

# Diurnal variation in central corneal thickness and intraocular pressure in eyes with pseudoexfoliation syndrome without glaucoma

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**Aim:** The aim was to ascertain if any differences exist in diurnal central corneal thickness (CCT) and intra-ocular pressure (IOP) between eyes with pseudoexfoliation (PXF) syndrome without glaucoma and eyes with no ocular pathology. A secondary aim was to determine whether there was a significant relationship between CCT and IOP. **Settings and Design:** This study was a prospective design conducted within a hospital setting. **Materials and Methods:** The experimental group consisted of seven participants with bilateral PXF (14 eyes) and the control group comprised of 15 participants (30 eyes). Testing included CCT and IOP measured at four different times on one given day (8.00 a.m.; 11 a.m.; 2 p.m. and 5 p.m.). **Statistical Analysis:** The data were analyzed with the generalized linear latent mixed model. **Results:** PXF eyes displayed a significantly thinner overall mean CCT (520  $\mu\text{m}$ ) compared to controls (530  $\mu\text{m}$ ). Furthermore, a significant reduction in CCT and IOP occurred in the PXF group from 8 a.m. to 5 p.m. The mean overall IOP in PXF eyes was significantly lower than the control group. A significant association between IOP and CCT was also found in PXF eyes. **Conclusions:** Displaying a significantly thinner mean CCT highlights the importance of measuring CCT in an ophthalmic clinical setting as to avoid falsely underestimated IOP measurements in such a high-risk glaucoma population. Furthermore, a statistically significant correlation between IOP and CCT in PXF eyes suggests that the reduction in CCT that occurred in PXF eyes between 8 a.m. and 5 p.m. may be partly responsible for the reduction in IOP measurements.

**Key words:** Central corneal thickness, diurnal variation, glaucoma, intra-ocular pressure, pseudoexfoliation syndrome

Pseudoexfoliation (PXF) syndrome results in the accumulation of pseudoexfoliative material particularly at the pupillary margin of the iris and throughout various structures in the anterior chamber of the eye.<sup>[1-3]</sup> Substantial research has been conducted to determine the effect PXF syndrome has on central corneal thickness (CCT).<sup>[4-9]</sup> To date, numerous studies<sup>[4,6-9]</sup> have reported that those eyes with PXF (with or without glaucoma) display thinner central corneas compared to those eyes with no ocular pathology, with only one study suggesting that an increase in CCT occurs.<sup>[5]</sup> Displaying a thinner cornea in itself is considered to be a significant risk factor in the development of glaucoma due to the underestimation of intra-ocular pressure (IOP) recordings.<sup>[10]</sup> Assessing the diurnal variation in CCT could also enhance the earlier detection of those individuals who are at risk of developing glaucoma as a variation in CCT throughout the day would cause a correspondingly different IOP measurement.

It has been well-established throughout the literature that significant diurnal fluctuations in CCT occur in subjects with no ocular pathology when CCT has been assessed over

a 12–48 h period.<sup>[11-14]</sup> The consensus in the literature is that CCT is thickest in the morning upon awakening and gradually thins as the day progresses, with the greatest proportion of this variation occurring in the 3 h after awakening.<sup>[11-14]</sup> More recent studies on individuals with no ocular pathology,<sup>[15-17]</sup> and those with glaucoma,<sup>[18,19]</sup> who have explored daytime (circadian) variations in CCT and its relationship to the circadian variations in IOP have not been in agreement of the importance of regular CCT examination. To date, no study has assessed the diurnal variation of CCT in PXF eyes without glaucoma which could prove pivotal so that the timing of glaucoma diagnosis for an individual is not overlooked.

## Materials and Methods

Based on previous literature,<sup>[4,18]</sup> a power calculation had confirmed that to have a 95% probability of identifying a difference of 18  $\mu\text{m}$  in mean CCT between PXF eyes and non-PXF eyes, the study required 6 participants (3 with PXF and 3 without PXF). To detect a 9  $\mu\text{m}$  change in diurnal CCT, these figures rose to 12 participants (based on a population with an SD of 30  $\mu\text{m}$ ).

