Sjögren’s syndrome (SS), a review of the subject and saliva as a diagnostic method

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Abstract

Sjögren’s syndrome is a chronic autoimmune disease whose main clinical manifestation is oral dryness (xerostomia) and ocular dryness (xerophthalmia). It is characterized by progressive mononuclear infiltration of the exocrine glands and can affect a variety of organ systems. The prevalence of primary Sjögren’s syndrome varies from 0.01 up to 4.8%; this variability reflects differences in definition, application of diagnostic criteria, and geographic differences in age groups. The etiology of primary Sjögren’s syndrome is unknown, but the interaction between genetic and environmental factors (viruses, hormones, vitamins, stress) is important. There are few reported cases of concordance in monozygotic twins, and it is common for patients with primary Sjögren’s syndrome to have relatives with other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, thyroid disease, psoriasis, and multiple sclerosis. Among the most common findings is hypergammaglobulinemia. Elevated levels of γ-globulins contain autoantibodies directed against nonspecific antigens such as rheumatoid factor, antinuclear antibodies, and cellular antigens SS-A/Ro and SS-B/La. Regarding diagnosis, there have been 11 different published criteria for Sjögren’s syndrome since 1965; none have been approved by the American College of Rheumatology or the European League Against Rheumatism. The current criteria were published in 2012 jointly with the progressive advance in the knowledge of the human salivary proteome that has gained wide acceptance in Sjögren’s syndrome, with the possibility of using saliva as a useful tool in both diagnosis and prognosis in this field because the analysis of salivary proteins may reflect the state of locally underlying disease of the salivary glands, which are the target organs in this disease. (Gac Med Mex. 2016;152:333-41)

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Introduction

Sjögren syndrome (SS) is a chronic autoimmune disease whose main clinical manifestation is mouth (xerostomia) and eye dryness (xerophthalmia). It is characterized by exocrine glands progressive mononuclear infiltration and can affect a variety of organs and systems. It occurs as an isolated condition known as primary (pSS) or secondary Sjögren syndrome (sSS) when it is associated with other autoimmune disease such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), etc.1.

pSS affects essentially women during the fourth and fifth decades of life (female:male ratio 9:1), but it can occur at any age, including childhood; it is the second most common rheumatic disease after RA2.
Epidemiology

pSS prevalence can vary from 0.01 to 4.8%. This variability reflects differences in terms of definition, diagnostic criteria application, geographic areas and age groups. A study conducted in Olmsted County, Minnesota, reported an annual incidence of pSS diagnosed by a physician of 4 cases per 100,000 inhabitants. Another cross-sectional study conducted in Paris in 2007 identified adults with pSS diagnosed based on the American-European Consensus Group (AECG) criteria and found a pSS prevalence of 1.52 per 10,000 adults.

Etiology

pSS etiology is unknown, but the interaction between genetic and environmental factors (viruses, hormones, vitamins, stress) is important. Few cases of concordance in homozygote twins have been reported and it is common for patients with pSS to have relatives with other autoimmune diseases such as SLE, RA, thyroid disease, psoriasis and multiple sclerosis. The genetic factor in pSS is complex and involves HLA-associated and non-associated genes. Among the HLA-associated genes, DR and DQ account for most part and their prevalence varies between ethnic groups. The DQA1*05:01, DQB1*02:01 and DQB1*03:01 are associated with high risk for the disease, whereas DQA1*03:01, DQA1*05:01 and DQB1*05:01 have been related to protection.

Within the non HLA-associated genetic factors, it has been linked with susceptibility to the interferon a (IFNα) pathway through the interferon regulatory factor 5 (IRF5), which codifies for type I IFN transcription and for the signal transducer and activator of transduction 4 (STAT4), which induces T cells differentiation into Th1 subtypes (other non-HLA genes that codify for interleukin 6 [IL6] have not been significantly related to predisposition, but rather to extraglandular manifestations and disease severity). Genes that codify for IL16 have been related to protection.

