Original study.

Histologic changes in eccrine apparatus in patients with Sjogren syndrome and no evidence of anhidrosis.

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Abstract: Sjogren syndrome (SS) is an autoimmune disease that involves salivary and lacrimal glands.   There are rare articles describing hypohidrosis or anhidrosis in SS patients. Little is known about the histologic findings seen in patients with SS who do not report decreased sweating. We analyzed skin samples from 31 patients with SS who had undergone skin biopsies for reasons not related to SS. We assessed the number, distribution and pathologic changes within and around the eccrine ducts, glands and acrosyringia. The composition of the inflammatory infiltrate was additionally accessed utilizing immunohistochemical stains. Overall, in 60% of cases we observed some degree of inflammation. In virtually all cases the inflammatory infiltrate was located around the eccrine ducts at a distance from the non-SS pathology. It was mainly composed of CD3+ T cells with increasing number of B and plasma cells as periductal inflammation intensified. This study is the first histologic analysis of asymptomatic skin in patients with SS. It demonstrates the presence of skin involvement in the majority of SS patients. Our results emphasize the importance of systematic skin evaluation in this category of patients as well as counseling, early preventive measurements and timely treatment when indicated.

 Sjogren syndrome; xerosis; anhidrosis; eccrine apparatus

1. Introduction

Sjogren's syndrome (SS) is an autoimmune disease with a broad range of clinical manifestations. It was first described in 1930 by a Swedish ophthalmologist, Henrik Sjogren, as a case of keratoconjunctivitis sicca associated with rheumatoid arthritis [1]. This syndrome can present as a primary disease or as secondary SS by accompanying other autoimmune conditions.   It is more frequent in women, peaking around 50 years old [2].

Central to the pathogenesis of SS is the production of autoreactive B and T cells, anti-Ro/SSA and anti-La/SSB autoantibodies, formation of ectopic lymphoid aggregates and hypergammaglobulinemia [3]. Disease-associated autoantibodies can be found several years before the diagnosis is established and first symptoms manifest.

The exact cause of SS is not definite and most probably multifactorial. Genetic susceptibility to SS has a strong association with certain HLA loci, particularly IRF5 and STAT4. Moreover, development of SS is reported in association with viral agents, particularly the Herpesviridae family [4, 5], imbalances in the gastrointestinal microbiome, squalene-adjuvanted anti-H1N1 influenza vaccine [6], development of menopause [7], low vitamin D [8], stress [9], exposure to silica [10] and silicone [11].

SS targets exocrine glands throughout the body, primarily salivary and lacrimal apparatus, leading to severe dryness of eyes and mouth. Extra-glandular manifestations include fatigue, and arthralgias as well as impairments in skin, vascular and neurologic

systems [12]. SS patients can develop various types of exocrine manifestations such as autoimmune thyroiditis, interstitial nephritis, or pneumonitis.

In a case that we described recently, a patient presented with markedly diminished axillary sweating.   The biopsy demonstrated extensive fibrosis and a marked diminution in the number of eccrine structures, with glandular ectasia deep in the specimen.   [13]

Although xerosis is an extremely common symptom in advanced SS, we know very little about the exact cutaneous processes in the affected skin, its chronology and its evolution in relation to salivary and lacrimal impairments. As with other autoimmune conditions, one might expect SS to cause systemic damage to all susceptible tissues. Although clinical manifestations begin due to salivary and lacrimal glands impairment, it is unknown if these glands are the initial targets of the disease or simply the most sensitive to the inflammatory damage. It is possible that cutaneous damage might happen concurrently with damage to other exocrine glands but not demonstrate clinical manifestations for a long time. There is no data in the literature nor any documented study of subclinical skin health in patients with already manifested SS. These patients may not yet exhibit cutaneous symptoms but steadily develop microscopic skin impairments. In order to investigate for the presence of a peri-eccrine, focally destructive autoimmune process involving eccrine structures, we conducted evaluation of 31 skin biopsies performed for other reasons on patients with diagnosed SS and asymptomatic skin.

2. Materials and Methods

The study protocol was approved by the Institutional Review Board at the University of Rochester School of Medicine and Dentistry. This study was based on the retrospective analysis of archival tissue. The study utilized surgical pathology specimens of patients who had been diagnosed with SS over the past 15 years (January 1st, 2004 to December 31, 2019). We found 162 such patients. From these 162 initial patients, 31 patients had skin biopsies for reasons not related to their SS diagnosis and were considered to be applicable to the study. Patients who were biopsied with an aim of diagnosing or staging of SS were excluded. All analyzed cases had primary pathology previously diagnosed by the dermatopathology unit in our institution and were reviewed for inclusion in this study by one of us (BRS).

