Association of salivary *S. mutans* colonisation and mannose-binding lectin deficiency with gender in Behçet's disease

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ABSTRACT

Objective. *The present study aimed to investigate the interactions among salivary S. mutans colonisation, serum mannose binding lectin level (MBL), oral ulcer activity and disease course in patients with Behçet's disease (BD).*

Methods. *One hundred and six BD patients, 25 patients with rheumatoid arthritis (RA) and 42 healthy controls (HC) were included in the study. BD patients were grouped as active (n=52) or inactive (n=54) according to oral ulcer status of the previous 3 months. Salivary colonisation of S. mutans levels were investigated by standard Caries Risk Test (CRT) Bacteria kits (Ivoclar, Vivadent). S. mutans colonies were categorized as high (≥10^5 colony forming unit (CFU)/ml of saliva) or low (<10^5 CFU/ml). Serum mannose binding lectin (MBL) levels were measured by ELISA.*

Results. *High levels of salivary S. mutans colonisation was significantly more present in BD (50%) than HC (28.6%)(p=0.039), whereas no significant difference was observed between RA and other groups (p>0.05). S. mutans presence in saliva was associated with oral ulcers (61.5% in patients with active oral ulcers vs 38.9% in inactives) (p=0.020). S. mutans colonisation in saliva was significantly higher among male BD patients with a severe disease course than a milder disease (p=0.04). Increased salivary S. mutans colonisation was also related to very low serum MBL (<100 ng/ml) in BD compared to controls (p=0.04).*

Conclusion. *The relationship between increased presence of S. mutans and MBL deficiency with active disease pattern may indicate an impaired innate immune response in BD patients which may predispose to oral infections and a severe disease course.*

Introduction

Behçet's disease (BD) is a systemic vasculitic disorder with mucutaneous ulcers and can also affect ocular, neurological, musculoskeletal and central nervous systems. Pathogenetic tissue inflammation in BD is suggested to be activated through infectious agents such as streptococcia or herpes simplex virus (1-6).

Since oral ulcers are usually the initial symptom, oral environment regarding oral microbial flora (7-13) and impaired oral health (14-19) have been implicated in the pathogenesis of BD. Increases in dental caries and tonsillitis originating from streptococcal infections, flare-up of disease symptoms by dental treatments (1-12, 20) and the beneficial effect of antibacterial treatments on disease symptoms (21-23), are among the important clinical findings supporting the association between streptococcal infections and BD. Oral ulcer occurrence after oral trauma and *S. sanguis* inoculation is previously reported by Isogai *et al.,* in a germ-free mice model (8). *S. mutans* is a cariogenic microorganism and a member of streptococci family (24). However, although *S. mutans* colonisation was studied in tongue, plague and saliva samples, its exact role in disease pathogenesis is still unclear (9, 12).

Mannose binding lectin (MBL), a serum protein originating from hepatic cells, activates innate immune response through binding various carbohydrate structures on the surface of microorganisms. Serum MBL levels are affected with genetic polymorphisms in MBL gene or promoter region and severe MBL deficiency associated with these polymorphisms may cause decreased clearance of invading microorganism or apoptotic debris (25-28). In a previous study, low serum MBL levels were observed in patients with BD compared

to healthy controls and are associated with a severe disease course (28).

The aim of this study was to investigate *S. mutans* colonisation in saliva with its relationship to serum MBL levels, oral ulcer activity and disease severity in patients with BD.

Material and methods

Patients and controls

One hundred and six BD patients (M/F: 41/65, mean age: 38.5±11.7 years), classified according to the International Study Group Criteria (29), age-matched 25 patients with rheumatoid arthritis (RA, F/M: 23/2, mean age: 44.1±6.8 years) and age- and sex-matched 42 healthy controls (HC, M/F: 20/22, mean age: 34.8±11.6 years), without any symptoms of a systemic disorder or a dental disease and who were not close relatives of patients, were recruited in the study. Patients using antibiotics or local antimicrobial mouthwashes within last month were also excluded as these agents could affect the salivary *S. mutans* colonisation.

