

RELATIONSHIP OF TNF- α -308, IL-10-1082 GENE POLYMORPHISMS WITH THE SEVERITY AND SUSCEPTIBILITY OF RHEUMATIC HEART DISEASE IN TURKISH CHILDREN

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Aim. Acute rheumatic fever is an inflammatory disease developing after upper respiratory tract infection with group A streptococci and its most important complication is rheumatic heart disease (RHD). Tumor necrosis factor (TNF)- α and interleukin (IL) -1-1082 gene polymorphisms were associated with susceptibility to develop rheumatic heart disease. The aim of our study is to determine the frequency of IL-10-1082 A/G and TNF- α -308 G/A gene polymorphism in Turkish population and to investigate the relationship between these polymorphisms and rheumatic heart disease.

Material and Methods. Genotypes of 57 unrelated children with rheumatic heart disease and 99 controls were determined by use of PCR-RFLP.

Results. No significant differences were found in genotypes or allele frequencies of TNF- α -308 and IL-10-1082 genes between RHD and control group. There was no relation between TNF- α -308 genotype and allele distribution with valvular involvement ($p>0.05$). IL-10-1082 GG and AG genotypes were seen more frequent in patients with multiple valvular disease but there was no statistical significance ($p>0.05$).

Conclusion. As a result, there was no relationship between TNF- α -308, IL-10-1082 gene polymorphisms and rheumatic heart disease or valvular involvement in the study population ($p>0.05$). Our results are thought that TNF- α -308 polymorphisms are silent and may become important only with some certain HLA alleles. Further studies checking both cytokine polymorphism and HLA alleles are needed.

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Key words: polymorphism, rheumatic heart disease, IL-10-1082, TNF- α -308.

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IL — interleukin, RF — rheumatic fever, RHD — rheumatic heart disease, SNP — single nucleotide polymorphisms, TNF — tumor necrosis factor.

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ОТНОШЕНИЯ ПОЛИМОРФИЗМОВ ГЕНОВ TNF- α -308 И ИЛ-10-1082 С ТЯЖЕСТЬЮ И ВОСПРИИМЧИВОСТЬЮ К РЕВМАТИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА У ТУРЕЦКИХ ДЕТЕЙ

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Цель. Острая ревматическая лихорадка — это воспалительное заболевание, которое развивается после инфицирования верхних дыхательных путей группой стрептококка A и его важнейшими осложнениями являются ревматические пороки сердца (РПС). Фактор некроза опухоли (TNF) - α и интерлейкин (IL) -1-1082 полиморфизмы генов были связаны со склонностью к развитию ревматической болезни сердца. Целью нашего исследования является определение частоты генного полиморфизма IL-10-1082 A/G и TNF- α -308 G/A у турецкого населения, и исследование отношений между этими полиморфизмами и ревматической болезнью сердца.

Материал и методы. Генотипы 57 неродственных детей с ревматическими пороками сердца и 99 детей (группа контроля) были определены с использованием ПЦР-ПДРФ.

Результаты. Не обнаружено значимых различий в генотипах или частоте аллеля TNF- α -308 и IL-10-1082 генов между РПС и контрольной группой. Не было никакой связи между генотипом TNF- α -308 и аллелью распределение с вовлечением клапанов ($p>0.05$). Генотипы IL-10-1082 GG и AG наблюдались

чаще у больных с множественными клапанными пороками, но не было никакой статистической значимости ($p>0.05$).

Заключение. Как следствие, не существует никакой связи между полиморфизмами генов TNF- α -308, IL-10-1082 и ревматической болезнью сердца или вовлечения клапанов в исследуемой популяции ($p>0.05$). Наши результаты предполагают, что полиморфизмы TNF- α -308 неактивны и могут стать актуальными только при некоторых определенных аллелях HLA. В дальнейших исследованиях необходима проверка обоих цитокинов и полиморфизма аллелей HLA.

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Ключевые слова: полиморфизм, ревмокардит, IL-10-1082, TNF- α -308.

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Introduction

Rheumatic heart disease (RHD) is an inflammatory disorder and autoimmune sequel of group A streptococcal infection complicated by rheumatic fever (RF). Autoimmunity induced by antigenic mimicry between the streptococcal glycoprotein and human cardiac myosin may be responsible for the pathogenesis of RHD [1]. High familial incidence of RF suggests that genetic factors play a role in susceptibility to RF and RHD. RF is still public health burden in Turkey as in developing countries [2].