This study was a prospective experimental design conducted within a hospital setting in Melbourne, Australia. Seven (2 males and 5 females) nonglaucomatous subjects with bilateral PXF (mean age, 69.86 years  $\pm$  8.4) and 15 (4 males and 11 females) healthy age-matched participants with no ocular pathology (mean age, 66.73 years  $\pm$  9.2) were included in this study. All participants undertook visual screening prior to the participation which consisted of relevant history questions, assessment of their visual function and both IOP and CCT measurements. Individuals who possessed one or more of the following; diabetes, uncontrolled hypertension, ocular injury, severe dry eye, corneal disease or corneal surgery were excluded

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from participation in this project as all of these factors have been proven to influence CCT.<sup>[6,11]</sup> Criterion for participation in the PXF group was the presence of characteristic granular deposits on the anterior segment structures of the eye on slit lamp and (or) gonioscopy examination. Furthermore, these participants showed no signs of the presence of glaucoma as determined via a comprehensive glaucomatous investigation performed by an ophthalmologist and imaging. Informed consent was obtained from subjects recruited for the study.

The participants that were eligible to participate in the study (both control and experimental) were required to undertake 4 separate testing sessions throughout 1-day during the time period of 8.00 a.m. to 5.00 p.m. (8.00 a.m.; 11 a.m.; 2 p.m. and 5 p.m.). The times chosen are in accordance with similar studies in this area of research<sup>[15,17]</sup> and were selected as they are within routine ophthalmic clinical consulting hours and if diagnoses or treatment is prescribed or carried out it is customarily based on the clinical measures taken during this time frame.

During each of the four testing sessions all participants (experimental and control) had their CCT and IOP measured. CCT was assessed using the DGH 55 pachmate handheld ultrasonic pachymeter. This pachymeter rapidly obtains and stores a total of 25 measurements of each eye, and an average of these is given. IOP measurements were obtained using the Perkins applanation tonometer. Previous studies<sup>[20,21]</sup> that have compared the accuracy of the Perkins tonometer to that of the “gold standard” in IOP evaluation, the Goldmann tonometer, have found IOP readings from the two instruments to be highly correlated. All measurements were undertaken by the same individual.

With the complex data structures in mind, the generalized linear latent mixed model (GLLMM)<sup>[22,23]</sup> was proposed. This is a general class of multiple parametric regression model designed for analyzing complex data structures. Unlike the conventional linear regression which is only appropriate for nonhierarchical data structure without multi-stage features, GLLMM is more versatile, robust, and precise. While there were 22 subjects involved, the data were collected from 44 eyes (coded 1: Right, 2: Left). With CCT and IOP measured longitudinally from 2 eyes on four sessions (SESSION; a: 8 a.m., b: 11 a.m., c: 2 p.m., d: 5 p.m.), there were a total of 176 data

points. This can be visualized as a multi-level or hierarchical structure because the 176 measurements were nested in the 44 eyes, which in turn nested within the 22 patients. As depicted in Fig. 1, Group (1: Control, 2: PXF) and IOP were the covariates included to explain the difference in CCT, and the variations in IOP were in turn to be explained by Group, while considering the time effects (SESSION). The proposed analysis could ascertain (1) if there was a difference in CCT between the controls and those on PXF, (2) how IOP affected CCT, (3) whether there was a difference in IOP between Groups, and (4) if CCT varied over time. Analyzed with Stata 12.0 (Stata Corporation, Texas, USA), all statistical tests were performed with 95% confidence intervals (CIs) (or equivalently, 5% level of significance).<sup>[24]</sup> A covariate is deemed to be statistical significant if the 95% CI does not contain 0.

This study had institutional ethics approval and strictly adhered to the principles of the Declaration of Helsinki.

## Results

The sample characteristics of the control and experimental groups over the four sessions are summarized in Table 1.

