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Increased Corneal Epithelial Permeability After Overnight Sleep

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Purpose. To investigate factors of ethnicity, sex, age, and diurnal variation on human corneal epithelial permeability.

Methods. Data of corneal epithelial permeability to sodium fluorescein (P_{dc}) were collected from 374 noncontact lens wearers at various times after awakening throughout the day. Mixed-effect models were developed to investigate the association between P_{dc} and factors of interest, including time awake (T_A), age, sex, ethnicity, and interactions of these factors.

Results. Two models evaluated the "P_{dc} recovery period" from awakening to 2 hours (Model 1) and the "P_{dc} plateau" period after T_A of 2 hours (Model 2). In Model 1, P_{dc} declined significantly with length of awake time ($P = 0.000$), and showed higher P_{dc} with males ($P = 0.098$), although this sex difference was not observed after 2 hours (Model 2). Both models showed significantly higher P_{dc} in Asians than in non-Asians ($P = 0.000$) and increased P_{dc} with age ($P = 0.048$, $P = 0.001$).

Conclusions. Baseline corneal epithelial barrier function increases after overnight sleep and varies significantly by ethnicity and age.

Keywords: cornea, race, ethnicity, corneal epithelial permeability, fluorometry, diurnal, sex, age

The corneal epithelium contributes to the innate defense system of the eye by acting as protective environmental barrier. A variety of factors have been shown to compromise corneal epithelial barrier function, including contact lens–induced hypoxia, physical presence of a contact lens, preservatives in ophthalmic solutions or medications, and age.[1–5] Previous studies of the human cornea with contact lens wearers have also suggested that corneal epithelial permeability changes significantly after initial eye opening upon awakening and that decreased corneal epithelial barrier function during overnight contact lens wear is associated with increased incidence of adverse event.[1,2]

Identifying factors that affect corneal epithelial permeability can potentially provide useful information for clinical applications. Specifically, determining the diurnal variation of corneal epithelial barrier function will also provide information for slow and controlled release of ophthalmic drugs, like dissolvable capsules or embedded microfluidic devices in soft contact lenses[6,7] and assessment of the efficacy of drug-assisted ocular lubrication and water soluble topical medications, as well as the safety and risks of extended contact lens wear.

In this current study, we investigate ethnic, sex, and age differences as well as diurnal variation on human corneal epithelial permeability.

Methods

Subject Recruitment

We recruited 374 subjects (51% male and 49% female) aged 18 to 38 years (mean ± SD = 22 ± 3.7 years) from the student population of the University of California, Berkeley, California (UCB). All participants had either never worn contact lenses or had discontinued lens wear a minimum of 1-year prior to study enrollment. Subjects had no history of ocular disease, surgery, or injury. Individuals with seasonal allergies or who were taking systemic drugs known to affect the tear film or ocular surface were excluded through a screening phone questionnaire. Subjects were asked to sleep for at least 6 hours the night before each visit and to avoid swimming, smoking, and smoky areas the day before their appointment. Subjects' self-reported ethnicity was categorized into Asians (Chinese, Korean, Vietnamese, and Taiwanese) and non-Asians (Caucasians and Latinos). Consent was obtained before the start of the study. This study adhered to the tenets of the Declaration of Helsinki and all procedures were approved by the institutional review board (Committee for Protection of Human Subjects, UCB).

Instrumentation

Central corneal epithelium permeability to sodium fluoroscein (P_{dc}) measurements were taken using Fluorotron Master automated scanning fluorometers (Ocumetrics, Mountain View, CA, USA) with the single-drop method.[8–10] The Fluorotron Master excites and collects light from opposite sides of an objective lens, while scanning along the optical axis of the eye. The 'focal diamond' of this instrument is the intersection between the excitation (420–490 nm) and emission (530–630 nm) beams, which measures 0.05 × 0.05 × 0.85 mm (height, width, depth).[11] Due to the limited instrument spatial resolution, one must emphasize the need for a pristine central cornea for accurate measurements.[11]

Table 1. Descriptive Statistics of P_{dc}.

