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Research Article

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Effect of Age on Individual Corneal Epithelial Thickness in Normal Eyes as Measured with Ultrasound Pachymeter

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Abstract

Purpose: *The goal of this study was to determine age-relatedvariation in the thickness of the corneal epithelium using ultrasound pachymeter.*

Methods: *One hundred three patients were enrolled in this study and grouped according to age: Group A (< 30 years), group B (31-40 years), group C (41-50 years), group D (51-60 years), group E (61-70), and group F (> 71). Total corneal and corneal epithelial thickness measurements were made using the SonogageCorneo-Gage Plus 2 (Cleveland, Ohio) ultrasound pachymeter. Correlations of central epithelial thickness with central total corneal thickness, age, and gender were calculated. In addition, mean central epithelial thickness (CET) was measured. One-way ANOVA testing and post hoc analysis with the Tukey test and Pearson correlation were performed to analyze data.*

Results: *The mean epithelial thickness at the central cornea was 47.88±1.15 µm, with no statistically significant difference between right and left eyes, and no significant differences in gender or central total corneal thickness. The difference in mean epithelial thickness across age groups was statistically significant (p <0.008). The mean epithelial thickness of the > 71 years group was significantly thinner than that of the < 30 years, 31-40, 41-50, and 51-60 years age groups.*

Conclusions: *Ultrasound pachymeter of the corneal epithelium demonstrated that the oldest age group (> 71 years) had significantly thinner central corneal epithelial thickness than the younger age groups. There was no correlation between epithelial thickness, total corneal thickness, gender, or laterality.*

Keywords: Aging, Cornea, Epithelium, Pachymeter, Ultrasound

Introduction

The outermost layer of stratified corneal epithelium is five to seven cells thick and measures approximately 50 µm [1,2]. Bowman's layer, a dense collagenous layer approximately 8 to 10 µm thickness lies between the epithelium and stroma. The anterior margin of Bowman's layer presents a sharp interface with the lamina densa of the basement membrane of the overlying epithelium [3]. It has been calculated that the epithelium accounts for an average of 1.03 diopters of corneal power at the central 2-mm diameter zone [4].

Different methods have been used to measure corneal epithelial thickness: optical coherence tomography (OCT), confocal microscopy, optical pachymetry, and through focusing confocal microscopy [5-10]. All of these studies measured average central epithelial thickness.

High-frequency ultrasound provides sufficient resolution to resolve the cornea and its constituent layers, including the epithelium. The sharp anatomic discontinuity and density change between the epithelium and Bowman's layer results in a high-amplitude reflection at the epithelial–Bowman's layer junction. Although the cornea has evolved to minimize abrupt optical refractive changes between layers to prevent internal reflections, this is not the case for ultrasound. Reinstein, et al. previously described the use of very high-frequency (VHF) digital ultrasound to measure corneal epithelium, with the first confirmed measurement of the epithelium of the cornea in vivo using a prototype rectilinear VHF digital ultrasound scanning system in 1993 [11,12].

Reinstein, et al. demonstrated that acoustic interfaces detected were indeed located spatially at the epithelial surface and the interface between epithelial cells and the surface of Bowman's layer [13]. There is an increasing interest in the effects of aging in general and parameters of the aging process that can be objectively measured. The eye is of interest in that respect, and several ocular parameters have been defined in a review by Pathai, et al., such as the corneal endothelial cell layer, but changes in the epithelial thickness could be of interest as well [14,15].

Particularly interesting from the perspective of senescence is the recent observation that removal of the corneal epithelium can trigger apoptosis in the underlying anterior keratocytes [16]. Epithelial disease has the potential to contribute to cell loss with in the endothelial layer [17]. We conducted a study to determine changes in epithelial thickness that occur as a result of aging process. This was achieved by examining the corneas of 103 patients whohad no ocular pathology other than refractive error.

Methods

In this prospective, cross-sectional observational study, the subjects were recruited from a population of patients seeking routine eye examination at the ophthalmology outpatient clinic of the Altintepe Medical Center, Istanbul, Turkey, between November 2012 and June 2013.

Exclusion criteria included subjects with a history of corneal surgery, contact lens wear, suspected clinical and subclinical keratectasia, active anterior segment disease, ocular pathology that might alter the optical quality or preclude proper viewing of the fixation target of the instruments, and a best-corrected visual acuity below 20/40. Before inclusion in the study, all eyes underwent a complete ophthalmic examination that included measurement of the manifest refraction, corneal topography, slit-lamp microscopy, applanation tonometry, and indirect ophthalmoscopy. Topography and keratometry were assessed using the Orbscan II (Bausch & Lomb, Salt Lake City, Utah).

