ASSESSMENT OF CORNEAL ENDOTHELIAL LAYER IN CONTACT LENS WEARERS WITH THE AID OF AN ENDOTHELIAL MICROSCOPE

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SUMMARY

Aims: The main aim of our study was to demonstrate the difference in endothelial cell density between a group of keratoconus contact lens users and non-contact lens users (without keratoconus).

Material and methods: In our study we had data from 96 subjects with an average age of 40.5 ±14.05 years. For the purposes of our study, we worked with each eye separately for each subject (n = 192). Keratoconus (research group) was diagnosed in 97 eyes. The mean age of the patients in the research group was 41.9 ±10.6 years. Keratoconus was not diagnosed in the remaining 95 eyes (control group). The mean age of the patients in the control group was 39.5 ±16.6 years. In the keratoconus group, the patients wore hard contact lenses (HCL) in a total of 43 eyes, hybrid contact lenses (HbCL) in 48 eyes and soft contact lenses (SCL) in 6 eyes. The average total period of contact lens use in the research group was 10.6 ±2.36 years.

Results: The average number of endothelial cells in the research group was 2607.11 ±298.45 cells per mm^2. The average number of endothelial cells in the control group was 2831.94 ±523.51 cells per mm^2. We tested these two variables using a T-test, which showed a statistically significant difference ($p < 0.001$). A statistically significant difference in the mean endothelial cell count related to patient age and use / non-use of contact lenses of two types was demonstrated only in the under-40 group in a comparison of the non-contact and keratoconus group with the keratoconus group using HCL ($p = 0.02$). A statistically significant difference in the length of contact lens wear was demonstrated between the keratoconus-free group with or without SCL and the keratoconus group when wearing HCL or HbCL for more than 20 years ($p = 0.01$ and $p = 0.02$). For HbCL users, this difference was demonstrated also after 15 years of wearing ($p = 0.001$).

Conclusion: From our results we can conclude that there is a difference in endothelial cell density between patients with and without keratoconus.

Key words: endothelial cell, endothelial microscope, soft contact lens, hard contact lens, hybrid contact lens

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INTRODUCTION

Measurement of central corneal thickness (CCT) and the number of endothelial cells per mm^2 (cell density – CD) is important for several surgical procedures on the cornea. It is also significant for example for patients with endothelial dystrophy, and for patients who wear contact lenses continually. It is recommended that measurement of these corneal parameters is performed on patients before an intraocular surgical procedure, for example in the case of cataract surgery [1] or planned keratoplasty procedures [2]. The corneal endothelium covers the posterior side of the cornea and contains metabolically active cells which are responsible for the regulation of fluids between the chamber fluid and corneal stroma. It is known that only proper hydration of the corneal stroma can ensure its transparency. Knowledge of the proper functioning of the corneal endothelium enables us for example to evaluate the transplantation of a donor cornea or the risk of intraocular or corneal procedures in clinical practice [3]. The cornea, like other tissues, is also subject to changes in relation to age. The corneal endothelium has only a limited capacity for regeneration [4]. Insufficient proliferation of endothelial cells leads to a decline in their number. The number of endothelial cells decreases with age, and the remaining cells have a tendency to enlarge and change their shape in order to fill the empty space on the posterior side of the cornea [5]. During the course of ageing of the cornea and endothelium, there is also a deterioration of the function of the endothelial pump [6]. The thinning of the cornea influences the quality of measurement of intraocular pressure, resulting in false low values of intraocular pressure [7]. It is therefore very important to know the normal values of corneal thickness and endothelial cell density in various different groups within the population [8,9].

The endothelial layer of the cornea is a layer of cells whose chief role is to ensure the hydration and transparency of the cornea [10]. In the past the corneal endothelium was evaluated primarily biomicroscopically, and was limited by the quality of imaging of the cells. It was very difficult to estimate the quality and reserve capacity in the number of individual endothelial cells [11]. Since the discovery of methods such as specular endothelial microscopy, fluorophotometry and pachymetry, it has been possible to perform qualitatively and qualitatively better evaluation of the morphological and functional properties of the endothelial cells and their density. Specular endothelial microscopy enables us to determine and compare information about the endothelium in various different age and ethnic groups [12,13].

