

β_2 Microglobulin measurements in saliva of patients with primary Sjögren's syndrome: influence of flow

S A P van der Geest, H M Markusse, A J G Swaak

Abstract

Objectives—To determine the diagnostic value of β_2 microglobulin in parotid saliva, its concentration in relation to salivary flow was determined in 29 patients with primary Sjögren's syndrome and in 30 normal controls. The specific secretion rate of β_2 microglobulin was calculated.

Methods—Parotid saliva samples were collected within a 20 minute period directly from Stensen's duct with Lashley cups: sample 1 without gustatory stimulation during the first 10 minutes and samples 2 and 3 during the next five to 10 minutes, when saliva production was stimulated by a 500 mg vitamin C tablet. The sample volumes were measured and the β_2 microglobulin concentration was determined by radioimmunoassay.

Results—During gustatory stimulation the mean β_2 microglobulin secretion rate in patients with primary Sjögren's syndrome was 0.31 $\mu\text{g}/\text{min}$; in normal controls it was 0.14 $\mu\text{g}/\text{min}$. The sensitivity and specificity of this test were 56 and 87% respectively. The mean salivary flow without stimulation in patients with primary Sjögren's syndrome was lower than that in normal controls, but no difference was found during stimulation.

Conclusions—Our results support the hypothesis of local β_2 microglobulin production in the parotid gland of patients with primary Sjögren's syndrome. The test cannot be used as a screening test owing to low sensitivity, but it may be used as a supplementary diagnostic test as it has the advantage of being non-invasive.

(*Ann Rheum Dis* 1993; 52: 461-463)

Primary Sjögren's syndrome is a chronic inflammatory disorder of the exocrine glands.^{1 2} It is characterised by keratoconjunctivitis sicca and xerostomia, which result from lymphocytic infiltration of the lachrymal and salivary glands.

β_2 Microglobulin was isolated in 1968 by Berggard and Bearn.³ It is a non-glycosylated protein with a low molecular weight of 11 800 and has been identified as the invariant light chain of the class I major histocompatibility antigens HLA-A, HLA-B, and HLA-C.⁴ The

HLA- β_2 microglobulin complex is found on the surface of nearly all nucleated cells.⁵ The surfaces of lymphocytes and monocytes are particularly rich in β_2 microglobulin, and lymphocytic synthesis and expression are further augmented by stimulation with mitogens or interferon.⁶ As a result of HLA turnover, β_2 microglobulin is dissociated in its 'free' form in the interstitial fluid.^{4 7}

It has been suggested that measurement of the β_2 microglobulin concentration in saliva is a simple method of quantifying the extent of glandular inflammation.^{8 9} In clinical situations in which quantitative comparisons of salivary proteins are made, the effect of flow alterations must be considered, especially when altered flows are characteristic of the disease.¹⁰ The flow of salivary fluid has not been taken into account in previous studies comparing salivary β_2 microglobulin concentrations in patients with primary Sjögren's syndrome.

To determine the diagnostic value of the measurement of β_2 microglobulin in parotid saliva, we measured its concentration in parotid saliva in relation to the salivary flow in patients with primary Sjögren's syndrome and in normal controls and calculated the secretion rate of β_2 microglobulin.

Patients and methods

The patient group consisted of 29 patients with primary Sjögren's syndrome (two men, 27 women), mean (SD) age 53 (10) years, selected from the department of rheumatology, Dr Daniel den Hoed Clinic, Rotterdam. They all met the modified criteria of Fox *et al.*¹¹ The control group consisted of 26 healthy women and four men, with a mean (SD) age of 50 (15) years. None of the subjects fulfilled the classifying criteria for another connective tissue disease.

Parotid saliva samples were collected directly from Stensen's duct with Lashley cups.¹² Three samples were taken within 20 minutes: sample 1 without gustatory stimulation during the first 10 minutes; sample 2 after stimulation of saliva with 500 mg vitamin C during the next five minutes; and sample 3 during the last five minutes, with the same stimulation. The sample volumes were measured by comparison with standard pipette samples. The collected samples were stored at -20°C . A blood sample was concurrently centrifuged at 3000 rev/min and stored at

Department of
Rheumatology,
Dr Daniel Den
Hoed Clinic,
PO Box 5201,
3008 AE Rotterdam,
The Netherlands
S A P van der Geest
H M Markusse
A G J Swaak

Correspondence to:
Dr Swaak.

