The impact of smoking on clinical features of Behçet's disease patients with glutathione S-transferase polymorphisms

H.T.E. Özer[1], R. Günesaçar[1], S. Dinkçi[1], Z. Özbalkan[2], F. Yildiz[1], E. Erken[1]

[1]Department of Medicine, Division of Rheumatology-Immunology, Çukurova University, Faculty of Medicine, Adana, Turkey; [2]Rheumatology Clinic, Ankara Numune Training and Research Hospital, Ankara, Turkey.

Hüseyin T.E. Özer, MD, Assoc. Prof.
Ramazan Günesaçar, PhD, Assoc. Prof.
Suzan Dinkçi, MSc
Zeynep Özbalkan, MD, Assoc. Prof.
Fatih Yildiz, MD
Eren Erken, MD, Professor

Please address correspondence to:
Hüseyin T.E. Özer,
Cukurova University Faculty of Medicine, Balcali,
01330 Yuregir (Adana), Turkey.
E-mail: teozer@cu.edu.tr

Received on October 5, 2011; accepted in revised form on December 21, 2011.

Clin Exp Rheumatol 2012; 30 (Suppl. 72): S14-S17.

Key words: Behçet's disease, smoking, glutathione S-transferase, polymorphism

Funding: this study was supported by the Çukurova University Research Fund.

Competing interests: none declared.

ABSTRACT

Objective. *Various cancer studies have suggested that polymorphism of GSTM1 may influence the ability to detoxify chemicals in cigarette smoke. In the present study the effect of smoking on clinical features of Behçet's disease were investigated in patients having GST-M1 and/or -T1 null polymorphisms.*

Methods. *Ninety-seven patients meeting International Study Group Criteria for Behçet' Disease (63 male, 34 female) and 172 healthy controls (94 male, 78 female) were included into the study. GST-M1 and -T1 polymorphisms were investigated using polymerase chain reaction-restriction fragment length polymorphism technique.*

Results. *Frequency of GSTM1- and/or GSTT1-null polymorphisms were comparable between the Behçet and the control groups. Smoking patients with GSTM1 null-polymorphism have decreased risk of developing papulopustuler lesions (OR=0.227 [0.063–0.818], χ^2=5.463, p=0.019). Non-smoking patients with GSTM1 null-polymorphism has increased risk for having chronic arthritis (OR=5.988 [0.845–43.478]) but smoking patients with GSTM1 null-polymorphism have decreased risk (OR=0.741 [0.593–0.926]). GSTT1 null-polymorphism is associated with the presence of venous insufficiency (χ^2=6.273, p=0.012, OR=2.740 [1.224–6.135]); smoking further increases the risk (χ^2=7.840, OR=3.333 [1.412–7.874], p=0.005). GSTM1 null-polymorphism seemed to effect development of large vessel vasculitis (OR=1.158 [0.981-1.367], χ^2=4.760, p=0.029). Male smoker Behçet patients even have more risk (OR=1.250 [0.971–1.610]).*

Conclusion. *Several manifestations of Behçet's disease may be influenced by smoking, and this effect can be augmented in patients carrying GST gene polymorphism, which code enzymes crucial for the detoxification of chemicals.*

Background

Behçet's disease (BD) is a multisystem vasculitis with unknown etiology manifesting as mucocutaneous, ocular, articular, vascular, gastrointestinal and neurologic involvement. It was first described by a Turkish dermatologist Hulusi Behçet in 1937 (1).

Abnormalities in lipid peroxidation and erythrocyte antioxidant defence system has been reported in BD. Reduced erythrocyte glutathione, glutathione reductase, glutathion peroxidase, catalase, malondialdehyde, and serum Zn values has been found to be lower in Behçet patients (2-4). Glutathion S-tranferase activity has been found to be comparable between Behçet patients and controls (2).

Glutathione S-transferases (GST) are induced under conditions of oxidative stress and play an important role in oxidative stress related syndromes. Their mu (M), pi (P), theta (T) GST variants were reported to be active in the detoxification of numerous products resulting from reactive oxidant damage to DNA and lipids (5). GSTM1, GSTT1, and GSTP1, have been found to have functional polymorphisms. These variants are frequently present in the general populations. Individuals homozygous for the GSTM1 null allele were reported to have a complete absence of GSTM1 activity (6-8).

