

Transcriptional activity of tumor necrosis factor-alpha gene in peripheral blood mononuclear cells in patients with coronary slow flow

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Short Communication

Abstract

BACKGROUND: Coronary slow flow (CSF), an angiographic phenomenon that is characterized by a delayed coronary blood flow in the absence of obstructive coronary artery stenosis, is known as a disorder of the coronary microcirculation. Inflammation has an important role in the vascular hemostasis and endothelial dysfunction especially regarding monocyte adhesion and infiltration. Pro-inflammatory cytokines released by inflammatory cells result in endothelial cell dysfunction and cardiovascular diseases. It has been demonstrated that tumor necrosis factor-alpha (TNF- α) mainly influences the vascular homeostasis and endothelial dysfunction. In the present enquiry the transcriptional activity of TNF- α gene in peripheral blood mononuclear cells (PBMCs) of patients with CSF was compared with healthy controls in order to further survey the role of TNF- α in pathophysiology of CSF.

METHODS: The study was carried out on 30 patients with CSF and 30 matched healthy controls. To analysis gene expression of TNF- α , total mRNA was isolated from PBMCs. The quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was used to compare the transcriptional activity of TNF- α gene between patients with CSF and controls.

RESULTS: The mean \pm standard error of mean of fold in CSF patients and controls were 0.20 ± 0.04 and 1.38 ± 0.27 , respectively. The mRNA mean expressions of TNF- α (fold) were different in tested groups, which indicated a significant decrease in TNF- α in patients with CSF group ($P = 0.0001$).

CONCLUSION: Expression of TNF- α was decreased in patients with CSF. Changes in TNF- α expression suggest a potential role for altered immune function in the pathophysiology of CSF.

Keywords: Inflammation, Tumor Necrosis Factor-alpha, Cytokines, Slow Flow Phenomenon, Coronary Angiography

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Introduction

Coronary slow flow (CSF) is defined as an angiographic phenomenon that is identified by the delayed opacification of the distal vessel in the absence of coronary artery stenosis. Etiology of this phenomenon is controversial.¹ The overall prevalence of CSF is 1%-7% among patients undergoing diagnostic angiography as the consequence of clinical distrust of cardiovascular

disorders.² CSF is frequent among current smokers and has several clinical findings such as unstable angina, metabolic syndrome, high resting microvascular endothelial tone, and high-minded aortic stiffness.³ CSF has been correlated to obesity as well as male gender.⁴ Predictors of CSF are gender, body mass index (BMI), hypertension, a low level of high-density lipoprotein cholesterol, and high hemoglobin.⁵ Pathogenic mechanism of CSF is

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complex and related to coronary microcirculation,⁶ endothelial dysfunction,^{7,8} atherosclerosis,^{8,9} inflammatory parameters¹⁰, and anatomic properties of coronary arteries.¹¹ The results of a recent study showed that endothelial function was impaired in CSF.¹² Also, plasminogen activator inhibitor-1 (PAI-1), angiotensin-converting enzyme (ACE) and endothelial nitric oxide synthase (eNOS) genes polymorphisms have not been associated with the risk of CSF. Several RNA based biomarkers have been studied in the case of human disease such as coronary heart disease,^{13,14} and CSF.¹⁵ The objectives of different studies were to investigate the pathophysiology of CSF.¹²⁻¹⁷ The coronary microcirculation is under control of anatomical factors of pre-arterioles, arterioles, capillaries, and venules as well as several systemic factors.¹⁸ Inflammatory cells and inflammation has an important role in the vascular homeostasis and endothelial dysfunction especially regarding monocyte adhesion and infiltration.¹⁸ Outcome of troubled balance because of inflammation in the endothelial cells changes from an anti-inflammatory state to a pro-inflammatory condition. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) and interleukin-1 (IL-1) are important mediators released by inflammatory cells and result in endothelial cell dysfunction and cardiovascular diseases.¹⁹ Several inflammatory factors have been increasingly recognized regarding vascular dysfunction and vascular disease including cytokines and cell adhesion molecules and C-reactive protein and other markers.²⁰⁻²³ TNF- α as a pro-inflammatory cytokine has several roles such as induction of expression of cell adhesion molecules including receptor for advanced glycation end products,²⁴ intercellular adhesion molecule-1 (ICAM-1) and E-selectin,²⁵ oxidized low-density lipoprotein (ox-LDL) receptor-1 by nuclear factor kappa B (NF-kappa B) activation²⁶ and eNOS activation.²⁷ It has been demonstrated that TNF- α mainly influences the vascular homeostasis and endothelial dysfunction with definite pathologies.²⁸⁻³¹ The genetics of human cardiovascular disease is complex and include several genetic risk factors.³² Gene expression profiling in human cardiovascular disease shows an important role for IL-1 β in coronary artery disease.³³ TNF- α is known as an ultimate mediator of the acute phase response and is involved in production of other inflammatory mediators including chemokines with important role in recruitment of leucocytes to the site of

