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Quantitative Assessment of Basement Membrane in Patients with Sjögren's Syndrome and Association with Laminin

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Abstract

There is little known about the role of basement membrane and matrix proteins in the pathogenesis of salivary gland disease associated with Sjögren's Syndrome (SS). Histologically, at the level of the light microscope, lymphocytic infiltration is by CD4 T cells.

Our laboratory has studied the basement membrane in salivary gland disease. Preliminary work indicates abnormalities in the major component of the basement membrane, laminin. This protein appears to be "trapped" intracellularly in the ductal and acinar epithelial cells as demonstrated by immunohistochemistry using specific monoclonal antibodies. It remains unclear whether the laminin dysregulation results from an anabolic or catabolic issue but investigations are ongoing at several levels. Preliminary transmission electron microscopic (TEM) studies on 6 patients have revealed variations in the basal lamina are suggestive of a general "narrowing" of this structure. In contrast, previously published studies report basal lamina "thickening" which is inconsistent with our observations and published data. The objective of the present study, therefore, is to attempt to resolve this discrepancy by carrying out sequential measurements on the basal lamina of ductal and acinar epithelium of diseased salivary glands by TEM from a larger group of patients (12) and compare them with controls. Results showed that there is a loss of the lamina lucida portion of the basal lamina, in spite of overall "thickening" that was characterized as structural disorganization. There was also a marked increase in intracellular acinar cell laminin, shown by immunohistochemistry and TEM, compared with healthy controls. The potential role of laminin dysregulation is discussed.

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Introduction

Salivary gland dysfunction leading to xerostomia may be seen in various autoimmune diseases, notably in both primary and secondary Sjögren's Syndrome (SS) and AIDS, as well as polymyositis/dermatomyositis and chronic thyroiditis [1,2]. The typical histological picture reported in SS is of chronic infiltration by mononuclear cells into salivary acini, ducts and regional perivascular areas. The predominant lymphocytes in each case are of a different phenotype suggesting a different pathological manifestation. In SS, the lymphocytes are predominantly $CD4^+$ T-helper cells while in a process of salivary gland disease, as seen in AIDS, for example, they are $CD8^+$. This infiltration is followed by periductal and perivascular sclerosis and fibrosis, expansion of the lesion and eventual glandular atrophy or acinar "drop-out".

Further studies have shown thickening of the ductal basement membrane, which is composed of laminin, type IV collagen, fibronectin and heparin sulfate proteoglycans [3-5]. Transmission electron microscopy (TEM) in the areas of irregular thickening demonstrate thickening of the lamina densa with high electron density [6]. However, this irregular thickening of the lamina densa is accompanied by the disappearance of the lamina lucida. We have noted in our previous observations that the same phenomenon of thickening of the lamina densa and the disappearance of the lamina lucida occurs in minor salivary glands of patients who were infected with HIV.

In a previous study, preliminary TEM studies of minor salivary glands in 6 patients with primary SS revealed variation in the basement membrane suggestive of a general narrowing of this structure rather than thickening. Using immunohistochemistry and a monoclonal antibody against the B1 chain of laminin, we have shown that this narrowing of the basement membrane is accompanied by a marked increase in laminin expression in minor salivary gland ductal epithelial cells in SS.

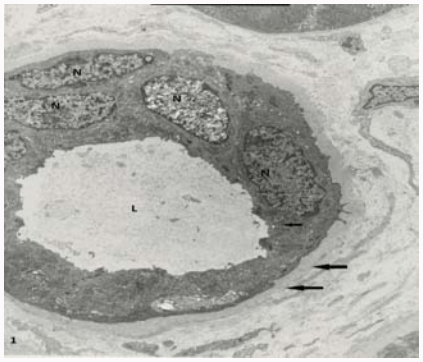

Figure 1: Electron micrograph showing a duct from a normal minor salivary gland. N: Nucleus; L: Lumen; Large arrows: Basal Lamina; Small arrows: Mitochondria.

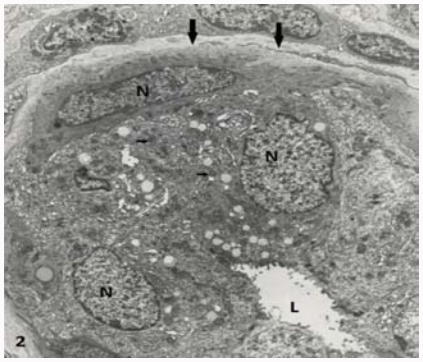

Figure 2: Electron micrograph showing a minor salivary gland from an individual with Sjögren's Syndrome. Note the thinning of the basal lamina compared to that shown in Figure 1. N: Nucleus; L: Lumen; Large arrows: Basal Lamina; Small arrows: Mitochondria.

