Sjogren Syndrome with Nasal Dryness: An Ultrastructural Study of Nasal Mucosa

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Abstract

Background: Sparse data are available in literature about pattern of nasal affection in Sjogren syndrome (SS).

Objective: To study the ultrastructural changes in the nasal mucosa in SS patients with nasal dryness.

Methods: Light microscopy and transmission electron microscopy (TEM) of anterior end of inferior turbinate in 14 patients with SS experiencing nasal dryness and five healthy controls who underwent turbinate reduction surgery were included in this study. Nasal symptoms were assessed according to visual analogue score. Patients were subjected to nasal endoscopy and computerized tomography of paranasal sinuses when indicated.

Results: Light microscopy showed mild to severe lymphocytic infiltration of nasal submucosa with dilated ducts, sparse seromucinous acini and mild fibrosis. The overlying epithelium showed variable squamocolumnar hyperplasia or atrophy with prominent goblet cell depletion. The basal lamina zone appeared apparently thickened and irregular with hyalnosis. TEM revealed disorganized surface epithelium. The basal lamina was frequently very thin atrophic and breached. Dense collagen bundles occupied the submucosa. Collagen bundles frequently extended through breached basal lamina (BM) to the surface epithelium in a process resembling cirrhosis. Blood vessels showed vasculitis. Sparse seromucinous glands showed minimal mucin and apoptotic myoepithelial and glandular cells.

Conclusion: This study is the first to describe ultrastructural changes of nasal mucosa in SS, especially nasal cirrhosis. Ultrastructural changes were generally indicative of an underlying autoimmune process and may add to better understanding of path physiology of SS. Lastly nasal affection in Sjogren syndrome is underestimated.

Key Words: Sjogren syndrome – Nasal mucosa – Electron microscopy.

Introduction

SJÖGREN'S Syndrome (SS) is an autoimmune disorder characterized by diffuse exocrinopathy that involves salivary and lachrymal glands. The term SS refers usually to keratoconjunctivitis sicca and xerostomia. However the clinical spectrum is broad and it may range from reduction of lacrimation and xerostomia to joint, pulmonary and renal impairment. Primary SS occurs isolated while secondary SS manifests concomitantly with other autoimmune diseases, especially lupus and rheumatoid arthritis. The current used criteria for diagnosis of primary SS is the American-European consensus [1]. The pathophysiology of SS is characterized by lymphocytic infiltrates and destruction of the salivary and lachrymal glands and systemic production of autoantibodies to the ribonucleoprotein particles SS-A/Ro and SS-B/La. The infiltrating cells (T- and B-cells, dendritic cells) interfere with glandular function at several points: Destruction of glandular elements by cell-mediated mechanisms; secretion of cytokines that activate pathways bearing the signature of type 1 and 2 interferons; production of autoantibodies that interfere with muscarinic receptors; and secretion of metalloproteinases that interfere with the interaction of the glandular cell with its extracellular matrix, which is necessary for efficient glandular function. As the process progresses, the mucosal surfaces become sites of chronic inflammation and the start of a vicious circle. Despite extensive study of the underlying cause of SS, the pathogenesis remains obscure [2]. There is sparse and confusing data in literature about incidence and pattern of rhinologic manifestation in Sjogren syndrome (SS). Henkin, et al., were first to emphasize the frequent occurrence of xerorhinia, nasal crusting, and decreased olfactory acuity (hyposmia) and assumed that this probably resulted from decreased nasal mucous
secretion [3]. Rasmussen, et al., found among SS patients 39% complained of dryness of the nose and 44% of nasal crust formation whereas none of healthy controls had such complaints [4]. Freeman, et al., reviewed 196 patients with SS classified into primary and secondary according to the revised international classification. Approximately 50% of patients in each group complained of nasal symptoms, but only 20% had abnormal findings on rhinoscopy [5]. Takeuchi, et al., reported a dissociation of mucociliary flow between sol and gel phase with the latter showing lowered transport rate. In comparison Rasmussen, et al., reported no difference in saccharin test for mucociliary clearance or quantitative olfactorymetry between SS patients and controls. Furthermore mucociliary clearance did not correlate with nasal dryness or encrustations [6]. Powell, et al., used light microscopy for examining nasal mucosa in SS. They reported periglandular infiltration with chronic inflammatory cells and glandular atrophy [7]. Further attempt is needed to elucidate the pattern of rhinologic affection in SS. The authors therefore aimed to study the ultrastructural changes in SS with nasal dryness.

