Vascular endothelial growth factor and tumor necrosis factor genes polymorphisms in Turkish patients with sarcoidosis

Ekrem Cengiz SEYHAN[1], Erdoğan ÇETİNKAYA[1], Zeki GÜNLÜOĞLU[1], Sedat ALTIN[1], Vedat YÜKSEL[2], Halim İŞSEVER[3]

[1] Yedikule Göğüs Hastalıkları ve Göğüs Cerrahisi Eğitim ve Araştırma Hastanesi,
[2] Burç Genetik Laboratuvarı,
[3] İstanbul Üniversitesi İstanbul Tıp Fakültesi, Biyoistatistik Anabilim Dalı, İstanbul.

ÖZET

Sarkoidozlu Türk hastalarda vasküler endotelyal büyüme faktörü (VEGF) ve tümör nekroz faktörü (TNF) gen polimorfizmi

Çalışmamızda, sarkoidoz gelişiminde predispozan bir faktör olduğu düşünülen ve hastalığın patogenezinde önemli bir rolü olan, tümör nekroz faktörü (TNF) genindeki -857 gen polimorfizmi ile sarkoidoza yatkınlığı azalttığı düşünülen vasküler endotelyal büyüme faktörü (VEGF) genindeki +813 polimorfizmi varlığını, sarkoidozlu ve sağlıklı Türk popülasyonunda karşılaştırmalı olarak incelemek amaçlanmıştır. Sarkoidoz tanısı histopatolojik olarak konulmuş 70 olgu ile herhangi bir kronik hastalık öyküsü olmayan sağlıklı 80 olgu çalışmaya alınmıştır. Olgulardan 5 cc EDTA'lı kan örnekleri alındıktan sonra DNA izolasyonunu takiben PCR + RFLP yöntemiyle TNF-857 ve VEGF+813 gen polimorfizmlerinin varlığı araştırılmıştır. TNF genindeki -857 gen polimorfizmi açısından hastalar ve kontrol olguları arasında fark görülmezken, bu polimorfizme sahip olan hastalarda daha fazla relaps olduğu belirlenmiştir. VEGF genindeki +813 polimorfizminin sağlıklı olgularda anlamlı şekilde yüksek olduğu saptanmıştır. VEGF geninin +813 lokalizasyonunda polimorfizminin sarkoidoza olan yatkınlığı azaltabileceği, TNF geninin -857 polimorfizminin ise hastalık şiddetini arttırdığı düşünülmektedir.

Anahtar Kelimeler: *Sarkoidoz, gen polimorfizm, TNF, VEGF.*

Yazışma Adresi (Address for Correspondence):

Dr. Ekrem Cengiz SEYHAN, Ressam Salihermez Sokak İnta-a Blok Daire: 18 Göztepe
İSTANBUL - TURKEY

e-mail: drekremcs@yahoo.com

SUMMARY

Vascular endothelial growth factor and tumor necrosis factor genes polymorphisms in Turkish patients with sarcoidosis

Ekrem Cengiz SEYHAN[1], Erdoğan ÇETİNKAYA[1], Zeki GÜNLÜOĞLU[1], Sedat ALTIN[1], Vedat YÜKSEL[2], Halim İŞSEVER[3]

[1] Department of Chest Diseases, Yedikule Chest Diseases and Thoracic Surgery Education and Research Hospital, İstanbul, Turkey,
[2] Burç Genetical Laboratory, İstanbul, Turkey,
[3] Department of Biostatistical, İstanbul Faculty of Medicine, University of İstanbul, İstanbul, Turkey.

Polymorphism at -857 locus of tumor necrosis factor (TNF)-α gene is considered to be a predisposition factor in sarcoidosis and held responsible for pathogenesis of the disease and polymorphism at +813 locus of vascular endothelial growth factor (VEGF) gene is thought to decrease predisposition to sarcoidosis. In our study, we examined and compared these polymorphisms in healthy Turkish control subjects and Turkish patients with sarcoidosis. We examined gene polymorphisms in 70 cases which were histopathologically diagnosed as sarcoidosis and 80 healthy subjects without any history of a chronic disease. 5 cc of blood were collected in tubes with EDTA from all of the cases. TNF-α-857 gene polymorphism and VEGF+813 gene polymorphism were determined using PCR + RFLP method after DNA isolation. TNF-α promoter polymorphism, at position -857, revealed no differences in genotype and allele frequency between patients and control subjects but more relapses were found in sarcoidosis patients who have this polymorphism. Considering the VEGF polymorphism at position +813, we observed a significant increase in the frequency of rarer T allele at this position in healthy subjects compared with sarcoidosis patients. VEGF gene polymorphism at +813 locus may diminish susceptibility to sarcoidosis. TNF-α-857 gene polymorphism influence severity of the disease.

