β₂ Microglobulin in tear fluid from patients with primary Sjögren’s syndrome

H M Markusse, J C Huysen, E J Nieuwenhuys, A J G Swaak

Abstract
β₂ 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 β₂ 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 β₂ microglobulin determinations may be useful as an adjunctive diagnostic test for primary SS.

Primary Sjögren’s syndrome (SS) is a multisystem 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

β₂ Microglobulin is a polypeptide (molecular weight 117 000) secreted by both B and T lymphocytes. ⁴ ⁵ It forms part of the light chain of the major histocompatibility class 1 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 β₂ microglobulin have been detected in the saliva of patients with SS, ⁶-¹⁰ and determination of β₂ 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 β₂ microglobulin concentrations in tear fluid in primary SS to determine whether this might be of diagnostic significance.

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 unaesthetised and the wetness of the strip was measured after five minutes. The production of tear fluid was not stimulated and all samples (two wettet filter papers strips from each patient) were stored at 0°C until assayed.

ASSAY OF β₂ MICROGLOBULIN IN HUMAN TEAR FLUID
β₂ Microglobulin was eluted from the filter paper strip by dissolving the strip in 400 μl phosphate buffered saline. Aliquots (100 μl) of the solution were tested for β₂ microglobulin by a β₂ 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
5 Laboratory evidence of a systemic autoimmune disease
   a Positive rheumatoid factor or
   b Positive antinuclear antibodies or
   c Positive antibodies to SS-A or SS-B antigens, or both
6 Exclusions: another connective tissue disease according to established criteria, pre-existing lymphoma, graft v host disease, acquired immunodeficiency disease, sarcoidosis

Patients and methods
Patiets
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, anti-nuclear 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.
**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 \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) microglobulin. On linear regression analysis of the patient group no correlation could be found between the tear fluid \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) 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. \( \beta_2 \) Microglobulin has been proposed as a marker for inflammatory and malignant conditions,\(^8\) but no data on tear fluid \( \beta_2 \) 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 \( \beta_2 \) microglobulin concentration in the saliva of patients with SS.\(^8\)-\(^10\) A positive correlation was found between the concentration of \( \beta_2 \) microglobulin in the saliva of patients with SS and the severity of the lymphocytic infiltration of minor salivary glands,\(^10\) and plasma \( \beta_2 \) microglobulin may serve as a marker for lymphocyte activation. As both in lachrymal and in salivary glands lymphocyte infiltrations are the characteristic abnormalities in SS\(^10\) it is conceivable that lymphocytes infiltrated in the lachrymal glands are the source of \( \beta_2 \) microglobulin in the tear fluid of patients with SS.

In this study several patients with SS had normal tear fluid \( \beta_2 \) 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 \( \beta_2 \) microglobulin simultaneous histopathological examination of the lachrymal gland and tear fluid \( \beta_2 \) 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 \( \beta_2 \) 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 \( \beta_2 \) microglobulin in tear fluid in some patients with SS, and in the diagnosis of primary SS simultaneous measurement of lactoferrin, lysozyme, and \( \beta_2 \) microglobulin in tear fluid may prove to be complementary.

As far as we know, this is the first study to show increased concentrations of \( \beta_2 \) microglobulin in the tear fluid of patients with SS. Further examination of the relation between the concentration of \( \beta_2 \) 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 \( \beta_2 \) microglobulin is needed to discover whether measurement of \( \beta_2 \) microglobulin in tear fluid is useful as an adjunctive diagnostic marker of the ophthalmic component of primary SS.

\[ \beta_2 \text{-Microglobulin concentration in tear fluid from a group consisting of 35 patients with primary SS (SS), 28 control subjects (Contr), and 18 suspect patients with arthralgias/myalgias and sicca complaints in whom the diagnosis primary SS had been excluded (Non-SS).} \]
Tear fluid $\beta_2$-microglobulin in Sjögren’s syndrome

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