Time From Awakening	*N* of Eyes	Mean (SD)	95% CI
1 h	173	0.136 (0.090)	0.123, 0.150
2 h	285	0.090 (0.052)	0.084, 0.096
3 h	83	0.099 (0.051)	0.088, 0.110
4 h	60	0.089 (0.058)	0.074, 0.104
5 h	32	0.095 (0.041)	0.080, 0.110
6 h	16	0.065 (0.031)	0.048, 0.081
7 h	22	0.066 (0.033)	0.051, 0.081
8 h	35	0.090 (0.033)	0.078, 0.101
9 h	31	0.086 (0.055)	0.066, 0.106
10 h	19	0.090 (0.062)	0.059, 0.120
11 h	17	0.105 (0.063)	0.072, 0.137
12 h	43	0.086 (0.059)	0.068, 0.104
13 h	59	0.082 (0.047)	0.070, 0.094
14 h	33	0.095 (0.066)	0.071, 0.118
15 h	46	0.090 (0.056)	0.074, 0.107
16 h	32	0.084 (0.036)	0.071, 0.097
17 h	11	0.079 (0.033)	0.057, 0.101

Procedures

Each visit consisted of a P_{dc} measurement conducted by a trained technician. A licensed clinician performed a corneal assessment with white light on a slit lamp prior to the P_{dc} measurements, as well as a corneal punctate staining examination after P_{dc} measurements. Two μL of 0.35% fluorescein was instilled onto the subject's central superior bulbar conjunctiva for permeability measurements. Staining was assessed and assigned grade 0 if no punctate staining points were present, grade 1 if fewer than four points were present, grade 2 if 5 to 10 points were present, grade 3 if 11 to 25 points were present, and grade 4 if 26 or more points were present.[12] For accurate measurement of P_{dc}, data with central corneal staining greater than grade 1 were excluded from analysis.

The difference between subjects' awake time and the beginning of their P_{dc} measurements was calculated to determine the subjects' time-awake (T_A). A predetermined randomization scheme was employed for the order of measurements between fellow eyes and machines. Study visits were scheduled at different T_A to ensure that individual eyes were measured at different time points in their diurnal cycle. Specifically, subjects were asked to report to the UCB-CRC (University of California, Berkeley Clinical Research Center) for up to a total of six measurements; four during the day at T_A between 0 and 2 hours, 2 and 4 hours, 4 and 8 hours, and 8 and 12 hours, one collected immediately before sleeping, and one immediately after awakening.

Statistical Analysis

Histogram, Quantile-Quantile (Q-Q) plot, and Kolmogorov-Smirnov test were used to examine the underlying distribution of P_{dc}. The lower boundary of potential outliers was set as the detection limit of the instrument (numbers that could not be rounded up to at least 0.01). The upper boundary was set as 2.5 SDs plus the P_{dc} hourly mean. The difference between the hourly mean P_{dc} during the 17-hour period was examined by post hoc test with Tukey adjustments. Scatterplot of P_{dc} against awakening time with a nonparametric LOESS curve was used to aid the assessment of the P_{dc} diurnal pattern. The LOESS curve smoothed the scatterplot by summarizing the central tendency of P_{dc} distribution at each localized subsets of data, thus providing guidance for our parametric model specification. Mixed-effect models were developed to investigate the association between P_{dc} and factors of interest, including T_A, age, sex, ethnicity, and interactions of the above. The correlation over repeated measurements and between fellow eyes was accounted for as random effects.