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Among the environmental factors implicated, viruses are considered the main candidates in the development of autoimmunity in pSS, either by local invasion, lymphocyte migration induction or molecular mimicry. Although a strict correlation of pSS with a particular virus has not been found, genetic material of the Cock-sakie virus has been found in minor salivary gland (MSG) biopsies of patients with pSS, and other studies have found evidence of Epstein-Barr virus (EBV) DNA in epithelial cells. Additionally, other viruses implied are the human immunodeficiency virus (HIV) and the hepatitis C virus (HCV).

Different components of the endocrine system have been implicated in the development and clinical expression of pSS, when hypoactivity becomes evident in the hypothalamic-pituitary-adrenal axis, either due to a pituitary defect and/or adrenal gland dysfunction. Anti-21-hydroxylase (OH) antibodies were found in the serum of approximately one fifth of patients with pSS in association with B cells activation and adrenal reduced function. As a result of this adrenal reduced function, there is a decrease in androgen levels, particularly dehydroepiandrosterone (DHEA-S). With androgens and estrogens decrease, glandular epithelial cells undergo apoptosis, since salivary gland tissue remodeling is under androgenic control, and androgen deficiency in these patients can explain the alterations observed in glandular architecture. In the absence of a compensatory action, the menopausal status can lead to an apoptotic process triggering an aberrant immune response. Another neuroendocrine hormone such as prolactin was found to be elevated in 16-46% of patients with pSS.

The role of proteins as immunologic modulators has been studied, especially the association between vitamin D levels and clinical manifestations of the disease, with no differences being found between patients and healthy control subjects, but vitamin D deficiency (≤ 15 ng ml⁻¹) was associated with neurologic manifestations and with the presence of lymphoma in patients with pSS.

Patients with pSS experience high levels of psychological stress with a significant number of negative events in their lives in the absence of satisfactory adaptive responses to confront stress. The lack of social support can contribute to the relative risk for the development of the disease.

Intrinsic stimulation of glandular epithelial cells by an unknown causative agent can lead to their activation, increased apoptosis and presence of neo-antigens to the immune system. These events lead to T and B memory cells accumulation and immune response perpetuation by auto-antigens released from apoptotic cells.

The histopathologic hallmark in pSS is the presence of cell aggregates around the ducts and acini of exocrine glands, mainly the salivary and lacrimal glands, with progressive deterioration of the secretory function. For a long time, lymphocyte infiltrates were regarded as the main cause of the secretory dysfunction observed in pSS; however, the association between clinical symptoms and the degree of glandular destruction, together with findings derived from animal models, suggest the existence of alternative pathways that contribute to this...
dysfunction such as epithelial glands apoptosis induction, aquaporins altered distribution or neurotransmission inhibition by antimuscarinic antibodies.

Exocrine glands are composed of ductal epithelial cells and acinar cells with secretory function. There are changes on these cells in pSS animal models in the absence of functional lymphocytes, and glandular epithelial cells have been proposed as the main components in the generation of autoimmune response by exhibiting an activated phenotype, since they express large quantities of HLA-DR, co-stimulating molecules (CD80, CD86, CD40), adhesion molecules (ICAM-1), innate response receptors such as Toll-type receptors and B cell activating factor (BAFF). Acinar epithelial cells also express auto-antigens originating in the cytoplasm, an represent a pathway by means of which these are presented to the immune system.

MSG infiltrates cell composition strongly depends on the severity of the histopathological lesion: CD4+ T lymphocytes predominate in mild early lesions and B lymphocytes are predominant in advanced stages of the disease.

**Local clinical manifestations**

**Oral manifestations**

Major salivary glands and MSG involvement leads to a decrease in salivary secretion, which manifests as xerostomia (mouth dryness or oral sicca) with an increase in oral infections, mucosal friability and dental caries due to the loss of lubrication and antimicrobial capacities of saliva. Oral candidiasis is common, and it appears in the form of erythematous mucosal lesions, lingual fissures, filiform papillae atrophy and angular cheilitis. Asymptomatic and self-limited growth of the parotid glands or other exocrine glands are composed of ductal epithelial cells with secretory function. There are changes on these cells in pSS animal models in the absence of functional lymphocytes, and glandular epithelial cells have been proposed as the main components in the generation of autoimmune response by exhibiting an activated phenotype, since they express large quantities of HLA-DR, co-stimulating molecules (CD80, CD86, CD40), adhesion molecules (ICAM-1), innate response receptors such as Toll-type receptors and B cell activating factor (BAFF). Acinar epithelial cells also express auto-antigens originating in the cytoplasm, an represent a pathway by means of which these are presented to the immune system.

