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INTERLEUKIN-6 PROMOTER POLYMORPHISM (-174 G/C) IN EGYPTIAN PATIENTS WITH BEHCET's DISEASE

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ABSTRACT

Behçet's disease (BD) is a multisystem inflammatory disease characterized by recurrent orogenital ulcerations, ocular inflammations, and skin lesions. The etiology of the disease is currently unknown but evidences suggested that there is a strong genetic component mediating the chronicity of the disorder. Cytokines seem to have important roles in the pathogenesis of BD. Its production could be affected by genetic polymorphisms. Thus, this study aimed at investigating the associations between BD in Egyptian population and IL-6 (-174) promoter polymorphism. We genotyped IL-6 (-174) position in the promoter region of IL-6 using Mutagenically Separated PCR (MS-PCR) in 61 Egyptian patients with BD and 97 healthy-matched control. No significant association between IL-6 (-174) polymorphism and BD was found. Only two genotypes were seen in the gel GC and GG there was no CC in BD or control. No significant association between IL-6 (-174 G/C) polymorphisms and BD was demonstrated. Moreover, there was no significant difference between G or C allele distribution between BD and controls. Comparing patients with various disease manifestations, we found no significant association with the -174 G/C genotypes. A remarkable increase (even not statistically

significant) in the frequency of IL-6 -174 GG (92.6%) in BD patients in active state as compared to BD inactive (73.5%) was found. In conclusion, our data revealed that IL-6 -174 promoter polymorphism does not contribute significantly to BD disease susceptibility or to distinct clinical feature.

INTRODUCTION

BD is a systemic vasculitis of unknown etiology characterized by recurrent oral ulcers, genital ulcers, skin lesions, and uveitis. It may also involve vessels of all types and sizes, arthritis, central nervous system disease, and gastrointestinal tract diseases. Patients with BD may manifest all or only some of these clinical features [(1–4).] BD has a worldwide distribution, but it is more prevalent in the regions along the ancient trading route known as "Silk Road," extending from Mediterranean countries such as Turkey and Iran to the Far East including Korea and Japan. The prevalence of BD in Turkey is particularly high at 80–420 ⁄ 100,000 [(5)]. In Egypt the prevalence is at 7.6/100,000 [(6)].

Although its etiopathogenesis remains elusive, the most widely accepted hypothesis is that the excessive inflammatory response is triggered by an infectious agent in a genetically susceptible host [(1)]. Human leukocyte antigen (HLA)–B51 has been identified as the genetic marker most strongly associated with BD, but it has also been reported that the highest contribution made by the HLA-B locus to overall genetic susceptibility to BD is 19%.This is why other susceptibility genes have been extensively investigated in BD. [(7-11)].

The disease is characterized by infiltration of lymphocytes and neutrophils into the affected organs. It is now well known that cytokines play critical roles in the pathogenesis of BD, because they mediate many of the effectors and regulatory functions of immune and inflammatory responses [(12-17)]. cytokines are considered to cause one of two types of responses: T-helper cell type 1 (Th1) responses, produces interleukin (IL)-2, IFN-γ, TNF-α and facilitates cell-mediated immune responses and elicit delayed hypersensitivity reactions; or Th2 response, produces mainly IL-4, IL-5, IL-6 , IL-10

and IL-13 and assists in antibody production and suppress cell mediated immunity [14,18-20]. IL-6 is an important mediator of inflammatory and immune responses, and IL6 gene polymorphisms are known to play a part in chronic inflammatory and autoimmune disorders [21,22]. Increased IL6 plasma levels and enhanced IL6 mRNA expression have been found in patients with BD especially those in active state [23,24]. Cytokine production has been shown to be under genetic control. Polymorphisms in several cytokine genes influence gene transcription, leading to interindividual variations in cytokine production [25-27]. This study aimed to ascertain the possible involvement of IL6-174 promoter polymorphisms on the susceptibility to BD and whether it has correlation with any of the clinical findings.

