ECRONICON OPEN ACCESS

EC OPHTHALMOLOGY
Research Article

Meibum Lipid Composition and Conformation in Parkinsonism

Solomon Blinchevsky[1], Aparna Ramasubramanian[2], Douglas Borchman[1]*, Shanzeh Sayied[1] and Krithika Venkatasubramanian[1]

[1]Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA

[2]Phoenix Children's Hospital, Phoenix, AZ, USA

***Corresponding Author:** Douglas Borchman, Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA.

Received: February 16, 2021; **Published:** March 31, 2021

Abstract

Purpose: Patients with Parkinson's disease (PD) exhibit unstable tear films. Tear film lipid composition and structure are related to tear film stability and dry eye and tear lipids have not been characterized in people with PD. The aim of this study is to characterize Meibum tear lipids in donors with PD using ^{1}H-NMR and infrared spectroscopy.

Methods: Three cohorts were compared: meibum from donors with PD (Mp) n = 10, meibum from donors with PD and dry eye (Mpd) n = 3, meibum from donors without PD (Mn) n = 29.

Results: There were no significant differences, $P > 0.05$, in hydrocarbon branching for Mp compared with Mn. Mn contained twice as much cholesteryl esters compared with Mp, $P < 0.0001$. The cooperativity of the phase transition was significantly 37% lower for Mp compared with Mn, $P < 0.0001$. Mpd was much more ordered (stiffer) with compared with Mp and Mn, $P < 0.0001$.

Conclusion: Changes in meibum lipid composition and structure could be a marker for and/or contribute to increase the susceptibility of dry eye in patients with PD. A less cooperative phase transition for Mp compared with Mn indicates that Mp was more heterogeneous and/or contained more contaminants than Mn. The data support the idea that more ordered lipid contributes to dry eye.

Keywords: *Dry Eye; Infrared Spectroscopy; Lipids; Meibum; NMR Spectroscopy; Parkinson's Disease*

Abbreviations

BR: Blink Rate; FTIR: Fourier Transform Infrared Spectroscopy; Mpn: Meibum from Donors with Parkinson's Disease; Mpd: Meibum from Donors with Parkinson's Disease and Dry Eye; Mn: Meibum from Donors without Parkinson's or Dry Eye Disease; M_{MGD}: Meibum from Donors with Meibomian Gland Dysfunction; PD: Parkinson's Disease; ^{1}H-NMR: Proton-Nuclear Magnetic Resonance Spectroscopy; TBUT: Tear Break Up Time; $\tilde{\nu}_{sym}$: Vibrational Frequency of the C-H Symmetric Stretch

Introduction

Parkinson's disease (PD) affects 1% of the population above 60 years of age that diminishes voluntary movements [1]. In most cases, its cause is unknown. One of the earliest studies to report ocular signs of PD in 1982, showed that individuals with PD that were treated

with anticholinergic drugs showed signs of dyskinesia associated with a decrease in blink rates, (BR), blinks per minute (16 ± 9, blinks per minute, n = 24) compared with controls (32 ± 9, n = 21) [2]. BR are a measure of tear film stability. Meta-analysis has confirmed this finding [3]. A lower blink rate associated with PD was correlated with levodopa levels used to treat PD [4]. Tear breakup time (TBUT) is another measure of tear film stability and was found to be higher in individuals with PD compared with those without PD [5-7], however, a study showed no difference [8]. Both BR and TBUT are highly variable from person to person with standard deviations of about 60% of the mean, so a large number of samples are necessary to test statistically significant differences. Patients with PD produce significantly less tears compared with age-matched controls [6,9-11]. Tear production measured using Schirmer's strips are highly variable from person to person with standard deviations of about 80% of the mean, so like BR and TBUT measurements, a large number of samples are necessary to test statistically significant differences.

