Functional and Morphologic Changes of Meibomian Glands in an Asymptomatic Adult Population

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Purpose. The aim of the study was to understand natural changes of meibomian glands (MG) that occur with aging in the absence of any ocular pathology or ocular discomfort symptoms, to differentiate between "age normal" and pathologic or dysfunctional changes of the MG.

Methods. A total of 185 subjects (109 females) with no pre-existing ocular and systemic abnormalities were recruited and divided into four age groups: 25 to 34, 35 to 44, 45 to 54, and 55 to 66 years. At a single visit, the following MG measures were collected: meibum quality (MQ) and MG expressibility (MGE) of the lower lid, and MG drop-out score (meiboscale) using infrared meibography of the upper and lower lids. Assessments of anterior eye, tear function variables, noninvasive and invasive tear breakup time (TBUT), corneal integrity, and lid wiper epitheliopathy were also performed during the visit. An Ocular Surface Disease Index (OSDI) questionnaire was used to record dry eye symptoms. Meibum lipids samples were collected and analyzed.

Results. A majority of the study population (61%) was asymptomatic. There was a significant worsening in the MQ ($P < 0.048$), MGE ($P < 0.03$), and meiboscale ($P < 0.01$) with increasing age. Significant increase was observed in anterior blepharitis ($P < 0.001$) and telangiectasia ($P < 0.02$) with aging. Interestingly, tear osmolarity decreased significantly ($P < 0.001$), while tear meniscus height ($P < 0.001$) and invasive TBUT ($P = 0.02$) increased with increase in age. There was no significant association between MG variables and sex, ocular discomfort symptoms, or meibum lipids classes.

Conclusions. Progressive MG loss occurs normally with age accompanied by reduced quality and quantity of the meibum produced. However, clinical presentation of ocular discomfort symptoms is stalled without corresponding disruption to tear function.

Keywords: meibomian gland, aging, gland dropout, meibum quality, gland expressibility

The International Dry Eye Work Shop (DEWS) committee reported that the worldwide prevalence of dry eye lies between 5% and 30% in the population aged 50 years and older.[1] The Dry Eye Work Shop definition of dry eye clearly states that it results in tear film instability with potential damage to the ocular surface.[2] One of the leading causes of dry eye disease is considered to be meibomian gland dysfunction (MGD),[3] which leads to the evaporative type of dry eye. Previous studies suggest that MGD is responsible for approximately 66% to 78% of dry-eyed patients.[4,5]

Meibomian gland dysfunction is defined as a chronic, diffuse abnormality of the meibomian glands (MGs), commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion, which may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease.[6] Several population-based studies have reported an MGD prevalence ranging from 3.5%,[7] 19.9%,[8] 46.2%,[9] 56.3%,[10] 60.8%,[11] 61.9%,[12] to 69.3%.[13] The disparity in MGD prevalence is partly due to the different definitions of MGD used in these various studies, an issue that was addressed by the Tear Film and Ocular Surface (TFOS) workshop of MGD in 2011, which successfully brought the definition and classification of MGD to a consensus. This notwithstanding, the prevalence of MGD has been found to be higher in Asian populations compared to Caucasians and is believed to increase radically after the age of 50.[14]

Aging is considered to have a major role in MGD by affecting the structure and/or functions of the MGs.[14,15] Hykin and Bron[16] reported that, in a cross-sectional study of a healthy cohort aged 5 to 87 years, lid margin vascularity, cutaneous hyperkeratinization, telangiectasia, MG orifice narrowing and pouting, and the viscosity of meibum increased with age. Similarly in a study including 398 healthy participants, Hom et al.[17] found that the percentage of people with cloudy or no meibum increased with age and was noted in approximately 40% of the population. Supporting clinical evidence was reported earlier by Norn,[18] where the number of active or expressible MGs was found to gradually decrease with increasing age. Lid margin and MG abnormalities also were shown to be associated with aging in another cross-sectional study involving a population between 21 and 93 years old, in

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which only a few patients aged < 50 years were observed with these changes.[19] Aging also is associated with changes in the lipid profiles of human meibum and the opacity of these secretions along with the lid margin changes.[20] Obata et al.[21,22] suggested that age-related pathologic alterations in human MGs include primary acinar atrophy leading to decreased meibum secretion. Furthermore, MG dropout has been demonstrated to increase with aging in the general population[23] and in patients with dry eye disease.[24] A detailed study of MG morphology assessment in 37 human eyes (age range, 10–79 years) showed that with an increase in age, there is significant reduction in the MG duct length and percent area of the gland acini along with an increase in gland dropout of the upper and lower eyelids.[25]

Considering aging as a strong risk factor for MGD, it is important to document the natural history of MGs as a means of understanding the process of developing MGD, associated symptoms, and the time course of the disease. Studies reporting age-related changes in MGs have attempted to demonstrate clinical and/or biochemical associations with MGD. However, less is known about the causation or mechanisms that underpin the relation between age and MGD in the absence of any ocular disease. Therefore, the present study investigated MG changes in people without any reported eye disease or prominent dry eye complaints. The aim of the study was to understand preliminary changes in MGs that usually are well tolerated by patients before they begin to precipitate more severe symptoms. We looked into the structural, functional, and physiologic variability in MGs associated with age, sex, and dry eye symptoms. We also examined other relevant eyelid and ocular surface signs that are likely to coexist with changes in MGs.

Methods

Study Design

This was a single-visit, cross-sectional, cohort study.

Participants

The study was conducted in accordance with the Declaration of Helsinki with approval of the Bellberry Human Research Ethics Committee. Written informed consent was obtained from 188 participants aged 25 to 66 years with normal ocular health. The upper age limit was set considering the feasibility of recruiting sufficient numbers of suitable older participants as the research study clinic was based on University campus, with a relatively young population in its catchment area. Participants with any systemic disease that might adversely affect ocular health, for example, diabetes, Graves' disease, and autoimmune diseases, such as ankylosing spondylitis, multiple sclerosis, Sjögren's syndrome, acne rosacea, or systemic lupus erythematosus, were excluded. Please note that although subjects with any pre-existing ocular pathology were excluded, ocular discomfort symptoms were not a part of the exclusion criteria as the objectives of the study were to examine MG health status in the otherwise healthy eyes. The exclusion criteria also included history of hormone replacement therapy, antiandrogen therapy, or corneal refractive surgery.