MATERIALS AND METHODS

Patients and Controls:

Blood samples, collected in Ethylene Diamine Tetraacetic acid (EDTA) sterile tubes, from 61 BD patients (48 men and 13 women; mean age 34.4 ± 10.69) recruited from the Department of Rheumatology at El-Kasr El-Aini hospital. All patients were diagnosed according to the International Study Group for Behcet's Disease (ISG) [28]. Patients who had other autoimmune disease, infection or malignancy were excluded from the study**.** The onset of the syndrome was defined as the time when the patient fulfilled the diagnostic criteria. At time of blood sampling, patients with two or more lesions in the previous 4 weeks (including oral ulcers, genital ulcers, skin lesions, uveitis, vascular, arthritis, gastrointestinal lesions, central nervous system lesions, and pulmonary involvement) were regarded to have active disease [29]. A control group was composed of 97 healthy donors unrelated to each other or to the patients, matched for age and sex. The study was approved by our institutional ethics committee and written consent was obtained from all patients**.**

DNA isolation and cytokine genotyping:

Genomic DNA was extracted from blood samples by using GeneJET™ Genomic DNA Purification Kit according to the manufacturer's instructions.

IL-6 - 174 G/C Genotyping :

Single nucleotide polymorphism (SNP) was analyzed in the promoter region of the IL-6 gene at position -174 by Mutagenically separated PCR (MS-PCR) assay [30]. The reaction is down in one tube, so at least one PCR product was found in a single reaction, thus avoiding false negative result. Additionally, base substitutions at different mutagenic positions were introduced into the allele specific primers, this allow the clear separation between the two alleles during amplification steps by reduction of the cross reactions.

Briefly, we used three primers mixes, two allele specific primers of different length forward primer G: 5'-GCACTTTTCCCCCTAGTTGTGTCTTACG-3'; forward primer C: 5'GACGACCTAAGCTTTACTTTTCCCCCTAGTTGTGTCTTGAC-3'; and nonspecific reverse primer: 5'ATAAATCTTTGTTGGAGGGTGAGG-3'. The final volume for each PCR reaction 25 μL; the PCR mixture consist of PCR Master Mix (2X) (Fermentas). 20 mM of each forward primer, 40 mM of reverse primer and 40 ng of DNA. PCR cycling conditions consisted of 95°C, 10 min [1 cycle]; followed by 94°C, 30 seconds, 66°C, 45 seconds, 72°C, 45 seconds [40 cycles]; and finally 72°C, 7 min [1 cycle]. The PCR products were visualized by 4% agarose gel electrophoresis with ethidium bromide staining. The PCR products were 121 bp (G allele) and 136 (C allele) (Figure 1).

Statistical analysis:

All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 11 (LEAD Technology Inc). Data were presented as means with corresponding standard error (SD). Comparisons among different groups were performed by independent T test. Allele frequencies were estimated by direct gene counting. Each polymorphism was examined in the control population to confirm that the distribution of the genotypes conformed to Hardy–Weinberg expectations (available at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The distribution of alleles and genotypes for each polymorphism was compared between cases and controls using a chi-square test (X^2). The odds ratio (OR) and 95% confidence intervals

(CI) were calculated to assess the risk associated with particular alleles or genotypes in both patient and control groups.

RESULTS

Patient's characteristics:

Sixty-one patients with a mean age of 34.4±10.69 years (range 18-67) years were included in our study. The mean disease duration from the onset time to study entry was 7.36 ±6.27 years (range 1-28 years). There was no significant difference in age or sex between the selected group of patients and healthy controls. None of the patients had a recent history of infection or malignancies. The detailed demographic and clinical characteristics of our patients are presented in Table (1).

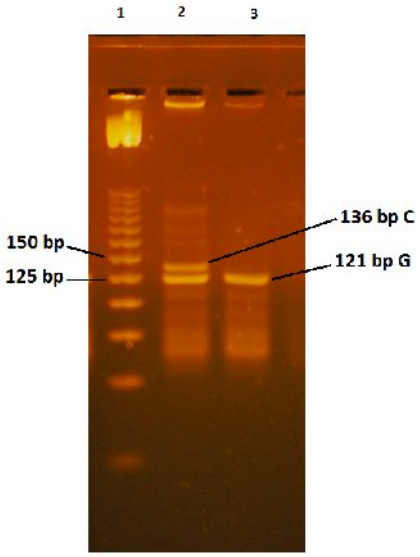

Figure (1): Polymerase chain reaction (PCR) products of IL-6 promoter polymorphism (-174 G/C) (4% agarose stained with

ethidium bromide). Lane (1) 25 bp ladder; lane (2) GC genotype and lane (3) GG genotype. There is no CC genotype.

