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Tumor Necrosis Factor Alpha Gene Polymorphism in Serbian Patients with Sarcoidosis

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SUMMARY

Introduction Sarcoidosis is a multisystemic disease of unknown etiology. Genetic factors play a considerable role in the onset of the disease. Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine which plays an important role in the pathogenesis of the disease and the formation of granuloma by regulating cellular proliferation and apoptosis.

Objective The aim of this study was to investigate the role of TNF-α-308 G/A polymorphism in the development of sarcoidosis and to evaluate the association between the aforementioned type of polymorphism and the clinical course of the disease.

Methods Seventy patients with sarcoidosis and 50 healthy volunteers were genotyped for the TNF-α-308G/A polymorphism. Polymorphism variants were examined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) on the DNA isolated from blood leukocytes.

Results There were no significant differences in TNF-α-308A allele frequency distribution between sarcoidosis patients and the control group, but the TNF-α-308A allele was observed significantly more frequently in the sarcoidosis patients with Löfgren's syndrome when compared with non-Löfgren's patients.

Conclusion We have found that the TNF-α-308A variant is associated with Löfgren's syndrome in Serbian patients with sarcoidosis.

Keywords: sarcoidosis; gene; polymorphism; TNF-α

INTRODUCTION

Sarcoidosis is a multisystemic disease of unknown etiology, characterized by the formation of lymphocytes, mononuclear phagocytes and epithelioid cell granuloma in the affected organs. The pathological changes are most often localized in the lungs. However, all organs within the body can be affected [1, 2]. Although the etiology of the disease is unknown, it is generally accepted that genetic factors play an important role in the onset of the disease. The more frequent occurrence of the disease within certain races and ethnic groups, as well as the described cases of familial sarcoidosis confirms this assumption.

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine which plays an essential role in the appearance of granuloma by regulating cell proliferation and apoptosis [3]. Given the fact that an increased production of TNF-α during a prolonged period of time can result in an uncontrolled inflammation and have considerable negative side-effects on the organism, control production of this cytokine is essential. The regulation is mostly performed at the level of transcription. The TNF-α gene is located on the chromosomal region 6p21.3-21.1, within the highly polymorphic HLA region. The TNF-α gene harbors different types of polymorphisms. Several single nucleotide polymorphisms (SNP) on the TNF-α gene are known to affect TNF-α production. Depending on their location, polymorphisms can result in an increased or a decreased production of TNF-α. The polymorphism of TNF-α-308, at position in the promoter region of the TNF-α gene, occurs in two variants: -308G and -308A alleles. The TNF-α-308A allele is present in the population to a much lesser extent, and it is associated with an increased production of TNF-α [4]. Moreover, it is often connected to autoimmune and inflammatory diseases such as systemic lupus erythematosus, insulin-dependent diabetes mellitus, inflammatory bowel diseases and rheumatoid arthritis [5]. The TNF-α-308A allele is associated with fourfold increased risk for cerebral malaria, the most severe outcome such as mucocutaneous leishmaniasis, severe septic shock and sepsis [6]. In autoimmune diseases, the majority of studies found no association between TNF-α-308 polymorphism and disease predisposition, but the TNF-α-308A allele was more common in patients with severe rheumatoid arthritis, juvenile arthritis, and poor response to anti TNF-α treatment in this disease [7]. In breast cancer, the TNF-α-308A allele is found to be a protective factor in the European populations, but the TNF-α-308 AA genotype may be a risk factor for breast cancer in African women [8]. Previous studies showed controversial results regarding the role of this type

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of polymorphism in the predisposition and clinical course of sarcoidosis [9]. Although previous studies on sarcoidosis have reported TNF-α-308 polymorphism association with a different clinical course of the disease, no such study is available in the Serbian population.

OBJECTIVE

The aim of this research was to investigate the role of TNF-α-308 G/A polymorphism in sarcoidosis and to estimate the association between this polymorphism and the course of the disease.

METHODS

Seventy patients with sarcoidosis (52 women, 18 men), average age 49.9±12.8 (22-67 years) were included in the study. All patients have been under follow-up for at last two years. Acute sarcoidosis was defined as one course of the disease which totally resolved within two years. Chronic sarcoidosis was defined as the disease lasting for over two years or at least two episodes of the disease in the lifetime. All patients were diagnosed at the Clinic of Pulmonary Diseases, Clinical Center Niš, and Clinic of Pulmonary Diseases, Belgrade, Clinical Center of Serbia. The diagnosis of sarcoidosis was based on the ETS/ARS/WASOG criteria [10], including histological confirmation, except in patients with Löfgren's syndrome (n=21), where the diagnosis was based on clinical, radiological and bronchoalveolar lavage fluid findings. This policy is consistent with the World Association of Sarcoidosis and Other Granulomatous Disease (WASOG) guidelines. A written patient's consent was obtained from all subjects. The study was approved by the local Ethical Committee of the Medical Faculty in Niš.

