

The findings of corneal specular microscopy in patients with keratoconus

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Abstract

Aim: The aim of this study was to determine the correlation of corneal endothelial cell density, coefficient of variation and hexagonal cell ratio with the stages of keratoconus and corneal thickness.

Material and Methods: The study included 102 eyes from 51 patients. The corneal topography was performed using the Sirius corneal topographic device and the examination of the endothelium was performed using Nidek CEM-530. The patients were divided into groups in accordance with keratometry values (group 1: K2<45 D, group 2: K2 45-52 D, group 3: K2> 52 D) and thinnest cornea thickness (group 1:> 450 µm, group 2: 400-450 µm, group 3: <400 µm). Specular microscopic findings were compared according to the severity of keratoconus and corneal thickness.

Results: Of the patients, 29 (50%) were male and 22 (50%) were female. The mean age was 29.8 ± 7.7 (15-45) years. Endothelial cell count and hexagonal cell ratio were significantly lower in severe keratoconus compared to mild and moderate keratoconus and the coefficient of variation was higher in severe keratoconus compared to the other groups (p <0.001). When the patients were compared according to the pachymetry value at the thinnest point of the cornea, the endothelial cell number and the hexagonal cell ratio were found to be lower in the group with pachymetry less than 400 µm (p <0.001). The coefficient of variation was higher in the same group compared to the others (p <0.001).

Conclusion: It was revealed that endothelial cell density, hexagonal cell ratio decreased and the coefficient of variation increased with the progression of keratoconus. Specular microscopic examinations in patients with keratoconus provide important data in addition to topography.

Keywords: Endothelial Cell Density; Keratoconus; Specular Microscopy.

INTRODUCTION

Keratoconus is a disease that develops in the puberty period, which affects both sexes and is frequently bilateral (1,2). Although its pathogenesis has not been clarified yet, it has been shown that environmental and genetic factors play a role in the onset of the disease (3,4). The disease leads to progressive thinning of the corneal stroma, ectasia and irregular astigmatism associated with it and decreases visual acuity (3,5). It is characterized by changes in the structures of corneal epithelium and stroma and stromal thinning associated (3). In addition, specular microscopy also revealed some differences in terms of cell number and morphology in the endothelium compared to healthy

individuals (6-8). Glasses and contact lenses are used for visual rehabilitation in keratoconus. Corneal cross-linking and intracorneal ring segments are also used to prevent progression in the early and middle stages of the disease (9 -11). In the late stages, Penetrating Keratoplasty (PKP) and Deep Anterior Lamellar Keratoplasty (DALK) can be performed to increase the patient's visual acuity (11).

Some studies revealed that ultraviolet rays led to endothelial toxicity and the number of endothelial cells decreased in the patients following the Corneal Cross-Linking Treatment for Keratoconus, especially the ones with less than 400 µm of corneal thickness and underwent epithelial debridement (12,13). Evaluation of endothelial cell counts and morphology before treatment can help

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determine whether cross-linking treatment will cause endothelial damage or not and what crosslinking modality should be pursued for the patient (8,13). A healthy corneal endothelium is crucial for patients receiving cross-linking treatment in keratoconus, as well as for patients undergoing keratoplasty. Healthy endothelium is an important factor in maintaining long-term transparency of the graft in patients undergoing keratoplasty (14). The impairment of endothelial cell counts and morphology leads to surgical failure in the long-term especially in patients undergoing DALK. Therefore, PKP is recommended for this patient group (14). In this group of patients with early diagnosis of keratoplasty, the preoperative evaluation of endothelial structure will have a direct affect in the surgical technique and success rate. Endothelial count and morphology are important factors in determining the keratoplasty or corneal crosslinking techniques to be performed on patients (12).

The aim of our study is to determine the quantitative and morphological differences in corneal endothelium in different stages of Keratoconus and to give Ophthalmologists an idea about which technique to apply before treatment.