Table (1): Demographic, clinical features and laboratory data of 61 patients with BD.

Parameter	Value
Demographic Data	
Age (mean ± SD)	34.4 ± 10.69
Disease Duration (mean ± SD)	7.36 ± 6.27
Male/ Female	48/ 13
Clinical Involvement	
Oral Ulcers (%)	61 (100%)
Genital Ulcers (%)	57 (93.4%)
Ocular Involvement (%)	36 (59%)
Skin Lesion (%)	35 (57.4%)
Vascular (%)	16 (26.2%)
Neuro (%)	13 (21.3%)
Arthritis	16 (26.2%)
GIT (%)	3 (4.9%)
Chest (%)	4 (6.5%)
Active patients (%)	27 (44.3%)
Laboratory Investigations	
ESR mmHg/hr (mean ± SD)	37.85 ± 20.40
Heamoglobin gm% (mean ± SD)	13.34 ± 1.3
WBC 1000/mm^3 (mean ± SD)	8.86 ± 4.4
Neutrophils 1000/mm^3 (mean ± SD)	58.82 ± 11.4
PLT 1000mm^3 (mean ± SD)	269.51 ± 57.3

IL-6 promoter polymorphisms -174 G/C in BD patients and controls:

IL-6 (- 174) genotypes and allele frequencies in patients with BD and healthy controls are shown in Table (2). Only two genotypes

were seen in the gel GC and GG there was no CC in BD or control. IL-6 - 174 GG and -174 GC genotypes were higher in healthy controls than in BD patients (73.3% and 22.7% versus 82% and 18%; respectively). However, no significant association between IL-6 (-174 G/C) polymorphisms and BD was demonstrated. Moreover, there was no significant difference between G or C allele distribution between BD and controls. The frequency of SNP genotype frequencies in patients and control was in Hardy-Winberg equilibrium (75%, 22%, 0% (observed) versus 76.25%, 19.51%, 1.25% (predicted).

Table (2): Genotype distribution and allelic frequencies of the IL-6 promoter polymorphism (-174 G/C) in patients with BD and control.

Cytokine gene	Control group (N=97)	BD group (N=61)	*P*	OR (95% CI)
Genotype				
G/G	75 (77.3%)	50 (82%)	NS	1.333 (0.595-
G/C	22 (22.7%)	11 (18%)	NS	2.989)
C/C	-	-	-	0.750 (0.335-
GCCC	22 (22.7%)	11 (18%)	NS	1.682)
				-
Allele				0.750 (0.335-
Frequency	172 (88.7)	111 (90.9)	NS	1.682)
G	22 (11.3)	11 (9.1)	NS	
C				
				-
				0.750 (0.335-
				1.682)

IL-6 promoter polymorphisms -174 G/C in clinical manifestation of BD:

The genotype distribution of IL-6 promoter polymorphism -174 G/C in BD patients with different clinical manifestations are shown in Table (3). Although, GG genotype is generally increased (as compared to GC genotype) in all clinical manifestations, no significant change in genotype frequency in BD patients was

demonstrated. A remarkable increase (even not statistically significant) in the frequency of IL-6 -174 GG (92.6%) in BD patients in active state as compared to BD inactive (73.5%) was found.

Table (3): Association between clinical findings and IL-6 -174 gene polymorphism in the patients with Behcet's disease.