Accepted for publication
10 February 1993

Salivary concentration, flow, and secretion rate of β_2 microglobulin

Parameter	Conditions*		
	Unstimulated (1)	Stimulated (2)	Stimulated (3)
Mean β_2 microglobulin concentration (mg/l)			
Normal controls	0.77	0.38	0.34
Patients	1.42	0.98	1.03
p Value	<0.004	<0.001	<0.001
Mean salivary flow (ml/min)			
Normal controls	0.023	0.37	0.25
Patients	0.011	0.22	0.28
p Value	0.01	0.14	0.14
Mean β_2 microglobulin secretion rate ($\mu\text{g}/\text{min}$)			
Normal controls	0.018	0.14	0.085
Patients	0.026	0.31	0.19
p Value	0.04	<0.001	<0.001

*See text for details of conditions.

-20°C. β_2 microglobulin was measured by a radioimmunoassay (Abbott Laboratories). Measurements were performed in duplicate. The β_2 microglobulin secretion rate was calculated as the product of β_2 microglobulin concentration and salivary flow.

Comparative analysis of the mean β_2 microglobulin concentrations was carried out after log transformation in an unpaired *t* test. Means were considered to be statistically different when $p < 0.05$. The sensitivity was calculated as the ratio of the number of patients with primary Sjögren's syndrome with a β_2 microglobulin level above the mean+SD of normal controls, and the total number of patients with primary Sjögren's syndrome. The specificity was calculated as the ratio of the number of normal controls with β_2 microglobulin levels less than the mean+SD of normal controls, and the total number of normal controls. The Pearson correlation test was used to analyse the correlation between serum levels of β_2 microglobulin and the secretion rate of β_2 microglobulin into saliva.

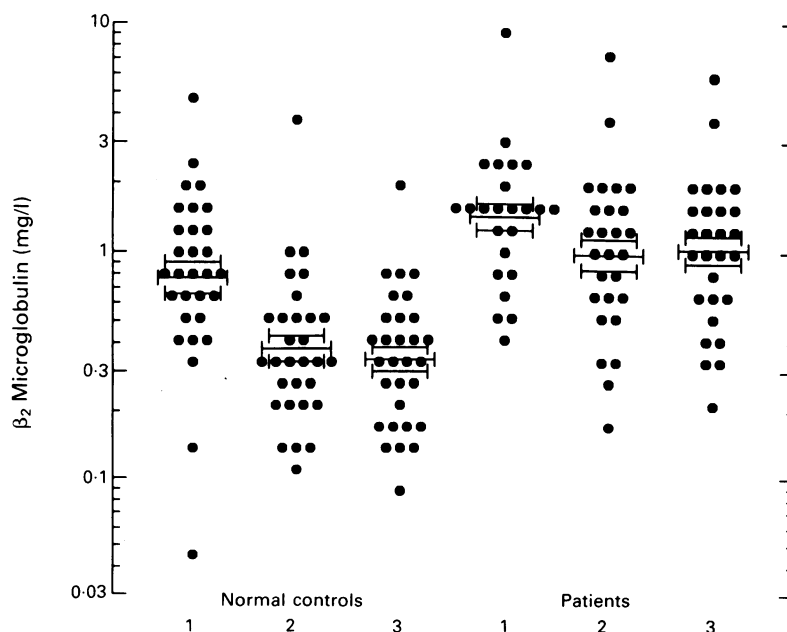


Figure 1 Concentrations of β_2 microglobulin in salivary fluid in normal controls and patients with primary Sjögren's syndrome under unstimulated (1) and stimulated conditions (2, 3). The concentration is higher in patients with primary Sjögren's syndrome ($p < 0.001$). The β_2 microglobulin concentration decreases during stimulation in both groups, but the difference remains statistically significant. The lines represent the mean (SEM) values in normal controls.

Results

The mean β_2 microglobulin concentration in the saliva of patients with primary Sjögren's syndrome was higher than in normal controls ($p < 0.001$) (table). During stimulation of salivary flow the β_2 microglobulin concentration decreases in the two groups (table; fig 1). The mean salivary flow in patients with primary Sjögren's syndrome was lower than that in normal controls, reaching statistical significance only under unstimulated conditions ($p < 0.01$) (table).

The β_2 microglobulin secretion rate increases in the two groups after stimulation (table; fig 2). The percentage of patients with primary Sjögren's syndrome with a β_2 microglobulin secretion rate greater than the mean (1 SD) of the controls under unstimulated conditions is 21%, and increases to 56 and 50% after stimulation.