MATERIAL and METHODS

The study was approved by the Ethics Committee (number: E.2731) of Erzincan Binali Yıldırım University Hospital and it was performed in the Ophthalmology Department of the same hospital following the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients. The legal guardians gave consent for the patients under age 18.

The record of each patient diagnosed with Keratoconus was taken including best-corrected visual acuity based on the Snellen chart, findings of biomicroscopic and fundoscopic examination and intraocular pressure measurements obtained with Goldmann applanation tonometer. Exclusion criteria were as follows: patients with history of intraocular surgery, contact lens use, additional ocular disease, central corneal opacity and hydrops history. Keratoconus was diagnosed only in the presence of at least one or more findings such as Munson's sign, A Fleischer ring, Vogt's striae, Rizutti's sign, apical corneal thinning accompanied by the presence of topographic findings (keratometry value greater than 45 D, inferior-superior asymmetry ratio greater than 1.8 D, decreased corneal thickness, keratoconus index over 1.07).

Topography of the patients was performed using the Scheimpflug camera system and Sirius corneal topography device combined with Placido's disc topography (Costruzione Strumenti Oftalmici, Italy). The flattest and steepest keratometry values (K1 and K2), central corneal thickness (CCT) and pachymetry at the thinnest corneal thickness were obtained with topographic measurements.

The cornea endothelium was examined using the Nidek CEM-530 noncontact specular microscope (NIDEK Co., Ltd. Japan). Endothelial cell density, hexagonal cell ratio

and variation coefficients were obtained using the device.

The patients were divided into three groups according to the study Collaborative Longitudinal Evaluation of Keratoconus (CLEK). The patients were classified into stages based on steepest keratometric value as follows: mild <45 D; moderate 45-52 D; severe >52 D (K2). Patients were also categorised into three groups according to thinnest cornea thickness. Accordingly, eyes with pachymetry value less than 400µm were classified as group 1, those between 400-450 µm were classified as group 2, and those with greater than 450µm were classified as group 3. The number of endothelial cells, hexagonal cell ratio and variation coefficients obtained by specular microscopy were compared in the eyes categorised according to the severity of Keratoconus and thinnest cornea thickness.

Statistical Analysis

Results are presented as number and percentage for categorical variables and as mean \pm standard deviation for continuous variables. The chi-square test was used for the comparison of categorical variables between groups and Shapiro-Wilk was used to test the normality distribution for the continuous variables. Either one-way ANOVA or Kruskal-Wallis test were used to compare the independent continuous variables between 3 groups depending on whether statistical hypotheses were fulfilled. Dun's test was used for Kruskal Wallis as a post-hoc test. The correlation coefficient of Pearson or Spearman was used to evaluate the correlation between the measurements. The statistical significance level for all tests was accepted as 0.05. Statistical analysis was performed using IBM SPSS 20 software (IBM Corp.). Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

RESULTS

The study included 102 eyes of 51 patients. 29 (50%) of the patients were male and 22 (50%) were female. The mean age was 29.8 ± 7.7 years (15-45). When the patients were classified according to K2, mild keratoconus was detected in 33 eyes (32.4%) of 19 patients, moderate keratoconus in 40 eyes (39.2%) of 28 patients and severe keratoconus in 29 eyes (28.4%) of 19 patients. There was no statistically significant difference in terms of age between the groups ($p = 0.944$). The specular microscopy findings by the severity keratoconus are shown in Table 1. When the endothelial cell density was compared among the groups, it was found that the number of endothelial cells in severe keratoconus was less than in the mild and moderate keratoconus ($p < 0.001$) (Table 1). The coefficient of variation was higher in severe keratoconus compared to the other groups ($p < 0.001$). Hexagonal cell ratio and the thinnest-point pachymetry of the cornea were found to be lower in severe keratoconus compared to the other groups ($p < 0.001$).

When the patients were compared according to the

thinnest-point pachymetry of the cornea, the mean average age was similar between the groups ($p = 0.112$). The endothelial cell count and the hexagonal cell ratio were found lower in the group with pachymetry less than 400 μm ($p < 0.001$). The coefficient of variation was higher in the same group compared to the others ($p < 0.001$). The specular microscopy findings according to the thinnest-point pachymetry of the cornea are given in Table 2.