Key Words: *Sarcoidosis, genetic polymorphism, TNF, VEGF.*

Sarcoidosis is a systemic granulomatous disease characterized with multiple organ involvement. It is believed that environmental antigenic stimulation and predisposing genetic factors play complementary roles in the development of the disease (1). Although the responsible environmental factors are not fully identified, there is some evidence supporting a possible genetic predisposition. The presence of familial sarcoidosis cases, some associations between the disease and HLA system, occurrence of diverse clinical pictures in different races support the assumption that some predisposing genes are engaged in sarcoidosis (2,3).

The essential pathologic element of sarcoidosis is a chronic granulomatous inflammation that is mediated through complex interactions between T lymphocytes, mononuclear phagocytes, fibroblasts, B lymphocytes and dendritic cells. The relations between these cells are achieved with some cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α (4). Recent data prove that genetic polymorphisms between individuals result in different regulations of cytokine production. Therefore, it is thought that some gene polymorphisms may take part in the increase or decrease of disease susceptibility and moreover in the gravity and progression of the disease, and studies on this subject have been reported (5,6).

Because of its crucial part in granuloma formation, TNF-α is believed to be the essential cytokine in the pathogenesis of sarcoidosis (7). It is demonstrated that the amount of TNF-α production is influenced by polymorphism in the promoter region of the TNF gene, categorizing individuals as high TNF-α producers and low producers (8). On the other hand, vascular endotheli-

al growth factor (VEGF) is another cytokine affecting the granuloma formation in sarcoidosis by activation of monocytes and contributing to angiogenesis and increased vascular permeability (9). Polymorphisms within the VEGF gene have been shown (10,11). Of these polymorphisms, two single-nucleotide polymorphisms (SNPs) at -627 and at +813 have been associated with VEGF protein production.

Under the light of these data, TNF and VEGF genes are considered as candidates for genetic predisposition researches in sarcoidosis. In our study, we aimed to investigate the incidences of a polymorphism at -857 of TNF-α gene, which is thought to be a predisposing factor for sarcoidosis and to play a role in the pathogenesis of the disease, and a polymorphism at +813 of VEGF gene, which is believed to decrease the susceptibility for the disease, with a comparative approach among Turkish patients with sarcoidosis and healthy population.

MATERIALS and METHODS

Study Population

Seventy patients with sarcoidosis (25 males, 45 females; mean age: 44.5 ± 11.2 years, range: 17-58 years) were included in the study. All patients in the study were Turkish. In patients with sarcoidosis the diagnosis and extent of disease have been determined on the basis of the typical clinical, radiological, and laboratory criteria, together with the finding of noncaseating granulomas in biopsy specimens. None of the patients had a history of exposure to organic or inorganic materials known to cause lung diseases. The mean follow-up time of patients was 34 ± 8.8 months (15.3 to 52.5 months). Eighty healthy subjects who had requested annual physical examinations were randomly selected, all were Turkish (54 females, 26 males; mean age: 45.9 ± 13.1 years; range: 20-78 years). The control population consisted of women and men from the same geographical region who were matched for age, sex, and ethnic origin. None had a history of lung disease or showed any symptoms of lung or other disease. All showed normal findings on chest radiography and laboratory examination, which included complete blood counts, urinalysis, and assays for hepatic enzyme activities and BUN levels. General characteristics of the patients and healthy subjects were seen in Table 1. Written informed consent was obtained from each patient and healthy subject. The research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and this study was approved by the Ethics Committee of our hospital.

Assessment of Clinical Outcome

The follow-up evaluation of disease activity included an inquiry into symptoms, a physical examination, an ophthalmologic examination by fundoscopy, plain chest radiographs, pulmonary function tests, including measuring vital capa-

Table 1. Characteristics of study subjects.

