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Research Article

The effects of interleukin (IL)1, 6, 10 and tumor necrosis factor (TNF) gene polymorphisms on CRP levels in coronary artery disease

Nil Dogruer Unal[1,*], Hatice Yildirim Yaroglu[2], Dilek Çiçek, Yilmaz[3], Lülüfer Tamer[4]

[1]*Mersin University Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey*
[2]*Mersin University Health Science Institute Stem Cell and Regenerative Medicine, Mersin, Turkey*
[3]*Mersin University Faculty of Medicine, Departments of Cardiology, Mersin, Turkey*
[4]*Mersin University Faculty of Medicine, Department of Medical Biochemistry, Mersin, Turkey*

*Corresponding author: nildogruer@mersin.edu.tr

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Abstract

Atherosclerosis is a dynamic and progressing inflammatory pathology. Studies have demonstrated that genetic variants that directly or indirectly alter the inflammatory system raise the risk of coronary artery disease (CAD). Genetic variations account for the variances in how the disease develops and progresses as well as the disclosure of poor prognostic information linked to extreme inflammatory reactions in individuals. In our investigation, we aimed to determine whether there may be a connection between CAD and the IL-1 C-889T, IL-6 G-174C, IL-10 G-1082A, and TNF G-308A polymorphisms. In the study, 80 patients with coronary artery stenosis of 70% or more and 80 people with normal coronary arteries were both evaluated using coronary angiography. An enzymatic colorimetric approach was used to quantify fasting blood glucose (FBG), the serum lipid profile, and an immunoturbidimetric method was used to measure CRP levels. Real-time PCR was used to identify IL-1 C-889T, IL-6 G-174C, IL-10 G1082A, and TNF G-308A polymorphisms. CRP (mg/L) levels varied in the CAD group for IL-1 C-889T and IL-10 G-1082A ($p = 0.031$ and 0.018, respectively). For IL-1, wild (CC) type CRP levels were 18.93 16.38, mutant (TT) genotype CRP levels were 53.12 21.03, and wild (GG) type CRP levels were 23.09 16.64. As a result, it was discovered that atherosclerosis significantly depends on the balance between pro and anti-inflammatory cytokines. In the presence of mutations in these cytokines, we identified variations in the levels of inflammatory markers.

Keywords: Atherosclerosis, CAD, CRP, IL-1α, IL-6, IL-10, TNF-α

Introduction

Cardiovascular diseases are one of the most common causes of death in the world [1]. Coronary artery disease (CAD) has a multifactorial basis, characterized by interactions between genetic and environmental factors. These interactions largely define the degree of susceptibility of the individual to CAD [2]. Recently, many candidate genes and chromosomal loci have been identified that may be associated with CAD, and most of these genes were involved in the etiopathogenesis of the inflammation process [3]. Inflammatory markers can be used to prevent prospective risks in cardiovascular events. The use of these markers alone or in combination may be protective for cardiovascular diseases. Therefore, genes encoding inflammatory cytokines are candidate genes in determining the risk of CAD. Although major risk factors leading to the disease have been determined by extensive epidemiological studies, these classical risk factors alone are not sufficient to explain the prevalence of CAD in the community and to explain the cause of premature CAD in some patients [4,5]. Cytokines are biological mediators that regulate the immunological, local or systemic inflammatory and reparative host responses and act as signals between cells [6]. Differential response to cytokine stimulation in the immune system may control the balance of T helper lymphocytes (Th1/Th2). Disruption of this balance in both directions significantly affects the clinic of many immune and infectious diseases [6,7]. Today, nearly 50 inflammation-related cytokines have been identified, and a significant portion of them have been detected in human atherosclerotic plaques [6,8,9]. Interleukin-1 (IL-1), which induces acute and chronic inflammation, has been shown to act a key role in atherogenesis and thrombus formation. IL-1, which plays a major role in CAD, regulates the inflammatory response during atherogenesis. IL-1, which enables many target cells to differentiate and synthesize specific products,

especially activates T cells differentiation and interleukin-1 (IL2) production, inducing inflammatory responses such as prostaglandins synthesis as well as inducing fever by acting as an endogenous pyrogen. IL-1 supports smooth muscle cell proliferation in the vascular space and prevents a wide spectrum of macrophage/monocyte functions (e.g. monokine synthesis, nitric oxide (NO) production, expression of molecules such as class II major histocompatibility complex (MHC), IL-12, and