Clinical Involvement	Yes	NO
Oral Ulcers N (%)	61 (100%)	0 (0%)
GG (%)	50 (82%)	0 (0%)
GC (%)	11 (18%)	0 (0%)
Genital Ulcers N (%)	57 (93.4%)	4 (6.6%)
GG (%)	46 (80.7%)	4 (100%)
GC (%)	11 (19.3%)	0 (0%)
Ocular Involvement N (%)	36 (59%)	25 (41%)
GG (%)	30 (83.3%)	20 (80%)
GC (%)	6 (16.7%)	5 (20%)
Skin Lesion N (%)	35 (57.4%)	26 (42.6%)
GG (%)	29 (82.9%)	21 (80.8%)
GC (%)	6 (17.1%)	5 (19.2%)
Vascular N (%)	16 (26.2%)	45 (73.8%)
GG (%)	13 (81.2%)	37 (82.2%)
GC (%)	3 (18.8%)	8 (17.8%)
Neuro N (%)	13 (21.3%)	48 (78.7%)
GG (%)	10 (77%)	40 (83%)
GC (%)	3 (23%)	8 (16.7%)
Arthritis N (%)	16 (26.2%)	45 (73.8%)
GG (%)	13 (81.2%)	37 (82.2%)
GC (%)	3 (18.8%)	8 (17.8%)
GIT N (%)	3 (4.9%)	58 (95.1%)
GG (%)	3 (100%)	47 (81%)
GC (%)	0 (0%)	11 (19%)
Chest N (%)	4 (6.5%)	57 (93.5%)
GG (%)	3 (75%)	47 (82.5%)
GC (%)	1 (25%)	10 (17.5%)
Active patients N (%)	27 (44.3%)	34 (55.7%)
GG (%)	25 (92.6%)	25 (73.5%)
GC (%)	2 (7.4%)	9 (26.5%)

*CC genotype excluded from the table because it is not present.

DISCUSSION

IL-6 (26 kDa) is a multifunctional pro-inflammatory cytokine with an important role in the regulation of immune response [(31)]. It is produced by monocytes, epithelial cells and fibroblasts, and causes polyclonal B-cell activation, hypergammaglobulinaemia, and autoantibody production with T-cell activation [(32).] The major effect of IL-6 described is the proliferation and differentiation of cells, as well as increasing secretion of acute-phase proteins by the liver [(33).] Abnormal IL-6 production has been implicated in some autoimmune diseases and chronic inflammatory reactions [(34-36)]. BD has been labeled as a Th1 driven disease. Conversely IL-6, which promotes Th2 cell differentiation, inhibits IFN-γ production and inhibits Th1 cell differentiation was shown in many studies to be elevated in serum of BD compared to control [(37-43)].

Active BD patients are characterized by a higher levels of IL-6 compared to that found in remission [(23,39-41,44)]. It seems that IL-6 plays a unique role in BD with central nervous system involvement. Early in 1992; it was reported that a significantly higher level of IL6 in cerebrospinal fluid (CSF) had been observed in Chinese BD patients in active stage of the disease with central nervous system (CNS) involvement, the level of IL6 decreased along with the ameliorating of the disease [(45)]**.** The Same results have been verified in patients from both Turkey and Japan population and showed the association of IL6 with clinical findings of BD [(46,47)]**.** Production of cytokines could be affected by genetic polymorphisms within promoter regions of cytokine genes. Therefore, this study aimed at investigating the associations between BD disease in Egyptian people and IL-6 gene polymorphism as a single nucleotide polymorphism (SNP) at -174 (G/C) in the IL-6 gene promoter region.

According to our findings, there was no evidence for genetic association between any genotype of IL-6 (-174) SNP and the susceptibility to BD. This result is consistent with the results of Dilek et al. [(48)] and Chang et al. [(49)] in Turkish and Korean populations, respectively. Consistency of our results with previous investigations approved a considerable interethnic variability in the distribution of

the IL6 -174 G/C genotypes. Although, a distinguish difference in the frequency of IL-6 -174 G/C genotypes in healthy controls (CC 0%, GC 22.7% and GG 77.3% in Egyptian, CC 0%, GC 96.7 and GG 3.3% in Korean and CC 8.2%, GC 30.3% and GG 61.5% in Turkish populations) was detected. These variations in the frequency of healthy control genotype stressed the importance of recording the frequency of different genotype in healthy control of distinct population even from the same geographic region and they should be compared to patients.

