Decreased Central and Limbal Epithelium Thickness After Corneal Crosslinking Evaluated by Anterior Segment Optic Coherence Tomography

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Research Article

Keywords: Anterior segment-OCT, Crosslinking, keratoconus, limbal corneal epithelium, corneal thickness, stromal thickness

Posted Date: April 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-329553/v1

Abstract

Aim: To Investigate the central cornea, limbal epithelium thickness and stroma thickness after corneal cross-linking by Anterior Segment Optical Coherence Tomography (AS-OCT) and Scheimpflug topography.

Methods: Fifteen keratoconus patients treated with cross-linking(CXL) and fifteen untreated keratoconus patients was included to the study. Corneal central, limbal epithelial, stromal and total thickness with was analyzed by using Anterior Segment Optical Coherence Topography (AS-OCT) and keratometric values were analyzed by Scheimpflug topography.

Results: There was a statisticallly significant difference between treated and untreated keratoconus patients according to limbal epithelial thickness (LET). Limbal epithelial thickness was 30.7 ± 5.5μm in the treated keratoconus patients and 45.6 ± 11.5μm in the untreated patients (p = 0.04). Central corneal epithelial thickness (CCET) was 38.27±3.5 in the treated group and 60.8 ± 10.9μm in the untreated group. There was a statistically signifficant difference the between two groups (p=0.01). The posterior astigmatism value was 0.7 ± 0.3D in the treated group and 0.9 ± 0.5D in untreated group by Scheimpflug topography (p=0.03).

Conclusion: Our study have shown that the central corneal epithelium and limbal epithelium were significantly thinned as a result of corneal cross-linking. Corneal posterior astigmatism value decreased among the keratometric values. Epithelial thickness and limbal thickness alterations detected with Ant-OCT could be useful for monitorizing the keratoconus patients treated with crosslinking and could show the effectivity of the treatment.

Introduction

Keratoconus, is an idiopathic and non-inflammatory disease characterized by bilateral, asymmetrical, central and paracentral thinning of the cornea and an irregular conical shape. The prevalence of 1/2000, keratoconus progresses until the age of 30-40 [1, 2]. The main symptoms are; monoocular diplopia, progressive visual impairment, photophobia, ocular irritation symptoms, constant change in eyeglass number, irregular astigmatism, and inability to achieve full vision with recovery [3, 4]. The progression of the disease usually stops in the 40s, but 20% of the patients continue to progress [5]. Various treatments are applied to stop the disease.

Corneal epithelium located in the limbal region is thinner than the central corneal epithelium. However, the limbal corneal stroma and total corneal thickness are thicker than the central one. Epithelium, stroma and total corneal thickness in keratoconus corneas are known to be thinner than normal eyes. Corneal cross-linking treatment is applied to increase the resistance of the stromal thickness in keratoconus patients [6, 7].

Corneal cross-linking therapy has been widely used, especially in cases of progressive keratoconus. With the corneal cross-linking technique, it is aimed to photopolymerize the stroma fibers by the combined effect of riboflavin and ultraviolet A (UVA) rays. It has been reported that corneal epithelial thickness decreases as a result of corneal cross-linking in keratoconus patients [8]. Technical and technological developments in the ophthalmological field have gained momentum in recent years. In particular, specular microscopy, Anterior

Segment Optical Coherence Topography (AS-OCT), provided detailed examination of all layers of the cornea [9].

Optical coherence tomography is a non-invasive imaging method that provides a cross-sectional view using the intensity of infrared light at ~ 800 nm wavelength reflected back from the biological tissue layers and the reflection delay time. It provides two or three dimensional images with high axial resolution (8-15 μm). The anterior segment corneal epithelium also enables the examination of the other eye structures. Anterior Segment Optical Coherence Tomography is a device to examine various anterior segment components and ocular surface problems. It contributes to the diagnosis of lesions in the cornea and clarification of pathological conditions and includes anterior segment parameters, tear film thickness , corneal epithelial thickness, corneal stromal thickness, corneal limbal thickness, , anterior chamber depth and angle. AS-OCT is a valuable device for early diagnosis and follow-up in the treatment of ocular diseases. It provides cross-sectional images of ocular tissues, and provides more precise and reproducible measurements in examining the corneal structure compared to biomicroscopy [10, 11].