inflammation.^{34,35} Elevated plasma and myocardial levels of TNF- α have been recognized in patients with heart failure.^{36,37} The human TNF- α gene maps on chromosome 6, at 6p21.33 between the class I HLA-B and the class II HLA-DR genes.³⁸ TNF- α gene has 1 transcript and 4 exons.³⁸ In the present study, the transcriptional activity of TNF- α gene in peripheral blood lymphocytes (PBMCs) of patients with CSF was compared with healthy controls to assess the role of TNF- α in pathophysiology of CSF.

Materials and Methods

This case-control investigation was approved by Medical Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (IR.umsu.rec.1393.26). The case group contained of 30 patients with CSF. The study subjects included individuals who had a history of chest pain and angina and thrombolysis in myocardial infarction (TIMI) frame count (quantitative way of assessing coronary artery flow) greater than 23 frames. One proficient cardiologist checked all individuals in case group via angiography and confirmed the diagnosis of CSF. The control group contained of 30 healthy subjects who were matched with the case group in terms of gender, age, and BMI. Exclusion criteria were positive medical history regarding coronary ectasia, coronary intervention associated with slow flow, myocardial inflammation, surgery and infectious diseases within past six months, familial hypercholesterolemia, and congenital heart defects.³⁵ Study subjects completed the written informed consent. Ten ml whole blood was obtained from study subjects and collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes. RNA was isolated from PBMCs using RNXTM Plus solution per manufacturer's instructions (CinaGen Co., Tehran, Iran). RNA samples were stored at -80 °C upon the next stage. The quality of extracted RNA was tested by BioPhotometer (Eppendorf AG, Hamburg, Germany). The RNA with poor quality (OD 260/280 ratio < 1.6, normal ratio > 1.8) was discarded. First strand cDNA synthesis was carried out using RevertAid First Standard synthesis kit (Fermentas, Lithuania) according to the manufacturer's instruction under settings of 65 °C for 5 min, 25 °C for 5 min, 42 °C for 60 min and 70 °C for 5 min. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was done via MaximaTM SYBR Green qPCR Master Mix (Thermo Scientific), primer pairs (Table 1) (GenFanavaran Co., Tehran, Iran)³⁵ on a real-time

Table 1. Oligonucleotide primers used for quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Gene	Sequences	Amplicon size	Reference
HPRT	5'-cctggcgtcgtgattagtgat-3' (forward)	131 bp	35
HPRT	5'-agacgttcagtcctgtccataa-3' (reverse)		
TNF- α	5'-cccaggcagtcagatcatcttc-3' (forward)	85 bp	
TNF- α	5'-agctgccctcagcttga-3' (reverse)		

HPRT: Hypoxanthine-guanine phosphoribosyl transferase; TNF- α : Tumor necrosis factor-alpha

PCR machine (Bio-Rad iQTM5 Multicolor Real-Time PCR Detection System). The PCR settings were as follows: initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 seconds and 58°C for 60 seconds. Real time PCR reactions were performed in duplicate. The cycle threshold (Ct) values were normalized against to endogenous reference gene of hypoxanthine-guanine phosphoribosyltransferase (HPRT).³⁵ Livak method was used for 2^{- $\Delta\Delta$ Ct} analysis.³⁹

All parameters were expressed as means \pm standard deviation (mean \pm SD) or mean \pm standard error of mean (SEM). Relative amounts of mRNA expressions were compared between two groups using the independent sample t-test. Statistical analyses were done by SPSS for Windows (version 16, SPSS Inc., Chicago, IL, USA). In order to check the normality of the distribution, Kolmogorov-Smirnov test was performed. Differences were considered to be significant at P-value < 0.0500.

Results

The demographic data of the tested groups is summarized in table 2. In tested groups, the mean differences in systolic and diastolic blood pressures were statistically significant, but for other parameters, the difference was not significant. About 60.7% of cases were regular smokers and 26.7% of cases had family history of coronary heart disease.