**Systemic manifestations**

There are systemic manifestations in 30 to 70% of patients before or after the pSS diagnosis. The presence of circulating anti-RO and anti-LA antibodies is common in comparison with a group of patients with isolated local disease.

Up to 70% of patients may experience fatigue with a moderate correlation between depression and fatigue. One possible explanation is that both share an underlying biological mechanism.

Approximately half the patients with pSS experience symptoms such as arthralgia and/or arthritis in the course of their disease, and they can occur before the onset of glandular symptoms. Other manifestations include morning stiffness and fibromyalgia-type conditions. There is a non-erosive, typical polyarthropathy that occasionally drives to Jaccoud’s arthropathy. Muscular involvement is mainly in the form of myalgias. Clinical presentations of proximal muscular weakness of insidious onset and/or mild inflammatory myopathy (polymyositis or inclusion body myositis) have been described.

There is a skin dryness condition known as “xerosis”, accompanied by pruritus; other rare manifestations include annular erythema and pernio-type lesions.

Cutaneous vasculitis affects 5-10% of patients in the form of palpable purpura; it is related to gammaglobulinemia with involvement of middle and small caliber vessels and is considered as an adverse prognostic marker for the development of lymphoma. Other cutaneous vasculitis rare manifestations include papules, ulcers and urticarial lesions predominantly at lower limbs.

Larynx, trachea and exocrine bronchial glands lymphocytic infiltration leads to dryness of the respiratory tract.
tree, which manifests as irritative and persistent dry cough. Pulmonary function tests reveal an obstructive pattern of the small airways. On high resolution computed tomography (HRCT) scan, it appears as a segmental thickening of the bronchi and on chest X-ray, as a “dirty lung”. Transbronchial biopsies reveal peribronchial and/or peribronchiolar mononuclear infiltration.

Interstitial lung disease (ILD) is rare and its clinical presentation includes dyspnea, cough, bilateral rales and interstitial infiltrates on chest X-ray; most common HRCT findings include ground-glass attenuations, small subpleural nodules, non-septal linear opacities, interlobular septal thickening and honeycomb in subpleural areas. Most common histological patterns are: Nonspecific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), organizing pneumonia (OP) and lymphocytic interstitial pneumonia (LIP). Lymphoma should be ruled out in case pulmonary nodules and hilar and/or mediastinal lymphadenopathies are found. Pleural effusions are very rare in pSS but common when associated with other rheumatic diseases.

Cardiac complications such as myocarditis and pericarditis are very rare in pSS. There is dysphagia due to pharyngeal and esophageal dryness or to esophageal motility alteration, and it can be accompanied by nausea and epigastric pain. Typical histological pattern is chronic atrophic gastritis with lymphocytic infiltration; hyperamylasemia is common, although very rarely it is the expression of acute or chronic pancreatitis. Autoimmune hepatitis is diagnosed in 1.7-4%, while autoimmune cholangitis (with histological changes similar to stage I of primary biliary cirrhosis) is diagnosed in 5-10% of patients together with antimitochondrial antibodies (AMA). There is anemia, erythrocyte sedimentation rate (ESR) elevation with C-reactive protein (CRP) normal levels, possibly owing to type I IFN elevated levels, which represses its production in addition to cytopenias (lymphopenia, thrombocytopenia and neutropenia) that are correlated with extraglandular manifestations. Neutropenia has been proposed as a negative prognostic marker for the development of lymphoma.

Central nervous system involvement is much less common, with multiple sclerosis-type changes, convulsions, transverse myelitis and multifocal white matter lesions. Neurological manifestations range from 2 to 60%, with the main being predominantly sensory polyneuropathy (sensory ataxia or painful small fiber neuropathy). Other less-frequent manifestations include: Sensory-motor polyneuropathy, polyradiculopathy, mononeuritis multiplex, cranial neuropathies (trigeminal neuralgia) and autonomic neuropathy (Adie’s tonic pupil and orthostatic hypotension).