All patients had oral ulcers (100%). Patients were categorized as active (n=52) or inactive (n=54) according to oral clinical status of the previous 3 months. The frequency and the healing time of oral ulcers were noted in active patients. Clinical characteristics of BD patients were genital ulcers and skin lesions in 100%, ocular disease in 33% (n=35), arthritis in 67.9% (n=72), vascular disease in 19.8% (n=21) and neurological involvement in 2.8% (n=3). A positive pathergy reaction was present in 74.5% (n=79). Disease severity score was calculated according to Krause *et al*. (30). Patients were categorized into severe (severity score ≥4) and mild (severity score < 4) groups. Treatment modalities included colchicine (n=71, 67%) and immunosuppressives with or without corticosteroids (n=35, 33%).

The colonisation of *S. mutans* levels were investigated in whole paraffin-stimulated saliva by Caries Risk Test Bacteria (CRT Bacteria test) (Ivoclar, Vivadent), a commercially available evaluation kit. Stimulated saliva samples were collected by paraffin chewing that removes bacteria from the biofilm around teeth. According to the test protocol, saliva was incubated with a CRF bacteria kit for 48 hours at 37°C and the number of colonies on the strip was compared with a predetermined scale. The levels were classified semi-quantatively according to the manufacturer's instructions. *S. mutans* colonies were categorized as high (≥ 10^5 colony forming unit (CFU)/ml of saliva) or low level (<10^5 CFU/ml) indicating the growth of colonies. Salivary *S. mutans* levels were re-examined in 16.9% of patients with BD (n=18) and 19% of HC (n=8) who were randomly selected in between 3 to 12 months. Stimulated and unstimulated salivary flow rates (31) were also evaluated in patients and controls. Salivary buffer capacity was examined by a standard kit (Dento-Buff, Ivoclar, Vivadent).

Serum MBL levels were measured by MBL oligomer ELISA (Bioporto, Denmark). The cut-off value of serum MBL level was 500 ng/ml in our previous study (high: ≥500 *vs*. low: <500 ng/ml). A second cut-off value of 100 ng/ml was also selected to examine the effects of severe MBL deficiency in the present study.

The study was approved by the Ethics Committee of Marmara University Medical School and informed consent was obtained from all patients and controls.

Statistical analysis

Data were analysed by using SPSS 11.5 statistic programme (SPSS Inc, Chicago, IL). Unpaired *t*-test and Chi-square test were used in the evaluation of data. Mann Whitney U test was used for non-normally distributed data. A *p*-value equal or less than 0.05 was accepted as statistically significant.

Results

High salivary *S. mutans* colonisation was observed significantly more frequent among BD patients (n=53, 50%,) compared to HC (n=12, 28.6%) (p=0.039). Yet, no significant difference was observed between RA (n=8, 32%) and others (BD *vs*. RA p=0.11, RA *vs*. HC p=0.77). Active oral ulcers within last 3 months were present in 49.1% of BD patients (n=52) whereas other patients were inactive (n=54). The mean healing time of oral ulcer was 8.1±2.3 days in active ones.

S. mutans colonisation in saliva was significantly higher in patients with active oral ulcers (32/52, 61.5%) compared to inactives (21/54, 38.9%) (p=0.020). However, no significant difference was observed in the healing time of oral ulcers according to high and low salivary *S. mutans* colonisation (7.9±2.7 *vs*. 8.4±1.2 days, p=0.35).

Mean disease severity score was 5.8±2.4 in patients with BD (males: 5.4±2.2 *vs*. females: 5.9±2,3 p=0.3). Salivary *S. mutans* colonisation was significantly higher in male BD patients with a severe disease course (63.3%) than the milder group (27.3%) (p=0.04). However, a similar relationship was not observed in female patients (p=0.42) (Table I). When the relationships between *S. mutans* colonisation and immunosuppressive treatment in males and females were examined separately, no significant association was observed in males and females (p=0.57 and p=0.15, respectively).

Daily tooth brushing frequency was similar in BD (1.7±0.5), RA (1.3±0.4) and HC (1.4±0.8) groups (p=0.43). No significant difference was also observed in tooth brushing frequency according to salivary colonisation of *S. mutans* both in BD (high: 1.3±0.8 *vs*. low: 1.2±0.9, p>0.05), RA (high: 1.1±0.4 *vs*. low: 1.3±0.5) or HC (high: 2.0±0.1 *vs*. low: 1.5±0.6, p>0.05).