The mean serum concentration of β_2 microglobulin of patients with primary Sjögren's syndrome is 1.78 mg/l and in normal controls

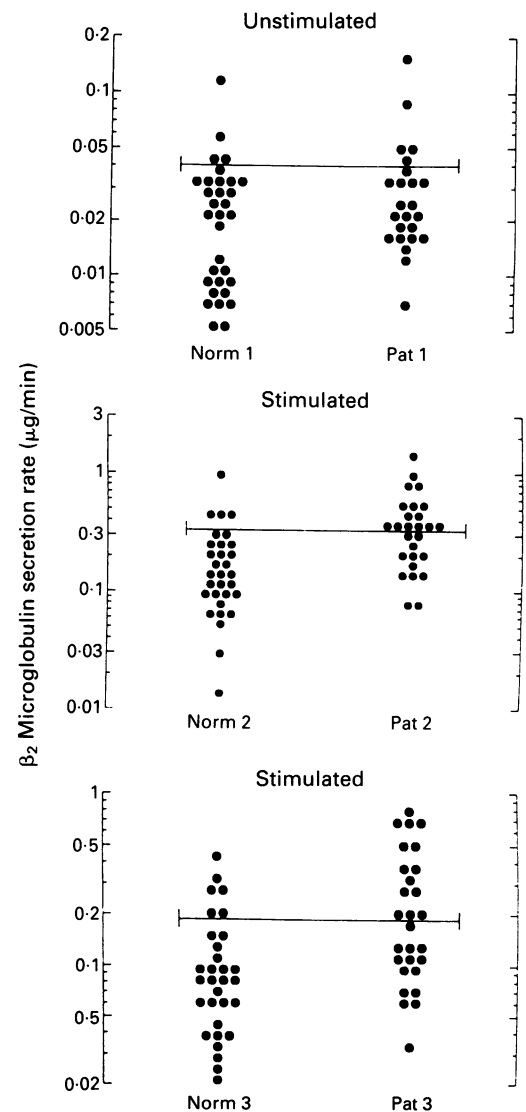


Figure 2 Secretion rates of β_2 microglobulin in salivary fluid in normal controls and patients with primary Sjögren's syndrome under unstimulated and stimulated conditions. The lines represent the mean+SD in the normal controls. The mean secretion rate in patients with primary Sjögren's syndrome is higher than in normal controls under stimulated conditions ($p < 0.001$).

1.02 mg/l ($p < 0.001$). No correlation was found between the serum β_2 microglobulin concentration and the secretion rate of β_2 microglobulin in saliva under stimulated conditions in patients and normal controls.

Discussion

For patients with unequivocal rheumatoid arthritis and a dry mouth, measurement of unstimulated whole salivary flow is reported to be a sufficient screening test to establish a diagnosis of secondary Sjögren's syndrome.¹³ Whole unstimulated salivary flow reflects the basal flow by measuring the total contribution from all the glands. Our results support these observations with respect to the parotid glands. The diagnosis of primary Sjögren's syndrome is often more difficult and requires the absolute presence of salivary gland lesions. To confirm lesions in the salivary gland in primary Sjögren's syndrome, a biopsy sample is taken from the labial glands and diagnostic sialography tests performed.¹¹ Measurement of the β_2 microglobulin concentration has been proposed as a less invasive test for the oral component in primary Sjögren's syndrome.¹⁴

Secretion rates are usually used to study the secretion of proteins into saliva because differences in concentration as a result of an alteration in dilution by different salivary flows are thus eliminated.¹⁰ From this study it appears that the β_2 microglobulin concentration in saliva increases when flow decreases. The salivary flow without stimulation in patients with primary Sjögren's syndrome is lower than that in normal controls, but no difference was found during stimulation. Higher concentrations of β_2 microglobulin in the saliva of patients could therefore be a result of an alteration in flow. We propose that secretion rates are used instead of concentrations for comparative analyses of β_2 microglobulin values.

Our results indicate that the mean secretion rate of β_2 microglobulin in salivary fluid is higher in patients with primary Sjögren's syndrome than in normal controls ($p < 0.001$) (table). The sensitivity of an increased β_2 microglobulin secretion rate is 56% and the specificity is 87%. As the difference between patients with primary Sjögren's syndrome and controls is most prominent during stimulation, we propose that the β_2 microglobulin secretion rate is measured during gustatory stimulation. Although the test cannot be used as a screening test due to its low sensitivity, it may be used as a supplementary diagnostic test, especially as it is non-invasive and other tests used for the diagnosis of primary Sjögren's syndrome are inconclusive. The sensitivity and specificity should be studied in larger groups of patients with sicca disorders.