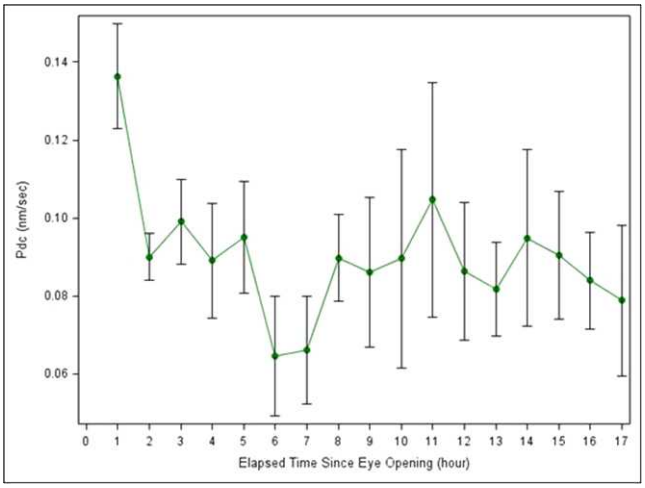

Figure 1. Hourly mean P_{dc} measured in a 17-hour period from awakening. P_{dc} showed a significant reduction from awakening to 2 hours ($P < 0.05$). The P_{dc} variation after 2 hours was not statistically significant ($P > 0.05$). Points represent mean. *Error bars* represent 95% CI.

Results

A total of 1097 P_{dc} data points were collected. On average, each subject provided two sets (SD = 2; range, 1–16) of P_{dc} measurements of both eyes, where 10% of subjects provided five sets or more; 21% of 2 to 4 sets, and the remaining 69% only one set of data. Fifty-nine observations with central corneal staining greater than 1 were excluded. Based on an a priori criterion, 11 P_{dc} data below the lower limit and 30 beyond the upper limit were excluded from analysis. The final data set pooled into statistical analysis contained 997 observations from 276 subjects (42% Asians, 58% non-Asians; 49% females, 51% males). The P_{dc} measurement time from awakening ranged from 3 minutes to 17 hours.

The descriptive statistics of hourly P_{dc} are summarized in Table 1. The highest mean P_{dc} was 0.136 [0.123, 0.150] nm/sec, observed within 1 hour of awakening. The hourly mean P_{dc} with 95% confidence interval (CI) is plotted in Figure 1. Results from post hoc *t*-test with Tukey adjustments showed that mean P_{dc} at 1 hour was significantly higher than all subsequent hourly means ($P < 0.05$). There was no significant P_{dc} change after 2 hours ($P > 0.05$) from awakening. A scatterplot of P_{dc} with a nonparametric LOESS curve in Figure 2 characterized the P_{dc} dynamics: a fast drop of P_{dc} from awakening up till nearly 2 hours, which can be regarded as a recovery period, followed by a plateau.

The results from mixed-effect modeling are presented in Table 2. P_{dc} values were transformed to natural logarithm to approximate normal distribution. Model 1 investigated the P_{dc} recovery period from awakening to 2 hours. Model 2 examined the P_{dc} plateau period. Covariate candidates included age, sex, ethnicity, awakening time, and a quadratic term of awakening time. Other covariates considered the interaction variables of age and sex, age and ethnicity, sex and ethnicity, awakening time and age, awakening time and sex, and awakening time and ethnicity. Due to the long duration of the study, a variable "Days *x* machine," representing the number of days from the

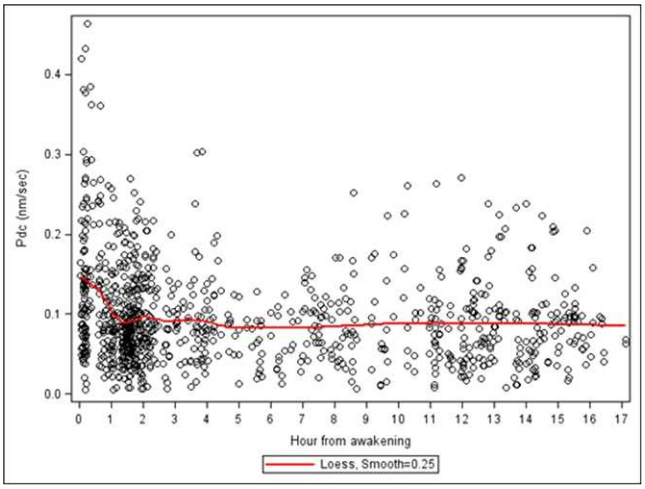

FIGURE 2. P_{dc} measurements in a 17-hour period from awakening fitted with LOESS curve.