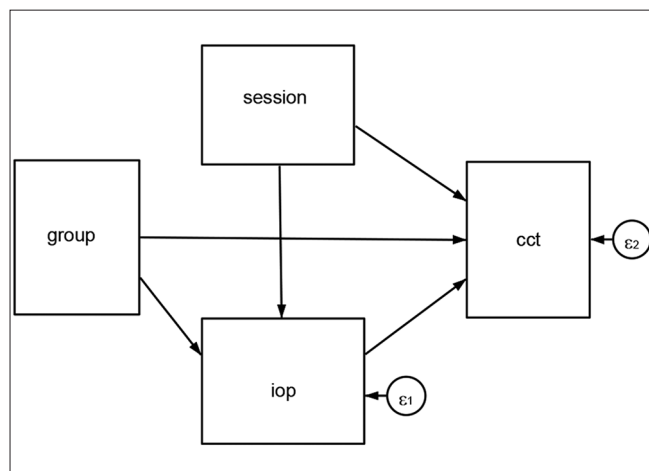
Central corneal thickness values were significantly thinner in PXF eyes at all time periods when compared with the control eyes. PXF eyes displayed a significantly thinner overall mean CCT of 9.87  $\mu\text{m}$  when compared with the controls. CCT values declined significantly over time when combining all control and experimental data. It is clear that this trend was more significant in PXF eyes when compared to control eyes [Fig. 2b] suggesting that a significant reduction in CCT occurred in the PXF group from 8 a.m. to 5 p.m. The mean overall IOP in PXF eyes was significantly lower than the control group. That is, PXF eyes displayed a lower overall mean IOP of 2.27 mmHg when compared with the controls. There was also a significant decline in IOP over time when combining all experimental and control data [Table 2]. It is clear that this trend was more significant in PXF eyes (4 mmHg) when compared to control eyes (0.8 mmHg) [Fig. 2a] which suggests that a significant reduction in IOP occurred in the PXF group from 8 a.m. to 5 p.m. The spread of CCT and IOP for the right and left eyes is demonstrated in Fig. 2.

A statistically significant correlation between IOP and CCT was revealed in PXF eyes at a 5% level. On average, a 1 mmHg increase in IOP was associated with a 1.13  $\mu\text{m}$  increase in CCT. It was observed that a reduction in IOP in the PXF group correlated to a reduction in CCT [Table 2].

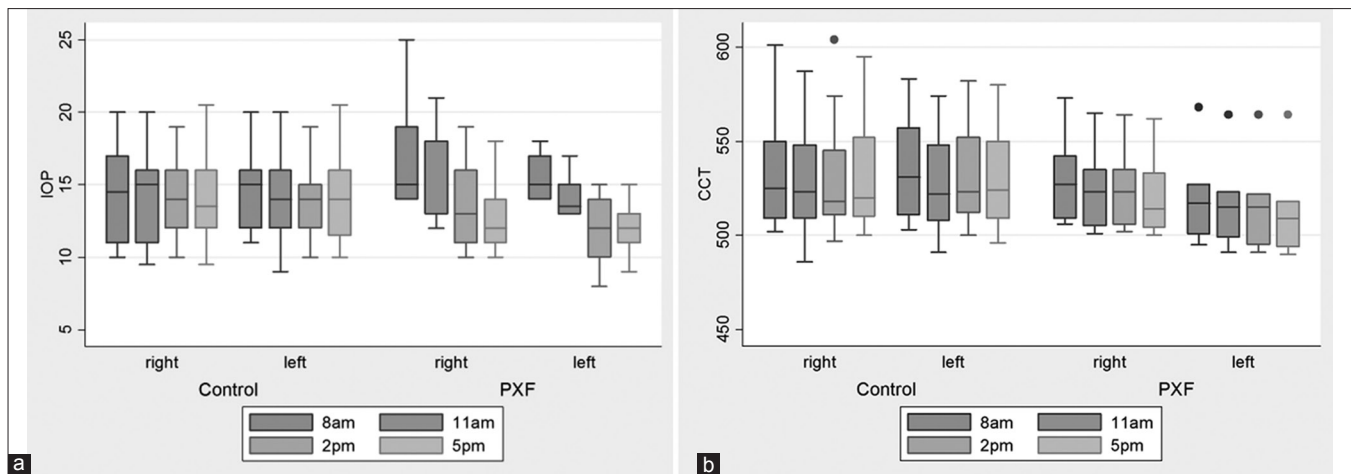
## Discussion

To the best of our knowledge, this study is the first to explicitly investigate the diurnal variation of CCT in patients with PXF without glaucoma.

