· Original article ·

Corneal densitometry and its correlation with anterior and posterior elevation in keratoconus

Mohammad Mirzaei, Nazli Taheri, Hossein Akhlaghi, Hesam Sadat Hashemi

Department of Ophthalmology, Nikookari Eye Hospital, Tabriz University of Medical Sciences, Tabriz 5154645395, Iran

Correspondence to: Nazli Taheri. Department of Ophthalmology, Nikookari Eye Hospital, Tabriz University of Medical Sciences, Tabriz 5154645395, Iran. Nazli.taheri81@yahoo.com

Received: 2017-10-29 Accepted: 2018-04-02

角膜密度测定法及其与角膜前、后仰角的相关性

Mohammad Mirzaei, Nazli Taheri, Hossein Akhlaghi, Hesam Sadat Hashemi

(作者单位:5154645395 伊朗,大不里士,大不里士大学医学院,Nikookari 眼科医院,眼科)

通讯作者:Nazli Taheri. Nazli.taheri81@yahoo.com

摘要

目的:评估在初治圆锥角膜患者中的角膜密度及其与前和后角膜仰角的相关性。

方法:使用 Pentacam 检测圆锥角膜。角膜密度测量是通过直径 12 mm 的区域测量,分为环形同心区域和深度,测量更多的角膜前后仰角。根据地形圆锥角膜分类对圆锥角膜进行分类。

结果:研究包括 152 例患者[72 例圆锥角膜患者(46 例男性,26 例女性)和 80 例健康对照受试者(50 例男性,30 例女性)]。两组间角膜 2 mm 中心处和角膜环状 2~6 mm 直径处角膜密度测量值差异有统计学意义(K=16.40±2.18 GSU, N=15.31±1.25 GSU, $P<0.0001$)。当不同深度的密度测量值时,前部层呈现最高值,明显高于中央层和后层的值。当测量不同深度密度值时,前层呈现最高值(KC=23.69±3.71 GSU, N=20.91±2.52 GSU, $P<0.0001$),显著高于中央层和后层值(KC=14.34±1.70 GSU, N=13.61±1.21 GSU, $P=0.001$;KC=11.40±1.23 GSU, N=12.35±0.88, $P=0.002$)。各层深度(前、中、后)角膜密度测量值与后角膜高度值的相关性分析显示两者显著相关(分别为 $r=0.293$, $r=0.278$ 和 $r=0.294$)。角膜光密度测定每层深度和角膜前角抬高之间没有发现类似的相关性(分别为 $r=-0.211$, $r=-0.101$, $r=0.99$)。在对照组受试者中,未发现每层深度角膜前/后角膜高度和角膜后向散射的显著相关性。

结论:光密度图显示前圆锥角膜中央区域的光后向散射较高。圆锥角膜患者的角膜光密度值与角膜 0~2、6~10、10~12 mm 环状区域前、中、后各层角膜高度相关。

关键词:角膜密度测定法;光后向散射;圆锥角膜;Scheimpflug 成像

引用:Mirzaei M, Taheri N, Akhlaghi H, Hashemi HS. 角膜密度测定法及其与角膜前、后仰角的相关性. 国际眼科杂志 2018;18(6):985-993

Abstract

• AIM: To evaluate the corneal densitometry in treatment-naive patients with keratoconus and its relevance with anterior and posterior corneal elevation.

• METHODS: Keratoconic corneas were examined using the Pentacam. Corneal densitometry was measured over a 12-mm diameter area, divided by annular concentric zones and depths, more over anterior and posterior corneal elevation was measured. Keratoconus was classified according to the topographic keratoconus classification.

• RESULTS: A total of 152 subjects [72 patients with keratoconus (46 male, 26 female) and 80 healthy control subjects (50 male, 30 female)] were included in the study. There were significant differences in corneal densitometry values of the groups in central 2 mm keratoconus (KC) = 19.62±4.17 gray scale unit (GSU), N=15.38±1.54 GSU ($P<0.0001$), and in annulus of 2 to 6 mm in diameter (K=16.40±2.18 GSU, N=15.31±1.25 GSU, $P<0.0001$). When densitometry values for different depths were examined, the anterior layer presented the highest value (KC=23.69±3.71 GSU, N=20.91±2.52 GSU, $P<0.0001$), which was significantly higher than the values obtained in the central and posterior layer (KC=14.34±1.70 GSU, N=13.61±1.21 GSU, $P=0.001$ and KC=11.40±1.23 GSU, N=12.35±0.88, $P=0.002$ respectively). Analysis of the correlation between corneal densitometry for each layer depth (anterior, central and posterior) with posterior corneal elevation values demonstrated significant association ($r=0.293$, $r=0.278$ and $r=0.294$ respectively). The similar correlation was not found between corneal densitometry for each layer depth and anterior corneal elevation ($r=-0.211$, $r=-0.101$, $r=0.99$ respectively). In the control patients such a significant correlation between posterior/anterior corneal elevation and corneal light backscatter for each layer depth was not found.

• CONCLUSION: The densitometry map reveals that light backscatter was higher in the central portion of the anterior keratoconic cornea. Corneal densitometry values

of keratoconus patients were correlated with posterior corneal elevation in 0-2, 6-10 and 10-12 annuli and in all anterior, central and posterior layers. The densitometry level was higher in more advanced stages.