We can examine the corneal endothelium also with the aid of a slit lamp if we use direct lighting using the technique of an optical or parallel cross-section, or a mirror reflection. In 1918 Alfred Vogt performed the first imaging of a corneal endothelium using the mirror reflection technique [14]. These techniques enable only referential examination. Specular endothelial microscopy enables analysis and measurement of the parameters of the corneal endothelium. The first specular microscope was developed by David Maurice in 1968 [14]. In principle it records light, which is reflected and dispersed onto the optical interface of the endothelium – chamber fluid. The light is reflected from the corneal endothelium onto the microscope lens and subsequently onto the detector. Here the path difference between the reflected rays is measured, and an image of the endothelium is produced which can then be observed or photographed. The main advantage of this imaging is the greater magnification of the image and the quality detail of the imaging of the individual cells. With the aid of the appropriate software, it is possible to conduct an analysis of the size of the cells, their number per spatial unit, and to evaluate their shape [15].

Wearing contact lenses has an influence on the cell density (CD), size (which is also evaluated with the aid of coefficient variation – CV) and shape of the endothelial cells (proportion of hexagonal cells – HEX), as documented in several studies. In their study on the population in Lithuania, Galgauskas et al. [3] demonstrated a difference in the endothelial parameters (CV, HEX) between a group of wearers and non-wearers of hard contact lenses. By contrast, they did not demonstrate this difference between wearers and non-wearers of soft contact lenses.

In our practice we use hard contact lenses (HCL), which are classified within the group of RGP (rigid gas permeable) lenses. Their main advantage is that they are oxygen permeable. The permeability value (Dk) is stated within the range of 8–80. The disadvantage is that this material is not water permeable. RGP lenses are manufactured as corneal lenses from a copolymer named siloxanylalcyl-perfluoralkyl-methyl methacrylate. These lenses are most often used for patients with an irregular shape of the anterior surface of the cornea, in some cases following injuries or surgery. They are also used for patients who have keratoconus, keratoglobus or pellucid marginal degeneration [14].

Hybrid contact lenses (HbCL) are produced by combining two types of materials. The central optical part consists of RGP material, while the periphery is formed by a silicone-hydrogel ring. This lens thereby effectively combines the need to correct the irregular shape of the cornea with comfort during wear. The lens has a soft edge and the dimensions of a sclerocorneal lens. The latest version of these lenses has been available on the market since 2005, and is manufactured for example by the SynergEyes company. The disadvantage of these lenses is potential damage to the silicone-hydrogel periphery of the lens. This type of lens can be used for wearers with irregular astigmatism, keratoconus or pellucid marginal degeneration [14].

Of soft contact lenses we use either hydrogel (pHEMA) or silicone-hydrogel lenses. Soft contact lenses made from polyhydroxyethyl methacrylate (pHEMA) have a high biocompatibility, especially due to the fact that they contain a large quantity of water and are oxygen permeable. Standard lenses of this type have an approximately 40 % water content, which ensures a permeability value of Dk 8. The disadvantage of these lenses is their negative impact on the lachrymal film, which during the course of the day frequently leads to dehydration of the lens and a feeling of dry eyes. At present lenses are available on the market made of Hypergel material, the purpose of which among other factors is to eliminate the feeling of dry eyes.

Soft contact lenses made of silicone-hydrogel combine the properties of hydrophilic and hydrophobic material. The purpose of the silicone components is primarily to ensure high oxygen permeability. The permeability of these lenses may even exceed the value of Dk 100. The disadvantage is their greater rigidity, which together with the higher abrasion coefficient may cause discomfort during wear of this type of contact lens [14].

The objective of our study was to demonstrate a difference in cell density (CD) of endothelial cells between a group of patients with contact lenses (hard contact lenses – HCL, hybrid contact lenses – HbCL, soft contact lenses – SCL) with keratoconus and a group of patients without contact lenses (without keratoconus). We also aimed to demonstrate a decrease in the number of endothelial cells in correlation with advancing age, in both subjects without keratoconus who did not wear contact lenses, and subjects with keratoconus with two types of contact lenses (HCL, HbCL). Last but not least, we also aimed to confirm a decline in the number of endothelial cells in correlation with the length of wear of three types of contact lenses (HCL, HbCL and SCL).