In our study, we aimed to investigate the central, limbal corneal epithelial thickness alterations in keratoconus patients before and after corneal crosslinking by AS-OCT and Scheimpflug topography.

Methods

Study Population

This prospective matched case-control study was performed in the Trakya University Faculty of Medicine, Ophthalmology Department. The study was approved by the Ethics Committee of the Trakya University and it followed the tenets of the Declaration of Helsinki. Informed consent was taken from all subjects after a statement of the possible consequences of the study. Fifteen patients with keratoconus who had epithelium-off corneal crosslinking operations and 15 patients with keratoconus without crosslinking treatment had included to the study. Postoperative 3th month measurements were included. Corneal thickness less than 400 μm before the operation, with corneal and ocular surface diseases, using contact lenses, and ocular disease and surgical history were the exlusion criteria keratoconus study. From the study. All patients was performed full ophthalmologic examination including; best corrected visual acuity (BCVA), intraocular pressure (IOP) measurement with applanation tonometer, biomicroscopy, funduscopy. Scheimpflug topography, AS-OCT was applied to all patients.

Surgical Technique

As local anesthesia, 0.5% proparacaine HCl ophthalmic solution (Alcaine®, Alcon, Fort Worth, Texas, USA) was dropped three times at intervals of 5 minutes. The site was cleaned with 10% diluted olivinyl pyrrolidone-iodine, and the lids had cleared with wire blepharosta. Corneal epithelium was debrided with a spatula. Riboflavin drop (10 mg riboflavin-5-phosphate in 10 ml dextran 20%) was applied to the stromal bed with sufficient thickness every three minutes for 30 minutes on average. After the parameters of the UVA device (UV-X; IROC AG, ZURICH, SWITZERLAND) had adjusted, UVA treatment was initiated for 30 minutes at a distance of 5 cm from the corneal apex. During this time, the riboflavin solution was continued to be dropped for 30 minutes every three minutes. At the end of the procedure, the procedure was completed by placing the

therapeutic soft contact lens. Maxifloxacin 0.5% ophthalmic solution 4x1 (1 week) and artificial tear drops containing no preservatives 4x1 (1 month) had applied to the patients in the postoperative period.

Anterior Segment OCT Measurement Technique

Cirrus high resolution (HD) OCT (model 5000; Carl Zeiss Meditec, Dublin, CA, USA) anterior segment module was used for corneal and limbal measurements. All the AS-OCT measurement taken from nasal quadrant. The Anterior Segment Line Raster mode was used to capture images of the central corneal epithelium and limbal epithelial region. All images had performed at least 3 scans at the same time of examination by 2 independent technicians. Technicians determined that the clearest image was captured, and limbal and corneal epithelial analysis was performed for the clearest image (signal strength ≥7/10). Technicians analyzing the images did not know the categories of subjects.

Scheimpflug Measurement Technique

Corneal topography was carried out with Pentacam (Schiempflug imaging system Oculus, Wetzlar, Germany). All measurements had performed under equal light conditions with a nondilated pupil. All the measurements had performed by two blind examiners (İA, ÖK). Only only high-quality topographical measurements approved by the Pentacam software system had accepted. The corneal topography measurements obtained until 3 acceptable “OK” quality measurement is achieved. Best Fit Sphere selection was done automatically to evaluate patients.