• KEYWORDS: corneal densitometry; light backscatter; keratoconus; Scheimpflug imaging
DOI:10.3980/j.issn.1672-5123.2018.6.03

Citation: Mirzaei M, Taheri N, Akhlaghi H, Hashemi HS. Corneal densitometry and its correlation with anterior and posterior elevation in keratoconus. *Guoji Yanke Zazhi*(*Int Eye Sci*) 2018;18(6):985-993

INTRODUCTION

Keratoconus is a progressive ectatic corneal disorder characterized by progressive thinning and protrusion, resulting in irregular astigmatism and decreased visual acuity[1-2]. The exact pathophysiology of the disease has not been clarified, but the primary lesion in the keratoconic eye is supposed to be located in the basal epithelial cell layer, leading to its disappearance and thinning of epithelial layer[3-4]. Elevation - based corneal imaging techniques provide valuable information about the anterior and posterior corneal surface properties which were not generated by placido disk - based topography. The knowledge of these indices is important in the preoperative examination of refractive surgery candidates, the diagnosis of early stages and progression of keratoconus, and keratoconus patients undergoing collagen cross - linking or ring implantation for treatment. The Pentacam HR (Oculus Optikgeräte GmbH, Wetzlar, Germany) employs the Scheimpflug imaging technique and provides information regarding corneal surface irregularity and asymmetry in addition to elevation data, and uses them to compute indices for the diagnosis of keratoconus[5-7].

Scheimpflug imaging has a large number of applications in corneal diagnosis. Corneal and anterior segment tomography based on a rotating Scheimpflug camera allows for imaging of both the anterior and posterior corneal surfaces, a full pachymetric map, and thus additionally, with new software, it is possible to measure the amount of backscattered light in the different regions of the cornea[8-9].

A normal cornea is expected to scatter light mostly in the interfaces air/cornea and cornea/water where there are higher differences in light refraction indices with only minimum scatter by the structures of corneal tissue. This optically transparent tissue results from regular spacing, small uniform diameter of orthogonally arranged collagen fibers, and balanced keratocyte components[10-11].

Keratoconus is an ectatic disease that produces several changes in the cornea. These changes disturb the fragile balance of well - distributed corneal extracellular matrix and cells and lead to the thinning and anterior protrusion of the cornea. In advanced cases, this disarrangement may lead to corneal scars with evident opalescence. But mild cases are supposed to present increased backscatter detectable by noninvasive Scheimpflug analysis[12-13].

Pentacam is a noninvasive optical system that uses a rotating Scheimpflug camera and has been designed to assess the anterior segment of the eye from the anterior corneal surface to the posterior lens surface[14]. This relatively new imaging modality can also provide precise and reproducible data regarding corneal densitometry that represent valuable information about the clarity of the cornea[9]. Corneal densitometry demonstrates pixel luminance per unit volume in a Scheimpflug image, which correlates with light scatter and transparency. The densitometry measurements are expressed in gray scale units and measurements range from 0 (no clouding, maximum transparency) to 100 (completely opaque cornea, no transparency) depending on the degree of light scatter from the cornea[15].

The purpose of this study was to evaluate the corneal densitometry in keratoconus patients and its relevance with anterior and posterior corneal elevations. We suppose that early changes in corneal tissue can be elucidate much more earlier with densitometric changes than elevation changes. It seems that there is not any similar research in the literature in the context of relationship between corneal elevation and densitometry.

SUBJECTS AND METHODS

Patient Inclusion The cross sectional observational study reviewed the chart of patients examined at the Nikookari Eye Hospital (Tabriz, Iran). A total of 72 consecutive subjects were recruited between March 2016 and February 2017 for this study.

The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Tabriz University of Medical Sciences. The confidentiality of the patients data were secured all over the study.

Patients were selected from a database of cases diagnosed as having bilateral keratoconus (KC). All eyes were examined by a fellowship trained cornea and refractive surgeon (Mirzaei M). Patients with KC were defined as those having the following ocular findings in at least one eye[7,16]: typical Placido disc-based videokeratographic findings as paracentral inferior - superior asymmetry and/or asymmetric bowtie pattern, with or without skewed axes and at least 1 clinical sign including stromal thinning, conical protrusion of the cornea, Fleisher ring, Vogt striae, enlarged corneal nerves, increased intensity of the corneal endothelial reflex, subepithelial fibrillary lines, and Munson and Rizzuti signs. The amount of refractive error and keratometry recorded for each patient. Contact lens wear was discontinued at least 3wk for rigid contact lens and 1wk for soft contact lens before the assessment. Patients with a history of ocular surgery, any corneal pathology other than KC such as corneal scarring due to keratitis, dystrophy or trauma, hydrops, glaucoma, uveitis, connective tissue disease, diabetes mellitus, pregnancy at the time of the measurements, and with a history

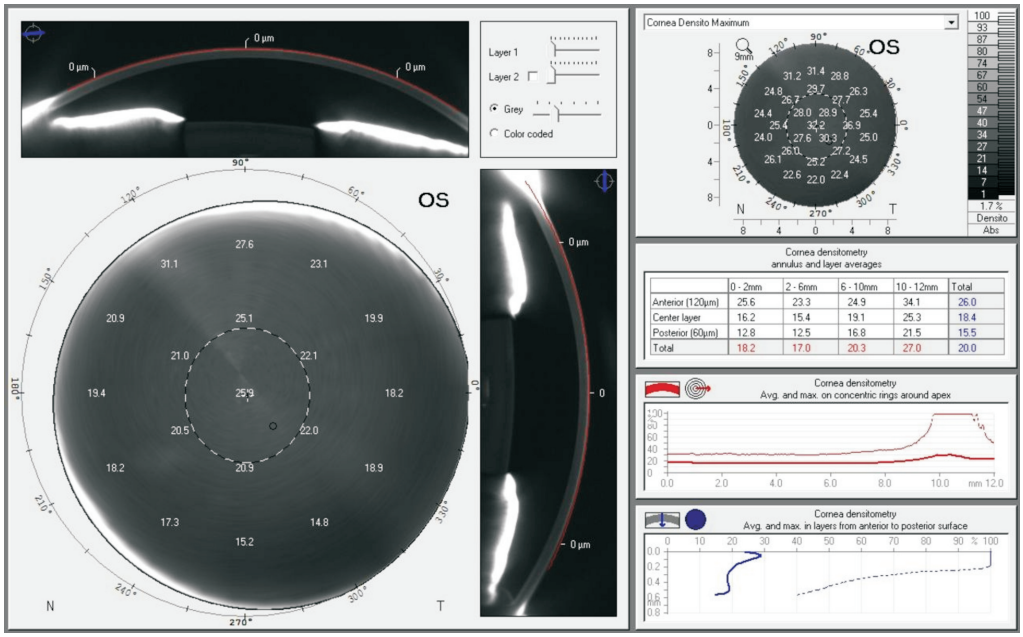

Figure 1 Corneal densitometry display in the Pentacam.

of ocular medication within 1wk were excluded.

