Original Investigation
Orijinal Araştırma

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Is there any association between mycosis fungoides and T_h-1, T_h-2 cytokines and transforming growth factor-beta 1 gene polymorphisms?

Mikozis fungoides ile T_h-1, T_h-2 sitokinleri ve transforming growth factor-beta 1 gen polimorfizmleri arasında herhangi bir ilişki var mıdır?

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Abstract

Background and Design: Cytokines are considered to play a crucial role in the development of mycosis fungoides (MF). Cytokine production is under genetic control and allelic variations of cytokine genes are associated with lower or higher production of cytokines. The aim of this study was to determine any possible cytokine gene polymorphisms that may be a risk factor for the development or progression of MF.
Materials and Methods: Genotyping of interferon-gamma (IFN-γ)+874, interleukin (IL)-10-1082, IL-6-174, transforming growth factor-beta 1 (TGF-β1), codon 10 and codon 25, and tumor necrosis factor-alpha (TNF-α)-308 was undertaken in 55 Turkish patients with MF and compared to 50 healthy controls. Polymerase chain reaction (PCR)-restriction fragment length polymorphism was applied for IFN-γ+874, IL-10-1082, IL-6-174 and TNF-α-308 gene polymorphisms. TGF-β1 was genotyped by direct DNA sequencing of PCR amplified gene products for codon 10 and codon 25 polymorphisms.
Results: Genotype distributions showed no significant difference between the patients and the controls for any of the five investigated cytokines. There was no significant difference between advanced stage and early stage cases and healthy controls.
Conclusion: None of the studied cytokine gene polymorphisms are risk factors for the development or progression of MF in the Turkish population, however, further studies are needed.
Keywords: Cytokines, mycosis fungoides, polymorphism

Öz

Amaç: Sitokinlerin mikozis fungoides (MF) gelişiminde önemli rol oynadığı düşünülmektedir. Sitokin üretimi genetik kontrol altındadır ve sitokin genlerinin allelik varyasyonları düşük veya yüksek sitokin üretimi ile ilişkilidir. Bu çalışmanın amacı MF gelişimi veya progresyonunda risk faktörü olabilecek herhangi bir sitokin gen polimorfizminin belirlenmesidir.
Gereç ve Yöntem: Interferon-gamma (IFN-γ)+874, interlökin (IL)-10-1082, IL-6-174, transforme edici büyüme faktör-beta 1 (TGF-β1) kodon 10 ve kodon 25 ve tümör nekroz faktörü-alfa (TNF-α)-308'in genotiplemesi, MF'li 55 Türk hastada yapıldı ve 50 sağlıklı kontrol ile karşılaştırıldı. IFN-γ+874, IL-10-1082, IL-6-174 ve TNF-α-308 gen polimorfizmleri için polimeraz zincirleme reaksiyonu (PCR) - kısıtlama parçası uzunluk polimorfizmi uygulandı. TGF-β1, kodon 10 ve kodon 25 polimorfizmleri PCR ile çoğaltılmış gen ürünlerinin doğrudan DNA dizilemesi ile genotiplendi.
Bulgular: Araştırılan beş sitokinden herhangi birinin genotip dağılımı hasta ve kontrol grubu arasında anlamlı farklılık göstermedi. İleri evre ve erken evre olgular ve sağlıklı kontroller arasında anlamlı fark yoktu.
Sonuç: Çalışılan sitokin gen polimorfizmlerinin hiçbiri, Türk popülasyonunda MF gelişimi veya progresyonu için risk faktörleri değildir, bununla birlikte daha ileri çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Sitokinler, mikozis fungoides, polimorfizm

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Introduction

Cytokines regulate the growth, differentiation and function of immune cells, and immune responses. It has been shown that pro-inflammatory T_h-1 interferon-gamma (IFN-γ) and anti-inflammatory T_h-2 [interleukin 4 (IL4), IL-5, IL-6, and IL-10] cytokines play a role in the pathogenesis of many infectious, autoimmune and malignant diseases[1]. Basal and stimulated cytokine production shows individual and geographic differences, and is affected by genetic factors. Allelic variations of cytokine genes may influence the susceptibility to and prognosis of lymphoproliferative malignancies, including non-Hodgkin's lymphomas (NHL)[2]. Mycosis fungoides (MF) is a mature T-cell NHL, and the most common form of cutaneous T-cell lymphomas. Although the exact pathogenesis of MF is still unknown, several environmental and immunological factors in individuals with genetic predisposition had been suggested. Several chromosomal aberrations have been reported in tumour cells in MF, and rare familial cases support the role of a potential genetic predisposition[3]. Continuous antigenic stimulation of T-cells leads to chronic inflammation and ultimately to the formation of malignant T-cell clones[4,5]. Cytokines play a crucial role in the pathogenesis of MF. $CD8^+$ cytotoxic T-cells and IFN-γ play a major role in the anti-tumour response in patients with early-stage MF. Shift from a T_h-1 (IFN-γ) to a T_h-2 (IL-6, IL-10) cytokine profile accompanies disease progression in MF, and it has been reported that T_h-1/T_h-2 dysfunction plays a key role in the lymphomagenesis[6,7].

