Pergamon

Thrombosis Research 100 (2000) 263–269

THROMBOSIS RESEARCH

ORIGINAL ARTICLE

Gene Polymorphisms in the TNF Locus and the Risk of Myocardial Infarction

Juliano C. Padovani[1], Antônio Pazin-Filho[1],
Marcus V. Simões[1], José A. Marin-Neto[1], Marco A. Zago[1,2] and Rendrik F. Franco[1,2]
[1]Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo; and [2]Blood Centre of Ribeirão Preto - FUNDHERP, Ribeirão Preto, São Paulo, Brazil.

(Received 24 February 2000 by Editor A. Estellés; revised/accepted 16 May 2000)

Abstract

We investigated two genetic polymorphisms in the tumor necrosis factor locus (TNF-α −308 G→A and LT-α +252 A→G) as risk factors for coronary atherothrombotic disease (CAD) by determining its prevalence in 148 survivors of myocardial infarction (MI) with angiographically-proven severe CAD, and in 148 age-, gender- and race-matched controls. The odds ratio (OR) for MI related to the mutant TNF-α and LT-α alleles was 0.8 (CI95: 0.4–1.3) and 1.3 (CI95: 0.8–2.0), respectively. We also sought interaction of smoking and metabolic risk factors for MI with each mutant genotype. Smokers not carrying the LT-α +252 A→G mutation had a risk of MI of 2.7 (CI95: 1.4–5.4) whereas in smoking carriers the risk was 6.9 (CI95: 3.4–14.1). An interactive effect of the LT-α mutation may also exist with dyslipidemia (OR for MI in non-carriers was 12 [CI95: 3.2–41.3] and in carriers the OR was 39, [CI95: 5.1–301] and with obesity (OR for MI was 2.7, [CI95: 1–7.2] in non-carriers and in carriers the OR was 6 [CI95: 2.1–16.8]). Lastly, the OR for MI in obese non-carriers of TNF-α −308 G→A was 2.8 (CI95: 1.3–6) and in obese carriers the OR was 14.5 (CI95: 1.8–113). Although significant interactive effects could not be detected, the findings suggest that interaction of polymorphisms in the TNF locus with major risk factors for CAD may exist, and should be explored in larger studies.

Key Words: TNF-α; LT-α; Myocardial infarction; Atherosclerosis; Coronary artery disease; Risk factor.

There is a close relation between atherothrombosis and inflammation [1–4]. Inflammatory mediators not only can contribute to atheroma formation, but may also be involved in the rapid evolution of the atheromatous injury, leading to rupture of the plaque and intraluminal thrombosis [1]. In this sense, it is worth noting that several cytokines may play a role in determining the degree of inflammation and contributing to atherothrombosis.

Tumor necrosis factor-α (TNF-α) is a macrophage- and lymphocyte-derived immune mediator that regulates the inflammatory response, modulates growth and cellular differentiation, and activates blood coagulation [5]. In general, increased TNF-α plasma levels and activity are also associated to increased production of other interleukins [2]. Previous studies using in situ hybridization techniques showed increased levels of TNF-α messenger RNA in the atherosclerotic plaque of symptomatic patients [6]. These data pointed to a local rise in the expression of this inflammatory mediator, which may therefore contribute to arterial

Corresponding author: Rendrik F. Franco, Laboratory of Hematology, Department of Clinical Medicine, School of Medicine of Ribeirão Preto University of São Paulo 14048-900 Ribeirão Preto (SP) Brazil. Tel: +55 (16) 602 22 23; Fax: +55 (16) 633 1144; E-mail: <rffranco@netsite.com.br>.

0049-3848/00 $–see front matter
PII S0049-3848(00)00315-7

thrombosis. Recently, a polymorphism directly affecting TNF-α expression has been identified in the promoter region of the gene, at nucleotide position −308 [7,8]. This genetic variation results in two possible allele forms, in which the presence of guanine defines the common variant (TNF1), whereas adenine defines the less common allele (TNF2). The presence of the TNF2 allele is associated with increased transcription of the gene and higher plasma TNF-α levels [7,8]. Of note, this polymorphism appears to influence clinical outcome of several diseases in which inflammation plays a role, such as malaria and non-Hodgkin lymphoma [9,10].