CD80/CD86). The main effects of pro-inflammatory TNF-α produced by macrophages in the cardiovascular system are to ensure the expression of adhesion molecules and leukocyte antigen proteins, the increasion of endothelial cytokine and NO release as well as vascular permeability, hepatic fatty acid synthesis, plasminogen activator inhibitor expression, von Willebrand factor synthesis and, the decreasing of lipoprotein lipase activity and suppression of protein-C, which has anticoagulant properties [13]. Today, in addition to inflammation, genetics is accepted as another important mechanism in atherosclerosis and arterial thrombosis. It is important to determine the genetic background of coronary artery diseases in order to describe the predisposition of individuals for CAD in the early periods and to take precautions according to the information obtained. In our study, we aimed to investigate the possible relationship between IL-1α C-889T, IL-6 G-174C, IL-10 G-1082A, TNFα G-308A polymorphisms, and CAD.

Materials and Methods

Study subjects

This study comprised 160 patients with chest pain who were referred to Cardiology at Mersin University Medical Faculty Hospital. The coronary artery disease group consisted of 80 individuals with an average age of 36 and stenosis in the coronary arteries of at least 70%, including 23 women and 57 males. Following coronary angiography, a control group of 80 individuals, including 32 women and 48 men with normal coronary arteries and ages ranging from 36 to 79, was assembled. Approval was obtained from the Mersin University Faculty of Medicine Ethics Committee for this study (2008/69), which was conducted according to the specifications of the Declaration of Helsinki. All individuals participating in the study were informed about the study and their written consent was obtained.

Blood sampling

Peripheral venous blood samples of the individuals participating in the study were taken into tubes containing ethylenediaminetetraacetic acid (EDTA), and their serums were taken into biochemistry tubes for separation. In the blood samples taken into EDTA tubes, erythrocyte sedimentation rates (ESR) were studied and stored at +4ºC until the study day for DNA isolation. Peripheral venous blood samples taken into uncontained biochemistry tubes for lipid profile, C-reactive protein (CRP), and fasting blood glucose (FBG) levels were kept for 10 minutes and then centrifuged at 3000 rpm for 10 minutes and their serums were separated. Serum FBG enzymatic hexokinase, lipid profile enzymatic colorimetric, and CRP levels were studied in a Cobas 501 autoanalyzer (Roche Diagnostics GmbH Mannheim, Germany) by particle-enhanced immunoturbidimetric methods. ESR was measured in a sedimentation device (ALIFAX, Italy) with the photometric capillary flow kinetic analysis method.

DNA extraction and genotyping

To perform genetic analysis, peripheral blood was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA). DNA extraction was performed from circulating leukocytes by utilizing a high pure PCR template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany, catalog no: 1 796 828). Mutations were detected by Real-Time PCR using Light Cycler (Roche Diagnostics, Manheim GmbH, Germany) of IL-1α C889T, IL-6 G-174C, IL-10 G-1082A, TNF α G-308A after DNA isolation of samples (Sample preparation kit, Roche Diagnostics, Manheim GmbH, Germany) (Cat. No. 2 015 102, Cat. No. 2 015 102, Cat. No. 2 015 102, Cat. No. 2 015 102 Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

IBM SPSS 20.0 package program was used for statistical analysis (IBM SPSS Inc. Free Download, Chicago, Illinois, USA). The Shapiro-Wilk test was used to determine whether FBG, TC, LDL, HDL, VLDL, TG, CRP, and ESR, showed normal distribution. Those with normal distribution were compared with the

Independent sample t-test and those without normal distribution with the Mann-Whitney U test. In the examination of the distribution of alleles in genotypes and the agreement of this distribution with expected values (HardyWeinberg equilibrium), it was determined that all genotypes were in balance in all groups. Chi-square or Likelihood Ratio test was used to examine the relationship of genotypes with other parameters.

