Asian Biomedicine Vol. 6 No. 2 April 2012; 159-165 DOI: 10.5372/1905-7415.0602.042

Original article

Influence of IL-10 polymorphism on the development of coronary artery disease in Pakistan

Muhammad Sohail Afzal, Sadia Anjum, Zia-ur-Rehman Farooqi, Mamoona Noreen, Sher Zaman Safi, Muhammad Ashraf, Ishtiaq Qadri

NUST Center of Virology and Immunology (NCVI), National University of Science and Technology (NUST), H-12, Islamabad 44000, Pakistan

Background: Coronary Artery Disease (CAD) is the most frequent form of heart diseases. IL-10 is an anti-inflammatory cytokine and down regulates the Th1 response by suppression of pro-inflammatory cytokine. There are interindividual variations in IL-10 production, which are genetically contributed by polymorphisms within IL-10 promoter region.

Objectives: We investigated the association of IL-10 gene promoter -1082 G/A, -819 C/T, and -592 C/A polymorphism with CAD susceptibility in Pakistani individuals.

Methods: Ninety-three CAD patients and ninety-nine controls were enrolled in the study. IL-10 (-1,082 G/A, -819 C/T, -592 C/A) genotyping was performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).

Results: There is an indication that IL-10 -1082 GG (p = 0.033, OR = 0.26, 95%, CI = 0.07-0.97) has positive association while -1082 GA (p = 0.031, OR = 2.7, 95%, CI = 1.07-6.90) has negative association with CAD. IL-10 -819 TT (-592 AA) were significantly higher in control than in patients (p = 0.008, OR= 3.1, 95%, CI = 1.09-9.02). We have not found any significant association between IL-10 alleles and haplotypes and CAD. GTA/ATA was the diplotype, which showed the protective effect (p = 0.006, OR = 3.6, 95%, CI = 1.16-10.57) in CAD susceptibility.

Conclusion: We found a significant distributional variation in IL-10 promoter SNPs in healthy individuals and the disease group. This difference may be manifested in IL-10 production and disturb the pro- and anti- inflammatory cytokine balance hence influencing the susceptibility of CAD.

Keyword: CAD, IL-10, polymorphism, susceptibility

Coronary Artery Disease (CAD) is one of the most frequent forms of heart diseases in which plaque builds up in the blood vessels and is deposited on arterial walls creating an obstruction in blood flow, a process referred as arthrosclerosis. Globally it is the leading cause of mortality today [1], with the maximum incidence rates in the developing countries such as Pakistan. Estimated increase in deaths between 1990 and 2020 from heart diseases in developing countries (137% in men and 120% in women) is much higher than that in developed countries (48% in men and 29% in women) [2]. According to National Health Survey of Pakistan, cardiac diseases are responsible for about 12% of all deaths in Pakistan today [3].

Atherosclerosis can be triggered by multiple molecular pathways and the genetic variants within an individual play a crucial role in the determination of susceptibility to CAD [4]. The presence of polymorphism in genes, which are involved in the atherosclerotic plaque formation, may influence the disease development [5] and a significant role of both the pro inflammatory and the anti-inflammatory cytokines has been observed in the pathogenesis of atherosclerosis [6]. Interleukin-10 (IL-10) is a cytokine, which have both inflammatory and anti-inflammatory functions [7]. It functions as anti-inflammatory by down regulating the Th1 response and suppression of pro-inflammatory cytokine e.g., IL-1, -6, IFN-γ, and tumor necrosis factor-α. It consequently shifts the Th1/Th2 balance [8]. The pro-inflammatory properties

Correspondence to: Muhammad Sohail Afzal, NUST Center of Virology and Immunology (NCVI), National University of Science and Technology, Islamabad 44000, Pakistan.
E-mail: sohail.ncvi@gmail.com

of IL-10 are mainly due to promotion of IFN- γ production by natural killer cells [9]. IL-10 also plays a key role in the development of atherosclerosis as it inhibits the synthesis of metalloprotenases and stimulates the production of its inhibitor, TIMP-1. Moreover, IL-10 promotes the stability of atherosclerotic plaque by preserving the extracellular matrix and fibrous cap [10].