In contrast to our study, Amirzargar et al., [50], found a significant decrease in the heterozygous IL-6 (-174) GC genotype in Iranian patient. This difference could be returned to the ethnic variation of 2 populations as many previous investigations showed considerable interethnic variability in the distribution of the IL-6 (-174) [21, 22,51].

Polymorphisms do not exist in isolation, and it could be the combination of several substitutions in different parts of whole cytokine gene, *i.e.* the haplotype. In this regard, interpretation of results just based on single changes is over simplification and could be misleading [42]. Chang et al., [49] found significant differences in the IL6 variable number tundum repeats (vntr) genotype and allele frequencies between patients with BD and controls. These differences were particularly apparent in the HLA-B51 negative subjects or female patients. In addition, the same study found that susceptibility to BD was increased significantly in subjects carrying the IL6 -174 G/IL6vntr/C haplotype. They showed that IL6 -174 is associated with BD not alone as a separate SNP but as part of a haplotype.

Frequency of IL-6 -174 GG (92.6%) was increased in BD patients in active state as compared to BD inactive (73.5%) ones, although it did not reach statistically significance. Many studies showed that the genotype GG is corresponds to a higher production of IL-6 [21,52-55], or at least is part of the haplotypes of IL-6-promoter associated with increased transcription level [52]. This may explains the high level of IL-6 in the serum of patients with active BD because the genotype GG which is more dominated in active BD patients is associated with higher level of IL-6 production.

In conclusion, our data approved that different IL-6 -174 G/C genotypes has no association with BD occurrence indicating that IL-6 -174 G/C gene polymorphism may be not involved in the susceptibility to BD. Our results give a shed of light that IL-6 -174 G/G may have a role in activity state of BD disease. To confirm our findings, further investigations are required on larger population and with detection of serum IL-6 levels and their correlation with documented polymorphism.

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REFERENCES

1- Wallace, G.R.; Kondeatis, E.; Vaughan, R.W.; Verity, D,H.; Chen, Y.; Fortune, F.; Madanat, W.; Kanawati, C.A.; Graham, E.M. and Stanford, M.R. (2007): IL-10 genotype analysis in patients with Behcet's disease. Hum Immunol 68(2):122–127.

2- Harmouche, H.; Maamar, M.; Sahnoune, I.; Tazi-Mezalek, Z.; Aouni, M. and Maaouni, A. (2007): Fever revealing Behcet's disease: two new cases. Eur J Intern Med 18(2):146–147.

3- Schwartz, T.; Langevitz, P.; Zemer, D.; Gazit, E.; Pras, M. and Livneh, A. (2000): Behcet's disease in familial Mediterranean fever: characterization of the association between the two diseases. Semin Arthritis Rheum. 29(5): 286–295.

4- Yates, P.A. and Michelson, J.B. (2006): Behçet disease. *Int Ophthalmol Clin.* 46(2):209-33.

5- Azizlerli, G.; Kose, A.A.; Sarica, R.; Tutkun, I.T.; Kulaç, M.;
Tunç, R.; Urgancioğlu, M. and Dişçi, R. (2003): Prevalence of Behcet's disease in Istanbul, Turkey. Int J Dermatol 42(10):803–6.

6- Assaad-Khalil, S.H.; Kamel, F.A. and Ismail, E.A. (1997): Starting a regional registry for patients with Behçet's disease in North West Nile Delta region in Egypt. In: Hamza M (ed) Behçet's disease. Pub Adhoua, Tunis (Tunisia), pp 173–176.

7- Gul, A.; Hajeer, A.H.; Worthington, J.; Barrett, J.H.; Ollier, W.E.R. and Silman, A.J. (2001): Evidence for linkage of the HLA–B locus in Behçet's disease, obtained using the transmission disequilibrium test. Arthritis Rheum 44:239.

8- Gul, A. (2001): Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol.* 19: S6-12.

