

Bacterial Diversity in Patients with Oral Ulceration in Behcet's Disease

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Abstracts: Background: Behcet's disease is a chronic, relapsing, multi-system inflammatory disorder, clinically characterized by recurrent oral and genital ulcers, skin lesions, and uveitis. Environmental factors such as infectious agents have also been implicated in its pathogenesis. Aims and Objectives: to isolate different types of bacteria from patients with oral ulceration of Behcet's disease that may contribute in pathogenesis of these oral ulcers and tissue typing was done for those patients. Design: Cross-sectional study. Setting: Al-Karamma Teaching Hospital from January 2000 – September 2000 at Baghdad city. Patients and methods: Study included 40 patients with Behcet's disease (BD). The other set was control group, consisted of 30 healthy volunteer's age- and sex-matched with first group from staff employees. Two swabs were taken from basal area of oral ulcer for direct exam and culture. Human Leukocytes Antigens typing was done for them by serological method. Results : Direct examination of their oral ulcer swabs showed significant difference regarding pus cells 87.5% (p= 0.0001) and red blood cells 75% (p= 0.0001). Gram stain showed significant increased in G+ cocci 95% (p= 0.0001). Other swab was cultured on Blood, Chocolate and MacConkey agar. The isolated bacteria were Streptococcus Pyogenes group A 75% (p= 0.0001) and Staphylococcus aureus 47% (p= 0.0001). HLA typing were done for them and 95% of them were HLA-B5 positive. Conclusions: Microbial infection by Streptococci has been implicated in the development of Behcet's disease by trigger cross-reactive autoimmune responses in those patients. The frequency of the HLA-B51 allele is high among patients with Behcet's disease [Mahdi A et al NJIRM 2011; 2(4) : 91-94]

Key Words: Behcet's , oral, ulcer, Streptococci.

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Introduction: Behcet's disease is an inflammatory disorder of unknown cause, named after the Turkish dermatologist Hulusi Behcet who first described the syndrome in 1924, characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions¹. There are many causes have been suggested for the causation of recurrent oral ulceration like food allergy², infection with virus like Herpes simplex³, Streptococcus sanguis or its L form has been isolated from the ulcers eliciting a delayed type hypersensitivity^{4,5}. The possibility of Mycoplasma pneumoniae may be associated with oral ulceration of Behcet's disease⁶. In addition to that, abnormal immune responses are implicated in this disease⁷ like increased serum level of C9⁸, IgA (serum and salivary)⁹ and IgG against B-Cell epitopes of the 65 kDa HSP (heat shock protein)¹⁰ mediating immune complex formation that modulate cellular immunity¹¹. Antigenic cross reactivity between oral mucosa and some microbial agent (Lipoteichoic acids) for example Streptococcus sanguis is responsible for the autoimmunity¹². There is an association

between HLA-B5 (Human Leukocyte Antigen) and Behcet's disease¹³ suggesting that the susceptibility to this disease is associated with a gene near the HLA region, which is in linkage disequilibrium with the HLA genes. This HLA may function as specific receptors for pathogens or that the antigenic determinants of some exogenous pathogens might mimic the HLA antigens¹⁴.

The aims of this study were to isolate different types of bacteria from patients with oral ulceration of Behcet's disease and HLA typing done for those patients.

Material and Methods: This cross – sectional study included 40 patients with Behcet's disease (BD). They were defined and diagnosed as BD by their specialist physicians according to the clinical examination. They were consulted Al-Karamma Teaching Hospital from January 2000 – September 2000 at Baghdad city.

Criteria for Diagnosis of indeterminate colitis: Patients with clinical features of BD: recurrent oral and genital ulceration, uveitis, arthritis, central

nervous system manifestation were included in this study.

Patients with recurrent oral ulceration, aphthous, positive history of antibiotics intake were excluded from this study. The second set was control group, consisted of 30 healthy volunteer's age- and sex-matched with first group from staff employees.

Two swabs were taken from basal area of oral ulcer, one was examined directly under light microscope and Gram stain was done and examined. Other swab was cultured on Blood agar, Chocolate agar and MacConkey agar. Further culturing and tests were done for isolation of specific species of bacteria according to its type¹⁵. HLA typing was done for them by serological method.

The Ethical Committee of Al-Karamma Teaching Hospital approved the study, and all samples were obtained with informed consent in accordance with the Al-Karamma Teaching Hospital Declaration.