Single nucleotide polymorphisms (SNPs) in IL-10 genes have been shown to be associated with the carotid artery intima-media [11], with atherosclerotic renal artery stenosis [12] and with restenosis after coronary stenting [13]. However, two other studies show no difference in IL-10 genotype prevalence in angiographically proven CAD or myocardial infraction compared with healthy control [14, 15]. Expression of IL-10 gene is regulated in part by three SNPs located at -1082, -819 and -592 of the gene promoter. These SNPs form haplotypes, including GCC, ACC, and ATA, have been described in Caucasian population [16]. A fourth GTA only in Chinese [17] and Pakistani Population [18, 19] has been seen. Based on these, haplotypes individuals are divided into high producers (GCC/GCC), intermediate producers (GCC/ACC, GCC/ATA), and low producers (ATA/ATA, ACC/ATA, ACC/ACC) of IL-10 [20]. In this study, we investigated three SNPs of IL-10 promoter region at -1082 G/A (rs1800870), -819 C/T (rs1800871) and -592 C/A (rs1800872), that are involve in inter individual differential IL-10 production. The objective of the study was to determine if there is any association between IL-10 promoter polymorphisms and incidence of CAD in Pakistani population.

Materials and methods

Patients and control

For determining the association between IL-10 promoter polymorphisms and CAD, blood samples were collected from 93 CAD patients from Hearts International Hospital, Rawalpindi. These samples were stored in EDTA tubes. Alongside these, 99 age-matched healthy control subjects were enrolled in this study, none of which had any history of heart disease. Patient and control populations were of the same ethnicity and from the same geographical area. The Ethical Committee of NUST Center of Virology and Immunology (NCVI), Islamabad, Pakistan approved the study and written consent was obtained, from each participant.

DNA extraction

Genomic DNA from venous blood of subjects includes in the study were extracted using genomic DNA extraction kit according to manufacturer protocol (Gentra, USA). DNA quantification was done by Bio Photometer (Eppendorf, USA). DNA was stored at -20°C.

Genetic analysis

For IL-10 haplotypes determination Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR) method was used as described by Perrey et al. [21]. For each polymorphism two separate reactions were performed. Each reaction contained one of the two-allele specific forward primers and a generic anti sense primer. PCR amplification was performed in 15 µl reaction volume containing 40 ng genomic DNA, 1.5 µl 2mM dNTP, 25 mM $MgCl_2$, 1 □l 10 pmol each primer and 0.7 units of Taq polymerase in 1X reaction buffer with cycling conditions 94°C for 5 min, followed by 35 cycles at 94°C for 45 sec, 58°C for 40 sec, 72°C for 50 sec and finally 7 minutes extension at 72°C. To ensure PCR success, an internal control region was amplified from the human growth hormone. The amplified products were analyzed on 2% agarose gel.

Statistical analysis

Statistical analysis was performed by Study Result Software Version 1.0.4 (CreoStat HB Frolunda, Sweden). The frequencies of alleles, genotypes and haplotypes of the IL-10 polymorphic sites in CAD patient and healthy control groups were determined by counting and compared by the χ^2 or Fischer's exact test. *p*-values smaller than 0.05 were considered significant.

Results

It was found that frequency of GG genotype at IL 10 -1082 in CAD patients was higher than controls (3% vs. 11%) thus, a significant susceptible association was found between CAD and -1,082 GG genotype ($p = 0.033$, OR = 0.26, 95%, CI = 0.07-0.97). Whereas IL-10 -1082 genotype GA was more common in controls then in CAD patients (93% vs. 83%) and has a protective association with CAD ($p = 0.031$, OR = 2.7, 95%, CI = 1.07-6.90). IL-10 -1082 AA genotype was equally prevalent between both study groups. There was a protective relationship found between

CAD and -819 TT polymorphism genotypes (p = 0.008, OR = 3.1, 95%, CI = 1.09-9.02) -819 and -592 are in linkage disequilibrium. There was no association between CAD and -819 CC and CT genotypes (-819 and -592 are in linkage disequilibrium) as shown in **Table 1**.