Introduction

Cigarette smoke contains numerous oxidising substances along with more than 4000 chemicals (9). Cigarette smoke also have been shown to elicit recruitment and adhesion of phagocytes to periferal vascular walls and increased amounts of activated phagocytes in smokers can generate large amounts of free radicals (9).

Various cancer studies have suggested that polymorphism of GSTM1 may influence the ability to detoxify chemicals in cigarette smoke (7, 10,

11). HLA-B51 and cigarette smoking, and especially their combination were reported to be risk factors for chronic progressive NB (12). In patients with rheumatoid arthritis smoking was associated with the most severe disease in patients who carried the GSTM1-null polymorphism (13). In the present study the effect of smoking on clinical features of Behçet's disease were investigated in patients having GST M1 and/or T1 null polymorphisms.

Patients and methods

Ninety-seven patients meeting the International Study Group Criteria for Behçet' disease (63 male, 34 female) and 172 healthy controls (94 male, 78 female) were included into the study (Table I). Patient's past and present clinical features along with smoking history were taken from present visits and hospital records. "Smoker" was assigned for patients who have ever smoked, "non-smoker" were defined as patients who have never smoked. Clinical manifestations were considered to be positive if the patient have ever had the sign in their medical visits or history. Acute arthritis is defined if its duration is less than 6 weeks. Chronic arthritis is defined as arthritis lasting more than 6 weeks. All the patients were given their informed consent and the study was approved by the institutional review board. Drugs taken by the patients in their disease history are listed in Table II.

After blood was drawn to the EDTA containing tubes, genomik DNA was isolated according to the method described by Miller *et al*. (14). GST-M1 and -T1 polymorphism were investigated using polymerase chain reaction-restriction fragment length polymorphysm technique (15). SPSS statistical package program was used for statistical analyses. Chi-square and Fisher exact tests were used for determining clinical associations with GST polymorphysms and chi-square test was used for comparisons between the groups.

Results

Demographic and genetic features of the study groups are shown in Table I.

Frequency of GSTM1- and/or GSTT1-null polymorphisms were comparable between the Behçet and the control groups. However there are a number of positive and negative correlations between the clinical findings and the studied polymorphisms.

In the Behçet group GSTM1-null polymorhysim is associated with decrease risk of developing papulopustuler lesions (OR=0.629, [0.422–0.938]). This effect is also observed in smokers and males (OR: 0.227 [0.063–0.818] and OR=0.660 [0.416–1.046], respectively). In female non-smoker patients with this polymorphysim have decreased the risk of erythema nodosum (OR=0.500 [0.225–1.113]. This polymorphysim also decrease the risk acute arthritis in non-smokers as whole group (OR=0.250 [0.066–0.943]. Risk of developing chronic arthritis also decreased in smoker group (OR=0.741 [0.593–0.926], while non-smoker with this null-polymorphism have increased risk (OR=5.988 [0.845–43.478].

While GSTM1 null-polymorphism seemed to effect development of large vessel vasculitis (OR=1.158 [0.981-1.367], χ^2=4.760, p=0.029). Male smoker Behçet patients even have more risk (OR= 1.250 [0.971–1.610]).

In non–smoking patients with GSTM1 null-polymorphism may confer reduced risk for developing acute arthritis (OR=0.250 [0.066–0.943], χ^2=6.171, p=0.013). While this is not the case for chronic arthritis, non-smoking patients with GSTM1 null-polymorphism has increased risk for having chronic arthritis (OR=5.988 [0.845–43.478]) and smoking patients with GSTM1 null-polymorphism have decreased risk (OR=0.741 [0.593–0.926]).

GSTT1 null-polymorphism is associated with the presence venous insufficiency (χ^2=6.273, p=0.012, OR= 2.740 [1.224–6.135]). When subanalysis of gender is made effect of GSTT1 polymorphism is apparent only in the male patients (OR=4.367 [1.575–12.195], χ^2=9.979, p=0.002). Smokers (only male) also have increased risk of developing venous insufficiency if they have GST-T1 null-polymorphism.

Association studies are summarised in Table III.

Table I. Demographic and genetic features of the study population.

	Behçet	Control	*p*-value
n.	97	172	
Age	36.8 ± 10.4	28.8 ±8.7	
Gender (M/F)	63/34	94/78	0.114
Disease duration (year)	9.6 ± 8.2		
Duration of diagnosis (year)	7.5 ± 6.4		
Smoker	65		
Smoking intensity (package-year)	15 (.02-60) ± 11.5		
GSTM1 null-polymorphism	47	80	0.800
GSTT1 null-polymorphism	27	40	0.463
Both GSTM1 and T1 null- polymorphisms	12	24	0.201

Table II. Drug history of Behçet patients.

