THE VALUE OF LABIAL BIOPSY IN THE DIFFERENTIAL DIAGNOSIS OF SARCOIDOSIS AND SJÖGREN’S SYNDROME AND IMMUNOHISTOCHEMICAL ANALYSIS

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ABSTRACT
The aim of this study was the evaluation of the value of labial biopsy in the differential diagnosis of sarcoidosis and Sjögren’s Syndrome and the value of immunohistochemical markers such as CD3, CD4, CD8, and CD20 at the immunopathogenesis. Fifteen labial salivary gland biopsies from patients affected by primary Sjögren’s syndrome, and 15 labial salivary gland biopsies from patients affected by sarcoidosis, were included in the study. In all patients, biopsies were carried out as a diagnostic procedure either for sicca syndrome or sarcoiosis. Sarcoidosis and Sjögren’s Syndrome were compared according to immunohistochemical markers. It was found that CD3 and CD4 immunoreactivity were statistically higher in sarcoidosis patients, and CD 20 immunoreactivity was statistically higher in SS patients. CD8 did not show any significance between groups. We also compared labial biopsy with radiological stage, and could not find any significant correlation.

When the microscopic methods are not enough to differentiate between sarcoidosis and Sjögren’s Syndrome, or when they are seen together, then obtaining the subgroups of lymphocytes with immunohistochemical methods can be helpful for diagnosis.


Keywords: Sarcoidosis, Sjögren’s Syndrome, labial biopsy and immunohistochemical markers

Introduction
Sarcoidosis is a multisystemic granulomatous disease of obscure etiology that predominantly affects the lungs (1). The many forms and presentations of this disease and the lack of a single diagnostic test make the diagnosis challenging (11, 22). A definitive diagnosis of sarcoidosis is best achieved by integrating clinical data with the presence of noncaseating granulomas. Histologically, the granuloma is characterized by tightly grouped epithelioid cells interspersed with a small number of giant cells. Accumulations of lymphocytic cells may be seen at the periphery of the granuloma. To further support the diagnosis of sarcoidosis, clinicians must use special stains to rule out other granulomatous conditions (for example, histoplasmosis, blastomycosis, berylliosis, foreign bodies, tuberculosis, leprosy) (8, 20). Furthermore, the clinician should be aware that noncaseating granulomas are occasionally seen in cases of tuberculosis (7, 18, 19). It has been demonstrated that labial salivary gland (LSG) biopsy had a high discriminatory value as a diagnostic tool in the differentiation of sarcoidosis from tuberculosis (18). Sjögren’s syndrome (SS) is a complex autoimmune rheumatic disease characterized by the presence of mononuclear cell infiltration of the salivary and lacrimal glands and B cell hyperreactivity leading to production of circulating autoantibodies (14, 15). The histopathological finding in LSG biopsies of SS patients is epithelial cell destruction by infiltrating lymphocytes, leading to the subsequent replacement of the salivary gland tissue by mononuclear cells (5). The extent and composition of the lymphocytic infiltrate in the LSG biopsy specimens can be considered as target organ specific parameters. However, the degree of focal infiltration is not directly related to the target organ dysfunction and is not specific for patients with SS, also it can be found in association with lymphomas, sarcoidosis, rheumatoid arthritis, graft-versus host disease and the the acquired immunodeficiency syndrome (13). The number of lymphocytic foci per 4 mm² in the LSG biopsy specimen is expressed as a focus score. However, it is well known that the focus score lacks both sufficient sensitivity and specificity for the diagnosis of SS, depending on whether a focus score of >1 (leading to decreased specificity) or >2 (leading to decreased sensitivity) is taken as the criterion for diagnosis. In contrast to the focus score, immunohistological examination of the infiltrate has been shown to have both high disease specificity and sensitivity (6). Sarcoidosis can also affect exocrine glands and cause sicca symptoms (2, 5, 9, 12). In such patients, LSG biopsy has been considered an important consideration among other procedures to help confirm the diagnosis of both sarcoidosis and SS. The coexistence of sarcoidosis and SS is
another dilemma and has been occasionally reported in the literature (2, 5, 6, 9, 12). When the microscopic methods are not enough to differentiate sarcoidosis and SS, or when they are seen together, obtaining the subgroups of lymphocytes with immunohistochemical methods can be helpful for diagnosis. The aim of this study was to investigate the value of LSG biopsy in the differential diagnosis of sarcoidosis and SS and the value of immunohistochemical markers such as CD3, CD4, CD8 and CD20 at the immunopathogenesis.