The 31 skin biopsies contained a total of 84 eccrine structures and were reviewed in order to assess the number, distribution and pathologic changes within and around the eccrine ducts and glands. Eccrine structures were interpreted as separate clusters of eccrine ducts and glands. Acrosyringia were examined as part of the eccrine apparatus. Biopsies were analyzed for the extent, distribution and nature of any inflammatory infiltrate.   We also observed for presence of any peri-eccrine fibrosis or other histologic changes that might hint at a prior inflammatory process.

Inflammation was scored from 0 to 4+. A score of "0" was given when less than 5 inflammatory cells were found in close proximity to the eccrine structures (<0.1 mm), score "1+" corresponded to 5 - 25 inflammatory cells, "2+" similarly to 25 - 40 inflammatory cells, "3+" to 40 - 50 inflammatory cells and "4+" when more than 50 inflammatory cells were found in inflammatory infiltrate surrounding the sweat glands. We considered positive cases exhibiting "1+' and higher degree of inflammation.

To evaluate the composition of inflammatory infiltrate, we stained all positive specimens with CD4, CD20 and CD 138.

The control group included 31 random skin biopsies with no evidence of lupus erythematosus (LE), lichen striatus, Sjogren's syndrome or other diseases in which peri-eccrine infiltrate would be expected as a part of the disease process.

3. Results

Out of 84 eccrine structures 1+ inflammation was noted in 25, 2+ in 19, 3+ in 2, 4+ in 4 and no inflammation appeared to be in 34 structures (see Table 1).

Table 1. Data from evaluation of eccrine structures according to study protocol (P - plasma cells, L - lymphocytes, EOS - eosinophils).

Specimen number	Type of biopsy (P/S/E)	Biopsy site	Number of eccrine structures	Degree of inflammation (1-4+)	Composition of inflammatory infiltrate	Distribution of inflammation	Pattern of fibrosis	Other findings	Stains
1	punch	leg	1	1+	L	peri eccrine	no	septal pan- niculitis	CD3
2	excision	back	4	1+	L	peri eccrine	no	peri eccrine edema	CD3
3	excision		1	2+	L	peri eccrine	no	dermal nevus	none
4	punch	arm	4	1+	L, P	peri eccrine	no	dermal edema	CD3 CD138 CD20
5	shave	face	2	1+	L	peri eccrine	no	one dy- ing keratino cyte in the ec- crine duct	CD20 CD3
6	punch		25	4+	P,L	peri eccrine	2+ intra eccrine		elimi- nated - sali- vary gland

|									
7	punch	acral	2	1+	L	peri eccrine	2+ intra eccrine		none
8	excision		6	1+	L	peri eccrine	no	more in-flamma-tion around ducts	none
9	punch		2	2+	L, EOS	peri eccrine	no		CD3 CD20
10	excision		5	1+ in one group only	L	peri eccrine	no		CD3
11	excision		4	0					
12	excision		1	0					
13	excision		1	1+	L	peri eccrine	no		CD3
14	punch		5	2+	P,L, EOS	peri eccrine	no	edema	CD3 CD138 CD20
15	punch		2	3+	L,EOS	peri eccrine	no	hemor-rhage periec-crine	CD3 CD20
16	shave		2	2+	L,P	peri eccrine	no		CD138 CD20 CD3
17	punch		2	0				no ducts	
18	punch		1	1+	L	peri eccrine	no		CD3
19	punch		2	2+	L, EOS	peri eccrine	no		CD3 CD20
20	punch		5	0					
21	excision		2	0				dying keratino cyte	
22	punch		3	1+	L	peri eccrine	no		CD3
23	shave		1	0					

24	shave		1	0					
25	punch		8	2+	L	peri eccrine	no		CD3 CD20
26	punch		4	0			3+ peri and intra ductal		
27	punch		1	1+	L	peri eccrine	no		CD3 CD20
28	punch		5	1+	L	peri eccrine	no		CD3
29	shave		1	1+	L	peri eccrine	no		CD3 CD20
30	shave		1	1+	L	peri eccrine	no	dying keratinocyte	CD3
31	punch		4	4+	L,P,Eos	peri eccrine	no		CD3 CD20