**Material and Methods**


All patients were provided with written consents. Fourteen female patients were included in the study. Age ranged from 34 to 65 years. Ten patients were secondary and 4 were primary SS. Diagnostic Criteria for SS used were that according to American-European consensus (Chart 1). For primary SS, there should be 4 positive criteria out of 6. For secondary SS, the presence of 3 specific criteria is enough. Schirmer test was positive in all patients. Xerophthalmia and xerostomia were present also in all patients. Serum autoantibodies was measured and proved to be elevated in 2 primary SS patients. Xerophthalmia and xerostomia were present also in all patients. Serum autoantibodies was measured and proved to be elevated in 2 primary SS patients. Positive labial biopsy was obtained in two primary patients. The 10 secondary SS patients, 9 had rheumatoid arthritis and 1 had SLE. All patients presented with nasal dryness. Nasal encrustations were found in 2 primary and 9 secondary SS patients. Two patients presented with symptoms of chronic rhinosinusitis and CT scan of paranasal sinuses revealed ethmoiditis and maxillary sinusitis. Eleven patients rated their nasal symptom score as severe and ten patients rae their nasal symptoms as moderate. All patients received saline nasal wash on daily basis for management of nasal dryness especially those with nasal encrustation. Control biopsies were obtained from inferior turbinates of volunteered patients who underwent turbinate reduction surgery for treatment of nasal obstruction. Five controls were all females with age ranged from 35 to 52 years. Exclusion criteria for patients and controls included previous head and neck radiotherapy; Hepatitis C; AIDS; preexisting lymphoma; sarcoidosis; use of anti-cholinergic drugs. A consent was taken from both patients and controls.

**Chart (1):** Criteria for classification of Sjögren’s syndrome.

| I- Ocular symptoms, positive response for at least one of the following questions: |
| Have you felt your eyes dry for the past 3 months? |
| Do you have a recurrent feeling of sand in the eyes? |
| Do you use tear substitutes more than 3 times a day? |

| II- Oral symptoms, positive response to at least one of the following questions: |
| Have you felt your mouth dry for the past 3 months? |
| Have you had recurrent of persistent increase in salivary glands in adult life? |
| Do you normally drink liquids to help you swallow dry foods? |

| III- Ocular impairment signs, positive results in one of the following tests: |
| Schirmer I test (< or = 5mm within 5min): |
| Rose bengal or other dye test (> or = 4). |

| IV- Histopathology: Presence of 1 or more foci (agglomerate of 50 or more inflammatory cells) by 4mm² of gland tissue in minor salivary gland biopsy. |

| V- Salivary gland involvement, positive result for one of the following diagnostic tests: |
| Sialometry with total non-stimulated flow < or = 1.5ml within 15 minutes. |
| Parotid sialography showing diffuse sialectasia, without evidence of major duct obstruction. |
| Salivary scintigraphy with delay in recording, reduction in concentration and/or delay in tracing secretion. |

| VI- Auto-antibodies, presence of one or both: |
| Anti-Ro antibodies (SS-A) or anti-La antibodies (SS-B). |

Source: Vitali et al. (2002).

**Ultrastructural TEM study:**

Inferior turbinate biopsies were put immediately in a test tube containing the fresh cold fixative. The specimens were fixed in modified Karnofsky solution (2.5% gluteraldehyde and 2% paraformaldehyde in 0.1mol/L phosphate buffer solution at
pH 7.4) for 72 hours at 4°C, the specimens were rinsed with distilled water, osmicated, dehydrated in a graded series of acetone and embedded in Epon 812 resin in no. O gelatin capsules. Polymerization of the plastic capsules was done for at least 48 hours in an oven at 50°C. One-micrometer-thick plastic sections were cut with a Reichart Ultramicrotome and stained with 1% toluidine blue solution. Ultra thin sections were cut from the selected regions of the tissue blocks with diamond knife. The selected ultra thin sections were picked up on Formvar-coated copper grids and stained with 2% uranyl acetate followed by lead citrate for 30 minutes each and examined with JEOL (Tokyo, Japan) transmission electron microscope. All specimens were reviewed blindly by two separate pathologists.

Results

Light microscopy:

Hematoxylin and Eosin staining revealed mild to severe plasmalymphocytic infiltration, mild fibrosis and congestion of the submucosa. The epithelium showed either variable squamous hyperplasia or atrophy with prominent goblet cell depletation. The basement membrane zone appeared irregular thickened and hyalinised (Figs. 1-3). Apart from mild lymphocytic infiltration of the submucosa, all these changes were absent in healthy controls.

Transmission electron microscopy:

Disorganized atrophic epithelium with lost cilia with cellular debris and interstitial oedema was present in all specimens with few goblet cells and inflammatory cells (Fig. 4). The epithelial cells show clumped nuclei, compact cytoplasm and distorted cell processes. There was interstitial fluid and cell debris i.e parts of cells. Basal lamina was thinned or lost although foci of increased thickness were sometimes present (Figs. 4,6,7). The subepithelium showed degenerated collagen bundles and elastic fibres. Collagen bundles are laid below BL and were noticed creeping between epithelial cells and goblet cells reaching the surface. Foci of elastic fibres were commonly seen among collagen fibres (Figs. 6,7). The Glands were sparse and atrophic (Fig. 5). Myoepithelial cells were markedly degenerated and even apoptotic. Blood vessels in Lamina propria showed vasculitis with swollen endothelial cells, wide basal lamina and degeneration of smooth muscle cells (Fig. 8). Vasculitis involved both capillaries and arterioles.
Fig. (4): Disorganised epithelium (left): Epithelial cells (E1-E5), goblet cells (G1-G3), inflammatory cells; macrophage (m), fibroblast (f) and lymphocyte (L). In epithelial cells: Clumped nuclei, compact cytoplasm and distorted cell processes. There is interstitial fluid (*) and cell debris i.e parts of cells (arrows). Right: Epithelial cells, oedema, cell debris and balls of degenerated collagen and elastic fibres (thick arrow). BL is lost.

