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Tear Film Lipid Layer Thickness in Subjects with Increasing Severity of Meibomian Gland Dysfunction

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Keywords: Dry eye disease; Meibomian gland disfunction; Meibum quality; Tear film lipid layer.

Abstract

Objective: Meibomian Gland Dysfunction (MGD) affects Tear Film (TF) stability, causing ocular irritation and dry eye. We conducted a prospective clinical study that included evaluating the TF Lipid Layer Thickness (LLT) in subjects with increasing severity of MGD.

Methods: In an exploratory clinical trial (NCT01979887), TF LLT was measured with an ocular surface interferometer at two subject visits. Pairwise comparisons were done using ANOVA model with cohort as a factor. All statistical comparisons were made at the $\alpha = 0.05$ level without adjustment for multiple comparisons.

Results: The mean (SD) TF LLT at Enrollment/Day 1 for Non-MGD, Mild/Moderate MGD, and Severe MGD was 75.86 (15.466) nm, 72.88 (15.486) nm, and 73.86 (20.088) nm, respectively. The mean (SD) TF LLT at Exit/Day 22 for Non-MGD, Mild/Moderate MGD, and Severe MGD were 68.10 (20.753) nm, 70.91 (17.888) nm, and 69.25 (19.049) nm, respectively. No significant differences were noted between MGD cohorts in TF LLT.

Conclusions: We previously reported from this clinical trial quantitative differences in lipid composition of meibum secretions across the 3 cohorts and demonstrated that molar ratios of cholesteryl ester to wax ester were lowest and aldehyde to wax ester were highest in the severe MGD cohort. While the range of LLT values was large and did not statistically differ between cohorts, the quantity of lipid and LLT of the TF may not be as critical as the quality of the lipid composition. The findings in this exploratory study may further contribute to the understanding of the pathophysiology behind MGD.

Introduction

Aqueous-deficient dry eye and evaporative dry eye are two potentially overlapping categories of dry eye disease (DED) [1]. Evaporative dry eye is most commonly associated with meibomian gland dysfunction (MGD), which is a chronic, diffuse abnormality of the meibomian glands. MGD is characterized by terminal duct obstruction and/or qualitative or quantitative changes in glandular secretion. This may cause changes to the Tear Film (TF), eye irritation, clinically apparent inflammation,

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and ocular surface disease including dry eye [2]. Age is an important risk factor for MGD [3], and in one large review, approximately 80% of subjects who presented with MGD-associated DED were over 40 years of age [4]. However, unlike aqueous deficient dry eye, which increases exponentially and then levels off after about 50 years of age, MGD seems to increase across all age ranges [5].

The TF serves as a liquid barrier between the environment and the eye by coating the ocular surface. Tear film lipid layer (TFLL) refers to the top layer of the TF, which is primarily made up of lipids [6]. A highly recognized and accepted function of the lipid layer is its role in stabilizing the TF and preventing aqueous evaporation [7-9]. The lipid layer of the TF slows evaporation of the aqueous component, keeps the optical surface clear and intact, and protects the eye from microbial agents and organic matter [10]. The TF consists of a mixture of proteins, enzymes, lipids, mucins, and salts that allow the TF to perform its functions [11]. Meibum produced by the meibomian glands is the major source of lipids on the surface of tears [12]. The lipid secretion of the meibomian glands is delivered to the skin of the lid margin as a clear fluid that forms shallow reservoirs on the upper and lower lid margins from which the TFLL is formed and replenished [13]. The composition of the meibum lipid layer is thought to be responsible for lowering the surface tension at the ocular surface allowing for spreading of the lipid layer and TF restoration between blinks [14].

The LipiView interferometer (Johnson and Johnson Vision, Irvine, CA, USA) has been used in clinical trials to assess TF Lipid Layer Thickness (LLT) with variable findings. We conducted a noninterventional clinical investigation to examine the variations in LLT recordings utilizing LipiView measurements in participants with and without progressively more severe MGD.

Material and Methods

This prospective, multicenter (2 sites in the United States, 1 site in the United Kingdom), noninterventional clinical study consisted of 2 study visits after screening activities in individuals with and without MGD. The study was conducted in accordance with the Declaration of Helsinki and applicable regulations. An institutional review board or ethics committee approved the study protocol before the study was initiated, and all participants provided written informed consent before screening.

