β2 Microglobulin measurements in saliva of patients with primary Sjögren’s syndrome: influence of flow

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Abstract

Objectives—To determine the diagnostic value of β2 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 β2 microglobulin was calculated.

Methods—Parotid saliva samples were collected within a 20 minute period directly from Stensen’s duct with Lashey cups: sample 1 without gustatory stimulation during the first 10 minutes and sample 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 β2 microglobulin concentration was determined by radioimmunoassay.

Results—During gustatory stimulation the mean β2 microglobulin secretion rate in patients with primary Sjögren’s syndrome was 0.31 μg/min; in normal controls it was 0.14 μg/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 β2 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.

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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.

β2 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-β2 microglobulin complex is found on the surface of nearly all nucleated cells.5 The surfaces of lymphocytes and monocytes are particularly rich in β2 microglobulin, and lymphocytic synthesis and expression are further augmented by stimulation with mitogens or interferon.6 As a result of HLA turnover, β2 microglobulin is dissociated in its ‘free’ form in the interstitial fluid.7 8 It has been suggested that measurement of the β2 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 β2 microglobulin concentrations in patients with primary Sjögren’s syndrome.

To determine the diagnostic value of the measurement of β2 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 β2 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 Lashey 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
Salivary concentration, flow, and secretion rate of $\beta_2$ microglobulin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
<th>Unstimulated (1)</th>
<th>Stimulated (2)</th>
<th>Stimulated (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $\beta_2$ microglobulin concentration (mg/l)</td>
<td>Normal controls</td>
<td>0.77</td>
<td>0.38</td>
<td>0.34</td>
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<td></td>
<td>Patients</td>
<td>1.42</td>
<td>0.98</td>
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<td></td>
<td>$p$ Value</td>
<td>&lt;0.004</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean salivary flow (ml/min)</td>
<td>Normal controls</td>
<td>0.023</td>
<td>0.37</td>
<td>0.25</td>
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<tr>
<td></td>
<td>Patients</td>
<td>0.011</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>$p$ Value</td>
<td>0.01</td>
<td>0.14</td>
<td>0.14</td>
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<tr>
<td>Mean $\beta_2$ microglobulin secretion rate (µg/min)</td>
<td>Normal controls</td>
<td>0.018</td>
<td>0.14</td>
<td>0.085</td>
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<td></td>
<td>Patients</td>
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<td>0.31</td>
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</tr>
<tr>
<td></td>
<td>$p$ Value</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*See text for details of conditions.

$\beta_2$ microglobulin was measured by a radioimmunoassay (Abbott Laboratories). Measurements were performed in duplicate. The $\beta_2$ microglobulin secretion rate was calculated as the product of $\beta_2$ microglobulin concentration and salivary flow.

Comparative analysis of the mean $\beta_2$ 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 $\beta_2$ microglobulin level above the mean$\pm$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 $\beta_2$ microglobulin levels less than the mean$\pm$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 $\beta_2$ microglobulin and the secretion rate of $\beta_2$ microglobulin into saliva.

Results

The mean $\beta_2$ 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 $\beta_2$ 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 $\beta_2$ microglobulin secretion rate increases in the two groups after stimulation (table; fig 2). The percentage of patients with primary Sjögren’s syndrome with a $\beta_2$ 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 $\beta_2$ microglobulin of patients with primary Sjögren’s syndrome is 1.78 mg/l and in normal controls...
Microglobulin was determined in saliva of patients with Sjögren's syndrome. \( \beta_2 \) microglobulin was found 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.\(^9\) 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 \( \beta_2 \) microglobulin concentration has been proposed as a less invasive test for the oral component in primary Sjögren's syndrome.\(^12\)

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 \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) microglobulin values.

Our results indicate that the mean secretion rate of \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) microglobulin enters the saliva, and how this transport mechanism is regulated. The translocation of \( \beta_2 \) microglobulin into saliva could be a process of constant active secretion or related to 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 \( \beta_2 \) microglobulin concentration and the salivary flow suggests that \( \beta_2 \) microglobulin is probably not actively secreted and if the secretion of \( \beta_2 \) microglobulin was directly related to the production of salivary fluid, its concentration should remain constant, which it does not.

Serum \( \beta_2 \) microglobulin levels in patients with primary Sjögren's syndrome are higher than in normal controls. It seems, however, improbable that \( \beta_2 \) microglobulin diffuses from serum into saliva because no correlation between serum \( \beta_2 \) microglobulin levels and secretion rates of \( \beta_2 \) microglobulin into saliva was found.

Evidence for increased local production in the salivary gland can now be based on the observation of increased \( \beta_2 \) microglobulin secretion rates in the saliva of patients with primary Sjögren's syndrome. Our data support the hypothesis of local \( \beta_2 \) microglobulin production, and therefore the secretion rate of \( \beta_2 \) microglobulin might be useful as a diagnostic tool.