Thyroid disease occurs in nearly 20% of patients with pSS as autoimmune thyroiditis (mainly Hashimoto’s thyroiditis and less frequently Graves disease), with more than 50% having subclinical hypothyroidism. Antibodies against thyroid peroxidase (anti-TPO) and thyroglobulin (anti-TG) can be used as predictors for the development of thyroid disease.

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There is dyspareunia in 40% of postmenopausal women, associated with loss of lubrication in comparison with 3% in healthy controls.

**Laboratory findings**

**General**

The most common finding is hypergammaglobulinemia. Gamma-globulin elevated levels contain autoantibodies directed against non-specific antigens such as RF, ANA, SSA/Ro and SSB/La cell antigens, and antibodies targeting organ-specific antigens such as salivary ductal cells, AMA, anti-thyroid gland cells antibodies and gastric mucosa antigens.

There is anemia, erythrocyte sedimentation rate (ESR) elevation with C-reactive protein (CRP) normal levels, possibly owing to type I IFN elevated levels, which represses its production in addition to cytopenias (lymphopenia, thrombocytopenia and neutropenia) that are correlated with extraglandular manifestations. Neutropenia has been proposed as a negative prognostic marker for the development of lymphoma.

There can be hypokalemia and hyperchloremic acidosis present due to distal renal tubular acidosis-driven renal involvement.

High levels of monoclonal immunoglobulin can be detected in 10 to 15% of patients with pSS, depending on the technique used. Approximately in 20% there are cryoglobulins present in their serum. RF and cryoglobulins play an important role in the pathogenesis of pSS, since they have been shown to be indicators...
of increased risk for lymphoma. Complement levels are likely to be decreased, especially C4, either due to genetic predetermination or secondary to their consumption (immune complexes) or cryoglobulinemia.

**Specific autoantibodies**

RF is positive in 40-50% of patients with pSS. This is an autoantibody that binds to the IgG Fc portion. ANAs are positive in 50-90% of patients. They are detected by indirect immunofluorescence in Hep-2 cells and display a dotted pattern. Anti-SSA/Ro and anti-SSB/La are the 2 most useful antibodies to establish the pSS diagnosis. Anti-SSA/Ro is present in 50 to 90% of patients with pSS. These antibodies are not pSS-specific, since they can be found in 30 to 50% of patients with SLE. Anti-SSB antibodies are detected exclusively in patients with anti-SSA. Anti-SSB are more pSS-specific, since they are detected in 30 to 60% of patients with pSS, but only in 20-30% of patients with SLE. Anti-SSA/Ro antibodies can bind to several antigenic epitopes expressed by 2 proteins with different molecular masses, 52 and 60 kDa. It appears that the reactivity profile can vary for patients with pSS (52-kDa anti-SSA isolated reactivity) and those with SLE (60-kDa anti-SSA reactivity). The presence of anti-Ro (SSA) and/or La (SSB) antibodies is associated with disease early onset and longer duration, parotid glands recurrent inflammation, splenomegaly, lymphadenopathy and vasculitis in patients with pSS. Anti-SSA and anti-SSB antibodies-secreting B lymphocytes are present in exocrine glands mononuclear infiltrates, often associated with SSA/SSB epitopes-specific T lymphocytes; therefore, SSA and SSB could become immunogenic owing to their abnormal location in the cytoplasm or to increased expression in epithelial cells apoptotic vesicles membrane.

**Other autoantibodies**

Anticentromere antibodies (ACA) are found in a limited number of patients with pSS and are characterized by higher prevalence of the Raynaud phenomenon and dysphagia in comparison with ACA-negative patients. Antibodies against the α-fodrin cytoskeleton protein were found in 41 of 43 patients with pSS in contrast with healthy subjects or patients with other autoimmune diseases. A commercial enzyme-linked immunosorbent assay (ELISA) was subsequently developed for the diagnosis of these autoantibodies; however, its low sensitivity and specificity preclude the use of this test for diagnostic purposes. Autoantibodies targeted against M3R acetylcholine receptors (main type of muscarinic receptor detected in salivary glands) have also been described in patients with pSS. These antibodies wave been associated with neuroglandular transmission inhibition, which results in sicca and pSS extraglandular features related to autonomic dysfunction. AMAs regarded as diagnostic markers for primary biliary cirrhosis (PBC) are present in 6.6% of patients with pSS. Ninety-two percent of pSS patients with hepatic involvement and chronic colangitis histological characteristics similar to those observed in stage I of PBC are AMA-positive.

