Meibomian Gland Dysfunction in Primary and Secondary Sjögren Syndrome

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Keywords

Primary Sjögren syndrome · Secondary Sjögren syndrome · Systemic lupus erythematosus · Rheumatoid arthritis · Meibomian gland · Lacrimal gland · Tear film

Abstract

Purpose: We hypothesized that women with primary (pSS) and secondary Sjögren syndrome (sSS; with systemic lupus erythematosus [SLE] or rheumatoid arthritis [RA]) have meibomian gland dysfunction (MGD). We sought to test our hypothesis. **Methods:** Subjects with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD were recruited from the Sjögren's Syndrome Foundation or outpatient clinics at Tufts University School of Dental Medicine or Brigham and Women's Hospital. The control population was recruited from the Greater Boston area. After providing written informed consent, the subjects underwent an eye examination and/or completed two questionnaires that assess symptoms of dry eye disease (DED). **Results:** Our results demonstrate that pSS and sSS patients have MGD. These subjects had meibomian gland orifice metaplasia, an increased number of occluded

meibomian gland orifices, and a reduced quality of meibomian gland secretions. Further, patients with pSS, sSS + SLE, sSS + RA, and MGD had significant alterations in their tear film, lid margin, cornea, and conjunctiva. Symptoms of DED were increased ~10-fold in all pSS, sSS, and MGD groups relative to controls. **Conclusions:** Our findings support our hypothesis and show that individuals with pSS, sSS + SLE, and sSS + RA have MGD. In addition, our study indicates that patients with pSS and sSS have both aqueous-deficient and evaporative DED.

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Introduction

Sjögren syndrome (SS) is a complex and currently incurable autoimmune disorder which occurs almost exclusively in women and is one of the leading causes of aqueous-deficient dry eye disease (DED) in the world [1]. This syndrome may be either primary (pSS; no associated connective tissue disease) or secondary (sSS; e.g., patients with systemic lupus erythematosus [SLE] or rheumatoid

arthritis [RA]) [2, 3]. SS is associated with an extensive accumulation of lymphocytes in lacrimal tissue; the generation of autoantibodies to glandular antigens; marked alterations in the expression of cytokines, chemokines, adhesion molecules, proto-oncogenes, and nitric oxide; an impairment in neurotransmitter release; an immune-mediated destruction and/or dysfunction of acinar and ductal epithelial cells; and a precipitous decline in aqueous tear output [1–3]. The result is DED, which is characterized by a vicious cycle of tear film hyperosmolarity and instability and ocular surface stress, leading to increased friction, inflammation, eye damage, visual impairment, and discomfort [1, 3].

We hypothesized that the impact of SS on the ocular surface is not limited to aqueous-deficient DED, but that the primary and secondary SS forms also are associated with meibomian gland dysfunction (MGD) and evaporative DED. Our rationale was two-fold.

First, we had previously discovered that women with pSS, sSS + SLE, and sSS + RA are androgen deficient [4]. Androgen insufficiency, in turn, is a significant risk factor for the development of MGD [1, 5–7]. Androgens stimulate meibomian gland function, which plays a critical role in the health of the eye [5, 7]. These glands produce and release a proteinaceous lipid mixture (i.e., meibum) that promotes stability and prevents evaporation of the tear film [1, 3, 8]. Conversely, MGD, and the resulting meibum loss, destabilizes the tear film, enhances its evaporation and osmolarity, and is the most common cause of DED [1, 3, 5, 8–12].

Second, lymphocyte accumulation is known to occur in the conjunctiva of SS patients [13–16] and to lead to signs of tarsal and periglandular inflammation [17]. Such conjunctival inflammation may create a toxic environment, leading to the release of proinflammatory cytokines that impact the terminal duct of the meibomian gland and cause other pathophysiological sequelae on the ocular surface. These effects are known to occur in severe atopy [18], Stevens-Johnson syndrome [19–21], and graft-versus-host disease [22, 23], conditions that are sometimes (e.g., graft-versus-host disease) [24–27], but not necessarily, associated with androgen deficiency.

The purpose of this study was to begin to test our hypothesis. We recruited women with pSS, sSS + SLE, and sSS + RA as well as a sex-matched control population. For comparative purposes, we also recruited women who had previously been diagnosed with non-SS-related MGD, and evaluated all subjects for the signs and/or symptoms of MGD and DED. The individuals underwent an evaluation of their meibomian glands as well as an examination

of their ocular surface and/or were asked to complete questionnaires that assessed the frequency and severity of DED symptoms.

Subjects and Methods

Human Subjects

Women with pSS, sSS + SLE, and sSS + RA were recruited from the outpatient clinics at Brigham and Women's Hospital (BWH; Boston, MA, USA) and Tufts University School of Dental Medicine (Boston, MA, USA), as well as from the Sjögren's Syndrome Foundation (Bethesda, MD, USA). All patients with SS met the ≥ 4 criteria required by the European Study Group on Diagnostic Criteria for Sjögren's Syndrome [28], whereas the women with SLE [29] and RA [30] fulfilled the criteria set by the American College of Rheumatology. Women previously diagnosed with MGD, but without SS, were recruited from BWH. Control subjects who had no sign of anterior segment disease, no punctal occlusion, no complaints of dry eye (e.g., foreign body sensation, grittiness, and burning), and no history of pSS or sSS were recruited from the BWH Bulletin, from the cornea practice of the Massachusetts Eye and Ear/BWH specialist (R.D., MD, MSc, MPH), the Harvard Cooperative Program on Aging (Boston, MA, USA), and the Greater Boston metropolitan area. Subjects were excluded if they had any active ocular infection, used topical antiglaucoma or anti-inflammatory medications, or could not understand the study purpose or procedure.