Corneal Tomography All eyes were examined using rotating Scheimpflug corneal and anterior segment tomography (Pentacam HR, Oculus GmbH, Wetzlar, Germany). An expert examiner, who was masked to the clinical condition of the patient, acquired Pentacam images as follows: the patient's chin was placed on the chin rest, and the forehead was placed against the forehead strap. After blinking a few times, the patient was asked to open both eyes and stare at the fixation target. Proper alignment was obtained using a joystick, and then the automatic release mode started the scan using 25 single Scheimpflug images captured within 2s for each eye. Image quality was checked, so that only cases with acceptable quality images were included in the study. The Pentacam provides a topographic keratoconus classification for staging the disease in grades 1 to 4 with 3 subgrades (1-2, 2-3, and 3-4). Cases were divided into mild, less than grade 2; moderate, from grades 2 to 3; and severe, grades higher than 3 (3-4 and 4). An add-on to standard software of pentacam provides corneal densitometry analysis. It measures the backscattered light over a 12-mm-diameter area. Zonal densitometry can be measured by 4 annular concentric zones centered in the apex of the cornea. The first central zone covers the annulus with a 2-mm diameter, the second covers the annulus that extends from a 2- to 6-mm diameter, the third covers the one from a 6- to 10-mm diameter, and the last zone is composed of the annulus that extends from a 10- to 12-mm diameter. The analysis is also performed by depth in 3 layers: the anterior layer, which corresponds to the 120- mm superficial corneal thickness; the posterior layer, which corresponds to the most posterior 60 mm; and the central, which has no fixed thickness value, and is the zone between the anterior and posterior layers (Figure 1). Densitometry is expressed in gray scale units, ranging from a minimum light scatter of 0 (maximum transparency) to a maximum light scatter of 100 (minimum transparency). Corneal elevation in anterior and posterior corneal surface (AEmax and PEmax) was measured with pentacam in microns.

The important pentacam sings that confirmed the diagnosis was the Kmax, thinnest point, belin-ambrosio enhanced ectasia display (BADD), mean keratometry, IVA and ISV, ARTmax indices which has a desirable diagnostic validity.

Statistical Analysis Statistical analyses were performed by using Statistical Package for the Social Sciences 20.0 version for Windows software (SPSS Inc., Chicago, IL, USA). Normality of the data distribution was evaluated using the Kolmogorov-Smirnov test. Independent- sample t-test was used to compare quantitative data and Chi-square analysis was used for qualitative data. One-way analysis of variance (ANOVA) was used to compare quantitative data between multiple groups. Descriptive statistics were expressed as frequency and percentage for categorical variables whereas quantitative data were expressed as mean±SD error of mean for normally distributed variables and median (minimum-maximum) for non-normally distributed data. The correlation coefficient was used is Pearson and the test of significance is two-tailed. A $P < 0.05$ was considered statistically significant.

Compliance with Ethical Standards This study was not funded by university. Authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

RESULTS

One hundred and twenty eyes from 72 patients with keratoconus and 80 healthy control subjects were included in the study. There were 46 men and 26 women in the study group and 50 men, 30 women in the control group. The subjects' age in the study group was 20.4±4.7 (range from: 11-36) y and in the normal group was 22±3.8 (range from: 17-29) y. And 15 (12.5%) of the eyes were classified as stage 1, 20 (16.7%) as stage 1-2, 40 (33.3%) as stage 2, 14 (11.7%) as stage 2-3, 28(23.3%) as stage 3 and 3 (2.5%) as stage 3 - 4 based on Amsler - Krumeich classification. Stage 2 keratoconus eyes had the most frequency between others.

There was bilateral involvement in 48 (67%) patients and 24 (33%) had unilateral involvement.

Total corneal light backscatter was higher in the KC group (KC = 16.62 ± 2.09 GSU, N = 15.63 ± 1.31 GSU, P < 0.0001). The densitometry map demonstrated a statistically significant difference between the groups in stratified analysis with less overlap between the groups. When densitometry values for different depths were examined, the anterior layer presented the highest value (KC = 23.69 ± 3.71 GSU, N = 20.91 ± 2.52 GSU, P < 0.0001), which was significantly higher than the values obtained in the central and posterior layer (KC = 14.34 ± 1.70 GSU, N = 13.61 ± 1.21 GSU, P = 0.001 and KC = 11.40±1.23 GSU, N = 12.35±0.88, P = 0.002 respectively). These results are summarized in Table 1.

Considering the total thickness of the cornea, separating the densitometry by concentric annular zones, the densitometric values at the first 2 annuli (center to 2 mm and 2 - 6 mm diameter) was statistically higher in the KC group (KC = 19.62±4.17 GSU, N = 15.38±1.54 GSU, P<0.0001; KC = 16.40 ± 2.18 GSU, N = 15.31 ± 1.25 GSU, P < 0.0001, respectively).

Separating the cornea into 3 layers, they showed the same result with a higher backscatter in the central cornea in the KC group. The differences were marked in the 2 central annuli (0-2 and 2-6 mm annuli) specially in the anterior and central layers (P<0.0001).

Total corneal densitometry was significantly correlated with age. The Pearson correlation coefficient for total densitometry was r = 0.042 (P<0.05). A significant correlation with age was found for all three depth layers: r = 0.035 (P<0.05), r = 0.045 (P<0.05), and r = 0.038 (P<0.05) for the anterior, central, and posterior layers, respectively.