9-Mizuki, N.; Ota, M.; Katsuyama, Y.; Yabuki, K.; Ando, H.; Goto, K.; Nakamura, S.; Bahram, S.; Ohno, S. and Inoko, H. (1999): Association analysis between the MIC-A and HLA-B alleles in Japanese patients with Behçet's disease. *Arthritis Rheum.* 42:1961-6.

10- Verity, D.H.; Marr, J.E.; Ohno, S.; Wallace, G.R. and Stanford, M.R. (1999): Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. *Tissue Antigens*. 54(3): 213-20.

11- Chang, H.K.; Kim, J.U.; Cheon, K.S.; Chung, H.R.; Lee, K.W. and Lee, I.H. (2001): HLA-B51 and its allelic types in association with Behçet's disease and recurrent aphthous stomatitis in Korea. *Clin Exp Rheumatol.* 19(5 Suppl 24):S31-5.

12- Yazici, H. (2004): The lumps and bumps of Behçet's syndrome. Autoimmun Rev. 3: S53–4.

13- Ahn, J.K.; Yu, H.G.; Chung, H. and Park, Y.G. (2006): Intraocular cytokine environment in active Behçet uveitis. *Am J Ophthalmol.* 142: 429-34.

14- Ben, A.M.; Houman, H.; Miled, M.; Dellagi, K. and Louzir, H. (2004): Involvement of chemokines and Th1 cytokines in the pathogenesis of mucocutaneous lesions of Behçet's disease. Arthritis Rheum. 50:2291–2295.

15- Alpsoy, E.; Cayirli, C.; Er, H. and Yilmaz, E. (1998): The levels of plasma interleukin-2 and soluble interleukin-2R in Behçet's disease: a marker of disease activity. *J Dermatol.* 25: 513-6.

16- Imamura, Y.; Kurokawa, M.S; Yoshikawa, H.; Nara, K.; Takada, E.; Masuda, C.; Tsukikawa, S.; Ozaki, S.; Matsuda, T. and Suzuki, N. (2005): Involvement of Th1 cells and heat shock protein 60 in the pathogenesis of intestinal Behçet's disease. Clin Exp Immunol. 139: 371–378.

17- Lacomba, M.S.; Martin, C.M.; Chamond, R.R.; Galera, J.M; Omar, M. and Estevez, E.C. (2000): Aqueous and serum interferon gamma, interleukin (IL) 2, IL-4, and IL-10 in patients with uveitis. *Arch Ophthalmol.* 118: 768-72.

18- Beutler, B. and Cerami, A. (1989): The biology of cachectin/ TNF--a primary mediator of the host response. *Annu Rev Immunol.* 7: 625-55.

19- Nicholson, L.B. and Kuchroo, V.K.; (1996): Manipulation of the Th1/Th2 balance in autoimmune disease. Curr Opin Immunol. 8:837–842

20- Rajendram, R. and Rao, N.A. (2003): Molecular mechanisms
in Behc¸et's disease. Br J Ophthalmol. 87:1199–1200

21- Fishman, D.; Faulds, G.; Jeffery, R.; Mohamed-Ali, V.; Yudkin, J.S.; Humphries, S. and Woo, P. (1998): The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 102:1369–76.

22- Murray, R.E.; McGuigan, F.; Grant, SF.; Reid, D.M. and Ralston, S.H. (1997): Polymorphisms of the interleukin-6 gene are associated with bone mineral density. Bone. 21:89–92.

23- Hamzaoui, K.; Hamzaoui, A.; Guemira, F.; Bessioud, M.; Hamza, M. and Ayed, K. (2002): Cytokine profile in Behcet's disease patients. Relationship with disease activity. Scand J Rheumatol. 31:205–10.

24- Koarada, S.; Haruta, Y.; Tada, Y.; Ushiyama, O.; Morito, F.; Ohta, A. and Nagasawa, K. (2004): Increased entry of CD4_ T cells into the Th1 cytokine effector pathway during T-cell division following stimulation in Behçet's disease. Rheumatology (Oxford). 43:843.

25- Turner, D.M.; Williams, D.M.; Sankaran, D.; Lazarus, M.; Sinnott, P.J. and Hutchinson, I.V. (1997): An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*. 24: 1-8.