Subjects were recruited for participation in one or more clinical studies detailed in an Institutional Review Board protocol entitled "Role of androgens in dry eye syndromes." These studies included the assessment of anterior segment signs, or ocular symptoms, or both signs and symptoms. The mean age and number of subjects evaluated for ocular signs and/or symptoms are shown in Table 1. These studies were approved by the Human Studies Committees of BWH and the Schepens Eye Research Institute (Boston, MA, USA), and were conducted in accordance with the guidelines established by the Declaration of Helsinki.

Clinical Assessments

After providing informed consent, the subjects were asked to answer questions related to their medical histories and current medications (see online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000487487). If they were being evaluated for symptoms, the subjects were then requested to complete without supervision two questionnaires that assess symptoms of ocular irritation consistent with DED (i.e., the Dry Eye Severity Questionnaire [DESQ] and Ocular Surface Disease Index [OSDI]; Allergan, Inc., Irvine, CA, USA). The DESQ evaluates eight ocular surface symptoms – i.e., dryness, sandy or gritty feeling, burning/stinging, pain, itching, sensitivity to light, blurred vision, and tired eyes – and also asks subjects to grade other unspecified symptoms. The DESQ grades the severity of symptoms experienced over the previous week on a 0–4 scale. A score of 0 reflects the absence of a specific symptom, whereas a score of 4 indicates that the subject always noticed the symptom, and that it was uncomfortable and interfered with his/her activities. The OSDI is a validated set of questions that measures the frequency of experiencing ocular symptoms, environmental triggers, and changes in vision-related quality of life during the prior week.

Table 1. Number and average age of the subjects evaluated for the signs and symptoms of DED

Group	Controls	pSS	sSS + SLE	sSS + RA	sSS combined	MGD
<i>Signs</i>						
Subjects, <i>n</i>	17	11	9	7	16	14
Age, years	46.7±4.2	53.8±5.1	59.1±4.8 ^a	52.4±7.0	56.2±4.0	54.4±3.5
<i>Symptoms</i>						
Subjects, <i>n</i>	18	22	13	13	26	9
Age, years	56.3±4.0	54.3±3.9	55.5±3.3	53.3±4.4	54.4±2.7	52.9±4.2

Age is expressed as the mean ± SD. In these groups, 6 controls, 9 pSS patients, 6 sSS + SLE patients, and 7 sSS + RA patients had both signs and symptoms recorded (see online suppl. Table 1). DED, dry eye disease; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; MGD, meibomian gland dysfunction. ^a Significantly ($p < 0.05$, one-tailed) higher than in the controls.

The subjects being evaluated for MGD and DED signs, as well as the control individuals, then underwent a complete anterior segment examination of both eyes (Table 2) by a corneal and external disease specialist at Massachusetts Eye and Ear/BWH. This examination, which was based upon standard protocols [31–35], included a slit lamp evaluation of

1. the tear film, for its volume and the presence of mucus and debris;
2. the tear meniscus: a normal meniscus appears as a clear and continuous tear film over the ocular surface adjacent to the lower lid margin, and is typically more than 0.3 mm in height [34]; an abnormal meniscus exists when a clearly continuous tear film cannot be identified over the ocular surface; an intermediate meniscus is distinguished as a level between normal and abnormal;
3. the lids, for the identification of erythema, notching, irregular posterior margins, neovascularization, telangiectasia, keratinization, misdirected or lost lashes, scurf, sleeves, collarettes, and the number of expressible meibomian glands;
4. the conjunctiva, for the appearance of erythema, bulbar and tarsal injection, and papillary hypertrophy;
5. the tear film breakup time, which is an indicator of tear film stability [31]; in brief, 5 µL of 2% sodium fluorescein solution was instilled in the inferior cul-de-sac of the subject's eye; examinations were performed with the slit lamp at ×10 magnification, and the tear film breakup time was measured over the cornea using cobalt blue illumination; the subjects were asked to blink normally, and the time for tear breakup was measured up to 10 s;
6. corneal fluorescein staining [31]; and
7. conjunctival rose bengal staining [31]; for calculation of the staining results, the grading scores from both eyes were summed, thereby yielding a total value for each patient. Additional parameters that were assessed included
 1. an examination for meibomian gland orifice metaplasia, a condition defined as an abnormal growth and keratinization of duct epithelium [36];
 2. an analysis of the quality of meibomian gland secretions, according to a specific classification system [35]; the grading scheme was “0” for clear excreta with small particles, “1” for opaque excreta with normal viscosity, “2” for opaque excreta

with increased viscosity, and “3” for excreta that retained their shape after digital expression by applying gentle pressure against the lower eyelid; and

3. an evaluation of the appearance of the anterior chamber and iris [33].

Statistical Analysis

Statistical analyses of the data were conducted by using the unpaired, two-tailed Student *t*, Mann-Whitney U, and χ^2 tests.

Results

Impact of pSS, sSS + SLE, and sSS + RA on the Anterior Segment

To determine whether primary and secondary forms of SS are associated with alterations of the meibomian glands and ocular surface, we examined the anterior segment of the women with pSS ($n = 11$), sSS + SLE ($n = 9$), and sSS + RA ($n = 7$), as well as of the sex-matched individuals with MGD ($n = 14$) and controls ($n = 17$). Our results demonstrate that pSS, sSS + SLE, and sSS + RA are associated with MGD and the signs and symptoms of DED.

An evaluation of the meibomian glands showed that the frequency of orifice metaplasia and the viscosity of the meibomian gland secretions were significantly increased, and that the number of expressible meibomian glands was significantly reduced, in both eyes of women with pSS, sSS + SLE, and sSS + RA relative to controls. The extent of these changes was similar to that in non-SS women with MGD (Table 3).