The proinflammatory cytokine lymphotoxin-α (LT-α, or TNF-β) is also a key mediator in the initiation of a local vascular inflammatory response. Its action is characterized by the stimulation of adhesion molecule production, thrombogenesis, smooth muscle proliferation, platelet activation, and release of vasoactive agents [1–4]. Hence, expression of this cytokine may theoretically contribute to the initiation and progression of atheromatous plaques. Recently, a polymorphism in the LT-α was reported: an A→G transition at nucleotide position+252, in the first intron of the gene. The presence of guanine at this position defines the mutant allele known as LT-α (5.5 kb), whereas adenine defines the wild-type allele, LT-α (10.5 kb). The mutant allele results in a significantly increased production of LT-α in in vitro-stimulated mononuclear cells, related to increased gene transcription [9].

The background information available on the role of inflammatory mediators in CAD encouraged us to test the hypothesis that the mutation −308 G→A in the promoter region of TNF-α and the mutation+252 A→G in the LT-α gene might be related to increased predisposition to CAD and MI.

1. Subjects and Methods

1.1. Patients and Controls

One hundred and sixty patients aged less than 55 years and with a diagnosis of MI were admitted for coronary angiography in the University Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo, Brazil, between June 1996 and December 1997. Of these, DNA samples from 148 unrelated individuals (114 men [mean age 42 years; range 25–55 years] and 34 women [mean age 46 years; range 30–55 years]) were available for analysis in the present investigation. Myocardial infarction was diagnosed on the basis of clinical, enzymatic, and electrocardiographic criteria. At least two of the following criteria were necessary to confirm this diagnosis: typical chest pain (longer than 30 minutes); an increase in creatine kinase of more than twice the baseline level; and characteristic EKG changes in two or more adjacent leads. Only patients submitted to coronary angiography, which demonstrated stenosis of 50% or higher in a major artery, were included in the current investigation. While the samples from patients were being collected, 148 unrelated, asymptomatic and apparently healthy subjects (blood donor candidates) without a personal history of arterial disease or MI were selected as controls. Each case was matched to a control for gender, age (+4 years) and for race. Both patients and controls came from the same geographic region, i.e., the city of Ribeirão Preto, state of São Paulo, Southeastern Brazil.

Table 1. General characteristics of myocardial infarction survivors with angiographically demonstrated severe atherosclerosis and of healthy controls

Variable	Patients (n=148)	Controls (n=148)
Male/female ratio	3.3/1.0	3.3/1.0
Mean age; range	43 (25–55)	42 (22–55)
Hypertension[a]	90 (60.8%)	21 (14.3%)
Diabetes[a]	21 (14.3%)	3 (2%)
Dyslipidemia[a]	49 (33.2%)	4 (2.7%)
Obesity[b]	41 (27.7%)	12 (8.1%)
Current smoking	99 (66.9%)	34 (22.9%)

[a] Physician's diagnosis and/or drug-treatment.
[b] Body mass index ⩾30 kg/m^2 (men) or ⩾27.3 kg/m^2 (women).

Table 1 shows general characteristics of the patient and control groups. As expected, major risk factors for MI were present in most of the cases and were rarer among controls. All data showed in Table 1 were collected by reviewing records and interviewing each patient and control subject included in the study.

1.2. Mutation Analysis

Peripheral blood was collected and genomic DNA extracted from mononuclear leukocytes by the

salting-out method [11]. For identification of the −308 TNF-α polymorphism, the following primers were used: 5′-AGGCAATAGGTTTTGAGGGC-CAT-3′ and 5′-TCCTCCCTGCTCCGATTCCG-3′ *Nco*I restriction-enzyme digestion was employed after PCR amplification, to determine the TNF-α genotypes. For identification of the +252 LT-α polymorphism, the following primers were used: 5′-CTCCTGCACCTGCTGCCTGGATC-3′ and 5′-GAAGAGACGTTCAGGTGTCAT-3′ *Nco*I restriction-enzyme digestion was employed after PCR amplification, to define the LT-α genotypes. Details regarding the PCR and restriction-digestion protocols have been published [9,12].

1.3. Statistical Analysis

Allele frequencies were calculated by counting genes from the observed genotypes. Odds ratios (OR) were calculated to estimate the relative risk of MI in an exposed category of subjects in relation to a reference category (e.g., wild type genotypes and subjects not exposed to major risk factors for MI), for which the OR is arbitrarily 1.0, indicating neutral risk. To assess a possible influence of the polymorphisms on the risk of MI conferred by major risk factors, stratified analyses were also performed. Thus, OR for MI were calculated relative to subjects not carrying a mutation and with neither of the classical risk factors. Confidence intervals of 95% (CI95) were calculated by standard methods [13].

