intima, thereby, promoting cholesterol

Table III. Serum levels of IFN- γ and IL-10 (pg/ml, $\bar{x} \pm SD$).

Cytokines	CHD group	Control group	<i>t</i>	<i>p</i>
IFN- γ	235.73 $\pm$ 46.52	186.84 $\pm$ 52.77	7.53	< 0.001
IL-10	134.43 $\pm$ 38.24	164.38 $\pm$ 36.45	6.08	< 0.001

Table IV. mRNA levels of IFN- γ and IL-10 in peripheral blood

Cytokines	CHD group	Control group	<i>t</i>	<i>p</i>
IFN- γ	1.26 $\pm$ 0.32	0.92 $\pm$ 0.25	8.914	< 0.001
IL-10	0.77 $\pm$ 0.26	1.08 $\pm$ 0.22	9.733	< 0.001

loading and the formation of foam cells at the core of atherosclerotic plaques. Lipid-laden macrophages produce a variety of inflammatory mediators, reactive oxygen species (ROS) and pro-coagulant, promoting the development of inflammation and thrombosis.

IFN- γ is mainly synthesized and secreted by Th1 cells and macrophages. It is an important factor for macrophage activation involved in both innate and adaptive immunity. IFN- γ can stimulate the generation of chemokines and cytotoxic molecules from macrophages and induce the expression of genes regulating lipid absorption^{10,11}. Also, IFN- γ exerts different functions in various stages of atherogenesis. Particularly, at the early stage, IFN- γ promotes the development of atherosclerosis through activating the release of adhesion molecules from the endothelial cells and regulating the proliferation of the smooth muscle cells (SMC)¹². At the late stage, IFN- γ induces the rupture of atherosclerotic plaques by accelerating the apoptosis and extracellular degeneration of macrophages¹³. The results of the present study showed that both the protein level and mRNA level of IFN- γ in the serum of CHD patients were higher than those of controls, indicating that the elevation of IFN- γ has a certain correlation with the development of CHD. Furthermore, the correlation analysis revealed that IFN- γ level in CHD patients was positively correlated with TG concentration, thereby further supporting the above hypothesis.

IL-10 is an important anti-inflammatory cytokine, primarily produced by Th2 subtype T lymphocytes and macrophages. Studies^{14,15} have shown that IL-10 exerts an anti-atherosclerotic effect by inhibiting the activation of macrophages, suppressing the expression of matrix metalloproteinase (MMP), pro-inflammatory cytokines and cyclooxygenase-2 in lipid-laden foam cells, and altering the lipid metabolism of macrophages. Another study³ found that over-expression of IL-10 is achieved by transplantation of bone marrow cells (BMCs) that are transduced by a macrophage-specific retroviral vector. IL-10 expressed by macrophages derived from transduced BMCs inhibits the development of atherosclerosis in LDLR^{-/-} mice by reducing cholesterol ester accumulation. *In vitro* experiments show that macrophage-derived IL-10 stimulates both the uptake (by up-regulating scavenger receptors) and efflux of cholesterol (by activating the PPAR γ -

LXR-ABCA1/ABCG1 pathway), thereby reducing inflammation and cell apoptosis in atherosclerosis³. Results of this study showed that serum IL-10 levels in CHD patients were significantly lower than those of controls, indicating that reduced expression of endogenous IL-10 was not sufficient to cease the initiation and development of CHD. Hence, appropriate intervention to elevate IL-10 levels in vivo appears to be particularly important. In addition, correlation analysis revealed that serum IL-10 level in CHD patients was negatively correlated with TG concentration, which is a further evidence to confirm the above speculation.

Conclusions

The present work investigated the relationship of IFN- γ and IL-10 with CHD, further revealing the relationship between inflammation and CHD. However, further study is required. Besides, studying the relationship between inflammation and CHD extends our understanding of CHD and provides insights into optimal therapy of CHD.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

- 1) ESMON CT. Molecular circuits in thrombosis and inflammation. *Thromb Haemost* 2013; 109: 416-420.
- 2) ANOGEIANAKI A, ANGELUCCI D, CIANCHETTI E, D'ALESSANDRO M, MACCAURO G, SAGGINI A, SALINI V, CARAFFA A, TETE S, CONTI F, TRIPODI D, SHAIK-DASTHAGIRISAHEB YB. Atherosclerosis: a classic inflammatory disease. *Int J Immunopathol Pharmacol* 2011; 24: 817-825.
- 3) HAN X, KITAMOTO S, WANG H, BOISVERT WA. Interleukin-10 overexpression in macrophages suppresses atherosclerosis in hyperlipidemic mice. *FASEB J* 2010; 24: 2869-2880.
- 4) LITTLE PJ, CHAIT A, BOBIK A. Cellular and cytokine-based inflammatory processes as novel therapeutic targets for the prevention and treatment of atherosclerosis. *Pharmacol Ther* 2011; 131: 255-268.
- 5) TUTTOLOMONDO A, DI RAIMONDO D, PECORARO R, ARNAO V, PINTO A, LICATA G. Atherosclerosis as an inflammatory disease. *Curr Pharm Des* 2012; 18: 4266-4288.
- 6) WEBER C, HRISTOV M. Atherogenesis and inflammation. From cellular mediators to regulatory mechanisms of inflammation in atherosclerosis. *Hamostaseologie* 2015; 35: 99-101.

- 7) CHARO IF, TAUB R. Anti-inflammatory therapeutics for the treatment of atherosclerosis. *Nat Rev Drug Discov* 2011; 10: 365-376.
- 8) LIBBY P, RIDKER PM, HANSSON GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011; 473: 317-325.
- 9) MOORE KJ, TABAS I. Macrophages in the pathogenesis of atherosclerosis. *Cell* 2011; 145: 341-355.
- 10) BILLIAU A, MATTHYS P. Interferon-gamma: a historical perspective. *Cytokine Growth Factor Rev* 2009; 20: 97-113.
- 11) NIEDZIELSKA I, CIERPKA S. Interferon gamma in the etiology of atherosclerosis and periodontitis. *Thromb Res* 2010; 126: 324-327.
- 12) FOTEINOS G, XU Q. Immune-mediated mechanisms of endothelial damage in atherosclerosis. *Autoimmunity* 2009; 42: 627-633.
- 13) VOLOSHYNA I, LITTLEFIELD MJ, REISS AB. Atherosclerosis and interferon-gamma: new insights and therapeutic targets. *Trends Cardiovasc Med* 2014; 24: 45-51.
- 14) HAN X, BOISVERT WA. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. *Thromb Haemost* 2015; 113: 505-512.
- 15) HAN X, KITAMOTO S, LIAN Q, BOISVERT WA. Interleukin-10 facilitates both cholesterol uptake and efflux in macrophages. *J Biol Chem* 2009; 284: 32950-32958.