The ocular surface examinations also revealed that the patients with pSS, sSS + SLE, and sSS + RA had significant alterations in their tear film (Table 4), lid margin (Table

Table 2. Clinical evaluation of the anterior segment

Parameter	Clinical assessment	Grading scheme
<i>Tear film</i>		
Schirmer test without anesthetic	mm wetting in 5 min	
Schirmer test with anesthetic	mm wetting in 5 min	
Mucus	Present or absent	
Debris	Present or absent	
Tear meniscus	Grade 0–2	0 = normal; 1 = intermediate; 2 = abnormal
Tear film breakup time		Seconds
<i>Lid and lid margin</i>		
Erythema	Grade 0–3	0 = none; 1 = mild, with redness of most of the lid margin; 2 = moderate, with redness of the lid margin and skin; 3 = severe, with marked redness of the lid and skin color to palpebral/bulbar conjunctiva
Notching	Present or absent	
Irregular posterior margins	Present or absent	
Misdirected lashes	Grade 0–3	0 = none; 1 = mild; 2 = moderate; 3 = severe
Loss of lashes	Grade 0–2	0 = none; 1 = some; 2 = extensive
Neovascularization	Grade 0–3	0 = none; 1 = micropannus; 2 = pannus <180°; 3 = pannus >180°
Telangiectasia	Present or absent	
Collarettes	Grade 0–2	0 = none; 1 = some; 2 = extensive
Sleeves/scurf	Grade 0–2	0 = none; 1 = some; 2 = extensive
Keratinization	Grade 0–2	0 = none; 1 = some; 2 = extensive
Metaplasia of meibomian gland orifices	Present or absent	
Number of expressible meibomian glands	Out of 10 lower lid glands	
Quality of meibomian gland secretions	Grade 0–3	0 = clear excreta with small particles; 1 = opaque excreta with normal viscosity; 2 = opaque excreta with increased viscosity; 3 = excreta that retained their shape after digital expression
<i>Conjunctiva</i>		
Erythema	Grade 0–3	0 = none; 1 = slight localized; 2 = pink color to conjunctiva; 3 = red color to palpebral/bulbar conjunctiva
Bulbar injection	Present or absent	
Tarsal injection	Present or absent	
Papillary hypertrophy	Present or absent	
Rose bengal staining	Grade 0–3 for 6 areas	
<i>Cornea</i>		
Fluorescein staining	Grade 0–3 for 5 areas	
<i>Other</i>		
Anterior chamber	Normal or abnormal	
Iris	Normal or abnormal	

Ocular surface evaluations of the right and left eyes were conducted following standard procedures, as described in Subjects and Methods.

5), and cornea and conjunctiva (Table 6). As compared to the controls, all SS patients had significantly decreased tear volumes and tear film breakup times, a significantly increased frequency of tear film mucus and debris, and an abnormal tear film meniscus (Table 4). The slit lamp as-

sessments of the lid and lid margin identified a significant increase in telangiectasia, keratinization, irregular posterior margins, erythema, neovascularization, notching, misdirected and lost lashes, and sleeves/scurf in SS patients (Table 5). An analysis of the ocular surface showed

Table 3. Signs of MGD in the women with pSS and sSS

Measure	Controls	pSS ¹	sSS + SLE	sSS + RA	sSS combined	MGD
Patients, <i>n</i>	17	11	9	7	16	14
Meibomian gland orifice metaplasia OD	0%	36.4% ^b	33.3% ^a	28.6% ^a	31.3% ^a	50% ^c
Meibomian gland orifice metaplasia OS	5.9%	36.4% ^a	33.3%	42.9% ^a	37.5% ^a	50% ^b
Meibomian gland metaplasia OD and OS	2.9%	36.4% ^c	33.3% ^b	35.7% ^b	34.4% ^c	50% ^d
Meibomian glands expressed OD, <i>n</i>	7.7±0.7	4.8±1.0 ^e	5.0±1.1 ^e	5.3±1.4	5.1±0.8 ^e	4.3±0.7 ^f
Meibomian glands expressed OS, <i>n</i>	7.7±0.7	4.7±1.0 ^e	5.0±1.0 ^e	5.4±1.5	5.2±0.8 ^e	4.4±0.8 ^f
Meibomian glands expressed OD and OS, <i>n</i>	7.7±0.5	4.8±0.7 ^g	5.0±0.7 ^f	5.4±1.0 ^e	5.2±0.6 ^f	4.4±0.5 ^h
Viscosity of meibomian gland secretions OD	0.4±0.1	1.4±0.3 ^c	1.2±0.2 ^c	1.0±0.3 ^a	1.1±0.2 ^c	1.6±0.2 ^d
Viscosity of meibomian gland secretions OS	0.4±0.1	1.3±0.3 ^b	1.2±0.2 ^c	1.1±0.3 ^a	1.2±0.2 ^c	1.6±0.2 ^d
Viscosity of meibomian gland secretions OD and OS	0.4±0.1	1.3±0.2 ^d	1.2±0.2 ^d	1.1±0.2 ^c	1.2±0.1 ^d	1.6±0.1 ^d

Clinical signs of MGD in the right (OD) and left (OS) eyes of the women with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD, as well as data from the controls. The “sSS combined” group includes the sSS + SLE and sSS + RA groups. Values for the number of expressed glands and their secretion viscosity equal the mean ± SE. Meibomian gland orifice metaplasia was evaluated on a present (i.e., yes) or absent (i.e., no) basis, and is reported in terms of the frequency (%) of “yes” answers. MGD, meibomian gland dysfunction; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis. ^{a-d} Significantly (^a $p < 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$, ^d $p = 0.0001$) greater than in the controls. ^{e-h} Significantly (^e $p < 0.05$, ^f $p \leq 0.01$, ^g $p \leq 0.001$, ^h $p = 0.0001$) less than in the controls. Significantly ($p < 0.05$, one-tailed) different signs, as compared to the controls, are italicized.