	Patients (n= 70)	Controls (n= 80)	p
Age, mean ± SD	44.5 ± 11.2	45.39 ± 13.1	NS
Age, range	20-78	17-58	NA
Disease stage (0/I/II/III/IV)	4/15/27/19/5	NA	NA
Prognosis of patients			
Spontaneous remission	23	NA	NA
Remission with treatment	36	NA	NA
Chronic sarcoidosis	11	NA	NA
Males/females	25/45	26/54	NS
Nonsmokers/smokers	59/11	65/15	NS

NA: Not available, NS: Statistically non-significant, p< 0.05: Statistically significant.

city, FEV_1/FVC, and diffusion capacity of the lung for carbon monoxide corrected for alveolar volume, and measurement of hepatic enzyme activities, serum calcium level, and serum angiotensin converting enzyme (ACE) activity. All these evaluations were repeated at three-months intervals. When all the symptoms and physical and radiographic manifestations had disappeared, and results of pulmonary function, and biochemical tests were found to be within the normal range, cases were judged to be in remission and length of the time from the onset of sarcoidosis was recorded. Relapse of the disease is defined as deterioration of the clinical and laboratory findings mentioned above, following a remission period.

Genetic Analysis

VEGF (+813 C→T) and TNF-α (-857 C→T) gene polymorphisms were analyzed by polymerase chain reaction-based methods. Genomic DNA was isolated from the peripheral blood samples according to a standard protocol. PCR reaction was performed in a total volume of 25 µL containing approximately 100 ng DNA, 2.5 µL of 10 X polymerase buffer, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.4 µmol/L of each primer and 1 U of *Taq* polymerase (MBI Fermentas). The PCR reaction was performed on PTC-150 Minicycler™ (MJ Research) termal cycler (Figure 1).

A 208-bp product from the VEGF gene was amplified using primers VEGF813-F: 5'-aag gaa gag gag act ctg cgc aga gc-3' and VGEF813-R: 5'-taa atg tat gta tgt ggg tgg gtg tgt cta cag g-3'. The PCR program was as follows: An initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30 sec at 94°C, 30 sec at 59°C, 30 sec at 72°C, and a final extension step of 8 min at 72°C. A total of 5 µL from the PCR product was run on 2% agarose to check for any nonspecific bands. A 10 µL of the PCR product was digested with 3U of the *Nla* III (Hsp92) enzyme and 2 µL of its 10 X reaction buffer in a 20 µL reaction volume (Promega). The mixture was incubated at 37°C for 3 hours or overnight. The digestion of the 208 bp product was resulted in 86 and 122 bp fragments for 813TT allele, whereas remained undigested for 813CC allele. The digested products were electrophoresed on 3% agarose gels at 100 Volt for 30 min. The gel and running buffers were 1 x TBE (0.89 M Tris-Base, 0.89 M Boric Acid, 20 mM Na_2EDTA adjusted to pH 8). The fragments were visualized by ethidium bromide under UV transilluminator. The TNF-α polymorphism was analyzed by ARMS method using the primers TNF857-C: 5'-aag gat aag ggc tca gag ag-3', TNF857-N: 5'-cta cat ggc cct gtc ttc g-3' and TNF857-M: 5'-t cta cat ggc cct gtc ttc a-3'. PCR reaction was performed in a total volume of 25 µL as above. Conditions used were as follows: 94°C for 5 min, then 5 cycles of 94°C for 25 s, 70°C for 45 s, 72°C for 25 s, 21 cycles of 94°C for 25 s, 65°C for 50 s, 72°C for 25 s, 4 cycles of 94°C for 30 s, 55°C for 60 s, 72°C for 90 s, and finally, 72°C for 10 min. The 270 bp PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

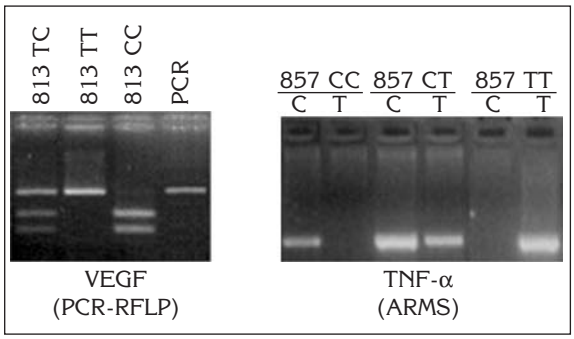

Figure 1. An electrophoretic image of TNF position -857 and VEGF position +813 genotyping.