Materials and Methods
Specimens from labial salivary glands were obtained from 15 subjects affected by primary Sjögren’s syndrome (14 women and 1 man, mean age±standard deviation (SD): 45.66±14.49 yr) diagnosed according to the clinical-laboratory criteria proposed by the American-European Consensus Group. Specimens from labial salivary glands were obtained from 15 subjects affected by sarcoidosis (11 women and 4 men, mean age±standard deviation (SD): 38.46±12.24 yr) diagnosed according to the clinical features together with histopathological evidence of noncaseating granuloma and admitted to Istanbul University, Faculty of Dentistry, Department of Oral Surgery and Medicine. In all patients, the LSG biopsies were carried out as a diagnostic procedure either for sicca syndrome or sarcoidosis. Before the surgical procedure the patient was asked to sign the consent form, following the ethical principles in the Helsinki Declaration (2000). Minor salivary glands from the mucosa of the lower lip were sampled in the oral surgery clinic on an ambulatory basis. The lower lip was everted, a random area was selected. Following local anesthesia injection, an incison of 6 to 10 mm in length and 1 to 2 mm in depth was made, and two or three salivary glands were excised. The incison was closed with 3.0 silk sutures. The sutures were removed a week later and no complications due to the biopsy procedure were observed. The immunohistochemical studies were performed on available sections from these biopsies. For the histopathologic examination, each biopsy was fixed in 10% buffered formalin and embedded in paraffin; the approximately 4μm thick histological section obtained was placed on individual glassess coated with poly-l-lysine (sigma catalog no:8920). Tissue sections were dried at 37˚C incubation overnight and at 55˚C incubation for one hour to provide glass adherence. Tissue sections were dewaxed in Tris buffer (pH 7.6) was used as washing solution. For each antigen and for each test, a negative control test was performed in parallel with nonimmune mouse serum, by incubation in the absence of the primary antibody and always yielded negative results. Slides were observed with Olympus BX50 (Olympus Corporation, Tokyo, Japan). 10X ocular and 40X objective was used for counting the cytoplasmic membrane immunoreactivity in the intensive immunoreactivity area. Reactivity index was calculated for each antigen by counting the number of lymphoid cells that showed cytoplasmic membrane immunoreactivity in one large magnification field of view.

For data processing and statistical analyses, the Statistical Package for Social Sciences (SPSS) software (SPSS for Windows, version 10, Chicago, IL, USA) was used. The Mann-Whitney U-test, t-test and chi-square test were used as appropriate to assess the statistical significance. A P-value<0.05 was considered statistically significant.

Results and Discussion
The characteristics of the sarcoidosis and SS patients is shown at Table 1. Noncaseating granulomas were present in the labial biopsies obtained from the 15/15 sarcoidosis suspected subjects. The study of lymphocytic cells in the diffuse infiltrate showed a predominance for the CD3 lymphocytic population, and a predominance of CD4 over CD8 T cells (almost 2:1 ratio) was seen in patients with sarcoidosis. In salivary glands with sarcoid infiltration, the number of cells intensively stained with anti-CD3 antibodies was 337±178 (mean±SD) cells per magnification field (Fig. 1), with anti-CD4 antibodies, 209±114 cells per field (Fig. 2), and with anti-CD8 antibodies, 119±58

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age±SD</th>
<th>Mean CD3±SD</th>
<th>Mean CD4±SD</th>
<th>Mean CD8±SD</th>
<th>Mean CD20±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis (n:15)</td>
<td>38.46±12.24*</td>
<td>337±178*</td>
<td>209±114*</td>
<td>119±58*</td>
<td>78±88*</td>
</tr>
<tr>
<td>Sjögren’s Syndrome (n:15)</td>
<td>45.66±14.49*</td>
<td>226±242*</td>
<td>121±63*</td>
<td>81±44*</td>
<td>302±187*</td>
</tr>
</tbody>
</table>

