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MEHMET YALTIRIK

HÜLYA KOÇAK BERBEROĞLU

KIVANÇ ERGEN

BARIŞ AYDIL

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CLINICAL INVESTIGATION

Sjögren's Syndrome: A Study of Salivary Electrophoresis*

Mehmet YALTIRIK¹, Hülya KOÇAK BERBEROĞLU¹, Kıvanç ERGEN², Barış AYDIL¹

¹Department of Oral Surgery and Medicine, Faculty of Dentistry, İstanbul University, 34093, Çapa, İstanbul - Turkey

²Department of Biophysics, Faculty of Medicine, Kocaeli University, Kocaeli - Turkey

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Abstract: Many diagnostic tests exist to assess the salivary and lacrimal involvement in Sjögren's syndrome (SS), but there is still disagreement.

The purpose of this study was to examine salivary proteins in patients with primary Sjögren's syndrome and healthy control subjects by the polyacrylamide gel electrophoresis, as well as the applicability of the method.

The polyacrylamide gel electrophoresis, applied in our examinations, is a simple and rapid method, requiring only a minute amount of saliva for the analysis. Saliva were collected from patients suffering from primary Sjögren's syndrome (n = 10) and a control group (n = 10). All saliva samples were stored at -70 °C until use. The diagnosis of Sjögren's syndrome was verified by a biopsy of the minor glands of the lower lip in the patient group.

Most of the patients with Sjögren's syndrome exhibited an electrophoretic profile that was different from healthy control subjects. Electrophoresis systems are most reliable for Sjögren's syndrome.

Our study suggests that the salivary electrophoresis are most reliable for Sjögren's syndrome. Salivary electrophoresis can provide a simple, noninvasive, inexpensive, and comfortable method for the diagnosis of Sjögren's syndrome.

Key Words: Sjögren's syndrome, saliva, salivary electrophoresis, polyacrylamide gel electrophoresis, syndrome, polyacrylamide gel.

Introduction

Sjögren's syndrome (SS) is a chronic inflammatory disorder of the salivary and lacrimal glands that leads to functional impairment (1). The prevalence of SS is thought to equal or exceed that of rheumatoid arthritis, which affects from 1% to 3% of the World's population (2). Each case of SS is classified as either primary or secondary on the basis of the clinical manifestation (1). In both forms of the disease, the salivary glands are the targets, as evidenced by the reduction in salivary output and the lymphocytic infiltration of the salivary glands (1,3).

Sjögren's syndrome affects primarily middle-aged women, and is the second most common rheumatic

disease in the World (4). The female-to-male ratio of occurrence is approximately 10 to 1 (5).

With many patients, the complaints are not of dry mouth itself, but rather an unpleasant taste, difficulty eating dry foods such as biscuits, soreness, or difficulties in controlling dentures. The salivary glands may swell in approximately one third of patients, but this is rarely the initial feature. Salivary gland enlargement may occur in more patients with primary SS (5-7).

The tongue typically develops a characteristic lobulated, usually red, surface with partial or complete depapillation. There is also a decrease in the number of taste buds, which are abnormal, and an impaired sense of smell. Soreness and redness of the mucosa are usually the

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result of candidial infection, found in 70% of patients; angular stomatitis and denture stomatitis also are common (5,6).

Patients with SS who have low salivary flow rate often experience oral infections such as candidiasis and rampant caries because of change in the oral microflora, salivary flow rate and salivary composition (4). The diagnosis of Sjögren's syndrome requires several clinical and laboratory tests, including a salivary gland biopsy (4).

There are also several reports that suggest a change in the electrophoretic profile of salivary proteins in the patient with SS (3-7). Currently, the diagnostic potential of these observations has not been fully investigated. Although serum electrophoresis is frequently used for the diagnosis of systemic diseases, the clinical application of salivary electrophoresis has not been pursued (1,2,8). The purpose of this study was to investigate the potential applicability of salivary protein electrophoresis in the diagnosis of SS.

Materials and Methods

Patient selection

A total of 20 subjects participated in the study. Saliva samples were collected from 10 patients with a diagnosis of primary Sjögren syndrome (SS) and from 10 age/sex/race-matched healthy control subjects (HCS) with no signs or symptoms of dry mouth or dry eyes, and with normal saliva functions. The diagnosis of SS was based on the European Community criteria (1,7). All the patients had dry mouth, dry eyes involving at least one eye, and a positive minor gland biopsy of the lower lip. None of the patients had a history of primary liver cirrhosis, graft-versus-host disease, sarcoidosis, amyloidosis, HIV infection, or radiation therapy for malignancy. A comprised 10 patients with SS and 10 HCS subjects sex range was shown Table 2, 3, age range was 18 to 72 years.