2. Results

2.1. TNF-α −308 G→A and LT-α +252 A→G and the Risk of MI

Similar frequencies of the two polymorphisms were found in patients and controls. The mutant TNF-α allele was found in 34 (1 homozygous and 33 heterozygous) out of 148 controls (allele frequency 11.8%, carrier frequency 22.9%) and in 28 (2 homozygous and 26 heterozygous) out of 148 patients with MI (allele frequency 10.1%, carrier frequency 18.9%). These data yield an overall OR for MI related to the TNF-α polymorphism of 0.8 (CI95: 0.4–1.4) (Table 2). The OR for heterozygous was 0.7 (CI95: 0.4–1.3), and for homozygous the OR was 1.9 (CI95: 0.2–21).

The mutant LT-α allele was found in 77 out of 148 controls (allele frequency 29.7%, carrier frequency 52%) and in 85 out of 148 patients with MI (allele frequency 35.1%, carrier frequency 57.4%). These data yield an OR for MI related to the LT-α polymorphism of 1.3 (CI 95: 0.8–2.0) (Table 2). The OR for heterozygous was 1.1 (CI95: 0.7–1.8), and for homozygous the OR was 1.9 (CI95: 0.9–4.4).

Table 2. Prevalence of TNF-α −308 G→A and LT-α +252 A→G in patients with MI and in controls

Genotype	Patients (n=148)	Controls (n=148)	OR (CI95)
−308 G/A TNF-α			
GG	120 (81.1%)	114 (76.7%)	1.0[a]
GA	26 (17.6%)	33 (22.3%)	0.7 (0.4–1.3)
AA	2 (1.4%)	1 (0.6%)	1.9 (0.2–21)
GA+AA	28 (18.9%)	34 (22.9%)	0.8 (0.4–1.4)
252 A/G LT-α			
AA	63 (42.6%)	71 (47.9%)	1.0[a]
AG	66 (44.6%)	66 (44.6%)	1.1 (0.7–1.8)
GG	19 (12.8%)	11 (7.4%)	1.9 (0.9–4.4)
AG+GG	85 (57.4%)	77 (52.0%)	1.3 (0.8–2.0)
Any mutation			
Absent	58 (39.2%)	69 (46.6%)	1.0[a]
Present	90 (60.8%)	79 (53.4%)	1.4 (0.9–2.1)

[a] Reference category.

Table 3. TNF-α −308 G→A and LT-α +252 A→G: interaction with major cardiovascular risk factors

Risk factor	LT-α/TNF-α mutations	Patients (n=148)	Controls (n=148)	OR (CI95)
Metab. RF (–)	Non-carrier	10 (6.7%)	54 (36.5%)	1.0[a]
	Carrier	21 (14.2%)	64 (43.9%)	1.7 (0.8–4.4)
Metab. RF (+)	Non-carrier	47 (31.7%)	13 (8.8%)	19.5 (7.8–48.6)
	Carrier	70 (47.3%)	17 (11.4%)	22.2 (9.4–52.4)
Non-smokers	Non-carrier	23 (15.4%)	48 (32.2%)	1.0[a]
	Carrier	26 (16.8%)	66 (44.3%)	0.8 (0.4–1.5)
Smokers	Non-carrier	33 (22.1%)	18 (12.7%)	3.8 (1.7–8.0)
	Carrier	66 (46.6%)	16 (10.7%)	8.1 (3.9–17)

[a] Reference category.

We also calculated the OR for homozygous carriers of one polymorphism who also carried the other polymorphism (data not showed in tables). In TNF-α −308 G→A homozygotes carrying the LT-α mutant allele, the OR for MI was 2.3 (CI95: 0.2–26). In LT-α +252 A→G homozygotes carrying the TNF-α mutant allele, the OR for MI was 1.9 (CI95: 0.6–5.4).

2.2. TNF-α −308 G→A and LT-α +252 A→G: Interaction with Metabolic Risk Factors and Smoking

We examined the possibility that TNF-α −308 G→A and LT-α +252 A→G might interact with major established risk factors for MI; therefore, the OR for MI were re-calculated in several contexts. Hypertension, diabetes, dyslipidemia, and obesity were considered "metabolic" risk factors. Smoking was analyzed separately as a "non-metabolic" risk factor.

