Whole Saliva Flow Rates and Saliva Proteins in Patients with Burning Mouth Syndrome

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Purpose: The aim of this study was to compare the amount of resting and stimulated whole saliva flow, the concentration of total salivary protein and the molecular weight profile of salivary proteins in patients suffering from burning mouth syndrome (BMS) with those in a control group.

Study Design: The study group consisted of 12 BMS patients; 11 subjects not suffering from burning mouth sensation were enrolled as controls. Resting and stimulated whole saliva flow rates were evaluated by spitting method; the amount of salivary protein was estimated using the LP-200 spectrophotometer and a total protein kit (Sigma 690-A) whereas their molecular weight profile was investigated using the sodium dodecylsulfate-polyacrylamide gel electrophoresis system (SDS-PAGE).

Results and Conclusions: No statistical differences were found between BMS patients and controls with respect to the flow rates of whole saliva, its protein concentration, or the electrophoretic profile of the salivary proteins.

Key words: burning mouth syndrome, saliva, spectrophotometer analysis, electrophoresis

Oral Biosci Med 2004; 1: 109-115 *Submitted for publication 3 February 2004; accepted for publication 9 May 2004.*

The term 'burning mouth syndrome' (BMS) refers to a chronic oral pain, diagnosed in the absence of any mucosal abnormality and where psychogenic factors are a possible cause. BMS patients describe their oral symptoms using words such as 'discomfort', 'tender' and 'annoying' which, in most cases, intensify during the course of the day (Hakeberg et al, 1997). Typically the tongue is affected but lips and the anterior part of the palate are also frequently involved (Savage, 1996). BMS patients often complain of xerostomia and dysgeusia. The cause of BMS is still unknown but traditionally its aetiology is considered to be multifactorial (Pedersen et al, 2004).

Several systemic, local or psychological factors have been pointed out as precipitating causes. These include: diabetes (Gibson et al, 1990); hematinic deficiencies (Basker and Main, 1991); menopause (Tarkkila et al, 2001; Frutos et al, 2002; Grushka et al, 2002) peripheral nerve damage (Jaaskelainen et al, 1997); oral candidiasis (Samaranayake et al, 1989); xerostomia (Glick et al, 1976; Grushka, 1987; Gorsky et al, 1991); parafunctional oral habits (Paterson et al, 1995); dental allergies (Lamey et al, 1994); inadequate denture design (Thomson, 1971); and anxiety, depression and cancerophobia (Rojo et al, 1993). In particular subjective oral dryness has been found to be related to depression frequently associated with BMS (Maresky et al, 1993; Bergdahl and Bergdahl, 2000, 2002).

Epidemiological studies on BMS reported different ranges of prevalence in investigated populations: the reasons for these differences may be related to the criteria used to diagnose BMS. In fact few studies include clinical examinations to distinguish between burning mouth as a symptom or as a syndrome. Reported prevalence rates in general populations vary from 15% (Tammiala-Salonen and Soderling, 1993) to 0.7% (Lipton et al, 1993) and relate to burning mouth as a symptom. BMS predominantly affects females (female/male=7/1) with an increased prevalence with age and following menopause (Lamey et al, 1996).

Table 1 Patients' characteristics

Cases ID	Age	Gender	Duration of burning sensation (months)	Complaints of xerostomia	Medication	Hormone substitution	Smoking habit	Alcohol consumption
1 FC	64	F	24	Yes	Lorazepam Nimesulide[1]	No	Non-smoker	Occasional
2 NM	85	F	12	Yes	N/A	N/A	N/A	N/A
3 CS	59	F	5	No	N/A	N/A	N/A	N/A
4 TG	57	F	72	Yes	Propranolol hydrochloride	No	Non-smoker	None
5 TA	75	F	30	Yes	Salycilic acid Digoxin Triazolam	No	Non-smoker	Regular wine
6 DA	57	F	12	Yes	2	No	Non-smoker	None
7 CE	42	M	3	No	No	N/A	Ex-smoker Stopped 6 yrs ago	Occasional
8 TV	56	F	7	No	Atorvastatin	No	Non-smoker	Occasional
9 SV	77	F	60	Yes	Anti-hypertensive[3] Alprazolam	No	Non-smoker	Regular wine Occasional spirits
10 LA	83	F	3	No	Indapamide Lorazepam	No	Non-smoker	Regular wine
11 MM	43	M	7	Yes	N/A	N/A	N/A	N/A
12 ST	59	F	72	Yes	Lormetazepam Reboxetine	No	Non-smoker	None

N/A: not available
[1]nimesulide is a non steroidal anti-inflammatory drug (NSAID)
[2]oxerutins, furosemide, fluticasone propionate, omeprazole, lorazepam, digoxin, clodronate
[3]Anti-hypertensive: patient could not remember the name

The correct management of patients suffering from a burning oral sensation should ensure that underlying possibly treatable causes of the symptom of burning mouth are initially identified; in the case of BMS it is important that the clinician provides an explanation about the syndrome's condition and its benign nature (Pedersen et al, 2004).