Mean serum MBL levels were not significantly different in BD patients compared to RA and HC (1782±1644, 2103.7±1737.3 and 2135±1556 ng/ml, respectively) (p=0.59 and p=0.23, respectively). High *S. mutans* colonisation also did not effect mean MBL levels in either group (BD: high: 1609±1571 ng/ml vs low: 1885±1709 ng/ml, p=0.53, RA: high: 2051±1548 ng/ml *vs*. low: 2160±1990 ng/ml, p=0.88, HC: high: 2490±1459 ng/ml *vs*. low: 1992±1594 ng/ml, p=0.28). Low serum MBL levels (<100 ng/ml) reflecting severe MBL deficiency were seen in 22.6% of BD patients (n=24), 12 % of RA (n=3) and 19% of HC (n=8), without a significant difference between the groups (p=0.48). Among low serum MBL group, high levels of *S. mutans* colonies in saliva was more prevalent in BD compared to

Table I. Disease severity status in Behçet's disease according to *S. mutans* colonisation and gender.

		Disease severity status				
		Mild		Severe		
		n	%	n	%	p^*
Male patients	*S. mutans* Low	8	72.7	11	36.7	0.04
	S. mutans High	3	27.3	19	63.3	
	Total	11	100	30	100	
Female patients	*S. mutans* Low	6	42.9	28	54.9	0.42
	S. mutans High	8	57.1	23	45.1	
	Total	14	100	51	100	

*Statistical comparison was done with Chi-square test.

HC (54.2% and 12.5%, respectively) (p=0.04). A similar relationship was not observed in high serum MBL group (p=0.53) (Table II).

As salivary flow rates might influence *S. mutans* colonisation, we also measured stimulated and unstimulated salivary flow rates in our study group Stimulated and unstimulated salivary flow rates of BD patients (2.2±1.2 ml/min and 0.7±0.3 ml/min, respectively) and healthy controls (2.1±0.8 ml/min and 0.5±0.2 ml/min, respectively) were observed to be similar (p=0.39 and p=0.69, respectively). Low buffer capacity was also equally present in both groups (18% in BD *vs*. 15% in HC) (p=0.45).

Discussion

Oral health as a focal infection focus plays a critical role in human health as a part of general health status (32). Since oral cavity provides an excellent environment for the growth and survival of bacteria, the flora and their metabolites have been suggested to contribute to health and disease (24, 32). Saliva serves as a medium for transporting bacteria in the mouth as many bacteria survive and grow in saliva (31, 32). Low salivary flow rates and buffer capacity are risk factors for colonisation of microorganisms in the oral environment (24, 32). *S. mutans* is a major etiological factor for the development of dental caries and is a member of oral biofilm (24). A significant increase was observed in salivary *S. mutans* colonisation in BD patients in our study. This increase was associated with oral ulcer presence, disease severity, male gender and very low MBL levels. However, salivary properties did not seem to predispose to this elevated colonisation of *S. mutans* in our patient group.

In previous studies, we observed an association between biofilm accumulation around teeth and the healing time of oral ulcers (21) and a severe disease course in BD (14). Oral streptococcia may enter the bloodstream after chewing, brushing of teeth or dental procedures and can avoid the clearance by the reticuloendothelial system (33). In oral infections, major immune responses appear to be directed primarily to streptococcal antigens such as *S. mutans* adhesion (AgI/II), glycosyltransferases and glucan-binding protein B, which facilitate the colonization and accumulation in biofilm and interact with human cells (33, 34). Antigen AgI/II of *S. mutans* interacts directly with human monocytes, epithelial and endothelial cells through lectin-like receptors and stimulates the production of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-8, and TNF-α. Glycosyltransferases of *S. mutans* can also modulate the production of Th-1 type cytokines and stimulate IL-6 (35). These immunopathological reactions of *S. mutans*-related antigens may suggest a possible link between disease course and *S. mutans* colonisation in patients with BD.

Gender is an important factor for a poor prognosis in BD (36, 37). Poor oral health was also observed in male

Table II. Salivary *S. mutans* colonisation in Behçet's disease and healthy controls according to serum MBL levels.