The analysis, considering different stages of KC, showed the same pattern when comparing the 3 groups with normal corneas. A statistically significant difference was present at the 2 central annuli and 2 - 6 diameter in the anterior and central layers (P<0.001 and P = 0.008 respectively); in the posterior layer it was significant in center and 6 - 10 mm diameter (P = 0.010) and with a trend to increase densitometry as the KC stage evolves. The same behavior was present in total thickness densitometry values. Comparison of corneal densitometry measurements of keratoconus stages and healthy control subjects is shown in detail in Table 2.

The values of anterior and posterior elevation in keratoconus and control groups was demonstrated in the Table 3.

Analysis of the correlation between corneal densitometry for each layer depth (anterior, central and posterior) with posterior corneal elevation values demonstrated significant association (r = 0.293, r = 0.278 and r = 0.294 respectively).

Table 1 Cornel densitometry values in KCN and control groups

	Group	Mean	Std. Deviation	P
Anterior layer 0-2	KCN	29.365	6.9599	<0.0001
	Control	20.663	3.0080	
Anterior layer 2-6	KCN	23.836	4.2935	<0.0001
	Control	21.288	2.8156	
Anterior layer 6-10	KCN	18.488	3.8500	0.105
	Control	19.236	1.7181	
Anterior layer 10-12	KCN	29.279	9.6760	0.123
	Control	27.543	3.1877	
Anterior layer total	KCN	23.694	3.7174	<0.0001
	Control	20.912	2.5290	
Central layer 0-2	KCN	16.897	3.6323	<0.0001
	Control	13.587	1.0691	
Central layer 2-6	KCN	13.942	1.9558	0.006
	Control	13.288	0.9119	
Central layer 6-10	KCN	11.870	1.6577	0.1
	Control	11.517	1.1579	
Central layer 10-12	KCN	18.404	5.9164	0.079
	Control	17.164	2.5546	
Central layer total	KCN	14.343	1.7039	0.001
	Control	13.616	1.2195	
Posterior layer 0-2	KCN	12.722	3.7917	0.078
	Control	11.914	1.8314	
Posterior layer 2-6	KCN	11.502	0.9982	0.540
	Control	11.360	2.2142	
Posterior layer 6-10	KCN	10.513	1.3376	<0.0001
	Control	11.203	1.0645	
Posterior layer 10-12	KCN	15.322	4.5403	0.300
	Control	14.772	1.5762	
Posterior layer total	KCN	11.840	1.2393	0.002
	Control	12.350	0.8859	
Total layer 0-2	KCN	19.628	4.1711	<0.0001
	Control	15.388	1.5445	
Total layer 2-6	KCN	16.402	2.1852	<0.0001
	Control	15.312	1.2513	
Total layer 6-10	KCN	13.615	2.1346	0.144
	Control	13.985	0.8958	
Total layer 10-12	KCN	21.000	6.1703	0.103
	Control	19.826	2.0552	
Total layer total	KCN	16.612	2.0981	<0.0001
	Control	15.626	1.3173	

KCN: Keratoconus.

Table 2 Comparison of corneal densitometry measurements of keratokonus satges and healthy control subjects (ANOVA)

	P	KCN				Post hoc test
		Severe	Moderate	Mild	Control	
Anterior						
0-2	<0.001	30.74±7.22	29.16±7.38	28.48±6.00	20.66±3.00	C- mL, mod, s
2-6	<0.001	24.77±5.19	23.45 ± 3.95	23.62±3.94	21.28±2.81	C-mL, mod, s
6-10	0.004	20.12±5.98	17.85±2.64	18.06±2.61	19.23±1.71	Mod-s
10-12	0.198	30.93±11.38	28.25±8.26	29.49±10.24	27.54±3.18	-
Total	<0.001	24.56±4.47	23.30±3.19	23.54±3.73	20.91±2.52	C-mL, mod, s
Central						
0-2	<0.001	18.07±5.08	16.30±2.94	16.75±2.81	13.58±1.06	C-mL, mod, s
2-6	0.012	14.36±2.70	13.70±1.36	13.94±1.93	13.28±0.91	C-S
6-10	0.053	12.36±2.11	11.76±1.37	11.58±1.55	11.51±1.15	-
10-12	0.189	19.39±8.76	18.15±4.80	17.94±4.33	17.16±2.55	-
Total	0.008	14.61±1.97	14.24±1.44	14.25±1.84	13.61±1.21	C-S
Posterior						
0-2	0.011	14.03±6.91	12.03±1.50	12.61±1.49	11.91±1.83	C-S
2-6	0.867	11.62±1.12	11.50±0.93	11.38±0.98	11.36±2.21	-
6-10	0.001	10.65±1.41	10.55±1.31	10.32±1.32	11.20±1.06	C-mL
10-12	0.183	16.30±6.26	15.28±4.18	14.54±3.04	14.77±1.57	-
Total	0.010	12.06±1.48	11.77±1.06	11.74±1.25	12.35±0.88	C-miL, mod
Total						
0-2	<0.001	20.85±6.15	19.17±3.21	19.28±3.19	15.38±1.54	C-mL, mod, s
2-6	0.001	16.82±2.75	16.21±1.88	16.32±2.08	15.31±1.25	C-mod, s
6-10	0.024	14.35±3.02	13.39±1.63	13.32±1.78	13.98±0.89	-
10-12	0.169	22.23±8.45	20.56±5.14	20.66±5.40	19.82±2.05	-
Total	0.001	17.07±2.59	16.41±1.71	16.51±2.16	15.62±1.31	C-S

C: control; mL: mild; Mod: Moderate; S: Severe; KCN: Keratoconus.

Table 3 Anterior and posterior elevation values in KCN and control groups Mean±SD

Group	Post. elevation	Ant. elevation
KCN (*n*=120)	47.29±19.016	21.14±9.962
Control (*n*=80)	4.87±0.786	5.13±1.173
P	<0.0001	<0.0001

The similar correlation was not found between corneal densitometry for each layer depth and anterior corneal elevation ($r=-0.211$, $r=-0.101$, $r=0.99$ respectively). In the control patients such a significant correlation between posterior/anterior corneal elevation and corneal light backscatter for each layer depth (anterior, central and posterior) was not found. Correlation of corneal densitometry measurements with posterior and anterior corneal elevation in keratoconus patients and healthy control subjects is given in detail in Figure 2 and Table 4.