A Cochrane review (Zakrzewska et al, 2002) emphasizes that there is little evidence of an effective treatment for BMS patients and that further trials of high methodological quality need to be undertaken. Several studies have aimed to investigate salivary components as a possible implicating factor in BMS aetiology (Glick et al, 1976) because BMS patients often refer to salivary changes (Pedersen et al, 2004).

The purpose of the present study was therefore to investigate if there are any differences in the amount of saliva production, the protein concentration and electrophoretic patterns of resting and stimulated whole saliva between patients suffering from BMS and a group of subjects with similar ethnic origin, gender and age without an oral mucosal complaint.

MATERIALS AND METHODS

Patient Selection

Twelve patients, who were previously diagnosed as suffering from BMS at the Department of Oral Medicine of the University of Milan, were enrolled between Jan-

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Table 2 Controls' characteristics

Controls ID	Age	Gender	Diagnosis	Complaints of xerostomia	Medication	Hormone substitution	Smoking Habit	Alcohol consumption
1 FM	64	F	Leukoplakia	No	Indapamide	No	Smoker	Regular
2 ME	66	F	Leukoplakia	No	No	No	Non-smoker	None
3 QV	53	F	Leukoplakia	No	No	No	Smoker	Regular
4 BM	36	F	Epulis	No	No	No	Smoker	None
5 BF	63	F	Leukoplakia	No	No	No	Non-smoker	None
6 PM	56	F	Pseudo-fibroma	No	No	No	Smoker	Occasional
7 TM	67	F	Leukoplakia	No	No	No	Ex-smoker Stopped 4 yrs ago	Occasional
8 MM	50	F	Restorative dentistry	No	No	No	Smoker	Occasional
9 AO	49	M	Leukoplakia	No	No	No	Smoker	None
10 BF	78	F	Restorative dentistry	No	No	No	Non-smoker	None
11 PA	59	F	Hyperplastic foliate papillae	No	Fluvastatin	No	Smoker	None

uary and June 2002 to participate in the present study.

The diagnosis of BMS was based on a patient's symptoms referred to as: spontaneous burning, stinging or itching sensation, oral dryness, oral paraesthesia, atypical odontalgia and altered taste or smell – in spite of a patient presenting an otherwise healthy looking oral mucosa on clinical examination. Patients were enrolled for this study subject to the following criteria: absence of clinical abnormality of the oral mucous membranes; absence of abnormal laboratory findings such as blood cell count; blood glucose level, serum iron and transferrin levels; serum vitamin B_{12} and folate levels; and absence of clinically detectable salivary gland diseases (before sialometry was performed). They were 10 females and 2 males with a median age of 59 years (range 44-85 years). The patients had suffered from a burning sensation for a median of 12 months (range 3-72 months). Eight out of 12 BMS patients complained of oral dryness, and the majority of the BMS patients where taking medications with a xerogenic potential (Table 1).

The control group comprised 11 adults (median age of 59 years (range 36-78 years) who were not suffering from burning mouth sensation. The subjects were attending the department of oral medicine of the University of Milan for control of oral mucosa diseases. They were not suffering from burning sensation or clinically detectable salivary gland disease. None of the controls complained of oral dryness (Table 2).

Sialometry

All saliva samples were collected in the morning. Resting (unstimulated) whole saliva was collected from patients and control subjects using the following method: patients were asked to not swallow but to allow the unstimulated saliva to accumulate in their mouth for 2 min. and then to spit out into a graduated collecting vessel. Three further collections were performed at 1 min. intervals. Thus the amount of whole saliva that was collected represented the saliva produced in the mouth over a 5 min. period. The total amount of saliva collected in the graduated vessel (in ml) was then divided by 5 to give a secretion rate in ml/min.

