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PhD THESIS

DIAGNOSTIC VALUE OF SALIVARY GLAND
ULTRASONOGRAPHY IN RELATION TO THE CLINICAL AND
BIOLOGICAL PROFILE OF SINDROM SJÖGREN PATIENTS

- ABSTRACT –

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Key words: Sjogren syndrome, sialadenitis, ultrasonography, elastography

INTRODUCTION

Sjogren syndrome (SS) is a chronic autoimmune disease, which predominantly involves the exocrine glands, thus being also mentioned as an autoimmune epithelitis. Exocrine hypofunction produces a generalized desiccation, which leads to the development of xerostomia and xerophthalmia in the majority of patients. The body of knowledge concerning this disease has increased significantly in the last decades, from the first attempts of standardizing the diagnostic criteria [1, 2, 3], continuous efforts for establishing main etiopathogenic factors, up to the therapeutic studies of the newly developed biologic agents [4]. The relative high prevalence of sicca symptoms in patients with autoimmune diseases, which is cited up to 30% by some authors [5], and also overlap of some serology markers, for example SS-specific autoantibodies, led to the differentiation between the primary and secondary SS clinical forms.

In general, SS is considered a chronic disease, without vital risks, and a clinical setting dominated by sicca symptoms, fatigability and non-specific pain. Nevertheless, the clinical spectrum of SS can be highly complex, with high prevalence of systemic involvement at the time of diagnosis of up to 70-80% [6]. The current diagnostic algorithm combines both subjective ocular and oral symptoms, objective items including tests for salivary and lacrimal hypofunction, specific autoantibodies and evidence of lymphocytic sialadenitis on histopathology exam. Salivary gland (SG) biopsy is a frequently used test in SS work-up, which offers the advantage of a direct examination of the affected tissue. The characteristic histopathology feature of labial SG in SS is the focal lymphocytic sialadenitis, which can be graded by quantifying the number of lymphocytic foci. The immunohistochemistry (IHC) study, although not included in the diagnostic criteria for SS, can offer additional information through the description of an immunophenotype profile of the lymphocytic infiltrate. Thus, numerous studies have tried to establish correlations between the immunophenotype and disease severity, or several prognostic factors related to the risk of malignant lymphoproliferation.

Major SG ultrasonography (US) is an accessible, non-invasive evaluation, frequently used for the examination of morphologic changes in the glandular tissue, and also functional features related to SS. Studies in the past decades have provided promising results regarding the diagnostic potential of US and, also, it's applicability in the therapeutic follow-up. Furthermore, US can facilitate the major SG real-time US-guided biopsy. The global sensitivity of US in the SS diagnosis varies in studies between 40-90% [7, 8]. Doppler mode can offer information related to the parotid

gland vascularity features of both morphology and functional status by means of pre- and post-stimulation salivary flux measurements.

Real-time elastography (RTE) is a US method used for the evaluation of soft tissue elasticity. The underlying principle for this technique resides in the deformity capacity (i.e. *tissue displacement or tissue strain*) of the examined tissue tested through external compression by the US probe [9]. Thus, tissues with increased stiffness will have a lower strain, compared with softer tissues. Acoustic Radiation Force Imaging (ARFI) is another elastography method which consists in the emission of an acoustic impulse at the region of interest, by which tissue strain can be measured, similarly to the RTE method. The advantage of ARFI resides in the fact that the emitted impulse by the probe eliminates the need of external compression, thus reducing in principle the examiner – dependent variations.

Inclusion of US in the diagnostic criteria has been supported by numerous studies [10, 11], which has led to the foundation of international expert groups working on the standardization of US evaluation and also attempts to establish a quantification system for structural changes [12]. From this standpoint, I have formed the main research objectives, in the attempt to examine some well-established concepts regarding SS, analyze some clinical and biologic correlations and study the imaging features of the disease centered on the US examination.

OBJECTIVES

By structuring the research hypotheses aforementioned, I delineated the main study objectives as follows:

- Study of the clinical, biologic and imaging profile of primary SS and a comparative study in relation to the results obtained in the patient group with secondary SS;
- Semi-quantitative US evaluation in gray-scale using the parenchymal inhomogeneity as study parameter and further analysis of correlation with clinical and histopathology profile;
- RTE and ARFI comparative study with the healthy subjects and analysis of correlations with measurements obtained with conventional US methods.

The following were secondary study objectives:

- IHC profile of sialadenitis in a subgroup of patients with primary SS;
- Evaluation of US features in subjects with hepatitis C related sicca symptoms.

