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A correlation study between a corneal endothelial count in patients with different stages of keratoconus using specular microscopy

Um estudo de correlação entre a contagem endotelial da córnea em pacientes com diferentes estágios de ceratocone usando a microscopia especular

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ABSTRACT

Objective: *To evaluate the corneal endothelial count in patients with keratoconus (KCN) by specular microscopy and correlate with the stage of keratoconus.* ***Methods:*** *Ninety-three eyes from 61 patients with KCN were included in this cross-sectional study. The eyes were classified into KCN stages 1 to 4 according to the Amsler-Krumeich classification using keratometry obtained by corneal topography and pachymetry readings obtained by specular microscopy.* ***Results:*** *Age ranged from 12 to 43 years, mean ± (standard deviation) 22.1 ± 6.7 years. The average keratometry ranged from 42.25 to 71.4 D, (53.0 ± 6.1 D). Pachymetry ranged from 350 to 606 µm, (461.7 ± 47.1 µm). Regarding the Amsler classification, 23 patients (24.7%) had stage 1, 24 (25.8%) stage 2, 5 (6.5%) stage 3 and 41 patients (44.1%) stage 4. No linear correlation was observed between mean keratometry and endothelial cell count (Pearson's correlation coefficient = -0.05). In the early to moderate stages of KCN, the mean endothelial cell count was 2738.3 ± 285.4 cell / mm2, while in the advanced KCN group (stages 3 and 4) it was 2670.6 ± 262.7 cell / mm2 , p = 0.24.* ***Conclusions:*** *No correlation was found between the endothelial cell count and the KCN stage.*

Keywords: *Corneal endothelium; Keratoconus; Specular microscopy; Endothelial cell density; Keratometry*

RESUMO

Objetivo: Avaliar a contagem endotelial da córnea em pacientes com ceratocone (KCN) por microscopia especular e correlacionar com o estágio do ceratocone. **Métodos:** Noventa e três olhos de 61 pacientes com KCN foram incluídos neste estudo transversal. Os olhos foram classificados nos estágios 1 a 4 de KCN de acordo com a classificação de Amsler-Krumeich utilizando ceratometria obtida pela topografia de córnea e leituras de paquimetria obtidas pela microscopia especular. **Resultados:** A idade variou de 12 a 43 anos, média ± (desvio padrão) 22,1 ± 6,7 anos. A ceratometria média variou de 42,25 a 71,4 D (53,0 ± 6,1 D). A paquimetria variou de 350 a 606 µm, (461,7 ± 47,1 µm). Em relação a classificação, 23 pacientes (24,7%) apresentavam estágio 1, 24 (25,8%) estágio 2, 5 (6,5%) estágio 3 e 41 pacientes (44,1%) estágio 4. Não foi observada correlação linear entre ceratometria média e contagem de células endoteliais (Coeficiente de correlação de Pearson = -0,05). Nos estágios iniciais a moderados de KCN, a média da contagem de células endoteliais foi 2738,3 ± 285,4 cel/mm2, enquanto no grupo de KCN avançado (estágios 3 e 4) foi 2670,6 ± 262,7 cel/mm2, p= 0,24. **Conclusões:** Não há correlação entre a contagem de células endoteliais e o estágio do KCN.

Descritores: Endotélio da córnea; ceratocone; microscopia especular; densidade celular endotelial; Ceratometria

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Introduction

Keratoconus (KCN) is a progressive disorder featured by central or paracentral corneal stroma (usually inferior) thinning followed by apical protrusion and irregular astigmatism.[1] According to epidemiological studies, annual estimates on the incidence of new KCN cases ranges from 1:3000 to 1:80000 a year,[1,2] . Such variation can be attributed to the following factors: different definition criteria applied to the cases, great sensitivity of modern diagnostic devices, differences in study design, ethnic differences and other differences related to the assessed sample. A recent study has estimated KCN prevalence in the general population of 1:375 (265 per 100,000) based on its annual incidence, patients' mean age at diagnosis and life expectancy.[2]