Analysis of the correlation between corneal densitometry for each annulus (in total thickness) and posterior corneal elevation values demonstrated significant association in the center to 2 mm, 6-10 and 10-12 mm diameters ($r=0.309$, $r=0.354$ and $r=0.235$ respectively); such a correlation was not found in the 2-6 annulus ($r=0.243$). There was not such a significant correlation between corneal light backscatter and for each annulus and anterior corneal elevation except in 6-10 mm diameter ($r=0.354$). In the control group we didn't find any significant correlation between posterior or anterior corneal elevation and corneal light back scatter in none of the annuli.

DISCUSSION

Corneal densitometry enables the objective assessment of corneal clarity. The total corneal densitometry measured with Scheimpflug corneal topography is the sum of epithelial, stromal, and endothelial light scattering[13]. The anterior superficial corneal epithelial cell layer and the corneal endothelium are the major sources of light scatter, while corneal stroma retains low scattering due to regular arrangement of collagen fibrils and to the precise organization of the extracellular matrix[10,17-19].

The analysis of light backscatter has gained increasing relevance in corneal diagnosis. It has been described in post refractive surgery[10-12], infectious keratitis[13], corneal dystrophies[20], corneal graft surgery[21-22], and for evaluating the crosslinking result in patients with KC[19,23].

In the clinic it is also used complementarily to endothelial cell count in monitoring the disease progression in cornea guttata patients[24]. Corneal transparency is caused by complex mechanisms; regular - arrangement and size regularity of

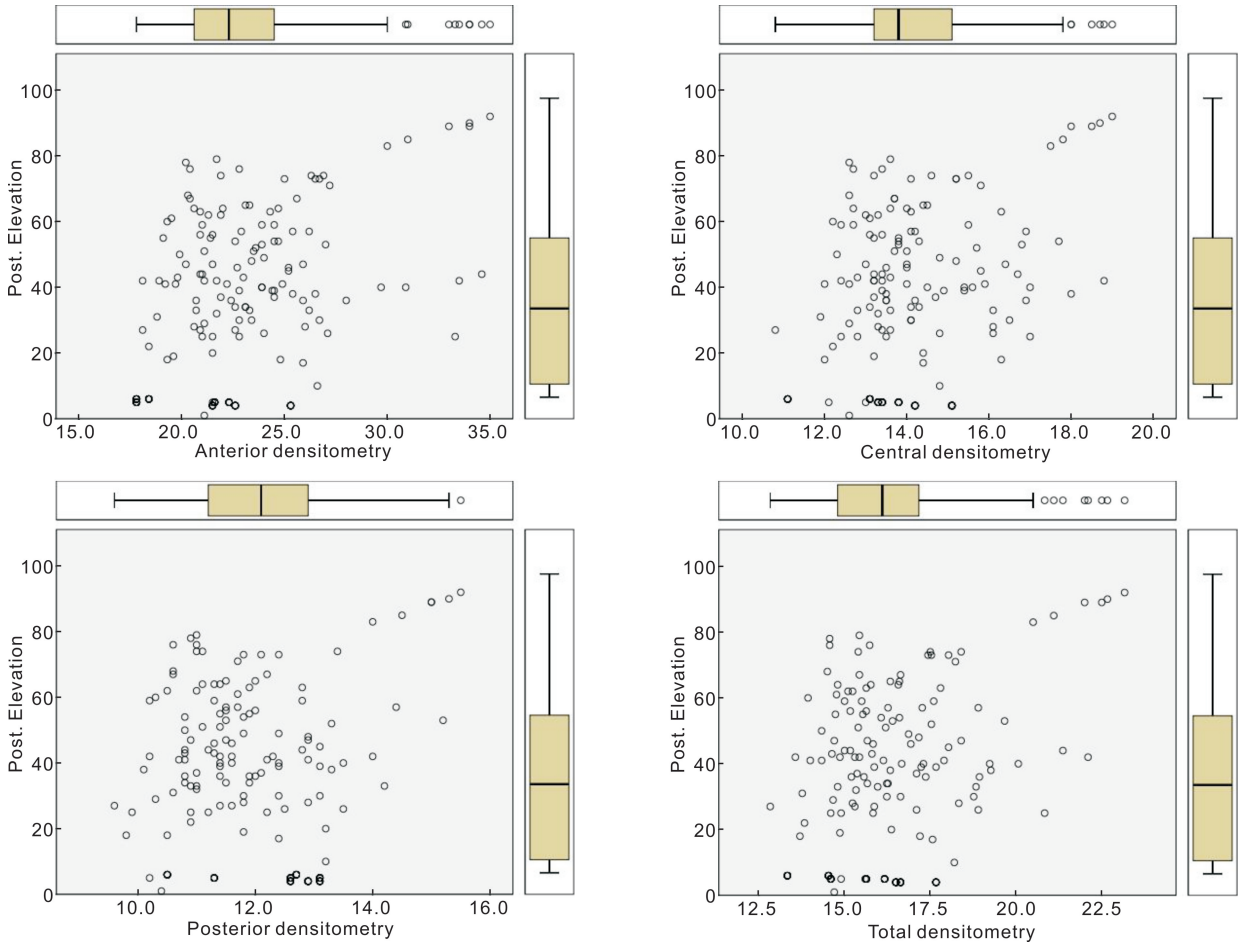

Figure 2　Scatter plot of correlation between posterior elevation and densitometry.