Clinical Signs and Symptoms Assessment

The following clinical tests were performed in the same order on both eyes (except for Tear Volume which was performed on the left eye only).

Tear Osmolarity. This was measured with a TearLab Osmometer (OcuScience, Inc., San Diego, CA, USA). The participant was seated with the chin tilted upwards and the tip of the TearLab pen was placed in the lower tear meniscus for 2 to 3 seconds. After successful tear collection, the pen was docked into the reader to provide an osmolarity measurement.

Meibography. This is a technique for observing and documenting the morphology of MGs in vivo. For MG imaging, a slit-lamp fitted with an infrared (IR) transmitting filter (950 nm) and an IR charge-coupled device (CCD) camera[23] was used. Custom software was used to capture images of the upper and lower everted eyelids. Meibomian gland dropout was graded using a 0 to 4 scale based on the area of gland loss (0, 0%; 1, <25%; 2, 25%–50%; 3, 51%–75%; and 4, >75%).[26] The score was recorded as "meiboscale" for each eye by summing grades from the upper and lower eyelids, with the score ranging from 0 to 8.

Symptomatology. The Ocular Surface Disease Index (OSDI) questionnaire was administered as a reliable and valid instrument for assessing dry eye symptoms. The index is also considered to assess the disease severity by quantifying the frequency and vision-related impact of symptoms.[27] Scores on the OSDI range from 0 to 100, with higher scores indicating greater discomfort (normal, <12; mild, 12–21; moderate, 22–33; and severe, >33).

Anterior Eye Assessment. Slit-lamp biomicroscopy was performed under diffuse illumination using ×10 to ×16 magnification. The examination included grading of anterior blepharitis, bulbar and limbal redness using 0 to 4 scales,[28] and the presence or absence of telangiectasia (yes/no). The palpebral conjunctiva also was assessed for redness and roughness after everting the upper and lower lids. To reduce the risk of interaction between measures, this assessment was delayed until after Corneal Integrity Assessment and Fluorescein Tear Breakup Time (FTBUT) had been completed.

Tear Film Assessment. The Tearscope (Keeler, Berkshire, UK) was used for these procedures.

Lipid Layer Appearance. The precorneal lipid layer was graded using ×16 magnification with the biomicroscope when the tear film was stationary for 2 to 3 seconds after a blink. The following scale was used for the lipid layer evaluation[29]: (0) absent—no lipid layer is visible, (1) open meshwork—lipid layer is very thin in large patches, (2) tight meshwork—thin lipid patches are very small, (3) flow—near parallel lines of thin and thick lipid, (4) amorphous—even thickness of lipid, and (5) colored fringes—interference colors are observed easily.

Noninvasive Tear Breakup Time (NITBUT). The time in seconds between the full eye opening after a blink and the appearance of a first visible break or the initiation of a reflex blink was recorded. Three consecutive readings were taken on each eye, in a randomized order, and the average of the three readings was recorded as the final NITBUT reading.

Tear Meniscus Height (TMH). This is a guide to the basal tear volume and was measured in the lower lid tear reservoir. The measurement was performed using the Tearscope and the graticule in slit-lamp biomicroscope at ×16 magnification, in increments of 0.1 mm.

Tear Volume. This was assessed using the Phenol red thread (Zone Quick Test; Menicon Ca, Ltd., Nagoya, Japan), which is a 70-mm long cotton thread impregnated with the phenol red dye, which is pH sensitive and changes from yellow to red when wet by tears. The test was performed only on the left eye by pulling down the lower lid slightly and placing the folded 3-mm portion of the thread at a point approximately one-third of the distance from the lateral canthus of the lower lid for 15 seconds. During the test, the participant was instructed to look straight ahead and blink normally. The entire length of the red portion of the thread (i.e., the length of the

Table 1. Clinical Grading Scales for MQ and MGE[70]

Grade	MQ	MGE
0	Clear meibum expressed, fluid-like	All (≥5) glands expressible
1	Cloudy meibum expressed	Mild (3–4 glands expressible)
2	Cloudy with debris (granular)	Moderate (1–2 glands expressible)
3	Thick meibum like toothpaste	Severe (no glands expressible)
4	No meibum expressed	–

thread wetted by tears) was measured in millimeters from the very tip, regardless of the fold.

MG Assessment. Meibomian gland expressibility (MGE) was assessed with the Korb MG evaluator (TearScience, Morrisville, NC, USA) for lower lids only.[30] With the participant seated at the slit-lamp biomicroscope under diffuse illumination and ×16 magnification, the Korb evaluator was placed onto the skin immediately below the lash line of the lower eyelid so that the shaft (white end) of the device was tangential to the eyeball. For the purpose of assessing MGE and meibum quality (MQ), the central 8 MGs (i.e., from the 4th to 11th gland lateral to the inferior punctum) were observed, as recommended by the TFOS MGD workshop.[31] The device was in place for approximately 10 to 15 seconds. Gland expressibility was assessed regardless of the qualitative appearance of meibum. Meibum quality and MGE were graded as shown in Table 1.

Following expression, a meibum sample was collected aseptically from nasal, central, and temporal regions of the lower lid margins of both eyes with a sterile metal spatula (one spatula per eye). Meibum was pooled from both eyes and dissolved in 1 mL chloroform, and then stored in glass vials at –80°C before being extracted for lipid analysis using nano-electrospray ionization tandem mass spectrometry.[32]

Corneal Integrity Assessment and FTBUT. Following the careful addition of sodium fluorescein (1 mg, Fluorets; Chauvin Pharmaceuticals Ltd., UK), the corneal region (i.e., the visible iris with the eye in primary gaze) was observed with cobalt blue illumination using a Wratten 12 filter in front of the slit-lamp biomicroscope under ×10 to ×16 magnification. Corneal staining was graded for the cornea as a whole using a 0 to 4 scale. Staining type was graded in 0.5 steps, while extent and depth of staining were graded in 1 steps.[28]

The fluorescein or invasive TBUT was measured using a stopwatch. The BUT was assessed after two full blinks and stopped after the first tear film break was observed. Three consecutive readings of TBUT were taken on each eye, in a randomized order and the average of the three readings was recorded as the final reading.