The control group consisted of 50 healthy blood donors (28 women, 22 men), mean age 47.9±14.2. None of them had any evidence of lung disease, autoimmune disease or any symptoms of other diseases. All subjects were of Serbian origin, and were not related.

Genetic analysis

Genetic tests were performed using the patients' whole peripheral blood. TNF-α-308 G/A allelic variants were determined by PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) on DNA isolated from blood leucocytes by a commercial kit (Fermentas UAB, Vilnius, Lithuania), as previously described by Wilson et al. and Yamaguchi et al. [4, 11]. The presence of PCR products was checked on the 2% agarose gel, and visualized under UV light. PCR products were digested with restriction enzyme NcoI. After digestion three genotypes of TNF polymorphism could be identified; homozygote G/G had one band of 97 and one of 20 base pairs (bp), homozygote A/A had only one band of the 117 bp and heterozygotes G/A had three bands of 117, 97 and 20 bp fragments.

Statistical analysis

The statistical analyses were performed using SPSS 17.0 program (SPSS Inc., Chicago, IL) for Windows. The allele ratios and genotype distributions in patients with sarcoidosis and healthy control subjects, radiographic stages, as well as the form of the disease were analyzed using the χ^2 test. The Fisher exact test was also applied for the comparison of small populations with expected values of <5. Tests for Hardy-Weinberg equilibrium were performed by the χ^2 test. Odds ratio was calculated by logistic regression analyses. Probability values less than 0.05 were considered statistically significant.

RESULTS

All patients and control subjects were genotyped for the TNF-α-308 polymorphism. Table 1 shows the demographic characteristics of the analyzed patients and the control group. Chest radiographic staging at the time of diagnosis showed that 35 patients (50%) were in stage I, 24 patients (34.29%) in stage II, and 11 (15.71%) patients in stage III. Löfgren's syndrome was manifested in 21 (30%) patients. Thirty-four patients (48.57%) had acute and 36 (51.43%) chronic course of the disease. Fifty-one patients were administered corticosteroid therapy during the follow-up period. Allele and genotype frequencies in patients and the control group are summarized in Table 2. Genotype frequencies in patients and in the control group were in agreement with Hardy Weinberg's equilibrium. We found no significant differences in the allele and genotype frequencies between the patients with sarcoidosis and healthy subjects.

Table 3 illustrates the distribution of genotype and allele frequencies in patients with and without Löfgren's syndrome. Allele A was statistically more frequent in the patients with Löfgren's syndrome (30.95%) than in the patients without this manifestation (13.26%) ($p<0.05$). There was no statistically signifficant difference in genotype distribution between men and women with Löfgren's syndrome.

Allele A was observed more frequently in patients with acute form of the disease, but this difference was not statistically significant (Table 4).

Table 1. Demographic characteristics of study population

Characteristics		Patients	Control
Number of patients	Man	18	22
	Women	52	28
Age (years)	Mean	49.9±12.8	47.9±14.2
	Range	22–68	28–69
Löfgren's syndrome		21	
Disease	Acute	34 (48.6%)	
	Chronic	36 (51.4%)	
Radiological stage	I	35 (50.0%)	
	II	24 (34.3%)	
	III	11 (15.7%)	
	IV	0	

Values are expressed as number of patient with per cent and mean value ± standard deviation.

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Table 2. Allele and genotype frequencies in sarcoidosis vs. control

Genotype		Patients	Control	OR	95% CI	p
GG		46 (65.71%)	30 (60%)	1.00	Standard	-
GA		22 (31.43%)	15 (30%)	0.96	0.42–2.13	0.53
AA		2 (2.86%)	5 (10%)	0.26	0.05–1.43	0.11
GA+AA		24 (34.29%)	20 (40%)	0.78	0.37–1.66	0.33
Allele	G	114 (81.43%)	25 (25%)	1.00	Standard	-
	A	26 (18.57%)	75 (75%)	0.68	0.36–1.27	0.15

Table 3. Allele and genotype frequencies in patients with Löfgren's syndrome vs. patients without Löfgren's syndrome

Genotype		Löfgren's syndrome	Non Löfgren's syndrome	OR	95% CI	p
GG		10 (47.62%)	36 (66.67%)	1.00	Standard	-
GA		9 (42.85%)	13 (33.33%)	2.49	0.83–7.49	0.09
AA		2 (9.52%)	0	-	-	-
GA+AA		11 (52.37%)	13 (33.33%)	3.05	1.05–8.84	0.03
Allele	G	29 (69.05%)	85 (86.74%)	1.00	Standard	-
	A	13 (30.95%)	13 (13.26%)	2.93	1.22–7.04	0.01