The aim of this study was to determine any possible cytokine gene polymorphisms (IFN-γ, IL-6, IL-10, TGF-β1, TNF-α cytokines) of the immune regulatory genes that may present as risk factors for the development or progression of MF.

Materials and Methods

Patients

A total of 55 MF patients and 50 age- and sex-matched healthy control subjects were enrolled in the study after providing informed written consent. All patients were diagnosed with MF on the basis of clinical, histopathological and immunohistochemical findings according to the International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer (ISCL/EORTC) criteria. Forty-seven patients had early-stage MF (IA-IIA), and eight had advanced-stage MF (IIB-IV) according to the Bunn-Lambert staging system[8]. Patients who had a history of atopic disease, family history of MF, autoimmune disorders or any other malignancies were excluded from the study. None of the patients progressed to the advanced stage during the study period. The study was approved by the Bursa Clinical Research Local Ethics Committee (approval number: 2009-7/12) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Clinical and demographic features of the groups are summarized in Table 1.

Cytokine gene polymorphism

The polymerase chain reaction (PCR)-restriction fragment length polymorphism method was applied for genotyping of IFN-γ+874, IL-10-1082, IL-6-174 and TNF-α-308 gene polymorphisms. TGF-β1 was genotyped by direct DNA sequencing of the PCR amplified gene products for codon 10 and codon 25 polymorphisms[9-11].

DNA extraction and genotyping of IFN-γ (T+874A), TNF-α (G308A), IL-10(A-1082G), IL-6 (G-174C) and TGF-β1 (codon 10, codon 25)

Blood samples of the patients were stored in EDTA tubes. Genomic DNA was extracted from the samples using a DNA isolation kit (Dr. Zeydanlı Hayat Bilimleri, Turkey) according to the manufacturer's instructions and then stored at -20 °C until the PCR was performed. Genomic DNA was amplified by PCR using the primers listed in Table 2. All of the restriction enzymes used were purchased from New England Biolabs, Inc. [Beverly, MA, United States of America (USA)]. Genotyping of the subjects is summarized in Table 3. For the TGF-β1 (codon 10, codon 25) polymorphism, forward 5'-TTCCCTCGAGGCCCTCCTA-3' and reverse 5'-CAGGCAGTTTCCTCTGGAAGG-3' primers were used[11]. Direct sequencing of the PCR products was performed. Genotyping of TGF-β1 was carried out by 3130 Genetic Analysers (Applied Biosystems, USA) (Figures 1,2).

Statistical Analysis

SPSS 13.0 for Windows program was used for statistical analysis. Data were summarized and organized into tables. Descriptive statistics are presented as the mean ± standard deviation, minimum, maximum, and median. The chi-square test or the Fisher's exact test was used to analyse the differences between the genotype distribution of cytokines, as well as frequencies of genotype variations among the patients with MF and among healthy controls. The Mann-Whitney U test was used to compare differences between early- and advanced-stage MF patients. A p value of less than 0.05 was considered statistically significant.

Results

A total of 55 MF patients (31 males, 24 females) with a mean age of 49.07±14.69 (15-78) years participated in the study. The mean age of the control group (25 males, 25 females) was 49.04±6.07 (40-64) years. Forty-seven patients were in early patch/plaque stages and eight were in advanced stages according to the ISCL/EORTC criteria (Table 1). Phenotypic expression of the cytokines in patients with MF and healthy controls are shown in Table 3. Genotype distributions showed no significant differences between the patients and the controls for any of the five investigated cytokines. There was no significant difference between advanced- and early-stage cases or healthy controls (Table 4).

Table 1. Clinical and demographic characteristics of the groups

	Mycosis fungoides	Control	p
n	55	50	-
Sex (m/f)	31/24	25/25	0.988
Age£	49.07±14.69	49.04±6.07	0.519
Stage			
Patch/Plaques	47	-	-
Tumour/Sézary syndrome	8	-	-

p<0.05 was considered to be statistically significant, n: Number of the patients, m: Male, f: Female; £: Age was presented as mean ± standard deviation

Table 2. Polymerase chain reaction primers and polymerase chain reaction products