Lid Wiper and Bulbar Conjunctival Integrity Assessment. Lissamine green dye (Green Glo; Hub Pharmaceuticals, Rancho Cucamonga, CA, USA) was used for this purpose. Before instillation, a 500 μL solution was prepared by soaking the Lissamine green strip in 500 μL of unit dose sterile saline for 1 minute. A 10 μL volume of the Lissamine green solution was instilled using a pipette into the lower conjunctival cul-de-sac of each eye.[33] Lid wiper epitheliopathy (LWE) of the upper lid was graded based on the following two parameters: horizontal length of the lid wiper, extending from the superior punctum to the lateral canthus, and sagittal height (width) of the lid wiper, extending from just proximal to the line of Marx to the subtarsal fold. The Korb grading scale was used as shown in Table 2. Following LWE assessment, bulbar conjunctival staining was assessed using the Oxford Grading system.[34]

Table 2. Clinical Grading Scale for LWE[71]

Grade	Horizontal Length of Staining, mm	Sagittal Height (Width) of Staining, %
0	<2	<25
1	2–4	25–50
2	5–9	50–75
3	>10	>75

Statistical Analyses

Data are reported as means ± SD. The association of demographic factors, such as age, sex, and contact lens (CL) wear, with clinical variables was analyzed using linear mixed model with subject random intercepts and multinomial logistic regression with robust estimate of variance to account for both eyes of the same participant. Linear mixed model was used for clinical variables on an interval scale while logistic regression was used for categorical variables. Strength of association was described using odds ratio (OR) and its 95% confidence interval (CI). Raw lipid data were normalized and log transformed before further analysis. Post hoc multiple comparisons were adjusted using Bonferroni correction. The level of significance was set at 5%. Analysis was performed using SPSS v21 and STATA 10.

Results

A total of 185 participants (109 females and 76 males) were enrolled in the study, with a mean age of 43.9 ± 11.8 years (range, 25.2–66 years). Three subjects who gave consent were not enrolled in the study as they failed to meet the inclusion criteria. The participants were distributed into four age groups and their distribution as per OSDI symptoms is shown in Table 3. There were 78 CL wearers in total in the study, with the following distribution across age groups: $n = 23$ (25–34), 16 (35–44), 26 (45–54), and 13 (55–66).

Figure 1 shows the distribution of MG variables across the population used in the present study. Approximately one-third of the population did not have any abnormality of MG structure or function (meiboscale 0, 28%; MQ 0, 39%; and MGE 0, 35%), while a small proportion demonstrated severe MG abnormalities (meiboscale 7–8, 2%; MQ 4, 6%; and MGE 3, 6%). Approximately two-thirds of the study population illustrated either flow or amorphous types of lipid layer pattern.

The clinical variables collected in the present study were analyzed to observe associations with the following factors: age, sex, CL wear, symptoms, and lipids classes.

Table 3. Distribution of Participants Across Age Groups and OSDI Symptoms

	N				
Age Group	Normal	Mild	Moderate	Severe	Total
25–34	35	10	4	1	50
35–44	28	10	7	2	47
45–54	25	14	8	2	49
55–66	25	9	2	3	39
Total	113	43	21	8	185

Table 4. Clinical Variables Across Age Groups (Both Eyes Included)

Variables	Age Group	Mean	SD	*P* Value	Post Hoc			
					25–34	35–44	45–54	55–66
Osmolarity	25–34	308.4	14.5	**0.000**		***0.001***	1.000	***0.000***
	35–44	300.1	11.0		***0.001***		0.051	1.000
	45–54	305.9	11.2		1.000	0.051		***0.006***
	55–66	298.1	12.0		***0.000***	1.000	***0.006***	
Anterior blepharitis	25–34	0.5	0.6	**0.000**		1.000	0.719	***0.000***
	35–44	0.7	0.6		1.000		1.000	***0.010***
	45–54	0.8	0.7		0.719	1.000		***0.033***
	55–66	1.2	0.8		***0.000***	***0.010***	***0.033***	
Bulbar redness	25–34	1.8	0.4	**0.000**		1.000	1.000	***0.003***
	35–44	1.7	0.3		1.000		0.155	***0.000***
	45–54	1.9	0.4		1.000	0.155		0.069
	55–66	2.1	0.4		***0.003***	***0.000***	0.069	
Limbal redness	25–34	1.7	0.5	0.482				
	35–44	1.5	0.5					
	45–54	1.6	0.5					
	55–66	1.5	0.4					
NITBUT	25–34	15.1	8.5	0.118				
	35–44	17.4	11.1					
	45–54	15.3	10.7					
	55–66	20.0	14.2					
TMH	25–34	0.2	0.1	**0.000**		0.263	1.000	***0.000***
	35–44	0.2	0.1		0.263		0.304	0.146
	45–54	0.2	0.1		1.000	0.304		***0.000***
	55–66	0.3	0.1		***0.000***	0.146	***0.000***	
Phenol red thread test	25–34	29.8	13.3	0.488				
	35–44	28.7	11.0					
	45–54	26.2	11.4					
	55–66	28.1	11.2					
Corneal staining–extent	25–34	0.2	0.2	0.379				
	35–44	0.1	0.2					
	45–54	0.1	0.2					
	55–66	0.1	0.3					
Corneal staining–depth	25–34	0.1	0.2	0.404				
	35–44	0.1	0.1					
	45–54	0.1	0.1					
	55–66	0.1	0.2					
Corneal staining–type	25–34	0.2	0.3	0.181				
	35–44	0.1	0.2					
	45–54	0.1	0.2					
	55–66	0.1	0.3					
FTBUT	25–34	13.5	9.4	**0.02**		1.000	0.868	0.659
	35–44	14.0	7.9		1.000		0.126	1.000
	45–54	12.0	10.3		0.868	0.126		***0.020***
	55–66	17.2	13.1		0.659	1.000	***0.020***	
Palpebral redness	25–34	1.9	0.6	**0.001**		***0.004***	1.000	0.073
	35–44	1.6	0.5		***0.004***		***0.010***	1.000
	45–54	1.9	0.6		1.000	***0.010***		0.143
	55–66	1.6	0.4		0.073	1.000	0.143	
Palpebral roughness	25–34	1.2	0.8	**0.004**		0.676	1.000	***0.034***
	35–44	0.9	0.7		0.676		0.208	1.000
	45–54	1.3	0.8		1.000	0.208		***0.007***
	55–66	0.7	0.7		***0.034***	1.000	***0.007***	
Lid wiper length	25–34	1.4	1.1	0.358				
	35–44	1.4	1.1					
	45–54	1.6	1.1					
	55–66	1.2	1.0					
Lid wiper height	25–34	1.1	0.9	0.244				
	35–44	1.1	1.0					
	45–54	1.2	1.0					
	55–66	0.9	0.8					