Results

The study included 80 healthy individuals with a mean age of 59.66 ± 9.80 (mean±SD), 23 females and 57 males, 80 CAD patients with a mean age of 57.80 ± 11.66 (mean±SD), 32 females and 48 males of the coronary artery patients, 14 were diagnosed with stable angina pectoris (SAP), 29 with unstable angina pectoris (USAP), and 37 with acute myocardial infarction (AMI). Descriptive information and distribution of risk factors of coronary artery patients and control groups are given in Table 1. Both groups were homogeneous in terms of age (p=0.276). It was determined that coronary artery disease is more common in males than females (p=0.001). While the presence of type 2 diabetes (p=0.081) and hypertension (p=0.999) did not differ between the groups, it was determined that cigarette consumption carries a risk for the formation of CAD (p=0.001). In the patient group consisting of coronary artery patients, serum FBG (p=0.005) and CRP (p=0.001) levels were determined to be significantly higher than the control group (Table 2). Although serum total cholesterol (TC) (p=0.248), LDL(p=0.406), VLDL(p=0.433), triglyceride (TG) (p=0.433), and ESR (p=0.184) levels in the patient group were higher than in the control group, they were not significant. While serum HDL-C (p=0.040) was significantly higher in the control group (Table 2).

The distribution of mutation analyses of IL-1, IL-6, IL-10, and TNF-α genes in CAD and control groups between genotypes are given in Table 3. There was no significant difference between the patient and control groups and IL-1α C-889T, IL-6 G-174C, IL-10 G-1082A, and TNFα G-308A genotypes (table 3) (p>0.05). CRP (mg/L) levels for IL-1α C-889T (p=0.031) and IL-10 G-1082A (p=0.018) were different in the patient group. CRP levels were found 18.93±16.38 in IL-1 C-889T wild (CC) genotype; 53.12±21.03 (p=0.031) in IL-1 C-889T mutant (TT) genotype and 23.09±16.64 in IL-10 G-1082A mutant (AA) genotype, 78.36±19.33 in IL-10 G-1082A wild genotype (GG) (p=0.018) (Table 4).

Table 1. *Descriptive information and distribution of risk factors in coronary artery patients and control groups*

		CAD Group	**Control Group**	**p value**
Age		59,66 ± 9,80	57,80 ± 11.66	0,276
BMI[#]		28,08 ± 2,93	28,34 ± 3.60	0.628
Gender	**Female**	23 (28,8)	32 (40)	0.001
	Male	57 (71,2)	48 (60)	
Diabetes (Type 2)	+	28 (35,0)	18 (22,5)	0,081
	-	52 (65,0)	62 (77,5)	
Hypertension	+	42 (52,5)	42 (52,5)	0,999
	-	38 (47,5)	38 (47,5)	
Smoking	+	28 (35,0)	15 (18,8)	0.020
	-	52 (65,0)	65 (81,2)	
Family History	+	25 (31,2)	19 (23,8)	0,288
	-	55 (68,8)	61 (76,2)	
Alcohol consumption	+			
	-	80 (100)	80 (100)	
	0		80	
	1	25 (31,3)		
	2	22 (27,5)		
	3	29 (36,2)		
	4	4 (5,0)		

[#]BMI: body mass index (kg/m2) Continuous variables are given as mean±standard deviation, categorical variables as n (%). p: degree of significance between groups

Table 2. *Serum FBC, lipid profile, CRP and ESR levels of coronary artery patients and control groups*