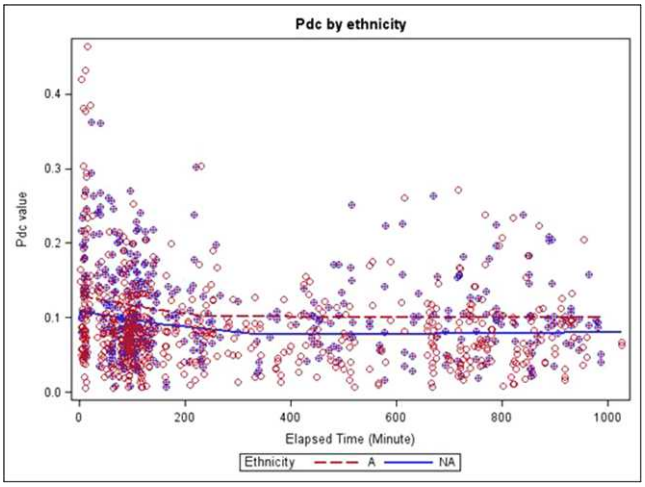

FIGURE 3. P_{dc} measurements for Asians (*red circles*) and non-Asians (*blue circles*) with LOESS fit (*red dash line* for Asians and *blue line* for non-Asians) in a 17-hour period from awakening.

first use of each machine, was added in the models to adjust for the effect of potential instrument and observer bias.

Model 1 indicated a significant decline of P_{dc} with length of awake time ($P = 0.000$). Model 2 showed no significant evidence of association between P_{dc} and awake time after 2 hours, confirming the observation of plateau. Both models showed that P_{dc} in Asians was significantly higher than in non-Asians and the difference was relatively constant (Fig. 3). P_{dc} in males was higher than in females during the first 2 hours with marginal significance ($P = 0.098$). Such sex difference was not observed after 2 hours. Age was positively associated with P_{dc} during the whole 17-hour period, indicating a higher P_{dc} (i.e., weaker epithelial barrier function) with age in the study age group of 18 to 38 years. None of the interaction terms between ethnicity, sex, age, and T_A showed statistical significance. Both models suggested a need to adjust for the duration of instrument usage of one of the fluorotrons ($P = 0.003$ in Model 1, $P = 0.016$ in Model 2).

DISCUSSION

The goal of our study was to gain a better understanding of diurnal fluctuations in corneal epithelial barrier function and its association with age, sex, and ethnicity.

Our study found that the overall trend of corneal epithelial permeability was highest within the first 2 hours after awakening and with the greatest decrease in P_{dc} occurring in the first hour. Beyond the first 2 hours, our results suggest that the corneal epithelial barrier function remains relatively stable.

TABLE 2. Mixed-Effect Models of ln(P_{dc}) Parameter Estimates

	Model 1, Within 2 h		Model 2, After 2 h	
	Estimate	*P*	Estimate	*P*
Intercept	−2.50	0.000	−3.33	0.000
Ethnicity, reference = non-Asian	0.30	0.000	0.25	0.000
Sex, reference = male	−0.12	0.098	0.002	0.972
Age	0.02	0.048	0.03	0.001
Time awake, h	−0.35	0.000	−0.007	0.128
Days × machine A	−0.0005	0.003	−0.0003	0.011
Days × machine B	−0.0002	0.370	0.0001	0.707