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Table 4. Continued

Variables	Age Group	Mean	SD	*P* Value	Post Hoc 25–34	Post Hoc 35–44	Post Hoc 45–54	Post Hoc 55–66
Conjunctival staining	25–34	0.8	0.8	**0.000**		1.000	***0.000***	***0.046***
	35–44	0.8	0.8		1.000		***0.000***	***0.046***
	45–54	1.4	0.8		***0.000***	***0.000***		1.000
	55–66	1.2	0.9		***0.046***	***0.046***	1.000	

Bold numbers indicate significant *P* values. Italic numbers indicate significant *P* values in post hoc tests.

Associations Between Age and Clinical Signs

Table 4 shows summary statistics for all the clinical variables assessed. There was a statistically significant association between age and osmolarity, anterior blepharitis, bulbar redness, TMH, FTBUT, palpebral redness and roughness, and conjunctival staining ($P \leq 0.02$). A significant negative association was observed in osmolarity with increasing age. Post hoc analysis revealed that the statistical significance was driven by the youngest and oldest age groups in the study (mean osmolarity, 308.4 and 298.1; SD, 14.5 and 12, respectively). The TMH and FTBUT increased with age, with the TMH being greater in the oldest age group by 0.1 mm relative to the other groups. Fluorescein TBUT was significantly higher in the oldest age group by approximately 5 seconds compared to the 45 to 54 age group. Conjunctival staining also was more distinct in the older age groups compared to the younger age groups by approximately 0.5 grade. There was no significant effect of age on NITBUT, tear volume (measured with the Phenol Red thread test), and LWE ($P \geq 0.12$). No other ocular surface or lid variable showed a significant association with age, except for anterior blepharitis ($P < 0.001$) and telangiectasia ($P \leq 0.02$), with the odds at each age group being significantly higher than the reference age group of 25 to 34 (Table 5). Telangiectasia was present in 270 of 370 eyes examined in the study.

Table 6 shows the results of the meiboscale analysis, which was conducted by grouping dropout scores as 0 (no gland dropout), 1 and 2, 3 and 4, and 5 to 8. The amount of MG dropout increased significantly and approximately linearly with age (Fig. 2). Figure 3 shows the distribution of MQ scores and Table 7 the OR for MQ across age groups, with clear MQ (grade 0) and age group 25 to 34 as the reference. The oldest participants (55–66) had significantly more risk of reduced MQ than the youngest group. While the same generally was true for the next oldest group (45–54), the finding was less consistent. Figure 4 shows the distribution of MGE and Table 8 shows the OR for MGE across age groups with grade "none" (all glands expressible) as the reference. The risk of having moderate and severe grades of MGE was significantly higher in the two age groups beyond 44 years ($P \leq 0.025$); however, a mild grade of MGE was significantly higher only in the oldest age group ($P = 0.029$).

Lipid layer appearance did not show a significant overall change across age groups (Table 9) and there was no specific pattern of distribution in the five grades of lipid layer within any age group (Fig. 5). Nevertheless, the ORs for an amorphous pattern relative to open or tight meshwork (which served as a reference pattern) were significantly lower in age groups 45 to 54 ($P = 0.01$) and 55 to 66 ($P = 0.001$), while the OR for colored fringes was significantly lower in age group 35 to 44 ($P = 0.02$) compared to age group 25 to 34.

Associations Between Sex and Clinical Signs

Males showed significantly higher NITBUT, FTBUT, and LWE than females, with the mean differences being 5.2 seconds, 4.4

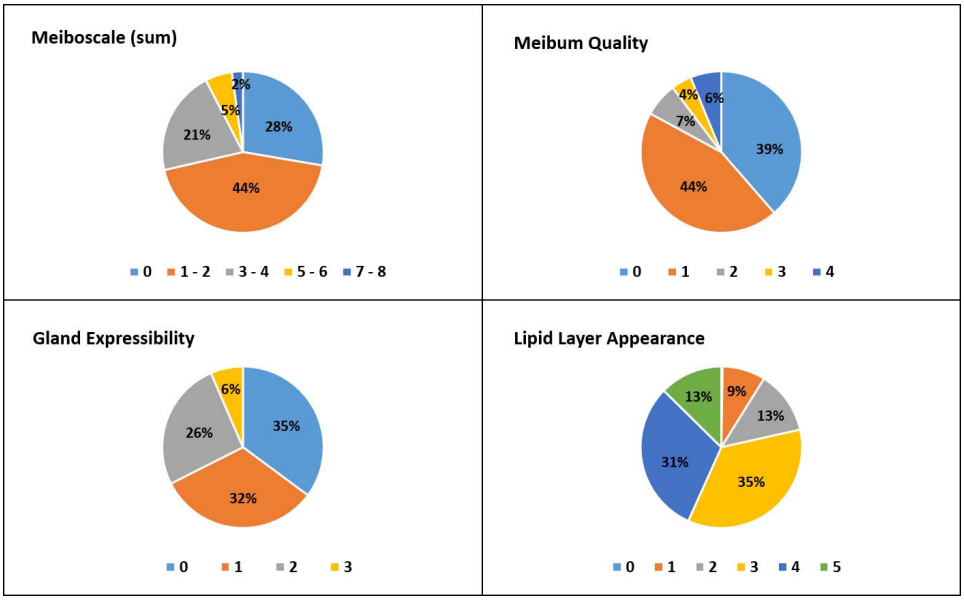

Figure 1. Distribution of MG variables in the population.