Statistical Analysis

Statistical analyses were performed with SPSS 10.0 program. Data were presented as mean value and standard deviation. Genotype distributions were in agreement with the Hardy-Weinberg equilibrium both in sarcoidosis patients and healthy subjects. Numerical values were compared with Student's t-test and ordinal values with chi-square analysis. Comparisons of the cases on the bases of genotype and allele frequency were carried out with the use of Yates' corrected chi-square. Analyses of pulmonary function data were performed using the Kruskal-Wallis tests. Correlation analysis was realized with Spearman correlation test. Remission periods of the cases were evaluated with repeated measures in gene-

ral linear model (Pillai's trace). $p< 0.05$ was considered as statistically significant.

RESULTS

TNF-α Polymorphism

Allele frequencies and genotypes for TNF-α SNP are summarized in Table 2. As for -857 genotypes, of the 70 patients with sarcoidosis, 35 patients had the CC genotype (50%), 33 patients had the CT genotype (47%) and two patients had the TT genotype (3%). The frequency of T allele was 26%. Of the 80 healthy control subjects; 44 subjects were type CC (55%), 30 subjects were type CT (38%), and 6 subjects were type TT (7%). The frequency of C allele was 27%. We found no significant differences in the genotype distribution or allele frequencies between the patients and control subjects.

VEGF Polymorphism

Allele frequencies and genotypes for VEGF SNP are summarized in Table 3. As for +813 genotypes, of the 70 patients with sarcoidosis, 59 patients had the CC genotype (84%), 10 patients had the CT genotype (14%) and 1 patient had the TT genotype (2%). The frequency of T allele was 8%. Of the 80 healthy control subjects; 42 subjects were type CC (52%), 36 subjects were type CT (45%) and 2 subjects were type TT (3%). The frequency of T allele was 24%. We ob-

Table 2. TNF-857 gene polymorphism allele and genotype frequency in sarcoidosis and control population.*

Polymorphism	Patients (n= 70)	Controls (n= 80)	p**	Odds ratio (95% confidence interval)
Position -857				
Genotype				
CC	35 (50)	44 (55)	0.84	0.62 (0.6-1.2)
CT	33 (47)	30 (38)	0.25	0.67 (0.3-1.2)
TT	2 (3)	6 (7)	0.28	0.69 (0.4-1.6)
Allele frequency				
C	103 (74)	118 (73)	0.86	1.05 (0.5-2.1)
T	37 (26)	42 (27)	0.88	0.94 (0.4-1.1)

* Values in parentheses are percentages.
** Chi-square analysis.

Table 3. VEGF+813 gene polymorphism allele and genotype frequency in sarcoidosis control population.*

Polymorphism	Patients (n= 70)	Controls (n= 80)	p**	Odds ratio (95% confidence interval)
Position +813				
Genotype				
CC	59 (84)	42 (52)	< 0.001	0.38 (0.22-0.66)
CT	10 (14)	36 (45)	< 0.001	0.42 (0.09-0.44)
TT	1 (2)	2 (3)	0.63	1.76 (0.1-19.9)
Allele frequency				
C	128 (92)	120 (75)	0.01	0.2 (0.1-0.6)
T	12 (8)	40 (25)	0.001	3.37 (1.5-7.1)

* Values in parentheses are percentages.
** Chi-square analysis.

served a significant increase in the rarer VEGF+813 T allele frequency in the control subjects compared with the sarcoid patients (χ^2= 12.4, p< 0.001). The less-common genotypes CT were found more often in control subjects than in patients (OR= 0.42, 95% CI= 0.095-0.44, p< 0.001).