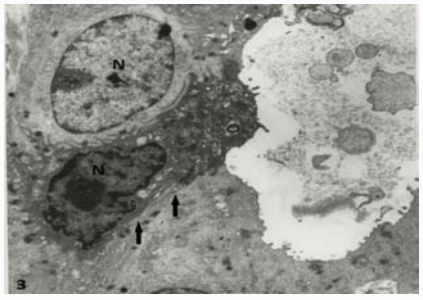

Figure 3: Electron micrograph showing a minor salivary gland from an individual with Sjögren's Syndrome. Note the degeneration of the epithelial cell indicated by large arrows. N: Nucleus; L: Lumen.

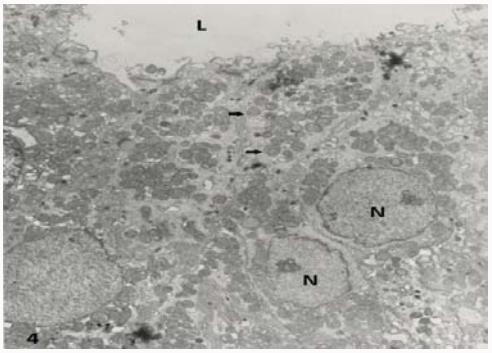

Figure 4: Electron micrograph showing a duct from a normal minor salivary gland. N: Nucleus; L: Lumen; Small arrows: Mitochondria.

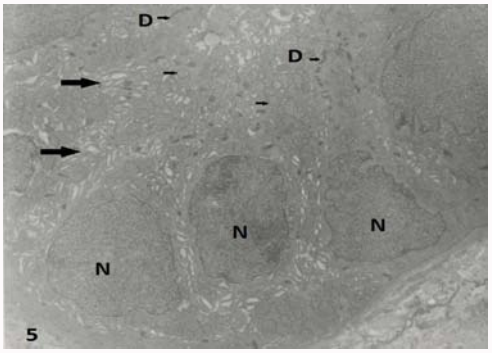

Figure 5: Electron micrograph showing a minor salivary gland duct from an individual with Sjögren's Syndrome. Note the tubal ectasia, indicated by large arrows and also the presence of increased number of desmosomes. N: Nucleus; D: Desmosomes; Small arrows: Mitochondria.

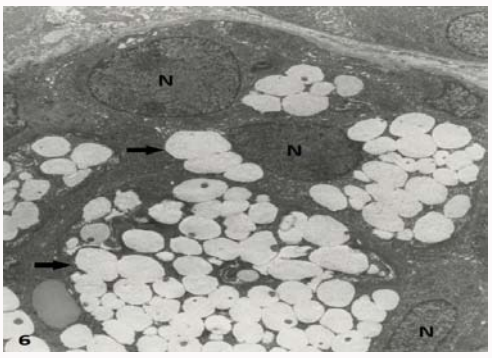

Figure 6: Electron micrograph showing a normal minor salivary gland acinar. N: Nucleus; Large arrows: Secretory granules.

Although laminin is characteristically an extracellular matrix protein, light microscopy demonstrates that there is abnormal intracellular accumulation of laminin in ductal epithelial cells as compared with fibronectin [7].

Laminin is the main component of the basement membrane. It is involved in structural support of the salivary acini and ducts. Laminin is also involved in maintaining cell polarity. It is hypothesized that changes in the basement membrane occur as a result of disorganization brought about by degradation of laminin and type IV collagen. This process may result from an over expression of matrix metalloproteases-9 and 3 (MMP-9 & 3) which, in turn, may result from cytokines released by tissue-infiltrating inflammatory cells [8]. It has also been demonstrated that the fragmented products of laminin that are produced due to degradation by MMPs also promote further MMP expression, possibly in a positive feedback loop [9]. Thus, it is possible that changes within the basement membrane, producing irregular areas of thickening or thinning, may be the result of laminin degradation. There is evidence to support this weak expression of laminin in the basement membrane of patients with Sjögren's Syndrome [10]. Furthermore, the role that MMPs play in the degradation of laminin and other extracellular matrix proteins within the basement membrane along with the subsequent disorganization of the basement membrane that they cause has been demonstrated [9-11]. Yet, since laminin is an inflammatory mediator, as well as being involved in maintaining cellular polarity, it may act as a stimulus for lymphocytic infiltration. The subsequent degradation

Figure 7: Electron micrograph showing minor salivary gland acinar from an individual with Sjögren's Syndrome. Note the formation of mucus island, pushing the nucleus toward the basal pole indicated by large arrows. N: Nucleus; Small arrows: Secretory granules.