Statistical analysis: Data were analyzed statistically using descriptive statistics (Frequencies for tables, mean and standard deviation, inferential statistics (Chi- square test) was used. All of these were done using MiniTab statistical software program 13.20. A P- value ≤ 0.05 was considered significant.

Result: The study group consists of forty patients with BD. Mean age allocation (BD patients $45.67 \pm SD 15.54$ and control $44 \pm SD 15.54$). Male to female ratio was 22:18 in BD patients and in the control group was 20:10.

Direct examination of their oral ulcer swabs showed significant difference regarding pus cells 87.5% ($p= 0.0001$) and red blood cells 75% ($p= 0.0001$). Gram stain showed significant increased in G+ cocci 95% ($p= 0.0001$). Other swab was cultured on Blood, Chocolate and MacConkey agar. The isolated bacteria were Streptococcus Pyogenes group A 75% ($p= 0.0001$) and Staphylococcus aureus 47% ($p= 0.0001$). HLA typing were done for them and 95% of them were HLA-B5 positive

Table-1- Illustrated finding in oral swab from BD patients and control group.

Findings in wet mount and Gram stain	BD patients No.=40		Control group No.=30		P- value
	No.	%	No.	%	
Red blood cells	30	75	2	6.6	0.0001
Pus cells	35	87.5	3	10	0.0001
Epithelial cells	36	90	28	93.3	0.6941
Gram positive bacilli	4	10	3	10	1.000
Gram positive cocci	38	95	5	16.6	0.0001
Gram negative cocci	2	5	3	10	1.000
Trichomonas tenax	0	0	0	0	-----
Budding yeast	0	0	0	0	-----

Table-2- Types of bacteria that isolated from patients with BD and control group.

Types of bacteria	BD patients No.=40		Control group No.=30		P- value
	No.	%	No.	%	
Staphylococcus aureus	19	47.5	1	3.33	0.0001
Streptococcus Pyogenes group A	30	75	1	3.33	00001
Streptococcus viridans	1	25	5	16.66	0.2278
Moraxella catarrhalis	1	25	4	13.33	0.1567
Diphtheroid	1	25	3	10.00	0.3067

Discussion: Behcet disease is a rare disease characterised by painful mouth ulcers¹⁶. The cause of this disease is unknown but it is presumed to be an autoimmune disorder. This is where an individual's immune system starts reacting against his or her own tissues. The reason for this remains a mystery but it is possible bacterial or viral infection may have a role in its development¹⁷. In this study we can isolate Streptococcus Pyogenes group A in 75% of patients ($p= 0.0001$) and Staphylococcus aureus in 47% of patients ($p= 0.0001$). This is in agreement with other studies that Streptococcus is the causative agent but the difference is the types of these bacteria. Other study found delayed hypersensitivity reaction to a

group of streptococcal bacteria was the causative agent by deposition of anti-streptococcal group D at vessel walls and infiltrated by inflammatory cells mainly composed of activated T-cells and macrophages in association with natural killer cells suggesting antigen-antibody mediated cytotoxicity may play an important role in causing the lesions of Behçet's disease¹⁸. Yoshikawa et al 1996¹⁹ found that *Streptococcus sanguis* is responsible for autoimmune response of Behçet's disease. Other study indicates that *H. pylori* may be involved in the pathogenesis of Behçet's disease or disease activity might be enhanced due to induced inflammation or altered immunity²⁰.

Other report demonstrate that *saccharomyces cerevisiae* may be responsible for this disease because they found that high prevalence of anti *saccharomyces cerevisiae* antibodies (ASCA) in BD. ASCA levels are also increased in healthy family members of BD patients, and are probably influenced by genetic as well as environmental factors. ASCA were significantly associated with a more severe oral ulcer disease²¹. Thus the relationships between microbial agents streptococci or microbial antigens and immune mechanisms such as innate and adaptive responses against microorganisms had a role in the pathogenesis of this disease²². Our study showed the association of BD with HLA-B51 in 95% of patients and with streptococci infection that is agreed with Kaneko et al 2008²³ who focused on the hypersensitivity against oral streptococci and HLA-B51 restricted CD8 T cell response was clearly correlated with the target tissues. There fore, exaggerated proliferative response gamma delta T-cells to products released by microorganisms present in oral ulcers may play a role in this disease²⁴. There is a difficulty in the follow-up those patients after treatment that limit this study and we suggest to do other autotiters in the serum of those patients.

Conclusion: Microbial infection by Streptococci has been implicated in the development of Behçet's disease by trigger cross-reactive autoimmune responses in those patients. The frequency of the HLA-B51 allele is high among patients with Behçet's disease

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