Drug	n.	%
Colchicine	61	62.8
Prednisolon	15	15.4
Azathioprin	7	7.2
Cyclosporine	8	8.2
Cyclophosphamide	5	5.1
Sulphasalazine	2	2.1
Aspirin	6	6.2
Coumadin	2	2.1

Discussion

In the literature several studies investigated the impact of smoking on clinical manifestations of Behçet's disease. HLA-B51 and smoking especially their combinations were found to be risk factors for chronic progressive neuro-Behçet's disease. To our knowledge, this is the first study investigating the composite effect of smoking and GST null-polymorphisms in Behçet patients.

Gluhathione dependent enzymes has been shown regulate defence against oxidative stress (5) and detoxify some of the chemicals in cigarette smoke. In the present study Behçet's patients having GSTM1 null-polimorphism was postulated to be more prone to the effects of cigarette smoke.

Table III. Clinical correlations of glutation S-transferase polymorphisms in Behçet's patients.

			GSTM1 null	GSTT1 null	
		χ^2	OR (95% CI)		*p*-value
Papulopustular lesions		5.426	0.629 (0.422–0.938)		0.015
	Male	3.873	0.660 (0.416–1.046)		0.049
	Smoker	5.463	0.227 (0.063–0.818)		0.019
Erythema nodosum	Non-smoker female	5.091	0.500 (0.225–1.113)		0.024
Venous insufficiency		6.273		2.740 (1.224–6.135)	0.012
	Male	9.979		4.367 (1.575–12.195)	0.002
	Smoker	7.840		3.333 (1.412–7.874)	0.005
	Male smoker	7.748		3.690 (1.368–10)	0.005
Large vessel vasculitis	Male	4.760	1.158 (0.981–1.367)		0.029
	Smoker	3.922	1.158 (0.981–1.367)		0.048
	Male smoker	4.788	1.250 (0.971–1.610)		0.029
Acute arthritis	Non-smoker	6.171	0.250 (0.066–0.943)		0.013
Chronic arthritis	Smoker	6.654	0.741 (0.593–0.926)		0.010
	Non-smoker	5.042	5.988 (0.845–43.478)		0.025

In the present study GSTM1 null-polymorphism was found to decreased the risk of developing papulopustulous lesions, if the patient smokes risk decreases more than two times. However GSTT1 null-polymorphism appear to be risk factor for venous insufficiency in the whole group, smoker group, male patients and male smokers patients. Similarly GSTM1 null-polymorphism appear to increase risk for large vessel vasculitis in male and/or smoker Behçet's patients.

Effects of the smoking on the occurence of chronic and acute arthritis are different in patients having GSTM1 null-polymorphism. Non-smokers (predominantly males) seem to have decrease risk for acute arthritis but have increase risk for chronic arthriris. But smokers (male) with null-polymorphism have decreased risk for chronic arthritis.

Some clinical findings of Behçet's disease became manifest and some become silent with the effect of cigarette smoke and presence of GSTM1 and T1-null polymorphisms. These findings cannot be explained solely by the antioxidant effects of GSTs. GSTs also have role in metabolism of various chemicals in the body; and cigarette smoke contains >4000 chemicals some of which may suppress inflammatory activity in Behçet's disease. This concept is also supported by the increase number of oral aftous lesions after quiting smoking (16); and the casual relationship between not smoking and ulcerative colitis and smoking and Crohn's disease (17).

There is gender difference in the presence of clinical findings in the present study, male patients were more effected by the GST polymorphisms or combined effect of smoking and polymorphisms. It is known that Behçet's disease may follow more severe course in male patients (18). Also in the present study 79% of males and 46% of the female patients were smokers. Male patients have also smoked longer and heavier than female counterparts (17.7±12.7 *vs*. 11.3±8.2 package years, respectively, p=0.150)

Some chemicals in the smoke may alleviate Behçet symptoms while some others and oxidative stress can aggrevate the disease. Further studies are needed to deliniate and isolate the chemicals that can be beneficial. Currently smoking cannot be recommended in any way for the management of BD.

Risk of some manifestations were also affected in patients with null-polymorphism who do not smoke. This can be explained by the fact that GST system does not only detoxify chemical in the cigarette smokes, but also various enviromental toxins, and oxidative stress products.