Table 4. Allele and genotype frequencies in patients with acute vs. patients with chronic form of the disease

Genotype		Acute disease	Chronic disease	OR	95% CI	p
GG		19 (55.88%)	27 (75%)	1.00	Standard	-
GA		13 (38.24%)	9 (25%)	2.05	0.73–5.77	0.17
AA		2 (5.88%)	0	-	-	-
GA+AA		15 (44.12%)	9 (25.0%)	2.37	0.86–6.52	0.07
Allele	G	51 (75%)	63 (87.5%)	1.00	Standard	-
	A	17 (25%)	9 (12.5%)	0	0.06–5.67	0.06

DISCUSSION

TNF-α plays several important roles in the body. The production of this cytokine can result both in cell proliferation and cell destruction. In experimental models of granulomatous inflammation TNF-α has a key role in granuloma formation, as it is involved in the early pathogenesis cascade of sarcoidosis [12]. It is generally considered that TNF-α has a double role in the development of sarcoidosis. On one hand, it maintains the chronic inflammation with clustering of inflammed cells, while on the other hand it reduces the apoptosis of T lymphocytes, which contributes to the maintenance of granuloma. Increased levels of TNF-α were detected in sarcoidosis patients' lavage samples [13]. The TNF-α gene is a strong candidate gene for sarcoidosis. Promoter polymorphisms of this gene are of great interest, since there is *in vitro* evidence that they have a role in interindividual variations in the TNF-α production of immune response in sarcoidosis [9].

In this study, we found no influence of the TNF-α-308 genotype as risk for sarcoidosis development in general. Kieszko et al. [14] investigated Polish patients with sarcoidosis and found no association between this type of polymorphism and susceptibility to sarcoidosis. Sharma et al. [15] obtained the same results working on the Indian population. So did Rybicki et al. [16] on African Americans patients and Mrazek et al.[17] when testing Czech patients with sarcoidosis. Only one study found the association between the TNF-α-308A polymorphism and the risk for sarcoidosis, but the study groups were small and consisted of 26 Japanese patients with cardiac sarcoidosis [18].

Our research showed the association of the TNF-α-308A allele with one clinical phenotype in sarcoidosis, Löfgren's syndrome. We found that in our patients carriers of the TNF-α-308A allele had three times higher risk for Löfgren's's syndrome in sarcoidosis. Similar findings were reported by Seitzer et al. [19] in German patients with Löfgren's syndrome. A studa by McDougal et al. [20] showed eight times higher risk for sarcoidosis with erythema nodosum in Caucasian carriers of the TNF-α-308AA genotype. Labunski et al. [21] found a strong association between the TNF-α-308A allele and erythema nodosum in sarcoidosis, but this association was not encountered in erythema nodosum in other diseases. Wijnen et al. [22] showed that the carrier of the TNF-α-308A allele had a favorable prognosis of sarcoidosis.

CONCLUSION

We have established that TNF-α-308-A variant is associated with Löfgren's syndrome in Serbian patients with sarcoidosis. Further investigations performed on a larger number of patients would additionally highlight the importance of this type of polymorphism for the clinical presentation of the disease.

NOTE

This article is a part of research conducted in the process of working on the PhD thesis of Tatjana Radjenović Petković.

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Полиморфизам гена за фактор туморске некрозе алфа код болесника са саркоидозом у Србији

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КРАТАК САДРЖАЈ

Увод Саркоидоза је системска болест непознате етиологије. Сматра се да генетички фактори имају значајну улогу у настанку болести. Фактор туморске некрозе алфа (*TNF-α*) је проинфламаторни цитокин који има значајну улогу у патогенези болести и настанку гранулома регулацијом пролиферације и апоптозе ћелија.

Циљ рада Циљ испитивања је био да се утврде улоге полиморфизма гена за *TNF-α-308G/A* у настанку саркоидозе и њеном клиничком току.

Методе рада Испитано је 70 особа са саркоидозом и 50 здравих добровољаца. Код свих испитаника одређиван је генотип полиморфизма гена за *TNF-α-308G/A* методом *PCR-RFLP*.

Резултати Није примећена значајна разлика у дистрибуцији генотипова и алела између експерименталне и контролне групе испитаника. Међутим, алел *TNF-α-308A* био је знатно чешћи у групи болесника код којих се саркоидоза манифестовала Лефгреновим (*Löfgren*) синдромом у односу на групу болесника без ове клиничке манифестације.

Закључак Може се рећи да је алел *TNF-α-308A* удружен с појавом Лефгреновог синдрома код особа оболелих од саркоидозе.

Кључне речи: саркоидоза; гени; полиморфизам; *TNF-α*

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