The frequencies of -819 C/T and -592 C/A alleles (-819 and -592 are in linkage disequilibrium) did not differ significantly between CAD patients and controls (**Table 2**). Our results suggested a lack of association of IL- 10, ACC, ATA, GTA, and GCC haplotypes with CAD.

Table 1. Relationship between IL-10 Polymorphic Genes, Alleles, and CAD.

IL-10 locus	Control (n = 99)	Frequency (%)	Patients (n = 93)	Frequency (%)	P value	OR (CI95%)
-1082 G/A						
G/G (high)	3	3.03	10	10.75	0.033	0.26 (0.07-0.97)
G/A (Intermediate)	92	92.93	77	82.80	0.031	2.7 (1.07-6.90)
A/A (Low)	4	4.04	6	6.45	NS	
Allele Frequency						
G (High)	98	49.49	97	52.15	NS	
A (Low)	100	50.51	89	47.85		
-819 C/T (-592 C/A)						
T/T (A/A)	15	15.15	5	5.37	0.008	3.1 (1.09-9.02)
C/T (C/A)	81	81.82	84	90.3	NS	
C/C (C/C)	3	3.03	4	4.30	NS	
Allele frequency						
T (A)	111	56.06	92	49.46	NS	
C (C)	87	43.94	94	50.54		

Table 2. Relationship between IL-10 Haplotypes, Diplotypes, and CAD.

IL-10 locus	Control (n = 99)	Frequency (%)	Patients (n = 93)	Frequency (%)	P value	OR (CI95%)
Haplotypes						
GCC (High)	83	41.92	86	46.24	NS	
GTA (High)	18	9.09	11	5.91	NS	
ACC (Intermediate)	4	2.02	8	4.30	NS	
ATA (Low)	93	46.97	81	43.55	NS	
Diplotypes						
GCC/GCC (High)	-	-	1	1.08	NS	
GCC/GTA (High)	3	2.02	9	9.67	NS	
GTA/GTA (High)	1	1.01	-	-	NS	
GCC/ACC (Intermediate)	3	3.03	2	2.15	NS	
GCC/ATA (Intermediate)	74	75.76	73	78.49	NS	
GTA/ACC (Intermediate)	-	-	-	-	-	
GTA/ATA (Intermediate)	14	14.14	4	4.30	0.006	3.6 (1.16-10.57)
ACC/ACC (Low)	-	-	1	1.08	NS	
ACC/ATA (Low)	4	4.04	6	6.45	NS	
ATA/ATA (Low)	-	-	1	1.08	NS	

In the diplotypes (Haplotype Zygosity) distribution, there was no significant association found in diplotypes in controls and CAD except GTA/ATA that was higher in healthy controls compared with CAD (14.1 vs 4.3%), and significant difference was observed between controls and CAD (p = 0.006, OR = 3.6, 95% CI = 1.16-10.57). In this study, we have not found any GTA/ACC haplotypes inherited in our local Pakistani population.

Discussion

The process of inflammation plays a key role in the progression of arthrosclerosis and cardiovascular diseases [22]. Inflammation seems to be involved in every step of artherosclerotic development and consequently, it is a major contributor in the development of coronary artery diseases (CAD). The magnitude of inflammation and the severity of CAD are evaluated by measuring plasma levels of acute phase proteins and cytokines such as fibrinogen, C reactive protein (CRP), IL-6, and IL-10 etc. The genetic polymorphisms associated with the expression of these proteins are therefore major contributors in predisposing an individual to any cardiovascular condition. Our focus is on IL-10, its polymorphisms and its association with the incidence of cardiovascular disease in the Pakistani population. Production of IL-10 is thought to be genetically controlled [23], and hence IL-10 polymorphisms may be a crucial factor in the differential production of this protein and thus, determination of these polymorphisms can be beneficial in predicting the probability of incidence of CAD. In this study, we have explored the association between the three promoter polymorphisms in IL-10 gene (*-1082 A/G, -819 C/T,* and *-592 A/C*) that have been shown *in vitro* to affect transcriptional strength and the incidence of CAD.