Interestingly, the proteomic analysis of tears from patients with PD show a dysregulation of proteins related with lipid metabolism [9]. Approximately 80% of the lipid in tears originates from the meibomian glands located in the eyelids [12,13]. Upon blinking, lipid from the meibomian glands called meibum, is deposited on the tear film surface and help to stabilize the tear film [14,15]. Almost all individuals with PD have blocked meibomian glands with a dry eye type termed meibomian-gland dysfunction [8], however, another study indicated that there was no relationship between meibomian gland dysfunction and PD [5].

As tear film lipid composition and structure are related to tear film stability and dry eye [14,15] and since tear lipids have not been characterized in people with PD, in this pilot study, meibum lipids, the major source of tear lipids, were characterized in donors with PD using ^{1}H-NMR and infrared spectroscopy.

Methods

Written informed consent was obtained from all donors. Protocols and procedures were reviewed by the University of Louisville Institutional Review Board (# 11.0319, August 2016) and the Robley Rex Veterans Affairs Institutional Review Board. All procedures were in accordance with the Declaration of Helsinki. Subjects were recruited from the Kentucky Lions Eye Center, the Robley Rex Veterans Affairs Medical Center and a Parkinson's patient exercise facility, all in Louisville, KY. Inclusion criterion for normal donors was donors older than 25 years-of-age who were given a 'normal' clinical diagnosis as described in the Clinical Diagnosis section below. Excluded were donors who did not meet the 'normal' clinical diagnosis criterion. Inclusion criterion for donors with PD was donors present at an exercise class that were diagnosed with PD by a clinician.

Meibomian gland expression and sample collection are presented in table 1. It is interesting that two of the three donors with dry eye were female. The demographics of the meibum from 19 are presented in table 2. Meibum from the following cohorts were compared: meibum from donors with PD (Mp).

Briefly, meibomian gland expression for donors without PD or dry eye (Mn) was done by compressing the eyelid between cotton-tipped applicators, with strict attention to avoid touching the eyelid margin during expression. All four eyelids were expressed, and approximately 1 mg of meibum was collected per individual for direct spectroscopic study. Sample collection of meibum from donors with PD and dry eye (Mpd), and donors with PD and no dry eye (Mpn) was identical to that previously reported [16]: Meibomian glands were gently expressed by pressing the eyelid with a fingertip with strict attention to avoid touching the eyelid margin during expression. All four eyelids were expressed, and approximately 0.5 mg of meibum lipid was collected per individual for direct spectroscopic study. The expressate was collected with a platinum spatula and immediately dissolved into 0.5 mL of $CDCl_3$ in a 9-mm micro vial with a Teflon cap (Microliter Analytical Supplies Inc., Suwanee, GA). Argon gas was blown over the samples to prevent oxidation. The sample in the vial was capped and frozen under argon gas until analysis Analyses were performed within 3 weeks of collection of the sample. The samples never were exposed to any plastic to avoid plasticizers Control $CDCl_3$ spectra were run with the meibum samples to insure no impurities were present.

Clinical diagnosis

Normal status was assigned when the subject's meibomian gland orifices showed no evidence of keratinization or plugging with turbid or thickened secretions, and no dilated blood vessels were observed on the eyelid margin. Meibum from 10 donors stating they had PD were collected on site at a Parkinson's patient exercise facility. Three of the 10 donors stated to have dry eye symptoms. Visual observation by a clinician at the time of donation suggested that the dry eye was moderate.

Spectroscopic measurements and statistics

Spectral data were acquired using a Varian VNMRS 700 MHz nuclear magnetic resonance (NMR) spectrometer (Varian, Lexington, MA) equipped with a 5 mm $^{1}H\{^{13}C/^{15}N\}$ ^{13}C enhanced PFG cold probe (Palo Alto, CA). Spectra were acquired with a minimum of 250 scans, 45° pulse width, and a relaxation delay of 1000 second. All spectra were obtained at 25°C. The TMS resonance was set to 0 ppm. Commercial software (GRAMS 386) Galactic Industries Corp, Salem, NH) was used for phasing, curve fitting and integrating hydrocarbon chain branching [16], saturation [17,18] and the molar ratio of cholesteryl ester (CE) to wax ester (WE) [19] were calculated from as described previously using the formula: (16+17)/(4+(1+15)/3) where the numbers are the intensities of the resonances numbered in table 2 except for the number 3 which is a constant.