It has been reported that the external ocular defense system changes its role during prolonged states of eye closure, presumably to be more proactively defensive in a hypoxic, warm, and moist closed-eye environment.[13] Since eye closure–induced hypoxia does not significantly increase P_{dc},[1] we speculate that the increase in P_{dc} immediately after overnight sleep is primarily attributed to changes in tear biochemistry. Within minutes of prolonged eye closure, a subclinical inflammatory state is initiated via the complement pathway, and a vast number of polymorphonuclear (PMN) cells and tissue-destructive enzymes are recruited to the tear film via chemotactic cytokines.[14] Further, our recent unpublished data (Lin MC, et al. *IOVS* 2013;54: ARVO E-Abstract 5403) showed that IL-8, among 11 cytokines, was the only cytokine that exhibited a significant increase in concentration immediately after overnight eye closure, followed by recovery to the baseline level within 2 to 3 hours after awakening. The increased concentration of IL-8 in tears upon awakening may be related to a substantial infiltration of PMN cells into the tears during sleep as IL-8 has been known as a potent PMN chemoattractant. Further investigation is warranted to understand the mechanisms responsible for compromising corneal epithelial barrier function due to overnight eye closure.

This study also shows that the Asian group had statistically significant higher corneal epithelial permeability within the first 2 hours of awakening as compared with the non-Asian group (Model 1), a finding in agreement with previously reported studies.[1,15] The inherent disparity in corneal epithelial permeability between Asians and non-Asians has been attributed to several differences in lid anatomy and physiology in previous studies. The volume of the preaponeurotic fat pad as well as volume of adipose tissue is known to be higher in Asians than in Caucasians.[16,17] In addition, the tighter positioning of the inferior and superior lids of Asians may lead to higher shear mechanical forces across the ocular surface.[18] As the eyelids open and blink with regularity upon awakening and throughout the remainder the day, the shear force provided by the lid wiper applies mechanical stress to the ocular surface,[19] resulting in a generalized depression in epithelial integrity in Asian corneas. Whether this inherent difference may place Asians at a higher susceptibility to ocular complications requires further investigation. Of interest, a recent longitudinal study has shown that contact lens induced adverse events during 30-day continuous wear was associated

with higher baseline corneal epithelial barrier function compared with corneas that did not develop complications.[2]

With respect to sex, higher corneal epithelial permeability was found in males than in females; however, it was not found to be statistically significant ($P = 0.098$). Previous studies have found differences between males and females in corneal epithelial gene expression that may affect the innate barrier function of the cornea.[20] It has been reported that the probability of an adverse event with contact lens wear has been found to be higher in males.[21–24] However, it is unknown whether this more frequent occurrence in males is due to an intrinsic characteristic of the inflammation system on the male ocular surface, or if there are other influences involved, such as compliance and hygiene.

Our study found that with increased age, P_{dc} values increased. Although a previous study[15] found no age effect on P_{dc} at least 4 hours after awakening, our study included a much larger sample size and included measurements immediately after awakening and beyond. However, a limitation of our study was the narrow age range between 18 and 38 years. Further investigation with a broader age range of the study cohort is needed to further understand the age effect on P_{dc}.

One clinical application when considering the corneal epithelial barrier function is to assess optimal instillation time for topical ophthalmic medications in order to provide patients with favorable drug absorption and efficacy. Low bioavailability requires topical glaucoma medications to have frequent dosing, which makes patient compliance a major challenge for effective topical therapy.[25] Our study findings suggest that in order to maximize the absorption of topical ophthalmic medications, patients can ideally administer their medications within 2 hours of awakening to maximize passage of the drug through the corneal epithelium. It is important to note that ocular surface toxicity is closely associated with many common preservatives in the topical medications, such as belzalkonium chloride, and increases corneal epithelial permeability by disrupting corneal epithelial intercellular tight junctions.[26,27] The question remains whether medications can be most effectively absorbed by optimally increased corneal epithelium permeability due to the physiologic corneal epithelial barrier function or with the use of preservatives disrupting epithelial tight junctions.

In conclusion, our results show that baseline corneal epithelial barrier function varies significantly by ethnicity, awake time, and possibly age. The impact of these differences must be considered in studies that use corneal epithelial permeability as a quantitative measure of the integrity of corneal surface physiology.

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