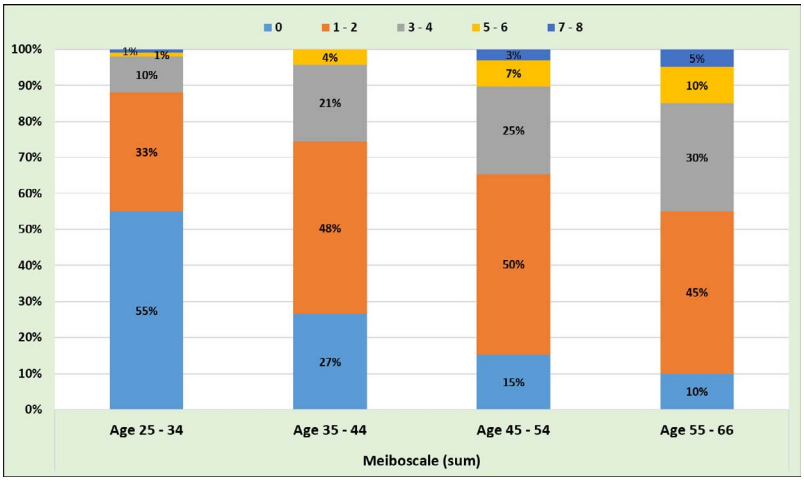

Figure 2. Distribution of frequency of meiboscale (sum) across age groups.

seconds, and grade 0.5, respectively ($P < 0.01$). Otherwise, there was no effect of sex on any other clinical signs.

Associations Between CL Wear and Clinical Signs

There was no association between CL wear and MG variables in the present study; however, an effect of CL wear was observed on some tear variables. The CL wear group demonstrated significantly lower TMH and FTBUT (difference of the means, 0.03 mm and 2.2 seconds, respectively) but higher osmolarity (difference of the means, 4.7) compared to non-CL wearers ($P \leq 0.04$).

Additional analysis was performed to determine any interaction between the significance of CL wear and age related changes in clinical variables. The results showed that there was no significant interaction of CL wear with MGE ($P = 0.39$), meiboscale ($P = 0.6$), TMH ($P = 0.69$), and FTBUT ($P = 0.23$). However, age-related changes in MQ interacted significantly with CL wear ($P = 0.002$).

To further understand the effect of CL wear and due to small sample sizes, the age groups were collapsed into two groups, namely 25 to 44 and 45 to 66 years. The distribution of MQ was not significantly different between CL and non-CL wearers within the age groups 25 to 44 ($P = 0.19$) and 45 to 66 ($P = 0.32$). Further multiple logistic regression analysis indicated that CL and non-CL wearers showed age-related changes, but the changes were observed to be more pronounced in the non-CL wearers (Table 10). For example, the odds of more cloudy meibum in the higher age group were 1.7 ($P = 0.29$) among CL wearers, but 2.7 ($P = 0.02$) in the non-CL wearers. Similarly the higher age group was 15.9 times more likely ($P = 0.01$) to demonstrate no meibum in CL wearers but this was 28.4 times more likely ($P = 0.003$) in the non-CL wearers.

Table 5. OR for Telangiectasia Across Age Groups

Telangiectasia	Age Group	OR	*P* Value	95% CI Lower	95% CI Upper
Yes vs. No, frequency %	25–34 (reference)	1.00			
	35–44	2.69	***0.019***	1.18	6.14
	45–54	3.84	***0.002***	1.64	8.96
	55–66	7.28	***0.000***	2.57	20.61

The bold and Italic numbers indicate *P* values that are statistically significant.

Associations Between Symptoms and Clinical Signs

There was no association between the OSDI symptoms and any clinical variable except meiboscale ($P = 0.03$), when the analysis included the best of two eyes (Table 11).

Associations Between Meibum Lipids Classes and Clinical Signs

Mass spectrometry analysis revealed the following major lipids classes were present in meibum samples taken from participants: cholesterol esters (CE) with free cholesterol (FC)—60.7%, wax esters (WE)—33.7%, (O-acyl)-omega-hydroxy fatty acids (OAHFA)—3.3%, tri-acyl glycerides (TAG)—2.2%, and ceramides—0.1%. Statistical analysis was applied to determine associations among the lipids classes, age, and clinical signs. Data from five participants were excluded due to excess ceramide content (average 2.5%), which indicates contamination from skin epithelial cells.[35] There was no significant effect

Table 6. OR for Meiboscale (Sum) Across Age Groups

Meiboscale, Sum	Age Group	OR	*P* Value	95% CI Lower	95% CI Upper
1–2 vs. 0	25–34 (reference)	1.00			
	35–44	3.00	***0.009***	1.32	6.81
	45–54	5.44	***0.000***	2.21	13.42
	55–66	7.50	***0.000***	2.63	21.39
3–4 vs. 0	25–34 (reference)	1.00			
	35–44	4.40	***0.009***	1.45	13.35
	45–54	8.80	***0.000***	2.73	28.36
	55–66	16.50	***0.000***	4.52	60.26
5–8 vs. 0	25–34 (reference)	1.00			
	35–44	4.40	0.135	0.63	30.72
	45–54	18.33	***0.001***	3.14	106.93
	55–66	41.25	***0.000***	6.47	262.91

The bold and Italic numbers indicate *P* values that are statistically significant.