Our results showed the shielding role of IL-10 SNP -819 TT (p = 0.008, OR = 3.1, 95% CI = 1.09-9.02) which is known to reduce IL-10 production. Protective role of IL-10 SNP -819 TT gene in stent restenosis was found by Mart□□nez-R□□os et al in Mexican population [13]. Corresponding studies from China [24] and Netherland [25] showed analogous results. In contrary to our results, these same polymorphisms of IL-10 promoter were not associated with CAD or myocardial infarction (MI) in a German study, which enrolled 998 CAD and 793 MI patients compared with 340 healthy control individuals [15]. Similar results were reported from France [14], Italy [26]. Therefore, overall it is seen that IL-10 promoter polymorphisms play divergent roles in MI and CAD. Even within a single population, this variance is observed like in Japan, a study by Hirashiki et al. showed a link between -819 C>T change and myocardial infarction [27] however, another investigation by Kanae Oda et al. gave contrary results [28].

The outcome of investigations concerning the effect of IL-10 expression and polymorphisms for the risks associated with atherosclerosis and CAD susceptibility is disconcordant when considering the haplotypes with respect to all three SNPs. Several studies have demonstrated that high IL-10 expression confers a protective effect against atherosclerosis or acute coronary events [25, 29-31], but this notion is not consistent with every population [32]. Contradictory or neutral effects of these polymorphisms have also been reported [15, 27, 33, 34]. The frequency of IL-10 -1082; -819; -592 ATA haplotype i.e., IL-10 low producer genotypes has been found to be higher in the patients with atherosclerotic renal artery stenosis (n = 66) than in the control subjects (n = 100), suggesting that IL-10 may protect against the development of atherosclerotic renovascular disease in Spanish population. However, in this study the overall levels of IL-10 mRNA and protein were higher in the patients than in the controls (irrespective of their genotype); this observation appears to be partly controversial [34]. In a study from North and South Italy ATA/ATA, ACC/ATA diplotypes, which were reported as low IL-10 producer, are more prevalent in CAD patients then control. Diplotype GCC/GCC (high producer) was less in patients then healthy subjects [26]. The conflicting effect of IL-10 polymorphisms could also be considered based on the recent study in which IL-10 -1082 G/-819 C/-592 C (IL-10 high-producer genotype) was more frequent in patients with aortic severe occlusive disease [34]. Identical IL-10 haplotype was associated with decreased arterial elasticity which is in disconcordance with the supposed anti atheromatous properties of IL-10 [7]. Coronary artery disease patients from Pakistan having GCC/GTA (high IL-10 producer) diplotype were genetically predispose to CAD while diplotype GTA/ATA showed protective effect from it.

Our results show that in Pakistani population, IL-10 haplotypes have no association with cardiovascular disease susceptibility as previously reported in Japanese [28], German [15, 35] and Caucasian patients

[21, 36]. In Pakistani CAD patients, IL-10 -1082 GG is frequent in patients while IL-10 -1082 GA is the genotype, which showed negative association with disease. It was reported by Estrella Blancoa et al. recently that IL-10 -1082 G does not have any role in transcription of IL-10 gene by its own [34]. A study on 3634 individuals predicts that genetic effect of these SNPs on IL-10 plasma level was relatively small [37]. Actually, IL-10 transcription depends on combination of different polymorphism into haplotypes [34]. A study on large number of 3634 patients from Sweden favor the Estrella Blancoa *et al* statement, IL-10 -1082 G/A showed no association with CAD but IL-10 plasma level were high in patients then control. Patients with high IL-10 showed high death rate in myocardial infraction as compared with patients having low IL-10 plasma level [37]. In contrast, two smaller studies showed elevation of IL-10 serum level associated with decreased risk of coronary events [38, 39]. Previously IL-10 -1082 GG was high in healthy in North Italy [26] and showed no significant association in studies from France [14], Japan [28], South Italy [26], Germany [15] and Caucasian population [21].

The limitation of our study is that we did not measure the plasma IL-10 levels, which could have been an interesting parameter for further analysis in the study. Accordingly, we were not able to confirm whether IL-10 -1082 G/A is associated with high or low IL-10 production. However, it has been reported that IL-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome [37]. Since this study is preliminary and based on a smaller sample size, we believe that these findings may stimulate further investigations on a larger scale to assess the association of these polymorphisms in CAD patients.