Samples were pooled for Fourier transform infrared spectroscopic (FTIR) analysis due to the paucity of sample (Table 1). Meibum was layered onto AgCl windows and lipid phase transitions were measured as described previously [20]: Curves were fit using Sigma plot 10 software (Systat Software, Inc., Chicago, IL), and the confidence levels were obtained from a critical value table of the Pearson product-moment correlation coefficient. Two of the phase transition parameters, the minimum and maximum $\tilde{\nu}_{sym}$, correspond to the most ordered and disordered states of hydrocarbon chains, respectively. Another parameter was the phase transition temperature, which is the temperature at which half of the lipid molecules undergo a change from the gel to liquid crystalline phase. The relative cooperativity of the phase transition describes how the order of a lipid influences that of its neighboring lipids. Broad phase transitions have a relatively smaller absolute value of the cooperativity. Lipid order was calculated at 33.4°C, the temperature at the surface of the eye, and at 36°C, the temperature of the eyelid [21]. Data are reported as the mean plus or minus the standard error" Averages were compared using the Student's t test. A value of $P < 0.05$ was considered statistically significant.

Results

Donor demographics and cohorts

The demographics of the Mp from 10 donors are presented in table 1. It is interesting that two of the three donors with dry eye were female. The demographics of the Mn from 19 donors are presented in table 2. Meibum from the following cohorts were compared: Mp, Mpd, Mpn and Mn.

NMR spectroscopic results

Donor Number	Age (y)	Sex	Dry Eye	Pool number for FTIR
1	64	Male	-	1
2	68	Male	-	1
3	71	Male	-	1
4	37	Male	-	2
5	58	Male	-	2
6	59	Male	-	3
7	86	Male	-	3
8	47	Female	+	4
9	75	Male	+	4
10	77	Female	+	4

Table 1: *Donor demographics of donors with Parkinson's disease. All donors were caucasian with Parkinson's disease.*

Cohort without dry eye (number of donors)	
Female	6
Male	13
Caucasian	15
Asian	1
Black	2
Hispanic	1
Average Age (y)	50 ± 5

Table 2: *Cohort demographics and data.*
Average ± standard error of the mean.

The major resonances were well resolved in the ^{1}H-NMR spectra of the meibum samples (Figure 1). Resonances for cholesterol/CE, hydrocarbon chain branching and =CCH_2 were evident in the 1 to 3 ppm region (Figure 1a and table 3). Resonances for =CH cis, CE and WE were evident in the 4 to 55 ppm region (Figure 1b and table 3). There was no significant difference (P > 0.05) in hydrocarbon branching for Mp compared with Mn (Table 4). Mp contained significantly (P < 0.0001) and relatively less, 0.26 ± 0.05 CE/WE (mole/mole), compared with Mn, 0.512 ± 0.05 CE/WE (mole/mole) n = 29. CE/WE increased slightly with age in the Mn group, with a slope of 0.0038 (CE/WE)/year. The 14 year difference in the average age between the normal and PD donors could only account for a small difference of 0.05 in the CE/WE ratio, so age cannot account for the large difference in the CE/WE ratio between Mn and Mp. The CE/WE molar ratio was not significantly different (P = 0.054) for Mpd, 0.19 ± 0.12 compared with Mn.