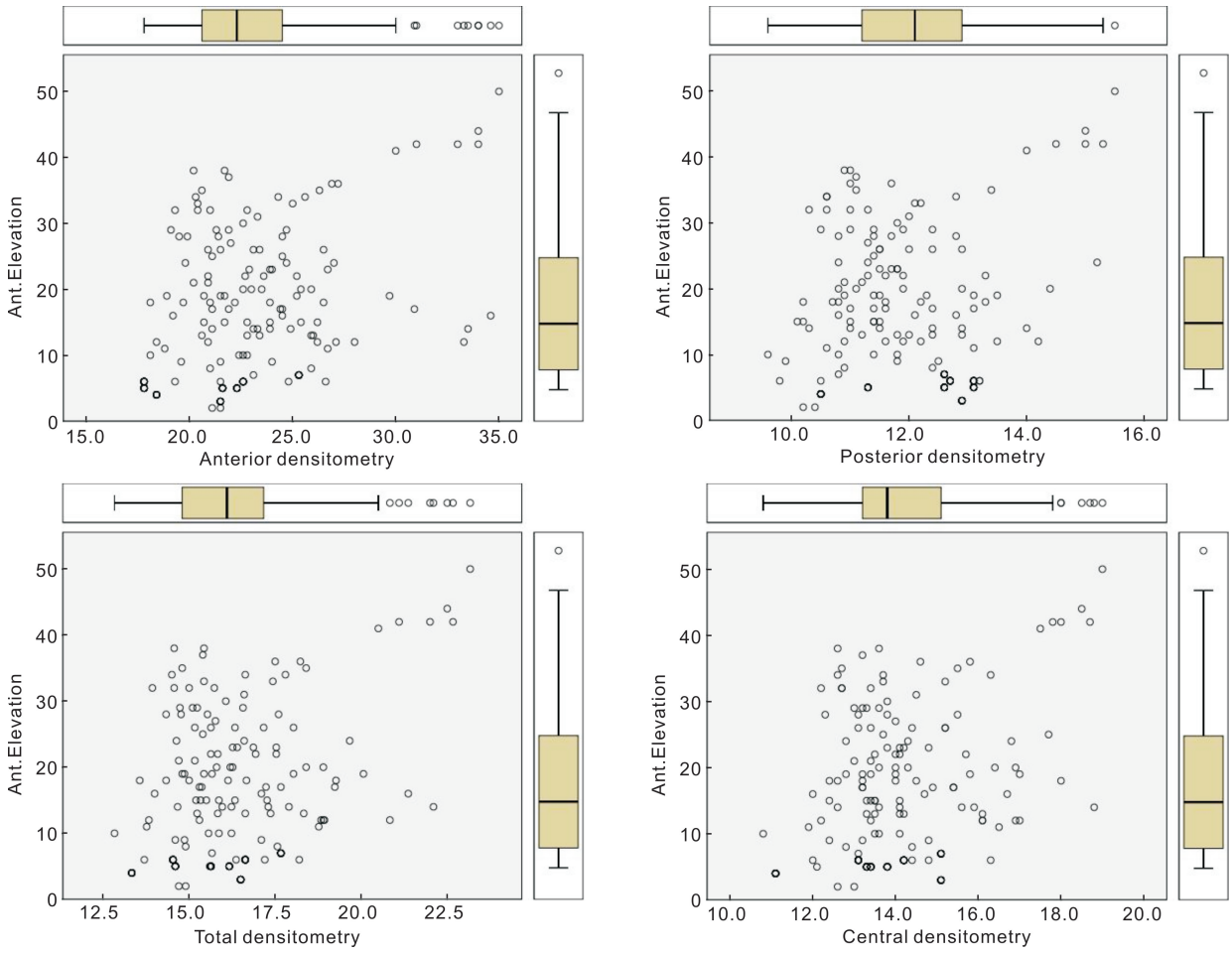

Figure 3　Scatter plot of correlation between anterior elevation and densitometry.

Table 4 Correlation of anterior and posterior elevation with corneal densitometry value in KCN and control group

		KCN		Control	
		Post. Elevation	Ant. Elevation	Post. Elevation	Ant. Elevation
Anterior layer 0-2	Pearson Correlation	0.242[a]	0.195[b]	-0.162	0.270
	P	0.008	0.033	0.897	0.607
Anterior layer 2-6	Pearson Correlation	0.242[a]	0.200[b]	-0.210	0.232
	P	0.008	0.029	0.723	0.605
Anterior layer 6-10	Pearson Correlation	0.390[a]	0.379[a]	-0.182	0.265
	P	0.000	0.000	0.34	0.22
Anterior layer 10-12	Pearson Correlation	0.197[b]	0.195[b]	-0.187	0.247
	P	0.033	0.034	0.21	0.41
Anterior layer total	Pearson Correlation	0.293[a]	0.243[a]	-0.211	0.271
	P	0.001	0.008	0.46	0.19
Central layer 0-2	Pearson Correlation	0.307[a]	0.304[a]	-0.095	0.156
	P	0.001	0.001	0.855	0.768
Central layer 2-6	Pearson Correlation	0.180[b]	0.193[b]	-0.111	0.201
	P	0.049	0.035	0.340	0.242
Central layer 6-10	Pearson Correlation	0.287[a]	0.262[a]	-0.158	0.214
	P	0.001	0.004	0.765	0.324
Central layer 10-12	Pearson Correlation	0.192[b]	0.206[b]	-0.177	0.209
	P	0.036	0.025	0.198	0.765
Central layer total	Pearson Correlation	0.278[a]	0.256[a]	-0.101	0.169
	P	0.002	0.005	0.886	0.721
Posterior layer 0-2	Pearson Correlation	0.280[a]	0.307[a]	0.89	0.287
	P	0.002	0.001	0.651	0.512
Posterior layer 2-6	Pearson Correlation	0.211[b]	0.196[b]	0.99	0.221
	P	0.021	0.032	0.348	0.349
Posterior layer 6-10	Pearson Correlation	0.214[b]	0.198[b]	0.95	0.209
	P	0.019	0.030	0.201	0.419
Posterior layer 10-12	Pearson Correlation	0.285[a]	0.293[a]	0.94	0.212
	P	0.002	0.001	0.427	0.381
Posterior layer total	Pearson Correlation	0.294[a]	0.295[a]	0.99	0.227
	P	0.001	0.001	0.378	0.418
Total layer 0-2	Pearson Correlation	0.309[a]	0.285[a]	-0.168	0.295
	P	0.001	0.002	0.393	0.398
Total layer 2-6	Pearson Correlation	0.243[a]	0.210[b]	-0.222	0.301
	P	0.008	0.022	0.187	0.506
Total layer 6-10	Pearson Correlation	0.354[a]	0.334[a]	-0.245	0.211
	P	0.000	0.000	0.673	0.209
Total layer 10-12	Pearson Correlation	0.235[b]	0.241[a]	-0.270	0.239
	P	0.010	0.009	0.249	0.711
Total layer total	Pearson Correlation	0.306[a]	0.269[a]	-0.213	0.248
	P	0.001	0.003	0.201	0.178