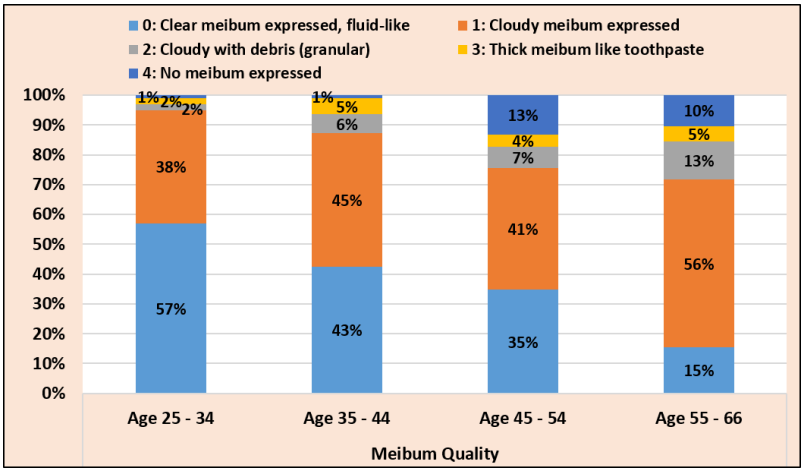

Figure 3. Distribution of frequency of MQ grading across age groups.

of age or sex on any lipids class. There was a statistically significant increase in CE lipids ($P = 0.03$) and a decrease in WE lipids ($P = 0.01$) as the MGE worsened. Other than this, there was no statistically significant association between any clinical variables or dry eye symptoms and meibum lipids classes in the present study.

Discussion

The present study aimed to characterize MG changes (structural, functional, and physiologic) that occur naturally with aging in the absence of any ocular pathology in an adult population without clinically presenting symptoms of ocular discomfort. The rationale for this approach is to provide age-appropriate reference points against which clinicians can judge the appearance and function of MG case presentations. The intention is to describe the "age normal" appearance and functional behavior of the MG so that potentially pathologic changes can be clearly identified.

Structurally, it was found that MG dropout increased approximately linearly with age, while the dropout was apparent from as young as 25 years of age. This finding is in agreement with other studies in which profound MG dropout was observed in older individuals[19] and was noted in younger population as well, but to a lower extent.[23,24] However, the present study could not confirm the suggestion by Arita et al.[36] that gland dropout occurs more commonly in upper, rather than lower eyelids, or is exacerbated by CL wear. Our study also showed reduction in MG function with increased age, consistent with many previous studies.[12,16,19,25] Some studies claim that structural and functional changes in the MGs are more severe in elderly men than in women aged >70 years,[19,23] which could not be assessed in the present study as the age limit was up to 66 years.

There was no sex-related effect on either MG structure or function in the present study. Previous work suggests that, while the prevalence of dry eye has been reportedly higher in women,[37,38] postmenopausal women were the major contributors to the population.[8,11,39] The decrease in the level of androgens in postmenopausal women is considered as a risk

Table 7. OR for MQ Grading Across Age Groups

MQ	Age Group	OR	*P* Value	95% CI Lower	95% CI Upper
Cloudy meibum, grade 1, vs. clear meibum, grade 0	25–34 (reference)	1.00			
	35–44	1.58	0.255	0.72	3.44
	45–54	1.76	0.171	0.78	3.98
	55–66	5.50	***0.001***	1.94	15.58
Cloudy with debris, grade 2, vs. clear meibum, grade 0	25–34 (reference)	1.00			
	35–44	4.28	0.102	0.75	24.37
	45–54	5.87	***0.048***	1.02	33.89
	55–66	23.75	***0.001***	3.92	144.08
Thick meibum, grade 3, vs. clear meibum, grade 0	25–34 (reference)	1.00			
	35–44	3.56	0.191	0.53	23.94
	45–54	3.35	0.188	0.55	20.30
	55–66	9.50	***0.021***	1.40	64.44
No meibum expressed, grade 4, vs. clear meibum, grade 0	25–34 (reference)	1.00			
	35–44	1.43	0.803	0.09	22.87
	45–54	21.79	***0.005***	2.59	183.30
	55–66	38.00	***0.002***	3.87	373.24

The bold and Italic numbers indicate *P* values that are statistically significant.

Table 8. OR for MGE Grading Across Age Groups

MGE	Age Group	OR	*P* Value	95% CI Lower	95% CI Upper
Mild vs. none	25–34 (reference)	1.00			
	35–44	1.43	0.366	0.66	3.13
	45–54	1.67	0.198	0.76	3.65
	55–66	2.82	***0.029***	1.11	7.16
Moderate vs. none	25–34 (reference)	1.00			
	35–44	2.04	0.143	0.79	5.31
	45–54	2.94	***0.025***	1.14	7.57
	55–66	4.78	***0.003***	1.73	13.21
Severe vs. none	25–34 (reference)	1.00			
	35–44	0.69	0.773	0.06	8.24
	45–54	11.61	***0.004***	2.21	60.86
	55–66	12.50	***0.005***	2.13	73.25

The bold and Italic numbers indicate *P* values that are statistically significant.

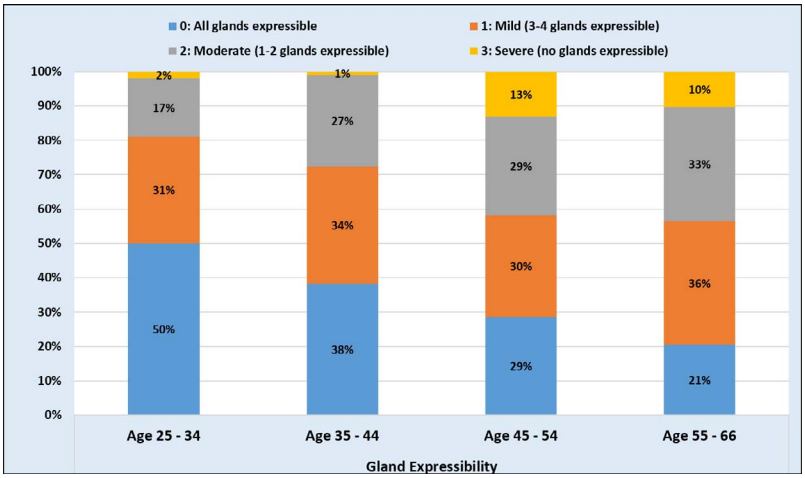

FIGURE 4. Distribution of frequency of MGE grading across age groups.

factor for MGD.[40,41] In the present study, sex did not appear to have any role in MG changes, perhaps because women who had undergone hormone replacement therapy were excluded from the study, which previously has been shown to have a strong correlation with higher prevalence of dry eye disease.[42–45] The same reasoning was explained by Den et al.[19] for the absence of association between sex and MG changes. Another possibility could be that men and women in the older age groups were becoming androgen-deficient[46] in the present study, which may have resulted in similar alterations in MGs among both sexes. The only variable that was possibly influenced by sex in the present study was the TBUT, which was significantly higher in men but with no corresponding changes in any other tear-related variables, such as osmolarity or TMH.