Central corneal epithelium (CCET), determined as the distance between the tear film(*TF*) and Bowman's membrane (*BM*). CCET was measured from corneal apex. Central corneal stromal thickness (CCST) was determined distance from the *BM* to the Descemets membrane (*DM*), Central corneal total thickness (CCTT) was determined from TF to the DM. (Fıgure-1). Limbal corneal epithelial thickness (LCET) was determined as the distance between the *TF* and *BM* on the *Line-2*. Limbal corneal stromal thickness (LCST) was determined as the distance from the *BM* to the *DM* on *Line-2*, limbal corneal thickness (LCT) was determined as the total corneal thickness on *Line-2* (Figure-2).

The *Line-1* is closest line to the central cornea and extends from *A* point to the *B* point. *A* point refers to the beginning of the corneal epithelium, **B** point refers to *DM* on *Line –1*. *Line –2* is the line extends 500 µm away from *Line-1* and *Line-3*, and Line-2 is the line in the middle of these two reference lines. *Line-3* extends from *E* point to *F* point.*E* point refers to the beginning of the corneal epithelium on *Line-3*, *F* point refers to Descement membrane on *Line –3*. (Fıgure-2).

Statistical Analysis

The data obtained as a result of the patients' examinations will be evaluated through the SPSS package program. Calculations regarding the frequency of demographic data and average values will be made with the SPSS program. The data to be scratched are numerical data. In comparing quantitative independent groups with each other, T-test will be used if normal distribution is detected in independent groups, and Mann Whitney U test will be used if normal distribution is not detected. In the comparison of dependent groups, if normal

distribution is detected, T-test will be used in dependent groups, and Wilcoxon test will be used if normal distribution is not detected. p□0.05 will be considered significant.

Results

15 eyes treated with corneal cross-linking and 15 eyes with keratoconus without any ocular operation were included to the study. The mean age of the treantment group was 28.2 ± 5.6, the mean age of the untreated group was 31.4 ± 8.5 (p=0.96). There was no gender difference (p = 0.61).

Table-1: *Scheimpflug tomography keratometric values of patients with corneal crosslinking therapy and without corneal crosslinking therapy.*

Keratometrıc Values	***GROUP-Treated (n:15) Mean/ Std. D.***	***GROUP-Untreated (n:15) Mean/ Std. D.***	***p***
Anterior K1 D	43,2±3,1	44,8±6,1	0,28
Anterior K2 D	42,4±14,2	49,4±7,2	0,28
Anterior Km D	44,8±2,6	47,0±6,5	0,96
Anterior Astigmatism D	3,4±1,9	4,5±2,5	0,12
Posterior K1 D	-6,7±0,6	-6,6±1,2	0,26
Posterior K2 D	-7,3±0,6	-7,5±1,4	0,22
Posterior Km D	-6,9±0,7	-7,0±1,3	0,33
Posterior Astigmatism D	0,7±0,3	0,9±0,5	0,03*
CCT µm	433±56,8	486±60,0	0,70
Kmax D	52,5±4,6	55,2±12,9	0,68
Corneal Diameter mm	11,8±0,3	11,8±0,5	0,29

GROUP-Treated; *Cross-linking treated keratoconus,* ***GROUP-Untreated;*** Keratoconus untreated. ***n;*** Number of patients, *Curvature and elevation data for anterior and posterior corneal surfaces in treatment and untreated keratoconus groups.* ***Anterior K1****;* anterior corneal dioptric power in the flattest meridian, ***Anterior K2****;* anterior corneal dioptric power in the steepest meridian, ***Anterior Km****;* anterior mean corneal power, ***Anterior Astıgmatısm***; anterior corneal astıgmatısm, ***Posterior K1****;* posterior corneal dioptric power in the flattest meridian, ***Posterior K2****;* posterior corneal dioptric power in the steepest meridian, ***Posterior Km***:posterior mean corneal power, ***Posterior Astıgmatısm****;* posterior corneal astıgmatısm, ***Pakimeter_Apex****;* Pupıl center cornea thickness, ***Kmax****;* maximum keratometry, ***CCT;*** Central Corneal Thickness.

*; Statistically significant (p=0.05).