¹ The meibomian glands in the left lid of 1 patient with pSS could not be expressed, and no value was recorded.

Table 4. Tear film changes in the women with pSS, sSS, and MGD

Measure	Controls	pSS	sSS + SLE	sSS + RA	sSS combined	MGD
Patients, <i>n</i>	17	11	9	7	16	14
<i>Tear film</i>						
Mucus OD	0%	54.6% ^c	11.1%	42.9% ^b	25% ^a	28.6% ^a
Mucus OS	0%	45.5% ^b	11.1%	42.9% ^b	25% ^a	21.4% ^a
Mucus OD and OS	0%	50% ^d	11.1% ^a	42.9% ^d	25% ^b	25% ^b
Debris OD	11.8%	72.7% ^c	55.6% ^a	71.4% ^b	62.5% ^b	78.6% ^c
Debris OS	17.7%	72.7% ^b	55.6% ^a	85.7% ^b	68.8% ^b	71.4% ^b
Debris OD and OS	14.7%	72.7% ^d	55.6% ^b	78.6% ^d	65.6% ^d	75% ^d
Meniscus OD	0.2±0.1	1.6±0.2 ^d	0.9±0.3 ^a	1.4±0.2 ^d	1.1±0.2 ^d	0.9±0.2 ^b
Meniscus OS	0.2±0.1	1.5±0.2 ^d	1.0±0.2 ^c	1.3±0.2 ^d	1.1±0.2 ^d	1.1±0.2 ^c
Meniscus OD and OS	0.2±0.1	1.5±0.1 ^d	0.9±0.2 ^d	1.4±0.1 ^d	1.1±0.1 ^d	1.0±0.2 ^d
<i>Tear volume</i>						
Schirmer test with anesthesia OD	13.0±1.9	4.2±1.7 ^f	7.4±2.1	5.6±1.2 ^e	6.7±1.3 ^e	8.9±1.9
Schirmer test with anesthesia OS	12.1±1.7	3.4±1.1 ^g	6.9±2.2	4.4±1.2 ^e	5.8±1.4 ^f	10.1±1.3
Schirmer test with anesthesia OD and OS	12.5±1.3	3.8±1.0 ^h	7.2±1.5 ^e	5.0±0.8 ^g	6.2±0.9 ^g	9.5±1.1
Schirmer test without anesthesia OD	25.4±2.7	6.3±1.6 ^h	12.3±3.5 ^f	6.8±3.2 ^g	9.9±2.4 ^g	17.2±3.0
Schirmer test without anesthesia OS	26.1±2.6	6.3±1.8 ^h	11.7±3.5 ^f	4.5±2.2 ^h	8.5±2.3 ^h	15.9±2.3 ^f
Schirmer test without anesthesia OD and OS	25.8±1.9	6.3±1.2 ^h	12.0±2.4 ^h	5.6±1.9 ^h	9.2±1.7 ^h	16.5±1.8 ^g

Tear film changes in the right (OD) and left (OS) eyes of the women with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD, as well as data from the controls. The “sSS combined” group includes the sSS + SLE and sSS + RA groups. Values equal the mean ± SE. Alternatively, clinical parameters that were evaluated on a present (i.e., yes) or absent (i.e., no) basis are reported in terms of the frequency (%) of “yes” answers. MGD, meibomian gland dysfunction; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis. ^{a-d} Significantly (^a $p < 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$, ^d $p = 0.0001$) greater than in the controls. ^{e-h} Significantly (^e $p < 0.05$, ^f $p \leq 0.01$, ^g $p \leq 0.001$, ^h $p = 0.0001$) less than in the controls. Significantly ($p < 0.05$, one-tailed) different signs, as compared to the controls, are italicized.