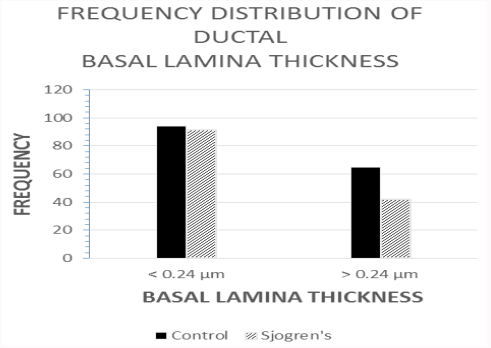

Figure 8: Frequency of ductal basal lamina thickness between diseased and non-diseased control specimens.

of laminin by MMP-9 & 3, following cytokine release, may contribute to epithelial cell death through apoptosis.

Overall, there is little known about the role of the basal lamina and matrix proteins in the pathogenesis of salivary gland disease associated with SS. Furthermore, there has been little explanation regarding the accumulation of laminin intracellularly following disorganization of the basal lamina. The objective of this research was to compare the ductal and acinar basal lamina of minor salivary glands in patients with SS and healthy individuals by TEM to elucidate pathogenesis and to confirm whether changes seen in the basal lamina disorganization in SS, and thereby the basement membrane at the light microscopic level, are due to an overall narrowing of the basal lamina.

Materials and Methods

Institutional Review Board (IRB) approval with signed patient consent was obtained from the UMKC IRB. Minor salivary gland (MSG) biopsies were obtained from 12 patients (11 Female, 1 Male; ages 30-78 years) with primary or secondary SS. The non-diseased control population, also contributing MSG biopsies, consisted of 14 age-matched patients. Criteria for SS were clinical symptoms of dry eyes and dry mouth, arthritis and a history of at least one positive serum SS-A (Ro), SS-B (La), anti-nuclear antibody (ANA) or rheumatoid factor (RF) test. Patients receiving steroid therapy were excluded from the study.

Around 3-5 labial salivary glands were obtained from each patient. A single gland from each patient was bisected and one-half was processed for light microscopy (fixation in 10% buffered

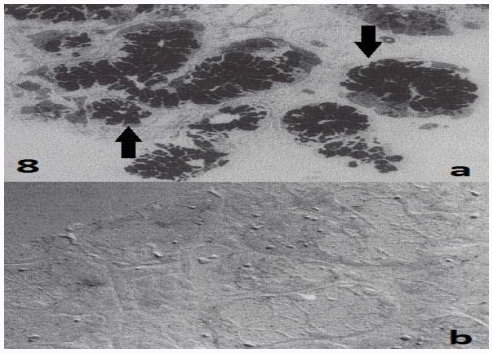

Figure 9: (A) Electron micrograph of minor salivary gland acinar from an individual with Sjögren's Syndrome. As shown with large arrows, there is significant staining of laminin with monoclonal antibody intracellularly. (B) Electron micrograph of minor salivary gland acinar from a healthy control. Note the absence of staining by immunohistochemistry for laminin intracellularly.

formalin solution) and Greenspan scoring. The remaining one-half was fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer at pH 7.0 for examination by TEM. The remaining glands (2 to 4) from each patient were processed and evaluated by light microscopy to provide a biopsy Grade for severity of SS.

Basal laminae adjacent to both ductal and acinar cells were measured at multiple points from photographs over a light-box. With respect to acinar cells, between 35 and 439 (mean 160) measurements were from each patient's biopsy specimen. Similarly, 7 to 111 (mean 31) measurements were made of the basal lamina associated with ductal epithelial cells. Measurements could not be obtained on ductal epithelium from 3 SS patients as there were no ducts present in these specific specimens. Ducts also could not be located in 1 control specimen. Analysis of frequency of distributions of thickness in both acinar and ductal basal lamina in SS and control patients was performed using the students T-test.

Results

Figures 1 to 7 are electron micrographs comparing minor salivary glands from SS and control patients. A comparison of a normal salivary gland (Figure 1) with that from a patient with SS (Figure 2) demonstrates the typical irregularities found within the basal lamina. Table 1 is a list of patients showing the number of measurements of ductal and acinar basal laminae from each patient and the histologic grade of each biopsied gland. The mean thickness of acinar basal laminae in patients with SS compared to control specimens was a mean of 190 nm ± 31.3 nm *vs.* a mean of 178 nm ± 40.3 nm, respectively. The mean thickness of ductal basal laminae for SS patients was 147 nm ± 11.5 nm *vs.* and a mean thickness of 189 nm ± 81.0 nm in non-diseased controls.