		Behçet's disease		Rheumatoid arthritis		Healthy controls		BD-HC	RA-HC	BD-RA
		n	%	n	%	n	%	p^*	p^*	p^*
MBL <100 ng/ml	*S. mutans* Low	11	45.8	1	33.3	7	87.5	0.04	0.07	0.68
	S. mutans High	13	54.2	2	66.6	1	12.5			
	Total	24	100	3	100	8	100			
MBL≥100 ng/ml	*S. mutans* Low	42	51.2	16	72.7	20	58.8	0.53	0.51	0.82
	S. mutans High	40	48.8	6	27.3	14	41.2			
	Total	82	100	22	100	34	100			
MBL <500 ng/ml	*S. mutans* Low	18	43.9	1	33.3	8	72.7	0.09	0.24	0.86
	S. mutans High	23	56.1	2	66.6	3	27.3			
	Total	41	100	3	100	11	100			
MBL≥500 ng/ml	*S. mutans* Low	35	53.8	16	72.7	19	61.3	0.58	0.30	0.67
	S. mutans High	30	46.1	6	27.3	12	38.7			
	Total	65	100	22	100	31	100			

*Statistical comparison was done with Chi-square test.

patients with a severe disease course, suggesting that gender is also an important factor for oral health-associated disease activation in BD (14). The interaction between high *S. mutans* colonisation and increased disease severity was observed mainly in our male patient group. According to the literature, *S. mutans* count are not increased in children undergoing renal transplantation at baseline and post-transplantation (38). In addition, growth-inhibitory effects of immunosuppresives on S. mutans could be observed (39). Therefore, a significant effect of immunosuppresives on *S. mutans* count was not presumed in our study.

In a previous study, an increase in *S. mutans* colonisation was seen in supragingival plaque samples compared to those of healthy controls (12). Yet, Isogai *et al*. found no significant difference in salivary *S. mutans* colonisations between BD patients and healthy controls (9). These discrepant results can be explained by methodological differences or patient selection. Also, in studies from Japan, *S. sanguis* is observed to be the dominant agent of oral flora in BD patients and geographical differences may lead to an association of different Streptococcal strains with BD pathogenesis (9, 11, 12). The relationship between *S. mutans* colonisation and MBL levels was not evaluated in these studies before.

In our previous study, MBL deficiency was associated with a severe disease course in BD (28). A significant increase in salivary *S. mutans* colonisation was observed in patients with very low serum MBL levels in the present study. MBL binds to carbohydrate structures on the surface of microorganisms and recognize a wide range of microorganisms including gram-positive and negative bacteria, yeasts, parasites, mycobacteria and viruses. It mediates an antibacterial effect by activating complement in an antibody and C1q-independent manner using MBL-associated serine protease 1 (MASP-1) and MASP-2 or opsonophagositosis without complement activation and deposition of opsonic C4 and C3 fragments (25, 26, 40). MBL deficiency may cause a decreased clearance of invading microorganisms, which might possibly lead to an extended presentation of exogenous antigens to the host. Long-term bacterial stimulation might then induce abnormal adaptive immune responses, such as tissue lymphocyte infiltrations and Th1-type cytokine profile with augmented expressions of IL-12 and interferon-γ, characteristically observed in tissue infiltrations of BD. Alternatively, low MBL might predispose to a decreased clearance of nuclear debris from the apoptotic neutrophils in BD tissues (1). Another crucial factor might also be the presence of MBL gene mutation at codon 54 which can predispose to bacterial infections such as streptococcal infection (41).

In our previous study, MBL levels in BD patients were significantly lower compared to controls, which did not reach significance in the present study. This partial discrepancy can be associated with the patient profile. In the current study, mainly, patients with mucocutaneous involvement were selected as the relationship between oral ulcers and *S. mutans* colonisations were investigated. Majority of these patients had a mild disease and treated with colchicine compared to our previous study, in which a significant patient subset had major organ involvement and use IS treatments.

In our study, levels of MBL in RA patients were similar to other groups. This is in accordance with some (42) but not other studies (43). However, a trend towards higher S. mutans colonisation is observed in low MBL group (<500 ng/ml). As periodontal disease is suggested to be increased in RA (44), this observation requires a study in larger samples. In addition, *Porphyromonas gingivalis,* another microorganism related to periodontal disease, is associated with disease progression in RA, recently (45).

In conclusion, we showed an interaction of gender, oral infectious foci and aberrant innate immune responses (through low MBL) in BD, suggesting that multiple etiological factors may be required in disease pathogenesis. Longitudinal studies on *S. mutans* colonisation and controlled clinical trials, aiming to modify pathogenic microbial factors in the oral environment, may clarify the exact role of infectious agents in BD pathogenesis.

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