The Evaluation of Saliva Flow Rate, pH, Buffer Capacity, Microbiological Content and Indice of Decayed, Missing and Filled Teeth in Behçet's Patients

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ABSTRACT

Background: Several lines of evidence indicate that oral microbial flora play a critical role in the pathogenesis of Behçet disease. Saliva flow rate, buffer capacity and microorganism content are very important in the maintenance of oral health.

Aims: We aimed to evaluate saliva flow rate, pH, buffer capacity and *Streptococcus mutans* and *Lactobacilli* content along with the decayed, missing, and filled tooth index in Behçet's patients.

Study Design: Case-control study

Methods: Forty patients with active Behçet disease [female:male (F/M) 21/19, mean age 31.05±11.08 years] and forty healthy persons (F/M 21/19, mean age 31.03±9.14 years) were included in the study. The oral region was first examined, and the decayed, missing, and filled tooth index was calculated for each person. Stimulated saliva was collected and divided into two separate millimetric tubes to calculate the buffer capacity of the saliva, determine *Streptococcus mutans* and *Lactobacilli* levels and measure the pH of the saliva. A Caries Risk Test buffer strip was used to calculate the buffer capacity. Caries Risk Test bacterial kit was used to determine *Streptococcus mutans* and *lactobacilli* levels. A pH meter was used to measure the pH of the saliva.

Results: The mean saliva pH of the patients was higher than the controls (7.76±0.51, 7.18±0.46, respectively) (p<0.001). The mean level of *Streptococcus mutans* and *Lactobacilli* in patients was higher than controls (p<0.05). The mean decayed, missing, and filled tooth index of patients was 9.82±6.59 (range 1-28), while the mean decayed, missing, and filled tooth index of the controls was 6.05±3.35 (range 1-16) (p<0.01). There were no significant differences between the patients and the controls with respect to saliva flow rate and saliva buffer capacity (p>0.05).

Conclusion: We think that the maintenance of oral health by effective, regular tooth brushing, regular dental check-ups and dental treatment for Behçet patients is very important for the prevention and therapy of Behçet disease.

Key Words: Behçet syndrome, saliva, DMFT index, *Lactobacilli*, *Streptococcus mutans*.

Introduction

Behçet's disease (BD), a multisystem inflammatory disorder, is characterised by mucocutaneous, ocular, arthritic, vascular, central nervous and gastrointestinal system involvement. It was first described by the Turkish dermatologist Hulusi Behçet in 1937 as "recurrent oral aphthous ulcers, genital ulcers, and 'hypopyon-uveitis" (1). The aetiology and pathogenesis of BD has not been fully clarified. Several lines of evidence indicate that oral microbial flora play a critical role in the pathogenesis of BD. Various clinical and basic data also strongly point to an infectious triggering agent in the pathogenesis of BD (2, 3). The relationship between streptococcal infection and BD can also explain the clinical observations, such as increased oral manifestations after dental treatment, hypersensitivity to streptococcal skin tests, elevated pro-inflammatory cytokine responses to streptococcal antigens (4-7) and recent reports of beneficial antibacterial therapy (8, 9).

In patients with BD, poor oral health, poor prognosis for natural dentition, an increased number of extracted teeth due to multiple carious lesions and changes in oral pH have been reported (10-12). In addition, an increased incidence of tonsillitis and aggravation of disease by dental treatment are seen in BD patients.

Saliva plays a critical role in the maintenance of oral health. Saliva provides many features, such as lubrication and protection, buffering action and clearance, maintenance of tooth integrity, and antibacterial activity (13). Saliva flow rate, buffer capacity and microorganism content are very important for oral health.

We aimed to evaluate saliva flow rate, pH, buffer capacity and *Streptococcus mutans* (*S. Mutans) and Lactobacilli* content and DMF index (index of decayed, missing, and filled teeth) in BD, since studies related to this issue are scant and results of these studies are inadequate.

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Materials and Methods

The study was performed together with the Atatürk University Dental Faculty Restorative Dental Department and the Medical Faculty Dermatology Department.

Forty active BD patients [female:male (F/M) 21/19, mean age 31.05±11.08 yr] diagnosed according to the International Study Group for BD (ISG) criteria (14) and forty healthy persons (F/M 21/19, mean age 31.03±9.14 yr) were also included in the study. If the patients had at least one clinical sign according to ISG criteria, they were accepted as having active BD.

The inclusion criteria for the patient group were: lack of other oral or systemic disorders from BD, persons not using an orthodontic apparatus, no colchicine medication in the last month, non-smoking, and non-pregnant.

The control group was selected randomly from healthy people who accompanied BD patients attending our clinics, and staff of our hospital. The inclusion criteria for the control group were: having no symptoms of any disorder, persons who had not used an orthodontic apparatus or taken any medicine during the last month, non-smoking, and non-pregnant. Healthy controls were matched with regard to age, gender and socio-economic status.