**Diagnosis**

Although there have been 11 different SS diagnostic criteria published since 1965, none has been approved by ACR or EULAR.

Through the years, several series of criteria have been proposed for SS classification, but none of them had been widely adopted by the scientific community until the preliminary classification European Criteria emerged in the year of 1993. These classification criteria have been largely employed both in clinical practice and observational and interventional studies for many years. In 2002, the preliminary European criteria were reexamined by an American-European joint Committee. The result of this review were the AECG criteria, which introduced more clearly-defined standards to classify patients with pSS or sSS, and provide more precise exclusion criteria.

Recent studies based on these criteria show prevalences of 0.1% with confidence intervals within < 0.1-0.4 ranges. These are the classification criteria proposed in 2002 (Table 1).

Primary Sjögren diagnosis is basically established in patients presenting with signs and symptoms of oral and ocular dryness, and who were positive to anti-SSA or anti-SSB antigen antibodies, or who have a positive salivary gland biopsy and have no other underlying autoimmune disease.

SS is found all over the world. Regional differences have not been really explored. There is a large preponderance in the female sex, with a female: male ratio of approximately 9:1. The disease can be found in all age groups, but it generally starts between the ages of 40 and 60 years, and it is rarely observed in children and adolescents.

AECG 2002 criteria have higher specificity than its predecessor, since they require autoimmunity evidence with anti-SSA/B positive serology or focal lymphocytic sialadenitis with a ≥ 1 score in a labial salivary gland biopsy. However, they have been criticized for including subjective tests (symptoms), psychological...
measurements lacking specificity and alternative diagnostic tests that are not equivalent. Therefore, it became necessary to generate new criteria, and for this purpose, the Sjögren International Clinic Collaboration Alliance (SICCA), which is funded by the National Health Institute, was created in order to develop new SS classification criteria, to better define the SS phenotype and to store data and clinical biological samples to support future research. They propose new classification criteria for SS following the ACR guidelines to the extent possible, a condition that requires multiple clinical specialties for diagnosis40.

In 2012, these new SS classification criteria were proposed, and are presented in table 2 40.

The rose Bengal test is usually carried out by an ophthalmologist; 1% of the rose Bengal dye is instilled in the eye, and ocular surface integrity is assessed by qualitatively weighing conjunctival staining. The rose Bengal dye will stain devitalized cells of the cornea and conjunctival epithelium. The test will identify keratoconjunctivitis sicca when ocular symptoms are minimal46,47.

A positive biopsy is defined as at least one of the dense inflammatory infiltrate foci containing at least 50 lymphocytes per 4 mm² 46 (Fig. 1).

These new SICCA classification criteria, developed based on data collected by means of the record of standardized measurements, appeared as a result of the analysis of 1,362 patients in a multicenter, multidisciplinary study, make a redefinition of the syndrome, since they are easy to apply, although the participation of at least 2 clinical specialties may be necessary, are exclusively based on objective tests. A series of validation exercises indicate better performance of the classification with regard to existing alternatives, making them more adequate for application in situations where classification errors can entail risks for health46.

Proteomic methods are expanding our capacity to determine changes in protein expression; the technology used has rapidly evolved over the past 10 years, which enables more accurate quantification of expressed proteins46.