It remains to be clarified how β_2 microglobulin enters the saliva, and how this translocation is influenced. The translocation of β_2

microglobulin into saliva could be a process of constant active secretion or related to with the production of salivary fluid. It could also be a process of diffusion from serum or from an infiltrate in the salivary gland.

The first two processes are unlikely: the absence of a complete inverse relation between the β_2 microglobulin concentration and the salivary flow suggests that β_2 microglobulin is probably not actively secreted and if the secretion of β_2 microglobulin was directly related to the production of salivary fluid, its concentration should remain constant, which it does not.

Serum β_2 microglobulin levels in patients with primary Sjögren's syndrome are higher than in normal controls. It seems, however, improbable that β_2 microglobulin diffuses from serum into saliva because no correlation between serum β_2 microglobulin levels and secretion rates of β_2 microglobulin into saliva was found.

Evidence for increased local production in the salivary gland can now be based on the observation of increased β_2 microglobulin secretion rates in the saliva of patients with primary Sjögren's syndrome. Our data support the hypothesis of local β_2 microglobulin production, and therefore the secretion rate of β_2 microglobulin might be useful as a diagnostic tool.

- Moutsopoulos H M, Chused T M, Mann D L, et al. Sjögren's syndrome (sicca syndrome): current issues. *Ann Intern Med* 1980; 92: 212-26.
- Bloch K J, Buchanan W W, Wohl M J, Bunim J J. Sjögren's syndrome. A clinical, pathological, and serological study of sixty-two cases. *Medicine (Baltimore)* 1965; 44: 187-231.
- Berggard I, Bearn A G. Isolation and properties of a low molecular weight β_2 globulin occurring in human biological fluids. *J Biol Chem* 1968; 243: 4095-103.
- Creswell P, Springer T, Strominger J L, Turner M J, Grey H M, Kubo R T. Immunological identity of the small subunit of HLA antigens and β_2 microglobulin and its turnover on the cell membrane. *Proc Natl Acad Sci USA* 1974; 71: 2123-207.
- Nissen M H, Plesner T, Rörth M. Modification of β_2 -microglobulin in serum from patients with small cell carcinoma of the lung: correlation to clinical course. *Clin Chim Acta* 1984; 141: 41.
- Acozar J, Essex M, Watson A, Garit E, Anderson D, Junis E. Changes in the expression of HLA and β_2 -microglobulin by cultured lymphoid cells. *Hum Immunol* 1982; 5: 83-93.
- Sidkey K, Walker R A. B2-microglobulin in non-malignant and malignant human breast: a feature of differentiation. *J Pathol* 1984; 142: 35-40.
- Talal N, Grey H M, Zaifler M, Michalski J P, Daniels T E. Elevated salivary and synovial fluid β_2 -microglobulin in Sjögren's syndrome and rheumatoid arthritis. *Science* 1975; 188: 1196-7.
- Swaak A J G, Visch L L, Zonneveld A. Diagnostic significance of salivary levels of β_2 -microglobulin in Sjögren's syndrome. *Clin Rheumatol* 1988; 7: 28-34.
- Rudney J D. Implications of a model from olfactory research for the use of secretion rates in salivary studies. *Arch Oral Biol* 1990; 35: 365-71.
- Fox R J, Robinson C A, Curd J G, Kozin F, Howell F V. Sjögren's syndrome: proposed criteria for classification. *Arthritis Rheum* 1986; 29: 577-85.
- Fox R I, Howell F V, Bone R C, Michelson P E. Primary Sjögren's syndrome: clinical and immunopathologic features. *Semin Arthritis Rheum* 1984; 14: 77-105.
- Speigt P M, Kaul A, Melsom R D. Measurement of whole unstimulated salivary flow in the diagnosis of Sjögren's syndrome. *Ann Rheum Dis* 1992; 51: 499-502.
- Markus H M, Otten H G, Vroom Th M, Smeets T J M, Fokkens N J W, Breedveld F C. The diagnostic value of salivary fluid levels of β_2 -microglobulin, lysosym and lactoferrin for primary Sjögren's syndrome. *Clin Rheumatol* 1993; 4: 521-5.