The mean \pm SEM of fold in patients with CSF and controls were 0.20 \pm 0.04 and 1.38 \pm 0.27, respectively. The mRNA mean expressions of TNF- α (fold) were different in tested groups, which

indicated a significant decrease in TNF- α in patients group (P = 0.0001). Figure 1 shows gene expression of TNF- α in CSF patients and controls.

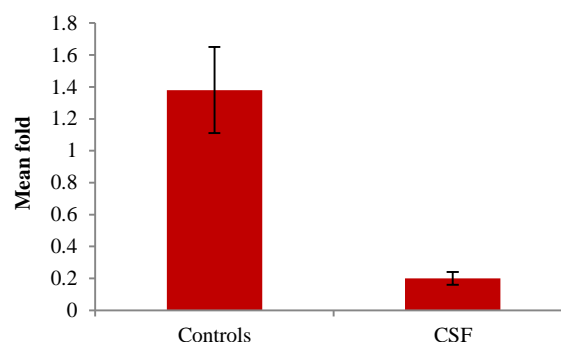


Figure 1. Tumor necrosis factor-alpha (TNF- α) mean fold changes in patients with coronary slow flow and controls CSF: Coronary slow flow

Discussion

In the present investigation, analysis showed a significant decrease in the expression of the TNF- α gene in PBMCs of patients with CSF compared to controls. To the best of our knowledge, this is the first study which assessed the quantitative expression of TNF- α gene in PBMCs of patients with CSF. Our findings showed that TNF- α might be donated in CSF in a distinct way. TNF- α as a pleiotropic cytokine is associated with the acute response of inflammation and critically occupied in the pathogenesis of atherosclerosis and heart complications.⁴⁰ The role of TNF- α is dose dependent and also partially associated with the signal transduction regarding certain receptor.

Table 2. Demographic characteristics of participants

Parameter	Controls (n = 30)	CSF (n = 30)	P
Age (year)	51.37 \pm 11.89	53 \pm 11.83	0.5960
Sex (female:male)	8:22	8:22	1.0000
Body mass index (Kg/m ²)	27.44 \pm 3.60	26.93 \pm 4.46	0.6260
Heart rate (n)	78.12 \pm 10.03	74.16 \pm 7.69	0.1040
Systolic blood pressure (mmHg)	137.48 \pm 13.79	128.03 \pm 15.85	0.0050
Diastolic blood pressure (mmHg)	88.72 \pm 10.31	81.86 \pm 22.08	0.0070

CSF: Coronary slow flow

TNF- α and its related signal transducers regarding two receptors participate in cardiovascular disorders.⁴⁰ TNF- α receptor type 1 (TNFR1) with a molecular mass of 60 kDa is expressed in all cell types; but, TNF- α receptor type 2 (TNFR2) with a molecular mass of 80 kDa is expressed on cells of the immune system and on the endothelial cells.^{4,40} In vascular dysfunction, TNF- α changes smooth muscle cell function and cell interactions and results in heart dysfunction.^{4,41} TNF- α inhibits eNOS in a dose dependent manner and leads to vascular inflammation.^{4,41} Patients with CSF were more likely to present with acute onset angina and abnormal ECG prompting emergency admission and rapid angiographic assessment.^{42,43} Several investigations studied the pathophysiological mechanisms of CSF with conflicting findings such as inflammation and endothelial dysfunction,⁴⁴ and oxidative stress.⁴⁵ Endothelial dysfunction and inflammation have also been understood as mechanisms related to the CSF.⁵ Several markers have also been shown to be associated with the CSF pathogenesis.⁴⁵ CSF is distinguished from cardiac syndrome X and microvascular angina and is understood as a separate clinical finding.⁴³⁻⁴⁶ Inflammation and oxidative stress mechanisms play an important role in acute CSF but not in the case of chronic CSF.^{6,18,46} As TNF- α levels are elevated in chronic inflammatory conditions, this suggests a potential role for altered immune function in the pathophysiology of CSF.

Future studies with big number of samples are essential to confirm the role of TNF- α in pathogenesis of CSF regarding TNF- α receptors and also more details including cytokines, as well as policies focusing on mechanisms involved in CSF.

Conclusion

Expression of TNF- α was decreased in patients with CSF. This phenomenon may affect the function of microvasculature and increases the risk.

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Conflict of Interests

Authors have no conflict of interests.

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