Table 5. Lid and lid margin alterations in the women with pSS, sSS, and MGD

Measure	Controls	pSS	sSS + SLE	sSS + RA	sSS combined	MGD
Patients, <i>n</i>	17	11	9	7	16	14
Telangiectasia OD	5.9%	54.6% ^b	66.7% ^c	71.4% ^c	68.8% ^c	71.4% ^d
Telangiectasia OS	5.9%	54.6% ^b	66.7% ^c	71.4% ^c	68.8% ^c	71.4% ^d
Telangiectasia OD and OS	5.9%	54.6% ^d	66.7% ^d	71.4% ^d	68.8% ^d	71.4% ^d
Keratinization OD	0.1±0.1	0.3±0.1	0.2±0.2	0.7±0.2 ^c	0.4±0.1 ^a	0.6±0.1 ^c
Keratinization OS	0±0	0.4±0.2 ^b	0.2±0.2 ^a	0.7±0.2 ^d	0.4±0.1 ^b	0.6±0.1 ^d
Keratinization OD and OS	0±0	0.3±0.1 ^b	0.2±0.1 ^a	0.7±0.1 ^d	0.4±0.1 ^d	0.6±0.1 ^d
Irregular posterior margin OD	11.8%	45.5% ^a	44.4%	71.4% ^b	56.3% ^b	85.7% ^d
Irregular posterior margin OS	17.7%	45.5%	33.3%	57.1%	43.8%	85.7% ^c
Irregular posterior margin OD and OS	14.7%	45.5% ^a	38.9% ^a	64.3% ^c	50% ^b	85.7% ^d
Erythema OD	0.3±0.1	1.1±0.3 ^a	1.0±0.2 ^b	1.4±0.4 ^c	1.2±0.2 ^c	1.1±0.3 ^b
Erythema OS	0.2±0.1	1.1±0.3 ^b	1.0±0.2 ^b	1.4±0.4 ^c	1.2±0.2 ^c	1.0±0.2 ^b
Erythema OD and OS	0.3±0.1	1.1±0.2 ^c	1.0±0.2 ^d	1.4±0.3 ^d	1.2±0.2 ^d	1.0±0.2 ^d
Neovascularization OD	0.1±0.1	0.4±0.2	0.4±0.2	0.4±0.2	0.4±0.2	0.4±0.2
Neovascularization OS	0.1±0.1	0.5±0.2	0.3±0.2	0.4±0.2	0.4±0.2	0.4±0.2
Neovascularization OD and OS	0.1±0.1	0.4±0.1 ^a	0.4±0.2	0.4±0.1 ^a	0.4±0.1 ^a	0.4±0.2
Notching OD	0%	18.2%	22.2% ^a	28.6% ^a	25% ^a	21.4% ^a
Notching OS	0%	18.2%	22.2% ^a	28.6% ^a	25% ^a	28.6% ^a
Notching OD and OS	0%	18.2% ^b	22.2% ^b	28.6% ^b	25% ^b	25% ^b
Misdirected lashes OD	0.5±0.1	1.2±0.3 ^a	1.3±0.3 ^b	1.1±0.3 ^a	1.3±0.2 ^b	1.3±0.2 ^b
Misdirected lashes OS	0.5±0.1	1.2±0.3 ^a	1.2±0.2 ^b	1.0±0.2	1.1±0.2 ^b	1.1±0.2 ^a
Misdirected lashes OD and OS	0.5±0.1	1.2±0.2 ^c	1.3±0.2 ^d	1.1±0.2 ^b	1.2±0.1 ^d	1.2±0.2 ^d
Loss of lashes OD	0.4±0.1	0.6±0.2	0.8±0.2	0.7±0.2	0.8±0.1 ^a	0.6±0.1
Loss of lashes OS	0.4±0.1	0.7±0.2	0.8±0.2	0.9±0.1 ^a	0.8±0.1 ^a	0.6±0.2
Loss of lashes OD and OS	0.4±0.1	0.7±0.1 ^a	0.8±0.2 ^a	0.8±0.1 ^b	0.8±0.1 ^b	0.6±0.1
Collarettes OD	0.1±0.1	0.2±0.1	0±0	0.1±0.1	0.1±0.1	0.1±0.1
Collarettes OS	0±0	0.3±0.1 ^a	0±0	0±0	0±0	0.2±0.1 ^a
Collarettes OD and OS	0±0	0.2±0.1 ^a	0±0	0.1±0.1	0±0	0.1±0.1
Sleeves/scurf OD	0±0	0.1±0.1	0.2±0.2 ^a	0.3±0.2 ^a	0.3±0.1 ^a	0.2±0.2
Sleeves/scurf OS	0±0	0.1±0.1	0.2±0.2 ^a	0.1±0.1	0.2±0.1	0.3±0.2
Sleeves/scurf OD and OS	0±0	0.1±0.1	0.2±0.1 ^b	0.2±0.1 ^b	0.2±0.1 ^b	0.3±0.1 ^a

Lid and lid margin alterations in the right (OD) and left (OS) eyes of the women with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD as compared to the controls. The “sSS combined” group includes the sSS + SLE and sSS + RA groups. Values equal the mean ± SE. Alternatively, clinical parameters that were evaluated on a present (i.e., yes) or absent (i.e., no) basis are reported in terms of the frequency (%) of “yes” answers. MGD, meibomian gland dysfunction; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis. ^{a-d} Significantly (^a $p < 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$, ^d $p = 0.0001$) greater than in the controls. Significantly ($p < 0.05$, one-tailed) different signs, as compared to the controls, are italicized.

a significant increase in corneal fluorescein and conjunctival rose bengal staining, as well as in conjunctival tarsal injection, in women with SS (Table 6). Some, but not all, patient groups with pSS or sSS had increased conjunctival erythema, papillary hypertrophy, and bulbar and tarsal injection. In contrast, the subjects with pSS, sSS + SLE, and sSS + RA had no discernable changes in their anterior chamber or iris relative to the controls.

The alterations in the tear film, lid, cornea, and conjunctiva were also found in the women with non-SS-related MGD. However, the extent of the aqueous tear de-

ficiency and ocular surface damage was far greater in the SS, as compared to the MGD, patients. Thus, relative to the patients with non-SS-MGD, the Schirmer test scores with and without anesthesia were 2.5- to 2.6-fold lower in the pSS patients, and 1.5- to 1.8-fold lower in the sSS patients (Table 4). In addition, as compared to the non-SS-MGD patients, the conjunctival rose bengal staining was 1.5- to 2.5-fold higher in the sSS and pSS patients, and the corneal fluorescein staining was 1.9- to 2.5-fold higher in the sSS and pSS patients (Table 6).