	Primers	Annealing temperature	PCR products (Base-pairs)	Restriction enzyme
IFN-γ (T+874A)	F:5′- TTCTTACAACACAAAATCAAGTC -3′ R:5′- AGTATTCCCAAAAGGCTTATGT -3′	50 °C	366 bp (for A allele→40+26)	Alw26 I
IL-10 (A-1082G)	F:5′- CTCGCTGCAACCCAACTGGC -3′ R:5′- TCTTACCTATCCCTACTTCC -3′	60 °C	139 bp (for G allele→106+33)	Mnl I
IL-6 (G-174C)	F:5′- GCTTCTTAGCGCTAGCCTCAATG -3′ R:5′-TGGGGCTGATTGGAAACCTTATTA -3′	55 °C	116 bp (for C allele→63+53)	Nla III
TNF-Alfa (G308A)	F:5′- AGGCAATAGGTTTTGAGGGCCAT -3′ R.5′-TCCTCCCTGCTCCGATTCCG-3′	60 °C	107 bp (for G allele→80+27)	Nco I

PCR: Polymerase chain reaction, IFN-γ: İnterferon-gama, IL: Interleukin, TNF: Tumor necrosis factor, F: Forward, R: Reverse

Table 3. Genotype distribution of the cytokines in patients with mycosis fungoides and healthy controls

Polimorphisms	MF	Control	Total	p
	n=55	n=50	n=105	
IFN-γ (T+874A)	-	-	-	0.456
TT*	22	19	41	-
TAδ	24	18	42	-
AAμ	9	13	22	-
TNF-α (G308A)	-	-	-	0.391
GG*	45	43	88	-
GAδ	8	7	15	-
AAμ	2	-	2	-
IL-10 (A-1082G)	-	-	-	0.872
AA*	26	25	51	-
AGδ	23	21	44	-
GGμ	6	4	10	-
IL-6 (G-174C)	-	-	-	0.153
GG*	29	17	46	-
GCδ	20	26	46	-
CCμ	6	7	13	-
TGF-β1 codon-10	-	-	-	0.853
TT*	15	15	30	-
TCδ	25	20	45	-
CCμ	15	15	30	-
TGF-β1 codon-25	-	-	-	0.342
GG*	50	47	97	-
GCδ	5	2	7	-
CCμ	-	1	1	-

MF: Mycosis fungoides, IFN-γ: Interferon-gama, n: Number of the patients, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin, TGF-β1: Transforming growth factor-beta, *: Normal cytokine production genotype, δ: Intermediate cytokine production genotype, μ: Low cytokine production genotype, p<0.05 was considered to be statistically significant

Table 4. Genotype distribution of the cytokines in patients with early- and advanced-stage mycosis fungoides patients

Polimorphisms	Early stage	Advanced stage	p
	n=47	n=8	
IFN-γ (T+874A)	-	-	0.228
TT*	17	5	-
TAδ	28	2	-
AAμ	8	1	-
TNF-α (G308A)	-	-	0.113
GG*	40	5	-
GAδ	6	2	-
AAμ	1	1	-
IL-10 (A-1082G)	-	-	0.414
AA*	24	2	-
AGδ	17	6	-
GGμ	6	-	-
IL-6 (G-174C)	-	-	0.153
GG*	25	2	-
GCδ	17	3	
CCμ	5	2	
TGF-β1 codon-10	-	-	0.305
TT*	14	1	-
TCδ	21	4	-
CCμ	12	3	-
TGF-β1 codon-25	-	-	0.338
GG*	42	8	-
GCδ	5	-	-
CCμ	-	-	-

p<0.05 was considered to be statistically significant, n: Number of the patients, IFN-γ: Interferon-gamma, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin, TGF-β1: Transforming growth factor-beta, *: Normal cytokine production genotype, δ: Intermediate cytokine production genotype, μ: Low cytokine production genotype

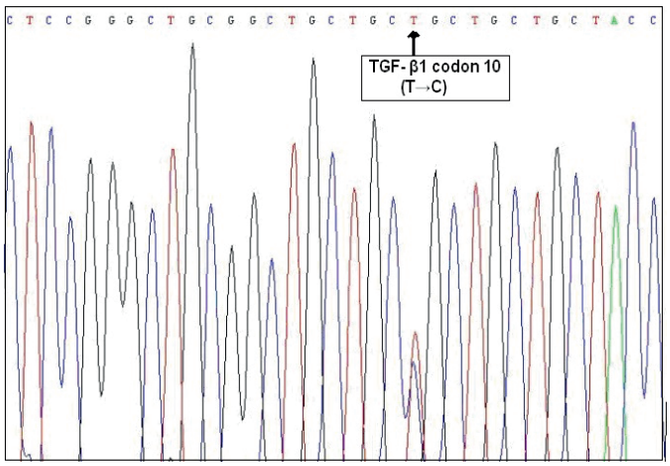

Figure 1. Representation of a subject with T/C genotype
TGF-β1: Transforming growth factor-beta