The study was approved by the Atatürk University Institute of Healthy Science and information consent was given by the patients at the time of study entry.

Assessment of general status

Patients and controls were instructed to collect data on a chart about their frequency of tooth brushing, degree of education and duration since the last dental visit.

Assessment of oral health

To determine oral health status, patients and controls were examined, and extracted teeth, carious teeth and filled teeth were recorded. We used the DMFT index that measures the amount of permanent teeth decayed, missing, and filled in the individual's mouth, ranging from 0 to 32. The DMFT index was calculated for each person.

Collection of saliva and calculation of saliva flow rate

Stimulated whole saliva was collected under resting conditions in a quiet room (to reduce any stressful conditions). The saliva samples were obtained two hours after breakfast, between 9-12 a.m. Two hours prior to the evaluation of stimulated whole saliva production, subjects were instructed not to eat, drink, chew gum, or rinse their mouths until the test was completed. Pre-stimulation was accomplished by chewing a piece of standard size paraffin, and the participants were asked to swallow the saliva pooled in the mouth after 60 s. Thereafter, whole stimulated saliva was collected for about 5 min into a dry, millimetric and sterilised plastic tube. The flow rate was calculated in ml/dk. The saliva collected was divided into two separate millimetric tubes to calculate the buffer capacity of the saliva, to determine *S. mutans* and *Lactobacilli* levels and to measure the pH of the saliva.

Calculation of buffer capacity and measurement of saliva pH

The saliva buffer capacity was measured using strips (Caries Risk Test (CRT) buffer strip, Ivoclar Vivadent Schaan/Liechtenstein) and measured against standards assigned by the manufacturer. A pH meter (Mettler Toledo MP 220 pH Meter, Schwarzenbach, Switzerland) was used to measure saliva pH.

Microbiological analysis

A CRT bacteria kit (Ivoclar Vivadent, Schaan, Liechtenstein) was used to determine *S. mutans* and *lactobacilli* levels. Saliva samples were dropped on the CRT bacteria kit media by pipette. After incubation at 37°C for 48 hours, viable colonies were measured. The salivary counts of *lactobacilli* and *S. mutans* determined by the number of colony forming units (CFU) were measured semi-quantitatively. The results were expressed as two values: $<10^5$ CFU and $\geq 10^5$ CFU.

Statistical analysis

Data were analysed using the SPSS 11.0 statistical program (SPSS Inc., Chicago, IL, USA). Chi-square test and Students t-test were used in the analysis. $P \leq 0.05$ was accepted as significant.

Results

The mean duration of BD was 3.7 months (range 1-120 months). In 9 patients, BD was diagnosed first. 25 patients were using colchicine; the remaining patients did not use any treatment. Clinical manifestations of the BD patients are given in Table 1.

According to the frequency of tooth brushing and degree of education, there were no significant differences between patients and controls (p>0.05). The duration since the last dental visit was significantly lower in patients than in controls (p<0.001).

The mean saliva pH of the patients was higher than controls (7.76±0.51, 7.18±0.46, respectively) (p<0.001). The mean level of *S. mutans and Lactobacilli* in patients was higher than controls (p<0.05). The mean DMFT index of patients was 9.82±6.59 (range 1-28), while the mean DMFT index of the controls was 6.05±3.35 (range 1-16). There was a significant difference between the patients and the controls with respect to DMFT index (p<0.05). There were no significant differences between the patients and the controls with respect to saliva flow rate and saliva buffer capacity (p>0.05) (Table 2-4).

Table 1. The clinical manifestations of Behçet's Disease patients

Finding	n (%)
Oral ulcer	40 (100)
Genital ulcer	40 (100)
Erythema nodosum	24 (60)
Arthritis	20 (50)
Vascular involvement	5 (12.5)
Uveitis	12 (30)
*BD: Behçet's Disease	

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Discussion

Behçet's disease usually starts in oral mucosa, and other clinical manifestations of the disease are seen after oral ulcers. The relationship between oral environment and BD pathogenesis can be summarised as: oral ulcers are the most common and usually the first clinical manifestation of BD, an augmented immune response to oral streptococci is observed in BD patients, and finally an increased incidence of tonsillitis and dental caries, and aggravation of the disease by dental treatment are important observational clues in clinical practice. Oral health is impaired in BD and associated with disease severity (3, 4, 8, 15).

Saliva plays an important role in oral health monitoring, and in regulating and maintaining the integrity of the oral hard tissues and some soft tissues. Saliva has many essential functions. Clinically, a more important role is in the maintenance of oral health, including the protection of teeth and mucosa from infections. Variations in salivary flow may be affected, reversibly or irreversibly, by numerous physiological and pathological factors. Decreased salivary flow results in clinically significant oral discomfort that may manifest as increased caries, susceptibility to oral infections, oral candidiasis, altered taste sensation or as a host of other problems. Similarly, decreased saliva flow rate may play a role in the pathogenesis of BD. Salivary buffering capacity has been identified as one of the many factors that may affect an individual's caries risk. The ability of saliva to buffer acids is essential for maintaining pH values in the oral environment above a critical pH, thereby protecting teeth against demineralisation (15-18).