The finding of a small, yet statistically significant diurnal variation in CCT during normal clinical consulting hours in the experimental group suggests that true IOP may be over- or under-estimated depending on an individual's scheduled appointment time. Furthermore, like previous studies conducted on individuals with no ocular pathology<sup>[11-14]</sup> CCT was found to be thickest in the morning and gradually thinned as the day progressed in the control group. Despite this, it remains unclear as to whether this amount of variation (experimental = 6.45  $\mu\text{m}$ , control = 5.70  $\mu\text{m}$ ) in CCT is



**Figure 1:** Proposed analysis: Correlation patterns among the variables



**Figure 2:** Intraocular pressure and central corneal thickness measurements of control and pseudoexfoliation group. (a) IOP from 8 a.m. to 5 p.m. (b) CCT from 8 a.m. to 5 p.m

ultimately too small to be clinically relevant. Therefore, more research is required to determine whether single or repeated measures of CCT are advantageous in individuals with PXF without glaucoma, particularly when first monitoring for glaucoma diagnosis.

Previous research suggests that displaying a thin cornea is considered a risk factor for glaucoma outcomes.<sup>[25,26]</sup> The finding of the PXF group displaying a significantly thinner CCT mean of 520 µm compared with the control group is consistent with previous studies.<sup>[4,6-9]</sup> This highlights the importance of measuring CCT in all PXF individuals without glaucoma in an ophthalmic setting as to avoid falsely underestimated IOP measurements caused by a thin CCT.

Similar to previous researchers that have assessed the diurnal variation in IOP in PXF subjects,<sup>[27]</sup> this study found that the IOP variation was higher in PXF eyes when compared to the control group at a statistically significant level. The difference between the mean IOP at 8 a.m. and the mean IOP at 5 p.m. in PXF eyes in this study was 4 mmHg when compared to that of 0.8 mmHg in the control group. The variation in IOP followed similar trends to those previously described<sup>[28]</sup> with IOP readings being higher in the morning and reduced later in the afternoon. This finding is noteworthy as a wide diurnal fluctuation in IOP is believed to be a major risk factor in glaucoma development and progression.<sup>[29]</sup>

Previous researchers are not in agreement regarding the relationship between diurnal fluctuations in CCT and IOP.<sup>[15-19]</sup> The results of this study showed a statistically significant correlation between mean IOP and mean CCT in PXF eyes. This is similar to the findings of Fogagnolo, Rossetti, and Orzalesi (2006) in patients with primary open-angle glaucoma and suggests that the variation in IOP that occurs throughout the period of a day may have a dependent relationship with the variation that occurs in CCT. That is, a statistically significant reduction in CCT that occurred in PXF eyes between 8 a.m. and 5 p.m. may be partly responsible for the reduction in IOP measurements that were found during the same time period.

Strengths of the current study include an age-matched control group who were free of any ocular disease and that a single examiner was utilized for all CCT and IOP measurements in

**Table 1: Sample characteristics**

	Mean (range)	
	IOP (mmHg)	CCT (µm)
Control group (n=15)		
Right eye		
8 am	14.6 (10.0-20.0)	537.3 (502-601)
11 am	13.9 (9.5-20.0)	528.0 (486-587)
2 pm	14.2 (10.0-19.0)	529.6 (497-604)
5 pm	13.8 (9.5-20.5)	530.7 (500-595)
Left eye		
8 am	14.8 (11.0-20.0)	532.7 (503-583)
11 am	14.0 (9.0-20.0)	527.7 (501-565)
2 pm	13.9 (10.0-19.0)	529.9 (500-582)
5 pm	14.0 (10.0-20.5)	527.9 (496-580)
PXF group (n=7)		
Right eye		
8 am	17.0 (14.0-25.0)	529.9 (506-573)
11 am	15.1 (12.0-21.0)	524.3 (501-565)
2 pm	13.7 (10.0-19.0)	525.0 (502-564)
5 pm	12.9 (10.0-18.0)	522.4 (500-562)
Left eye		
8 am	15.7 (14.0-18.0)	518.7 (495-568)
11 am	14.1 (13.0-17.0)	516.4 (491-564)
2 pm	11.9 (8.0-15.0)	516.1 (491-564)
5 pm	11.9 (9.0-15.0)	513.3 (490-564)

PXF: Pseudoexfoliation, IOP: Intraocular pressure, CCT: Central corneal thickness

order to counteract the effect of any inter-examiner variability. Despite this, operator bias as a result of human error cannot be excluded as it would not be possible for all measurements to be in exactly the same central location for all CCT measurements. However, as multiple CCT measurements were taken at each time point it is doubtful whether this would produce significant bias.