26- Pravica, V.; Asderakis, A.; Perrey, C.; Hajeer, A.; Sinnott, P.J. and Hutchinson, I.V. (1999): *In vitro* production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. *Eur J Immunogenet*. 26: 1-3.

27- Warlé, M.C; Farhan. A.; Metselaar, H.J.; Hop, W.C.; Perrey, C.; Zondervan, P.E.; Kap, M.; Kwekkeboom, J.; Ijzermans, J.N.; Tilanus, H.W.; Pravica, V.; Hutchinson, I.V. and Bouma, G.J. (2003): Are cytokine gene polymorphisms related to *in vitro* cytokine production profiles? *Liver Transpl.* 9: 170-81.

28- International Study Group (ISG) for Behcet's Disease. (1990): Criteria for dignosis of Behcet's disease. *Lancet.* 335: 1078-1080.

29- Aydintug, A.O.; Tokguz, G.; Ozoran, K.; Duzgun, N.; Gurler, A. and Tutkak, H. (1995): Elevated levels of soluble intercellular adhesion molecule-1 correlate with disease activity in Behcet's disease. *Rheumatol Int*. 15: 75-78.

30- Rust, S.; Funke, H. and Assmann, G. (1993): Mutagenically separated PCR (MS-PCR): a highly specific one step procedure for easy mutation detection. Nucleic Acids Res. 11;21(16):3623-9.

31. Lie.; J.T. (1992). Vascular involvement in Behc¸et's disease: arterial and venous vessels of all sizes. J Rheumatol; 19: 341-343.

32. Schmitz-Huebner, U. and Knop, J. (1984). Evidence for an endothelial cell dysfunction in association with Behc¸et's disease. Thromb Res; 34: 277-285.

33. Orem, A.; Cimsit, G.; Deger, O,; Vanizor, B. and Karahan, S.C. (1999). Autoantibodies against oxidatively modified low-density lipoprotein in patients with Behc¸et's disease. Dermatology; 198: 243-246.

34- Kallen, K.J. (2002): The role of transsignalling via the

agonistic soluble IL-6 receptor in human diseases. Biochem
Biophys Acta. 1592:323-43.

35- Evans, C.A.; Jellis, J.; Hughes, S.P.; Remick, D.G. and Friedland, J.S. (1998): Tumour necrosis factor-alpha, interleukin-6, and interleukin-8 secretion and the acutephase response in patients with bacterial and tuberculous osteomyelitis. J Infect Dis. 177:1582-7.

36- al-Dalaan, A.; al-Sedairy, S.; al-Balaa, S.; al-Janadi, M.; Elramahi, K.; Bahabri, S. and Siddiqui, S. (1995): Enhanced interleukin 8 secretion in circulation of patients with Behçet's disease. J Rheumatol. 22(5):904-7.

37- Yamakawa, Y.; Sugita, Y.; Nagatani, T.;Takahashi, S.; Yamakawa, T.; Tanaka, S.; Nakamura, S.; Ohno, S.; Sekihara, H.; Okuda, K. and Nakajima, H. (1996): Interleukin-6 (IL-6) in patients with Behçet's disease. J Dermatol Sci. 11(3):189-95.

38- Rincon, M.; Anguita, J.; Nakamura, T.; Fikrig, E. and Flavell, R.A. (1997): IL-6 directs the differentiation of IL-4 producing CD4+ T cells. J Exp Med. 185:461–9.

39- Adam, B. and Calikoglu, E. (2004): Seruminterleukin-6, procalcitonin and C-reactive protein levels in subjects with active Behçet's disease. J Eur Acad Dermatol Venereol. 18:318–20.

40- Evereklioglu, C.; Er, H.; Turkoz, Y. and Cekmen, M. (2002): Serum levels of TNF-alpha, sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behcet’s disease. Mediators Inflamm. 11:87-93.

41- Akdeniz, N.; Esrefoglu, M.; Keleş, M.S; Karakuzu, A. and Atasoy, M. (2004): Serum interleukin-2, interleukin-6, tumour necrosis factor-alpha and nitric oxide levels in patients with Behcet's disease. Ann Acad Med Singapore. 33(5):596-9.