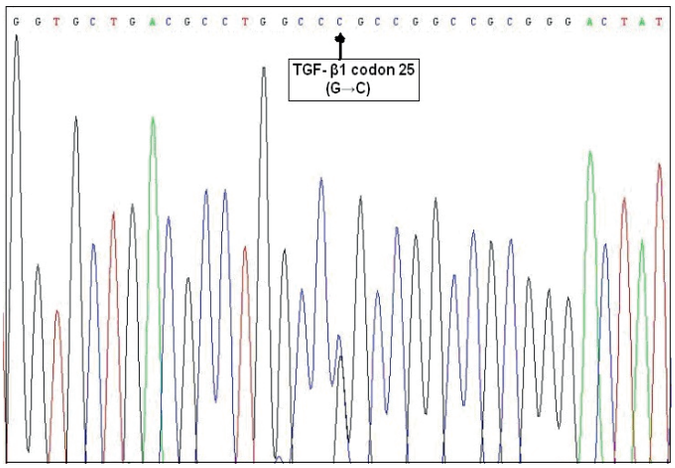

Figure 2. Representation of a subject with G/C genotype
TGF-β1: Transforming growth factor-beta

Discussion

Genetic variation results in altered structure or expression of a cytokine protein leading to pathological consequences (lower or higher production of cytokines), and a predisposition to diseases[12]. Three population-based case-control studies from the USA (1.946 cases and 1.808 controls) reported that genetic variation in T_h-1/T_h-2 cytokine genes might contribute to lymphomagenesis[13]. In their case-control study including 93 patients with NHL and 204 control subjects, Gu et al.[14] showed that polymorphic variations of inflammation - related genes including *TNF-α*, *IL-6* genes could be important for the NHL etiology in the Han Chinese population. Cytokines play a role in the pathophysiology of MF. Th-1 cells produce IFN-γ, which promotes cell-mediated immune response and regulates anti-tumour host defense in early-stage MF. Polymorphism analysis of IFN-γ at position +874 showed no genotype difference in Turkish lung cancer patients[15]. IL-6 is a major mediator of the inflammation, and thought to play a major role in disease progression and to have a prognostic significance in aggressive lymphomas. The common polymorphism reported in the IL-6 gene is -174 G>C[12,16]. Polymorphisms in the promoter region of the TNF-α gene at position-308 had been associated with the outcome of NHL[17]. A current meta-analysis including 136 articles showed that TNF-α -308 allele was associated with increased risk of NHL among Africans and Caucasians, but decreased risk among Asians[18]. IL-10 plays a crucial role in immune regulation by inhibiting pro-inflammatory mediators and promoting the T_h-2 responses. It has been shown that IL-10 plays a role in dendritic cell-related immunosuppression by tumour cells in MF patients[19]. IL-10 and its polymorphisms have been reported to play a role in both the susceptibility to and prognosis of various benign and malignant diseases, including NHL[20]. Two common single nucleotide polymorphisms in immunoregulatory genes (TNF-α G308A and IL-10 T3575A) have recently been reported to be risk factors for NHL in a large pooled analysis[21]. Although the IL-10 gene polymorphism (-1082 A>G) has been linked to the risk of developing lymphoma, in their meta-analysis which included 12 studies, including 5847 cases and 6016 controls, Yu et al.[22] suggested that the IL-10-1082A>G polymorphism was weakly associated with altered susceptibility to lymphoma. TGF-β1 is an immunosuppressive cytokine that inhibits the activity of both T helper cell types and is secreted by reactive T-cells, as well as by tumour cells in MF[23,24]. In their study including 33 patients with MF and 48 controls, Hodak et al.[25] from Israel suggested that patch-stage MF was not determined by a specific genotype polymorphism. Although our results are compatible with that of Hodak et al.[25], we report a different ethnic group, and this is the first report in Turkish patients with a larger study population (55 patients with MF and 50 controls). In addition, even though the number of advanced-stage patients limited in this study, it was suggested that advanced-stage MF is not determined by a specific genotype polymorphism. To the best of our knowledge, this is the first study to investigate the predictive value of possible cytokine gene polymorphisms of immune regulatory genes as a potential risk factor for the development and/or progression of MF with negative results in the Turkish population.

Study Limitations

Its small sample size is the main limitation of this study.

Conclusion

None of the studied cytokine gene polymorphisms are a risk factor for the development or progression of MF in the Turkish population, however, further studies with larger sample size are needed.

Ethics

Ethics Committee Approval: Bursa Clinical Research Ethics Committee (approval number: 2009-7/12).
Informed Consent: Written informed consent was obtained from all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: S.Y., K.A., Design: S.Y., K.A., Data Collection or Processing: S.Y., Analysis or Interpretation: S.Y., K.A., E.B.B., H.S., Ş.T., Literature Search: S.Y., K.A., Writing: S.Y.
Conflict of Interest: No conflict of interest was declared by the authors.
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