There are several limitations of the study. Principal of them the is the power of the study, making not possible for some subgroup analysis. Second, the smoking status of the patients were described as "ever smoked" or "never smoked". Oral ulcers may increase after cessation of smoking (10). But for this study the number for the patients who just stopped smoking was ignorable to take into consideration. In addition the activation status of the patients are not considered, clinical findings were described if they ever had the manifestation, so oral aftous lesions were not included into statistical analysis. Third there may be confounding factors such as treatment that the patients receive. The manisfestations were recorded to be positive if they ever had the finding in clinic visits as well as from their medical reports. Therefore activation status of the patients were not stated. Nevertheless treatment may be important for the fact that it may conceal some manifestations of the disease before they become apparent. Besides its limitations, this study to our knowledge was the first to show the additional impact of smoking to the presence of GST polymorphisms.

In conclusion, several manifestations of Behçet's disease may be influenced by smoking, and this effect can be augmented in patients carrying GST gene polymorphism, which code enzymes crucial for the detoxification of chemicals.

References

1. BEHÇET H: Über rezidiverende aphthöse, durch ein virüs verursachte geschwure am mund, am auge und an den genitelien. *Dermatol Wochenschr* 1937; 105: 1152-7.
2. DINCER Y, ALADEMIR Z, HAMURYUDAN V,

FRESKO I, AKCAY T: Superoxide dismutase activity and glutathione system in erythrocytes of men with Behçet's disease. *Tohoku J Exp Med* 2002; 198: 191-5.

3. TAYSI S, DEMIRCAN B, AKDENIZ N, ATASOY M, SARI RA: Oxidant/antioxidant status in men with Behçet's disease. *Clin Rheumatol* 2007; 26: 418-22.
4. KÖSE K, YAZICI C, AŞCIOĞLU O: The evaluation of lipid peroxidation and adenosine deaminase activity in patients with Behçet's disease. *Clin Biochem* 2001; 34: 125-9.
5. HAYES JD, MCLELLAN LI: Glutathione and glutathione dependent enzymes represent a coordinately regulated defense against oxidative stress. *Free Radical Res* 1999; 31: 273-300.
6. NEBERT D, VASILIOU V: Analysis of the glutathione S-transferase (GST) gene family. *Hum Genome* 2004; 1: 460-4.
7. REBBECK TR: Molecular epidemiology of the human glutathione Stransferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 733-43.
8. XU S, WANG Y, ROE B, PEARSON WR: Characterization of the human class Mu glutathione S-transferase gene cluster and the GSTM1 deletion. *J Biol Chem* 1998; 273: 3517-27.
9. CROSS CE, VAN DER VLIET A, EISERICH JP: Cigarette smokers and oxidant stress: a continuing mystery. *Am J Clin Nutr* 1998; 67: 184-5.
10. SEIDERGARD J, PERO RW, MARKOWITZ MM, ROUSH G, MILLER DG, BEATTIE EJ: Isoenzyme(s) of glutathione S-transferase (class mu) as a marker for the susceptibility to lung cancer: a follow up study. *Carcinogenesis* 1990; 11: 33-6.
11. CHENEVIX-TRENCH G, YOUNG J, COGGAN M, BOARD P: Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. *Carcinogenesis* 1995; 16: 1655-7.
12. ARAMAKI K, KIKUCHI H, HIROHATA S: HLA-B51 and cigarette smoking as risk factors for chronic progressive neurological manifestations in Behçet's disease. *Mod Rehumatol* 2007; 17: 81-2.
13. MATTEY DL, HUTCHINSON D, DAWES PT *et al*.: Smoking and disease severity in rheumatoid arthritis, association with polymorphism at the glutathione S-gransferase M1 locus. *Arthritis Rheum* 2002; 46: 640-6.
14. MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1998; 16: 1615.
15. BEEGHLY A, KATSAROS D, CHEN H *et al*.: Glutathione S-transferase polymorphisms and ovarian cancer treatment and survival. *Gynecologic Oncology* 2006; 100: 330-7.
16. SOY M, ERKEN E, KONCA K, OZBEK S: Smoking and Behçet's disease. *Clin Rheumatol* 2000; 19: 508-9.
17. CALKINS BM: A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci* 1989; 34: 1841-54.
18. KURAL-SEYAHI E, FRESKO I, SEYAHI N *et al*.: The long term mortality and morbidity of Behçet syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. *Medicine* (Baltimore) 2003; 82: 60-76.