Saliva collection

Citric-acid stimulated (2%) parotid saliva samples were collected between 9 AM and 11 AM. Food and drink were restricted for at least 2 hours before collection. All ten -minute saliva samples were stored at -70 °C until they were used .

Protein determination

Before being analyzed, each saliva sample was centrifuged on a bench centrifuge for 2 minutes at 4 °C for the purpose of removing insoluble material. Total salivary protein was determined on 20 µl aliquots of the clear supernatant through use of the Bicinchoninic acid method (9).

SDS-PAGE electrophoresis

SDS-PAGE (Sodium DodecylSulphate-polyacrylamide gel electrophoresis) electrophoresis was made according to Laemmli (10). Vertical electrophoresis system was used to investigate the samples. Saliva samples were mixed with loading buffer making the volume 30 µl, containing 10% volume in volume glycerol, 2% weight in volume (w/v) SDS, 10% w/v 2-mercaptoethanol, and 0.025% w/v bromophenol blue in total. After heating the samples for 2 minutes at 100 °C, samples were applied to 12% SDS-PAGE having 5% acrylamide stacking gel with 1mm thickness and run at a constant voltage (120 volts) for 90 minutes at room temperature.

Staining methods

Gel was stained with Coomassie Brilliant Blue (CBB), in staining solution containing 0.05% w/v CBB-R250 in 40% ethanol and 10% acetic acid and afterwards destained in 10% acetic acid.

Results

Ten patients with SS and ten HCS were involved in the study. The SDS-PAGE electrophoresis of the saliva in seven patients with SS showed an additional protein band (SDS-P) with an apparent molecular mass of approximately 90 kDa (Figure1), while this was not seen in three patients. SDS-P was observed in 70% of the samples from the SS group but in only 30% of the samples from HC group. SDS-P was also detected in three HCS. This accords with the previous findings (2) (Table 1).

Eight patients with SS did not smoke or drink alcohol; of these, six were positive in SDS-PAGE system. Only one patient who was smoking and drinking alcohol was positive in SDS-PAGE system. Five of the SS patients had prostheses, and all of them were positive in SDS-PAGE system (Table 2).

Table 1. Observed frequencies in the additional protein bands SDS-PAGE electrophoresis.

| | SDS-PAGE(+) | SDS-PAGE(-) |
|-------|-------------|-------------|
| SS | 7 | 3 |
| HCS | 3 | 7 |
| Total | 10 | 10 |

(+) Additional protein bands present

(-) No additional protein bands present

Four of the HCS had never smoked or drunk alcohol, in theal no SDS-PAGE positivity was observed. Three of the HCS group had drinking habits, and three of HCS were smoking and in those HCS the positivity in SDS-PAGE system was not observed. Three of the HCS had prostheses and all of them were positive in SDS-PAGE system (Table 3).

Discussion

One of the major difficulties clinicians who deal with patients with primary SS face is the lack of a simple diagnostic test for the condition. None of the autoantibodies (ANA,RF,SS-A,SS-B) is specific for SS. As to the focus score in a salivary gland biopsy, a major drawback of this criterion is that it only measures severity of inflammation (1,5).

Studies have indicated that people with SS tend to have increased rates of tooth loss; gingival recession and alveolar bone loss, and are at higher risk of having adult periodontitis than are age-and race-matched healthy controls (1,5,7).

On the other hand, in the late stages of SS, the increased fibrosis and atrophy of the acinar cells obscures the focus score and presents an additional obstacle to the diagnosis (11). Because of the difficulties associated with

Table 2. SDS-PAGE positivity of SS patients, due to age/sex/smoking, drinking habits and wearing prostheses are shown.

| SS no | Age | Sex | Smoking | Drinking | Prosthesis | SDS-PAGE |
|-------|-----|--------|---------|----------|------------|----------|
| 1 | 37 | Female | + | + | + | + |
| 2 | 48 | Female | - | - | - | - |
| 3 | 38 | Female | - | - | + | + |
| 4 | 45 | Female | - | - | + | + |
| 5 | 18 | Female | - | - | - | + |
| 6 | 56 | Female | - | - | + | + |
| 7 | 62 | Female | - | - | + | + |
| 8 | 26 | Male | + | + | - | - |
| 9 | 37 | Male | - | - | - | + |
| 10 | 72 | Female | - | - | - | - |

SS: Sjögren syndrome.