FTIR results

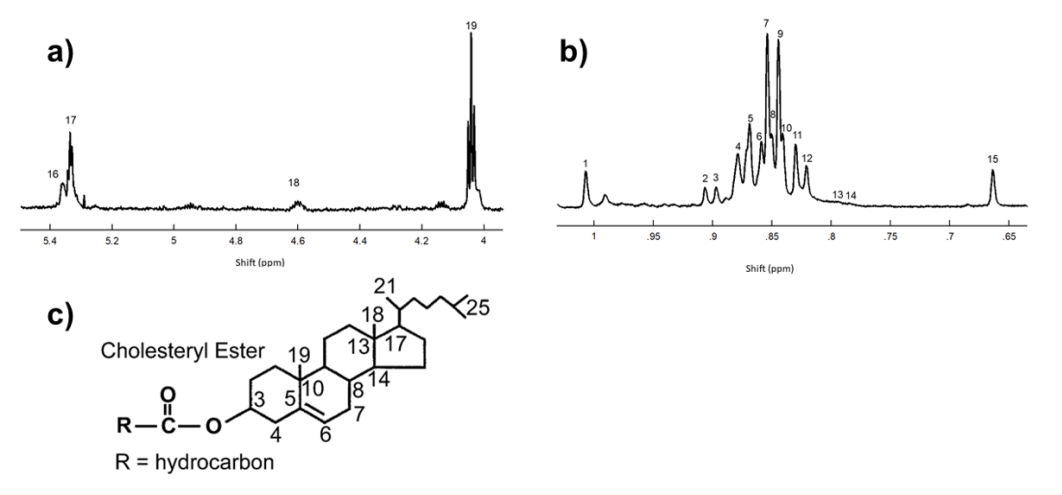

Figure 1: *A typical 1H-NMR spectrum of meibum from a 58 year-old Caucasian male who has Parkinson's disease. Numbers refer to the resonance assignments in table 2. a) The ester and =CH region. b) The CH3 / CH2 resonance region. c) Numbering of cholesteryl ester carbons.*

Figure 1 resonance #	Shift (ppm)	Resonance Assignment
1	100 to 0.996	Cholesterol Carbon # 19 (Figure 1c)
2	0.906	Cholesterol Carbon #21 (Figure 1c)
3	0.897	Cholesterol Carbon #21 (Figure 1c)
4	0.878	Straight-chain
5	0.868	Straight-chain
6	0858	Straight-chain
7	0.853	Iso-branched
8	0.850	Anteiso-branched
9	0.843	Iso-branched
10	0.839	Anteiso-branched
11	0.829	Anteiso-branched
12	0.821	Anteiso-branched
13	0.799	Not assigned
14	0.789	Not assigned
15	0.663	Cholesterol Carbon #18 (Figure 1c)
16	5.36	Cholesterol Carbon #6 (Figure 1c)
17	5.33	Hydrocarbon =CH- cis
18	4.6	Cholesteryl Ester Carbon #3 (Figure 1c)
19	3.9	Wax Ester -CH_2-O-(C=O)-

Table 3: *Assignments for resonances numbered in figure 1.*

Parameter	Mn[a] (n)	Mp (n = 10)	Mpn (n = 7)	Mpd (n = 3)	Mn vs Mpn P
Straight Chain (%)	57 ± 1 (65)	58 ± 2	58 ± 3	55 ± 2	0.7
Anteiso Branched (%)	20 ± 1 (65)	15 ± 1	15 ± 1	17 ± 1	0.09
Iso Branched (%)	23 ± 1 (65)	27 ± 2	27 ± 2	28 ± 2	0.2

Table 4: *Meibum composition from ^{1}H-NMR spectra.*

None of the values were significantly different (P > 0.05). [a]From [17]. Meibum from donors with Parkinson's disease (Mp). Meibum from donors with Parkinson's disease and no dry eye (Mpn), meibum from donors with Parkinson's disease and dry eye (Mpd) and meibum from donors without Parkinson's or dry eye disease (Mn).