MATERIAL AND METHODS

In our study we had data from 96 subjects with an average age of 40.5 ±14.05 years. For the purposes of our study, we worked with each eye separately on the individual subjects. We therefore had a total of 192 eyes available to us. Keratoconus (research group) was diagnosed in 97 eyes. The average age of the patients from the research group was 41.9 ±10.6 years. Keratoconus was not diagnosed in the remaining 95 eyes (control group). The average age of the patients in the control group was 39.5 ±16.6 years. In the group with keratoconus, the patients wore hard contact lenses (HCL) in a total of 43 eyes, hybrid contact lenses (HbCL) in 48 eyes and soft contact lenses (SCL) in 6 eyes. The average total length of wear of contact lenses in the research group was 10.6 ±2.36 years (minimum 2 years). For the purposes of this study, we did not observe the influence of the development of the disease (keratoconus) on the cornea on cell density (CD), namely the number of endothelial cells per square millimetre. We were interested mainly in the influence of various different types of contact lenses on CD. The results of this study will therefore be contaminated by changes in the progression or alternatively stagnation of this disease.

We performed an examination using a Nidek CEM-530 endothelial microscope, which is supplied to our market by the company OCULUS, spol. s r.o. This is a fully automatic contactless instrument for measuring the corneal endothelium and its thickness. Measurement is very quick and precise. A total of 16 images are produced upon each measurement. The scanned area is 0.25 x 0.55 mm. Scanning is possible in the centre of the cornea, in 8 points paracentrally and in 6 points in the corneal periphery. With the aid of an automatic analysis, an evaluation is performed not only of the number of endothelial cells per square millimetre (CD), but also of their size and shape (Oculus, 2022). In our study we focused primarily on an evaluation of the average number of endothelial cells per square millimetre (CD) in the centre of the cornea (Fig. 1). We compared this variable between the stipulated groups.

The results of the examination were recorded in an MS EXCEL table and subsequently statistically evaluated with the aid of the program Statistica version 12 by the