Relationship with organ involvement was also investigated. Cases of eye (n= 20), skin (n= 11), or involvement of three or more organs (n= 19), and initial chest radiographic stage II or higher (n= 51) were examined. Genotypes at loci VEGF+813 and TNF-857 were not associated with extrapulmonary organ involvement, Löfgren's syndrome, erythema nodosum, serum ACE levels and radiographic staging of the disease; on the other hand, the frequency of involvement of three or more organs was significantly higher in patients with rarer CT genotype at position -857 of the TNF-α gene compared with patients exposing more common CC genotype (p< 0.01) (Table 4). In addition, pulmonary function parameters among VEGF and TNF genotypes were compared. Genotypes at loci VEGF+813 and TNF-α-857 had no significant relations with pulmonary function parameters and values of diffusion capacity of lungs. Furthermore, the relationship between the genotypes and clinical course of sarcoidosis was examined. The cases were followed up at three-month intervals (average, for 33 months). Correlations between relapse rates and high stage (r= 0.29, p< 0.05) and also involvement of three or more organs (r= 0.37, p< 0.05) were determined. Genotypes at loci VEGF+813 showed no significant relation with use of steroid, relapse rates and duration times until reaching remission. Genotypes of TNF-α-857 did not show relation with use of steroid and duration time until reaching remission too, but, in patients with rarer CT genotype at localization TNF-α-857, relapse rates were significantly higher (p< 0.05).

DISCUSSION

There is a lot of marker known determinates activity and prognosis of sarcoidosis but, studies about genetic markers influenced susceptibility to the disease are seldom (12,13). Genetic predisposition for sarcoidosis has long been assumed because of familial occurrence of the disease and the prevalence of the disease in different ethnic groups. It is supposed that the genetic susceptibility is influenced by multiple immune regulatory genes (2,13). Numerous studies have been performed to clarify this aspect and, especially, the highly polymorphic HLA locus has been investigated intensely (2). The findings for HLA associations have not been consistent because of differences in the HLA allele distribution in different ethnicities and additional susceptibility genes adjacent to or within the MHC (14). A study investigated 122 affected siblings from 55 families showed highly significant evidence for the involvement of genes of the MHC region for genetic predisposition to sarcoidosis (15). The analysis suggested that no single gene may account for the significant result but multiple additive MHC gene effects, possibly some of them in linkage disequilibrium, may be considered in conferring susceptibility to sarcoidosis.

The inflammatory response in sarcoidosis is characterized by the production of increased amount of several proinflammatory cytokines at sites of disease, including TNF-α and IL-1 (4). Therefore, particular variant cytokine genotypes might contribute to the predisposition to sarcoidosis or modulate disease severity. There is growing evidence for the contribution of genetic polymorphisms to inter-individual differences in the regulatory mechanisms of cytokine production. TNF-α is thought to play pivotal role for granulomatous reaction of sarcoidosis (12). Yumogaci et al. have previously demonstrated increased TNF-α production by alveolar macrophages obtained by BAL in patients with sarcoidosis and it has been shown that sarcoid granulomas show increased expression of TNF-α messenger RNA (16). Because of the in vitro evidence of inter-individual variations in TNF-α production in the immune response, promoter polymorphism of the TNF gene are of great immunogenetic interest (8). These data support the idea that this gene polymorphism affects the process of disease in sarcoidosis through altered expression of TNF-α. The gene controlled this cytokine is localized at HLA zone of MHC gene

Table 4. Relationship of VEGF and TNF polymorphisms to organ involvement, pulmonary function parameters and relapse rates.

	Position -857 TNF gene				Position +813 VEGF gene			
	CC (%) (n= 35)	CT (%) (n= 33)	TT (%) (n= 2)	p*	CC (%) (n= 59)	CT (%) (n= 10)	TT (%) (n= 1)	p*
Involved organs								
Eye (n= 20)	10 (28)	9 (27)	1 (50)	NS	17 (28)	2 (20)	1 (100)	NS
Skin (n= 11)	4 (11)	7 (21)	0	NS	10 (16)	1 (10)	0	NS
Extrathoracic involvement (n= 31)	18 (51)	12 (36)	1 (50)	NS	28 (47)	3 (30)	0	NS
Löfgren's syndrome (n= 5)	3 (8.57)	2 (6.06)	0	NS	5 (8)	0	0	NS
Erythema nodosum (n= 19)	9 (25)	9 (27)	1 (50)	NS	18 (30)	1 (10)	0	NS
> 3 organs (n= 19)	5 (14)	14 (42)	0	< 0.01	15 (25)	3 (30)	1 (100)	NS
Initial chest radiograph stage II or higher (n= 51)	25 (71)	25 (75)	1 (50)	NS	42 (71)	8 (80)	1 (100)	NS
Relapse rates	3/35 (8.57)	11/33 (33)	0	<0.05	11/59 (18)	3/10 (30)	0	NS
Pulmonary functions parametres	CC (n= 35)		CT (n= 33)	p***	CC (n= 59)		CT (n= 10)	
VC, % predicted**	90 ± 16		87 ± 17	NS	91 ± 15		85 ± 20	NS
FEV_1 /FVC**	79 ± 11		77 ± 10	NS	78 ± 10		76 ± 11	NS
DLCO, % predicted**	76 ± 23		82 ± 17	NS	80 ± 18		77 ± 28	NS