When metabolic risk factors were taken in combination, as well as the two mutations in the TNF-α locus, no interactive effect of the mutations with the metabolic risk factors was observed (see Table 3). Specifically, carriers of either mutation without a metabolic risk factor had a risk of MI of 1.7 (CI95: 0.8–4.4). For non-carriers in the presence of a metabolic risk factor, the OR for MI was 19.5 (CI95: 7.8–48.6), whereas for carriers with a metabolic risk factor the OR for MI was 22.2 (CI95: 9.4–52.4). In non-smokers carrying a mutation, the risk of MI was 0.8 (CI95: 0.4–1.5). In smoking non-carriers, the OR was 3.8 (CI95: 1.7–8) and tended to be somewhat increased in smoking carriers (OR 8.1, CI95: 3.9–17), though this difference was not significant (Table 3).

Table 4 shows the OR for MI related to TNF-α −308 G→A in the presence and in the absence of metabolic risk factors (taken in combination) and

Table 4. TNF-α −308 G→A: interaction with major cardiovascular risk factors

Risk factor	TNF-α mutation	Patients (n=148)	Controls (n=148)	OR (CI95)
Metab. RF (−)	Non-carrier	26 (17.6%)	88 (58.8%)	1.0[a]
	Carrier	7 (4.7%)	29 (19.6%)	0.8 (0.3–2.1)
Metab. RF (+)	Non-carrier	94 (63.5%)	26 (18.2%)	12.2 (6.6–22.7)
	Carrier	21 (14.2%)	5 (3.4%)	14.2 (4.9–41.4)
No smoking	Non-carrier	39 (26.4%)	86 (58.1%)	1.0[a]
	Carrier	10 (6.8%)	28 (18.2%)	0.8 (0.4–1.9)
Smoking	Non-carrier	81 (54.7%)	27 (18.9%)	6.4 (3.6–11.3)
	Carrier	18 (12.1%)	7 (4.7%)	5.7 (2.2–14.7)

[a] Reference category.

Table 5. LT-α +252 A→G: interaction with major cardiovascular risk factors

Risk factor	LT-α mutation	Patients (n=148)	Controls (n=148)	OR (CI95)
Metab. RF (−)	Non-carrier	12 (8.1%)	57 (38.5%)	1.0[a]
	Carrier	20 (13.5%)	60 (40.5%)	1.6 (0.7–3.5)
Metab. RF (+)	Non-carrier	52 (35.1%)	14 (9.4%)	17.6 (7.5–41.6)
	Carrier	64 (43.2%)	17 (11.5%)	17.9 (7.9–40.6)
No smoking	Non-carrier	37 (25%)	56 (37.8%)	1.0[a]
	Carrier	12 (7.4%)	58 (39.2%)	0.3 (0.1–0.6)
Smoking	Non-carrier	35 (24.3%)	20 (13.5%)	2.7 (1.46–5.4)
	Carrier	64 (43.2%)	14 (9.5%)	6.9 (3.4–14.1)

[a] Reference category.

smoking. Interactive effects were not observed. Table 5 shows the OR for MI associated with LT-α +252 A→G in the presence and in the absence of metabolic risk factors (taken in combination) and smoking. Interaction was not detected with the metabolic risk factors. Non-carriers of the LT-α +252 A→G mutation who smoke had an OR for MI of 2.7 (CI95: 1.4–5.4), whereas in smoking carriers the OR was 6.9 (CI95: 3.4–14.1). However, there was an overlap between these CI95, showing that the difference was not significant.

Table 6 shows the OR for MI linked to TNF-α −308 G→A calculated by taking each metabolic risk factor separately. The data do not point to an interaction of the mutation with hypertension, diabetes, or dyslipidemia. An interactive effect with obesity seems to exist: obese not carrying the variant TNF-α allele had an OR of 2.8 (CI95: 1.3–6) whereas in obese carriers the OR increased (not significantly) to 14.5 (CI95: 1.8–113).