Cytokines appear to play a critical role in triggering inflammatory and immunologic reactions in RF. Increase

in tumor necrosis factor (TNF) - α and interleukin (IL) -1 levels in patients with RF and RHD have been known for a long time [3]. Even, that IL-1 α in carditis and IL-6 in arthritis may be helpful as minor criteria for diagnosis of RF has been considered [4]. IL-10 is another important immunoregulatory cytokine. The biological actions are mainly inhibitory including inhibition of pro-inflammatory cytokines [5].

Single nucleotide polymorphisms (SNPs) in regulatory regions of cytokine genes can lead to the susceptibility and onset of diseases. The human TNF- α gene is located in the HLA region of human chromosome 6 and contains several

polymorphic sites. TNF- α biallelic SNPs at position -238, -308, and -376 with respect to the TNF transcriptional start site are all substitutions of adenine (A) for guanine (G) [6]. Several SNPs in the promoter region of IL-10 have been described but one of the the most investigated are located at positions -1082 of the transcriptional start site [5]. In RHD, a relationship between TNF- α -308, and IL-10-1082 polymorphisms and multivalvar involvement has been determined [4]. Since cytokine gene polymorphisms are specific on the basis of population, there are very few studies about TNF- α -308 and IL-10-1082 gene polymorphism in Turkish children with RF of which results have also been conflicting. The aim of our study is to find out the possible influences of IL-10-1082 A/G and TNF- α -308 G/A gene polymorphisms on RHD development. Besides; on the basis of our sample, to determine the allele and genotype frequencies of IL-10-1082 A/G and TNF- α -308 G/A polymorphisms in Turkish population are also aimed.

Material and Methods

Patients

This study included 57 unrelated children with RHD recruited from the pediatric cardiology department at the Mersin University Medical Faculty. Patients were diagnosed according to Jones' modified criteria and the lesions of RHD were confirmed by echocardiography. The control group consisted of 99 healthy unrelated children diagnosed with innocent murmur who had normal echocardiography. The genotyping success rate was 97.8% for the TNF- α -308 polymorphism and 88.0% for the IL-10-1082 polymorphism. Demographic characteristics, personal or family history of rheumatic fever, and echocardiographic results were obtained from the groups. The exclusion criteria for control group consisted of subjects who have history of rheumatic fever or related persons. Besides, the subjects with the history of other autoimmune associated diseases were excluded. This study had received Ethical Approval from the Mersin University Medical Faculty Ethics Committee and inform consents were given.

Genotype Analyses

Genomic DNA was extracted by the standard phenol-chloroform methods from peripheral blood leukocytes. The polymorphisms were analyzed by using of PCR/RFLP. The primary sequences for the TNF- α -308 forward and reverse primaries were (5'-AGGCAATAGGTTTGTAGGGCCAT-3') and (5'-TCCTCCCTGCTCCGATTCCG-3'), respectively. In order to characterize the IL-10-1082 polymorphism, forward (5'-CTCGCTGCAACCCAAGTGGC-3') and reverse (5'-TCTTACGCAACCCAAGTGGC-3') primaries were used. PCR amplification was performed in a final volume of 23 μ l containing 30 ng genomic DNA, 50 pmol of each TNF- α -308 and IL-10-1082 primaries, 0.2

mM dNTPs, 1XPCR buffer with $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 10% DMSO, and 2.0 U of Taq DNA polymerase (Sigma-D4545).

The PCR conditions for the TNF- α -308 gene were initial denaturation at 96 °C for 2 min, 35 cycles of denaturation for 45 s at 96 °C, annealing for 1 min at 60 °C, and extension for 90 s at 72 °C, terminated by a 7 min elongation at 72 °C. The 107 bp PCR product was digested with *NcoI* restriction endonuclease (MBI, Fermentas, Vilnius, Lithuania) for overnight at 37 °C. The PCR conditions for the IL-10-1082 gene were initial denaturation at 95 °C for 5 min, 35 cycles of denaturation for 30 s at 95 °C, annealing for 45 s at 60 °C, and extension for 30 s at 72 °C, terminated by a 7 min elongation at 72 °C. The 139 bp PCR product was digested with *MnII* restriction endonuclease (MBI, Fermentas, Vilnius, Lithuania) for overnight at 37 °C. The digestion products were electrophoretically separated on a 3% agarose gel and visualized using ethidium bromide staining.

Statistical analysis

Continuous variables were presented as mean \pm standard deviation and categorical variables as numbers and percentages. The "independent sample t-test" was used for comparison of continuous variables between case group and control group. The distribution of genotype and allele frequencies between cases and controls was compared using the "chi-square test".