	CAD Group	Control Group	p value
Fasting Blood Sugar*	121.91 ± 54.01	101.84 ± 31.62	0.005
Total Cholesterol*	170.67 ± 43.45	178.77 ± 44.70	0.248
HDL-Cholesterol*	37.99 ± 11.99	41.54 ± 10.30	0.040
LDL-Cholesterol*	100.75 ± 37.64	105.34 ± 35.56	0.406
VLDL-Cholesterol*	30,42 ± 13.51	28.79 ± 12.78	0.433
Triglyceride *	152,12 ± 67,51	143,95 ± 63,93	0.433
CRP#	33,77 ± 15,21	7,09 ± 3,86	0.001
ESR$^{\Psi}$	14,70 ± 12,70	12,19 ± 10,56	0.184

* Continuous variables are given as mean±standard deviation, and concentration units are mg/dl. #mg/L, Ψ mm/hr, p: significance between groups

Table 3. *Distribution of mutation analysis between genotypes in CAD and control groups*

	Genotype	CAD	Control	P value
IL-1α C-889T	CC	40 (%50,0)	40 (%50,0)	0,841
	CT	32 (%40,0)	34 (%42,5)	
	TT	8 (%10,0)	6 (%7,5)	
IL-6 G-174C	GG	49 (%61,2)	45 (%56,2)	0,787
	GC	24 (%30,0)	28 (%35,0)	
	CC	7 (%8,8)	7 (%8,8)	
IL-10 G-1082A	GG	10 (%12,5)	9 (%11,3)	0,968
	GA	37 (%46,3)	38 (%47,5)	
	AA	33 (%41,2)	33 (%41,2)	
TNF α G-308A	GG	63 (%78,7)	65 (%81,2)	0,753
	GA	15 (%18,8)	12 (%15)	
	AA	2 (%2,5)	3 (%3,8)	

n: number of patients, p: degree of significance between groups

Table 4. *CRP levels according to IL-1α C-889T and IL-10 G-1082A genotype distribution in CAD group*

Mutasyon	GenotYPE	CRP#	p
IL-1α C-889T	CC	18,93 ± 16,38	0.031
	CT	47,02 ± 18,61	
	TT	53,13 ± 21,03	
IL-10 G-1082A	GG	78,36 ± 19,33	0.018
	GA	31,16 ± 16,98	
	AA	23,09 ± 16,64	

#Continuous variables are given as mean±standard deviation and concentration units are mg/dl

Discussion

Single nucleoid polymorphisms (SNPs) for IL-1 (-889 C/T), IL-6 (-174 G/C), and TNF (-308 G/A) have been linked to increased CVD risk in various groups [14,15]. Through a number of mechanisms, such as vascular endothelial damage and the production of adhesion molecules for monocytes in the arterial wall, these cytokines contribute to inflammation and atherogenesis [16]. IL-10 (-1082G/A) single nucleotide polymorphism plays an important role in anti-inflammation to balance the pro-inflammatory response with decreased levels shown in patients with CAD [17]. Pasqui et al. [18] demonstrated increased TNF-α and decreased IL-10 levels in patients with acute coronary syndrome. Cytokine production is under genetic control, and many single-nucleotide polymorphisms in cytokine genes affect gene transcription and cytokine function and were associated with decreased or increased cytokine production [17]. It has been found that IL-1 α (-889

C/T) and TNFα (-308G/A) polymorphisms cause increased IL-1 α and TNFα levels (19, 20). IL-6 (-174 G/C) polymorphism causes decreased IL-6 levels [21]. The effect of IL-10 (-1082G/A) polymorphism on IL-10 levels has also been reported to cause a decrease [22]. Most of the gene polymorphisms of cytokines and their receptors are located in the promoter, intron, and 3' untranslated regions. These sequence changes in the nontranslated regions of the gene can still affect gene expression and function by disrupting or stopping transcription regulatory elements. All four of these polymorphisms shown in IL-1, -6, -10, and TNFα included in the study are in the promoter region [19,21-23]. When we examined IL-1 α (-889 C/T), IL-6 (-174 G/C), TNFα (-308G/A) and IL-10 (-1082G/A) polymorphisms, the distribution between genotypes, there was no significant difference between the groups. For the first three polymorphisms, we determined that while individuals with mutant genotypes were less in both the patient and control groups, the number of mutants and heterozygous individuals for IL-10 was higher than the number of individuals with the wild genotype. Although our data do not support the hypothesis that there may be a relationship between polymorphisms in IL-1 α, IL-6, TNFα and IL-10 genes and the development of CAD, our negative findings may be related to factors limiting our study, such as the low number of individuals in the study groups. When Banarje et al. [24] examined IL-1 α (-889 C/T), IL-6 (-174 G/C), TNFα (-308G/A) polymorphisms in their study in which they included 442 individuals, they found no difference for CAD, similar to our findings. Francis et al. [25]with