Table 6. Conjunctival and corneal appearance in the women with pSS, sSS, and MGD

Measure	Controls	pSS	sSS + SLE	sSS + RA	sSS combined	MGD
Patients, <i>n</i>	17	11	9	7	16	14
<i>Conjunctiva</i>						
Erythema OD	0.6±0.2	1.4±0.3 ^a	1.0±0.2	1.4±0.2 ^b	1.2±0.2 ^a	1.2±0.2 ^a
Erythema OS	0.6±0.2	1.4±0.2 ^b	0.9±0.3	1.6±0.2 ^b	1.2±0.2 ^a	1.3±0.2 ^b
Erythema OD and OS	0.6±0.1	1.4±0.2 ^c	0.9±0.2	1.5±0.1 ^d	1.2±0.1 ^c	1.3±0.1 ^c
Bulbar injection OD	52.9%	72.7%	77.8%	100% ^a	87.5% ^a	85.7%
Bulbar injection OS	52.9%	72.7%	66.7%	100% ^a	81.3%	92.9% ^a
Bulbar injection OD and OS	52.9%	72.7%	72.2%	100% ^b	84.4% ^b	89.3% ^b
Tarsal injection OD	17.7%	72.7% ^b	66.7% ^a	57.1%	62.5% ^b	64.3% ^b
Tarsal injection OS	17.7%	63.6% ^a	33.3%	57.1%	43.8%	64.3% ^b
Tarsal injection OD and OS	17.7%	68.2% ^d	50% ^a	57.1% ^b	53.1% ^b	64.3% ^c
Papillary hypertrophy OD	5.9%	54.6% ^b	11.1%	28.6%	18.8%	50% ^b
Papillary hypertrophy OS	5.9%	45.5% ^a	11.1%	14.3%	12.5%	42.9% ^a
Papillary hypertrophy OD and OS	5.9%	50% ^d	11.1%	21.4%	15.6%	46.4% ^c
Rose bengal staining OD ¹	0.8±0.3	5.3±1.2 ^c	4.0±1.1 ^b	4.3±1.2 ^b	4.1±0.8 ^c	2.1±0.5 ^a
Rose bengal staining OS ¹	0.6±0.3	5.5±1.2 ^d	4.1±0.8 ^d	2.7±0.8 ^b	3.5±0.6 ^d	2.6±0.8 ^a
Rose bengal staining OD and OS ¹	0.7±0.2	5.4±0.9 ^d	4.1±0.6 ^d	3.5±0.7 ^d	3.8±0.5 ^d	2.4±0.5 ^b
<i>Cornea</i>						
Fluorescein staining OD	1.7±0.5	6.6±1.0 ^d	5.5±1.3 ^b	4.4±1.3 ^a	5.0±0.9 ^b	2.5±0.8
Fluorescein staining OS	1.2±0.4	6.6±1.0 ^d	5.3±1.3 ^c	4.3±1.5 ^a	4.9±1.0 ^c	2.6±0.8
Fluorescein staining OD and OS	1.4±0.3	6.6±0.7 ^d	5.4±0.9 ^d	4.4±1.0 ^c	5.0±0.7 ^d	2.6±0.6
Anterior chamber OD	0%	0%	0%	0%	0%	7.1%
Anterior chamber OS	0%	0%	0%	0%	0%	7.1%
Anterior chamber OD and OS	0%	0%	0%	0%	0%	7.1%
Iris OD	0%	0%	0%	0%	0%	0%
Iris OS	0%	0%	0%	0%	0%	0%
Iris OD and OS	0%	0%	0%	0%	0%	0%

Appearance of the conjunctiva, cornea, anterior chamber, and iris in the right (OD) and left (OS) eyes of the women with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD as compared to the controls. The “sSS combined” group includes the sSS + SLE and sSS + RA groups. Values equal the mean ± SE. Alternatively, clinical parameters that were evaluated on a present (i.e., yes) or absent (i.e., no) basis are reported in terms of the frequency (%) of “yes” answers. MGD, meibomian gland dysfunction; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis. ^{a-d}Significantly (^a $p < 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$, ^d $p = 0.0001$) greater than in the controls. Significantly ($p < 0.05$, one-tailed) different signs, as compared to the controls, are italicized. ¹One control patient declined to have rose bengal staining.

Influence of pSS, sSS + SLE, and sSS + RA on Ocular Symptoms

To assess whether patients with pSS and sSS display comparable ocular symptoms that are comparable to those with non-autoimmune MGD, the women with pSS ($n = 22$), sSS + SLE ($n = 13$), sSS + RA ($n = 13$), and MGD ($n = 9$), as well as the sex-matched controls ($n = 18$), completed the DESQ and OSDI questionnaires. Our findings show that pSS, sSS + SLE, and sSS + RA are associated with a significant increase in the severity and frequency of ocular symptoms, and that the extent of this change is analogous to that observed in non-SS MGD (Table 7).

All patients had a significant increase in their DESQ and OSDI scores (Table 7). These included a heightened severity of symptoms related to dryness, sandy or gritty feeling, burning/stinging, pain, itching, sensitivity to light, blurred vision, and tired eyes as compared to the controls. In addition, the patients had a significantly higher frequency of ocular symptoms including photophobia, painful feeling, reading difficulty, and sensitivity to windy and low-humidity conditions (Table 7). The women with pSS and sSS (combined), but not those with non-SS MGD, also had significant problems with night driving and had poor and blurred vision (Table 7).

