

## Chemical analysis of whole saliva in Sjögren's syndrome

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**SUMMARY** In some studies, but not in all, abnormally high concentrations of salivary Na<sup>+</sup>, K<sup>+</sup>, and IgA have been found in patients with Sjögren's syndrome (SS). The lack of agreement between various reports might be due to the different ways in which saliva was collected. Some analysed stimulated parotid or whole saliva, whereas others used unstimulated saliva. In this study, therefore, the rate of flow and Na<sup>+</sup>, K<sup>+</sup>, and IgA levels in unstimulated and stimulated whole saliva in normals and in rheumatoids with and without SS have been determined. The results confirmed significantly raised levels of Na<sup>+</sup>, K<sup>+</sup>, and IgA in unstimulated whole saliva in SS. In response to stimulation there was marked decrease in Na<sup>+</sup>, K<sup>+</sup>, and IgA levels, whereas normally, as shown by the other two groups, there is an increase in Na<sup>+</sup>, no change in K<sup>+</sup>, and a mild decrease in IgA. As a result, the differences between SS and normals became much less significant (K<sup>+</sup>, IgA) or were even completely obliterated (Na<sup>+</sup>). The abnormal response of SS to stimulation may be partially explained by the initially low rate of flow and by the extremely high IgA levels. Thus chemical analysis of unstimulated whole saliva is much more sensitive than analysis of stimulated whole saliva in the detection of salivary gland involvement in SS.

**Key words:** rheumatoid arthritis, sialochemistry.

Clinical diagnosis and evaluation of the ocular component in Sjögren's syndrome (SS) are easily carried out by the Schirmer test and rose bengal staining. Oral involvement needs more sophisticated (scintigraphy) or aggressive (labial biopsy) methods as the value of analysis of the saliva (sialochemistry) is still controversial. Several workers have reported a significant increase in salivary concentrations of Na<sup>+</sup>, K<sup>+</sup>, and IgA, either solely or in combination,<sup>1-3</sup> whereas others could not confirm it.<sup>4,5</sup>

One of the differences between the various reports is the method of collection of saliva, i.e., whether stimulated or unstimulated saliva was analysed. To elucidate the importance of this point we compared whole stimulated saliva with whole unstimulated saliva in patients with SS.

### Patients and methods

Forty people agreed to participate in the study; they were divided into three groups: (A) 20 healthy

subjects, matched for age and sex (control group); (B) 11 patients who fulfilled the American Rheumatism Association criteria for definite or classical rheumatoid arthritis (RA), without xerostomia or xerophthalmia (RA group); and (C) nine patients with either classical or definite RA who had xerostomia, positive Schirmer's test, and positive rose bengal staining of the cornea (SS group).

None were taking  $\beta$  blockers or monoamine oxidase inhibitors. The treatment for their RA was not changed for at least one month before saliva was tested.

All samples were collected at the same time of day, at least one hour after meals, in a quiet room. Whole unstimulated saliva was collected for 10 minutes by spitting into a test tube. The volume of the saliva was then measured.<sup>1</sup> The patient's response to stimulation was evaluated by applying a swab with a 2% solution of citric acid every 30 seconds on both sides of the tongue during the 10 minute period of saliva collection. The concentrations of Na<sup>+</sup> and K<sup>+</sup> were analysed by flame photometry. Salivary IgA levels were determined by the radial immunodiffusion method of Mancini *et*

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Table 1 Flow rate and composition of whole unstimulated and stimulated saliva

Group	Rate of flow (ml/min)		Na <sup>+</sup> (mmol/l)		K <sup>+</sup> (mmol/l)		IgA (mg/l)	
	Unst*	St*	Unst	St	Unst	St	Unst	St
A (n=20)	0.25 (0.1)	1.28 (0.47)	4.9 (0.48)	10.1 (1.1)	19.6 (1)	18.5 (0.85)	71 (19)	31 (11)
B (n=11)	0.27 (0.04)	1.03 (0.21)	5.8 (1.07)	13.8 (2.7)	23.1 (1.3)	19.9 (1.1)	98 (13.9)	44 (8.5)
C (n=9)	0.06 (0.02)	0.52 (0.28)	25.3 (7.07)	15.9 (4.5)	52.7 (16)	25.7 (2.3)	454 (62)	120 (31)
<i>p</i> Values								
B/A	NS	NS	NS	NS	0.05	NS	NS	NS
C/B + C/A	<0.01	<0.01	<0.01	NS	<0.001	<0.05	<0.001	<0.05
<i>Unst/St</i>								
A	<0.01		<0.05		NS		<0.01	
B	<0.01		<0.05		<0.01		<0.001	
C	<0.01		NS		<0.001		<0.001	

\*Unst=unstimulated whole saliva; St=stimulated whole saliva. The results are given as mean (standard error).

al,<sup>6</sup> on low level Meloy plates, using serum IgA standards. Student's *t* test for paired and unpaired samples was used for statistical analysis.

