β_2 Microglobulin in tear fluid from patients with primary Sjögren's syndrome

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Abstract

β_2 Microglobulin concentration in tear fluid was measured in 35 patients with primary Sjögren's syndrome (SS), in 28 normal control subjects matched for age and sex, and in 18 patients with arthralgias or myalgias and sicca complaints, in whom the diagnosis primary SS had been excluded. Increased β_2 microglobulin concentrations were found in the patients with SS, but no correlation was found with the duration of the disease, age, or tear fluid production. Tear fluid β_2 microglobulin determinations may be useful as an adjunctive diagnostic test for primary SS.

Primary Sjögren's syndrome (SS) is a multi-system autoimmune disease characterised by keratoconjunctivitis sicca and xerostomia. Secondary SS designates the presence of a well defined connective tissue disease together with keratoconjunctivitis sicca or xerostomia, or both.¹ The ophthalmic component of SS is usually established by Schirmer's test, rose bengal staining of the cornea, and the break up time of the tear film. Measurement of tear fluid components, such as lysozyme and lactoferrin, has also been proposed for the diagnosis of SS.²

β_2 Microglobulin is a polypeptide (molecular weight 117 000) secreted by both B and T lymphocytes.³⁻⁴ It forms part of the light chain of the major histocompatibility class I molecule, and its amino acid sequence shows close homology with the constant region of the immunoglobulin molecule, suggesting a possible role in the immune response.⁵⁻⁷ Increased concentrations of β_2 microglobulin have been detected in the saliva of patients with SS,⁸⁻¹⁰ and determination of β_2 microglobulin as an aid in the diagnosis of the oral component of SS has been suggested.⁹⁻¹¹

The present study aimed at obtaining data on β_2 microglobulin concentrations in tear fluid in primary SS to determine whether this might be of diagnostic significance.

Patients and methods

PATIENTS

Tear fluid was collected from three groups of subjects: (a) 39 patients with primary SS fulfilling all modified Californian criteria¹² (table); (b) 28 control subjects without sicca complaints matched for age and sex (controls). These participants were recruited from the hospital staff and from patients attending our outpatient clinic for treatment of soft tissue rheumatic

disorders; (c) 19 patients suspected of having primary SS, in whom the diagnosis primary SS had been excluded (non-SS). They had arthralgias or myalgias, or both, and dryness of the eyes or the mouth, or both. In these patients the diagnosis primary SS was excluded because they had no history of Raynaud's phenomenon or parotid gland swelling. These patients had normal or negative results for the following: erythrocyte sedimentation rate, peripheral white blood cell counts, rheumatoid factors, antinuclear antibodies, antibodies to SS-A and SS-B antigens, serum γ globulin, salivary flow, and histopathology of the minor salivary glands.

All participants gave informed consent.

COLLECTION OF TEAR FLUID

Tear samples were collected from all subjects using sterile paper strips (Schirmer tear test; IOLAB Pharmaceuticals, Claremont, CA, USA) which were placed under the lower eyelid. The eye was unanaesthetised and the wetness of the strip was measured after five minutes. The production of tear fluid was not stimulated and all samples (two wetted filter papers strips from each patient) were stored at 0°C until assayed.

ASSAY OF β_2 MICROGLOBULIN IN HUMAN TEAR FLUID

β_2 Microglobulin was eluted from the filter paper strip by dissolving the strip in 400 μ l phosphate buffered saline. Aliquots (100 μ l) of the solution were tested for β_2 microglobulin by a β_2 microglobulin radioimmunoassay (Abbott Laboratories, USA). A standard curve was constructed with stained known volumes of phosphate buffered saline, and a factor of 0.66 was found to convert the millimetre tear front of the Schirmer paper strip into microlitres of tears obtained.

Criteria used for the diagnosis of primary Sjögren's syndrome

A Criteria for inclusion of patients

- 1 Keratoconjunctivitis sicca
  - a Decreased tear flow rate using Schirmer's test (<10 mm/5 min) and/or
  - b Increased staining with rose bengal dye
- 2 Xerostomia
  - a Symptomatic xerostomia
  - b Objective evidence of salivary gland involvement
- 3 Extensive lymphocytic infiltrate on minor salivary gland biopsy (grade 4) on the Chisholm scale¹³ obtained through normal buccal mucosa or
- 4 Abnormal sialography according to established criteria¹⁴
  - a Positive rheumatoid factor or
  - b Positive antinuclear antibodies or
  - c Positive antibodies to SS-A or SS-B antigens, or both

- B Exclusions: another connective disease according to established criteria, pre-existing lymphoma, graft v host disease, acquired immunodeficiency disease, sarcoidosis

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β_2 -Microglobulin concentration in tear fluid from a group consisting of 35 patients with primary SS (SS), 28 control subjects (Contr), and 18 suspect patients with arthralgias/myalgias and sicca complaints in whom the diagnosis primary SS had been excluded (Non-SS).