[a]Correlation is significant at the 0.01 level (2-tailed); [b]Correlation is significant at the 0.05 level (2-tailed).

collagen fibrils are part of it[25]. High levels of corneal light backscatter can be seen even in corneas considered clinically clear[26]. The disarrangement in corneal histology caused by KC can also alter densitometry levels[27].

In the present study, we measured the corneal densitometry of keratoconus patients with Pentacam® HR and we evaluated its relevance with corneal anterior and posterior elevation and compared these results with those of healthy control subjects. We hypothesized that decreased corneal clarity may cause an increase in the corneal densitometry in keratoconus patients and this is correlated with corneal elevation. Disruption of the regular arrangement of collagen fibrils in keratoconus patients can produce high levels of corneal light backscatter and increased corneal densitometry even in corneas considered to be clinically clear[9].

Corneal densitometry measurement seems to provide a potential explanation related to the pathophysiology of the disease. Assessing corneal clarity with the Pentacam has a

potential role in describing the part of the cornea that is mostly affected in keratoconus.

Lopez *et al*[5] found an increase in light backscatter in the central cornea (6 mm in diameter) and total diameter in the KC group (2-mm central annulus: KC = 19.87±5.07, N = 16.58±1.8, $P<0.001$; 2- to 6-mm annulus: KC = 16.64±2.99, N = 14.89±1.61, $P=0.005$; total diameter: KC = 17.82 ± 5.57, N = 16.71±2.3, $P=0.033$). A higher densitometry in the central cornea was also seen in all 3 layers ($P<0.001$). The difference was marked in the central annulus of the anterior layer, where the difference was found in the different stages of KC (2-mm central annulus: KC severe 37.26±11.77, KC moderate: 30.53±8.16, KC mild = 25.34±3.49, $P<0.05$); in the central layer, the higher densitometry in the KC group (2-mm central annulus: KC = 16.84±5.16, N = 13.94 ±2.21, $P=0.0001$; 2- to 6-mm annulus: KC = 13.88±2.96, N = 12.39±1.93, $P=0.003$).

Our findings were consistent with Lopez *et al*[5] and Anayol *et al*[9] and the corneal densitometry of keratoconus patients was higher particularly in the central cornea (0-2 and 2-6 mm annular zones) when compared to healthy control subjects. Increased corneal densitometry was most prominent in the central and anterior cornea, which was consistent with the histopathology of keratoconus. The anterior central cornea is the first and most affected part of the cornea in keratoconus. The primary lesion in the keratoconic eye is supposed to be located in the basal epithelial cell layer, leading to thinning of epithelial layer.

At the anterior layer, the post hoc test revealed that the higher values of mild, moderate and severe keratoconus were statistically significant ($P<0.001$) in central cornea (0-2 mm and 2-6 mm annuli) in comparison with those of 6-10mm and 10-12 mm annuli.

In the central layer, the higher densitometry in the KC group is consistent with the findings of a previous study using the principle of Scheimpflug photography for the assessment of corneal light scatter, where most corneal light scatter is produced by the stroma[28], and with the haze seen in confocal microscopy studies[29]. The values of peripheral zones, however, must be interpreted with caution, especially the 10 to 12 mm, as the repeatability and reproducibility in this region were the weakest in a normative study[8].

In this study analysis of the correlation between corneal densitometry for each layer depth (anterior, central and posterior) with posterior corneal elevation values demonstrated significant association. The similar correlation was not found between corneal densitometry for each layer depth and anterior corneal elevation. In the control patients such a significant correlation between posterior and anterior corneal elevation and corneal light backscatter for each layer depth (anterior, central and posterior) was not found.

Analysis of the correlation between corneal densitometry for each annulus (in total thickness) and posterior corneal elevation values demonstrated significant association in the center to 2 mm, 6-10 and 10-12 mm diameters. There was not such a significant correlation between corneal light backscatter and for each annulus and anterior corneal elevation except in 6-10 mm diameter ($r=0.354$). In the control group we didn't find any significant correlation between posterior or anterior corneal elevation and corneal light back scatter in none of the annuli.

To our knowledge, this is one of the preliminary studies in the literature that evaluates corneal densitometry as a measure of corneal transparency in keratoconus and its relationship with anterior and posterior corneal elevation, which may be an objective measurement method for progression of the disease in the future. Corneal densitometry allows objective assessment of corneal clarity in keratoconus and may provide a baseline approach for an examiner - independent classification. Furthermore, it could play an important role in objective monitoring of corneal haziness following therapeutic interventions such as corneal collagen crosslinking. In conclusion, our results suggest that treatment - naive keratoconus patients have significantly higher corneal densitometry values at the central cornea when compared to healthy subjects. Corneal densitometry may be a useful index of disease progression and a prognostic indicator that could be used in the accompaniment with the corneal shape measures such as corneal elevation. Densitometry and shape changes are correlated and it seems that corneal light backscatter could be diagnostic even in mild and subclinical cases, so further study is required to prove this relationship and to determine whether densitometric changes add any additional useful information. Corneal densitometry as an objective measure of corneal clarity warrants further longitudinal study in order to monitor keratoconus progression and to further ascertain its clinical relevance.