Anterior Segment OCT Measurements

The mean tear film (TF) thickness was 19.11 ± 17.7 µm in the treated group and 16.7 ± 5.0µ in the untreated group (p=0.07) and there was not statistically significant.

The mean central corneal epithelial thickness was 38.2±3.5 µm in the treated group and 60.8±10.9 µm in the untreated group (p=0.01). The mean central corneal stromal thickness was 389.8±66.1 µm in the treated group and 423.2 ± 63.3 µm in the untreated group (p=0.88). Total corneal thickness 459.8±49.2 µm in the treated group and in the untreated group 502.3±66.1µm (Figure-3).There was no statistically significant (p=0.94).

The corneal measurements were taken on the *Line 1* (Figure-2). The mean limbal epithelial thickness (LET) was 35.2±3.8µm in the treated group and 54.3±9.6µm in the untreated group (p=0.38). The mean corneal stromal thickness was 538.8±61.8µm in the treated patients, 585.6 ± 57.7µm (p=0.86) in the untreated patients. The mean total corneal thickness was 583.2±70.1µm in the treated group, in the untreated group, 648.8 ± 50.6µm (p=0.22). There was no statistically significant

The corneal measurements were taken on the *Line 2* (Figure-2). The mean LET was 30.7±5.5µm in the treated group and 45.6±11.5µm in the untreated group (p=0.04). The mean corneal stromal thickness was 597.5±78.5µm in the treated patients and 643.1±66.9µm in the untreated patients (p=0.61). The mean total corneal thickness was 633.2±82.6µm in the treated group, 689.3±65.2µm in the untreated group (p=0.50) and there was no statistically.

The mean LET was 24.8 ± 7.5µm in the treated group, 29.3 ± 8.5µm in the untreated group on *Line-3* (p=0.74) (Figure-2) and no statistically significant difference was found. The mean stromal thickness closest to the sclera was 647.8 ± 68.2µm in the treated group, 706.8 ± 92.9µm in the untreated group (p=0.43). The mean total corneal thickness on the side closest to the sclera was 672.6 ± 69.4µm in the treated group and 735.6 ± 91.6µm (p=0.52) in the untreated group, and there was no statistically significant difference.

Table-2: *Correlation of measurements by Scheimpflug Tomography and AS-OCT in cross-link treated patients.*

Correlation	*Line 1 ET*		*Line 2 ET (LCET)*		*Line 3 ET*		*CCET*	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Anterior K1 D	-0.408	0.041*	-0.104	0.620	-0.108	0.599	-0.108	0.599
Anterior K2 D	-0.378	0.062*	-0.017	0.937	0.035	0.865	0.035	0.865
Anterior Km D	-0.446	0.026*	-0.110	0.621	-0.003	0.987	-0.117	0.568
Anterior Astigmatism D	-0.414	0.039*	-0.082	0.696	-0.056	0.792	-0.138	0.503
Posterior K1 D	-0.465	0.012*	0.185	0.377	0.174	0.406	0.262	0.196
Posterior K2 D	-0.511	0.001*	0.121	0.564	0.125	0.551	0.259	0.202
Posterior Km D	0.497	0.01*	0.143	0.496	0.130	0.535	0.224	0.229
Kmax D	-0.512	0.001*	-0.203	0.331	-0.138	0.510	-0.102	0.621
CCT µm	0.431	0.31	0.376	0.064	0.464	0.019*	0.497	0.010*

Line-1 ET; Line-1 epithelial thickness, ***Line-2 ET;*** Line-2 epithelial thickness, ***Line-3 ET;*** Line-3 epithelial thickness, ***CCET;*** central corneal epithelial thickness. ***Anterior K1****;* anterior corneal dioptric power in the flattest meridian, ***Anterior K2****;* anterior corneal dioptric power in the steepest meridian, ***Anterior Km****;* anterior mean corneal power, ***Anterior Astıgmatısm***; anterior corneal astıgmatısm, ***Posterior K1****;* posterior corneal dioptric power in the flattest meridian, ***Posterior K2****;* posterior corneal dioptric power in the steepest meridian, ***Posterior Km****:* posterior mean corneal power, ***Posterior Astıgmatısm****;* posterior corneal astıgmatısm, ***Kmax****;* maximum keratometry, ***CCT;*** central corneal thickness,