**Results**

The results are summarised in Table 1 and Fig. 1.

The flow rate and composition of unstimulated saliva were similar in the control and RA groups, except for marginally significantly higher levels of

K<sup>+</sup> in the RA group. The SS group had a significantly lower flow rate and a significant increase in Na<sup>+</sup>, K<sup>+</sup>, and IgA concentrations. IgA levels were so high that there was no overlap between the SS group and the other two groups; the same was true of K<sup>+</sup> levels, except for one patient who had a K<sup>+</sup> level within the normal range. Sodium levels were high in five patients and within the normal range in four (Fig. 1).

Stimulation led to a significant increase in flow rate in all groups; the flow rate in the SS group, however, was still significantly less than in the other two groups. After stimulation IgA levels were significantly lower in all groups; the most dramatic change occurred in the SS group, where the level of IgA was less than one third of the unstimulated value. As a result there was a considerable overlap between the SS group and the other two. Four patients of the SS group had IgA levels within the normal range. The highest concentration of IgA after stimulation was similar to the lowest one in unstimulated saliva. The difference became much less significant (*p*<0.05 instead of <0.001). In the SS group, but not in the control and RA groups, the K<sup>+</sup> level decreased significantly after stimulation. As with IgA, the difference between the SS group and the other two groups became much less significant.

Stimulation caused a significant increase in Na<sup>+</sup> concentration in both the control (except two subjects) and RA (except one subject) groups (Fig. 1). The effect of stimulation on Na<sup>+</sup> concentration in the SS group was in the opposite direction, i.e., there was a marked reduction of Na<sup>+</sup> level in most cases. Only two patients, who had normal Na<sup>+</sup> values in unstimulated saliva, had normal response to stimulation, i.e., increased Na<sup>+</sup> levels.

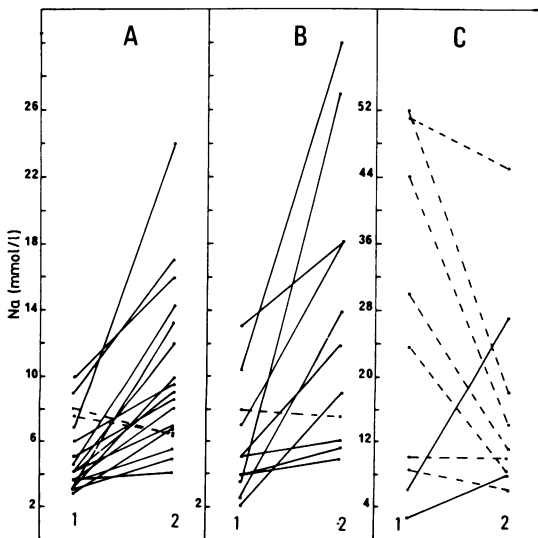


Fig. 1 Effect of stimulation on Na<sup>+</sup> levels in whole saliva. The dotted lines emphasise that the direction of the response is opposite to normal.

## Discussion

The normal response of healthy salivary glands to citric acid stimulation includes increased flow rate, decreased IgA concentration, marginal changes in  $K^+$  levels, and a significant increase in  $Na^+$  levels.<sup>7,8</sup> Both the control and RA groups showed the normal pattern of response after citric acid stimulation. Patients with SS had a different response, characterised by a marked decrease (40%) in  $Na^+$  concentrations, a significant decrease in  $K^+$  levels, and a much more dramatic reduction in IgA levels.

Normal saliva is hypotonic; the primary saliva in the acini of the major salivary glands is isotonic, and active  $Na^+$  reabsorption takes place in the ductal system (striated ducts, main duct), so that the final saliva is hypotonic, containing 4–9 mmol/l of  $Na^+$ . After stimulation the excreted primary saliva is still isotonic; but owing to higher flow rate there is less time for ductal  $Na^+$  reabsorption, leading to increased  $Na^+$  concentration in stimulated final saliva.<sup>9</sup> This pattern of response was shown by both the control and RA groups.

Based on experimental evidence from the sub-maxillary gland in rats, it has been suggested that there are sites in the terminal parts of the lobar duct tree where saliva equilibrates with the serum. The contact time necessary for re-equilibrium is so great, however, that it can take place only at the lowest flow rate. In this case the final saliva contains higher concentrations of  $Na^+$ .<sup>9</sup> The high  $Na^+$  concentrations in unstimulated saliva in SS are thus due to the very low flow rate, which enables partial re-equilibrium of the saliva with the serum. With stimulation, the flow rate is similar to that of unstimulated saliva in healthy subjects, with less available contact time to equilibrate, leading to more hypotonic saliva.