The literature about KCN is rich in publications on the anterior part of the cornea. These studies investigate the epithelium, Bowman's layer and stroma, and few of them focus the endothelium and Descemet's membrane.[1] However, changes in corneal microstructure can also lead to changes in the corneal endothelial layer.[3,4]

Corneal endothelium plays essential role in corneal physiology and transparency. Its morphological data, such as endothelial density, mean cell area, rate of hexagonal cells and coefficient of variation are important tools to assess the corneal physiology.[5] Specular microscopy is application in KCN cases, since it is useful in studies about transient and chronic changes in endothelial cell morphology in contact lens wearers, in pre- and postoperative corneal crosslinking surgical procedures, and in corneal transplants.[6,7] There are reports in the literature associating KCN to Fuchs endothelial dystrophy.[8,9]

The aims of the current research were to assess corneal endothelial cell counting in patients with keratoconus (KCN) based on specular microscopy and to correlate it to keratoconus stage.

Methods

Cross-sectional study to review the medical records based on the consecutive inclusion of all patients with keratoconus referred to Bonsucesso Federal Hospital, Contact Lens sector, from January to December 2019, who had undergone corneal topography and corneal specular microscopy exams before the first test and contact lens adaptation. The Ethics Committee of Bonsucesso Federal Hospital approved the research (n. 57,557,8166,5253). Cases with corneal scarring, hydrops, previous eye surgeries, cornea guttata or Fuchs endothelial dystrophy, and contact lens wearers were excluded from the sample.

The following data were collected from the medical records: sex, age, k1 and k2 obtained by corneal topography (Aladdin, Topcon Medical Systems, Inc. Oakland), endothelial cell count and corneal pachymetry obtained by non-contact specular microscopy (Tomey Corporation, Nagoya, Japan). This specular microscopy device automatically captures a sequence of 15 images during each measurement and counts up to 300 cells per image in the region of interest through an automated image processing. The image with the highest contrast and lighting quality is automatically selected by the instrument and subsequently manually checked by the examiner. The automated cell detection and counting implemented in the manufacturer's software was used in the xperiment.

Eyes were classified into KCN stages 1 to 4 according to the Amsler-Krumeich classification by using keratometry obtained through corneal topography and pachymetry readings through specular microscopy.

SPSS software (SPSS version 19.0, IBM, New York) was used to perform the statistical analyses. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were expressed as frequencies and percentages. Student's t-test was used to compare continuous variables with normal distribution between stages 1 and 2 to the advanced group (stages 3 and 4). The correlation between endothelial cell count and mean keratometry was assessed through Pearson's correlation coefficient (Values of $p < 0.05$ were considered significant).

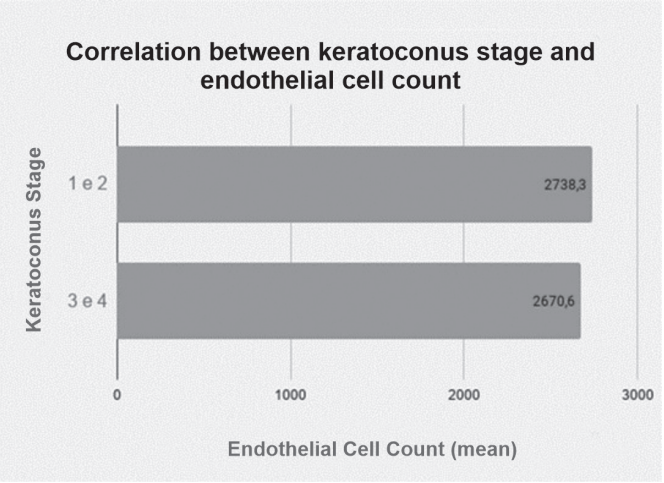

Figure 1: Comparison between endothelial cell count (cells/mm2) between patients with keratoconus at the early to moderate stages (stages 1 and 2) to advanced stages (stage 4), p = 0.24.