Fig. (5): Glands: Glandular epithelial cell E) showing few cell processes which are thin with few desmosomes. Variable sized mucin secretions are seen. Myoepithelial cells (My1-3) are markedly degenerated and even apoptotic (My 3).
Fig. (6): Epithelial cell (E) and goblet cell (G) and adherent lymphocyte (L). Collagen (Co) is seen between individual epithelial cells and also goblet cells. A nidus of elastic fibres (EL) is seen in centre of collagen fibres in lamina propria. The basal lamina is lost.

Fig. (7): Epithelial cells (E) and goblet cells (G) show loss of tight junctions, vacuolated cytoplasm and loss of cilia. Few segments of the basal lamina (BL) are pointed by arrows. Dense collagen is being laid down below the BL and is seeing creeping to epithelial cells. A mast cell (m) is also seen.
Discussion

In daily practice, the diagnosis of Sjögren’s Syndrome can be made based only on clinical impressions, but to include a patient in scientific studies, it is necessary to have diagnostic confirmation through the criteria obtained from the clinical history and objective complementary exams. The authors therefore examined a cohort of SS patients fulfilling the criteria according to American-European consensus [1]. These patients were complaining of nasal dryness rated moderate to severe according to visual analogue score. Control subjects had similar gender and age.

SS affects mainly salivary and lachrymal glands. The human turbinate glands clearly are different from either major or minor salivary glands. In salivary glands, secretory units consist of secretory endpieces that drain into a system of excurrent ducts that consist of morphologically distinct segments. In contrast, the turbinate glands are tubules that are secretory for most of their length and that gradually change into ducts of simple morphology. Like gastric glands, they lack encapsulation, simply forming an array in the lamina propria. Mucous cells in human turbinate glands cytologically resemble their counterparts in salivary glands although they lack filamentous bodies characteristic of virtually all human mucous cells with the exception of those at the surface of the stomach and of goblet cells in general. Unlike other tubular glands, the nasal one posses also myoepithelial cells like other salivary glands. The ducts of nasal glands do not show basal striations as they are not involved in electrolyte transfer but share in the formation of a glycoconjugate coat [8]. Similarities thus exist between salivary glands and nasal glands, therefore the latter can be involved in SS.

Light microscopy revealed mild to severe lymphocytic infiltration of nasal submucosa. Mild lymphocytic infiltration was also noted in nasal submucosa of controls. In comparison to study of Powell, et al., lymphocytic infiltration was not exclusively localized in areas of seromucinous glands [7]. Furthermore lymphocytic infiltration can also occur in a variety of disorders and therefore diagnosis of SS cannot rely exclusively on this finding. Glandular acini were generally sparse and replaced by dense collagen and lymphocytic infiltrates while ducts were dilated and could be identified in all specimens. TEM demonstrated well apoptosis of glandular and myoepithelial cells. Interstitial edema in our specimen probably reflects destruction of intercellular matrix which is essential of glandular function.

Submucosal fibrosis was more pronounced in our study in comparison to work of Powell, et al. [7]. In light microscopy it led to widening of BL zone. Creeping of collagen bundles through breached BL was noticed even in light microscopy but was more evident in TEM.
In this study the goblet cells were found subjectively decreased in nasal mucosa. This is in agreement of previous studies of conjunctival goblet cells in SS. Goblet cell activity is under neural regulation of parasympathetic and sympathetic nerves adjacent to human and mouse goblet cells; furthermore, muscarinic and adrenergic receptors were found on goblet. Muscarinic receptors are upregulated in SS while serum anti muscarinic autoantibodies is elevated [9].

Nerve fibres were not present in our specimens. We therefore could not search for an associated SS sensory neuropathy. SS neuropathy is claimed also as an etiological factor of sicca symptoms [10]. Ultrastructural changes in this study were coinciding generally with an autoimmune process. Vasculitis was evident in all specimens. This supports that nasal symptoms encountered in these patients are due to SS.

**Conclusion:**

This the first study to describe ultrasructural changes of nasal mucosa in SS. Creeping of collagen bundles through braeched BL to surface epithelium resembles a process of cirrhosis. These ultrastructural changes may lead to better understanding of SS pathophysiology. Lastly nasal affection in SS is underestimated.

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**References**