*; Statistically significant (p=0.05)

Table 3: *Correlation of AS-OCT measurements in cross-link treated patients.*

	LCET		*LCST*		*CCET*		*CCST*		*CCTT*	
Correlations	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LCET			0,158	0,450	0,603	0,001*	0,324	0,114	0,465	0,019*
LCST	0,158	0,05*			0,224	0,02*	-0,152	0,467	0,874	0,001*
CCET	0,603	0,001*	0,224	0,283			0,494	0,01*	0,486	0,014*
CCST	0,341	0,011*	-0,152	0,467	0,494	0,01*			0,049	0,816
CCTT	0,376	0,064	0,874	0,001*	0,497	0,01*	0,049	0,816		

LCET; Limbal corneal epithelial thickness, ***LCST;*** Limbal corneal stromal thickness, ***CCET***; Central corneal epithelial thickness,***CCST;*** Central corneal stromal thickness, ***CCTT***;Central corneal total thickness

Discussion

In the present study, the keratoconus patients who treated with CXL had statistically significant thinner CCET and LET. Total central epithelial thickness, stromal thickness decreased with CXL. However, there was no statistically significant difference.

Keratoconus is an asymmetrical, non-infective, non-inflammatory progressive corneal ectasia that usually affects both eyes [12]. It starts at an early age and progression usually stops in the middle ages. Compared to previous years, as a result of today's technical and technological studies, the patient is diagnosed early, thanks to measurements during routine examination, before the patient becomes aware of it and the chance of intervention before the disease progresses is achieved [13]. 20% of patients have progressive corneal ectasia. High myopia and irregular astigmatism limit patients' ability to see [14]. Science has sought cures for this disease. Various treatment protocols have been developed. Some of those; corneal cross-linking, keratoconus lens, intrastromal ring segments, keratoplasty[15, 16].

Corneal cross-linking therapy had first applied to humans in 1999-2002 and became a candidate for entry into the medical literature in 2003 [17, 18]. Corneal cross-linking therapy stops the progression of keratoconus. Riboflavin and Ultraviolet-A are used in cross-linking therapy [19]. Purpose in corneal cross linking; It is aimed to increase the number of cross-links in the cornea, biomechanical strength, increase in collagen fiber diameter, increase in shrinkage temperature, increase enzymatic resistance, and increase resistance to edema [20, 21]. At the same time corneal cross-linking and anti-collagenase effect, anterior and middle Apoptosis of keratocytes in the stroma and deep keratocytes in their follow-upit makes it reproduce again [22].

Various complications have been reported in the literature as a result of corneal cross-linking. These are complications; infection, corneal haze, stromal scar, corneal scar, encountered epithelization delay and uveitis [23]. Infection Presence of epithelial defect as facilitating reasons, contact lens wear and steroid drops may be demonstrated. Escherichia coli, Acanthamoeba, Staphylococcus, Pseudomonas aeruginosa or polymicrobial agents formed after crosslinking blamed for keratitis [24, 25]. In this study, no complications developed in keratoconus eyes that underwent corneal cross-linking.

In our study; keratometric values such as Kmax, Km, K1, and K2 was decreased in keratoconus patients who underwent corneal cross-linking. But there was no significant difference in terms of Schiempflug topography between the treated with untreated. However, by Schiempflug topography, the posterior astigmatism value was decreased in the treated group compared to the untreated group and it was statistically significant. Waszczykowska and Jurowski did not report significant improvement in visual acuity and any significant differences in pre- and postoperative astigmatism [26].