NS: Statistically non-significant, p< 0.05: Statistically significant.

* Chi-square analysis or Fisher exact test.

** Mean ± SD.

*** Kruskal Wallis test.

region (17). Identified gene polymorphisms in this locus associated with TNF-α production offer the opportunity of detecting new genes associated with sarcoidosis.

Genetic analysis has revealed a number of polymorphisms in these genes (18). New polymorphisms with potential functional consequences continue to be discovered. Wilson and colleagues were the first to describe a single base pair transition polymorphism (nucleotide G to A at position -307) in the promoter region of the human TNF-α gene (19). Reporter gene assays have suggested a small but significant effect of polymorphism on the TNF-α transcription with rarer -307 A allele (20). Yamaguchi and Swider found no significant difference between healthy subjects and sarcoidosis patients considering the polymorphisms in the intron 1 of TNF-β gene and -308, -244 and -238 loci of TNF-α gene (21,22). One of the more recently discovered polymorphisms in the promoter region of the human TNF-α gene is a change from C to T at position -857 (23). The transcriptional promoter activity of the rarer TNF-α-857 T allele was shown to be higher than that of the common allele in activated blood mononuclear cells from Japanese donors (24). Grutters et al. examined polymorphism in TNF-α promoter region among sarcoidosis patients and healthy subjects of two different ethnic origins (German and English). In this study, it was pointed out that rarer T allele at position -857 of the TNF-α gene were significantly more frequent in sarcoidosis patients compared with healthy subjects (%26 versus%14) independently of their ethnic origin (25). So, it is possible that the TNF-α -857 T allele may be thought a marker shown susceptibility to sarcoidosis. But, we did not detect a significant difference considering polymorphism at -857 locus of TNF-α gene between sarcoidosis patients and healthy subjects. Our data are in contradiction with those of Grutters et al. who published the only study in literature investigating TNF-α-857 gene polymorphism. We believe that this may be caused by interracial variations of genetic susceptibility to sarcoidosis and effects of polymorphism.

There is not any reliable genetic prognostic marker for a better assessment of sarcoidosis. (12). So, studies investigating the contribution of gene polymorphism into the prognosis of sarcoidosis provoke interest. Yamaguchi et al. performed a first prognostic study on 110 patients with a mean follow-up period of 67 months to evaluate the potential prognostic value of the TNF-α and TNF-β polymorphism (6). In this study, prolonged disease course was found in patients with the TNFB1 allele. Like this result, we detected tendency to more extensive disease and more relapse rate in sarcoidosis patients having rarer CT genotype at TNF-α-857. So, we think that, this genotype may be used as marker for prognosis of sarcoidosis. But, no correlation was detected between TNF-α-857 gene polymorphism and pulmonary function tests or specific organ involvement.