There was a significant correlation between endothelial cell count, coefficient of variation and hexagonal cell ratio in severe keratoconus compared to mild and moderate keratoconus ($p = 0.00$). There was a significant correlation between endothelial cell count, coefficient of variation and hexagonal cell ratio in the eyes with pachymetry value below 400 μm at the thinnest cornea thickness ($p = 0.00$).

Table 1. Specular microscopic findings by the severity of Keratoconus

	Mild ($K_2 < 45$)	Moderate ($45 \leq K_2 \leq 52$)	Severe ($K_2 > 52$)	P
Age	32.2 \pm 8.7	28.7 \pm 6.2	28 \pm 7.2	0.089
Endothelial cell count	2643.4 \pm 299.4	2707.5 \pm 264.2	2483 \pm 121.4	<0.001
Coefficient of variation	27.9 \pm 3.4	29.7 \pm 3.8	33.7 \pm 2.9	<0.001
Hexagonal cell ratio	67.8 \pm 5.6	64.7 \pm 5.8	61.2 \pm 5.3	<0.001
The thinnest-point pachymetry of the cornea	504.2 \pm 29.3	480.2 \pm 45.5	436.7 \pm 45.7	<0.001

Table 2. Findings of specular microscopy according to the thinnest-point pachymetry value of the cornea

	TCT <400	400 \leq TCT \leq 450	TCT > 450	P
Age	29.5 \pm 6.7	28.5 \pm 8.4	31.6 \pm 6.8	0.112
Endothelial cell count	2451 \pm 60.8	2632.5 \pm 204.5	2719.3 \pm 329.4	<0.001
Coefficient of variation	33.3 \pm 3.6	30.5 \pm 4	28.1 \pm 3.2	<0.001
Hexagonal cell ratio	58.1 \pm 2.2	65.8 \pm 5.1	67.7 \pm 5.6	<0.001
Pachymetry value of corneal apex	406.9 \pm 22.8	487.3 \pm 36.1	506.2 \pm 24	<0.001

DISCUSSION

It has been revealed that polymegatism and pleomorphism are more common in corneal endothelial cells of the patients with keratoconus who underwent penetrating keratoplasty, compared to healthy corneas (15). Endothelial cell density has been shown to decrease particularly in patients with advanced keratoconus, in addition to these findings (16-17). El-Agha et al. found a decrease in endothelial cell count, increased coefficient of variation and a decrease in hexagonal cell ratio in advanced keratoconus but could not obtain a statistically significant result from these variations (6). In the same study, the authors reported that statistically significant results could be obtained in studies evaluating larger cases. (6). In the study of Bozkurt et al., eyes diagnosed with keratoconus were classified according to CLEK criteria and it was found that endothelial cell density decreased in advanced keratoconus. However, no significant change was observed in pleomorphism and polymegatism as in the study by El-Agha et al. Ucakhan et al. compared patients with keratoconus with the healthy control group and found a decrease with no statistical significance. Again, in the same study, it was shown that the number of endothelial cells decreased significantly in advanced keratoconus in comparison of the keratoconus stages (16). In addition, the ratio of hexagonal cells was low compared to the control group in this study and they detected a decrease with no statistical significance between the stages of keratoconus (16). In our study, a statistically significant decrease in endothelial cell

density was found in patients with advanced keratoconus compared to mild and moderate keratoconus, similar to the studies in the literature. In addition, in our study, we detected a decrease in the ratio of hexagonal cells and increase in the coefficient of variation with pleomorphism and polymegatism, which were found to be statistically significant in contrast to these studies.