* cells per field

TABLE 1

Characteristics of the Sarcoidosis and Sjögren’s syndrome patients
Fig. 1. Many CD3+ lymphocytes in the histiocytic granulomatous infiltration zone, X100, AEC Cromogen

Fig. 2. Histiocytic granulomas, CD4+ cells on the periphery. Partially stained CD4+ cells in the histiocytic granulomatous infiltration zone, X100, AEC Chromogen

Fig. 3. CD8+ lymphocyte cells around the granuloma that includes histiocytic giant cell, X100, AEC Chromogen

Fig. 4. Lymphocytic infiltration around the histiocytic granuloma and few numbers of CD20+ lymphocytes, X100, AEC Chromogen

Fig. 5. Interstitial lymphocytic infiltration of the salivary gland parenchyma and a small CD3+ T-lymphocytes population at the infiltration, X100, AEC Chromogen

Fig. 6. CD4+ lymphocyte cells more centrally localized in the nodular lymphocytic infiltration zone. X200, AEC Chromogen
cells per field (Fig. 3). Unlike the other markers, CD8-stained cells were intensive in the outer part of the granulomas. The cells stained with CD20 were 78±89 cells per field (Fig. 4). In labial salivary glands with a sarcoid infiltrate, T cells stained with anti-CD3 antibodies intensively as expected but, in contrast, not too much with anti-CD20 antibodies. According to the radiographic appearance of lung, five patients were stage 1, nine patients were stage 2 and one patient was stage 1. No significant correlation between the radiographic stage and immunohistologic markers of the salivary glands was found (p<0.05).

All the labial salivary glands from the SS patients showed focal accumulation of mononuclear cells, localized predominantly around the ducts. In the salivary glands of patients with SS, the cells stained with anti-CD3 antibodies were 196±255 cells per field (Fig. 5), with anti-CD4 antibodies, 49±78 cells per field (Fig. 6) and with anti-CD8 antibodies, 81±44 cells per field (Fig. 7). The immunohistochemical analysis of the lymphocytic focus showed that SS patients had a predominance of CD20-positive cells (p<0.05) and the cells intensively stained with anti-CD20 antibodies were 302±187 cells per field (Fig. 8). The patients with sarcoidosis had a greater number of cells stained with anti-CD3 antibodies in their salivary glands than the patients with SS (p<0.05). The patients with sarcoidosis had significantly higher numbers of cells stained with anti-CD4 antibodies in their salivary glands than the patients with SS (p<0.05). No significant correlation was found between the patients with sarcoidosis and the patients with SS in terms of anti-CD8 antibody staining (p<0.05). The patients with sarcoidosis demonstrated a decreased numbers of cells stained with anti-CD20 antibodies in their salivary gland specimens compared to the patients with SS (p<0.05).