Our study was limited due to the small sample size, gender imbalance, and an uneven number of participants in

**Table 2: Multilevel path analysis of IOP and CCT with GLLAMM**

CCT	Coefficients	95% CI
Direct effects:		
Group		
Control	Reference	Reference
PXF	-9.87*	-12.94- -6.80
IOP	1.13*	0.46-1.80
Indirect effect:		
Control		
PXF	0.02	-0.23-0.28
Session		
8 am	Reference	Reference
11 am	-5.84*	-8.39- -3.29
2 pm	-5.19*	-7.74- -2.63
5 pm	-6.29*	-8.84- -3.74
<b>IOP</b>		
Direct effect:		
Group		
Control	Reference	Reference
PXF	-2.27*	-2.81- -1.72
Session		
8 am	Reference	Reference
11 am	-0.81*	-1.18- -0.44
2 pm	-1.09*	-1.46- -0.72
5 pm	-1.25*	-1.62- -0.88

\*Statistically significant at 5% level of significance. PXF: Pseudoexfoliation, IOP: Intraocular pressure, CCT: Central corneal thickness, GLLAMM: Generalized linear latent mixed model, CI: Confidence interval

the control and experimental groups. Despite this, the sample size exceeded that estimated from the power calculations. In both groups, more females than males were recruited for participation. It still remains unclear whether definite gender prevalence exists in the PXF syndrome. Some researcher's state that men and woman are equally affected<sup>[3]</sup> but others claim that the prevalence is greater in the male population.<sup>[30]</sup> As such, it is likely that these baseline imbalances in gender are not critical. Furthermore, the prevalence of PXF in Australia is only 0.98%<sup>[2,3]</sup> which is the lowest reported prevalence of PXF in any country and was a significant obstacle when recruiting participants for the experimental group in this study.<sup>[2,3,31]</sup> Further, to increase the sample size of participants, all IOP and CCT measurements were taken on both eyes in the control and experimental groups which may have confounded results.

Future research could focus on undertaking a study with a larger cohort of participants in order to establish whether this was a representative finding of the greater PXF Australian population. Moreover, it may be that short-term fluctuations in CCT that relate to changes in corneal hydration throughout the day do not influence corneal rigidity to the same extent as corneal thickness. For example, normal increases in corneal hydration that occur throughout the day due to aqueous ingress through the endothelium or changes in endothelial pump function will produce higher CCT readings, however, the force of the cornea that resists to flattening (corneal rigidity)

may not be influenced as the structure of the collagen lamellae does not change. Therefore, it may also be advantageous for future lines of research to correlate with dynamic contour tonometry (DCT).<sup>[32]</sup> DCT is an updated form of tonometry designed to measure IOP independent of corneal properties such as CCT and rigidity. Rather, it is based on a new physical principle that when the contour of the corneal surface and the tonometer match, the pressure measured at the surface of the eye equals the pressure inside the eye.<sup>[33]</sup> As a result, DCT may be less affected by the biomechanical corneal changes that can be associated with PXF and, therefore, may be a more accurate measure of IOP in these cases.<sup>[34]</sup>

The pathogenesis of glaucoma development in individuals with PXF still remains largely unclear. PXF with associated open angle glaucoma represents a somewhat severe and progressive form of secondary open angle glaucoma with high IOP levels and large fluctuation in IOP.<sup>[3,34]</sup> This notion in the literature is supported by our findings that the overall diurnal variation between the 8 a.m. and 5 p.m. IOP measurements were higher in PXF eyes compared to control eyes. An unresolved issue in the literature at present is discovering why some individuals with PXF go on to develop glaucoma while others do not. It may be that combinations of such factors as angle anatomy, the gradual build-up of exfoliative material over time, and the site and degree of pseudo-exfoliative material present that predispose to the development of glaucoma in PXF syndrome by contributing to a decreased facility of aqueous outflow.<sup>[34]</sup> Our study would suggest that diurnal variation has an influence on the CCT in individuals with PXF without glaucoma and may have a confounding effect on the underestimation of IOP in this population. All of these factors must be closely monitored so that glaucomatous damage does not go undetected.

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