The bar graph (Figure 8) demonstrates the frequency of ductal basal lamina thickness between diseased and non-diseased control specimens. Both SS and control patients had the same frequency of thickness when the basal laminae of the two groups were 0.24 µm or less in thickness. However, basal laminae that were greater than 0.24 µm proved to be thicker in healthy patients more frequently than that of SS patients. Tables 2 and 3 provide the frequency of basal lamina thickness between SS and non-diseased patients in more detail. As noted in the bar graph, ductal cell basal laminae in healthy controls were generally thicker on average compared to SS patients, when basal lamina 0.24 µm. In acini, however, the basal laminae were

Table 1: Patients' details.

	Basement Membrane		Group	Lesion Class/Grade
	Mean Acinar (N)	Mean Duct (N)		
E.R.	180 nm (416)	289 nm (111)	SS	IV, IV, IV
Gy	134 nm (50)	59 nm (9)	SS	IV, IV, IV
S.W.	235 nm (64)	183 nm (21)	SS	II
L.C.	180 nm (164)	340 nm (38)	SS	II, III, I
D.C.	189 nm (63)	167 nm (25)	SS	III, III
M.G.	222 nm (114)	ND	SS	I, I
G.W.	193 nm (89)	146 nm (41)	SS	I, II
By	140 m (194)	120 nm (19)	SS	III, IV
A.D.	180 nm (45)	ND	SS	I, IV, III
J.N.	230 nm (242)	250 nm (71)	SS	II, II
A.T.	200 nm (308)	ND	SS	III, III, I
D.J.	200 nm (493)	210 nm (97)	SS	I, I, III
A.S.	220 nm (82)	230 nm (67)	Control	I, I
T.K.	220 nm (35)	295 nm (7)	Control	I, I, I
K.C.	186 nm (261)	375 nm (60)	Control	I, I, I
Z.A.	148 nm (139)	140 nm (11)	Control	I, I, I
C.T.	190 nm (258)	170 nm (12)	Control	I, I, I
Bm	130 nm (48)	125 nm (50)	Control	I, I, I
K.B.	186 nm (160)	281 nm (17)	Control	I, I
M.M.	270 nm (48)	210 nm (8)	Control	I, I, I
A.S.	152 nm (61)	120 nm (9)	Control	I, I, I
J.B.	182 nm (139)	85 nm (21)	Control	I, I
S.R.	127 nm (172)	163 nm (46)	Control	I, I
W.M.	136 nm (228)	182 nm (32)	Control	I, I, I
D.W.	156 nm (38)	150 nm (21)	Control	I, I, I
J.S.	189 nm (170)	124 nm (28)	Control	I, I, I

Table 1 Results

Range	Range
Acinar SS 134 nm to 230 nm (#45-493) Control 130 nm to 270 nm (#35-261) SS Mean- 190 ± 31.3 Control Mean- 178 ± 40.3 P 0.4	Ductal SS 59 nm to 340 nm (#9-111) Control 85 nm to 375 nm (#7-67) SS Mean- 147 ± 115.6 Control Mean- 189 ± 81.0 P 0.28

thicker consistently for SS patient specimens vs. that of control group specimens, regardless of any parameter of thickness. Overall, this data reveals irregularities in basal laminae thickness when comparing acinar and ductal basal laminae in the two patient groups. Despite our observations, based on trends in the measurements, the present study was unable to show a statistically significant difference in the thickness of the basal laminae between SS *vs.* control patient biopsy specimens.

Figures 3 to 7 detail some of the structural changes that characteristically occur in SS patients. Figure 3 shows a degenerating epithelial cell, indicating a pathologic process that may be related to a dysfunctional disorganization within the basal lamina. Figures 4 and 5 show minor salivary gland ductal cells from SS and control patients. As can be seen in Figure 5, there is tubal ectasia and an increase in the number of desmosomes. Figures 6 and 7 allow comparison of acinar cells in SS and normal salivary glands, with emphasis on the fusion of granules forming "mucus islands", which displace the nucleus in the SS specimen. Figure 9A and 9B present the results of the immunohistochemical staining of laminin in minor salivary gland acini using a monoclonal antibody. Figure 9B shows a significant intracellular accumulation of laminin (dark staining) compared to that of the control specimen.