Table 7. Symptoms of DED in the women with pSS, sSS, and MGD

Measure	Controls	pSS ¹	sSS + SLE	sSS + RA	sSS combined	MGD
Patients, <i>n</i>	18	22	13	13	26	9
DESQ	1.4±0.5	13.5±1.4 ^d	19.0±1.6 ^d	14.8±1.7 ^d	16.9±1.2 ^d	12.6±2.6 ^d
Dryness	0.2±0.1	2.4±0.2 ^d	2.9±0.3 ^d	2.7±0.2 ^d	2.8±0.2 ^d	2.4±0.5 ^d
Sandy/gritty	0±0	1.4±0.2 ^d	2.2±0.3 ^d	1.9±0.3 ^d	2.0±0.2 ^d	1.6±0.5 ^d
Burning/stinging	0.2±0.2	1.6±0.2 ^d	2.6±0.3 ^d	1.9±0.3 ^d	2.2±0.2 ^d	1.7±0.4 ^c
Pain	0±0	1.1±0.3 ^c	1.4±0.3 ^d	0.6±0.2 ^b	1.0±0.2 ^c	0.7±0.4 ^a
Itching	0.2±0.1	1.4±0.2 ^d	2.3±0.3 ^d	1.5±0.4 ^b	1.9±0.2 ^d	1.6±0.4 ^c
Light sensitivity	0.5±0.2	2.0±0.3 ^c	3.2±0.2 ^d	2.5±0.4 ^d	2.8±0.2 ^d	1.9±0.5 ^b
Blurred vision	0.2±0.1	1.4±0.3 ^b	2.0±0.4 ^d	1.5±0.3 ^c	1.7±0.3 ^d	0.7±0.3
Tired eyes	0.1±0.1	2.1±0.2 ^d	2.5±0.2 ^d	2.0±0.3 ^d	2.2±0.2 ^d	2.1±0.5 ^d
<i>OSDI</i>	3.3±1.6	31.0±4.7 ^d	45.2±4.6 ^d	36.5±6.8 ^d	40.9±4.1 ^d	28.2±9.0 ^b
Poor vision	0.4±0.2	1.2±0.3	1.5±0.4 ^a	1.1±0.3	1.3±0.3 ^a	0.1±0.1
Light sensitive	0.4±0.2	1.6±0.3 ^b	3.2±0.3 ^d	2.0±0.4 ^c	2.6±0.3 ^d	1.4±0.5 ^a
Feel gritty	0±0	1.0±0.2 ^c	1.8±0.3 ^d	1.5±0.3 ^d	1.7±0.2 ^d	0.7±0.3 ^b
Feel painful	0±0	1.0±0.3 ^b	1.4±0.4 ^c	0.9±0.4 ^b	1.1±0.3 ^b	1.0±0.5 ^b
Blurred vision	0±0	1.2±0.3 ^b	1.4±0.3 ^c	1.1±0.2 ^c	1.2±0.2 ^d	0.3±0.2
Reading	0.1±0.1	0.8±0.2 ^b	1.4±0.4 ^b	1.0±0.3 ^b	1.2±0.3 ^b	1.2±0.6 ^a
Night driving	0.2±0.2	1.6±0.4 ^b	1.1±0.5	1.5±0.5 ^a	1.3±0.3 ^a	0.6±0.5
Computer/ATM	0±0	0.8±0.3 ^a	0.7±0.3 ^a	0.7±0.4 ^a	0.7±0.2 ^a	1.1±0.5 ^a
TV	0±0	0.5±0.2 ^a	1.0±0.4 ^b	1.0±0.4 ^b	1.0±0.3 ^b	0.6±0.4 ^a
Windy	0.4±0.2	1.9±0.3 ^c	2.8±0.4 ^d	1.7±0.4 ^b	2.3±0.3 ^d	2.2±0.6 ^b
Low humidity	0.1±0.1	2.1±0.4 ^d	3.0±0.4 ^d	2.4±0.4 ^d	2.7±0.3 ^d	2.1±0.6 ^c
Air conditioning	0±0	1.7±0.3 ^d	2.3±0.4 ^d	2.0±0.4 ^d	2.2±0.3 ^d	1.8±0.6 ^c

Symptoms of DED in the women with pSS, sSS + SLE, sSS + RA, and non-SS-related MGD as compared to the controls. The “sSS combined” group includes the sSS + SLE and sSS + RA groups. Values equal the mean ± SE. DED, dry eye disease; MGD, meibomian gland dysfunction; pSS, primary Sjögren syndrome; sSS, secondary Sjögren syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; DESQ, Dry Eye Severity Questionnaire; OSDI, Ocular Surface Disease Index. ^{a-d}Significantly (^a $p < 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$, ^d $p = 0.0001$) greater than in the controls. Significantly increased symptoms, as compared to the controls, are italicized ($p < 0.05$, one-tailed). ¹ One control did not answer the OSDI questions, and a number of subjects (controls: $n = 5$; pSS: $n = 11$; sSS + SLE: $n = 7$; sSS + RA: $n = 7$; MGD: $n = 1$) submitted incomplete OSDI forms. The most common absent answers were those related to driving at night and computer/ATM use, which may have reflected a lack of activity in these areas during the prior week.

Discussion

Our results demonstrate that women with pSS, sSS + SLE, and sSS + RA have a significant increase in signs of MGD as compared to controls. Our pSS and sSS subjects, like the MGD patients, presented with metaplastic and occluded meibomian gland orifices, as well as a significantly decreased quality of meibomian gland secretions. These signs are characteristic of obstructive MGD [36], which is caused primarily by hyperkeratinization of the terminal duct epithelium, reduced meibum quality, and consequent obstruction [1, 5]. In addition, as with the MGD patients, the women with pSS, sSS + SLE, and sSS + RA had significant alterations in their tear film, lid margin, cornea, and conjunctiva, and a marked increase in

DED symptoms [1, 37]. Our findings support our hypothesis that individuals with pSS and sSS have MGD. These women also display the signs and symptoms of both aqueous-deficient and evaporative DED.

Our observation that women with pSS, sSS + SLE, and sSS + RA have MGD and aqueous-deficient and evaporative DED is consistent with the results of other investigators. Researchers have reported that subjects with SS have decreased meibum expressibility and quality [38], as well as increased meibomian gland dropout [39, 40] and dysfunction [38, 40, 41]. Further, SS patients have a reduced volume [1, 42], stability [39], lipid layer [39], and surface activity [43] of tears, heightened tear film evaporation [44, 45], ocular surface damage [1, 42], and increased DED symptoms [1, 41]. In effect, as indicated by Akpek

et al. [46], SS patients have combined aqueous and evaporative DED. Also consistent with our findings is the report that patients with RA have symptomatic MGD [47]. It should be noted, though, that the magnitude of the aqueous tear deficiency and ocular surface damage was far greater in the SS, as compared to the MGD, patients. These differences may well have contributed to the worse symptoms of poor vision, blurred vision, and night driving difficulties in the SS individuals.