This exploratory study expands on previously published results examining the signs and symptoms associated with MGD that could be used as clinical trial endpoints [15] and the results from the patient reported outcomes used in the study [in press]. Lastly, the biochemical composition of the meibum secretions in subjects examined in this study with and without MGD was previously published [16]. The purpose of this study was to specifically report the results of the TF LLT that was assessed at 1 of the 3 clinical sites with a LipiView Ocular Surface Interferometer.

The selection criteria used for cohort assignment into Non-MGD, Mild/Moderate MGD, and Severe MGD were consistent with diagnostic criteria and severity grading established by the TFOS International Workshop on Meibomian Gland Dysfunction [13,17] and have been previously reported [16]. The criteria included the Maximum Meibum Quality Score (MMQS) obtained in the evaluation of 6 central meibomian glands in the lower lid, the sum of scores for the worst 2 symptoms on an ocular symptom questionnaire, and Schirmer test results.

Developed by Johnson and Johnson Vision, the LipiView Ocular Surface Interferometer is a device with ophthalmic imaging capabilities intended to capture digital images of specular (interferometric) observations of the TF. The LipiView operates on the principle of white light interferometry and provides a color assessment of the TF by specular reflection. Using images, the device measures the absolute thickness of the TFLL [18].

The TF LLT was measured at the enrollment and exit visits at one of the sites that had the LipiView Ocular Surface Interferometer instrumentation. Measurements were performed at 2 visits since there may be day-to-day variability in the LLT and the degree of concordance between visits could be assessed. Measurements from the device were recorded on the electronic case report form (eCRF) in Interferometry Color Units (ICU), where 1 ICU is equivalent to 1 nanometer (nm). These measurements included the ICU average of all the frames, the standard deviation of the frame averages, the maximum ICU recorded for a given frame, and the minimum ICU recorded for a given frame.

The study was conducted in accordance with the International Conference for Harmonisation guidelines, applicable regulations, and the Declaration of Helsinki. Institutional review board or ethics committee approval was obtained at each site before the study was initiated, and all participants provided written informed consent prior to any study-related assessments. The study was registered at ClinicalTrials.gov with the identifier NCT01979887.

Participant Selection & Assignment to Cohorts

Participant eligibility for the study was assessed at a screening visit and Enrollment/Day 1. The key inclusion criteria required male or female participants to be age 40 years or older before the enrollment visit. Key exclusion criteria included people with uncontrolled ocular disease (except for MGD) or uncontrolled systemic disease; history of LipiFlow® (Johnson and Johnson Vision, New Brunswick, NJ, USA) or other lid heating therapy, therapeutic gland expression, or meibomian gland probing within the last 12 months; use of any eyelash growth-stimulating product within the last 30 days; and use of systemic or topical macrolides, tetracyclines or tetracycline-derivative drugs, or systemic anti-histamines within the last 30 days. Individuals who currently used certain systemic vitamins or supplements (those containing omega 3 fatty acids; vitamins A, B, or E; fish oil; or evening primrose oil) were excluded if the dosing regimen had not been stable for the last 60 days.

Screened individuals who had recently used an artificial tear product or prohibited medication, worn eye makeup or contact lenses, or performed lid hygiene were required to undergo a washout interval, and eligibility was reassessed at the enrollment visit scheduled up to 90 days later. For individuals who met all eligibility criteria at the screening visit, screening and enrollment could occur on the same day (Day 1).

Study participants who met the enrollment criteria were assigned to a study cohort: Non-MGD, Mild/Moderate MGD, or Severe MGD.

Statistical methods

An ANOVA model with cohort as a factor was performed to analyze the TF LLT data at the study site. Pairwise comparisons between cohorts were obtained using the least square means from this model and two-sided 95% confidence intervals were

provided for the pairwise differences between cohorts. A paired t-test was used for the change between the enrollment and exit visits (exit minus enrollment visit). As this is an exploratory trial, all statistical comparisons were made at the $\alpha = 0.05$ level without adjustment for multiple comparisons, and nominal p values were provided.

Results

A total of 28 subjects at Enrollment/Day 1 and 27 subjects at Exit/Day 22 were evaluated at the selected site with the LipiView interferometer.