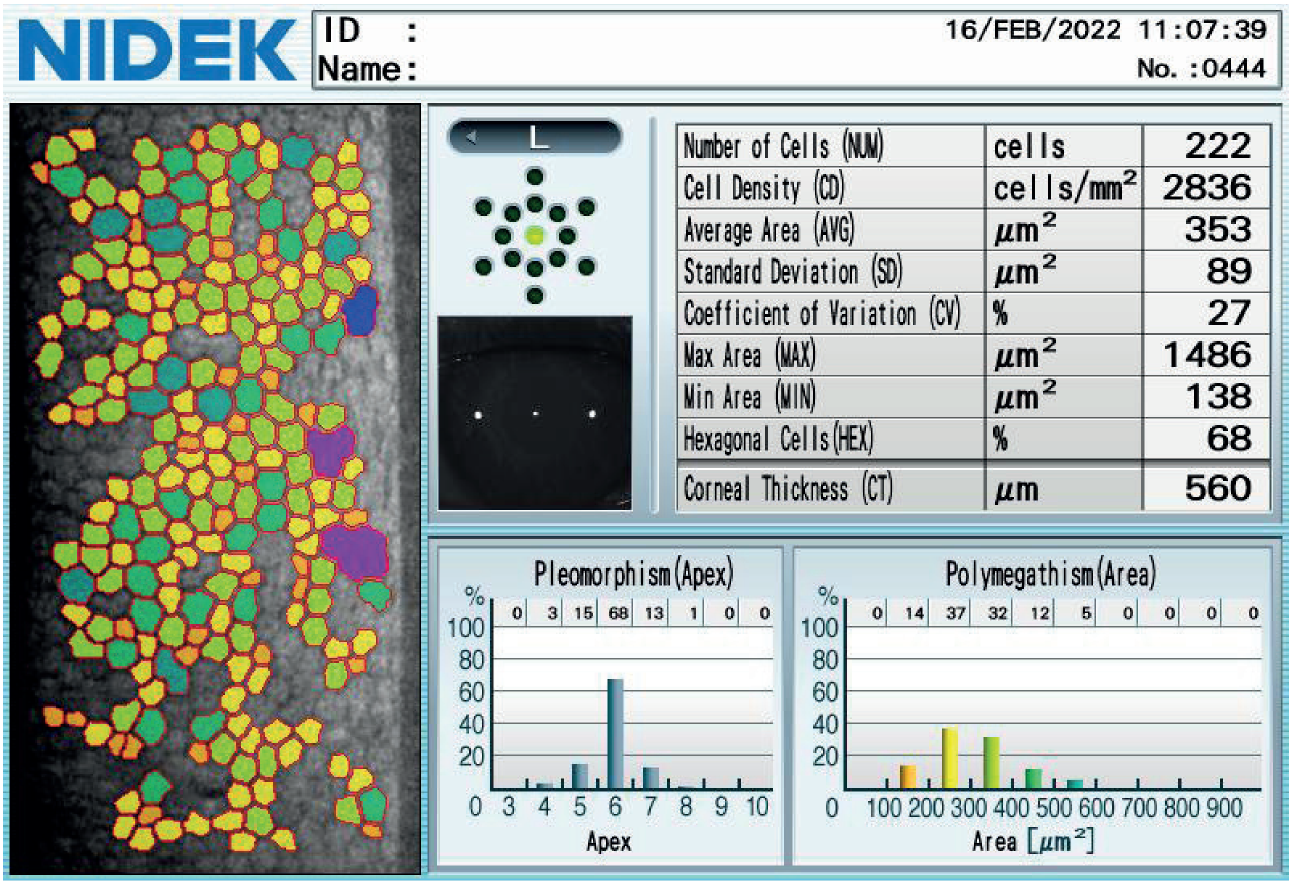

Figure 1. Example of endothelial cell count evaluation using a Nidek CM-530 endothelial microscope [16]

STATSOFT company, and MedCalc. The statistical level of significance was set at p = 0.05.

RESULTS

The average endothelial cell density in the research group was 2607.11 ±298.45 cells per mm^2. The average endothelial cell density in the control group was 2831.94 ±523.51 cells per mm^2. We tested these two variables with the aid of a T-test, which demonstrated a statistically significant difference ($p < 0.001$). By contrast, we did not demonstrate a statistically significant difference upon a comparison of the average endothelial cell density between the group of patients with keratoconus who were long-term wearers of HCL and the group of patients who were long-term wearers of HbCL (p = 0.83).

Secondarily, we were interested in changes to the average endothelial cell density in connection with patient age and the wear/non-wear of contact lenses of both types. Table 1 presents the results of the statistical comparison. A statistically significant difference was demonstrated only in the group of patients aged under 40 years, in a comparison between the group without contact lenses and without keratoconus, and the group with keratoconus who wore HCL (p = 0.02).

Finally, we dealt with the question concerning the influence of the type of contact lens and the length of its wear on the average endothelial cell density (CD). Table 2 presents a statistically significant difference between the group without keratoconus with or without SCL and the group with keratoconus wearing HCL or HbCL for a period of longer than 20 years (p = 0.01 and p = 0.02). In the case of HbCL wearers, this difference was demonstrated also after 15 years of wear (p = 0.001). No statistically significant difference in the average endothelial cell density per mm^2 was demonstrated in the group of HCL wearers after 15 years, or in the groups of HCL and HbCL wearers after 10 years of wear.

DISCUSSION

Endothelial microscopy enables us to compare normative data of the corneal endothelium between different ethnic groups or between the sexes. The average normative data about the corneal epithelium is presented in a study by Abdellah et al. [17], in which a total of 568 eyes of healthy Egyptians were measured. The age of these subjects was within the range of 20 to 85 years. Measurement was conducted with the aid of an endothelial microscope. Average central corneal thickness (CCT) was 514.45 ±43.04 μm. The average size of one endothelial cell was 350.49 ±149.94 μm^2 and the average endothelial cell density was 2647.50 ±382.62 per mm^2. In this study, the authors did not demonstrate any statistically significant difference between endothelial cell density per mm^2 between men and women (p = 0.171). Similarly, the size of the individual endothelial cells did not differ between the sexes (p = 0.09). However, a difference was demonstrated between men and women in the variable referred to as coefficient variability (CV) of the size of endothelial cells (p = 0.024), and hexagonal shape of cells (p = 0.015). The variables of CCT (p = 0.007, r = -0.113) and CD ($p < 0.001$, r = -0.357) showed a statistically significant negative correlation with patient age. By contrast, the variable CV ($p < 0.001$, r = 0.341) and the size of the individual endothelial cells (p = 0.008, r = 0.111) showed a statistically significant positive correlation with age. The authors of this study also calculated that the average loss of endothelial cells is approximately 0.3% per year.