STUDY GROUPS

The study included a total of 62 subjects, divided in 4 groups. The first group consisted of 22 patients diagnosed with primary SS according to the AECG 2002 criteria [1]. The second group comprised of 20 patients diagnosed with secondary SS. The US examination was extended, as secondary objective, to a group of 10 patients diagnosed with chronic hepatitis C and associated sicca symptoms. The fourth group comprised of 10 healthy, non-smoking subjects.

METHODS

The description of the clinical profile consisted in the subjective features and objective tests related to the glandular hypofunction, followed by the laboratory work-up. The first step involved collecting of detailed information related to demographics, heredity, onset, severity, evolution and association of extraglandular manifestations or any other autoimmune disease. For the lacrimal gland secretion I opted for the Schirmer test and OSDI questionnaire; xerostomia was assessed through the Xerostomia inventory. The laboratory work-up included: complete blood count, acute phase reactants, i.e. ESR, fibrinogen and C reactive protein. Renal function was evaluated through serum measurements of urea, creatinine and an urinalysis. Liver tests included levels of alanine transaminase and aspartate transaminase, gamma-glutamyl transferase, alkaline phosphatase and anti-VHC antibodies. Immunology panel consisted of the following: rheumatoid factor, serum protein electrophoresis, C3 and C4 complement fractions and screening for autoantibodies. In this regard, I opted for the ANA Blot test.

US examination was performed on all patients diagnosed with primary and secondary SS. Gray-scale and Doppler evaluation was done using a MyLab25Gold US system, using a linear probe, with work frequency of 12 MHz. For every examination, the patient was positioned in dorsal decubitus, with slight extension of the head and contralateral rotation in relation to the examined parotid gland (PG). The glandular parenchyma was examined both in longitudinal and transversal sections, in which the global homogeneity, echogenicity, contour, size, fibrosis bands, sialectasis and sialolithiasis were assessed. A subsequent Staging of the parenchymal inhomogeneity (PIH) into four grades was made according to a semi-quantitative scoring system described by Salaffi et al. [13]. RTE examination was performed using a Hitachi Preirus US system, and every recorded image was then processed by the Elasto_ver 1.5.1 incorporated software. Using a semi-quantitative scoring system described by Dejaco et al. [14], the grade of glandular stiffness was assessed as follows: 0=

lack of blue area, 1= $\leq 25\%$, 2= 26%-50%, 3= 51%-75%, 4= blue area occupies $>75\%$ area of interest. ARFI examination was performed on a Siemens Acuson US system, using a 9L4 linear probe, with work frequency of 8 MHz. RTE and ARFI assessment was made on 10 patients diagnosed with primary SS and compared with results obtained on healthy subjects.

Minor SG biopsy was performed by a maxillofacial surgeon, using the Daniel technique [15] for each tissue probe. Thus, specimens were obtained from the lower lip, in an area of healthy mucosa, containing at least 5 minor SG. Tissue probes thus obtained were analyzed by a pathology specialist in order to establish the histopathology profile. For the IHC study, the following immunostaining markers were used: CD3, CD4, CD8, CD20, CD68, Matrix metalloproteinases (MMP) 2, MMP8 and triptase.

RESULTS

Clinical, biological and imaging profile of primary Sjogren syndrome patients

Demographic analysis of the first patient group has shown a net superiority for women, with a 90.9% distribution, compared to 9.1% for male patients and a female to male ratio of 10:1. Mean age was 52.36 years (± 11.27 years). SS specific symptoms had a high prevalence in the first group, higher for xerophthalmia, with 90.9%, compared to xerostomia, with 68.2%. Parotidomegaly and keratoconjunctivitis sicca were also present at a significant number of patients, with the latter affecting almost half of the study group (45.5%). The high prevalence of xerophthalmia is also reflected in the high number of patients with positive Schirmer test. The extraglandular manifestations were present at over half of the patients, with predominance for arthritis, skin and nervous system involvement, with prevalence of 40.9%, 36.4% and 31.8%, respectively.

US study performed in the first group included all four methods, although it is worth mentioning that RTE and ARFI were done on a smaller subgroup of only ten patients. By analysing the data obtained through assessment of PIH in gray-scale US, a uniform distribution of the results was observed, ranging from minimum changes up to stage 4 PIH, and a maximum frequency for grade 2 PIH. Data obtained through doppler examination showed predominance of lower scores and normal features. RTE and ARFI elastography assessment performed on the ten patient subgroup, data regarding tissue stiffness of the PG were recorded. The results for RTE showed similar values

for the right and left PG, with a global mean of 41.40% ($\pm 14,56$). Also, uniform result were obtained on ARFI examination, with a global mean of 2.01 ($\pm 0,15$) m/s.