The CH stretching region of the infrared spectra of Mpn and Mpd were typical of meibum (Figure 2a). A plot of the vibrational frequency of the C-H symmetric stretch $\tilde{\nu}_{sym}$ verses temperature (Figure 2b) was used to follow the phase transitions of meibum from an ordered (stiffer) gel phase to a disordered liquid crystalline phase. There was no significant difference (P > 0.05) between the phase transition parameters of Mn and Mpn except that the cooperativity of the phase transition was significantly (P < 0.0001) 37% lower for Mpn compared with Mn (Table 5). Cooperativity decreased slightly but not significantly, P > 0.1, with age in the Mn group, with a slope of -0.029 units/year. The 14-year difference in the average age between the normal and PD donors could only account for a small difference of 0.4

cooperativity units, so age cannot account for the large difference, 2.9 units, between the cooperativity of Mn and Mp. Mpd was much more ordered (stiffer) with a higher phase transition temperature compared with Mpn and Mn (Figure 2b and table 5). Hydrocarbon chain order decreased slightly and significantly, P > 0.1, with age in the Mn group, with a slope of -0.111% trans units/year. The 14-year difference in the average age between the normal and PD donors could only account for a small difference of 1.6% trans, so age cannot account for the large difference, 47% trans, between the hydrocarbon chain order of Mn and Mp.

Discussion

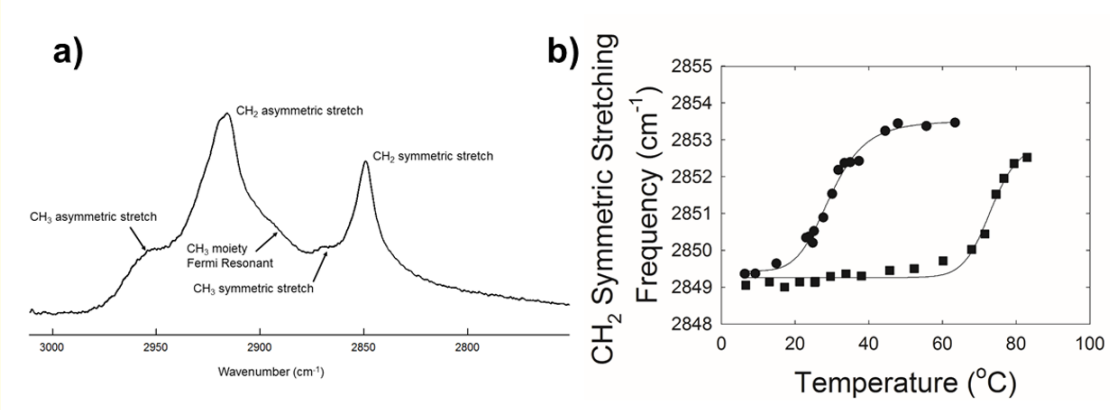

Figure 2: *a) A typical infrared spectrum of meibum from a de-identified donor with Parkinson's disease at 33.4oC. b) Lipid phase transitions for (•) pool 1 of meibum from donors with Parkinson's disease without dry eye and (■) pool 4 of meibum from donors with Parkinson's disease and dry eye.*

Parameter	Mn	Mpn	Mpd
Transition Temperature (°C)	29.7 ± 0.5	28.4 ± 2.9	73
Cooperativity (Hill coefficient)	7.9 ± 0.5	5 ± 1	19
Order 36.0 °C (% trans)	36 ± 1	37 ± 6	83
Order 33.4 °C (% trans)	38 ± 1	40 ± 4	83
Δ enthalpy (kcal/mol)	144 ± 10	112 ± 22	160
Δ entropy (kcalmol/degree)	0.48 ± 0.03	0.37 ± 0.07	0.46
Magnitude (cm^{-1})	4.01 ± 0.09	4.0 ± 0.1	3.7
Minimum Frequency (cm^{-1})	2849.75 ± 0.08	2849.6 ± 0.16	2849.3
Maximum Frequency (cm^{-1})	2853.7 ± 0.1	2853.57 ± 0.03	2852.9
N	19 individual	3 pools	1 pool

Table 5: *Phase transition parameters of meibum from infrared spectroscopy.*

± The standard error of the mean. The only significant difference between Mn and Mpn was for the cooperativity, P < 0.0001.