In the present study, central corneal epithelium and limbal corneal epithelium was found thinner often cross-linking. In the study of Kanellopoulos et al., healthy subjects normal eyes, with keratoconus and corneal crosslink treated patients with keratoconus were investigated and epithelial changes in the corneal center and peripheral zones were analyzed. As a result of the research, it was observed that the thinnest epithelial

thickness was found thinner in patients who underwent corneal cross-linking both centrally and peripherally. [27]. In a recent study central and regional thicknesses of the corneal epithelium of healthy subjects and keratoconus patients treated with after corneal cross-linking was compared. They found significant thining in treated patients comparing to healthy subjects [28].

The Fourier-field Optical Coherence Tomography (OCT) of Yan li et al. detected that there was a statistically significace between keratoconus patients and healthy subjects in CCET and süperior, inferior, epithelial thickness. Central corneal, inferior, temporal limbal epithelium was found thinner after CXL. [29]. In literature multiple studies, investigated the epithelial thickness of the same keratotic eyes before and after corneal crosslinking. While the thickness of the inferior and temporal limbal epithelium of eyes with keratoconus had low before the operation, epithelial thinning occurred in the cornea central and mostly in the quadrant after the operation [30-32].

In our study, we found that stroma and total corneal thickness decreased in keratoconus patients with cross linking with AS OCT, but there had no statistically significant difference. In the study of Kanellopoulos et al. investigated the stroma and total corneal thickness of all three groups. Both stroma and total corneal thickness of the patients who had cross-linking treatment was thinner than healthy eyes and untreated keratoconus [27].

Our results showed that there was a statistically significant correlation between CCT and CCET in treated patients. CCST correlates with CCET and was statistically significant. There was a sttatistically significant correlation between LCET and LCST. Also, LCET shows a strong correlation with CCET. This result reveals the limbal epithelium acts as a source for the central epithelial cells. The LCET thinning may cause the CCET thinning because of reduced migration of decreased number of stem cells to the center.

There are some limitations of the present study. First it has small sample size, second the OCT measurements had taken manually, third the thickness measurements could not taken with Schiemplug topography. However this study enlightens the limbal thickness and central thickness changes before and after corneal crosslinking.

Corneal cross-linking has gained an important place among the inventions that prevent the progression of keratoconus disease. Thanks to the latest technical and technological developments, the details of the corneal structure are gradually increasing. This leads to the development of new medical and surgical methods.

As a result epithelial thickness and limbal thickness alterations detected with Ant-OCT could be useful for monitorizing the keratoconus patients treated with crosslinking and could show the effectivity of the treatment.

Declarations

Funding ınfo: None

Author contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by **Hande GÜÇLÜ**, **İrfan AKARAY** and **Özlem KAYA**. The first draft of the

manuscript was written by **Hande GÜÇLÜ** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability: Data are available from the corresponding author upon request.

Animal Research (Ethics): The study was approved by the Ethics Committee of the Trakya University and it followed the tenets of the Declaration of Helsinki.

Consent to Participate (Ethics): Informed consent was obtained from all individual participants included in the study.

Consent to Publish (Ethics): Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Conflicts of interest/Competing interests: The authors have no conflict of interests and competing interests.