Histopathology and immunohistochemistry study

Histopathology study provided a quantification of the grade of mononuclear infiltrate at the site of minor salivary glands and; it was coupled with IHC study in a ten patient subgroup. The main histopathology features observed were focal lymphocytic sialadenitis, structural changes of the ductal system, which consisted mainly of sialectasis, desmoplastic reaction secondary to the chronic inflammatory process. Cellular populations identified by IHC study comprised of CD20+ B lymphocytes, CD3+ T lymphocytes, CD68+ macrophages. Prevalence of the lymphocytic subpopulations varied in relation to the grade of cellular infiltrate. Thus, predominance of CD3+ T lymphocytes was observed in specimens with low grade focus score. In cases with high grade lymphocytic aggregates, i.e. 2+ focus score, intense IHC reactions were obtained for CD20+ B lymphocytes. These features provided some correlations between significant intraglandular proliferation of B lymphocytes and advanced disease stage, marked secretory hypofunction and positive serology markers.

Descriptive study of subject groups with secondary Sjogren syndrome and patients with sicca symptoms related to hepatitis C virus infection

The study group attributed to secondary SS included 20 subjects. Inclusion in this group required that every patient had been diagnosed with another autoimmune disease. All the 20 patients had one of the following associated diseases: rheumatoid arthritis, systemic lupus erythematosus or systemic scleroderma. Of the three autoimmune diseases, rheumatoid arthritis was the most frequent, being diagnosed in over half of the study group. Sicca symptoms were encountered in the majority of patients, with similar frequency for xerostomia and xerophthalmia. Parotidomegaly was absent in this group and konjunctivitis sicca was detected in only two cases. Serology profile showed both specific autoimmunity markers for SS and non-specific markers as rheumatoid factor. Assessment of structural changes of the glandular tissue was done by means of PG US. In gray-scale examination, majority of cases had low PIH score of 1; only 25% of patients had a PIH score of 2; significant changes (PIH III-IV) were not detected. Structural changes of the vascular component evaluated through Doppler mode were observed in less than half of the subjects, theses being of low grad, i.e. Doppler score of 1.

US study was extended also to a small group of viral hepatitis C patients and it included only gray-scale and Doppler exam. Although structural features of PGs were mostly normal and some had minimum PIH in gray-scale US, there were 2 cases of patients with viral hepatitis C related sicca symptoms that developed severe structural abnormalities of the glandular parenchyma.

The fourth study group comprised of the 10 healthy subjects, which were assessed by PGUS using all four methods, i.e. gray-scale, Doppler, RTE and ARFI. In the majority of cases, recorded images in gray-scale and Doppler did not reveal structural changes. In only two subjects, a minimum PIH grade could be detected. Also, minimum Doppler signal, shown as isolated Doppler spots, was observed in two subjects. The mean scores for RTE mode were similar between the two PGs, with a global mean of 8.30% blue area. Parameters measured by ARFI technique showed slight differences between PGs, with a mean of 1.93 m/s on the left PG 1.81 m/s for the right PG and a global mean of 1.85 m/s.

Analytic study of clinical, biological and imaging parameters

Comparative study between groups with primary and secondary Sjogren syndrome

Several demographic characteristics, clinical and biological features of SS were compared between the first two groups, i.e. primary and secondary disease form. Significant statistical results obtained allowed for some associations to be noted between the clinical features and the classification as either primary or secondary disease. An initial analysis meant to point out the similar build-up of the two groups, with regard to the number of patients, sex distribution and also mean age at inclusion, did not yield significant differences. These results support the statistical compatibility of the two groups, which in turn validates the tests carried out in the next steps. One relevant clinical aspect that differentiates between the two groups is the time interval in years since the onset of sicca symptoms. It was noted that a longer history of the specific symptoms is linked to the first group, with a statistically significant difference ($p=0.034$), which can also influence the degree of glandular involvement in primary SS that is expressed either clinically or in the imaging features.