The transition temperature, cooperativity and order were significantly different P < 0.0001, for Mpd compared with Mn and Mpn.

The major findings of the current study were that there were differences in the composition and cooperativity of meibum from donors with PD compared with donors without PD. The relevance of the differences to the increased susceptibility of people with PD to dry eye, are discussed.

Phase transition cooperativity

The major structural difference between Mn and Mp was that the cooperativity of the phase transition of Mpn was lower compared with Mn. Cooperativity (Hill coefficient) is related to how the melting of a lipid influences the melting of adjacent lipids, the cooperative unit size [22] and the homogeneity of the meibum lipid. In terms of homogeneity, the phase transition cooperativity of pure meibum is much less than that of pure waxes and the phase transitions for pure waxes are more cooperative than for waxes mixtures [23,24]. Contaminants, proteins [25] and unsaturation [17,18,26] could also lower the cooperativity. Patients who underwent hematopoietic stem cell transplantations and are susceptible to dry eye have meibum lipid phase transitions that are about as cooperative, 5.4, as meibum lipid phase transition of Mp, 5, and lower than that of Mn, 7.9 [15,27-29]. Mp is therefore more heterogeneous compared with Mn, but this difference is not always a prerequisite to being more susceptible to dry eye as meibum from donors with dry eye due to Meibomian gland dysfunction have cooperativity values that are higher, 9, compared with Mp or Mn [27]. Like meibum from donors with meibomian gland dysfunction, Mpd was much higher compared to Mp, however due to the small sample size the result should be viewed cautiously. Whether increased cooperativity with dry eye is a sign or contributes to dry eye has yet to be determined.

Lipid conformational order

The lipid order or stiffness, at both the temperature of the Meibomian glands, 36°C and on the surface of the eye, 33.4°C [21], was significantly higher for Mpd compared with Mn and Mpn. The average ages of the donors of Mpd and Mpn were similar, so dry eye and not age may be a factor related to the difference. Note that Mpd order is among the highest measured and that meibum lipid order and tear film stability are related with age between 0 and 25 years old, Meibomian gland dysfunction, and with donors who have had hematopoietic stem cell transplantation [15,28,29]. It is intriguing that treatment to restore tear film stability also restored meibum lipid order to normal levels [30], suggesting the correlations may be more than coincidental. A more ordered lipid could contribute to the formation of a discontinuous patchy tear film lipid layer that is related to more ordered lipid, that which in turn was related to deteriorated spreading, and decreased surface elasticity [14,15]. Perhaps more ordered lipid attenuates the capability to restore tear film lipid layer structure between blinks Furthermore, more ordered lipids could block the Meibomian glands and be less likely to exit the gland onto the tear film upon blinking. Our finding that Mpd order is among the highest measured and that it correlates with a decrease in tear film stability measured by an increase in the blink rate supports the idea from other studies [15] that lipid order and blink rate are related. Clearly more studies are needed to confirm these ideas.

Remarkably, except for the difference in cooperativity, the phase transition parameters for Mn were similar to Mpn despite a large difference in CE content. This suggests that the structure of meibum is buffered against large changes in CE content, opposite of what we would expect from a model CE/WE study [23]. Synthetic CE and WE may not be good models for native CE and WE as native esters contain a complex mixture of hydrocarbon chain branching, saturation and length and synthetic CE and WE available commercially do not. Current studies are underway to test how the concentration of CE and WE isolated from human meibum affects rheology and structure.

Relative cholesteryl ester content

It is interesting that the Mn contained twice as much cholesteryl esters compared with Mp. Meibum from donors with meibomian gland dysfunction contained 35% less CE compared with Mn [15,19,24], so it is attractive to believe that lower levels of CE contribute to or make one more susceptible to dry eye. Based on model studies [24], it has been speculated [31] that lower levels of CE could contribute to a meibum that is more ordered, as with dry eye, however, confirmatory studies using natural CE from human meibum are necessary.