In Table 7 similar calculations are presented for the LT-α +252 A→G mutation. Non-carriers with dyslipidemia had a 12-fold risk for MI (CI95: 3.2–41.3), and in dyslipidemic carriers the OR was 39 (CI95: 5.1–301). The OR was 2.7 (CI95: 1–7.2) in obese non-carriers and it was 6.0 (CI95: 2.1–16.8) in obese carriers of the mutation. Interaction between LT-α +252 A→G with hypertension or diabetes was not observed. Conversely, a trend toward a decreased risk was observed when comparing hy-

Table 6. TNF-α −308 G→A: interaction with metabolic risk factors

Risk factor	TNF-α mutation	Patients (n=148)	Controls (n=148)	OR (CI95)
Hypertension (−)	Non-carrier	46 (31.0%)	98 (66.2%)	1.0[a]
	Carrier	12 (8.1%)	29 (19.6%)	0.9 (0.4–1.9)
Hypertension (+)	Non-carrier	74 (50.0%)	16 (10.8%)	9.8 (5.2–18.8)
	Carrier	16 (10.8%)	5 (3.4%)	6.8 (2.4–19.7)
Diabetes (−)	Non-carrier	105 (70.3%)	111 (75.0%)	1.0[a]
	Carrier	22 (15.5%)	34 (23.0%)	0.7 (0.4–1.3)
Diabetes (+)	Non-carrier	17 (10.8%)	3 (2.0%)	5.7 (1.6–20.1)
	Carrier	4 (3.4%)	0 (0%)	
Dyslipidemia (−)	Non-carrier	77 (52.0%)	114 (77.0%)	1.0[a]
	Carrier	22 (14.9%)	30 (20.3%)	1.0 (0.5–2.0)
Dyslipidemia (+)	Non-carrier	43 (29.0%)	4 (2.7%)	16.0 (5.4–46)
	Carrier	6 (4%)	0 (0%)	
Obesity (−)	Non-carrier	92 (62.1%)	103 (69.6%)	1.0[a]
	Carrier	15 (10.1%)	33 (22.3%)	0.5 (0.2–0.9)
Obesity (+)	Non-carrier	28 (18.9%)	11 (7.4%)	2.8 (1.3–6.0)
	Carrier	13 (8.8%)	1 (0.7%)	14.5 (1.8–113)

[a] Reference category (OR=1.0).

Table 7. LT-α +252 A→G: interaction with metabolic risk factors

Risk factor	LT-α mutation	Patients (n=148)	Controls (n=148)	OR (CI95)
Hypertension (−)	Non-carrier	23 (15.5%)	56 (37.8%)	1.0[a]
	Carrier	35 (23.6%)	71 (48.0%)	1.2 (0.4–2.2)
Hypertension (+)	Non-carrier	39 (26.3%)	6 (4%)	15 (5.9–42.0)
	Carrier	51 (34.4%)	15 (10.1%)	8.3 (5.9–42.5)
Diabetes (−)	Non-carrier	53 (35.8%)	61 (41.2%)	1.0[a]
	Carrier	73 (49.3%)	84 (56.7%)	1.0 (0.6–1.6)
Diabetes (+)	Non-carrier	9 (6.0%)	1 (0.6%)	10.3 (1.3–84)
	Carrier	12 (8.1%)	2 (1.3%)	6.9 (1.4–32.3)
Dyslipidemia (−)	Non-carrier	39 (26.3%)	59 (39.9%)	1.0[a]
	Carrier	60 (40.5%)	85 (57.4%)	1.0 (0.6–1.8)
Dyslipidemia (+)	Non-carrier	23 (15.5%)	3 (2%)	12 (3.2–41.3)
	Carrier	26 (17.6%)	1 (0.6%)	39 (5.1–301)
Obesity (−)	Non-carrier	46 (31.0%)	55 (37.1%)	1.0[a]
	Carrier	61 (41.2%)	81 (54.7%)	0.9 (0.5–1.5)
Obesity (+)	Non-carrier	16 (10.l8%)	7 (4.7%)	2.7 (1.0–7.2)
	Carrier	25 (16.9%)	5 (3.4%)	6.0 (2.1–16.8)

[a] Reference category (OR=1.0).

pertensive non-carriers (OR 15, CI95: 5.9–42) with hypertensive carriers (OR 8.3, CI95: 5.9–42.5), and diabetic non-carriers (OR 10.3, CI95: 1.3–84) with diabetic carriers (OR 6.9, CI95: 1.4–32.3). These differences were not statistically significant as the CI95 overlapped.

3. Discussion

The prevalence of LT-α and TNF-α was not significantly different between patients with MI and controls in the present study, suggesting that when isolated these mutations do not exert a major impact on the risk of atherothrombosis. When the OR for MI was recalculated by taking mutation combinations into account, still no increased risk for MI was verified. It must be emphasized, however, that the specific effect of the homozygous state for each mutation could not be fully addressed in our investigation because of the small number of homozygotes. This aspect deserves further attention, considering that the OR for MI tended to be higher (albeit not significantly) in homozygotes.