Sarcoidosis is also associated with non-granulomatous microangiopathic lesions (26). VEGF is a pluripotent growth factor has multiple physiological roles in the lung, including the regulation of vascular permeability and the stimulation of angiogenesis (9). VEGF has also been reported to enhance the activation and migration of monocytes which are key events in granuloma formation of sarcoidosis (26). In contrast, VEGF levels in BAL fluid from patients with sarcoidosis were significantly lower than normal controls (27). Low VEGF levels in lung parenchyma may reduce angiogenesis and induce apoptosis of vascular endothelial cells and play role in the pathogenesis of lung involvement of sarcoidosis. In the study of Sekiya et al., serum VEGF concentrations were found significantly higher in patients who received corticosteroid treatment compared to patients with spontaneous remission (28). In addition, VEGF levels were found higher in patients with extrathoracic involvement than in patients in which the disease was limited to the thoracic cage. Based on these findings, the authors suggested that VEGF may represent a marker of disease severity and of extrathoracic involvement in sarcoidosis (28). It is known that, at least two SNPs effect VEGF protein production (10,11). T allele of VEGF at position +813 was associated with significantly

lower VEGF plasma levels in healthy men (11). The polymorphic site at +813 was predicted to lie within a potential binding site for transcription factor activating enhancer binding protein 4 (11). Activator protein 4 is a helix-loop-helix transcription factor enhancing the expression of several viral and cellular genes by binding to specific enhancer sites (29). Therefore, the T allele at +813 might possibly reduce the binding specificity of this motif, resulting in a decrease of the VEGF expression. Individuals who carry the variant T allele might have decreased recruitment of monocytes, which is one of the key pathophysiologic features of sarcoidosis because of low VEGF productions. During our literature screening (Medline, 1996-2006), we encountered only one study investigating the relationship between VEGF gene polymorphism and sarcoidosis. In that study by Morohashi et al., the frequencies of VEGF+813(C/T) and VEGF-627(C/G) polymorphisms in Japanese sarcoidosis patients and healthy Japanese subjects were investigated and it was found that the rarer T allele at position +813 of the VEGF gene was significantly higher (20% versus 11%) in healthy subjects (30). It was concluded that VEGF+813(C/T) polymorphism could be validated as a marker of decreased susceptibility to sarcoidosis. No correlation was reported between VEGF+813 and -627 polymorphisms and extrathoracic involvement. We found that, rarer CT polymorphism at VEGF gene +813 position constituted susceptibility to sarcoidosis but no correlation was detected between this polymorphism and extent of the disease.

Morohashi et al. reported that the cases with the rarer GG genotype at VEGF-627 locus, which is alleged to increase VEGF levels in mononuclear cells, had mean FEV_1/FVC ratios significantly lower than those with other genotypes (GC, CC). However, they found no relationship between VEGF+813 polymorphism which is alleged to decrease VEGF levels and mean FEV_1/FVC ratios. In our study, we also found no association between genotypes at locus VEGF+813 and pulmonary function and diffusion capacity tests of the cases.

In conclusion, our results suggest that VEGF+813 gene polymorphism may constitute a part of susceptibility to sarcoidosis and TNF-α-857 gene polymorphism can be used as a marker indicating severity of the disease in Turkish population.

REFERENCES

1. *McGrath DS, Goh N, Foley PJ, du Bois RM. Sarcoidosis: Genes and microbes-soil or seed? Sarcoidosis Vasc Diffuse Lung Dis 2001; 18: 149-64.*
2. *Luisetti M, Beratta A, Casali L. Genetic aspects in sarcoidosis. Eur Respir J 2000; 16: 768-80.*
3. *Rybicki BA, Major M, Popovich JJ, et al. Racial differences in sarcoidosis incidence: A 5-year study in a health maintenance organization. Am J Epidemiol 1997; 145: 234-41.*
4. *Agostini C, Semenzato G. Cytokines in sarcoidosis. Semin Respir Infect 1998; 13: 184-96.*
5. *Petrek M, Drabek J, Kolek V, et al. CC chemokine receptor gene polymorphism in Czech patients with pulmonary sarcoidosis. Am J Respir Crit Care Med 2000; 58: 293-8.*
6. *Yamaguchi E, Itoh A, Hizawa N, Kawakami Y. The gene polymorphism of tumor necrosis factor-β, but not that of tumor necrosis factor-α, is associated with the prognosis of sarcoidosis. Chest 2001; 119: 753-61.*
7. *Bergeron A, Bonay M, Kambouchner M, et al. Cytokine patterns in tuberculosis and sarcoid granulomas: Correlations with histopathologic features of the granulomatous response. J Immunol 1997; 159: 3034-43.*
8. *Udalova IA, Richardson A, Denys A, et al. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promotor region. Mol Cell Biol 2000; 20: 9113-9.*
9. *Papaioannou AI, Kostikas K, Kollia P, Gourgoulianis KI. Clinical implications for vascular endothelial growth factor in the lung: Friend or foe? Respir Res 2006; 7: 128.*
10. *Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphism within vascular endothelial growth factor (VEGF) gene: Correlation with variation in VEGF protein production. Cytokine 2000; 12: 1232-5.*
11. *Renner W, Kotschan S, Hoffmann C, et al. A common 936 C/T mutation in the gene vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res 2000; 37: 443-8.*
12. *Muller-Quernheim J. Sarcoidosis: Immunopathogenetic concepts and their clinical application. Eur Respir J 1998; 12: 716-38.*
13. *Hunninghake GW, Costabel U, Ando M, et al. Statement on sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 1999; 16: 149-73.*