IL-1 α (-889 C/T), TNFα (-308G/A), and Keso et al. [26] with TNFα (-308G/A) found no relationship between these mutations and CAD in their study. Manginas et al. [17] found no relationship between TNFα (-308G/A) and IL-10 (-1082G/A) polymorphisms and CAD in their study on the Greek population but found a significant difference for IL-6 (-174 G/C). Sekuri et al. [21] did not find a significant difference for IL-6 (-174 G/C) as in our findings and their study included 225 people. Karaca et al. [27] also found no relationship between IL-10 (-1082G/A) polymorphisms and CAD, in line with our findings.

Factors such as aging, high blood pressure, hypercholesterolemia, diabetes (insulin resistance), smoking and obesity (adipose tissue), which trigger inflammatory/proliferative reactions in the vascular wall by stressing the endothelium involved in the development of atherosclerosis, have also been found to be associated with the release of proinflammatory cytokines such as IL-1 and TNF-α, which can lead to excessive CRP release. These cytokines are responsible for the expression of molecules that will increase the inflammatory cascade, such as ICAM-1, P and E selectin, MCP-1 and CSF (29-31). In our study, a significant difference was found between patient serum CRP levels and control serum CRP levels (p=0.001). In coronary artery patients, CRP levels for IL-1α C-889T were low in the wild (CC) genotype, but increased in the heterozygous (CT) genotype and were highest in the mutant (TT) genotype. CRP levels for IL-10 G-1082A in coronary artery patients were low in mutant (AA) genotype, but slightly increased in heterozygous (GA) genotype and highest in wild (GG) genotype. There may be two alternatives for this situation, which conflict with CRP levels and IL-1 data. The first is that IL-10 levels may not decrease in the homozygous genotype, as reported by Matsumoto et al. [22].

This alternative will align our findings with the pro-inflammatory anti-inflammatory balance state of IL-1 and IL-10. Kingo et al. [32] reported that there were different results given to different groups noting this contradictory situation. The second alternative, as we mentioned before, may be the unclear state of Th1/Th2 cytokines, as well as high levels of CRP due to statin use, smoking, and adipose tissue itself. Considering the heterozygous genotype levels, the first alternative seems more valid. Further studies are needed to explain this discrepancy.

The cardiovascular risk factor CRP was found to be high in the inflammatory cytokine IL-1α C-889T mutant (TT) genotype and low in the anti-inflammatory cytokine IL-10 G-1082A mutant (AA) genotype. We can say that IL-1α C-889T mutant (TT) genotype together with CRP can be considered a risk factor for CAD, and the IL-10 G-1082A mutant (AA) genotype can be considered protective in CAD.

As a result, it may be possible to determine if genetic variants that directly or indirectly affect the inflammatory system raise the risk of disease by elucidating other cellular interactions at the molecular level that will clarify the association between CAD and inflammation, particularly with CRP, IL-1, and IL-10. This may lead to the development of new approaches in the diagnosis and treatment of CAD.

References

[1] Gotlieb AI. (2005). Atherosclerosis and acute coronary syndromes. Cardiovascular Pathology. 14: 181- 184.

[2] Nordlie MA, Wold LE, Kloner RA. (2005). Genetic contributors toward increased risk for ischemic heart disease. Journal of Molecular and Cellular Cardiology. 39: 667–679.