The demographics at the site where LLT was tested are reported in Table 1. In brief, the mean (standard deviation, SD) age of the participants assigned to cohorts was 55.9 (8.65) years. More than one half of these participants were women (60.7% [17/28]), and most were either Black (50.0% [14/28]) or White (28.6% [8/28]); the remainder were Hispanic (14.3% [4/28]), Asian (3.6% [1/28]), or Other (3.6% [1/28]). There were some differences in the distribution of age and race among the three MGD cohorts due to the small sample size. It can be noted that the Non-MGD cohort had more male than female participants, while the Mild/Moderate and Severe MGD cohorts had more females. In addition, there was a paucity of Asian participants in all the cohorts.

At Enrollment/Day 1, there were 28 participants total with 5 in the Non-MGD group, 11 in Mild/Moderate MGD group, and 12 in the Severe MGD group. At Exit/Day 22, there were 27 participants total with 5 Non-MGD group, 11 in Mild/Moderate MGD group, and 11 in the Severe MGD group. The mean (SD) TF LLT at Enrollment/Day 1 for Non-MGD, Mild/Moderate MGD, and Severe MGD were 75.86 (15.466) nm, 72.88 (15.486) nm, and 73.86 (20.088) nm, respectively (Table 2). The mean (SD) TF LLT at Exit/Day 22 for Non-MGD, Mild/Moderate MGD, and Severe MGD were 68.10 (20.753) nm, 70.91 (17.888) nm, and 69.25 (19.049) nm, respectively. The mean (SD) TF LLT scores across all cohorts were 73.83 (17.023) nm at Enrollment/Day 1 and 69.71 (18.168) nm at Exit/Day 22. The p values for comparison at Enrollment/Day 1 between Severe MGD versus Non-MGD, Mild/Moderate MGD versus Non-MGD, and Severe MGD versus Mild/Moderate MGD were 0.833, 0.757, and 0.896, respectively. The p values for comparison at Exit/Day 22 between Severe MGD versus Non-MGD, Mild/Moderate MGD versus Non-MGD, and Severe MGD versus Mild/Moderate MGD were 0.911, 0.785, and 0.838, respectively. No significant differences were shown between cohorts in TF LLT at both Enrollment/Day 1 and Exit/Day 22 ($p > 0.05$ for all comparisons).

The mean (SD) change in TF LLT from Enrollment/Day 1 to Exit/Day 22 was -7.76 (21.895) nm for the Non-MGD cohort, -1.97 (22.105) nm for the Mild/Moderate MGD cohort, and -5.87 (17.481) for the Severe MGD cohort. No significant changes were noted in TF LLT from Enrollment/Day 1 to Exit/Day 22.

Table 1: Subject Demographic Characteristics at LipiView Testing Site.

Characteristics	Attributes	Non-MGD (N = 5)	Mild/Moderate MGD (N = 11)	Severe MGD (N = 12)	Total (N = 28)
Age (Years)	Mean	51.6	53.5	59.8	5.9
	SD	5.41	6.07	10.45	8.65
	Median	52.0	54.0	59.5	54.5
	Min	43	44	48	43
	Max	58	63	84	84
	<45	1 (20.0%)	1 (9.1%)	0	2 (7.1%)
	45 – 65	4 (80.0%)	10 (90.9%)	10 (83.3%)	24 (85.7%)
	>65	0	0	2 (16.7%)	2 (7.1%)
Sex	Male	3 (60.0%)	4 (36.4%)	4 (33.3%)	11 (39.3%)
	Female	2 (40.0%)	7 (63.6%)	8 (66.7%)	17 (60.7%)
Race	Caucasian	1 (20.0%)	4 (36.4%)	3 (25.0%)	8 (28.6%)
	Black	3 (60.0%)	5 (45.5%)	6 (50.0%)	14 (50.0%)
	Asian	0	0	1 (8.3%)	1 (3.6%)
	Hispanic	1 (20.0%)	1 (9.1%)	2 (16.7%)	4 (14.3%)
	Other [a]	0	1 (9.1%)	0	1 (3.6%)
	Caucasian	1 (20.0%)	4 (36.4%)	3 (25.0%)	8 (28.6%)
	Non-Caucasian	4 (80.0%)	7 (63.6%)	9 (75.0%)	20 (71.4%)

MGD: meibomian gland dysfunction; SD: standard deviation; min: minimum; max: maximum.
Other [a]: Mexican American.

Table 2: LipiView Lipid Layer Thickness Results.