14. *Mori M, Beatty PG, Graves M, et al. HLA gene and haplotype frequencies in the North American population. Transplantation 1997; 64: 1017- 27.*
15. *Rybicki BA, Kirkey KL, Major M, et al. Familial risk ratio of sarcoidosis in African-American sibs and parents. Am J Epidemiol 2001; 153: 188-93.*
16. *Yamaguchi E, Itoh A, Furuya K, et al. Release of tumor necrosis factor-α from human alveolar macrophages is decreased in smokers. Chest 1993; 103: 479-83.*
17. *Cytokine Gene Polymorphism in Human Disease On-Line Database. http://www.pam.bris.ac.uk/services/GAI/cytokine4.htm*
18. *Seitzer U, Gerdes J, Müller-Quernheim J. Genotyping in the MHC lokus: Potential for defining predictive markers in sarcoidosis. Respir Res 2002; 3: 6.*
19. *Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1992; 1: 353.*
20. *Kroeger KM, Carville KS, Abraham LJ. The -308 tumour necrosis factor-alpha promotor polymorphism effects transcriptions. Mol Immunol 1997; 34: 391-9.*
21. *Yamaguchi E, Itoh A, Hizawa N, Kawakami Y. The gene polymorphism of tumor necrosis factor-β, but not that of tumor necrosis factor-α, is associated with the prognosis of sarcoidosis. Chest 2001; 119: 753-61.*
22. *Swider C, Schnittger L, Bougunia-Kubik K, et al. TNF-alpha and HLA-DR genotyping as potential prognostic markers in pulmonary sarcoidosis. Eur Cytokine Netw 1999; 10: 143-6.*
23. *Ugliolara AM, Turbay D, Pesavento PA, et al. Idendification of three new single nucleotide polymorphism in the human tumour necrosis factor-alpha gene promoter. Tissue Antigenes 1998; 52: 359-62.*
24. *Higuchi T, Seki N, Kamizono S, et al. Polymorphism of the 5'-flanking region of the human tumor necrosis factor-alpha gene in Japanese. Tissue Antigenes 1998; 51: 605-12.*
25. *Grutters JC, Sato H, Pantelidis P, et al. Increased frequency of the uncommon tumor necrosis factor-857T allele in British and Dutch patients with sarcoiddosis. Am J Respir Crit Care Med 2002; 165: 1119-24.*
26. *Tolnay E, Kuhnen C, Voss B, et al. Expression and lokalization of vascular endothelial growth factor and its receptor flt in pulmonary sarcoidosis. Virchows Arch 1998; 432: 61-5.*
27. *Koyama S, Sato E, Haniuda M, et al. Decreased level of vascular endothelial growth factor in bronchoalveolar lavage fluid of normal smokers and patient with pulmonary fibrosis. Am J Respir Crit Care Med 2002; 166: 382-5.*
28. *Sekiya M, Ohwada A, Miura K, et al. Serum vascular endothelial growth factor as a possible prognostic indicator of sarcoidosis. Lung 2003; 181: 259-65.*
29. *Hu YF, Luscher B, Admon A. Transcription factor AP-4 contains multiple dimerization domains that regulate dimer specifity. Genes Dev 1990; 4: 1741-52.*
30. *Morohashi K, Takada T, Omori K, et al. Vascular endothelial growth factor gene polymorphism in Japanese patients with sarcoidosis. Chest 2003; 123: 1520-6.*