Stimulated whole saliva flows were induced by chewing on 1 gram of paraffin wax. Collection of saliva was performed as described above over a 5 min. period. After the amount of whole saliva production was determined, resting and stimulated saliva samples were centrifuged at 1200 rpm for 2

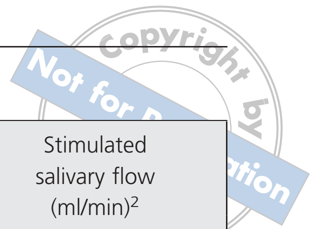

Table 3 Resting and stimulated salivary flow from cases and controls

Cases ID	Resting salivary flow (ml/min)[1]	Stimulated salivary flow (ml/min) [2]	Controls ID	Resting salivary flow (ml/min) [1]	Stimulated salivary flow (ml/min)[2]
1	0.3	0.5	1	0.5	0.7
2	0.7	1.0	2	0.6	0.8
3	0.3	0.7	3	0.1	0.2
4	0.2	0.3	4	0.5	0.7
5	0.1	0.1	5	0.3	0.7
6	0.3	0.4	6	0.3	0.5
7	1.0	1.5	7	0.2	0.8
8	0.3	0.6	8	0.3	0.6
9	0.4	0.7	9	0.4	0.9
10	0.3	0.6	10	0.3	0.8
11	0.2	0.4	11	0.1	0.4
12	0.1	0.1			
Mean	0.35	0.57	Mean	0.33	0.65
SD	0.26	0.39	SD	0.16	0.21

[1]Student's t-test not significant (P=0.8)
[2]Student's t-test not significant (P=0.7)

min. in order to eliminate sloughed cells and food debris. Samples were then stored at −20 °C until further use.

Total Protein Concentration Determination

Protein concentration in saliva samples was evaluated using the Biuret method: each saliva sample was mixed with a diluted Biuret reagent and later with folin and Ciocalteu's phenol reagent following the manufacturer's instruction (Micro Protein determination kit – Sigma Diagnostic Procedure No. 690). The colour formed was read at 620 nm (a suitable wavelength is between 550 and 750 nm) using the LP-200 spectrophotometer. Protein concentrations were determined from a calibration curve ($Y=0.43376+0.0118X$) previously defined using the protein standard provided in the kit. Absorbance values comprised between 0.5 and 2 were considered adequate to estimate protein concentration. Thus, 10^{-1} and 10^{-2} dilutions were performed and read four times at 620 nm wavelength to obtain the net product for each sample. For each sample the mean of the four values comprised in the interval was calculated and considered for statistical analysis.

Electrophoresis Conditions

For all salivary samples, the sodium dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE) was performed using a Mini-Protean electrophoretic unit (Bio-Rad®). Five μL of whole saliva were mixed with 5 μL of reducing electrophoretic sample buffer. Prepared samples were analysed using pre-cast gel (7 cm×8 cm) with 7.5% resolving gel and 4% stacking gel.

Molecular weight standard (Bio-Rad #161-0318) was used for all gels; the molecular weight standard used was composed of the following proteins: rabbit myosin (212 kDa), *E. coli* beta-galactosidase (116 kDa), bovine serum albumin (67 kDa), chicken ovalbumin (43 kDa), and bovine carbonic anhydrase (30 kDa). Electrophoresis was performed at 75 mA constant current for approximately 1 hour. Gels were fixed and stained in a solution of colloidal coomassie brilliant blue (Bio-Rad #161-0786). Digital picture of gels were taken using a Kodak EDAS digital camera and analysed using dedicated Kodak software (Kodak 1D LE 3.5). Visual inspections of gels revealed a single strong stained band in the size between 43 and 67 KDa, and in some cases a second and a third weaker band was observed. The proteins were tentatively identified according to Johnson (1984).

Statistical Analysis

Unpaired *t*-test was used to compare the means of the two groups. Significance was established for P values ≤0.005.

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Table 4 Protein concentration of resting and stimulated salivary flow for both cases and controls

Cases ID	Protein concentration in resting salivary flow (mg/dL)[1]	Protein concentration in stimulated salivary flow (mg/dL)[2]	Controls ID	Protein concentration in resting salivary flow (mg/dL)[1]	Protein concentration in stimulated salivary flow (mg/dL)[2]
1	101	79	1	54	42
2	54	43	2	91	56
3	128	130	3	90	64
4	74	78	4	80	76
5	108	61	5	83	77
6	49	70	6	70	109
7	53	50	7	112	116
8	117	90	8	76	49
9	116	77	9	77	91
10	104	66	10	86	84
11	43	53	11	115	115
12	65	112			
Mean	84	76	Mean	85	80
SD	31	25	SD	17	26

[1]Student's t-test not significant (P=0.9)
[2]Student's t-test not significant (P=0.7)

RESULTS

The comparison of resting and stimulated whole saliva flow rates between cases and controls did not show any statistically significant difference (Student's t-test with p values of 0.8 and 0.7 respectively) (Table 3). Moreover, the study group showed values of resting salivary flow higher than the control group despite the fact that many of the BMS cases were taking benzodiazepine on a daily basis (i.e. medication with a xerogenic potential). Interestingly 8 out of 12 cases and none of the controls complained about xerostomia and therefore, with BMS, the feeling of oral dryness did not seem to be related to impaired saliva secretion from the major salivary glands.