REFERENCES

1 Davidson AE, Hayes S, Hardcastle AJ, Tuft SJ. The pathogenesis of keratoconus. *Eye(Lond)* 2014;28:189-195

2 Basu S, Vazirani J. Keratoconus: current perspectives. *Clin Ophthalmol* 2013;7:2019

3 Kohlhaas M, Spoerl E, Schilde T, Unger G, Wittig C, Pillunat LE. Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. *J Cataract Refract Surg* 2006;32:279-283

4 Mathew JH, Goosey JD, Bergmanson JP. Quantified histopathology of the keratoconic cornea. *Optom Vis Sci* 2011;88:988-997

5 Lopez B, Ramos I, Ambrosio R Jr. Corneal Densitometry in Keratoconus. *Cornea* 2014;33(12):1282-1286

6 Hashemi H, Beiranvand A, Khabazkhoob M, Mehravaran S, Emamian MH, Yekta A, Shariati M, Fotouhi A. Corneal elevation and keratoconus indices in a 40- to 64-year-old population, Shahroud Eye Study. *J Curr Ophthalmol* 2015;27(3-4):92-98

7 Krachmer JH, Feder RS, Belin MW. Keratoconus and related noninflammatory corneal thinning disorders. *Surv Ophthalmol* 1984;28(4):293-322

8 Ni Dhubhghaill S, Rozema JJ, Jongenelen S, Ruiz Hidalgo I, Zakaria N, Tassignon MJ. Normative values for corneal densitometry analysis by Scheimpflug optical assessment. *Invest Ophthalmol Vis Sci* 2014;55(1):162-168

9 Anayol MA, Sekeroglu MA, Ceran BB, Dogan M, Gunaydin S, Yilmazbas P. Quantitative assessment of corneal clarity in keratoconus: a case control study of corneal densitometry. *Eur J Ophthalmol* 2016;26(1):18-23

10 Fares U, Otri AM, Al-Aqaba MA, Faraj L, Dua H. Wavefront-optimized excimer laser in situ keratomileusis for myopia and myopic astigmatism: refractive outcomes and corneal densitometry. *J Cataract Refract Surg* 2012;38(12):2131-2138

11 Rozema JJ, Trau R, Verbruggen KH, Tassignon MJ. Backscattered light from the cornea before and after laser-assisted subepithelial keratectomy for myopia. *J Cataract Refract Surg* 2011;37(9):1648-1654

12 Cennamo G, Forte R, Aufiero B, La Rana A. Computerized Scheimpflug densitometry as a measure of corneal optical density after excimer laser refractive surgery in myopic eyes. *J Cataract Refract Surg* 2011;37(8):1502-1506

13 Otri AM, Fares U, Al-Aqaba MA, Dua HS. Corneal densitometry as an indicator of corneal health. *Ophthalmology* 2012;119(3):501-508

14 Cho YK, Chang HS, La TY, Ji D, Kim H, Choi JA, Kim MS. Anterior segment parameters using Pentacam and prediction of corneal endothelial cell loss after cataract surgery. *Korean J Ophthalmol* 2010;24(5):284-290

15 Ruiseñor Vázquez PR, Galletti JD, Minguez N, Delrivo M, Fuentes Bonthoux F, Pförtner T, Galletti JG. Pentacam Scheimpflug tomography findings in topographically normal patients and subclinical keratoconus cases. *Am J Ophthalmol* 2014;158(1):32-40.e2

16 Rabinowitz YS. Keratoconus. Keratoconus Survey of Ophthalmology 1998;42(4):297-319

17 Takacs AI, Mihaltz K, Nagy ZZ. Corneal density with the Pentacam after photorefractive keratectomy. *J Refract Surg* 2011;27(4):269-277

18 Orucoglu F, Talaz S, Aksu A, Muftuoglu O. Corneal densitometry evaluation in archipelago keratitis. *Int Ophthalmol* 2014;34(1):99-102

19 Greenstein SA, Fry KL, Bhatt J, Hersh PS. Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: Scheimpflug and biomicroscopic analysis. *J Cataract Refract Surg* 2010;36(12):2105-2114

20 Elflein HM, Hofherr T, Berisha-Ramadani F, Weyer V, Lampe C, Beck M, Pitz S. Measuring corneal clouding in patients suffering from mucopolysaccharidosis with the Pentacam densitometry programme. *Br J Ophthalmol* 2013;97:829-833

21 Bhatt UK, Fares U, Rahman I, Said DG, Maharajan SV, Dua HS. Outcomes of deep anterior lamellar keratoplasty following successful and failed "big bubble". *Br J Ophthalmol* 2012;96(4):564-569

22 Koh S, Maeda N, Nakagawa T, *et al*. Quality of vision in eyes after selective lamellar keratoplasty. Cornea 2012;31(Suppl 1):S45-S49

23 Gutiérrez R, Lopez I, Villa-Collar C, González-Méijome JM. Corneal transparency after cross-linking for keratoconus: 1-year follow-up. *J Refract Surg* 2012;28(11):781-786

24 Weyns M, Rozema J, Koppen C, *et al*. Clinical applications of corneal Scheimpflug densitometry in DSAEK and cornea guttata patients. *Acta Ophthalmol* 2010;88:0

25 Freegard TJ. The physical basis of transparency of the normal cornea. *Eye (Lond)* 1997;11(Pt 4):465-471

26 Patel SV, McLaren JW, Hodge DO, Bourne WM. The effect of corneal light scatter on vision after penetrating keratoplasty. *Am J Ophthalmol* 2008;146(6):913-919

27 Rozema JJ, Koppen C, Bral N, Tassignon MJ. Changes in forward and backward light scatter in keratoconus resulting from corneal cross-linking. *Asia-Pacific J Ophthalmol* 2013;2(1):15-19

28 McCally RL, Farrell RA. The depth dependence of light scattering from the normal rabbit cornea. *Exp Eye Res* 1976;23(1):69-81

29 Efron N, Hollingsworth JG. New perspectives on keratoconus as revealed by corneal confocal microscopy. *Clin Exp Optom* 2008;91(1):34-55