Contact lens wear did not show any association with the MG dropout and MGE in the present study, while an effect on MQ was observed but at a clinically nonsignificant level. To ensure that the lack of CL effect was not due to insufficient power, as the study had relatively small number of CL wearers, a supplementary analysis was conducted that excluded all CL wearers and the results did not indicate that any substantial change in interpretation is required. The present study was not designed to address specific types of contact lens wear and the impact on the MGs, but to globally assess the MGs in a clinic sample across age. There have been conflicting reports in the past regarding the link between CL wear and MGD with some studies showing no effect of CL wear either on MG function[17,47] or MG dropout,[48] while other studies demonstrate poor MG expressibility[49] and increased MG dropout due to CL wear.[36,50] Alghamdi et al.[50] suggested that, while CLs do alter MG morphology and function, they only affect MGs during the early stages (first 2 years) of CL wear. Thus, if there are significant numbers of CL wearers in the population, it will tend to skew the effects toward a younger age group in general. So, our results (including CL wearers) will tend to show MG changes slightly earlier than they would occur in a population of nonwearers. While knowledge in this area is emerging, future large-scale studies are needed to further understand the link between CL wear and MGD.

Tear function also was measured in this study as an indicator of the impact that MG changes can have. Although changes were small in general, the overall impression was that tear production tended to increase for the oldest participants, as shown by the greater TMHs and TBUTs together with reduced osmolarity. Therefore, there was an inverse relationship between the tear functions and MG changes with aging in the present study, which is in accordance with previous work.[19,23,51] Arita et al.[51] illustrated that tear secretion in patients with MGD increased remarkably despite the MG loss and concluded that such a compensatory mechanism may be in place to stabilize the tear film and maintain ocular surface homeostasis. On the other hand, Feng et al.[24] suggested that TBUT may not be a good indicator of MG dropout as they did not find any correlation between the two variables in dry eye patients. The current study shows complementary changes in tear functions and MGs associated with age, which may account for the majority of the population being asymptomatic.

As a measure of the physiologic changes in MGs, meibum was collected and analyzed in the present study. There was no change in the lipid profiles due to aging, sex, or CL wear. The major component of the meibum across all age groups consisted of cholesterol and wax esters, consistent with previous findings for normal human meibomian secretions.[52–55] Accordingly, the lipid layer appearance was unaffected in the present study by any of the assessed risk factors with the majority of the population illustrating flow (35%) or amorphous (31%) lipid layer patterns. Meibomian gland dysfunction and chronic blepharitis have been associated

TABLE 9. OR for Lipid Layer Appearance Grading Across Age Groups

Lipid Layer Appearance	Age Group	OR	*P* Value	95% CI	
				Lower	Upper
Flow vs. open meshwork/tight meshwork	25–34 (reference)	1.00			
	35–44	1.13	0.827	0.38	3.33
	45–54	0.56	0.293	0.19	1.64
	55–66	0.56	0.302	0.19	1.68
Amorphous vs. open meshwork/tight meshwork	25–34 (reference)	1.00			
	35–44	0.40	0.118	0.13	1.26
	45–54	0.23	***0.010***	0.08	0.70
	55–66	0.13	***0.001***	0.04	0.42
Colored fringes vs. open meshwork/ tight meshwork	25–34 (reference)	1.00			
	35–44	0.14	***0.020***	0.03	0.73
	45–54	0.41	0.191	0.11	1.55
	55–66	0.63	0.483	0.18	2.27

The bold and Italic numbers indicate *P* values that are statistically significant.

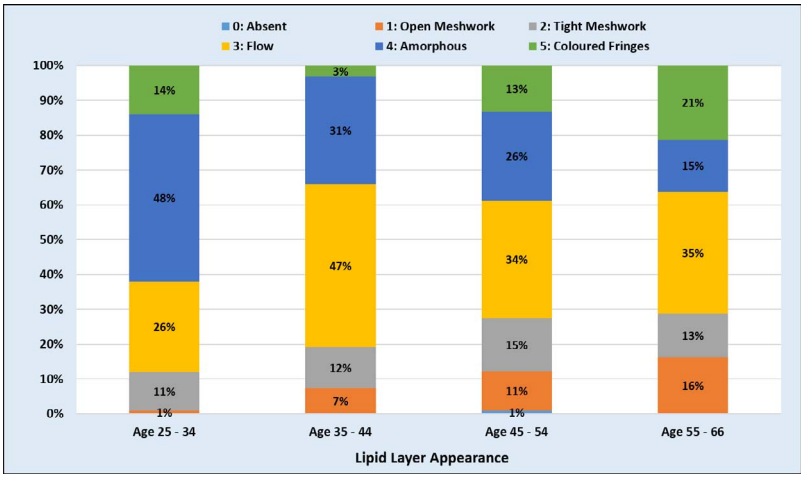

Figure 5. Distribution of frequency of lipid layer appearance grading across age groups.

with changes to the meibum lipid composition, with reduced levels of wax and cholesterol esters, and unsaturated fatty acids and triglyceride profiles.[56,57] A significant increase in the total free fatty acids levels has been reported in patients with blepharitis and MGD compared to normal human meibum.[58,59] Sullivan et al.[20] have shown significant age-related and sex-related differences between the ions identified in the neutral and polar lipid patterns of the meibomian secretions detected using mass spectrometry, along with an increase in meibum opacity with aging.[20] Given that barely 10% of the population illustrated severely worsened MQ and MGE in the present study, there were very few cases of MGD and, therefore, the lipid profiles were likely to be similar across the study cohort regardless of the age group or sex. Smaller age range (25–66 years) in the present study as opposed to a broader age range (27–83 years) in the study by Sullivan et al.[20] also could be a reason for the contrasting findings.