STATISTICS

The Mann-Whitney U test was used to determine the significance of the differences observed.

Results

In four patients with primary SS and in one suspect patient no tear fluid could be detected on Schirmer's paper after five minutes.

The tear fluid β_2 microglobulin concentration of 35 patients with primary SS (mean (SEM) 24 (3.14) mg/l) was significantly greater than that of the 28 controls (9.57 (0.77) mg/l; $p < 0.01$) or of 18 non-SS patients (12.38 (1.39) mg/l; $p < 0.05$) (figure). When two standard deviations above the mean were taken to represent the upper limit of normal β_2 microglobulin concentration in tear fluid 19 of 35 patients with primary SS (54%) had an increased concentration in tear fluid. By comparison, two of 18 non-SS patients (11%) had increased concentrations of tear β_2 microglobulin. On linear regression analysis of the patient group no correlation could be found between the tear fluid β_2 microglobulin concentration and duration of the disease, age, or the length of the moistened area on the paper strip. In two patients with primary SS and one control subject a second tear sample taken a few weeks after the first was analysed together with all other samples. No significant difference in β_2 microglobulin concentration between the first and the second sample was found (difference less than 5%).

Discussion

The results of this study show that patients with primary SS have increased β_2 microglobulin concentrations in tear fluid. If a cut off point at two standard deviations above the mean of the controls is used the sensitivity of the procedure is rather low (19/35 = 54%). As two of the 18 patients suspected of having primary SS showed slightly increased tear concentrations of β_2 microglobulin, however, the specificity in comparison with these patients is 89% (16 true negative patients out of 18). Thus in a rheumatological practice when primary SS is suspected measurement of β_2 microglobulin

concentration in tear fluid may be a useful adjunctive procedure. It is an attractive method because it is non-invasive, easy to perform, inexpensive, and does not require specific equipment. β_2 Microglobulin has been proposed as a marker for inflammatory and malignant conditions,^{8, 15} but no data on tear fluid β_2 microglobulin concentrations in other inflammatory conditions are available. Clearly, further studies of larger numbers of patients, including those with other connective tissue diseases and those with inflammatory eye diseases, are needed to determine the diagnostic value of this test.

Our findings are in agreement with previous studies, which invariably showed increased β_2 microglobulin concentration in the saliva of patients with SS.⁸⁻¹⁰ A positive correlation was found between the concentration of β_2 microglobulin in the saliva of patients with SS and the severity of the lymphocytic infiltration of minor salivary glands,¹⁰ and plasma β_2 microglobulin may serve as a marker for lymphocyte activation.⁴ As both in lachrymal and in salivary glands lymphocyte infiltrations are the characteristic abnormalities in SS¹⁶ it is conceivable that lymphocytes infiltrated in the lachrymal glands are the source of β_2 microglobulin in the tear fluid of patients with SS.

In this study several patients with SS had normal tear fluid β_2 microglobulin concentrations, which may be related to a relatively low degree of lymphocytic infiltration into the lachrymal glands of these patients. To determine whether the severity of lymphocytic infiltration in the lachrymal glands corresponds with the concentration of β_2 microglobulin simultaneous histopathological examination of the lachrymal gland and tear fluid β_2 microglobulin concentration should be performed. Gradual destruction of the infiltrated lachrymal glands in SS is assumed to be the reason for diminished concentrations of lactoferrin and lysozyme in the tear fluid of patients with SS.^{17, 18} In this patient group no relation between the concentration of β_2 microglobulin in tear fluid, age, or duration of the disease could be established. Consequently, senile atrophy or increasing destruction of lachrymal gland tissue does not seem to be responsible for the relatively low concentrations of β_2 microglobulin in tear fluid in some patients with SS, and in the diagnosis of primary SS simultaneous measurement of lactoferrin, lysozyme, and β_2 microglobulin in tear fluid may prove to be complementary.

As far as we know, this is the first study to show increased concentrations of β_2 microglobulin in the tear fluid of patients with SS. Further examination of the relation between the concentration of β_2 microglobulin in tear fluid and the severity of keratoconjunctivitis sicca—that is, rose bengal staining, tear film break up time and, more invasively, lachrymal gland histopathology—and simultaneous examination of the tear fluid concentration of lysozyme, lactoferrin, and β_2 microglobulin is needed to discover whether measurement of β_2 microglobulin in tear fluid is useful as an adjunctive diagnostic marker of the ophthalmic component of primary SS.

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