42- Nalbant, S.; Sahan, B.; Durna, M.; Ersanli, D.; Kaplan, M.; Karabudak, O. and Unal, M (2008): Cytokine profile in Behçet uveitis. Bratisl Lek Listy. 109:551–4.

43- Triolo, G.; Acardo-Palumbo, A.; Dieli, F.; Ciccia, F.; Ferrante, A.; Giardina, E.; Sano, C.D. and Licata, G. (2001): Humoral and cellmediated immune responses to cow's milk proteins in Behçet's disease. Ann Rheum Dis. 61:459,

44- Raziuddin, S.; al-Dalaan, A.; Bahabri, S.; Siraj, A.K. and Al-Sedairy, S. (1998): Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. J Rheumatol. 25:329.

45- Wang, C.R.; Chuang, C.Y. and Chen, C.Y. (1992): Anticardiolipin antibodies and interleukin-6 in cerebrospinal fluid and blood of Chinese patients with neuro-Behçet's syndrome. Clin Exp Rheumatol. 10:599–602.

46- Akman-Demir, G.; Tüzün, E.; Içöz, S.; Yeşilot, N.; Yentür, S.P.; Kürtüncü, M.; Mutlu, M. and Saruhan-Direskeneli, G. (2008): Interleukin-6 in neuro-Behçet's disease: association with disease subsets and long-term outcome. Cytokine. 44:373–6. 426

47- Central Hirohata, S. (2001): Nervous system involvement in Behçet's disease. Rinsho Shinkeigaku 41:1147–9.

48- Dilek, K.; Ozçimen, A.A.; Saricaoğlu, H.; Saba, D.; Yücel, A.; Yurtkuran, M.; Yurtkuran, M. and Oral, H.B. (2009): Cytokine gene polymorphisms in Behçet's disease and their association with clinical and laboratory findings. Clin Exp Rheumatol. (2 Suppl 53):S73-8.

49- Chang, H.K.; Jang W.C.; Park, S.B.; Han S.M.; Nam, Y.H.; Lee, S.S.; Kim, J.U. and Lee H.S. (2005): Association between interleukin 6 gene polymorphisms and Behc¸et's disease in Korean people. Ann Rheum Dis. 64:339–340.

50- Amirzargar, A.; Shahram, F.; Nikoopour, E.; Rezaei. N.; Saeedfar, K.; Ziaei, N. and Davatchi, F. (2010): Proinflammatory cytokine gene polymorphisms in Behçet's disease. Eur Cytokine Netw. 21(4):292-6.

51- Bowcock, A.M.; Kidd, J.R.; Lathrop, G.M.; Daneshvar, L.; May, L.T.; Ray, A.; Sehgal, P.B.; Kidd, K.K. and Cavalli-Sforza, L.L. (1988): The human ''interferon-beta 2/hepatocyte stimulating factor/interleukin-6'' gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 3:8–16.

52- Shahram, F.; Nikoopour, E.; Rezaei, N.; Saeedfar, K.; Ziaei, N.; Davatchi, F. and Amirzargar, A. (2011): Association of interleukin-2, interleukin-4 and transforming growth factor-beta gene polymorphisms with Behcet's disease. Clin Exp Rheumatol. 29(4 Suppl 67):S28-31.

53- Hulkkonen, J.; Pertovaara, M.; Antonen, J.; Pasternack, A. and Hurme, M. (2001): Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. Rheumatology (Oxford). 40: 656-61.

54- Cardellini, M,; Perego, L.; D'Adamo, M.; Marini, M.A.; Procopio, C.; Hribal, M.L.; Andreozzi, F.; Frontoni, S.; Giacomelli, M.; Paganelli, M.; Pontiroli, A.E.; Lauro, R.; Folli, F. and Sesti, G. (2005): C-174G polymorphism in the promoter of the interleukin-6 gene is associated with insulin resistance. Diabetes Care. 28: 2007-12.

55- Terry, C.F.; Loukaci, V. and Green, F.R (2000): Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 275: 18138-44.