SS diagnosis is complex and sometimes it cannot be easily established because there is not a sign, symptom

### Table 1. Sjögren Syndrome classification criteria proposed in 2002

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Ocular symptoms (at least one of the following):</td>
<td>– Daily, persistent, troublesome dry eyes for more than 3 months. – Recurrent sensation of sand or gravel in the eyes. – Use of tear substitutes more than 3 times a day.</td>
</tr>
<tr>
<td>Oral symptoms (at least one of the following):</td>
<td>– Daily feeling of dry mouth for more than 3 months. – Recurrent or persistent swollen salivary glands in adulthood.</td>
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<tr>
<td>Ocular symptoms (positive results in at least one of the following tests):</td>
<td>– Schirmer’s test. – Rose bengal test or other ocular dye test.</td>
</tr>
<tr>
<td>Histopathology (positive salivary gland biopsy)</td>
<td></td>
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<tr>
<td>Salivary gland involvement (positive results in at least one of the following tests):</td>
<td>– Unstimulated whole salivary flow (less than 1.5 ml in 15 minutes). – Parotid sialography showing the presence of diffuse sialectasias. – Salivary scintigraphy showing delayed uptake, reduced concentration and delayed excretion of tracer.</td>
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<tr>
<td>Presence of antibodies to antigens:</td>
<td>– Anti-SSA (Ro) – Anti-SSB (La)</td>
</tr>
<tr>
<td>Classification of primary Sjögren syndrome requires:</td>
<td>– 4 of the 6 criteria, including a minor salivary gland biopsy or SSA/SSB-positive antibodies. – Or 3 of the 4 objective criteria (criteria 3 to 6)</td>
</tr>
<tr>
<td>Classification of secondary Sjögren syndrome:</td>
<td>– It requires established connective tissue disease and one of the sicca symptoms (criteria 1 or 2), in addition to 3 of the 4 objective criteria (items 3, 4, 5).</td>
</tr>
<tr>
<td>Exclusions:</td>
<td>– Include previous head and neck radiotherapy, lymphoma, sarcoidosis, graft versus host disease, and hepatitis C virus or HIV infection, use of anticholinergic drugs.*</td>
</tr>
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Table 2. Sjögren Syndrome classification criteria proposed in 2012

The SS classification is applicable to individuals with signs/symptoms suggestive of SS. Two out of the following 3 objective findings:

Antibodies:
- Anti-RO (SSA)
- Anti-La (SSB)
- RF and ANA-positivity (with titers of at least 1:320)

Minor salivary gland biopsy with focal lymphocytic sialadenitis with > 1 focus/4 square millimeters.

Keratoconjunctivitis sicca with staining score ≥ 3 by (assuming the patient is not currently receiving topical treatment for glaucoma, and has NO history of corneal surgery or blepharoplasty in the last 5 years.

The following should have been excluded: History of head and neck radiation treatment, HIV, HCV, sarcoidosis, IgG4 syndrome, graft versus host disease, amyloidosis.


Saliva as a diagnostic tool

Human saliva is a fluid with different biological functions that are essential to the maintenance of oral health. Its recent use in the diagnosis of different conditions such as HIV-associated disease, heart conditions, different types of cancer and its possible role as an indicator of autoimmune diseases has demonstrated that it can be a useful diagnostic tool in the clinical field. Whole saliva is a mixture of secretions of the 3 pairs of major salivary glands (parotid, submandibular and sublingual), of the MSGs located beneath the oral mucosa, and of gingival fluid constituents, as well as many other oral microbial contaminants and oral epithelium desquamative cells. The contributions of all different salivary glands to the makeup of whole saliva are variable depending on the degree of stimulation and different hours of day, and many works have focused on the study of whole and ductal saliva, which has prompted the salivary proteome full analysis, which can offer important clues for the understanding of systemic diseases pathogenesis.

The wide variety of molecules present in salivary secretions places saliva as an attractive source of biomarkers, in addition to advantages such as easy collection, storage and low-cost transportation; unlike plasma, it does not coagulate and is a non-invasive method that this way reduces patient nonconformity when repeated sample taking is necessary. In comparison with blood, saliva can demonstrate more sensitive and specific markers for the diagnosis of oral diseases.