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Figures

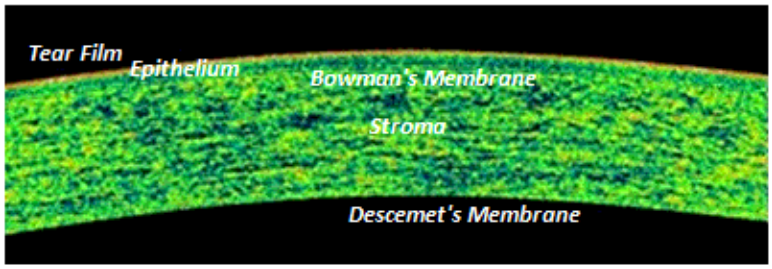

Figure 1

Anterior Segment OCT corneal layers

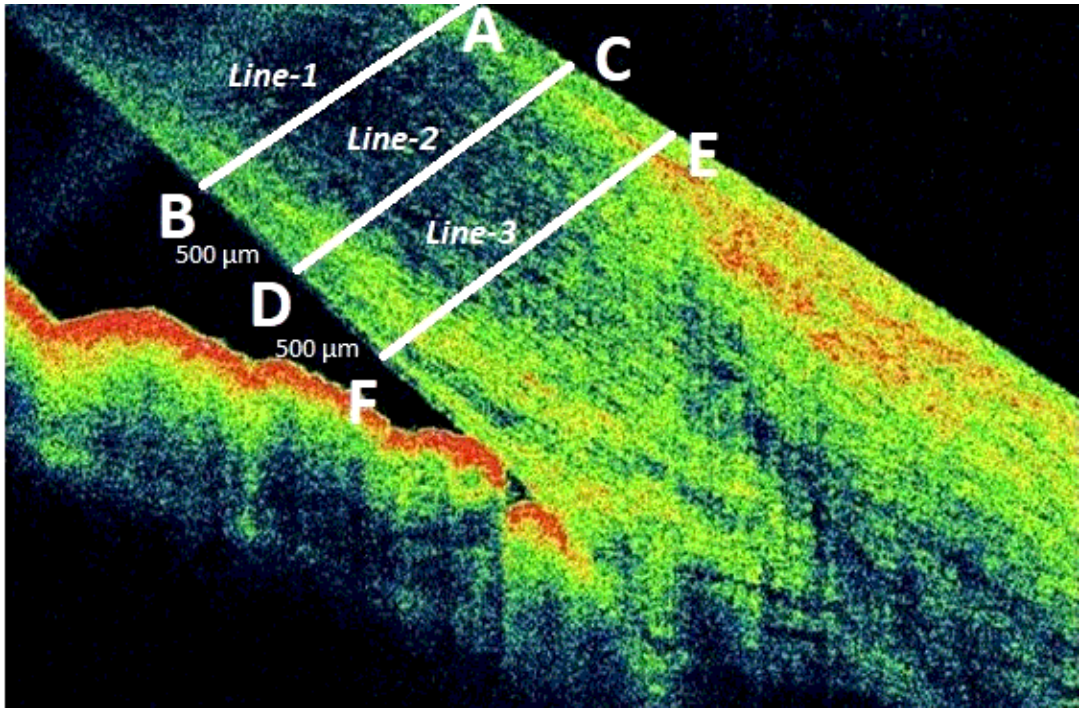

Figure 2

Anterior Segment OCT measurements were investigated on three lines from a distance of 500 µm: Line-1, Line-2, Line- 3

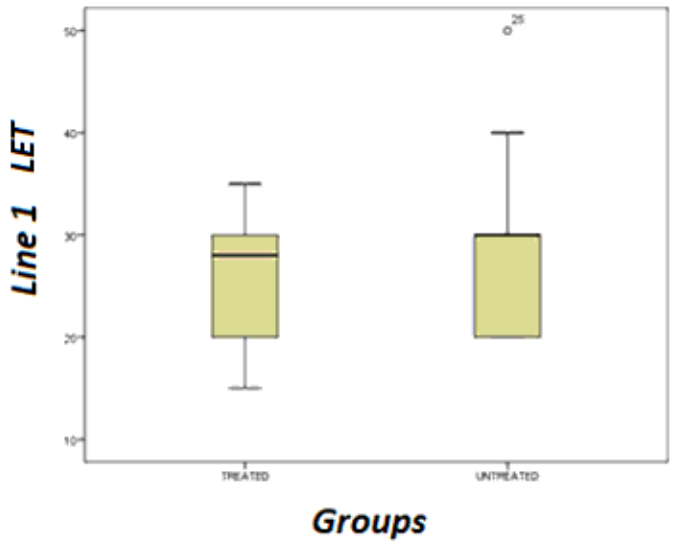

Figure 3

Histogram representation of limbal epithelial thickness (LET) on Line-1 in treated and untreated keratoconus patients