Discussion

Previous unpublished studies in our laboratory revealed a statistically significant narrowing of the basal lamina in acinar and ductal cells of SS patients *vs.* that of non-diseased controls. However, results of the current study do not support previous findings and, interestingly, are in contrast toother reports, which generally describe "thickening" of basal laminae in SS. Although our cases of SS consisted of 1 male (30 years) and 11 females (40-77 years) all with well-defined primary disease, it is pertinent that in none of the previous publications concerning the nature of the basal lamina were actual measurements of the basal lamina carried out as was in the present

Table 2: Frequency distributions of total ductal basal lamina thickness in 14 controls and 6 representative Sjögren's patients.

DUCTAL	0.6 µm	0.06 µm to 0.12µm	0.12 µm to 0.18 µm	0.18 µm to 0.24 µm	0.24 µm to 0.36 µm	0.36 µm to 0.48 µm	>0.48 µm	Total
CONTROL	6	38	15	35	23	20	22	159
SJÖGREN'S	6	25	8	52	25	7	10	133

Table 3: Frequency distributions of total acinar basal lamina thicknesses in 14 control and 6 representative Sjögren's patients.

ACINAR	0.6 µm	0.06 µm to 0.12µm	0.12 µm to 0.18 µm	0.18 µm to 0.24 µm	0.24 µm to 0.36 µm	0.36 µm to 0.48 µm	>0.48 µm	Total	<0.6 µm
CONTROL	27	99	47	113	15	36	28	5	370
SJÖGREN'S	6	76	24	116	13	33	11	5	284

study. Observations in previous reports are of a distinctly qualitative nature with statements describing basal laminae as "irregularly thickened". Such statements are open to a variety of interpretations and unsupported by actual measurements.

The lack of statistically significant narrowing in our present study suggests that it is possible that differences between our data and others are attributable to sampling differences or clinical criteria for diagnosis. The differences in thickness that have been attributed to this study may be coincidental, but we suggest that it reinforces the fact that the basal laminae are irregular in contour. We have concluded that the finding of a "narrow" basal lamina is consistent with the abnormal intracellular accumulation of laminin-1 isoform which we have recently demonstrated in SS [7]. This isoform appears to be "trapped" intracellularly in ductal and acinar cells and may prevent the transportation and incorporation of laminin into the basal lamina, thereby providing an explanation for the narrowing we found in some patients.

It has been suggested by Konttinen et al. [12] and Royce et al. [13], that laminin-1 is responsible for the maintenance of healthy acini and is weakly expressed in acini of patients with SS, resulting in acinar atrophy. The postulation is that the absence of laminin-1 from damaged or disorganized basal laminae in SS affects the normal migration and differentiation of acinar progenitor cells which depend on signaling from laminin for maturation and development. This reinforces our theory that laminin degradation coincides with basal lamina changes, which according to the current study, is a sign of irregularity of the basal lamina. As such, given the results from our previous study, intracellular accumulation of laminin may accompany its degradation in the basal lamina. It remains unclear whether any of these processes are attributable to a catabolic or anabolic process taking place in the ductal or acinar cells.

Acinar atrophy seen histologically in patients with salivary gland disease attributable to SS or other diseases, including AIDS, tend to coincide with scattered ductal cell hyperplasia within lymphoid tissue. Hyperplastic ducts have most often been observed in association with TEM studies that reveal islands of epithelial cell aggregates (epi-myoepithelial islands) extending from the ductal epithelium [6,14]. Other changes seen by TEM include dilation of intercellular spaces in acini and microvascular changes in periacinar microcirculation that occur at the time of pathogenesis of SS in patients presenting with xerostomia [3]. As the study by Perminova [3], it may be the disturbances in the periacinar microcirculation that are important in contributing to the on-set of Sjögren's Syndrome, thus, suggesting a persistent incomplete understanding of the sequence of steps in the pathogenesis of salivary gland disease in SS.

At this point, there may be other variables to consider, but what has been ascertained by our lab is the development of irregularities in the basal laminae of patients with SS due to changes that are concomitant with intracellular laminin accumulation. We have been able to show previously that high levels of laminin mRNA exist in labial salivary glands in Sjögren's Syndrome, and that this intracellular substance is laminin and not some laminin like substance [7]. As discussed earlier in this paper, the mechanism of laminin degradation in the basal lamina may occur as a result of the activation of MMPs by infiltrating lymphocytes into salivary gland tissues. Therefore, it may still be difficult to describe the changes in the basement membrane as just narrowing of the basal lamina, but rather a process of disorganization due to laminin degradation accompanied by intracellular accumulation leading to overall irregularities that are not consistently narrow or thick.

Acknowledgement

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