The average age of the donors with PD was about 64 years, slightly more than the average age, 50 years of the donors of the Mn samples. One might argue that factors other than PD, such as age or lid hygiene due to the slightly older age of the PD donors could influence the comparison between Mpn and Mpd. However, as pointed out in the Results, the age related differences in NMR and FTIR parameters were very small and could not account for the large differences between Mpn and Mpd. Although there was a sufficient quantity of meibum to measure NMR spectra from individual samples, a limitation of the current study was that samples had to be pooled for infrared spectroscopic measurements. Another study showed that the phase transition parameters from pooled samples of meibum were almost identical to the averages of the parameters from the same individual samples [17,29]. The current study shows changes in the composition and structure of meibum from individuals with PD, which justifies future studies involving greater numbers of samples, and the characterization of dry eye type and degree, disease duration, age and race of the participants, PD stage and drugs used by the participants. Disease duration does not correlate with BR, TBUT or tear production [6].

Conclusion

In conclusion, changes in meibum lipid composition and structure could contribute to and/or increase the susceptibility of dry eye in patients with PD. Changes in meibum lipid composition and structure should be considered when developing therapies to ameliorate dry eye in patients with PD. The data support the idea that ordered meibum lipids contribute to or are a marker of dry eye disease.

Author Contributions

Conceptualization, SB and DB, methodology, DB validation, DB, AR, SB, SS and KV, formal analysis, DB, SB, SS and KV, investigation, DB, AR, SB, SS and KV, resources, DB, data curation, DB, AR, SB, SS, and KV writing-original draft preparation, DB and AR writing-review and editing, DB and AR supervision, DB, project administration, DB, funding acquisition, DB

Funding

This research was funded by National Institutes of Health EYO RO126180 (DB) and an unrestricted grant from Research to Prevent Blindness, Inc New York, NY, USA GN151619B.

Conflicts of Interest

None of the authors had a conflict of interest.

Bibliography

1. Tysnes OB*., et al. "*Epidemiology of Parkinson's disease". *Journal of Neural Transmission* 124.8 (2017): 901-905.

2. Karson CN*., et al. "*Blink rates in Pakinsonism". *American Neurological Association* 12.6 (1982):580-583.

3. Fitzpatrick E*., et al. "*Case-control study of blink rate in Parkinson's disease under different conditions". *Journal of Neurology* 259.4 (2012): 739-744.

4. Iwaki H*., et al. "*Using spontaneous eye-blink rates to predict the motor status of patients with Parkinson's disease". *International Medicine* 58.10 (2019): 1417-1421.

5. Tamer C*., et al. "*Tear film tests in Parkinson's disease patients". *Ophthalmology* 112.10 (2005): 1795e1-1795e8.

6. Esin SS*., et al. "*Tear osmolarity, break-up time and Schirmer's scores in Parkinson's disease". *Turkish Journal of Ophthalmology* 45.4 (2015): 142-145.

7. Biousse V*., et al. "*Ophthalmologic features of Parkinson's disease". *Neurology* 62.2 (2004): 177-180.

8. Reddy VC*., et al. "*Corneal sensitivity, blink rate, and corneal nerve density in progressive supranuclear palsy and Parkinson disease". *Cornea* 32.5 (2013): 631-635.

9. Boerger M*., et al. "*Proteomic analysis of tear fluid reveals disease-specific patterns in patients with Parkinson's disease - A pilot study". *Parkinsonism Related Disorders* 63 (2019): 3-9.

10. Bagheri H*., et al. "*Lacrimation in Parkinson's disease". *Clinical Neuropharmacology* 17.1 (1994): 89-91.

11. Kwon OY*., et al. "*Schrimer test in Parkinson's disease". *Journal of Korean Medical Science* 9.3 (1994): 239-242.

12. Borchman D*., et al. "*Confirmation of squalene in human eye lid lipid by heteronuclear single quantum correlation spectroscopy". *Lipids* 48.12 (2013): 1269-1277.