Visit	Statistic	Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Pairwise Comparisons [b]		
						p value Difference 95% CI [c]	p value Difference 95% CI [d]	p value Difference 95% CI [e]
Enrollment/Day 1	N	5	11	12	28	0.833 -2.00 (-21.4, 17.4)	0.757 -2.98 (-22.6, 16.6)	0.896 0.98 (-14.2, 16.2)
	Mean	75.86	72.88	73.86	73.83			
	SD	15.466	15.486	20.088	17.023			
Exit/Day 22	N	5	11	11	27	0.911 1.15 (-19.9, 22.2)	0.785 2.81 (-18.2, 23.8)	0.838 -1.66 (-18.3, 14.9)
	Mean	68.10	70.91	69.25	69.71			
	SD	20.753	17.888	19.049	18.168			
Change	N	5	11	11	27	0.864 1.89 (-20.7, 24.5)	0.601 5.79 (-16.8, 28.4)	0.656 -3.90 (-21.7, 13.9)
	Mean	-7.76	-1.97	-5.87	-4.63			
	SD	21.895	22.105	17.481	19.615			
	p value [a]	0.472	0.773	0.291	0.231			

MGD: meibomian gland dysfunction; SD: standard deviation; CI: confidence interval.
[a] p values are based on paired t-test.
[b] The results are based on ANOVA model with cohort as factor.
[c] Comparison of Severe MGD versus Non-MGD. [d] Comparison of Mild/Moderate MGD versus Non-MGD.
[e] Comparison of Severe MGD versus Mild/Moderate MGD.

Discussion

In this study, no correlation was found between LLT and subjects' cohort (Non-MGD, Mild/Moderate MGD, or Severe MGD), indicating that other mechanisms may be contributing to the increase of severity observed in this condition. We hypothesize that although factors affecting the LLT and composition may be at play, the lipid composition may have a stronger influence on TF stability and the signs and symptoms experienced by subjects with MGD.

The literature surrounding the association between the LLT and Dry Eye Disease (DED) or MGD is mixed [7,8,19-21]. Some studies have shown that LLT is a good differentiator between MGD and aqueous deficient dry eye [19]. The expectation is that as LLT is reduced with MGD, more evaporation is observed; however, one study performed in adults assessed LLT correlations in MGD and Non-MGD subjects noted that paradoxically, the normal control group had significantly thicker LLTs than the MGD arm [20]. Another study assessed symptomatology of subjects with DED and compared them accordingly to their average LLTs (<60 nm, 60 to 99 nm, and 100 nm as thin, normal, and thick LLT, respectively) and results showed that subjects with thick LLTs can still exhibit severe dry eye symptoms [22]. A study examining tear evaporation rates demonstrated that rapid evaporation could occur in subjects that exhibit thick LLTs, however large variabilities in LLT across the entire tear film could limit the ability to sub-classify subjects into distinct categories [8]. Although the literature supports that the TFLL is an important component of the TF, it is not clear as to the whether the LLT has a direct impact on a subject's signs and symptoms of dry eye with or without MGD. There has been less discussion on the importance of lipid quality, which may be more of a factor to TF stability and the signs and symptoms observed in MGD.

Based on our previous research conducted in subjects with the same cohort assignment, compositional lipid changes were shown across groups suggesting that alterations in meibum composition may be a result of MGD pathophysiology, which can contribute to TFLL instability [17]. It was demonstrated that the molar ratio of CE to WE ($R_{CE/WE}$) were lowest and the molar ratio of aldehyde to wax ester ($R_{ald/WE}$) was highest among the most severe MGD cohort. In concordance, previous studies have also proposed a potential relationship between lipid composition, MGD, and TFLL functionality [7,8].