Informed consent was obtained from each subject after thorough explanationof the procedure and possible outcome. The study adhered to the tenets of the Declaration of Helsinki and was performed in accordance with an Institutional Review Board approved protocol. Subjects were divided into six age groups: <30, 31-40, 41-50, 51-60, 61-70, and > 71 years.

The central corneal thickness and epithelial thickness were measured with an ultrasound pachymeter (Corneo-Gage Plus, Sonogage, Cleveland, Ohio). Probe frequency was 50 MHz. Ultrasound epithelial thickness measurment was performed first. The subject was comfortably seated with the head upright and eyes in the primary position of gaze. After topical anesthetic drop (proparacaine0.5%) was instilled in the subject's eye, the probe was carefully aligned perpendicularly to and lightly touching the cornea. All measurements were performed by a trained ophthalmic technician between 10.00 am and 12.00 pm to avoid diurnal variation. The US pachymetry device takes multiple, rapid, and sequential readings during a single applanation of the probe. At least ten measurements of central epithelial thickness (CET) were made for each of the averaged recorded values. Central corneal thickness (CCT) was measured by determining the minimum of 10 consecutive central corneal measurements.

The statistical package used foranalyses was the NCSS (Number Cruncher Statistical System, Kaysville 2007, Statistical Software, Utah, USA). Descriptive statistics (average, minimum, maximum, standard deviation, and range) were calculated for each variable. One-way analysis of variance (one-way ANOVA) was employed to test for significance difference between groups. The post-hoc Tukey multiple comparison test was used to investigate which of the means were different by pairwise comparison of couples of groups. The Pearson correlation test was used to to determine relations between sets of data.

Linear regression analysis was performed to seek possible correlations between central epithelial thickness (CET) and age. Statistical significance was reached when p-value was < 0.05.

Results

A total of one hundred and three subjects (n = 103) comprising 72 females and 31 males took part in the study. The average age all subjects were 49.09 ± 16.50 years (range 16 - 82 years). Table 1 shows the descriptive statistics of the measured variables. images were obtained either with OCT 1000 (Topcon) or Nidek RS330 Scan Duo.

Table 1: Statistics of measured variables from all subjects

	Females	(n = 72)	Males	(n = 31)
Variable	Mean ± SD	Range	Mean ± SD	Range
CET, µm	47.93 ± 1.2	46 - 51	47.68 ± 0.99	46 - 50
CCT, µm	545 ± 34.43	479 - 648	545.55±33.34	459 - 598
Age, y	49.18 ± 15.77	17 - 81	48.87 ± 18.36	16 - 82

Analysis of the relationship between CCT and CET with age, gender and left/right eyes are tabulated in Table 2 and 3.

Table 2: Gender characteristics of subjects

Gender	Count	Age Mean ± SD	Range
Male	31 (30.10%)	48.87±18.36	16 - 82
Female	72 (69.90%)	49.18±15.77	17 - 81

Table 3: Statistics of measured variables according to laterality

Variable	RIGHT EYES Mean ± SD	LEFT EYES Mean ± SD	p
CET (µm)	47.91±1.16	47.8±1.12	0.316
CCT (µm)	544.8±34.35	545.53±33.87	0.052

Associations of CET and CCT using ultrasound pachymetry with other factors such as age, gender and left/right eyes were tested with the regression model.

Mean CCT measurements using ultrasound pachymetry were 545.55±33.34 and 545±34.43 µm in male and female subjects respectively. Mean CCT measurements were 544.8±34.35 and 545.53±33.87 µm in right and left eyes, respectively.

No statistically significant difference (ANOVA) in the mean value of CCT was found between right and left eyes (p=0.052). Also, there was no statistically significant difference in the mean values of CCT between male and female subjects (p=0.916).

Mean (±standard deviation) central epithelial thickness for all eyes was 47,88 ± 1,15 µm (95% confidence interval: 47.73 % to 48.04 %). Statistics of CET and CCT is shown in Table 4. Central epithelial thickness ranged from 46 to 52 µm for all eyes. Mean (± standard deviation) central epithelial thickness was 47,91±1,16 µm for right eyes and 47,8±1,12 µm for left eyes. No statistically significant difference between the mean central epithelial thickness of right and left eyes was noted (p=0.316). Mean CET measurements were 47,68±0,99 µm and 47,93±1,2 µm in male and female subjects respectively.