Results

The current research comprised 93 eyes from 61 patients - women accounted for 46 eyes (49.5%) and men for 47 eyes (51.0%). The sample consisted of 46 (49.5%) right eyes and 47 (51.0%) left eyes. Age ranged from 12 to 43 years, mean ± (standard deviation) was 22.1 ± 6.7 years.

Mean keratometry ranged from 42.25 to 71.4 D, mean 53.0 ± 6.1 D. Pachymetry ranged from 350 to 606 µm, mean 461.7 ± 47.1 µm. Based on the Amsler classification, 23 patients (24.7%) were stage 1, 24 (25.8%) were stage 2, 5 were (6.5%) stage 3 and 41 patients (44.1%) were stage 4.

No linear correlation was observed between mean keratometry and endothelial cell count (Pearson's correlation coefficient: -0.05, $p<0.05$). There was no correlation between more curved keratometry (k2) and endothelial cell count (Pearson's correlation coefficient: -0.08, $p <0.05$). Mean endothelial cell count was 2738.3 ± 285.4 cell/mm2 in keratoconus early to moderate stages (stages 1 and 2), whereas the advanced keratoconus group (stages 3 and 4) recorded 2670.6 ± 262.7 cell/mm2 ($p = 0.24$). (Figure 1)

Discussion

Specular microscopy is a non-invasive technique, easy to perform, based on corneal endothelium reflection. The specular microscope captures the specular reflection of part of the endothelium and presents endothelial image that is magnified by an electronic set.5

Individual suffer with natural decline in endothelial density

Table 1
Comparison between endothelial cell count studies at different keratoconus stages carried out through specular microscopy

	Current study	**Uçakhan et al.[14]**	**Niederer et al.[11]**	**Timucin et al.[6]**	**El-Agha et al.[10]**	**Goebels et al.[13]**
Sample Size with KCN (eyes)	93	48	52	65	90	712
Age (mean) in years	22	22,8	27,9	20,9	27	38
Adopted Classification	Asmler-Krumeich Stage 1: 24,7% Stage 2: 25,8% Stage 3: 0,5% Stage 4: 44,1%	Main Keratometry Mild (<47D) 10,4% Moderate (47-55D) 35,4% Severe (>55D) 54,2%	Greater Keratometry Mild (<45D) / Moderate (45-52D): 40.3% Severe (>52D) 61.5%	Greater Keratometry Mild (<45D) 29.2% Moderate (45-52D): 32.3% Severe (>52D): 38.4%	Asmler-Krumeich Stage 1: 27.5% Stage 2: 42.5% Stage 3: 30% Not observed: Stage 4	Amsler-Krumeich Stage 0 23.7% Stage 1: 13.2% Stage 2: 28.9% Stage 3: 23.3% Stage 4: 10.8%
Endothelial cell count (mean) cells/mm^2 based on stage	Initial and moderate 2738,3 Severe (stages 3 and 4): 2670,6	Not mentioned	Mild/Moderate: 2510,6 Severo: 2345,5	Mild: 2759 Moderate: 2747 Severe: 2698	Stage 1: 2404.5 Stage 2: 2455,4 Stage 3: 2214,8	Stage 0: 2611 Stage 1: 2624 Stage 2: 2557 Stage 3: 2487 Stage 4: 2401
Conclusion	No relationship was found between KCN stages and EC	There was significant difference in EC when it was compared to severe KCN, with mild to moderate KCN ($p < 0.05$)	There was no significant difference in EC between KCN stages	There was no significant difference in EC between KCN stages	Significant difference in EC, at stage 3 with lower EC ($p < 0.05$)	Significant difference in EC, at stage 3 with lower EC ($p < 0.05$)

KCN = keratoconus; EC = endothelial cell count

throughout life, and ith can be potentiated by corneal diseases.[5] Mean age in the assessed group was 22 years, and this age group is compatible to the age range accounting for the highest keratoconus prevalence; moreover, it is related to good endothelial cell reserve.[1,5] The sample was divided into stages, based on the Amsler-Krumeich Classification. There was an attempt to relate endothelial density to different KCN stages.