We calculated odds ratios and 95% confidence intervals using logistic regression. The allele and genotype frequencies are in "Hardy-Weinberg equilibrium". Statistical analyses were performed by use of the computer software SPSS for Windows, version 11.5 and the value of $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics

Our study population consisted of 57 RHD patients (27 female, 30 male) and 99 age-matched healthy control subjects (43 female, 56 male) without a history of heart failure. The mean age of the patients was 12.2 ± 2.5 years (range, 7–18 years) and the mean age of the control group was 12.0 ± 2.7 years (range, 7–17 years).

Demographical characteristics of the patients and controls are shown in Table 1. There were no significant differences in age, gender and socio-economic status, between the patients and controls. It is remarkable that positive family history of RHD was present in 19% of cases. Depending on the exclusion criteria, no family history has been reported in the control group. Carditis were only major manifestation of the disease in most of the patients. While 21 patients had arthritis and 3 patients had Sydenham's chorea as well as carditis. On the basis of valvular involvement, 16 (28,1%) patients had mitral regurgitation, 10 (17,5%) had aortic

Table 1

Demographical characteristics of patients and controls

	Patients (n=57)	Controls (n=99)	p
Age (year)	12,19±2,51	12,01±2,66	0,625
Gender (F/M) (%)	27 (47,4) / 30 (52,6)	43 (43,4) / 56 (56,6)	0,738
Parental consanguinity (%)	15 (26,3)	30 (30,3)	0,714
Family history of RF	19 (33,3)	-	-
Low socioeconomic status	28 (49,1)	40 (40,4)	0,318

Abbreviations: F — female, M — male, RF — rheumatic fever.

Table 2

Allele and genotype frequencies of TNF- α -308 and IL-10-1082 in patients and controls

	Patients (n=57)	Controls (n=97)	P	OR	95% CI
TNF-α-308					
Genotypes					
GG (%)	45 (78,9)	64 (66)		1,0 (ref)	
AG (%)	12 (21,1)	30 (30,9)	NS	0,569	0,263–1,230
AA (%)	0	3 (3,1)	NS	0,202	0,010–4,017
Alleles					
G (%)	102 (89,5)	158 (81,4)	NS	1,937	0,963–3,897
A (%)	12 (10,5)	36 (18,6)	NS	0,516	0,257–1,039
IL-10-1082					
Genotypes					
AA (%)	28 (51,9)	33 (40,2)		1,0 (ref)	
AG (%)	21 (38,9)	40 (48,8)	NS	0,618	0,298–1,283
GG (%)	5 (9,3)	9 (11)	NS	0,654	0,196–2,180
Alleles					
A (%)	77 (71,3)	106 (64,6)	NS	1,359	0,803–2,299
G (%)	31 (28,7)	58 (35,4)	NS	0,736	0,435–1,245

Abbreviations: OR — odds ratio, 95% CI — 95% confidence intervals, NS — not significant.

regurgitation and 31 (54,4%) had both aortic and mitral regurgitation.

TNF- α polymorphism analysis

The genotypic and allelic distributions of TNF- α -308 and IL-10-1082 polymorphisms for cases and controls are shown in Table 2. The frequencies of G and A alleles for the TNF- α -308 gene were 89,5% and 10,5% in the cases and 81,4% and 18,6% in the controls, respectively. These differences were not significant. Among the cases, the prevalence of the GG, AG and AA genotypes were 78,9%, 21,1% and 0%, while it was 66%, 30,9% and 3,1% among the controls, respectively. The homozygous AA genotype was not observed in cases our study. Significance of the deviation of observed genotype frequencies from those predicted by the Hardy-Weinberg law was determined using χ^2 analysis in both groups ($p=0,001$). No considerable differences were observed in allele or genotype frequencies for the IL-10-1082 gene between RHD and controls. The frequencies of A and G alleles for the IL-10-1082 gene were 71,3% and 28,7% in the cases whereas 64,6% and 35,4% in the controls, respectively.

The frequencies of the AA, AG, and GG genotypes in the control and case groups were 51,9%, 38,9%, and 9,3%, and 40,2%, 48,8%, and 11%, respectively. The genotype distributions were in accordance with the Hardy-Weinberg equilibrium among patient group and control group ($p=3,15$ and $p=0,58$, respectively).

We also analyzed relation between genotype and allele distribution of TNF- α -308 and IL-10-1082 gene polymorphism with valvular involvement (Table 3). However, there was no relation between TNF- α -308 genotype and allele distribution with valvular involvement ($p>0,05$). IL-10-1082 GG and AG genotypes were observed more frequently in patients with multiple valvular disease but there was no statistical significance ($p>0,05$).