The inflammatory infiltrate in all positive biopsies was peri-ductal without involvement of the eccrine glandular structures. In all cases, the peri-ductular inflammation was localized at some distance from the primary pathology for which the biopsy was performed and was judged to be an independent an unrelated process (see Figure 1).
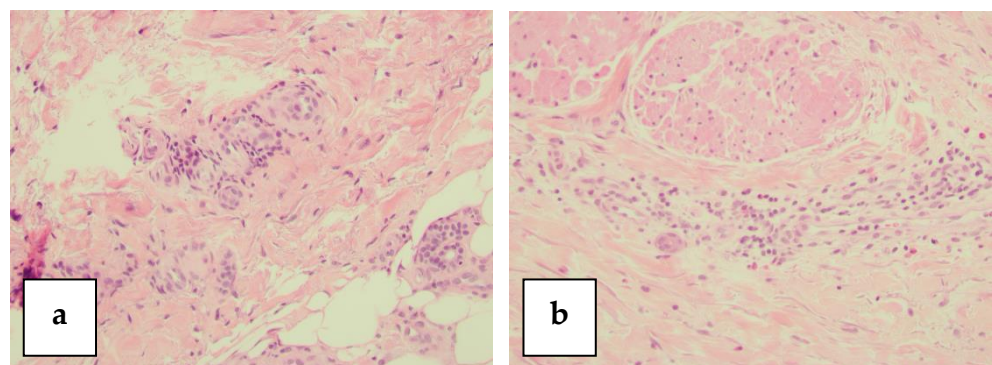
Figure 1. H&Ex20. Histological findings in skin biopsies from SS patient without cutaneous symptoms. Figure "a" represents example of 1+ peri-ductal inflammation, while figure "b" illustrates significantly higher number of inflammatory cells around the ducts and falls into category 4+ inflammation according to study protocol.

Immunohistochemical (IHC) stains revealed that the inflammatory infiltrates were purely composed of CD3+ T-cells in 14 structures, a mixture of CD3+ T-cells and CD20- B-cells in 24 structures, and a "cocktail" of inflammatory cells including CD3+ T-cells, CD20+ B-cells and CD138+ plasma cells was revealed in 12 structures (see Figure 2).
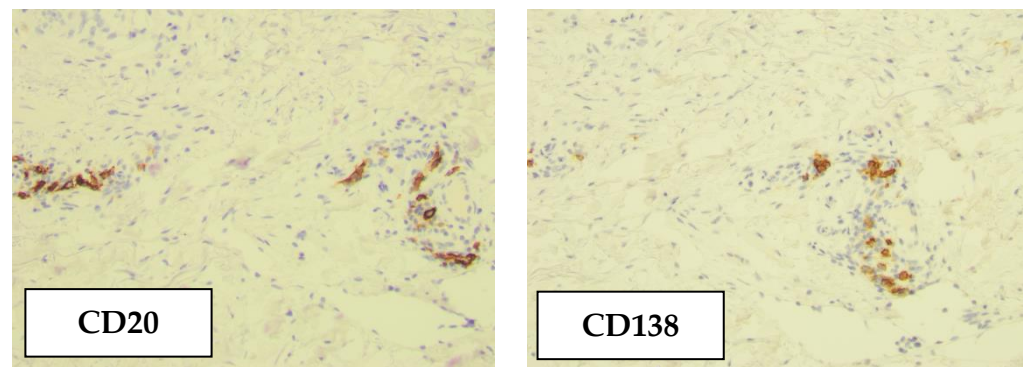
Figure 2.x200. Immunohistochemical analysis of peri - ductal inflammatory infiltrates. These figures represent example of 2+ infiltrate with B-cells highlighted by CD20+ and 4+ infiltrate that was found to contain CD138+ plasma cells

We noted that cases with mild peri-ductal inflammation consistently demonstrated T-cells as the sole components of the process, while the presence of B-cells and plasma cells was noted with higher degrees of inflammation (see Figures C&D).

Accompanied observations included peri-ductal eosinophils in 5 cases, peri-eccrine hemorrhage in 1 case and apoptotic keratinocytes in 2 eccrine ducts. Eosinophils were noted only in cases with higher degrees of inflammation and the presence of B – lymphocytes and plasma cells.

Overall, 60% of biopsies demonstrated some degree of inflammation related to the eccrine ducts. In contrast, all 31 skin biopsies in the control group showed less than five lymphocytes around the eccrine structures and were scored as "0" according to the criteria listed above.

4. Discussion

Cutaneous manifestations of Sjogren's syndrome are broad [14],[15-17],[18],[19] with autoimmune anhidrosis/xerosis being described in 26-67% of patients [15, 20].

These symptoms are not routinely recognized or assessed for in SS patients and may be underestimated and commonly neglected [18, 21]. To some measure, this is due to xerosis being perceived by clinicians as subjective and the fact that it is often associated with more attention demanding symptoms. This may also be attributed to a poor understanding of the mechanism of xerosis in SS patients. Herein, we demonstrated that most SS patients exhibit some degree of inflammation prior to developing any clinical cutaneous manifestations. These findings may play an important role in further understanding the pathophysiology of the disease in terms of its overall systemic nature, as well as the mechanism of disease progression, given the biological similarities between salivary and eccrine structures.