Table 4: Measured variables in the present study

Variable	Mean ± SD	Range	95% CI
CET (µm)	47.88 ± 1.15	46 - 52	47.73 – 48.04
CCT (µm)	545.17 ± 34.03	469 - 648	540.49 – 549.84

Gender was not correlated with CET measurements (p = 0.145) while age showed negative correlation with CET (r = -0.259, p= 0.0001).

Using linear regression analysis, the adjusted R^2 value was found to be 0,139 and the relationship between age and CET was formulated as follows (P 0.01):

Epithelial Thickness = 48.598 + (- 0.015*Age)

Figure 1 is a graphical representation of the regression of CET on age.

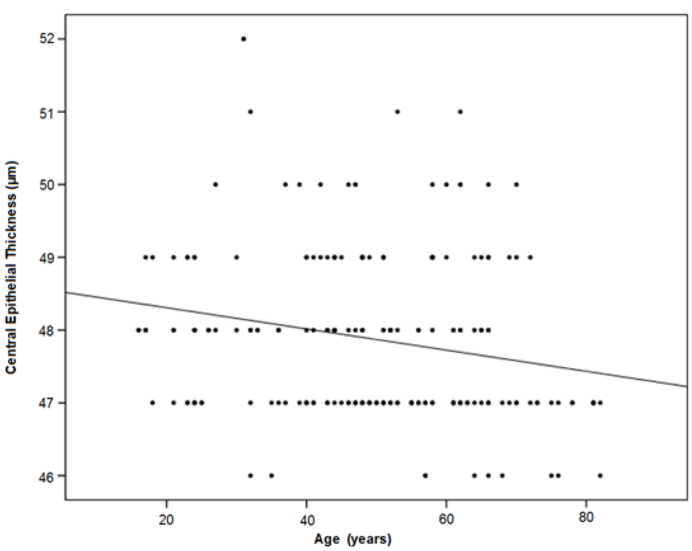

Figure 1: Regression of central epithelial thickness on age

The difference in mean CET across age groups was statistically significant (p 0.008) (Table 5).

Table 5: The effect of age group on measured variables in the present study

Age group (years)	Count	CET (µm)	CCT (µm)
< 30	32	48.03 ± 0.82	541.78 ± 31.15
		47 - 50	490 - 597
		47.73 – 48.33	530.55 – 553.01
31-40	26	48.19 ± 1.63	537.35 ± 39.14
		46 - 52	493 - 648
		47.54 – 48.85	521.54 – 553.15
41-50	48	48.02±1	548.06 ± 37
		47 - 50	479 - 648
		47.73 – 48.31	537.32 – 558.80
51-60	42	47.86 ± 1.1	554.31 ± 23.22
		46 - 51	518 - 598
		47.52 – 48.20	547.07 – 561.55
61-70	40	47.85 ± 1.23	539.65 ± 35.43
		46 - 51	469 - 609
		47.46 – 48.24	528.32 – 550.98
> 71	18	46.94 ± 0.64	545.67 ± 39.49
		46 – 49	490 - 618
		46.63 – 47.26	526.03 – 565.31
		p 0.008	p 0.295

Mean and standard deviation (top row), range (middle row) and 95% confidence interval (bottom row). CCT = central corneal thickness, CET = central epithelial thickness, µm = micron meter.

The mean CET of the > 71 years old was significantly thinner than that of the < 30 years, 31 - 40, 41 - 50, and 51 - 60 years age groups. Table 5 shows the mean value, the median, and the 95% CI for the mean at the various age groups. The general trend is the reduction of CET in the older age groups.

The correlation between age groups and epithelial thickness is shown in a box plot (Figure 2).

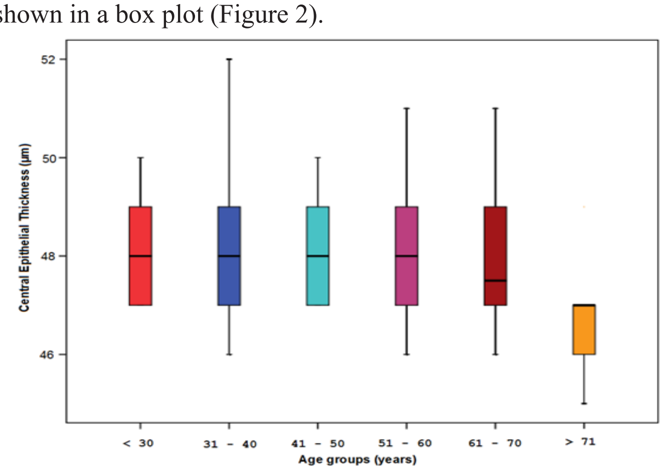

Figure 2: The Correlation between age groups and central epithelial thickness

A box plot displays statistical values for ranges of data. The line within the box marks the median. The boundary of the box indicates the 25th and 75th percentile. Results of Tukey multiple comparison test is tabulated in Table 6.

