



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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Uludağ University Faculty of Medicine, Department of Dermatology and Venereology, Bursa, Turkey

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- β 1), 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- β 1 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- β 1) kodon 10 ve kodon 25 ve tümör nekroz faktörü-alfa (TNF- α)-308'in genotiplenmesi, 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- β 1, 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 hiçbirisi, Türk popülasyonunda MF gelişimi veya progresyonu için risk faktörleri değildir, bununla birlikte daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Sitokinler, mikozis fungoides, polimorfizm

Address for Correspondence/Yazışma Adresi: Serkan Yazıcı MD, Uludağ University Faculty of Medicine, Department of Dermatology and Venereology, Bursa, Turkey Phone: +90 533 277 66 83 E-mail: serkanyazici@uludag.edu.tr **Received/Geliş Tarihi:** 27.09.2017 **Accepted/Kabul Tarihi:** 25.06.2018

ORCID ID: orcid.org/ 0000-0001-6407-0962

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 diseases¹. 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 predisposition³. 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- β 1, 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 system⁸. 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- β 1 was genotyped by direct DNA sequencing of the PCR amplified gene products for codon 10 and codon 25 polymorphisms⁹⁻¹¹.

DNA extraction and genotyping of IFN- γ (T+874A), TNF- α (G308A), IL-10(A-1082G), IL-6 (G-174C) and TGF- β 1 (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- β 1 (codon 10, codon 25) polymorphism, forward 5'-TTCCCTCGAGGCCCTCTA-3' and reverse 5'-CAGGCAGTTTCTCTGGAAGG-3' primers were used¹¹. Direct sequencing of the PCR products was performed. Genotyping of TGF- β 1 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 \pm 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 \pm 14.69 (15-78) years participated in the study. The mean age of the control group (25 males, 25 females) was 49.04 \pm 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 \pm 14.69	49.04 \pm 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 \pm 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'- AGTATTCCTCCAAAAGGCTTATGT -3'	50 °C	366 bp (for A allele→40+26)	Alw26 I
IL-10 (A-1082G)	F:5'- CTCGCTGCAACCCAAGTGGC -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- α (G308A)	F:5'- AGGCAATAGTTTTGAGGGCCAT -3' R:5'-TCCTCCCTGCTCCGATTCCG-3'	60 °C	107 bp (for G allele→80+27)	Nco I

PCR: Polymerase chain reaction, IFN- γ : Interferon-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	-
TA δ	24	18	42	-
AA μ	9	13	22	-
TNF- α (G308A)	-	-	-	0.391
GG*	45	43	88	-
GA δ	8	7	15	-
AA μ	2	-	2	-
IL-10 (A-1082G)	-	-	-	0.872
AA*	26	25	51	-
AG δ	23	21	44	-
GG μ	6	4	10	-
IL-6 (G-174C)	-	-	-	0.153
GG*	29	17	46	-
GC δ	20	26	46	-
CC μ	6	7	13	-
TGF- β 1 codon-10	-	-	-	0.853
TT*	15	15	30	-
TC δ	25	20	45	-
CC μ	15	15	30	-
TGF- β 1 codon-25	-	-	-	0.342
GG*	50	47	97	-
GC δ	5	2	7	-
CC μ	-	1	1	-

MF: Mycosis fungoides, IFN- γ : Interferon-gama, n: Number of the patients, TNF- α : Tumor necrosis factor-alpha, IL: Interleukin, TGF- β 1: 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	-
TA δ	28	2	-
AA μ	8	1	-
TNF- α (G308A)	-	-	0.113
GG*	40	5	-
GA δ	6	2	-
AA μ	1	1	-
IL-10 (A-1082G)	-	-	0.414
AA*	24	2	-
AG δ	17	6	-
GG μ	6	-	-
IL-6 (G-174C)	-	-	0.153
GG*	25	2	-
GC δ	17	3	-
CC μ	5	2	-
TGF- β 1 codon-10	-	-	0.305
TT*	14	1	-
TC δ	21	4	-
CC μ	12	3	-
TGF- β 1 codon-25	-	-	0.338
GG*	42	8	-
GC δ	5	-	-
CC μ	-	-	-

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- β 1: Transforming growth factor-beta, *: Normal cytokine production genotype, δ : Intermediate cytokine production genotype, μ : Low cytokine production genotype

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