Few studies in the literature compare endothelial changes at different keratoconus sages, but they present conflicting results.[10-14] If the literature has proven any correlation between endothelial abnormality in patients with KCN and disease stage, such a finding could affect the selection criteria set for corneal penetration or for lamellar anterior corneal transplantation.

No correlation was found between endothelial density and KCN stage in the current research, and this outcome corroborates findings in a previous study that has assessed 40 eyes from 29 Egyptian patients. This study has recorded the following results: mean 2404 ± 345 cell/mm2 at stage 1, 2455 ± 331 cell/mm2 at stage 2 and 2214 ± 748 cell/mm2 at stage 3; correlation coefficient was 0.018 ($p = 0.9$).[10]

Likewise, the study conducted by Niederer et al. used confocal microscopy in the assessments and did not record any significant difference between KCN stage and endothelial cell counting. The recorded findings were 2510.6 ± 334.4 cell/mm2 count at the mild and moderate stages (1 and 2) and 2345.5 ± 331.8 cell/mm2 at the advanced stages (with k> 52D, $p = 0.09$).(11)

Timocin et al. reported that changes observed in endothelial cell counting in KCN were not depend on central pachymetry and were not correlate to KCN stages.[6]

Ostadi-Moghaddam et al. conducted a study to assess specular microscopy in the contralateral eyes of patients with Vogt striae; they found no significant difference between then.[12] On the other hand, the study conducted by Goebels et al. with 712 patients, whose patients were in the mean age group 38 years, re-

ported significantly lower endothelial cell count in more advanced KCN stages: with 2624 ± 300 cell/mm2 at stage 1 and 2401 ± 464 cell/mm2 at stage 4 ($p < 0.01$).[13] It is important highlighting that most patients included in the study wore contact lenses. More advanced KCN stages can be associated with prolonged contact lenses' use throughout life. Such a profile can lead to endothelial changes; therefore, the inclusion of patients who wear contact lenses may have influenced the results in the current research. However, difference in endothelial density between KCN stages 1 and 4 remained significant, even when only the subgroup with no current history of contact lens use was assessed.[13]

Corneal endothelial cell count was evaluated based on KCN using. Different devices showed conflicting results in different studies, and it can be the consequence of different data collection protocols. Table 1 shows the comparison between published articles and the current study.

A limitation of the current research lies on the fact that specular microscopy did not capture any image in very curved and/or very thin corneas, because specular surface area depends on the curvature of the reflecting surface. The more curved the cornea, the smaller the image obtained in specular microscopy devices. There is additional restriction in the light reflection area due to the proximity of the two concentric surfaces (i.e., epithelium and endothelium). The epithelial surface is highly reflective due to the significant difference in the refractive index between air and the epithelium. The light beam crosses the cornea and reflects on both epithelium interface and the endothelial interfaces. The visible specular area is given by the relationship between beam width and cornea thickness. The viable endothelium area is a rectangle because of the aforementioned restriction; corneal curvature radius dominates the height of the rectangle.[5] Therefore, very curved and very thin corneas can decrease exam's reliability by reducing the visible area of the specular reflex or even by making it impossible to capture its image.

The current research did not have access to all data of the specular microscopy exam, such as endothelial mosaic pictures and other important indices that are not routinely registered in all medical records, in our service, like coefficient of variation, hexagonal cell rate and mean cell area, and this is a medical record review study

Conclusion

Specular microscopy is important for the follow-up of patients with keratoconus. despite the evidence in the current research about lack of direct correlation between disease classification stage and endothelial cell count, the evaluation of endothelial cell count is useful for both surgical monitoring and planning.

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