We hypothesized that one mechanism contributing to MGD in women with SS is androgen deficiency. We discovered that women with pSS, sSS + SLE, and sSS + RA are androgen deficient [4]. Similarly, others have found that pSS [48], as well as SLE and RA [49–57], are associated with a significant decrease in serum levels of androgen precursors (i.e., DHEA [dehydroepiandrosterone] and DHEA sulfate). These circulating precursors are the primary substrate for androgen synthesis in peripheral tissues, and this intracrine production generates the vast majority of androgens in women [58, 59]. Disruption of this intracrine process has also been found in SS, leading to further androgen insufficiency [60].

Androgen deficiency is a significant risk for the development of MGD and evaporative DED [1, 5, 7, 61–66], and is the key biomarker for DED [67]. We found that androgen deficiency in humans is associated with an increase in lid keratinization and the number of occluded and metaplastic meibomian gland orifices; reduced meibum quality; altered meibum lipid patterns; a decreased tear film breakup time; enhanced corneal fluorescein and conjunctival rose bengal staining; and increased DED symptoms [62–65, 68, 69].

The reason for these sequelae of androgen deficiency is that the meibomian gland is an organ targeted by androgen. We discovered that androgens stimulate the function of this tissue, including increasing its lipogenesis and suppressing its keratinization [62–65, 70–76]. Indeed, dihydrotestosterone regulates the expression of thousands of genes in human meibomian gland epithelial cells, many of which are linked to lipid- and keratin-related activities [70]. This influence of androgen may explain why androgen therapy has been reported to alleviate the signs and symptoms of human MGD and DED [77–85].

It is possible that androgen deficiency may also lead to a decrease in the influence of insulin-like growth factor 1 (IGF-1) on meibomian glands. Androgens have been reported to increase the production of IGF-1 [86, 87], up-regulate IGF-1 receptor expression [88, 89], and modulate IGF-1 signaling [90]. IGF-1, in turn, is known to stimulate the function of human meibomian gland epi-

thelial cells [91, 92]. Consequently, androgen deficiency could result in a loss of IGF-1 activity, which is known to be associated with the development of DED [93]. Of interest, others have reported that IGF-1 receptor levels decrease in the minor salivary glands in SS [94], and that salivary gland IGF-1 may even be a target of autoimmunity in SS [95].

Another possible mechanism contributing to the MGD in SS may be, as speculated by Shimazaki et al. [40], that the meibomian gland is an organ targeted by the immune system in SS. These authors suggested that lymphocytic infiltration adjacent to the tarsal conjunctiva in SS might trigger destruction of the gland [40]. However, unlike the lacrimal gland, there is no peer-reviewed evidence of inflammation within the meibomian gland in obstructive MGD [1, 5, 96–98]. It is possible, though, that conjunctival inflammatory cytokines secreted into the tear film may promote hyperkeratinization of the terminal duct epithelium of the meibomian gland [1, 5, 99, 100].

Yet another process that may be involved in the pathophysiology of MGD in pSS, sSS + SLE, and sSS + RA is a cytokine-induced disruption of neural-meibomian gland epithelial cell interactions [101]. The meibomian gland is the only sebaceous gland in the human body that has elaborate sensory, sympathetic, and parasympathetic innervation [5, 102]. We discovered that parasympathetic neurotransmitters and their agonists exert a significant influence on the activity of human meibomian gland epithelial cells [102]. These cells contain muscarinic acetylcholine and vasoactive intestinal peptide receptors, and ligands for these receptors stimulate the adenylyl cyclase pathway, enhance the intracellular $[Ca^{2+}]$, and may promote cell proliferation [102]. In SS, though, the lymphocyte infiltration in the conjunctiva could possibly cause the following:

1. A decrease in neural innervation; this response, which may occur in areas of extensive inflammation, has been observed in salivary glands of pSS and sSS patients [103, 104]
2. A cytokine-linked suppression of neurotransmitter release from cholinergic and adrenergic nerves, as has been found with IL-1 β during intestinal inflammation [105, 106]
3. Activated lymphocyte secretion of methionine-enkephalin [107], a transmitter known to inhibit the parasympathomimetic stimulation of lacrimal epithelial cell secretion [108]; the overall effect of these changes may be an immune-mediated interference with the neural regulation of meibomian gland epithelial cells, and a consequent alteration and/or decline in meibum output

There are several limitations to our current study. First, we did not measure the serum levels of androgens and their metabolites in the women with pSS, sSS + SLE, and sSS + RA, because we had previously performed, and published the results of, such analyses [4]. Of interest, other researchers found that patients with non-autoimmune MGD are also androgen deficient [109]. Second, several control patients had diabetes, hypertension, depression, and hypercholesterolemia. These conditions are risk factors for the development of MGD and DED [6, 61]. Consequently, we may have underestimated the extent of the differences between the signs and symptoms of the pSS and sSS patients and those of the control population. Third, a few sSS patients reported having both SLE and RA, but these dual conditions were not confirmed. How they might have influenced the data is unclear.

Overall, our research shows that women with pSS, sSS + SLE, and sSS + RA have MGD, as well as aqueous-defi-

cient and evaporative DED. Further research is required to elucidate the underlying mechanism(s) that promote(s) these pathological conditions.

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Disclosure Statement

The authors have no conflict of interests.

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