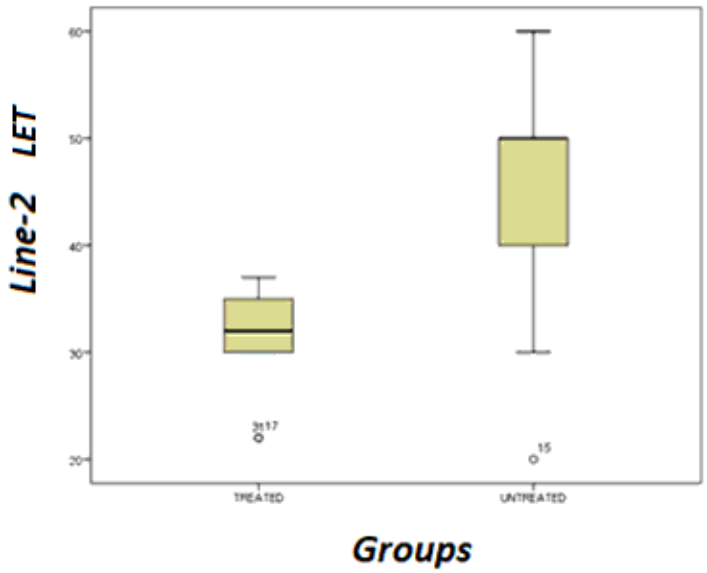

Figure 4

Histogram representation of limbal epithelial thickness (LET) on Line-2 in treated and untreated keratoconus patients

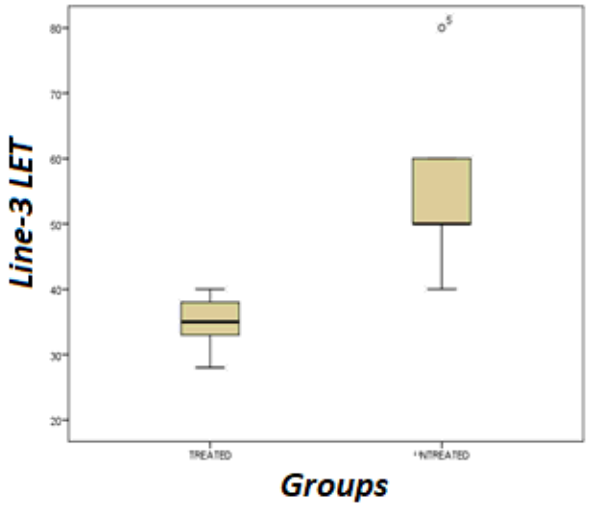

Figure 5

Histogram representation of LET on Line-3 in treated and untreated keratoconus patients

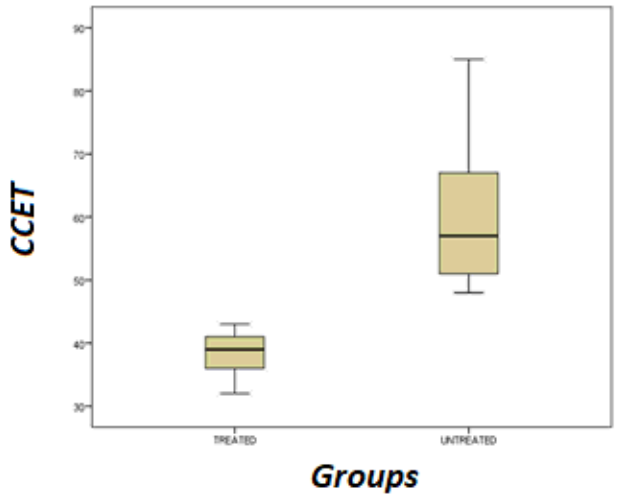

Figure 6

Histogram representation of Central corneal epithelial thickness (CCET) in treated and untreated keratoconus patients.