No differences were observed in protein concentration of resting and stimulated salivary flow for both cases and controls (Student's t-test showed p values of 0.9 and 0.7 respectively) (Table 4). Visual inspections of gels revealed a strong stained blue band and in some cases two higher bands between 67 and 43 KDa of molecular weight. The same pattern was observed in

Fig. 1 SDS-PAGE protein electrophoretic pattern of resting and stimulated salivary samples of some BMS cases and controls. Column 1 shows the molecular weight marker in both gels. Columns 2, 4, 6 and 8 of cases gel represent resting saliva of SV, LA, ST and MM respectively; Columns 3, 5, 7 and 9 of cases gel represent stimulated saliva of the same patients.
Columns 2, 4, 6 and 8 of controls gel represent resting saliva of FM, ME, QV and BM respectively; Columns 3, 5, 7 and 9 of controls gel represent stimulated saliva of the same subjects.
For all samples a strong stained band was evident between 67 and 43 KDa of molecular weight tentatively identified as amylase (Johnson et al, 1995).

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cases and controls in both unstimulated and stimulated saliva samples (Fig. 1). The stronger lower band was tentatively identified as amylase whereas the faint higher one could correspond to the major glycosylated proline-rich protein (PRP) as reported by Schwartz et al (1995). However, since equal amounts of saliva proteins were not added to the gels we could not make any quantitative comparisons between the protein profiles of the two groups.

DISCUSSION

Some studies (Grushka, 1987; Lamey and Lamb, 1988; Gorsky et al, 1991) suggested that reduction in salivary volume or changes in the composition of saliva can contribute to the complaint of burning mouth (Grushka, 2002). In particular, a reduction in salivary flow rate is believed to be associated with a burning oral sensation. Nevertheless most salivary flow rate studies in BMS subjects have not demonstrated a significant decrease in salivary output (Huang et al, 1996) – but contrasting findings have nonetheless been reported (Muzyka and De Rossi, 1999).

Our findings reported in the present study allow us to conclude that there is no significant difference in the unstimulated and stimulated whole saliva flow rates among BMS patients and controls. It should however be stressed that the study and control group sample size was very small. Interestingly 4 of the BMS patients and 3 of the controls had low levels of unstimulated salivary flow (≤0.2 ml/min), of which 2 BMS cases and 2 controls had unstimulated flow values of 0.1 ml/min thereby placing these individuals in the hyposalivation diagnosis category. Moreover, it is remarkable that the majority of the BMS patients (10 out of 12) and of the controls subjects (7 out of 11) should be considered low secretors as they showed a chewing stimulated whole saliva flow rate of ≤0.7 ml/min. Surprisingly in the control group none of the subjects with low unstimulated flow rates complained of oral dryness, a finding which stands in contrast to what is observed in the BMS group.

In the present study we did not evaluate the oral mucosal symptoms using visual analogue scales and therefore we cannot make any conclusion regarding the relations between the intensities of oral symptoms – i.e. burning sensation and oral dryness. Two previously published studies (Tammiala-Salonen and Soderling, 1993; Lundy et al, 1997) investigated the protein concentration and composition in saliva samples of BMS patients. The results of the present study agreed with the above findings in that we found that the total protein concentration did not differ between saliva in BMS cases and controls. Therefore BMS does not appear to be associated with a decrease in the protecting and lubricating properties of whole saliva.

In particular, Lundy et al (1997) evaluated major parotid glycoproteins in patients with BMS and did not observe differences in banding pattern between patients and controls. Correspondingly, no differences in a SDS-PAGE analysis of banding pattern were observed by Tammiala-Salonen et al (1993). This finding is supported by the present study in which the quality of protein concentration of saliva samples was assessed using SDS-PAGE in conjunction with a Mini-Protean electrophoretic unit.

CONCLUSIONS

On the basis of the findings of this present study we can conclude that BMS patients did not show quantitative alteration in salivary flow when compared to that of a control group. The concentration of total protein in resting and stimulated saliva samples was similar for cases and controls. Moreover, a similar and characteristic qualitative band pattern was observed for all samples. However, our methods did not allow for a complete characterization of the salivary proteins. If compared with other but similar methods as the one applied in our study (Schwartz et al, 1995), a two-dimensional method using a bigger unit would be more appropriate for accurate comparison of spots (Beeley and Khoo, 1999).

ACKNOWLEDGMENTS

The authors are grateful to Professor Eugenio Brambilla for his support on the use of the spectrophotometer and to Dr Andrea Felloni for his statistical advice in the definition of the calibration curve.

This paper was originally given as an oral presentation at the 6th Biennial Congress of the EAOM, 3–5 October 2002, Lisbon, Portugal.

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