Although when isolated the two polymorphisms were not found to influence the risk of MI, we sought to establish whether in conjunction with other (classical) established risk factors this risk might be increased. Although significant interactions could not be observed as the CI overlapped, our findings suggest that synergism may occur in some contexts. Firstly, the LT-α mutation may increase the risk of MI in smokers. Secondly, an interactive effect may also exist between the LT-α mutation and dyslipidemia and obesity. Thirdly, TNF-α −308 G→A may increase the risk of MI conferred by obesity.

One might speculate that the putative synergistic effects suggested by the results of the present study are related to the inflammatory roles of the two cytokines and their contributions to the atherogenic process. It should be mentioned, however, that the epidemiological data presented do not shed light into the mechanisms by which these polymorphisms may interact with major risk factors for MI. In fact, the pathophysiological basis for such interactions would deserve future exploration, should our data be confirmed in larger studies.

At least one aspect of the design of our study deserves an additional comment. This investigation was designed to specifically examine the impact of genetic risk factors on the risk of severe coronary atherothrombosis. Therefore, only relatively young patients submitted to coronary angiography, which demonstrated stenosis of 50% or higher in a major artery, were enrolled. Thus, possible influences of the TNF polymorphisms (isolated or in combination with classical risk factors) on the risk

of less severe forms of coronary atherosclerosis were not assessed in the current investigation. In addition, the findings should not be directly extrapolated to older patients suffering from CAD and MI.

The frequency of the LT-α mutant allele in the present study (0.30) is similar to those reported in European Caucasians (ranging from 0.30 to 0.32). In contrast, the frequency of the TNF-α mutant allele in the Brazilian control subjects was 0.10, which is lower than the frequency observed in Europeans (approximately 0.15 in different European populations analyzed). This may point to an ethnical heterogeneity linked to this polymorphism, a finding that deserves further confirmation and that should be taken into account when interpreting data on the prevalence of this polymorphism in unmatched case-control studies.

In conclusion, we investigated two common gene polymorphisms in the TNF locus as risk factors for MI. Although significant interactive effects could not be detected, our findings suggest that polymorphisms in the TNF locus may contribute to atherothrombosis by increasing the risk conferred by specific major classical risk factors. Future studies are warranted not only to confirm this possibility but also to further explore the role of genetic variations that influence inflammatory status in determining the risk of vascular thrombosis. In addition, our data should encourage the investigation of the contribution of these polymorphisms to clinical outcome in other entities in which inflammation plays a role.

J. Padovani was a recipient of a FAPESP Grant (98/10207-0). This research was supported by FAPESP (Grant 00/02623-5) and FUNDHERP.

References

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
2. DeGraba TJ. Expression of inflammatory mediators and adhesion molecules in human atherosclerotic plaque. Neurology 1997;49:S515–9.
3. Selwyn AP, Kinlay S, Ganz P. Atherogenesis and ischemic heart disease. Am J Cardiol 1997; 80:3H–7H.
4. Maseri A. Inflammation, atherosclerosis, and ischemic events – exploring the hidden side of the moon. N Engl J Med 1997;336:1014–6.
5. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. N Engl J Med 1996;334:1717–25.
6. Barath P, Fishbein MC, Cao J, Berensen J, Helfant RH, Forrester JS. Detection and localization of tumor necrosis factor in human atheroma. Am J Cardiol 1990;65:297–302.
7. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8 and DR3 alleles. J Exp Med 1993;177:557–60.
8. Wilson AG, Symons JA, Mcdowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proc Natl Acad Sci USA 1997;94:3195–9.
9. Warzocha K, Ribeiro P, Bienvenu J, Roy P, Charlot C, Rigal D, Coiffier B, Salles G. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. Blood 1998;91:3574–81.
10. McGuire W, Hill AVS, Kwjatkowski D. Variation in the TNF-α; promoter region associated with susceptibility to cerebral malaria. Nature 1994;371:508–11.
11. Miller AS, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215–8.
12. Dizon-Townson DS, Major H, Ward K. A promoter mutation in the tumor necrosis factor α gene is not associated with preeclampsia. J Reprod Immunol 1998;38:55–61.
13. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955; 19:251–3.