[3] Ozaki K, Sato H, Lida A, Mizuno H, Nakamura T, Miyamoto Y, Takahashi A, Tsunoda T, Ikegawa S, Kamatani N, Hori M, Nakamura Y, Tanaka T. (2006). A functional SNP in PSMA6 confers a risk of myocardial infarction in the Japanese population. Nature Genetics. 38:921–925.

[4] Ridker PM, Libby P. Risk factors for atherothrombotic disease. (2008). In: Libby P, Bonow RO, Mann DL, Zipes DP, Braunwald E. Eds. Braunwald's Heart Disease, A Textbook of Cardiovascular Medicine, 8th Ed. Philadelphia: Saunders Elsevier, pp 1003-1025.

[5] Libby P. (2002). Inflammation in atherosclerosis. Nature. 420: 868-874.

[6] Roitt IM, Delves PJ. (2001) Roitt's Essential Immunology, 1Oth ed., London: Blackwell Science, The Production of Effectors, pp 226-262.

[7] Keen LJ. (2002) The extent and analysis of cytokine and cytokine receptor gene polymorphism. Transplant Immunology. 10: 143-146.

[8] Larionov S, Dedeck O, Birkenmeier G, Thal DR. (2007) Expression of alpha2 macroglobulin, neutrophil elastase, and interleukin-1alpha differs in early-stage and late-stage atherosclerotic lesions in the arteries of the circle of Willis. Acta Neuropathologica.113(1):33-43.

[9] Durán MC, Martín-Ventura JL, Mas S, Barderas MG, Dardé VM, Jensen ON, Egido J, Vivanco F. (2007). Characterization of the human atheroma plaque secretome by proteomic analysis. Methods in Molecular Biology. 357:141150.

[10] Tone M, Powell MJ, Tone Y, Thompson SA, Waldmann H. (2000). IL-10 Gene Expression Is Controlled by the Transcription Factors Sp1 and Sp3. Journal of Immunology. 165(1):286-291.

[11] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. (2001). Interleukin-10 and the interleukin-10 receptor. Annual Review of Immunology. 2001; 19:683-765.

[12] D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. (1993). Interleukin 10 (IL10) inhibits human lymphocyte interferon gamma production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. Journal of Experimental Medicine. 178: 1041-1048.

[13] Azzawi M, Hasleton P. (1999). Tumour necrosis factor-alpha and the cardiovascular system: its role in cardiac allograft rejection and heart disease. Cardiovascular Research. 43(4): 850-859.

[14] Georges JL, Loukaci V, Poirier O, Evans A, Luc G, Arveiler D, Ruidavets JB, Cambien F, Tiret L. (2001). Interleukin-6 gene polymorphisms and susceptibility to myocardial infarction: the ECTIM study. Etude CasTemoin de l'Infarctus du Myocarde. Journal of Molecular Medicine. 79:300–305.

[15] Bernard V, Pillois X, Dubus I, Benchimol D, Labouyrie JP, Couffinhal T, Coste P, Bonnet J. (2003). The -308 G/A tumor necrosis factor-alpha gene dimorphism: a risk factor for unstable angina. Clinical Chemistry and Laboratory Medicine. 41:511–516.

[16] Rosa MS, Pinto AM. Cytokines. In: Burtis CA, Ashwood ER, Bruns DE. (2006). Eds. Tietz textbook of clinical chemistry and molecular diagnostics, 4th Ed. Missouri: Elsevier Saunders, pp645-744

[17] Manginas A, Tsiavou A, Chaidaroglou A. (2008). Inflammatory cytokine gene variants in coronary artery disease patients in Greece. Coronary Artery Disease. 19:575–582.

[18] Pasqui AL, Di Renzo M, Bova G, Maffei S, Pompella G, Auteri A, Puccetti L. (2006) Proinflammatory/anti-inflammatory cytokine imbalance in acute coronary syndromes. Clinical and Experimental Medicine. 6(1):38-44.