Discussion

In many autoimmune diseases, the role of TNF- α and IL-10 was investigated [7, 8]. In general, the effect of TNF- α is pro-inflammatory, IL-10 is immunosuppressive and anti-inflammatory. Especially, TNF- α has an active role in the pathogenesis of rheumatic diseases. Besides, in

Table 3

Allele and genotype frequencies of TNF- α -308 and IL-10-1082 in patients with different valve damage

	SVL	MVL	P	OR	95% CI
TNF-α-308	(n=26)	(n=31)			
Genotypes					
GG (%)	21 (80,8)	24 (77,4)	NS	0,816	0,225 \pm 2,961
AG (%)	5 (19,2)	7 (22,6)	NS	1,225	0,338 \pm 4,443
AA (%)	0	0			
Alleles					
A (%)	5 (9,6)	7 (11,3)		1,196	0,356 \pm 4,019
G (%)	47 (90,4)	55 (88,7)		0,836	0,249 \pm 2,808
IL-10-1082	(n=25)	(n=29)			
Genotypes					
AA (%)	15 (60)	13 (44,8)		1,0 (ref)	
AG (%)	9 (36)	12 (41,4)	NS	1,538	0,492 \pm 4,808
GG (%)	1 (4)	4 (13,8)	NS	4,610	0,456 \pm 46,673
Alleles					
A (%)	39 (78)	38 (65,5)	NS	0,536	0,227 \pm 1,267
G (%)	11 (22)	20 (34,5)	NS	1,866	0,789 \pm 4,413

Abbreviations: OR — odds ratio, 95% CI — 95% confidence intervals, SVL — single valvular lesions (mitral or aortic), MVL — multivalvular lesions, NS — not significant.

the cases of inflammatory involvements of the heart, the level of TNF- α has been increased [3]. It has also been demonstrated that TNF- α -308 polymorphism is associated with high TNF- α production [9]. Due to the fact that, several studies have been conducted to evaluate the association of the TNF- α -308 gene polymorphism and RHD. The results, however, are conflicting. One study has showed that A allele (OR=10,8) and AG genotype (OR=9,85) have been associated with RHD [10]; on the other hand, borderline association between RHD and TNF- α -308 gene polymorphism (OR=1,4) has been reported [11]. In addition, this study showed that TNF- α -308 polymorphism was related to the development of mitral valve lesions in a cohort of Brazilian RHD patients (OR=1,9) [11]. Furthermore, Settin et al. [4] demonstrated that all RHD cases had a significantly higher frequency of AA genotype of TNF- α -308 gene polymorphism (OR=5,7). Recently, Rehman et al. [12] observed that the TNF- α -308 AA and GA genotypes were associated with susceptibility to RHD (OR=9,94 and OR=1,97, respectively). There is only one study in the Turkish population where an association of TNF- α -308 A allele with RHD has been observed (OR=3,3) [13]. On the other hand, in two studies from Turkey no differences in the distribution of genotypes between cases and controls were observed [14, 15], and these results were similar to those of our study.

In the literature, there are only three studies on the association of IL-10-1082 gene polymorphism with RHD of which results were controversial. Settin et al. [4] found that the frequency of AA and GG genotypes was higher in RHD than controls, which supported a role for the IL-10-1082 polymorphism in determining the risk of

RHD. Rehman et al. [12] reported that no association was found between IL-10-1082 polymorphism and susceptibility to RHD. Another study from Turkey reported no association between IL-10-1082 genotype and RHD, which are similar to our results [15].

Previous studies examined associations between TNF- α -308 and IL-10-1082 polymorphisms with valvular involvement. We did not find an association between the TNF- α -308 and IL-10-1082 polymorphisms and valvular involvement in the Turkish population. An association between TNF- α -308 polymorphism and patients with multivalvular disease in a Mexican population has been reported. The TNF- α -308 AG genotype and A allele frequencies were increased in patients with multivalvular disease when compared controls (OR=14 and OR=11,7, respectively). There was also increased frequency of TNF- α -308 A allele in patients with multivalvular lesion (OR=8.65) [10]. Settin et al. [4], reported that valvular involvement is associated with TNF- α -308 and IL-10-1082 polymorphisms in the Egypt population. They found that increased frequencies of genotypes TNF- α -308 AA, GG with IL-10-1082 GG in cases with multivalvular disease. Also genotypes AA of both TNF- α -308 and IL-10-1082 were increased in patients with multivalvular lesions.

Conclusion

In this study, which was conducted in a Mersin sample, the results have demonstrated that IL-10-1082 A/G and TNF- α -308 G/A gene polymorphisms had no influence on the risk of RHD development. Besides, no relationship between TNF- α -308 and IL-10-1082 gene polymorphisms

with RHD and valvular involvement was detected. The rates of allele and genotypes of control group contribute to determine the frequencies of these polymorphisms in Turkish population.

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