Salivary proteins and peptides have been studied with different biochemical techniques, such as liquid chromatography, gel electrophoresis, mass spectrometry, immunoassays and ELISA. Over the past 40 years, a variety of salivary proteins and peptides have been identified, with a wide range of functional properties, including immunoglobulins with antimicrobial activity (lysozyme, lactoferrin, sialoperoxidase, hystatins and defensins), lubricants and physical protectors such as mucins, proline-rich proteins with precipitating functions of harmful chemicals of the diet and other salivary proteins that are subject-ed to the proteolytic activity of salivary and bacterial
proteases in the mouth\textsuperscript{56}. In a review by Hu et al., the discovery of approximately 1,380 proteins, detected using different techniques, was revealed, although only 100 of them were abundantly present\textsuperscript{57}.

In conjunction with the progressive advance in the knowledge of the human salivary proteome, the possibility of using saliva as a useful tool both in the diagnostic and prognostic field has earned great acceptance in SS, and this is because salivary protein analysis may locally reflect any underlying pathological state of the salivary glands, which are the target organ in this condition. Several studies have been conducted applying different techniques, using both stimulated saliva and non-stimulated saliva (whole saliva). Most of these preliminary studies, where different methods are used, have focused on the search of diagnostic biomarkers on order to validate and typify a salivary proteomic panel for early, non-invasive detection of SS\textsuperscript{58-66}. Data obtained from these studies indicate that acinar-origin secretory proteins are reduced, whereas inflammatory proteins are increased when compared with healthy control subjects\textsuperscript{58,64}. The inflammatory increase tends to correlate with salivary gland chronic inflammation and persistent damage in the oral microenvironment. \(\beta_2\)-microglobulin and \(\gamma\) and \(\kappa\) immunoglobulins light chains increased expression is thought to be a reflection of intraglandular immunoglobulin activation and increased synthesis. The decrease in secretory proteins is attributed to acinar cells damage and dysfunction and to the presence of fragmentation processes, which can be associated with an expression unbalance between proteases and protease inhibitors\textsuperscript{67}. Of all proteins that have been characterized in the saliva of patients with SS, Hu et al. have carried out \(\beta_2\)-microglobulin, \(\alpha\)-enolase and D cathepsin preclinical validation in patients with pSS in comparison with patients with sSS or SLE and healthy controls, using the ELISA and Western Blot method, as well as 3 mRNA biomarkers using the polymerase chain reaction (PCR)\textsuperscript{51}. In 2011, Baldini et al. studied 180 patients with pSS, sSS, RA-sSS with limited scleroderma, healthy control subjects and patients with xerostomia without autoimmune disease, who underwent proteomic analysis using two-dimensional electrophoresis, mass spectrometry, Western Blot and ELISA. In this study, significant differences were found in a-amylase precursors, carbonic anhydrase VI, \(\beta_2\)-microglobulin, glyceraldehyde 3-phosphate dehydrogenase (G3PDH), epidermal fatty acid binding protein (E-FABP) and immunoglobulin k light chain (IGK-light chain) in the saliva of patients with pSS in comparison with healthy volunteers and controls with xerostomia without SS\textsuperscript{59}.

\(\beta_2\)-microglobulin was isolated in 1968 by Berggård and Beam; it is a non-glycosylated protein with molecular weight of 11,800 and has been identified as an invariant light chain of the HLA-A, HLA-B and HLA-C class-histocompatibility antigens; this complex is found on the surface of all nucleated cells. The surface of monocytes and lymphocytes is particularly rich in \(\beta_2\)-microglobulin; its lymphocytic synthesis and expression are increased by stimulation with mitogens or IFN. As a result of HLA recycling, \(b_2\)-microglobulin is dissociated at its free form in interstitial fluid, and measurement of this protein in saliva has been suggested to be a simple method to assess the extent of glandular damage in patients with SS\textsuperscript{68}. Some reports show an increase of \(\beta_2\)-microglobulin expression in the saliva of patients with SS\textsuperscript{56,61,69-72}. Hu et al. indicate that this protein is a highly sensitive and specific marker to distinguish patients with SS\textsuperscript{71} and can be used as a non-invasive supplementary diagnostic test\textsuperscript{68}. The presence of this protein in the saliva of Mexican patients with SS has not been studied, and any such study will determine the levels of salivary \(\beta_2\)-microglobulin in a population of patients with SS in comparison with a healthy control group without immune disease.

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