Eyelid appearance has had an important role in MGD diagnosis in many previous epidemiologic studies. In the present study, the risk of having telangiectasia increased with age in agreement with previous findings.[16,19,23] Telangiectasia was considered as one of the MGD diagnostic factors in some studies reporting a diverse range in MGD prevalence: 46.2%,[9] 60.8%,[11] and interestingly 68% (asymptomatic) and 69.3% (symptomatic) in dry eye patients in the Beijing Eye Study.[13] Although telangiectasia is an important clinical sign in diagnosing MGD, it should be combined with the anatomic and functional changes of the MGs as recommended by the TFOS Committee for MGD Workshop.[31] Viso et al.[60] were perhaps the first to apply the TFOS recommended MGD diagnostic criteria to a general population and reported that asymptomatic MGD is more than twice as common as symptomatic MGD, with the prevalence being 21.9% and 8.6%, respectively.

A large proportion of the current study population was asymptomatic (61%) as per the OSDI questionnaire. The questionnaire measures the severity of discomfort mainly arising from ocular surface dryness, which is presented clinically as disruption to tear function and/or tear film instability. The fact that tear function as well as lipid layer appearance were normal in the majority of the study population, underpin the absence of symptoms in the present

Table 10. OR for MQ Across CL and Non-CL Wearer Groups

Group	MQ	Age Group	OR	*P* Value	95% CI	
					Lower	Upper
CL group	Cloudy meibum vs. clear meibum	25–44	1.00			
		45–66	1.67	0.294	0.64	4.39
	Cloudy with debris vs. clear meibum	25–44	1.00			
		45–66	–	–	–	–
	Thick meibum vs. clear meibum	25–44	1.00			
		45–66	0.40	0.458	0.03	4.53
	No meibum expressed vs. clear meibum	25–44	1.00			
		45–66	15.91	***0.013***	1.79	141.64
Non-CL group	Cloudy meibum vs. clear meibum	25–44	1.00			
		45–66	2.71	***0.020***	1.17	6.26
	Cloudy with debris vs. clear meibum	25–44	1.00			
		45–66	3.88	***0.033***	1.11	13.48
	Thick meibum vs. clear meibum	25–44	1.00			
		45–66	6.03	***0.047***	1.03	35.39
	No meibum expressed vs. clear meibum	25–44	1.00			
		45–66	28.42	***0.003***	3.21	251.64

The bold and Italic numbers indicate *P* values that are statistically significant.

Table 11. Sample Distribution as Per MG Variables Across OSDI Scoring Categorization

	OSDI Score				
MG Variables	None	Mild	Moderate	Severe	*P* Value
MQ–0	70.9%	15.2%	12.7%	1.3%	0.06
MQ–1	56.8%	26.1%	11.4%	5.7%	
MQ–2	37.5%	37.5%	0.0%	25.0%	
MQ–3	50.0%	25.0%	25.0%	0.0%	
MQ–4	50.0%	50.0%	0.0%	0.0%	
MGE–0	64.3%	17.9%	14.3%	3.6%	0.57
MGE–1	64.5%	21.0%	9.7%	4.8%	
MGE–2	51.5%	33.3%	9.1%	6.1%	
MGE–3	50.0%	50.0%	0.0%	0.0%	
Meiboscale–0	63.5%	23.8%	9.5%	3.2%	***0.03***
Meiboscale–2	61.7%	16.0%	17.3%	4.9%	
Meiboscale 3–4	61.3%	35.5%	0.0%	3.2%	
Meiboscale 5–6	62.5%	25.0%	12.5%	0.0%	
Meiboscale 7–8	0.0%	50.0%	0.0%	50.0%	

The bold and Italic numbers indicate *P* values that are statistically significant.

study. Previous studies show that elevated tear osmolarity levels activate corneal cold thermoreceptors,[61,62] which may lead to the sensation of ocular surface dryness, ocular discomfort, and pain in dry eye patients.[63,64] It has been reported that cold trigeminal neurons progressively die with aging in rats,[65] which presumably decreases the overall sensory input by the ocular cold afferent nerve fibers, responsible for evoking dryness sensations.[66] In the present study, the decrease in osmolarity with aging also may have reduced the activation of cold thermoreceptors of the cornea possibly causing minimal or absence of ocular discomfort symptoms among the population. Although we found that MQ and MGE decrease with aging, a presumption for the absence of symptoms could be that perhaps relatively less meibum output is required to maintain the tear film stability in older than in young adults. The proportion of the population that demonstrated dry eye symptoms did not demonstrate any association with ocular surface signs, a finding in agreement with several previous studies.[7,8,67,68] Some studies specifically claim that there is poor correlation between MG dysfunction signs and dry eye symptoms.[13,60,69] Therefore, ocular surface symptoms may not be a strong indicator of MGD. Further research is required to develop an MGD-specific questionnaire to study the association between MG-related symptoms and signs to facilitate a symptoms-based approach in diagnosing MGD in general optometry practice.

There were some limitations in the current study design, one being the upper limit of the age range as 66 years, which might have restricted the recruitment of people with more severe MG disorders. Previous studies have reported a greater prevalence of MG disorder between 70 and 90 years, and further work is required to establish if the ageing trends identified in the current work continue within this more elderly group. Another drawback of the study was the cross-sectional design, which could identify some associated factors with MG changes but not the causative factors; thus, we cannot comment about the natural course of MG dysfunction.

In summary, to the best of our knowledge, this is the first attempt to extensively investigate the functional, morphologic, and physiologic features of MGs across different age groups. Our findings suggested that MG structure and functions deteriorate with aging in a healthy population which was predominantly asymptomatic. The study also showed that gland dropout may not be a major concern if mild changes are observed in the MQ and MGE, as the meibum lipids profiles are unaffected with minor functional changes in the gland. The finding that tear functions are better in the older population compared to the younger age groups is intriguing, which suggests that a compensatory response to MG changes may be in place to prevent the clinical presentation of ocular irritation symptoms. To conclude, mild MG changes exist in the young age group and their occurrence increases with age; however, the ocular surface appears to be uninterrupted until these changes are combined with disrupted tear function, probably then leading to ocular discomfort symptoms.

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