Potassium concentrations in final saliva are higher than in the serum owing to active secretion of  $K^+$  in the ductal system, as has been shown in human parotid glands and in other species.<sup>9</sup> With high flow rates  $K^+$  levels remain constant, indicating increased secretion with increasing flow. At very low flow rates the increasing contact time of the saliva with the secretory areas leads to  $K^+$  levels higher than normal.<sup>9</sup> The high levels of  $K^+$  in the SS group were probably due to the low flow rate; when stimulated, the increasing flow rate led to a rapid decrease in  $K^+$  levels, approaching levels seen in normals.

In a normal population the concentration of IgA in the saliva is inversely related to the flow rate; increasing the flow rate by stimulation dilutes IgA levels in both parotid and whole saliva.<sup>7</sup> In SS extremely high concentrations of IgA were found

Table 2 Relation between salivary IgA concentration and salivary flow rate

Group	Flow rate ratio (St/Unst)*	IgA ratio (Unst/St)	Flow rate ratio/IgA ratio
A	5.12	2.3	2.23
B	3.8	2.2	1.73
C	8.7	3.8	2.29

\*St=stimulated saliva; Unst=unstimulated saliva.

which were greater than could be accounted for by the low flow rate. This supports the previous report of local oversecretion of IgA from the massive lymphocyte infiltrates.<sup>10</sup>

The reduction of IgA in all groups after stimulation was flow dependent. The greater absolute reduction of IgA levels in the SS group was due to the extremely high initial values. When the ratio of IgA reduction was related to the ratio of the increase in flow rate, similar proportions were found in all groups (Table 2). The high IgA levels in stimulated saliva in SS reflect its local overproduction in this disease.

This study has shown that chemical analysis of unstimulated whole saliva is preferable to analysis of stimulated saliva for the detection of salivary involvement in SS. Although the differences between the SS group and the other two groups in  $Na^+$ ,  $K^+$ , and IgA concentrations in unstimulated saliva were highly significant, stimulation tended to minimise or even cancel them. It is not surprising, therefore, that studies based on stimulated saliva, either parotid or whole saliva, reached inconclusive findings.

Like unstimulated whole saliva, unstimulated parotid saliva may also be significantly different in patients with SS. Even if this should prove to be so, however, the extreme difficulty of obtaining unstimulated parotid saliva from these patients makes such a test impractical.

The abnormally high levels of  $Na^+$ ,  $K^+$ , and IgA in unstimulated whole saliva were found to be correlated with lip biopsy abnormalities in SS.<sup>3</sup> It seems, therefore, that the analysis of whole unstimulated saliva for  $Na^+$ ,  $K^+$ , and IgA levels is not only simple and cheap but also a sensitive means for the detection of salivary gland involvement in SS.

## References

- 1 Ben-Aryeh H, Nahir A M, Scharf Y, Gutman D, Laufer D, Szargel R. Sialochemistry of patients with rheumatoid arthritis. *Oral Surg Oral Med Oral Pathol* 1978; **45**: 63–70.
- 2 Nahir A M, Ben-Aryeh H, Szargel R, *et al.* Sialochemistry in evaluating bromhexine treatment of Sjögren's syndrome. *Br Med J* 1979; **2**: 833.
- 3 Spielman A, Ben-Aryeh H, Lichtig H, *et al.* Correlation

- between sialochemistry and lip biopsy in Sjögren's syndrome patients. *Int J Oral Surg* 1982; **11**: 326-30.
- 4 Tsianos E B, Tzioufas A G, Mavridis A K, *et al*. Sialochemistry of patients with autoimmune rheumatic disease with and without histological manifestations of Sjögren's syndrome. *Ann Rheum Dis* 1985; **44**: 412-4.
  - 5 Benedek-Spat E, Berenji B, Schia A. A sialochemical study on patients with Sjögren's syndrome. *Arch Oral Biol* 1975; **20**: 649-52.
  - 6 Mancini G, Carbonara A O, Hermans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 1976; **2**: 235-46.
  - 7 Ben-Aryeh H, Naor H, Szargel R, Horowitz G, Gutman D. The concentration of salivary IgA in whole and parotid saliva and the effect of stimulation. *Int J Oral Surg* 1986; **15**: 81-2.
  - 8 Shanon I L, Suddik R P, Dowd F J Jr. *Saliva: composition and secretion*. Basel: Karger, 1974.
  - 9 Young J A, Fromter E, Schogel E, Hamann K F. Micropuncture and perfusion studies of fluid and electrolyte transport in the rat submaxillary gland. In: Schneyer L H, Schneyer C A, eds. *Secretory mechanisms of salivary glands*. New York and London: Academic Press, 1967.
  - 10 Ben-Aryeh H, Szargel R, Gutman D. Salivary IgA in Sjögren patients. *Int J Oral Surg* 1983; **12**: 120-3.