[19] Berrahmoune H, Herbeth B, Lamont JV. (2007). Association between TNF and IL-1 bloc polymorphisms and plasma MCP-1 concentration. Atherosclerosis. 192:348-353.

[20] Carter KW, Hung J, Powell BL, Wiltshire S, Foo BT, Leow YC, McQuillan BM, Jennens M, McCaskie PA, Thompson PL, Beilby JP, Palmer LJ. (2008). Association of Interleukin-1 gene polymorphisms with central obesity and metabolic syndrome in a coronary heart disease population. Human Genetics. 124:199–206.

[21] Sekuri C, Cam FS, Sagcan A, Ercan E, Tengiz I, Alioglu E, Berdeli A. (2007). No association of interleukin-6 gene polymorphism (-174 G/C) with premature coronary artery disease in a Turkish cohort. Coronary Artery Disease. 18:333–337.

[22] Matsumoto K, Oki A, Satoh T, Okada S, Minaguchi T, Onuki M, Ochi H, Nakao S, Sakurai M, Abe A, Hamada H, Yoshikawa H. (2010). Interleukin-10 -1082 gene polymorphism and susceptibility to cervical cancer among Japanese women. Japanese Journal of Clinical Oncology. 40(11):1113- 1116.

[23] Déniz-Naranjo MC, Muñoz-Fernandez C, Alemany-Rodríguez MJ, Pérez-Vieitez MC, Aladro-Benito Y, Irurita-Latasa J, Sánchez-García F. (2008). Cytokine IL-1 beta but not IL-1 alpha promoter polymorphism is associated with Alzheimer disease in a population from the Canary Islands, Spain. European Journal of Neurology. 15(10):1080-1084.

[24] Banerjee I, Pandey U, Hasan OM, Parihar R, Tripathi V, Ganesh S. (2009). Association between inflammatory gene polymorphisms and coronary artery disease in an Indian population. Journal of Thrombosis and Thrombolysis. 2009; 27:88–94.

[25] Francis SE, Camp NJ, Dewberry RM, Gunn J, Syrris P, Carter ND, Jeffery S, Kaski JC, Cumberland DC, Duff GW, Crossman DC. (1999). Interleukin-1 receptor antagonist gene polymorphism and coronary artery disease. Circulation. 99:861–866.

[26] Keso T, Perola M, Laippala P, Ilveskoski E, Kunnas TA, Mikkelsson J, Penttilä A, Hurme M, Karhunen PJ. (2001). Polymorphisms within the tumor necrosis factor locus and prevalence of coronary artery disease in middle-aged men. Atherosclerosis. 154:691–697.

[27] Karaca E, Meral Kayikçioglu M, Onay H. (2011).The effect of interleukin-10 gene promoter polymorphisms on early-onset coronary artery disease. Anadolu Kardiyoloji Dergisi. 11: 285- 289.

[28] Klein L, Greenland P. Other risk factors for coronary artery disease. (2007). In: Topol EJ, Califf RM, Thompson PD, Prystowsky EN, Thomas JD. Eds. Textbook of cardiovascular medicine, 3rd Ed. Philadelphia: Lippincott Williams&Wilkins, pp 141-152.

[29] Gomes F, Telo DF, Souza HF, Nicolau JC, Halpern A, Serrano CV Jr. (2010). Obesity and Coronary Artery Disease: Role of Vascular Inflammation. Arquivos Brasileiros de Cardiologia. 94(2): 255-261.

[30] Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB Jr, Haffner SM. (2005). Liver markers and development of the metabolic syndrome: the insulin resistance. Diabetes. 54(11):3140-3147.

[31] Davidson MH. (2005). Management of dyslipidemia in patients with complicated metabolic syndrome. American Journal of Cardiology. 96(4A):22E-25E.

[32] Kingo K, Kõks S, Silm H, Vasar E. (2003). IL-10 promoter polymorphisms influence disease severity and course in psoriasis. Genes and Immunity. 4(6):455-457.