The clinical profiles of the two study groups had similar characteristics, especially in regard to the prevalence of sicca symptoms. Presence of sicca phenomenon was an essential clinical feature that led to further work-up of patients diagnosed with other autoimmune diseases, and this in turn

provides for the high prevalence in the second group, similar to that in primary SS group. Clinical features that allowed for a differentiation with significant results were parotidomegaly ($p < 0.001$), keratoconjunctivitis sicca ($p = 0.011$) and mean scores obtained through the OSDI questionnaire ($p = 0.002$). Analysis of the biological profile provided a series of significant differences in regard to the SS-specific immune markers, i.e. anti-Ro and anti-La autoantibodies, hypergammaglobulinemia and hypocomplementemia. These results support the superior specificity of these markers for the primary disease form. Rheumatoid factor, although more prevalent in the second group (55% vs 31%), was attributed mainly to the predominance of rheumatoid arthritis patients in this group, without a statistically significant difference compared to the first group.

Study of ultrasonography evaluation of parotid glands

In order to evaluate the diagnostic value of PG US in SS patients, an initial analysis of potential correlations between imaging aspect in gray-scale and clinical or biologic features was performed for the subjects appointed to the primary SS group. The results offered a series of statistically significant correlations between US assessment of PIH grade and prevalence of xerostomia ($p = 0.045$), keratoconjunctivitis sicca ($p = 0.050$), hypergammaglobulinemia ($p = 0.039$), hypocomplementemia ($p = 0.049$) and a positive correlation with the mean score obtained through the OSDI questionnaire ($r = 0.565$, $p = 0.006$). Evaluation of the severity of glandular involvement of the PGs quantified through the PIH score in primary SS patients yielded a strong correlation with the scored obtained for vascularity index, examined in color Doppler mode ($r = 0.902$, $p < 0.001$). The mean PIH and Doppler scores obtained in the first study group were compared with those obtained in the second group, and mean scores for a subgroup of 10 primary SS patients was compared to those obtained in the third and fourth group, in order to account for the limited number of subjects in the latter two groups. Significant differences from the perspective of both US methods were noted between the primary SS patients, compared to secondary SS patients ($p = 0.007$ and $p = 0.002$, respectively) and healthy subjects ($p < 0.001$ and $p = 0.005$, respectively). The subgroup of 10 patients from the first study group, which were evaluated also through RTE and ARFI elastography, was compared with the fourth group of healthy subjects in regard to the parameters obtained by these methods. Both RTE and ARFI results provided statistically significant differences compared to the healthy subjects. In one last analysis of the data obtained through US imaging, a series of significant correlations were noted between elastography parameters and conventional gray-scale US method. Positive correlations were obtained between all three methods, with statistically significant results for gray sclae-RTE ($r = 0.846$, $p = 0.002$) and RTE-ARFI ($r = 0.782$, $p = 0.008$).

CONCLUSIONS

- Sjogren syndrome is systemic autoimmune disorders that can develop as either primary form or secondary form associated with another autoimmune disease;
- There are a series of clinical, immunological and, also, morphological features that contribute to the differentiation of the two clinical forms, in regard both to the clinical picture at presentation and also disease outcome; negative prognostic factors have been proven to be more prevalent in the primary disease form;
- Diagnostic approach of a patient with SS can be difficult, especially in cases with a clinical picture dominated by sicca symptoms, but without any serology markers, and vice-versa. In these cases the decision of undergoing a tissue biopsy, followed by pathology examination is salutary;
- Ultrasonography of the salivary glands is an accessible, non-invasive imaging method, which acquired significant interest in the last years, in the attempts to improve the diagnostic algorithm of SS. Thus, the detection of an imaging pattern specific to SS, and also grading of the structural changes observed provides the clinician with additional information in regard to the later diagnostic work-up, especially concerning the recommendation for minor salivary gland biopsy;
- A central element, with major importance for the research done in order to validate the diagnostic performance of salivary gland ultrasonography, relies in obtaining a significant correlation with the current “golden standard”, i.e. histopathology examination. This correlation was studied in the primary Sjogren syndrome study group. Between the PIH score and focus score quantified in this group a weak positive correlation was noted, without statistical significance. These results are in accordance with a series of revised studies, which in fact explains the current lack of scientific background supporting the inclusion of ultrasonography as an additional diagnostic criterion.
- The results obtained in this thesis support data in literature regarding the applicability of salivary gland ultrasonography both at presentation and in later follow-up. The main advantages of this imaging method reside both in the complementary data obtained for a confirmed case of Sjogren syndrome and, also, in the initial diagnostic work-up phase, when the clinician is required to recommend a minor salivary gland biopsy when facing a poor clinical picture or non-specific serology panel.

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