Studies performed with confocal microscopy have shown that the number of endothelial cells does not decrease in keratoconus (18-21). However, in the studies by Yeniad and Mocan et al. (19-20), they underlined the increase of polymegatism and pleomorphism although the number of endothelial cells did not decrease. In the study of Yeniad et al., they compared the patients according to the use of contact lenses and stated that the number of endothelial cells did not differ between the groups. In addition, the use of contact lenses showed that polymegatism and polymorphism were higher among those who did not use them, but they could not find a statistically significant difference (19). Weed et al. reported that the size of endothelial cells did not change in eyes with keratoconus compared to the control group. In the same study, it was shown that the size of endothelial cells increased with age in patients with keratoconus and this could be related to the age and location of the disease (20).

The pathogenesis of the decrease in endothelial cell density has not been completely explained in patients with advanced keratoconus. It has been argued that keratoconus is a degenerative disease and that apoptosis

of endothelial cells may lead to a decrease in endothelial cell density (22). In addition, it has been revealed that polymorphism and polymegatism develop in endothelial cells as a result of trying to compensate the damage caused by mechanical stress on the keratoconus cornea resulting in tears and cracks in the descemet and endothelial cell layer to prevent corneal edema (23). As a result, the decrease in the number of endothelial cells is not an indicator of the endothelial function on its own, but effective function can still be maintained with polymorphism and polymorphism. In our study, the decrease of endothelial cell density and the increase in the coefficient of variation (polymorphism and polymegathism) in the eyes with severe keratoconus, compared to the eyes with mild and moderate keratoconus, was attributed to the increased damage in the cell apoptosis and descemet membrane in the advanced stages. The fact that there is no visible acute hydrops and compensation in the endothelium indicates that the damage in Descemet's membrane is effectively compensated and corneal transparency can be maintained.

Studies have revealed some changes in endothelial cell density and morphology in patients who wear contact lenses upon the diagnosis of keratoconus (19,24-26). It is thought that hypoxia or contact lens-related mechanical epithelial trauma are responsible for these changes occurring after contact lens wear (25,26). Edmond et al. found that contact lenses with high oxygen permeability had less effect on the number of endothelial cells compared to those with less oxygen permeability, thereby proving that hypoxia directly affects corneal endothelium (24). In addition, the long span of contact lens wear has been shown to affect the polymorphism and polymatism of the endothelial cells (24). In the light of this data, it was revealed that the contact lenses with high oxygen permeability should be preferred for visual rehabilitation in the eyes with keratoconus, where endothelial changes were detected by specular microscopy screening.

Today, corneal crosslinking treatment is frequently used to prevent the progression of keratoconus (12,13). Although there are studies reporting a decrease in endothelial cell density and / or morphology following the cross-linking, this method can be applied reliably (28,29). Hypoosmolar riboflavin solution is recommended to prevent endothelial toxicity especially for the eyes with corneal thickness of less than 400 μm (30). The fact that endothelial cell density and morphology are known to be affected in patients with corneal thickness below 400 μm indicates that we should determine a crosslinking modality so as not to increase the damage currently present in this patient group. In the light of these findings, the evaluation of endothelial cell count and function before treatment in patients who have been recommended cross-linking treatment will be advantageous in terms of predicting and taking precautions for possible endothelial complications in the postoperative period.

Penetrating Keratoplasty (PKP) or Deep Anterior Lamellar

Keratoplasty (DALK) are frequently performed in patients diagnosed with advanced keratoconus. Performing DALK instead of PKP will prevent the graft preservation in the postoperative period and cause surgical failure in such cases with endothelial count and morphology affected in advanced stages as in our study. Therefore, the evaluation of endothel before keratoplasty will directly affect the surgical method to be applied (14).

CONCLUSION

In conclusion, in our study, endothelial cell density, coefficient of variation and hexagonal cell ratio were found to be negatively affected in patients with advanced keratoconus and corneal thickness below 400 μm according to the CLEK classification. Preliminary determining whether endothelial cell count and morphology are affected or not by a non-contact specular microscopy will directly contribute to the success of the treatment.

Declaration of interest: There are no conflict of interest in this study, all authors approved this information.

Competing interests: The authors declare that they have no competing interest.

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