Also, saliva flow rate of our patients was higher than controls, although the difference was not statistically meaningful. We thought that saliva flow rate might be increased with oral ulcers. Unfortunately, we could not identify any studies concerned with saliva flow rate in BD.

There have been a few studies of saliva buffering capacity and pH in BD. Şengün et al. observed that the mean salivary pH in BD patients was 7.15±0.39 and the buffering capacity was 3.68±0.80. The mean salivary pH in controls was 6.94±0.43 and the buffering capacity was 3.48±0.50. There were no significant differences in pH and buffering capacity between the groups. In our study, similar to this study, saliva pH was higher than in controls (19). Feslihan also observed that there was no significant difference between patients with BD and controls with respect to saliva buffering capacity (20).

Both Şengün et al. and Mumcu et al. observed that there was no significant difference in DMFT index between BD patients and controls. However, we found that the DMFT index of BD patients was significantly higher than controls (19, 21).

Oral health is impaired in BD and associated with disease severity (22). Less tooth brushing and a longer period since the last dental visit in BD may be associated with the poor oral hygiene conditions caused by active painful oral ulcers. Akman et al. observed that the daily frequency of tooth brushing was lower in BD patients than in controls, but the difference was not statistically significant, similar to our study (23).

Mumcu et al. (24) observed the frequency of tooth brushing to be significantly lower in Turkish patients when compared with those in the UK. We found that there was no difference between BD patients and controls according to the frequency of tooth brushing, but the duration since the last dental visit was significantly higher in BD patients than in controls.

As BD starts mostly from the oral mucosal surfaces (oral aphthae being the first manifestation in 70% of patients), oral microbial flora have long been implicated in the pathogenesis. The relationship between streptococcal infections and BD can also explain the clinical observations. Patients with BD have a significantly higher incidence of tonsillitis and dental caries. Systemic symptoms of BD could thus be induced after treatment of dental caries or even by intracutaneous injection of streptococcal antigens. Recent reports have shown that antibacterial treatment is beneficial in BD (8, 25).

Mumcu et al. (26) observed that high levels of salivary *S. mutans* colonisation was significantly more present in BD than controls. The presence of *S. mutans* in saliva was associated with oral ulcers (61.5% in patients with active oral ulcers vs. 38.9% in inactive ulcers; p=0.020). *S. mutans* colonisation in saliva was significantly higher among male BD patients with a severe disease course compared to a milder disease.

We also found that the mean level of *Lactobacilli* in BD patients was significantly higher than controls. We could not find any study related to lactobacilli in BD in the literature.

Table 2. The saliva flow rate, saliva pH, and DMFT* index of patients and controls

	Patient group	Control group	p value
Saliva flow rate mL/min	1.44±0.52	1.41±0.50	0.757
Saliva pH	7.76±0.51	7.18±0.46	0.000
DMFT index	9.82±6.59	6.05±3.35	0.017*
DMFT: Decayed, missing and filled teeth p<0.05(According to Mann Whitney U test)			

Table 3. The saliva buffer capacity of patients and controls

Group	Low		Medium		High		χ²	p
	n	%	n	%	n	%		
Patient	3	7.5	11	27.5	26	65	1.324	0.557**
Control	1	2.5	14	35	25	62.5		
Fisher's Exact Test*, p>0.05**								

Table 4. The level of *S. mutans* and *Lactobacilli* of patients and controls

Group	<10⁵ cfu n%	≥10⁵ cfu n%	χ²*	p
S. mutans				
Patient	13 32.5	27 67.5	6.084	0.014**
Control	24 60	16 40		
Lactobacilli				
Patient	17 42.5	23 57.5	4.073	0.044**
Control	26 65	14 35		
Pearson Chi-square*, p<0.05**				

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It is possible that chronic infection of oral mucosal structures in genetically susceptible patients could accelerate BD by promoting a chronic systemic inflammatory status through the release of bacterial products, heat shock proteins (HSP), acute-phase reactants and other inflammatory mediators. Since BD starts mostly from the oral mucosal surfaces, it is possible that the immune response to microorganisms in saliva might initially cause elevation of the inflammatory mediators locally, which consequently enters the systemic circulation and, by interacting with endothelial tissues, plays a role in the pathogenesis of BD. This view is supported in that BD usually begins with recurrent oral ulcers, followed by later signs of BD (27).

It is suggested that an immune response to streptococcal HSP might also be directed to epithelial and other human 60-kDa HSPs, although there was a lack of specificity. It is thus possible that antigens other than these HSP-related peptides might be involved in the pathogenesis of BD (28).

In conclusion, we believe that the maintenance of oral health by effective, regular tooth brushing and dental flossing, regular dental check-ups and dental treatments in patients with Behçet is very important for the prevention and therapy of BD.

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