13. Mudgil P*., et al. "*Sebum/meibum surface film interactions and phase transitional differences". *Investigative Ophthalmology and Visual Sciences* 57.6 (2016): 2401-2411.

14. Georgiev GA*., et al. "*Structure-function relationship of tear film lipid layer: a contemporary perspective". *Experimental Eye Research* 163 (2017): 17-28.

15. Borchman D. "Lipid conformational order and the etiology of cataract and dry eye". *Journal of Lipid Research 62* (2021): 10039.

16. Borchman D*., et al. "*Human meibum chain branching variability with age, gender and meibomian gland dysfunction". *Ocular Surface* 17.2 (2019): 327-339.

17. Sledge S*., et al. "*Human meibum age, lipid-lipid interactions and lipid saturation in meibum from infants". *International Journal of Molecular Science* 18.9 (2017): E1862.

18. Nencheva Y*., et al. "*Effects of lipid saturation on the surface properties of human meibum films". *International Journal of Molecular Science* 19.14 (2018): E2209.

19. Borchman D*., et al. "*Human meibum cholesteryl and wax ester variability with age, gender and meibomian gland dysfunction". *Investigative Ophthalmology and Visual Sciences* 60.6 (2019): 2286-2293.

20. Borchman D*., et al. "*Temperature-induced conformational changes in human tear lipids hydrocarbon chains". *Biopolymers* 87.2-3 (2007): 124-133.

21. Abreau K*., et al. "*Temperatures of the ocular surface, lid, and periorbital regions of Sjögren's, evaporative, and aqueous-deficient dry eyes relative to normal". *Ocular Surface* 14.1 (2016): 64-73.

22. Lu H*., et al. "*Differential scanning calorimetric evaluation of human meibomian gland secretions and model lipid mixtures: transition temperatures and cooperativity of melting". *Chemistry and Physics of Lipids* 170-171 (2013): 55-64.

23. Borchman D., *et al.* "^{13}C and ^{1}H-NMR ester region resonance assignments and the composition of human infant and child meibum". *Experimental Eye Research* 112 (2013): 151-159.

24. Hetman ZA., *et al.* "Concentration dependent cholesteryl-ester and wax-ester structural relationships and meibomian gland dysfunction". *Biochemical Biophysical Reports* 21 (2020): 100732.

25. Shrestha RK., *et al.* "Analysis of the composition of lipid in human meibum from normal infants, children, adolescents, adults and adults with meibomian gland dysfunction using H-NMR spectroscopy". *Investigative Ophthalmology and Visual Sciences* 52.10 (2011): 7350-7358.

26. Georgiev GA., *et al.* "Lipid Saturation and the Rheology of Human Tear Lipids". *International Journal of Molecular Science* 20.14 (2019): 3431.

27. Borchman D. "The optimum temperature for the heat therapy for meibomian gland dysfunction". *Ocular Surface* 17.2 (2019): 360-364.

28. Ramasubramanian A., *et al.* "Structural differences in meibum from teenage donors with and without dry eye induced by allogeneic hemopoetic stem cell transplantations". *Journal of Pediatric Hematological Oncology* 42.2 (2019): 149-151.

29. Ramasubramanian A., *et al.* "Structural differences in meibum from donors after hematopoietic stem cell transplantations". *Cornea* 38.9 (2019): 1169-1174.

30. Foulks GN., *et al.* "Topical azithromycin therapy of Meibomian gland dysfunction: clinical response and lipid alterations". *Cornea* 29.1 (2010): 781-788.

31. Borchman D., *et al.* "Differences in meibum and tear lipid composition and conformation". *Cornea* 39.1 (2020): 122-128.

Volume 12 Issue 4 April 2021