There are scattered publications describing the histology of xerotic skin in SS patients. Mitchell et al. described a skin biopsy in a patient with SS and hypohidrosis that showed a moderate number of eccrine glands and ductal structures in the reticular dermis and the presence of lymphocytes and plasma cells heavily infiltrating the epithelial structures [22]. Whaley et al. performed skin biopsies on seven patients with xerosis, showing a chronic inflammatory process destroying the sweat glands. A nonspecific perivascular lymphocytic infiltrate was found in this study [23]. In another study by Roguedas et al., axillary skin biopsies were performed on 12 of 22 patients previously diagnosed with SS to investigate the role of B and T-cells in the cutaneous pathogenesis of Sjogren's syndrome [24].

A lymphocytic infiltrate was identified in 8 cases (66%). In addition to T lymphocytes, numerous B lymphocytes (marginal-zone-like and memory-type) were found within the inflammatory infiltrate surrounding eccrine ducts. Our findings correlate with data reported by the Roguedas' team, as well as these other studies. Additionally, we observed the composition of the inflammatory infiltrate to correlate with the degree of peri-eccrine inflammation. We demonstrated that T lymphocytes are the main components of the inflammatory infiltrate when inflammation is mild and the disease process is presumably in its initial stages, while increasing severity of inflammation is associated with presence of B–cells and plasma cells in the infiltrate. Therefore, it is possible to speculate that xerosis might result from immunologic destruction of the eccrine apparatus by an organized group of inflammatory cells with clearly defined roles for each member. It begins with T cells and leads to the production of antibodies by an erroneously selected and differentiated population of B cells. This process would be analogous to the destruction of salivary or lachrymal glands [25]. Considering exclusively T - lymphotropic qualities of the epidermis, it would be very unusual to locate B- or plasma cells in the peri-eccrine zone in asymptomatic skin unless for a reason. Chemotactic factors for B-cells are described in labial salivary glands [26] and might similarly be produced by all exocrine tissue [27]. The location of the inflammatory infiltrate in our study suggests these chemotactic factors might be, in theory, secreted by ductular epithelium rather than gland itself. Further studies are necessary to test the validity of this speculation.

Our study is the first histologic evaluation of asymptomatic skin in SS patients. We demonstrated the majority of these patients to have mild inflammation in the peri-eccrine zone. In five cases with higher degree of inflammation (according to study protocol) we noted presence of eosinophils. Although not striking in samples from asymptomatic patients, these cells might have a meaningful role when inflammation gets serious. Eosinophils might influence secondary destruction of the ducts. They are recruited via T cells and IL-5 and might also regulate local tissue remodeling and fibrosis [28].

The diagnosis of SS is based on histopathological examination of the minor salivary glands. The diagnostic finding includes an inflammatory infiltrate surrounding salivary and lacrimal glands and ducts that is primary composed of lymphocytes and plasma cells (mostly in lacrimal glands). Additional features are parenchymal atrophy as well as fibrosis, ductal ectasia and acute inflammation. The most characteristic feature of SS on biopsy is focal lymphocytic sialadenitis, with a sensitivity and specificity of >80% [29]. This is described as the presence of dense aggregates (foci) of ≥50 mononuclear cells (mostly lymphocytes), in a peri-ductal or perivascular localization [30]. In our study, cases with 4+ inflammation (2 cases – 29 structures) met this criterion demonstrating a huge discordance in disease manifestation between minor salivary gland findings and cutaneous eccrine glands. This may mean that minor salivary glands might be more sensitive to the chronic inflammatory damage than eccrine glands and possibly have weaker abilities to self-repair. An appropriate grading scale for peri-eccrine inflammation and its prognostic or diagnostic significance must be determined with a larger study and long-term patient follow-up.

Obtaining skin samples from patients with suspected or established SS might be helpful for disease staging as well as evaluation of therapeutic measurements [24, 31]. Since most of our cases revealed some degree of inflammation, we suggest that a majority of patients have subclinical cutaneous involvement at the time of the diagnosis. We support the idea to include skin biopsy in the routine care of SS. Evaluation of cutaneous exocrine glands might be particularly helpful in challenging situations i.e. symptomatic patients without identifiable sialadenitis etc. [32]. Skin biopsy could also serve as a secondary prognostic and diagnostic factor in those who simply decline lip biopsy because of cosmetic reasons.

Early and a routine cutaneous evaluation in SS patients is a necessity due to therapy-induced immunosuppression and disease-associate immunodysregulation promoting skin malignancy. Also, patients with SS are known to have higher incidence of B-cell lymphomas compared to the general population or patients with other autoimmune diseases [34]. Skin lymphomas in patients with SS have been described and might be early detected on frequent skin checks in these patients. [35].

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