The average CD value of our control group was 2831.94 ±523.51 cells per mm^2 (average age was 39.5 ±16.6 years). We can compare these values with the value from the study by Abdellaha et al. [17], in which the average calculated CD value was 2933.75 ±345.92 cells per mm^2 for the group of individuals within the age range of 20–30 years. This is approximately 100 cells per mm^2 more. For comparison we can also present the average CD values for example for the American population within the

Table 1. Statistically significant differences in average endothelial cell density between non-users of contact lenses without keratoconus and users of two types of contact lenses with keratoconus depending on age of patients

T-test, $p < 0.05$	up to 30 years	up to 40 years	up to 50 years
without CL, without KTC (control)	2782,75	2901,50	2784,25
HCL + KTC	3108,67	2434,75	2714,63
HbCL + KTC	2445,86	2703,75	2748,25

CL – contact lenses, KTC – keratoconus, HCL – hard contact lenses, HbCL – hybrid contact lenses

Table 2. Statistically significant differences in average endothelial cell density between non-users of contact lenses without keratoconus and users of two types of contact lenses with keratoconus depending on length of use

T-test, $p < 0.05$	up to 10 years	up to 15 years	up to 20 years
SCL/without KC, without CL (control)	2753,92	2887,11	3046,75
HCL + KTC	2761,21	2582,20	2486,20
HbCL + KTC	2694,71	2488,25	1931,50

SCL – soft contact lenses, CL – contact lenses, KTC – keratoconus, HCL – hard contact lenses, HbCL – hybrid contact lenses age range of 31–40 years. The average value was 2739 ±208 cells per mm^2 [13], which by contrast is 100 cells per mm^2 less. Average endothelial cell density in our research group was 2607.11 ±298.45 cells per mm^2, which is 224 cells per mm^2 less than in the control group. In our study we succeeded in demonstrating a statistically significant difference in CD between the research and control groups.

The majority of studies demonstrate a natural decrease of CD in correlation with age. A study conducted by the authors Nemesure et al. [18] demonstrated a loss of CD by 5–6% every 10 years. Another study by the author Moller-Pedersen [19] shows a loss of 0.3% every year. The average loss of CD in our control group was 0.29% per year. We did not succeed in demonstrating a statistically significant loss in the individual groups in correlation in age. We detected a statistically significant difference ($p = 0.02$) in CD only in a single case, namely in the group within the age range of 30 and 40 years, between the group with and without keratoconus, who wore HCL.

Several studies have demonstrated a negative impact of long-term wearing of contact lenses. For example, the author Nieuwendaal [20] demonstrated a difference between the size of endothelial cells in contact lens wearers of 307 ±35 µm^2 versus 329 ±38 µm^2 in the control group. A study by the authors Setälä et al. [21] demonstrated a difference in the CD parameter between a group of hard contact lens wearers (longer than 10 years) and a control group. In the HCL group the value was 2846 cells per mm^2 and in the control group the measured value was 2940 cells per mm^2. The difference was statistically significant ($p < 0.05$). In our study we identified a statistically significant difference between the control group and the group of HbCL wearers after a wearing for a period of longer than 15 years. After 20 years of wear of HbCL and HCL, a statistically significant difference was recorded in both of these groups in comparison with the control group.

CONCLUSION

The results of our study demonstrated a statistically significant difference in endothelial cell density (CD) between the research and control groups. This means that the average number of endothelial cells in the group of patients with keratoconus and contact lenses (HCL, HbCL, SCL) was statistically significantly lower (by 224 cells per mm^2) than in the group without contact lenses and without keratoconus.

Secondly, we did not demonstrate any statistically significant difference in the loss of endothelial cells in the group of patients without contact lenses and without contact lenses in comparison with wearers of two types of contact lenses (HCL, HbCL) with keratoconus in correlation with their age.

Thirdly, we demonstrated a difference in corneal endothelial cell density between the group with keratoconus (with or without SCL) and the group with keratoconus wearing HCL or HbCL for a period of longer than 20 years ($p = 0.01$ and $p = 0.02$). In the group of HbCL wearers, this difference was demonstrated also after 15 years of wear ($p = 0.001$). No statistically significant difference in average endothelial cell density (CD) was demonstrated in the group of HCL wearers after 15 years of wear, or in the group of HCL and HbCL wearers after 10 years of wear.

From our results, we can therefore draw the conclusion that a difference exists in endothelial cell density (CD) between patients with and without keratoconus. Furthermore, we can conclude that the loss of CD in correlation with age is similar in patients both with and without keratoconus. Last but not least, we may assume that after 20 years of wearing HCL or HbCL, a pronounced decrease of CD occurs in patients with keratoconus in comparison with patients without keratoconus, and moreover that a loss of CD will occur earlier (after 15 years) in wearers of HbCL in comparison with wearers of HCL.

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