Table 3. SDS-PAGE positivity of HCS, due to age/sex/smoking, drinking habits and wearing prostheses are shown.

| HCS no | Age | Sex | Smoking | Drinking | Prosthesis | SDS-PAGE |
|--------|-----|--------|---------|----------|------------|----------|
| 1 | 30 | Female | + | - | - | - |
| 2 | 23 | Female | + | - | - | - |
| 3 | 21 | Male | - | - | + | + |
| 4 | 42 | Female | - | - | + | + |
| 5 | 28 | Female | - | + | - | - |
| 6 | 24 | Male | - | + | - | - |
| 7 | 26 | Female | + | - | - | - |
| 8 | 35 | Male | - | - | + | + |
| 9 | 38 | Female | - | + | - | - |
| 10 | 49 | Female | - | - | - | - |

HCS: Healthy control subject.

the diagnosis of SS, many clinicians are uncertain, and patient management is compromised (11). There is a recognized need for an alternative or complementary test for the diagnosis of primary SS (1,2,4,11).

In this study we have investigated the potential use of salivary electrophoresis in the diagnosis of primary SS. The electrophoretic profiles of saliva from patients with SS and control subjects were examined by SDS-PAGE system. SDS-PAGE system does not require extensive experience to implement, is sensitive for minute amounts of saliva, and do not require pretreatment of the sample (1-4,8,11,12).

The diagnostic value of SDS-PAGE differentiates between the salivary profiles of the patients with primary SS and those of the HCS.

The polyacrylamide gel electrophoresis, applied in our examinations, is a simple and rapid method, requiring only a minute amount of saliva for the analysis (5,7).

It is important to note that all of the patients who participated in the study did complain of dry mouth. It may be interesting, however, to examine the electrophoretic profile of saliva in patients who have primary SS with minimal salivary gland involment.

The saliva from most of the patients with primary SS in our study exhibited an electrophoretic profile that was different from the saliva of HCS on SDS-PAGE system (Figure 1).

Conclusion

Salivary electrophoresis of the patients with SS is simple and requires only a minute amount of saliva. However its specificity for SS requires further investigation. SS can be diagnosed much earlier than by other methods available today.

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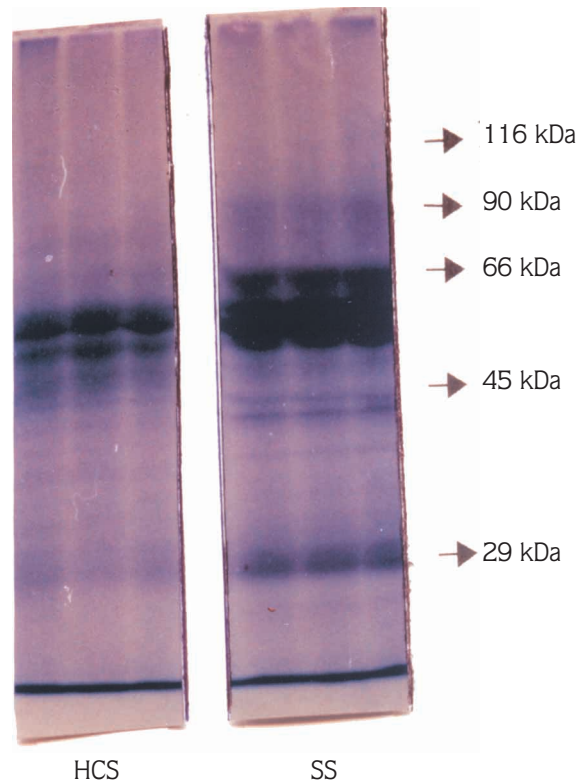


Figure 1. SDS-PAGE gel electrophoresis of saliva samples. 116,66,45,29 kDa indicate the molecular weight markers. Arrow corresponds to 90 kDa. The mean total salivary protein in patients was greater than in controls.

Corresponding author:

Mehmet YALTIRIK

İstanbul University Faculty of Dentistry,

Department of Oral Surgery and Medicine

34093, İstanbul - TURKEY

E-mail: myaltrk@yahoo.com

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