Lipid composition varies drastically from other lipid types within the body, but some similarities can be drawn to provide insights into its role as a barrier for aqueous evaporation and its association with MGD. Other lipid types in the body, such as sebum, reinforce the importance of maintaining balanced lipid levels to fulfill the function of the barrier [23,24]. Although meibum from meibomian glands, which are regarded as largely modified sebaceous glands, is often compared to sebum from the skin's sebaceous glands, their lipid level compositions vary drastically [24]. Human sebum contains very little cholesterol and is mainly composed of triglycerides, free fatty acids, wax esters, and squalene, which is a precursor to cholesterol [23]. The majority of human meibum consists of cholesteryl esters (CE) and Wax Esters (WE) [25]. Aside from cholesterol esters and wax esters, meibum is highly comprised of other non-polar lipids, such as free cholesterol and triglycerides, and far fewer polar compounds, including free fatty acids, (O-Acyl)-ω-hydroxy fatty acids (OAHFA) and phospholipids [24,26]. Cholesterol can either be present as free cholesterol, an active biologic form that in excess can have cytotoxic effects, or as cholesteryl ester, a more protective storage form within cells that can be used for transportation in plasma [27]. Although the various human sebaceous glands have adapted and produce lipids to best suit their function in the skin or the eye (despite their differences in lipid profiles), they all share a role in preserving their respective barriers, and therefore deviations in their composition can cause changes in their function and lead to disease [23,24].

Abnormal lipid composition in the meibum is correlated with MGD and can cause TF instability, epithelial damage, and meibum stiffness [16,28]. A recent study investigated the association between MGD, aging, and alterations in meibum lipid composition within three groups: MGD subjects, healthy elderly subjects, and healthy younger subjects. MGD subjects and healthy elderly subjects demonstrated low cholesteryl esters, but high cholesterol, OAHFAs, and fatty acids, in comparison to healthy younger subjects [26]. In addition, high triglycerides were significant only in the MGD cohort versus both healthy groups. No differences in WEs were expressed among the three groups, but the ratios of CE/WE were significantly lower in healthy elderly subjects and subjects with MGD [26]. Meibum rigidity could be attributed to the CE/WE ratio along with CE hydrocarbon chains [26]. A recent report in Soat1-null mice showed that a decrease in CE and an increase in free cholesterol were associated with increased meibum stiffness, melting temperatures, and plugging of the meibomian gland orifices [29]. These cases reinforce how meibum lipid composition, especially CE/WE ratio, and its impact on meibum rigidity, along with other factors such as advanced age, can play a key role in maintaining the barrier against aqueous evaporation [16].

Systemic factors, such as advanced age and dyslipidemia, may contribute to the different ratios seen in meibum lipid composition. A systematic review and meta-analysis were performed to assess the relationship between dyslipidemia and MGD prevalence [30]. Elevated total cholesterol and triglycerides were found to be significantly associated with MGD, implying that age-related dyslipidemia may be involved with MGD development. High cholesterol and triglyceride levels in the serum may be an underlying factor behind the increases of these lipids in the meibum [26]. The synthesis of cholesterol into cholesteryl esters may not occur fast enough in relation to the excessive intake of cholesterol, potentially leading to abnormal lipid profiles in the meibum [26,29]. Therefore, advanced age and age-related dyslipidemia may be factors that predispose individuals to an abnormal change in lipid composition and disruption of the TFLL. This highlights the importance of preserving the barrier by maintaining proper levels of key lipids and optimal molar ratios.

Limitations of this exploratory trial include a small sample size, a single investigational site, and all statistical comparisons made at the alpha = 0.05 level without any adjustment for multiple comparisons with the use of nominal p values. In addition, there were differences in the distribution of race and gender of the enrolled subjects in the different arms of the study and this may be a potential limitation. For example, a higher MGD prevalence has been described in Asians and very few Asians were enrolled in our study [31]. Possible considerations for future studies include a larger sample size and a diverse population to help provide a better representation of the general MGD-affected population.

In summary, given that the LLT did not differ between cohorts, the quantity of lipid and LLT of the TF may not be as critical as the quality of the lipid composition in the TF in the setting

of MGD. Previous data from this clinical trial showed quantitative differences in meibum lipid composition that correlated among the 3 cohorts (Non-MGD, Mild/Moderate MGD, and Severe MGD). Therefore, it is important to maintain balanced lipid levels as abnormalities to the lipid composition can lead to changes in the TFLL functionality and contribute to the signs and symptoms observed in subjects with MGD.

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AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g., protocols, clinical study reports, or analysis plans), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications.

These clinical trial data can be requested by any qualified researchers who engage in rigorous, independent, scientific research, and will be provided following review and approval of a research proposal, Statistical Analysis Plan (SAP), and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time after approval in the United States and Europe and after acceptance of this article for publication. The data will be accessible for 12 months, with possible extensions considered. For more information on the process or to submit a request, visit the following link: https://www.abbvieclinicaltrials.com/hcp/data-sharing/.

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