Conclusion

The pattern of these polymorphisms is in ethnic specific manner. Considering Pakistan, no one has ever reported any study on this issue and our conclusions based on the observations in this study is that these polymorphisms are very important in clarifying the role of genetics in CAD pathogenesis.

The authors declare no conflict of interest in conducting this study.

References

1. Tregou□t Tregouet DA, Konig RI, Erdmann J, Munteanu A, Braund PS, Hall AS, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nat Gen. 2009; 4:283-5.
2. Murray CJL, Lopez AD. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Boston, Mass: Harvard School of Public Health. 1996.
3. Pakistan Medical Research Council. National Health Survey of Pakistan 1990-94. Islamabad, Pakistan: Pakistan Medical Research Council, Network Publication Service. 1998.
4. Apostolakis S, Baritaki S, Kochiadakis GE, Igoumenidis NE, Panutsopulos D, Demetrios A. et al. Effects of polymorphisms in chemokine ligands and receptors on susceptibility to coronary artery disease. Thromb Res. 2007; 119:63-71.
5. Sarecka B , Zak I , Krauze J. Synergistic effects of the polymorphisms in the PAI-1 and IL-6 genes with smoking in determining their associated risk with coronary artery disease. Clin Biochem. 2008; 4:467-73.
6. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol. Rev. 2006; 86:515-81.
7. Heiskanen M, K□h□nen M, Hurme M, Lehtim□ki T, Mononen N, Juonala M, et al. Polymorphism in the IL-10 promoter region and early markers of atherosclerosis: The Cardiovascular Risk in Young Finns Study. Atherosclerosis. 2010; 208:190-6.
8. Thio C. Host genetic factors and antiviral immune responses to HCV. Clin Liver Dis. 2008; 12:713-26.
9. Rousset F, Garcia E, Defrance T, P□ronne C, Vezzio N, Hsu DH, et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA.1992; 89:1890-3.
10. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, et al. Protective role of interleukin-10 in atherosclerosis. Circ Res. 1999; 85:17-24.
11. Kahraman S, Yilmaz R, Arici M, Altun B, Erdem Y, Yasavul U, et al. IL-10 genotype predicts serum levels of adhesion molecules, inflammation and atherosclerosis in hemodialysis patients. J. Nephrol. 2006; 19:50-6.
12. George S, Ruan XZ, Navarrete C, Turner D, Reynard M, Sweny P. et al. Renovascular disease is associated with low producer genotypes of the anti-inflammatory cytokine interleukin-10. Tissue Antigens. 2004; 63:

470-5.
13. Martinez-Rios MA, Pena-Duque MA, Fragoso JM, Delgadillo-Rodriguez H, Rodriguez-Perez JM, Miranda-Malpica E, et al. Tumor necrosis factor alpha and interleukin 10 promoter polymorphisms in Mexican patients with restenosis after coronary stenting. Biochem Genet. 2009; 47:707-16.
14. Donger C, Georges JL, Nicaud V, Morrison C, Evans A, Kee F, Arveiler D, et al. New polymorphisms in the interleukin-10 gene-relationships to myocardial infarction. Eur J Clin Invest. 2001; 31:9-14.
15. Koch W, Kastrati A, B□ttiger C, Mehilli J, von Beckerath N, Sch□mig A. Interleukin-10 and tumor necrosis factor gene polymorphisms and risk of coronary artery disease and myocardial infarction. Atherosclerosis. 2001; 159:137-44.
16. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet. 1997; 24:1-8.
17. Mok CC, Lanchbury JS, Chan DW Lau CS. Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. Arthritis Rheum. 1998; 41:1090-5.
18. Afzal MS, Anjum S, Salman A, Ashraf S, Farooqi ZR, Ahmed T, et al. Interleukin-10 gene promoter polymorphism as a potential host susceptibility factor in Pakistani patients with pulmonary tuberculosis. Afr J Biotechnol. 2011; 10:14706-10.
19. Afzal MS, Tahir S, Salman A, Baig TA, Shafi T, Zaidi NS, et al. Analysis of interleukin-10 gene polymorphisms and hepatitis C susceptibility in Pakistan. J. Infect. Dev. Ctries. 2011; 5:473-9.
20. Hulkkonen J, Pertovaara M, Antonen J, Lahdenpohja N, Pasternack A, Hurme M. Genetic association between interleukin-10 promoter region polymorphisms and primary Sjogren's syndrome. Arthritis Rheum. 2001; 44:176-9.
21. Perrey C, Turner ST, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL- 10, TNF.a, TNF-B and TGF-B1 gene polymorphisms. Transplant Immunology. 1998; 7: 127-8.
22. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med. 1999; 340:115-26.
23. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 59 flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum. 1999; 42:1101-8.
24. Xie G, Myint PK, Zhao L, Li Y, Wang H, Liang L, et al. Relationship between -592A/C polymorphism of interleukin-10 [IL-10] gene and risk of early carotid atherosclerosis. Int J Cardiol. 2010; 143:102-4.
25. Trompet S, Pons D, DE Craen AJ, Slagboom P, Shepherd J, Blauw GJ, et al**.** Genetic variation in the interleukin-10 gene promoter and risk of coronary and cerebrovascular events: the PROSPER study. Ann N Y Acad Sci. 2007; 1100:189-98.
26. Lio D, Candore G, Crivello A, Scola L, Colonna-Romano G, Cavallone L, et al. Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. J Med Genet. 2004; 41:790-4.
27. Hirashiki A, Yamada Y, Murase Y, Suzuki Y, Kataoka H, Morimoto Y, et al. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol. 2003; 42:1429-37.
28. Oda K, Tanaka N, Arai T, Araki J, Song Y, Zhang L, et al. Polymorphisms in pro- and anti inflammatory cytokine genes and susceptibility to atherosclerosis: a pathological study of 1503 consecutive autopsy cases. Hum Mol Genet. 2007; 16:592-9.
29. Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Boersma E, Simoons ML, et al. Serum level of the anti-inflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation. 2003; 107: 2109-14.
30. Altun B, Yilmaz R, Kahraman S, Gen□toy G, Arici M, Onalan O, et al. Impact of cytokine gene polymorphism on cardiovascular risk in renal transplant recipients. Transpl Int. 2005; 18:681-9.
31. Ambrosius W, Kazmierski R, Michalak S, Kozubski W. Anti-inflammatory cytokines in subclinical carotid atherosclerosis. Neurology. 2006; 66:1946-8.
32. Lakoski SG, Liu Y, Brosnihan KB, Herrington DM. Interleukin-10 concentration and coronary heart disease [CHD] event risk in the estrogen replacement and atherosclerosis [ERA] study. Atherosclerosis. 2008; 197:443-7.
33. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl JMed. 2002; 347:1916-23.
34. Blanco E, Mo□ux G, Mas A, Serrano FJ, de la Concha

EG, Urcelay E. Role of IL-10 promoter polymorphisms in tmhe development of severe aorto-iliac occlusive disease. Hum Immunol. 2008; 69:651-4.

35. Koch W, Tiroch K, Beckerath NV, mig AS, Kastrati A. Tumor necrosis factor-a, lymphotoxin-a, and interleukin-10 gene polymorphisms and restenosis after coronary artery stenting. Cytokine. 2003; 24:161-71.
36. Densem CG, Hutchinson IV, Yonan N, Brooks NH. Influence of interleukin-10 polymorphism on the development of coronary vasculopathy following cardiac transplantation. Transplant Immunology. 2003; 11:223-8.
37. Ma□larstig A, Eriksson P, Hamsten A, Lindahl B, Wallentin L, Siegbahn A. Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome. Heart. 2008; 94:724-9.
38. Anguera I, Miranda-Guardiola F, Bosch X, Filella X, Sitges M, Mar□n JL, et al. Elevation of serum levels of the anti-inflammatory cytokine interleukin-10 and decreased risk of coronary events in patients with unstable angina. Am Heart J. 2002; 144:811-7.
39. Tziakas DN, Chalikias GK, Hatzinikolaou HI, Parissis JT, Papadopoulos ED, Trypsianis GA, et al. Anti-inflammatory cytokine profile in acute coronary syndromes: behavior of interleukin-10 in association with serum metalloproteinases and proinflammatory cytokines. Int J Cardiol. 2003; 92:169-75.