Table 6: Pairwise comparison of each group

Age groups (years)	p
< 30 / 31-40	0.994
< 30 / 41-50	0.998
< 30 / 51-60	0.986
< 30 / 61-70	0.983
< 30 / > 71	0.014
31-40 / 41-50	0.989
31-40 / 51-60	0.835
31-40 / 61-70	0.828
31-40 / > 71	0.005
41-50 / 51-60	0.982
41-50 / 61-70	0.980
41-50 / > 71	0.008
51-60 / 61-70	0.998
51-60 / > 71	0.047
61-70 / > 71	0.053

The correlation between age and epithelial thickness is shown in a scatter plot (Figure 2). The line in scatter plot is the linear regression (r^2 = 0.139,P = 0,0001).

There was no significant linear correlation between CET and CCT (Pearson correlation coefficient r = 0.087, P = 0,214).

Discussion

In this study, we investigated the corneal epithelial thickness differences among different age groups in a population of normal eyes.

Reinstein et al previously described the signal processing strategies for measurement of epithelial thickness. Two potential sources of uncertainty in ultrasound measurement of the thickness of the corneal epitheliufm are the epithelial speed of sound constant and the anatomic source of the echo we associate with the interface between the epithelium and Bowman's layer [13].

One other ultrasonic phenomenon that could theoretically affect accuracy is dispersion. In dispersion, the speed of sound increases with increasing frequency within the same medium; however, dispersion at frequencies between 30 and 60 MHz is negligible. Therefore, speed of sound constant errors in epithelial thickness measurements are justifiably negligible [18].

Bowman's layer is approximately 8-µm thick. Individual echo signals from the front and back surface of Bowman's layer are not resolved and Bowman's layer appears as a single reflection. 13 Frequency-dependent attenuation is of no practical significance in ultrasound biometry of the cornea at 50 MHz [18].

Central corneal epithelial thickness has been previously measured with the reported valuesvarying between 48±5 µm11 and 59.9±5.9 µm [19-22].

Artemis VHF digital ultrasound is carried out using an ultrasonic standoff medium, and so it is stated that it provides the advantages of immersion scanning (eg, the tear-film is not incorporated in the corneal or epithelial thickness measurement, and has no physical contact of the transducer with the cornea) [18].

Mean (± standard deviation) corneal vertex epithelial thickness for all eyes was 53.4 ± 4.6 µm. No statistically significant difference between the mean corneal vertex epithelial thickness of right and left eyes was noted [18].

The mean and standard deviation of central epithelial thickness found in this study are consistent with previously reported values obtained by a variety of measurement techniques.

Using a high frequency ultrasound pachymeter, we found the central epithelial thickness in 206 normal eyes to be 47,88±1,15 µm. Our mean central epithelial thickness is thinner than that reported by Reinstein, et al. who used immersion scanning technique that is widely accepted to provide more accurate measurementsas far as the effect of tear film thickness and physical corneal contact is concerned [18]. Thus we expected thicker mean values for central epithelial thickness but we have reached the opposite results.

Reinstein, et al. noted no statistically significant correlation between corneal vertex epithelial thickness and age. No statistically significant difference in corneal vertex epithelial thickness between the over and under 45-year-old patients was noted [18].

We found an average central epithelial thickness of 47,88±1,15 µm. The epithelial thickness was found to be thinner in the oldest age group.

Previous studies in humans have reported a reduction in central corneal thickness in Sjögren's and non Sjögren's dry eye; in these studies the central thickness of the corneal epithelium did not change or was not evaluated [23-27].

Kannelopoulos, et al. evaluated in vivo epithelial thickness in dry eye by anterior segment optical coherence tomography. This studyindicated increased epithelial thickness in dry eyes [28]. In the light of this study, we can eliminate dry eye as the cause of thinner epithelium in our old age group. Corneal epithelial thickness measurement may gain importance in forming normative database to be used in evaluating eye patients in near future. Large scale studies are required for this purpose.

To our knowledge, this is the first study to report the tendency of epithelial thinning in the human cornea in advanced ages. High frequency ultrasound thickness measurement of the corneal epithelium demonstrated that epithelial thickness is getting thinner in the oldest age groups. Knowledge of the age-related changes of epithelial thickness in the normal corneas should help in understanding corneal aging process, as well as improving the diagnosis in corneal diseases.

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