This study reports on LSG lymphocyte immunophenotyping and demonstrates the effective use of immunohistochemical markers such as CD3, CD4, CD8 and CD20 as an additional discriminative tool to distinguish sarcoidosis patients from patients with SS. Labial gland biopsy is a highly sensitive and specific diagnostic test in the histological assessment of SS, but it also can assist in the differentiation of SS from sarcoidosis when clinical presentations are similar (7, 10). Several studies have shown sarcoid infiltration of the minor salivary glands with or without clinical involvement of the major salivary glands (16). Therefore, when accessible clinically involved tissues are not available, the clinician can perform a biopsy of normally appearing tissue to confirm the histologic diagnosis in a patient with compatible clinical findings. The association of sarcoidosis with sicca symptoms is well known (6, 21). Drosos et al. (2) described the cases of five patients with xerophthalmia and xerostomia, documented by a positive rose bengal or Schirmer’s test and decreased salivary flow, whose diagnosis of sarcoidosis was made by minor salivary gland biopsy (3 patients) or transbronchial lung biopsy (2 patients). The presence of granulomata in minor salivary gland tissue allows clinicians to distinguish sarcoidosis from SS because noncaseating granulomata are found in the minor salivary glands of patients with sarcoidosis even when sicca symptoms are not present, but they are not seen in patients with known SS (2, 3). Giotaki et al. (7) found no noncaseating granulomata in the biopsy specimens of minor salivary glands of 28 patients with SS, all of whom showed typical lymphocytic infiltration, fibrosis, or both. While the cause or “trigger” of sarcoidosis remains unknown, the pathogenesis of its many manifestations is becoming more fully understood. A specific agent or trigger stimulates local macrophages. The stimulated macrophage secretes chemotactic factors such as lymphokines that then attract T-helper (T4) lymphocytes. The T4 cells, in turn, stimulate antibody production by B-lymphocytes. Together, the activated macrophage, B-lymphocytes, and antibodies form granulomas within the affected tissue (12). On the other hand, previous studies have shown that epithelial cell destruction in
LSG biopsies of SS patients is mediated by the mechanism of programmed cell death or apoptosis. Two major pathways have been implicated in this process: (i) interactions between epithelial cells expressing the Fas molecule and surrounding T-lymphocytes expressing Fas ligand (FasL); and (ii) perforin–granzyme secretion by cytotoxic T-lymphocytes (CTL), leading to subsequent degeneration of epithelial cells (23). Fox et al. (4) found that salivary gland cluster of differentiated CD4+ T-cells produce over 40-fold more IL-2 and IFN-gamma than peripheral blood CD4+ T-cells in patients with SS compared to that in normal controls. Their results showed activation of Th-1 lymphocytes at the site of organ-specific immune damage. Thus, the predominance of Th-1 lymphocytes may be associated with the coexistence of SS and sarcoidosis. Minshall et al. (2) reported a high expression level of interleukin (IL)-2 and interferon-gamma (IFN-gamma) mRNAs in bronchoalveolar lavage cells in sarcoidosis patients compared to that in normal patients, but no significant differences were observed in the percentages of IL-4 and IL-5 mRNA-positive cells between sarcoidosis patients and normal controls. These results showed that there is a preferential expression of T-helper type 1 (Th-1) cytokines in pulmonary sarcoidosis (13). T lymphocytes form the majority of the infiltrate, while B cells constitute 20-30% of the total cell population and macrophages are rarely observed. Approximately 70% of the T cells are CD4+, while 25% are CD8+. Most of the T cells are activated as they express HLA-DR and CD95 molecule (14, 15). Ramos-Casals et al. (17) demonstrated that standard histological analyses were insufficient when the infiltration is mild and scattered. When the differential diagnosis could not be made immunophenotyping may be helpful for diagnosing. Giotaki et al. (7) analysed the phenotypes of the cells that infiltrate the minor salivary glands of sarcoidosis patients and control group with indirect immunoperoxidase method and found that no infiltrating lymphocytes were seen in the control group, whereas intense T lymphocytes, especially (CD4+), were observed in sarcoidosis patients. Melsom et al. (12) reported that early lymphocytic infiltration of salivary glands at sarcoidosis can mimic that at SS and this may cause confusion in making the diagnosis. Lymphocytic infiltration was observed in the biopsy material of sarcoidosis patients in this study. In our study group 33.3% of the patients were stage 1, 60% were stage 2, and 6.6% were stage 3. Our findings were consistent with the literature. Although we could not find any significant association between the radiographic appearance and lymphocytic infiltration, 33% of our patients were stage 1. And this finding did not correlate with the findings of Marks et al. (11) who demonstrated the late involvement of salivary glands. In this study the sarcoidosis and SS patients were compared in terms of immunohistochemical markers such as CD3, CD4, CD8, and CD20. It was found that CD8 did not show any significant difference between the groups. At sarcoidosis CD3 and CD4 immunoreactivities were statistically higher than those at SS, and SS CD20 immunoreactivity was statistically higher than that for sarcoidosis. Our findings are consistent with the literature demonstrating that T cells are predominant in sarcoidosis, whereas B cells are predominant in SS.

Conclusions
According to our results we concluded that the cells playing a role in the etiopathogenesis of sarcoidosis and SS are different. LSG biopsy, which is simple, minimally invasive and associated with significantly lower morbidity than a parotid gland biopsy, has been considered an important choice among other procedures to help confirm the diagnosis of both sarcoidosis and SS. When the histopathological methods are not enough to differentiate between sarcoidosis and SS, or when they are seen together, obtaining the subgroups of lymphocytes with immunohistochemical methods can be helpful for diagnosis.

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